Pediatric Physiologically Based Pharmacokinetic (PBPK) Modeling to Advance Knowledge of Breastfeeding Infant Exposure to Maternal Medications

by

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Author’s Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

While there are benefits of breastfeeding to the maternal-infant pair, mothers taking medication may decide not to breastfeed amid unclear risks of exposing the infant to the drug through milk. Uncertainty arises mainly due to the fact that lactating mothers and breastfeeding infants are excluded in the drug development process. In lieu of necessary data for decision making, existing resources include metrics to help estimate risk to the breastfed infant and informational resources that aim to gather all sparsely available information in databases to increase accessibility and empower healthcare providers with knowledge. Current metrics such as the relative infant dose, solely estimate the dose the infant would intake. Before better understanding the potential adverse events an infant might experience (response), a step further to understand exposure is paramount. Yet, the availability of exposure information is difficult to ascertain due to the lack of critical information on the pharmacokinetics (PK; movement of drugs in the body describing dose to exposure) of drug secretion into breast milk, and the resultant levels or exposure of the drug in infant plasma.

Physiologically based pharmacokinetic (PBPK) modeling is a promising tool to fill in the gap of scant maternal medication exposure information in breastfeeding infants. PBPK models use a simulation-based approach to model drug kinetics in an organism using knowledge of anatomy and physiology and the physicochemical properties of the drug. Pediatric PBPK models can be developed with minimal *a priori* data in children because these models rely on a mechanistic understanding of the disposition of the drug typically learned from rich adult data. Thus, despite the lack of available data on drug PK in infants, pediatric PBPK modeling can be used to simulate virtual breastfeeding infant populations to predict exposure given proper estimated doses.

The aim of this thesis is to use PBPK modeling to produce a novel risk metric that advances the knowledge of breastfeeding infant exposure to maternal medications. The objectives are to (1) create and apply a workflow incorporating pediatric PBPK modeling to develop the novel metric with infants breastfed from mothers taking lamotrigine, cannabidiol (CBD), and ezetimibe, (2) identify potential maternal factors that may impact concentrations of drugs in milk for incorporation into the workflow established in objective 1 for CBD, and (3) optimize the utility of the novel metric for use in clinical practice. To arrive at the first objective, a literature review was used to develop a model to describe the weight-normalized volume of intake infants typically receive. The model was then used in combination with literature (lamotrigine) or collaborator collected (CBD and ezetimibe) drug

concentrations in breast milk to estimate infant daily doses. The doses were then given to virtual breastfeeding infants created through developed and evaluated pediatric PBPK models. For the second objective, linear regression was used to identify influential maternal factors on CBD milk concentrations and breastfeeding exposure predictions. Finally, qualitative interviews were conducted with healthcare providers to ascertain perspectives on the novel metric for use in practice.

Through this work, a milk intake model described weight-normalized milk intake with a maximum of 152.6 mg/kg/day at 19.7 days postnatal age. The greatest risk for breastfeeding infant exposure to maternal medications occurred during the 2-4 week postnatal age window. Pediatric PBPK models were developed for lamotrigine, CBD, and ezetimibe. For CBD, literature *in vitro* data informed the identity and percent contributions of metabolizing enzymes to clearance. These contributions were ascertained as UGT1A7 4%, UGT1A9 16%, UGT2B7 10%, CYP3A4 38%, CYP2C19 21%, and CYP2C9 11%. This information was used to populate the CBD pediatric PBPK model. Results from the linear regression analysis with maternal factors, including administration type, dose-frequency of use, and time after last dose of CBD, revealed that oil or pipe and joint/blunt or edible administrations produced the highest and lowest CBD concentrations in milk, respectively. Overall, the three PBPK models were able to adequately predict exposures of the drug administered in children. A novel risk metric termed the upper area under the curve ratio (UAR) was developed to describe the 95^{th} percentile of breastfed infant AUC divided by the median therapeutic AUC of adults or children for approved indications. Across all ages (0-1 years old), the UAR ranged from 0.18-0.44, 0.00022-0.0044, and 0.0015-0.0026 for lamotrigine, CBD, and ezetimibe, respectively. From the qualitative interviews with 28 healthcare providers, six main themes emerged: (1) Current Practice Approaches, (2) Advantages of Existing Resources, (3) Disadvantages of Existing Resources, (4) Advantages of the UAR, (5) Disadvantages of the UAR, and (6) Strategies to Improve the UAR. Multiple strategies to improve the UAR, such as combining the UAR with another resource and providing guidance to interpret the UAR were attained.

The work in this thesis developed the UAR to account for the relative exposure of breastfeeding infants to maternal medications and identify potential outliers who may be most vulnerable. Through healthcare provider interviews, it was evident that the UAR confers benefits over existing metrics and can be optimized for use in practice. With the workflow applied to further drugs, the UAR has the potential to improve our understanding of drug exposures in breastfeeding infants and be used by healthcare providers in their advising.

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Dedication

I would like to dedicate this thesis to my parents, Mabel and Andes, and my partner, David, for showing love and support for me and my efforts over the past few years. Your encouragement to look beyond studying and work has made me both a better researcher and person. Thank you for supporting my career goals and always believing in me.

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7-OH-CBD	7-hydroxy-cannabidiol
AAFE	absolute average fold error
AAP	American Academy of Pediatrics
ACOG	American College of Obstetrics and Gynecologists
ADME	absorption, distribution, metabolism, and excretion
AFE	average fold error
AUC	area under the curve
BCS	Biopharmaceutics Classification System
BLQ	below limit of quantification
CA	corrected age
$C_{avg,ss}$	average concentration at steady state
CBD	cannabidiol
CL_{int}	intrinsic clearance
CL_{spec}	specific clearance
C_{max}	peak concentration
C_{milk}	concentration of drug in milk
CNS	central nervous system
CUDDLE	Pharmacokinetics and Safety of Commonly Used Drugs in Lactating Women and Breastfed Infants
CV	coefficient of variation
CYP	cytochrome P450
DDI	drug drug interaction
DLAC	“Drugs in Lactation” Analysis Consortium

EBF	exclusively breastfeeding
ELBW	extremely low birth weight
EM	extensive metabolizer
EZE-glucuronide	phenolic ezetimibe glucuronide
F	bioavailability
FaSSIF	fasted state simulated intestinal fluid
FDA	Food and Drug Administration
FeSSIF	fed state simulated intestinal fluid
f_u	fraction unbound
GA	gestational age
GFR	glomerular filtration rate
HMB	Human Milk Biorepository
ICRP	International Commission on Radiological Protection
IDDM	insulin-dependent diabetes mellitus
IV	intravenous
K_p	tissue-to-plasma partition coefficient
L1-5	Lactation Risk Categories 1-5
LBW	low birth weight
LCMS/MS	liquid chromatography-tandem mass spectrometry
LLOQ	lower limit of quantification
M/P ratio	milk-to-plasma ratio
MMM	Medications and Mothers' Milk
MPPGL	microsomal protein per gram of liver

NHANES	National Health and Nutrition Examination Survey
NICU	Neonatal Intensive Care Unit
PBF	partially breastfeeding
PBPK	physiologically based pharmacokinetic
PDR	Physicians' Desk Reference
PI	prediction interval
PK	pharmacokinetics
PLLR	Pregnancy and Lactation Labeling Rule
PM	poor metabolizer
PN	parenteral nutrition
PNA	postnatal age
PO	taken by mouth
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PTN	Pediatric Trials Network
qd	once a day
RID	relative infant dose
RT-PCR	reverse transcription polymerase chain reaction
SD	standard deviation
SNP	single nucleotide polymorphism
SSRI	selective serotonin reuptake inhibitor
TAD	time after last dose
THC	tetrahydrocannabinol
UAR	upper area under the curve ratio

UGT	uridine 5'-diphospho-glucuronosyltransferase
UM	ultrarapid metabolizer
WHMI	weight-normalized human milk intake
WHO	World Health Organization

Chapter 1
Background

1.1 Benefits of Breastfeeding

Breastfeeding is the accepted standard for infant feeding and nutritional support and has been linked to improved health outcomes and neurodevelopmental advantages in both developed and developing countries. In fact, 823,000 annual deaths in children (13.8% of all deaths of children <2 years) could be prevented if breastfeeding practices reached universal levels (1). The American Academy of Pediatrics (AAP) and the World Health Organization (WHO) recommend that infants be exclusively breastfed for the first 6 months postpartum, after which complementary foods can slowly be introduced (2). Advantages of breastfeeding include, reduced incidence and severity of respiratory tract infections and otitis media in the newborn (3-5), and protection against allergic disease states (3, 6) and metabolic disorders such as obesity and diabetes later in life (7-10). Neurodevelopmental outcomes of preterm neonates who were breastfed have also shown improvement compared to their counterparts, as demonstrated by greater white matter and total brain volume, and increased intelligence quotients (11).

The act of breastfeeding can also provide benefits to the mother. In the short-term, breastfeeding decreases postpartum bleeding (12), leads to more rapid involution of the uterus (13), and promotes postpartum weight loss (14). Prospective cohort studies have shown an increase in postpartum depression in mothers who did not breastfeed or who weaned early as compared to those who breastfed and did not wean early (15). This benefit is likely due to bonding and skin-to-skin contact between the mother and infant promoted by the act of breastfeeding (16, 17). Breastfeeding has been found to positively influence lifetime maternal health. Studies have shown in women who breastfeed an associated risk reduction in a large range of diseases, including type 2 diabetes (18-21), rheumatoid arthritis (22), breast cancer (23), ovarian cancer (24), endometrial cancer (25), metabolic syndrome (26, 27), hypertension (28, 29), and myocardial infarction (28, 30).

1.2 Maternal Medication Use During Breastfeeding

Despite the many apparent benefits of breast milk, mothers taking medication often have difficulty deciding whether to breastfeed their infant. Uncertainty regarding the safety of breastfeeding while on medication has been frequently cited as a reason for mothers not to initiate or continue breastfeeding (31-33). This uncertainty affects women in many countries. In recent estimates, the prevalence of

breastfeeding mothers taking medications ranges from 50–96% depending on the location and its practices (34-37). In Canada, 90% of mothers initiate breastfeeding soon after their infant's birth, and assuming that 66% take medications (37), an estimated 221,000 mother-infant pairs may be affected annually.

Concerned mothers may choose not to breastfeed due to the risk of exposing the infant to the drug through milk, which has led to serious toxicity, including death in some reported cases (38-41). Alternatively, mothers may discontinue taking their medication even though the resultant infant exposure to medications may actually be low. As examples, breastfeeding mothers have been shown to be noncompliant to oral antibiotics when they were safe to take while breastfeeding (42) and to antidepressant therapies that may have been relatively safe for the infant after a risk-benefit ratio assessment (43). Moreover, these decisions may have been made as a result of advice from healthcare providers who did not have enough information to confidently make recommendations on breastfeeding while taking medication. For instance, a questionnaire at the American College of Physicians Annual Meeting demonstrated that 32% of attendees answered that they did not know whether mothers taking antiepileptic drugs could breastfeed safely (44).

This problem of maternal-infant health is in large part due to the uncertainty of risks associated with exposing the infant to the drug through milk from a lack of critical information on the pharmacokinetics (PK) of drugs in infants, information that is necessary for the risk assessment process and resulting clinical recommendations. Contributing to the lack of the PK of drugs in infants is the fact that lactating mother-infant pairs are largely excluded from the drug development process due to perceived ethical and practical challenges (45, 46). These challenges include the need for mothers to follow a strict and demanding instructed sampling schedule for formal PK studies. Currently, there is no existing workflow that focuses on drugs taken by breastfeeding mothers to adequately fill in the gap of information on the PK of drugs in breastfeeding infants. A novel workflow should consider allowing mothers to provide flexible milk samples and to factor in how the infant uniquely handles drugs as compared to adults, such as a lowered glomerular filtration rate (GFR) and liver metabolic capacity (47).

1.3 Factors to Consider in Breastfeeding and Medication Use

Understanding the PK of the drug in breastfeeding infants requires a consideration of several important factors in assessing the risk of the maternal medication. These factors can have influence

on the dose that infants could intake through breast milk and on the resulting exposure to the medication.

1.3.1 Physicochemical Drug Properties

For a drug to enter the breast milk, it must first overcome the mammary alveolar epithelium as a main barrier. Drugs can pass through this epithelium through passive diffusion, carrier-mediated transport, and transcytosis. Once in breast milk, it is immersed in an environment with a pH slightly lower than plasma (pH of 6.8-7.3 (48)) and approximately 2.1-3.1% fat in composition for the drug to partition into milk fat globules (49). Whether the drug transfers and remains in milk is mainly determined by its physicochemical properties. Generally, drugs that are low in molecular weight, non-ionized weak bases, unbound to plasma proteins, and lipophilic tend to distribute into breast milk (48).

1.3.2 Maternal Factors

1.3.2.1 Maternal Pharmacogenomics

Pharmacogenomics is a relatively new field that combines the study of drugs (pharmacology) with the study of genes and their functions (genomics) (50). These unified study topics allow pharmacogenomics to examine how genes can affect an individual's response to medications. In the context of breastfeeding mothers taking medications, metabolic or elimination pathways can be altered by maternal pharmacogenotype which can increase drug or active metabolite concentrations in breast milk and subsequent exposure to the infant (**Figure 1-1**).

The influence of maternal pharmacogenetics has been observed in breastfeeding women taking opioids for the treatment of acute pain and their infants. In 2005, a fatal case of a full-term healthy infant due to morphine poisoning from breastfeeding by a mother prescribed Tylenol T3 consisting of 30 mg codeine and 300 mg acetaminophen was reported (51). Codeine in Tylenol T3 is metabolized by cytochrome P450 (CYP) enzyme, CYP2D6, a polymorphic enzyme that can display poor metabolizer (PM), extensive metabolizer (EM), and ultrarapid metabolizer (UM) phenotypes. Morphine serves as the metabolite that is pharmacologically active and potent. Subsequent metabolism of morphine into inactive morphine-3-glucuronide and morphine-6-glucuronide is attributed to uridine 5´-diphospho-glucuronosyltransferase (UGT) enzyme, UGT2B7. Mothers with a CYP2D6 pharmacogenotype (i.e., UM) that could lead to an excess production of morphine can lead

to increased exposure in infants through breast milk. Potential effects in breastfed infants include drowsiness (52), apnea (53), and central nervous system (CNS) depression (54).

For the reported fatal case, it was later determined that the mother was heterozygous for a CYP2D6*2A allele with CYP2D6*2x2 gene duplication, also known as a CYP2D6 UM. Informed by the significant role maternal pharmacogenotypes can play in influencing infant drug exposure, a case-control study of 72 mother-infant pairs was conducted by Madadi, Ross (55). Of the 17 symptomatic infants, two mothers of infants displaying severe CNS depression were CYP2D6 UMs (55). These mothers were also of the UGT2B7*2/*2 genotype, where UGT2B7 is known catalyze the production of the active equipotent metabolite of morphine, morphine-6-glucuronide (55).

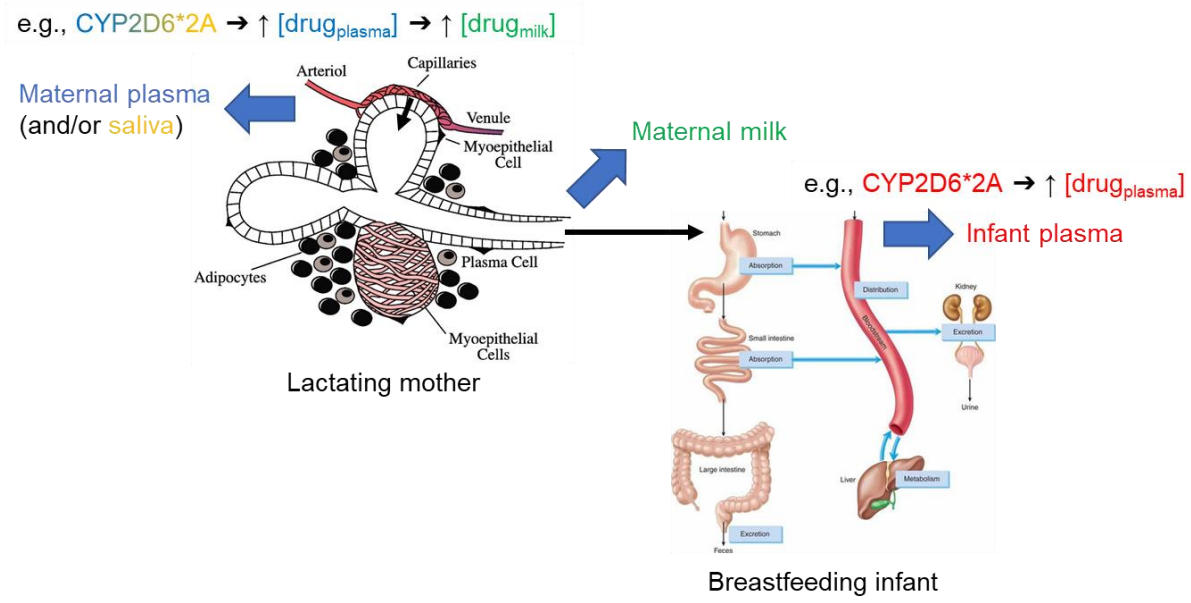

Figure 1-1. The proposed interplay between maternal and breastfed infant pharmacogenotypes. In this example, the CYP2D6*2A haplotype obtained from maternal plasma or saliva significantly increases drug plasma concentrations and therefore drug milk concentrations. In combination with the breastfed infant also possessing a CYP2D6*2A haplotype, increased drug plasma exposure may be observed.

The results of these pharmacogenomic studies in breastfeeding mothers taking codeine led to further assessments in mothers taking other medications. However, a study examining oxycodone with CYP2D6, CYP3A5, ABCB1, and OPRM1 polymorphisms showed that none of these genetic variants were associated with oxycodone-induced depression in neonates (56). In this study, mothers

were taking an average of 0.22 mg/kg/day of oxycodone for childbirth, headache/migraine, and dental/minor surgery indications. Similarly, a study by Berle, Steen (57) did not detect serum levels of sertraline and paroxetine in infants of mothers with CYP2D6 and CYP2C19 polymorphisms predicted to increase infant drug exposure levels. Although the studies in breastfed infants with mothers taking oxycodone, sertraline, or paroxetine showed inconclusive results, the sample sizes were likely not large enough to detect differences in the studied polymorphisms. Additionally, the samples did not carry some of the genotypes with potential to contribute to high drug concentrations in milk (e.g., homozygous mutant for OPRM1 118 G) (56).

The CYP2D6 and CYP2C19 genes are highly polymorphic and have been reported to influence the metabolism of selective serotonin reuptake inhibitors (SSRIs), fluvoxamine, paroxetine, citalopram, escitalopram, and sertraline (58). SSRIs are commonly prescribed antidepressants that act by increasing levels of serotonin in the brain by decreasing presynaptic serotonin reuptake. Escitalopram is an example of an SSRI that is cleared by both CYP2D6 and CYP2C19 enzymes. Since escitalopram is extensively metabolized by CYP2C19 into a compound that confers less serotonin reuptake inhibition, variants of the CYP2C19 gene may lead to altered drug exposure. In fact, CYP2C19 Ums (two increased function alleles, or one normal function allele and one increased function allele) have significantly lower exposure to escitalopram when compared to those with a "normal" rate of metabolism, or EM (two normal function alleles) (59-61). These findings prompt a closer examination into maternal pharmacogenotypes of CYP2C19, which may influence the probability of failing therapy and affect the breastfeeding infant.

1.3.2.2 Maternal Dose

The amount of drug excreted into breast milk is generally dependent on the dose of medication a mother receives. In the case-control study by Madadi, Ross (55), the authors found that in addition to maternal genotype, the dose of codeine that mothers consumed was also a significant factor to induce CNS depressed infants. Mothers of symptomatic infants (n = 17) were taking a mean 59% higher codeine dose than mothers of asymptomatic infants (n = 55) (1.62 ± 0.79 mg/kg/day vs. 1.02 ± 0.54 mg/kg/day, p = 0.004) (55).

1.3.2.3 Maternal Age and Body Weight

The effect of maternal age and body weight on the excretion of drug into breast milk has not been extensively studied. However, it is possible that milk fat content is a function of the age and body weight of the lactating mother. In a study by Lubetzky, Sever (62), macronutrient contents were measured in 38 older (≥35 years) and 34 younger (<35 years) lactating mothers at 72 hours, 7 days, and 14 days after delivery. Analyses on the milk composition of older as compared to younger mothers revealed that fat content in colostrum and carbohydrate content in mature milk were significantly higher (62). Although these results suggest increased maternal age increases milk fat content, longitudinal studies are needed to confirm this hypothesis. Variations in breast milk fat composition would affect concentration of drug in milk depending on the physicochemical properties of the drug with respect to interactions with breast milk lipids.

1.3.3 Infant Factors

1.3.3.1 Infant Pharmacogenomics

As discussed previously, maternal pharmacogenotypes can have an impact on the level of drug excretion into the milk compartment and therefore the concentration the infant receives. The pharmacogenotype of the infant is also of importance as certain haplotypes can directly affect their exposure to the drug (**Figure 1-1**). Madadi, Kelly (63) reported two cases of breastfed infants whose deaths were related to their mothers taking methadone. The first infant was a 3-week-old male born at 36 weeks' gestation who was exclusively breastfed by his mother taking 65 mg/day of methadone. The second infant was an 18-day-old male born at 35 weeks' gestation with a birthweight of 2.34 kg. His mother was prescribed 85-115 mg methadone and was also using cocaine and smoking cigarettes during pregnancy. Postmortem pharmacogenetic analyses for variants associated with methadone metabolism and response revealed that both infants were heterozygous for three single nucleotide polymorphisms (SNPs) in the ABCB1 gene and one infant homozygous for the CYP2B6*6 haplotype (63). The ABCB1 gene is known to encode for an efflux transporter (P-glycoprotein) expressed in the luminal membrane of the blood-brain barrier. Functional impairment of its activity due to heterozygous SNPs in the ABCB1 gene can lead to impaired efflux of methadone and thus a significant amount of the compound reaching the brain (64). The infant homozygous for the CYP2B6*6 haplotype was likely affected by an impaired ability to metabolize methadone which has been associated with adult mortality (65, 66).

Berle, Steen (57) were also interested in the potential role of infant pharmacogenotypes breastfed by mothers taking medications. In this study, six mothers were taking paroxetine with infants 2-33 weeks old (mean of 16 weeks). Of these pairs, a mother taking 20 mg/day paroxetine and her infant were both CYP2D6 poor metabolizers (PM). As the authors noted, having both the mother and infant as PM represents the "worst-case" scenario, with high levels of paroxetine present in maternal serum and milk, and a lowered capacity for the infant to metabolize the drug (57). However, in this case example, the resulting serum level of the infant for paroxetine was undetectable (lower limit of detection of <5 nmol/L). Nevertheless, these studies show the potential significance of infant pharmacogenotypes on drug exposure from maternal medication use during breastfeeding and the need for more research in this area.

1.3.3.2 Postnatal Age of the Infant

In a review of case reports, the number of reported adverse reactions tended to be related to the postnatal age of the infant. The review showed that approximately two-thirds of reported adverse reactions occurred during the first month postnatal age, and more than three-quarters occurred in the first two months postnatal age (67). It is important to note that these findings may suggest differences in drug in milk exposure may be related to the volume of milk an infant ingests on a weight-normalized basis. Infants tend to consume a larger volume of milk at 2-4 weeks of postnatal age and their intake decreases thereafter (68).

1.3.3.3 Gestational Age of the Infant

Infants who are born before 37 weeks of pregnancy are completed are classified as preterm. Breast milk is particularly beneficial to preterm infants in providing appropriate nutrition during their time growing ex-utero in a crucial period of accumulating nutrient reserves typical for the developing fetus (69-71), and reducing necrotizing enterocolitis and sepsis, which are more prevalent in this population as compared to term infants (72). Further attention to preterm infants is warranted since they are more vulnerable than term infants to toxicity from drug exposure through breast milk. Preterm infants have reduced capacities for drug metabolism in the liver and drug excretion in the kidneys, and as a result, eliminate drugs more slowly from the body (47, 73). In comparison to term infants, their further lowered ability to eliminate drugs may lead to high sustained drug concentrations in plasma, especially over multiple doses or feeds.

1.3.3.4 Ontogeny of Systems

Maturation of different organism systems, also known as ontogeny, has an important role in the disposition and action of drugs. The effect of ontogeny on the absorption, distribution, metabolism, and excretion (ADME) should be considered when assessing potential risk to the breastfeeding infant, as risk can differ based on age and stage of development. A review by Kearns, Abdel-Rahman (47) describes the effects of ontogeny on the ADME in infants and children. Developmental changes in absorptive surfaces and processes (i.e., age-dependent changes in biliary function) can affect the bioavailability and rate of absorption of drugs. For example, bioavailability can be altered by CYP1A1 ontogeny, where the intestinal activity of CYP1A1 is known to increase with age (74). Age-dependent changes in body composition including larger extracellular and total-body water spaces, as well as adipose tissue with higher ratio of water to lipid in neonates and young infants compared to adults can affect the extent of total distribution (V_{ss}). Ontogeny can also affect the extent and rate of organ uptake, for example, reduced transporter activity at the cellular membrane can limit uptake into specific sites (e.g., enterocytes). Developmental changes can impact clearance. Age-dependent organ-specific elimination is important to acknowledge, particularly since clearance is closely linked with exposure. A key component to organ maturation is change in metabolic capacity. Specifically, drug metabolizing enzymes each mature at different rates and thus have their own ontogeny profile (e.g., CYP3A4 activity in the liver increases as a function of age (75, 76)).

1.3.3.5 Volume and Frequency of Breast Milk Intake

The dose to the infant through breast milk is influenced by the volume of milk consumed by the infant. Generally, the volume of milk intake is proportional to the dose that is received by the infant. The daily milk intake volume of 150 mL/kg is commonly used to determine infant dose, a value first proposed by Wilson, Brown (77) in 1983 and solidified by the WHO in 1988 (78). However, as suggested by Anderson and Sauberan (79), and clearly demonstrated in longitudinal data from the United States (68), feeding volumes are not constant across postnatal ages and often have large inter-individual variability. It is therefore fundamental to capture representative intake volumes to inform more appropriate risk assessments. The number of feeds by the infant may also be important to account for in infant drug exposure. Total daily milk intake divided by the frequency gives volume per feed, which essentially determines the dose of the drug to the infant. This approximation is likely reasonable, although the volume of breast milk per feed (76 ± 12.6 g) can fluctuate based on which

breast was suckled, unpaired vs paired breastfeeding, first vs second breast of paired breastfeedings, time of day, and whether breastfeeding occurred at night (80). Higher doses lead to higher peak concentrations and may factor into a decision about risk during breastfeeding.

1.3.4 Milk Composition

The composition of milk changes during the first few days postpartum. Colostrum, a sticky yellow fluid that is high in protein content and low in lactose and fat, is secreted shortly after birth until approximately 5 days postpartum (81). Between 5-15 days postpartum, fat levels approximately double, while protein concentrations decrease by a factor of four and reach levels in mature milk. After 15 days postpartum, milk composition no longer varies significantly. The composition of milk also changes within an individual feeding. Initially, low in fat foremilk is expressed by the mother. The fat content increases disproportionately throughout a feeding, with high concentrations of fat at the end of the feeding, which is labelled as hindmilk (82). Therefore, based on the physicochemical properties of the drug, the concentration of drug in breast milk can vary during an individual feed (83).

1.4 Drug in Breast Milk Risk Assessment

Understanding the benefit-risk of maternal medication use while breastfeeding is important for clinicians to provide lactation recommendations. However, data informing these risk assessments are scarce. In a review of 213 new pharmaceutical approvals between 2003 and 2012, 47.9% of drugs had no data on breastfeeding, 42.7% had some animal data on breastfeeding, and only 4.7% had human data on breastfeeding (84). In addition, animal data are generally not useful in predicting drug concentrations in milk and human clinical data are typically derived from case studies that are considered insufficient to establish a risk or an absence of risk (85). The most direct method of assessing infant exposure is to measure the actual drug concentration in the plasma of breastfed infants. However, the procedure to take multiple plasma samples is invasive and painful for the infant. Alternatively, assessments can be performed by estimating the infant dose to determine the amount of drug an infant would ingest through milk (86). The following formula to calculate daily infant dose requires knowledge of the concentration of drug in milk (C_{milk}) and the volume of weight-normalized human milk intake (WHMI) on a daily basis:

$$Infant\ dose\ (mg/kg/day) = C_{milk}\ (mg/mL) * WHMI\ (mL/kg/day)$$

As discussed in section 1.3.3.5, the infant is often assumed to consume 150 mL/kg of breast milk per day. This value typically informs the WHMI in the infant dose calculation. Besides daily infant dose, other commonly used methods for determining the safety of a medication are the milk-to-plasma ratio (M/P ratio) and the relative infant dose (RID) which are discussed further in the next sections. There are a number of existing resources that report the M/P ratio, RID, and supporting data on the potential benefits and/or risks in databases of medications used in lactation. A summary of these main tertiary references for making clinical recommendations are presented in **Table 1-1**.

Table 1-1. Main tertiary references available for clinical recommendation

Reference	Description
LactMed (87)	LactMed is an online resource database that provides information on drugs used during lactation based on the available scientific literature. Where data are available, LactMed provides information on maternal and infant drug levels, effects in breastfed infants, effects on lactation and breast milk, and alternate drugs to consider. The database contains 1677 drugs to date.
Medication & Mothers' Handbook (88)	The Medication & Mothers' Handbook was designed to aid clinicians in determining risk to an infant from mothers taking medications. The main author of the handbook developed five risk categories (L1 to L5), collectively called, "Dr. Hale's Lactation Risk Category". The handbook is regularly updated and contains an extensive list of medications.
Drugs in Pregnancy and Lactation (89)	The Drugs in Pregnancy and Lactation textbook by Briggs and Freeman (89) is a reference guide to fetal and neonatal risk. The latest 11th edition contains more than 1200 commonly prescribed drugs taken during pregnancy and lactation and provides monographs with known or possible effects on the mother, embryo, fetus, and nursing infant.
MotherToBaby (90)	MotherToBaby is a counseling service by the nonprofit Organization of Teratology Information Specialists to provide evidence-based information to mothers, healthcare professionals, and the general public about medications and other exposure during pregnancy and breastfeeding. MotherToBaby have developed easily accessible fact sheets on frequently asked questions and provides a communication channel with mothers.
InfantRisk Center (91)	The InfantRisk Center is a world-wide call center in the Texas Tech University Health Sciences Centre in Amarillo, U.S. The center is used by physicians, nurses, lactation consultants, and mothers internationally. Up-to-date evidence-based information on the use of medications during pregnancy and breastfeeding are provided.

1.4.1 Milk-to-Plasma Ratio

The M/P ratio defines the extent to which a drug crosses from maternal plasma to the milk compartment. The M/P ratio is calculated as the ratio of the average concentration of a drug in breast milk divided by the average concentration of the drug in maternal plasma (79). An inherent problem with the M/P ratio is that maternal plasma and milk drug concentrations rarely rise and fall in parallel, thereby placing a large emphasis on the time of sampling with respect to the dose (79). To overcome this issue of variable M/P ratio values, the currently accepted method is to use the ratio of area under the curves (AUCs) of maternal plasma and breast milk drug concentrations. Although the use of AUCs is more advantageous, there are still sources of variability in this metric, such as the method of calculating AUC and the number of milk or plasma samples that need to be collected over a dose interval (92).

1.4.2 Relative Infant Dose

The RID is a widely used and generally accepted method for determining the safety of a drug to a breastfeeding infant. In this metric, the dose of the drug ingested by the infant is compared with the maternal dose or with doses of the reference drug used therapeutically in infants of similar age and weight (93). The RID is calculated by dividing the weight-normalized daily infant dose obtained through milk divided by the therapeutic dose or the weight-normalized dose received by the mother or infants of similar age and weight:

$$RID(\%) = \frac{infant\ dose\ (mg/kg/day)}{therapeutic\ dose\ (mg/kg/day)} \times 100\%$$

One of the main criticisms of the RID is the use of 10% as the cut-off where breastfeeding is considered safe, a threshold that was not developed based on experimental data. In fact, guidelines have suggested that 5% should be the cut-off for drugs with psychotropic effects (94). For drugs with a broad therapeutic range, using these standard proposed cut-offs may be a misleading safety threshold for infant exposure. For example, for drug with a therapeutic window spanning 10-fold or more, at a high maternal dose such as 10-fold higher than the low-end therapeutic dose, an acceptable RID of 5% estimates that the absolute infant dose through milk is as high as 50% of the low-end therapeutic dose (95). Furthermore, there have been instances where the RID was calculated to be <10% and yet an adverse event was reported in a bupropion-induced seizure (96). The variability in the “right” cut-off value for RID is likely related to the fact that the metric only considers drug dose

that is translated to level of risk, thereby not accounting for how infants uniquely handle drugs (e.g., drug exposure). Additionally, RID does not address the variability in milk intake volumes and drug concentrations in milk as seen in the population.

1.5 Physiologically Based Pharmacokinetic Modeling

In past two decades, physiologically based pharmacokinetic modeling (PBPK) modeling has become well-recognized for its utility in several contexts, such as predicting the interplay between drugs via drug-drug interactions (97). PBPK models have the ability to provide *in silico* estimates of drug exposure given the proper parameterization with host physiology and drug properties (98). Whole-body PBPK models consist of 15 organs and two blood organs (17 compartments) that describe drug transfer between compartments using ordinary differential equations (**Figure 1-2**). A common application of PBPK modelling is extrapolation to special populations by using relevant physiological information of the target population to make predictions based on information with high confidence (e.g., drug PK in healthy adults). This practice is particularly useful when predicting drug exposures in pediatrics <2 years of age when processes governing drug disposition are not fully mature. A workflow to scale from adult to pediatrics has been formalized by Maharaj, Barrett (99) and is described in **Figure 1-3**. Using these concepts, virtual breastfeeding infants can be created through pediatric PBPK modelling in order to aid in understanding the risk of drug exposure to infants. This approach is advantageous since current metrics, such as the RID, focus on assessing risk directly from maternal and infant dose. The existing metrics do not specifically account for: (a) the anatomy and physiology of the breastfeeding infant, (b) age dependent factors (e.g., milk intake volumes across age), and (c) the variability in infant and maternal population. A complete list of factors that differentiate a metric that incorporates PBPK modeling from the M/P ratio and RID are presented in **Table 1-2**. These factors are particularly important to identify outlier infants who may be most vulnerable to drug toxicity.

In contrast, integrating the infant dose with a PBPK model can lead to a metric of exposure that, when linked to a measure of safety, forms the basis for risk assessment. PBPK models can account for how an infant uniquely handles the drug and with a workflow that incorporates variability, particularly in milk intake and in drug concentrations in milk, it can be used to derive an improved measure of risk. To date, few PBPK models have been developed to simulate breast milk exposure to maternal drug therapy or chemical toxicants, although the practice is growing (100-113). None of

these models have attempted to create a workflow that can be used to derive a novel metric that could be used by healthcare providers advising mothers taking medication during breastfeeding.

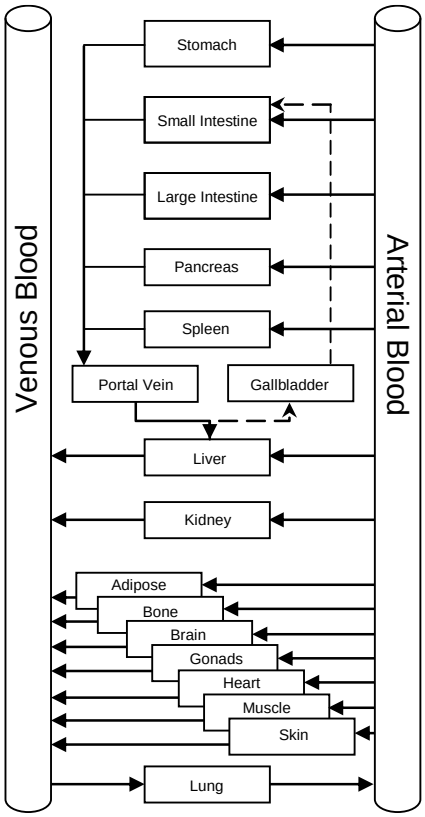

Figure 1-2. Whole-body PBPK model adapted from Edginton, Zimmerman (114). The 15 organs and two blood organs represent compartments. The arrows between the compartments describe drug transfer using ordinary differential equations.

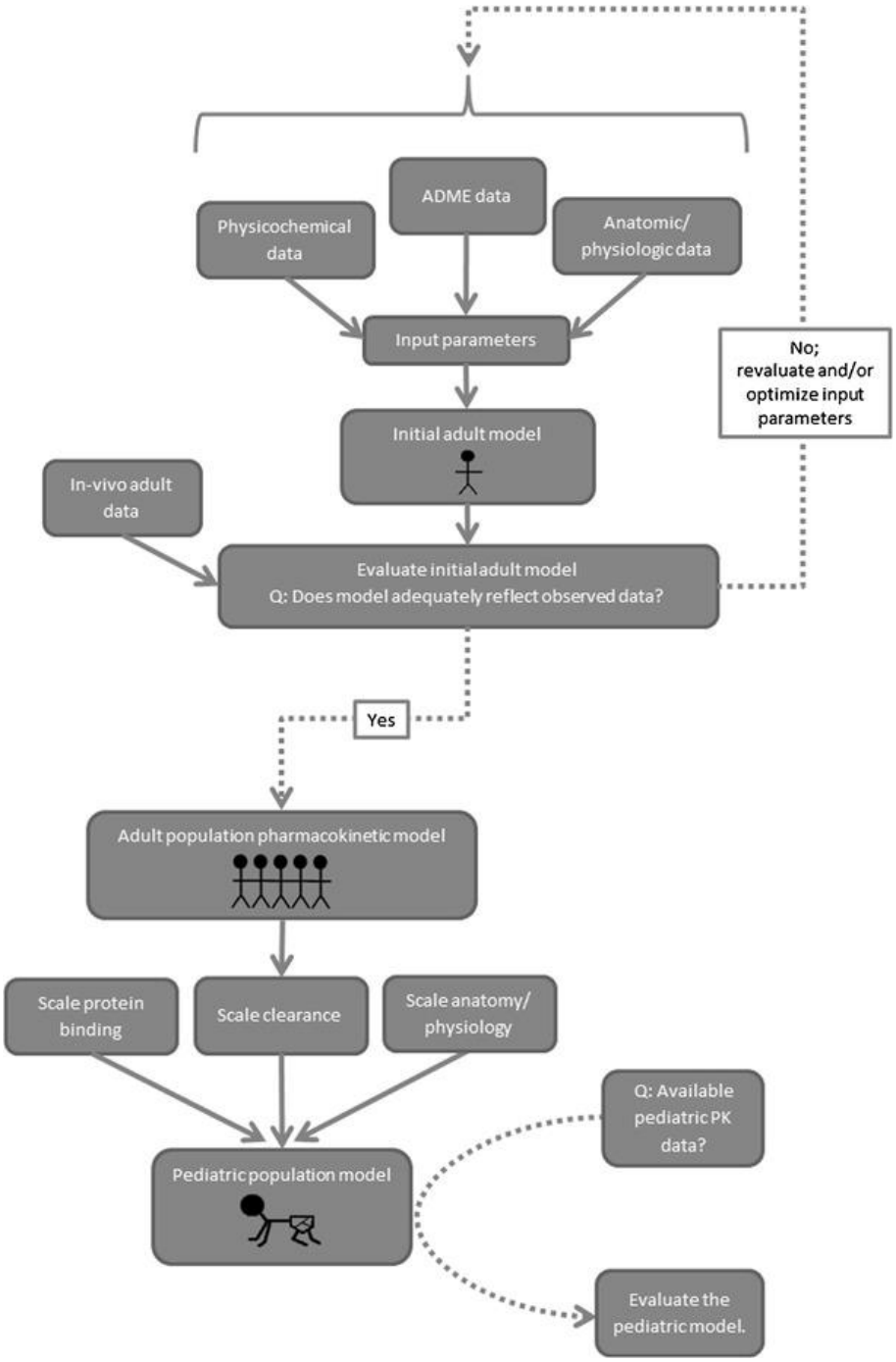

Figure 1-3. An established workflow to scale adult PBPK models to pediatric populations. Figure from Maharaj, Barrett (99).

Table 1-2. Factors accounted through PBPK modeling compared with existing metrics

Accounts for…	M/P ratio	RID	A metric incorporating PBPK modeling
Dose to infant via milk	No	Yes	Yes
Comparison to maternal therapeutic dose	No	Yes	Yes
Oral bioavailability in infants	No	No	Yes
Age of infant	No	No	Yes
Most vulnerable children with lowest clearance	No	No	Yes
Most vulnerable children receiving highest dose	No	No	Yes
Active metabolites	No	No	Yes
Systemic exposure of infants	No	No	Yes
Factors that may lead to high dose to infant (e.g., maternal pharmacogenotype)	No	No	Yes

1.6 Real World Data on Medications in Breast Milk

To address the lack of data on medications in milk, the Food and Drug Administration (FDA) provides recommendations on conducting clinical lactation studies (115). In the guidance three general study designs are proposed: (1) lactating woman (milk-only) study, (2) lactating woman (milk and plasma) study, and (3) mother-infant pair study. With respect to improving our understanding of PK of the drug in breast milk and in the breastfeeding infant, the latter two study types are especially useful. In milk and plasma studies, milk and plasma are collected from lactating women to obtain drug PK data. This information can provide information on the amount of drug transferred into breast milk. Next, mother-infant pair studies include measuring drug concentrations in both the breast milk and infant plasma. These studies are particularly advantageous when the drug is known to accumulate in breast milk and likely to be absorbed by the breastfed infant. Besides general study designs, the guidance reflects on additional considerations. Further factors for consideration include maternal factors (e.g., weight, age, gestational age at delivery, concomitant drugs) and infant factors (e.g., age, weight, history of prematurity, existing medical conditions), a majority of which were discussed in sections 1.3.2 and 1.3.3 of this thesis. Moreover, the FDA recommends including milk sampling

methods such as the type of milk collected (foremilk versus hindmilk, timing of dose and milk sample, and colostrum vs mature milk) which were mentioned in section 1.3.4.

Altogether, these recommendations are directed towards industry for pre- or post-marketing lactation studies. However, several academic groups have developed platforms to collect voluntary breast milk samples from lactating mothers using these principles. Real-world data collection serves as a practical approach to obtain an improved understanding of drug PK during lactation. Two ongoing studies performing such data collection are the "Drugs in Lactation" Analysis Consortium (DLAC) and Mommy's Milk Human Milk Biorepository (HMB).

1.6.1 The Drug Lactation Analysis Consortium

DLAC is a voluntary sample collection platform initiated and monitored by the Hospital for Sick Children (SickKids). The platform allows the Drugs in Breast Milk Study (PI: Shinya Ito) to have a multi-center, multi-drug, open-label, opportunistic PK study design. Recruitment sites for the study include SickKids, the Epilepsy Clinic (Toronto Western Hospital), the Diabetes and Endocrine in Pregnancy Program (Mount Sinai Hospital, Toronto), the Section of Endocrinology (Hôpital St-Boniface Hospital, Winnipeg), and the Reproductive Life Stages Program (Women's College Hospital, Toronto). Interested breastfeeding mothers taking medication as part of their medical care are directed to the DLAC website (http://www.thedlac.com/) to participate in the study. The study is designed to be open-label and focus on target drugs including escitalopram, lamotrigine, methotrexate, and levetiracetam. These medications were identified as currently feasibly used by mothers, and if discontinued would pose significant harm to mothers. Additionally, the drugs had published toxicity reports, or a clinical need was recognized and drug concentrations in milk data were non-existent or minimal. **Table 1-3** presents previously studied and current DLAC study drugs and summarizes the key physicochemical and ADME properties considered at infant PBPK modeling stages. In contrast with conventional tight-schedule PK study designs, the Drugs in Breast Milk Study employs opportunistic sampling, where breastfeeding mothers have flexibility in sample collection times and drug concentration profiles are generated at a population level.

Table 1-3. Basic physicochemical and ADME properties of DLAC study drugs

Study drug	M/P ratio	RID (%)	LogP	Plasma protein bound (%)	Main elimination	Substrate for transporters
Escitalopram	2.2	5.3	3.5	56	CYP2C19, CYP3A4, CYP2D6	PGP (weak)
Ezetimibe	NR	NR	4.14	>90	UGT1A1, UGT1A3, UGT2B15	PGP, ABCC2, ABCC3, ABCG2, OATP1B1
Lamotrigine	0.4	9.2	2.5	50	UGT1A4, UGT2B7	PGP, OCT1, OAT1, OAT3
Levetiracetam	1.05	6.9-13.8	-0.6	<10%	Type B esterases in the blood and other tissues	Pgp
Methotrexate	0.08	0.11	-0.85	50	Renal	ABCC3, ABCC4, ABCC1, SLC22A6, ABCC10

M/P ratio: milk-to-plasma ratio. RID: relative infant dose. NR: not reported.

As the Project Center, SickKids personnel perform the data collection process (**Figure 1-4**). For study enrolment, the inclusion criteria for mothers are that they are ≥18 years old, taking at least one of the study drugs as per standard of care, obtained informed consent, and have the ability to communicate in English. Mothers are excluded if they are taking any concomitant condition or medication judged by the SickKids Principal Investigator that would preclude the subject's participation in the study, and/or if they are pregnant during PK sampling. Infants are included if they are older than 1 week old and exposed to one of the study drugs through breast milk and are excluded if they have any concomitant condition or medication judged by the SickKids Principal Investigator that would preclude the subject's participation in the study. After inclusion of the participant, demographic information, milk, blood, and saliva samples from lactating mothers and/or their infants are collected.

Following data collection, SickKids provides the Drug Analysis Core team at the Centre Hospitalier Universitaire Sainte-Justine (Co-PI: Julie Autmizguine) with the samples for assessment (**Figure 1-4**). The Drug Analysis Core focuses on developing and validating assays for measuring the study drugs in the biological samples. Once drug concentrations are attained, de-identified data is

provided to the Modeling Core at the University of Waterloo (Co-PI: Andrea Edginton) to predict drug exposures in virtual breastfed infants (**Figure 1-4**).

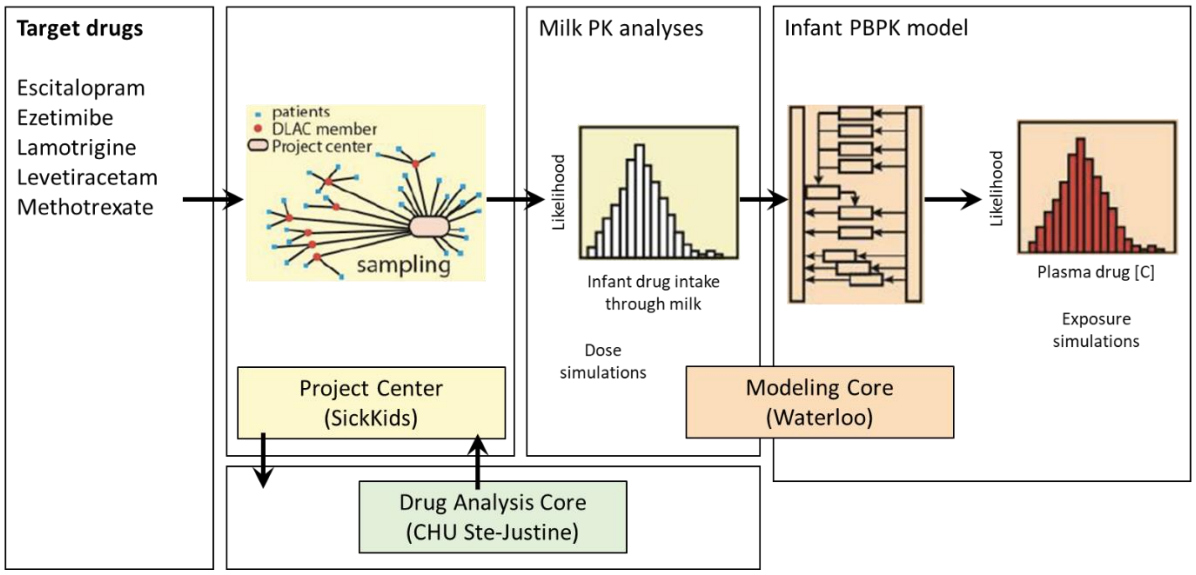

Figure 1-4. Overview of the Drugs in Breast Milk Study and the roles of collaborating centers. Adapted from CIHR Project Grant Proposal - RN# 354927.

1.6.2 Mommy's Milk Human Milk Biorepository

The Mommy's Milk HMB is a US and Canada-wide study that collects human milk samples from mothers who were or were not taking medications and recreational drugs, including marijuana, cannabidiol (CBD), and CBD-containing products. The biorepository was an initiative started in 2014 by investigators (PI: Christina Chambers) at the University of California San Diego to have an improved understanding of maternal exposure to various agents.

Detailed information on recruitment, data collection, and sample preparation and analysis methods have been reported by the Principal Investigators (116). Briefly, participants complete an interview to provide their demographics, maternal and child health history, breastfeeding habits, and all maternal exposures mainly in the previous two weeks prior to sample collection. Exposure information from women who medication or recreational drug use at any time since giving birth include route of administration, frequency of use, dose, and time since last use before milk sample collection.

Cannabis and its main cannabinoids, tetrahydrocannabinol (THC) and CBD, were specific drugs of interest by the Mommy's Milk study team. Through the HMB, milk samples from mothers taking products containing these substances have been collected and measured. Of particular interest is CBD, the cannabinoid that is rarely studied, especially in breastfeeding maternal-infant pairs.

1.7 Workflow with Breast Milk Real World Data and Pediatric PBPK Models

Real world data from mothers taking medications during breastfeeding can be leveraged when multiplied by a daily weight-normalized volume of milk intake that infants typically receive. As a result, doses are estimated and can be given to virtual breastfeeding infants created through evaluated pediatric PBPK models. With infants administered estimated doses via breast milk, exposures can be predicted across infant age groups and compared with exposures of individuals receiving therapeutic doses. This proposed workflow is outlined in **Figure 1-5**.

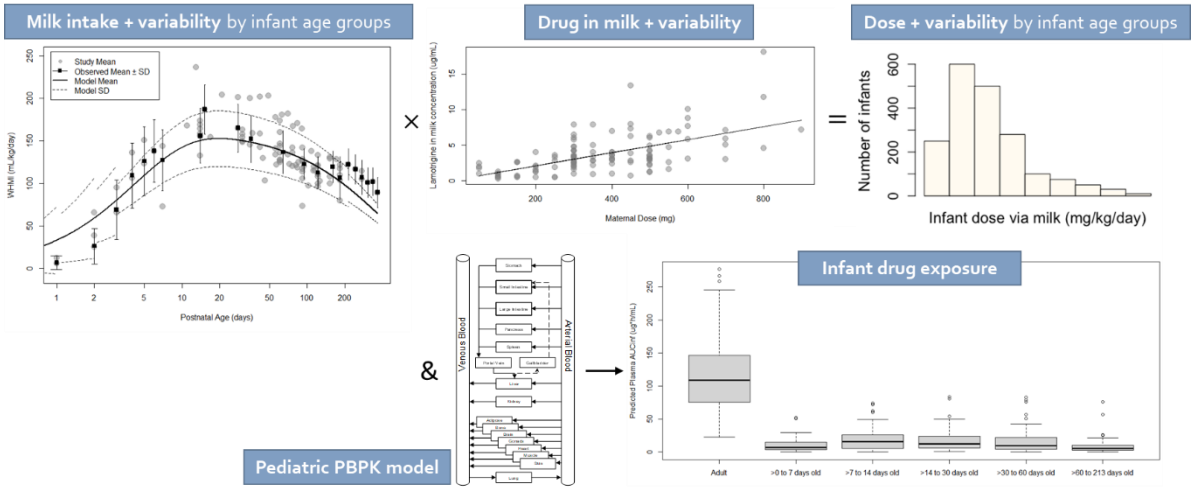

Figure 1-5. A workflow incorporating literature review derived weight-normalized volume of milk intake and variability across postnatal age and drug concentrations in milk and variability to estimate infant daily dose by age groups. Doses administered to virtual breastfeeding infants created through an evaluated pediatric PBPK model will result in predicted infant drug exposures.

1.8 Aim, Objectives, and Hypotheses

This thesis seeks to answer the research question, among infants breastfeeding from mothers taking medications, will a workflow incorporating pediatric PBPK models to derive a novel risk metric,

serve as an improvement over current metrics to advance our description of potential risk to the breastfed infant?

The aim of this thesis is to produce a novel risk metric to advance the knowledge of breastfeeding infant exposure to maternal medications.

1.8.1 Objective 1: Evaluate the Approach to Derive a Novel Risk Metric

Objective 1: Evaluate the accuracy of a new approach to predict drug exposure in populations of breastfeeding infants that combines measured drug concentrations in milk, weight-normalized volume of milk intake, and pediatric PBPK modelling.

Hypothesis: The new approach to predict drug exposure in populations of breastfeeding infants will be deemed accurate through evaluations with observed data.

1.8.2 Objective 2: Identify Contributing Influential Maternal Factors

Objective 2: Identify maternal factors in the general population that may contribute to variability in drug concentrations in breast milk for incorporation into the workflow established in **Objective 1**.

Hypothesis: Multiple maternal factors related to their medication administration will be used to explain the variability in drug concentrations in milk.

1.8.3 Objective 3: Determine and Optimize the Utility of the Novel Metric

Objective 3: Determine whether the novel pediatric PBPK-derived novel metric that incorporates considerations from **Objectives 1** and **2**, confers benefits over existing metrics and strategies to improve its use in practice by healthcare providers.

Hypothesis: The novel risk metric will confer multiple benefits over existing resources, and improvements in the metric and how it is described to healthcare providers will be ascertained.

Chapter 2

Quantifying breast milk intake by term and preterm infants for input into paediatric physiologically based pharmacokinetic models

This chapter is reflective of an original manuscript published by the PhD candidate (Cindy Hoi Ting Yeung) in *Maternal & Child Nutrition*. All pertinent dialogue in this chapter was written by the PhD candidate.

Information regarding applicable letters of copyright permission is presented in the **Letters of Copyright Permission**.

2.1 Introduction

Breastfeeding is the accepted standard for infant feeding and nutritional support and has been linked to improved health outcomes and neurodevelopmental advantages in both developed and developing countries. The American Academy of Pediatrics (AAP) and the World Health Organization (WHO) recommend that infants be exclusively breastfed for the first 6 months postpartum, after which complementary foods can slowly be introduced (117). Advantages of breastfeeding include reduced incidence and severity of respiratory tract infections and otitis media in the newborn (118-120), and protection against allergic disease states (119, 121) and metabolic disorders such as obesity and diabetes later in life (7-10). Neurodevelopmental outcomes of preterm neonates who were breastfed have also shown improvement compared with their counterparts, as demonstrated by greater white matter and total brain volume, and increased intelligence quotients (11).

Despite the many apparent benefits of breast milk, mothers taking medication often have difficulty deciding whether to breastfeed their infant. Uncertainty regarding the safety of breastfeeding while on medication has been frequently cited as a reason for mothers not to initiate or continue breastfeeding (31-33). Concerned mothers may choose not to breastfeed due to the risk of exposing the infant to the drug through milk, which has led to serious toxicity, including death in some reported cases (38, 40, 41, 55). Alternatively, mothers may discontinue taking their medication even though the resultant infant exposure to medications may actually be low. As examples, breastfeeding mothers have been shown to be noncompliant to oral antibiotics (42) and to antidepressant therapies that may have been relatively safe for the infant after a risk-benefit ratio assessment (43).

As a strategy to reduce uncertainties surrounding maternal drug use during breastfeeding, risk assessments can be performed. In these assessments, an infant dose is estimated to determine the amount of drug an infant would ingest through milk (86). Integrating the infant dose with a physiologically based pharmacokinetic (PBPK) model can lead to a metric of exposure that, when linked to a measure of safety, forms the basis for the risk assessment.

The incorporation of PBPK models in the process of drug development has become increasingly prevalent in the past two decades (97). PBPK models have the ability to provide *in silico* estimates of drug exposure given the proper parameterization with host physiology and drug properties (98). In order to fully exploit the utility of PBPK models in quantifying drug uptake in breastfed neonates, an accurate measure of infant feeding parameters, volume and frequency of maternal milk intake, is needed. Essentially, dose to the infant through breast milk is calculated by multiplying daily volume of milk intake by the drug concentration in milk (79). Knowledge of the total daily milk intake combined with information on intake frequency can help identify the peak serum concentration an infant would receive after feeding (C_{max}) and contribute to an assessment of drug safety. Total daily milk intake divided by the frequency gives volume per feed, which essentially determines the dose of the xenobiotic to the infant. Higher doses lead to higher peak concentrations and may factor in to a decision about risk during breastfeeding. The daily milk intake volume of 150 ml/kg is commonly used to determine infant dose, a value first proposed by Wilson, Brown (77) in 1983 and solidified by the WHO in 1988 (78). However, as suggested by Anderson and Sauberan (79), and clearly demonstrated in longitudinal data from the United States (68), feeding volumes are not constant across postnatal ages (PNAs) and often have large interindividual variability. It is therefore fundamental to capture representative intake volumes to inform more appropriate risk assessments.

Although feeding volumes have been captured by several reviews and reports (122-127), they focus solely on term infants, whereas milk intake by preterm neonates remains an unexplored area. Notwithstanding the lack of reviews for preterm infants, the need to study this population in relation to breast milk and maternal medication should be emphasized. Breast milk is particularly beneficial to preterm infants in providing appropriate nutrition during their time growing ex-utero in a crucial period of accumulating nutrient reserves typical for the developing fetus (69-71) and reducing necrotizing enterocolitis and sepsis, which is more prevalent in this population (72). Further attention to preterm infants is also warranted because they are more vulnerable than term infants to toxicity from drug exposure through breast milk. Preterm infants have reduced capacities for drug metabolism

in the liver and drug excretion in the kidneys, and as a result, eliminate drugs more slowly from the body (47, 73). In comparison to term infants, their further lowered ability to eliminate drugs may lead to high sustained drug concentrations in plasma, especially over multiple doses or feeds.

In this review, a comprehensive search of the literature was performed to retrieve estimates of human milk intake and frequency for term and preterm infants as a function of PNA, as inputs for PBPK models with the purpose of subsequent drug-in-milk risk assessments.

2.2 Methods

2.2.1 Eligibility Criteria

Studies reporting term or preterm infants receiving human milk with data on their volume or frequency of intake were of interest. Term infants were defined as >37 weeks gestational age (GA) at birth, and those of ≤37 weeks GA at birth were identified as preterm infants. For term infants, articles were included if data were provided on infants of any age who were exclusively breastfeeding (EBF) or infants >6 months PNA who were partially breastfeeding (PBF). These criteria were selected to reflect the AAP and WHO recommendations and produce conservative estimates for subsequent risk assessments. For preterm infants, articles were included if data were provided on infants who were exclusively human milk-fed or were PBF with a diet that consisted mainly of breast milk, where information regarding the proportion of human milk and other sources of nutrients in the diet were provided. Only articles with volume data presented in relation to individual infant's body weights, presented as weight-normalized human milk intake (WHMI), were included to reduce interinfant and intrainfant variability of daily milk consumption observed in absolute milk intake measurements. Included studies were those that measured volume and frequency of intake for at least 24 hr, as intake of breast milk tends to differ throughout the day (80). Articles were excluded if infants had significant birth complications or were otherwise unhealthy, and studies in which the intake of breast milk was influenced by the study investigators, including non-ad libitum feedings and interventions that significantly increased the milk expression of mothers.

2.2.2 Search Strategy

The search strategies consisted of MeSH headings and text words related to premature and term infants, breastfeeding and volume and frequency of intake. The Ovid MEDLINE and Embase databases were searched up to July 2, 2019. Results were limited to the English language. Complete

search strategies are provided in **Appendix A1**. In addition to the searches, reference lists of key studies on volume or frequency of breastfeeding and the grey literature were used to identify studies.

2.2.3 Study Selection and Data Extraction

Two investigators (C. H. T. Y. and S. F.) screened title, abstract and full text for inclusion. The results were not screened in duplicate. Data extraction was performed by one investigator (C. H. T. Y.).

2.2.4 Data Analysis

Daily WHMI and human milk feeding frequency from each study were presented as mean ± standard deviation (SD) ml/kg/day and number of feeds/day, respectively. When only a median value was reported for a feeding parameter, the mean was assumed equal to the median based on an assumption of normally distributed data as demonstrated previously with individual subject measurements in five of the included studies (128). Studies reporting volume of milk intake in grams were converted to millilitres using the density of milk (1.03 g/ml). PNA in days were approximated from alternative sources of infant age, such as corrected age (CA) and GA, where appropriate. Data without quantitative summaries in the literature were digitized using Plot Digitizer (v2.6.8 by Joseph Huwaldt). Data were graphically represented and analysed using MATLAB R2018b. A sample-size-weighted nonlinear regression was performed to quantify the WHMI by EBF infants across all studies using the Intiquan Toolbox (IQMTools v1.2.2.2 by Henning Schmidt and colleagues). The following function containing an integrated form of a first-order increase followed by a first order decline was selected to represent the general shape of the data using the least number of parameters:

$$WHMI = \theta_1 \cdot \frac{\theta_2}{\theta_2 - \theta_3} \cdot \left(e^{-\theta_3 \cdot t} - e^{-\theta_2 \cdot t}\right),$$

where WHMI is in ml/kg/day and t is age of the infant in days. The unknown parameters (θ_1, θ_2 and θ_3) were fitted using the observed data for breast milk volume, weighted by the sample size in each dataset. The cost function to be minimized was the sum of squared error. A simulated annealing temperature-based approach was used to explore the parameter spaces. The optimization was repeated 50 times with randomized parameter start values to confirm that a global minimum had been achieved and to explore any potential correlations between parameters.

2.3 Results

The literature search identified 2,257 nonduplicate results, and 17 articles were identified through other sources. Title and abstract screening of 2,274 records resulted in 2,054 articles excluded at this stage. In assessing the eligibility of full-text articles, 220 results were screened and 164 were excluded. The review process resulted in 52 studies presented in 56 articles. A Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) study selection flow diagram is provided in **Figure 2-1**, and characteristics of the included studies are described in **Table 2-1**.

2.3.1 Volume of Human Milk Intake

Twenty-eight and seven studies reported on the WHMI for term and preterm infants, respectively. The mean WHMI of term infants EBF (of all ages) and PBF (>6 months of age) according to age in days are presented in **Appendix A2: Supplementary Table 1**. **Table 2-2** reports the WHMI of preterm infants exclusively and partially breastfed across all PNAs. **Figure 2-2** shows mean WHMI plotted against PNA and log-transformed PNA for term (**Figure 2-2a** and **Figure 2-2c**) and preterm (**Figure 2-2b** and **Figure 2-2d**) infants.

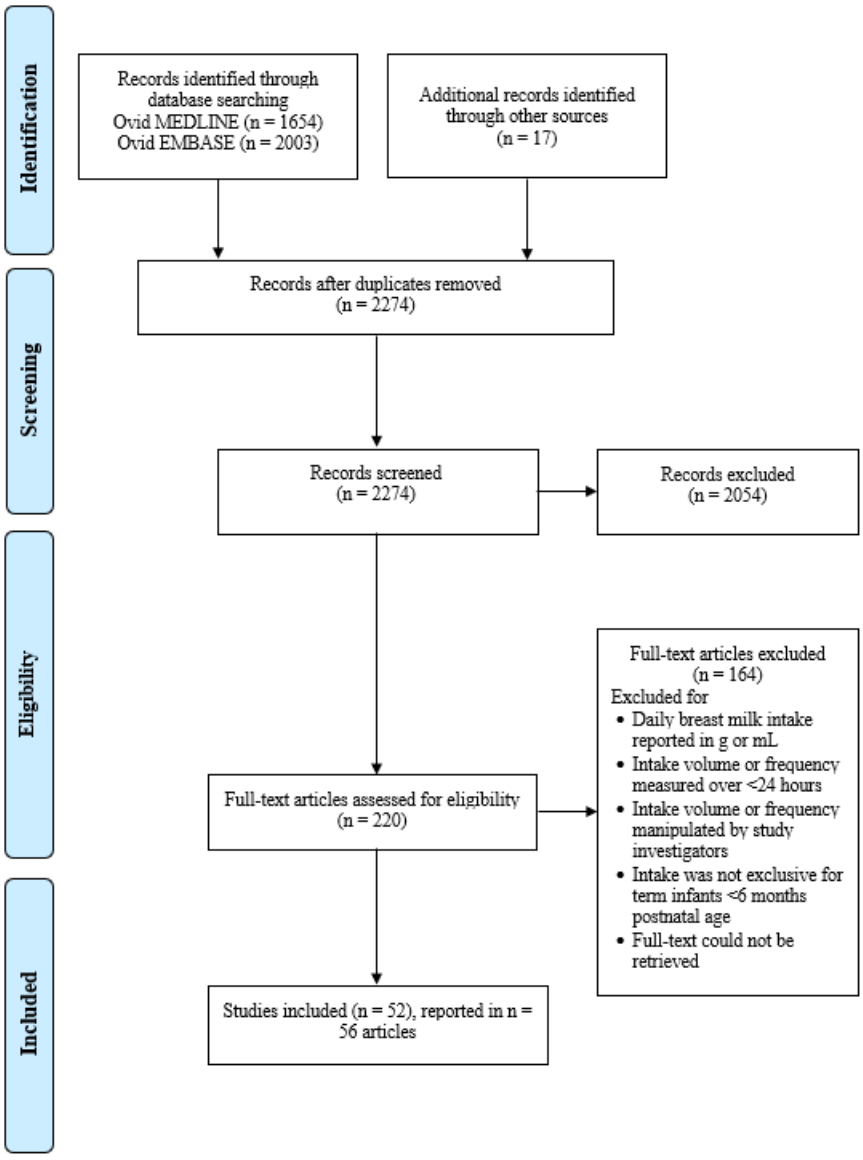

Figure 2-1. Preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow diagram of breast milk feeding parameter study selection.

Table 2-1. Characteristics of studies quantifying breast milk feeding parameters in term and preterm infants.

Study	Design	Country	Main study aim	Population	Feeding parameter(s)
Aimone, Rovet (129)	Longitudinal, randomized controlled	Canada	To examine the impact of feeding human milk containing extra nutrients for 12 weeks after discharge on premature infant bone mineralization, body composition and human milk use up to 1 year.	Thirty-four preterm infants (born <33 weeks GA, 750–1,800 g; control group: 47% male, intervention group: 74% male) who completed the 12-week randomized controlled trial by O'Connor, Khan (130) followed until 12 months CA regarding human milk feeding duration, exclusivity and time to introduction of solids.	PBF volume
Amatayakul, Wongsawasdi (131)	Longitudinal, observational	Thailand	To observe whether or not previous successful breastfeeding has any influence on subsequent breastfeeding behaviour.	Sixty breastfeeding infants (42% male) who were randomly selected from three subdistricts of the rural northern Thai population, received supplementary feeding with premasticated rice within the first month of life. The infant pairs were stratified by whether their mothers were primiparous or multiparous, and were followed until 360 days of age.	PBF volume, PBF frequency
Atkinson, Bryan (132)	Longitudinal, non-randomized controlled	Canada	To examine the adequacy of preterm infant's mother's milk as compared with donor milk or formula for the premature infant.	Of the 24 LBW premature infants (born <1,300 g) selected from all patients admitted to the NICU, eight received pooled breast milk and eight received their mother's own breast milk.	EBF volume, PBF volume

Study	Design	Country	Main study aim	Population	Feeding parameter(s)
				The infants were followed for 2 weeks during the NICU stay.	
Bandara, Hettiarachchi (133)	Cross-sectional, observational	Sri Lanka	To measure the human milk intake of infants during the first 6 months of age to assess their adequacy of human milk intake and to document the breastfeeding practices of their mothers.	Forty-eight exclusively breastfeeding healthy term infants randomly selected from maternal and child health clinics. Infants were stratified across three examined age groups: <2 months, 2 to <4 months and 4–6 months.	EBF volume
Bhutta, Abbass (134)	Longitudinal, observational	Pakistan	To survey and evaluate the growth pattern, and breast milk and fluid intake patterns of infants in Pakistan.	Twelve term infants who were a subset of the 112 surveyed mother–infant pairs recruited from a hospital medical centre, followed over 6 months.	EBF volume
Borschel, Kirksey (135)	Longitudinal, observational	U.S.	To validate the accuracy of the test weighing procedure on volume of formula intakes for formula-fed infants by comparing the test weighed measured values with those obtained by direct measurement.	Of the 35 healthy term infants of mothers from a university community who were followed for the first 6 months, 15 infants (67% male) were exclusively breastfed and test weighed. The infants were originally part of a larger study investigating vitamin B6 nutrition from consumed breast or formula milk.	EBF volume, EBF frequency
Brown, Robertson (136)	Longitudinal, observational	Bangladesh	To describe the amounts of breast milk-derived macronutrients consumed by	Fifty-eight infants of mothers from an underprivileged semiurban community were followed for 9 months. The infants generally had a lower	EBF volume

Study	Design	Country	Main study aim	Population	Feeding parameter(s)
			marginally nourished Bangladeshi infants and examine their patterns of growth.	body weight (approximating the U.S. National left for health Statistics (NCHS) 5th centile during the first 4 months and declining thereafter) than most exclusively breast-fed North American infants.	
Butte, Garza (137)	Cross-sectional, observational	U.S.	To compare the deuterium dilution technique against the test-weighing method for measuring breast milk intake.	Twenty-one infants who were breastfed exclusively (one infant customarily received water) and assessed at a mean of 3.3 ± 0.4 months of age.	EBF volume
Butte, Garza (138), Butte, Garza (139)	Longitudinal, observational	U.S.	Butte, Garza (138): To longitudinally document intakes and growth of breastfed infants to obtain normative data on human milk production and examine potential discrepancies between observed levels of human milk intake and U.S. National Center for Health Statistics (NCHS) nutrient recommendations. Butte, Garza (139): To examine the influence of maternal diet and body composition on lactational performance in a group of privileged, presumably wellnourished women to	Forty-five exclusively breastfed healthy term infants (born 37–42 weeks GA, 2,560–4,570 g; 60% male) of mothers recruited through a milk bank program, who were observed over the first 4 months.	EBF volume, EBF frequency

Study	Design	Country	Main study aim	Population	Feeding parameter(s)
			establish energy recommendations during lactation.		
Butte, Wong (140)	Cross-sectional, observational	U.S.	To compare the deuterium dilution technique against the test-weighing method for measuring breast milk intake.	Nine term infants, with five of the infants feeding exclusively on human milk and measured at approximately 1.5, 3 and 4 months of age. The remaining four infants were fed human milk and supplemental foods.	EBF volume
Butte, Wong (141)	Cross-sectional, observational	U.S.	To combine the doubly labelled water method with conventional indirect calorimetry to explore possible differences in energy utilization between breastfed and formula fed infants.	Forty term infants, of which 20 were exclusively breastfed human milk since birth. Ten of the breastfed infants were observed at 1 month, and a second set of 10 infants were observed at 4 months of age.	EBF frequency
Butte, Smith (142)	Cross-sectional, observational	U.S.	To investigate the energy utilization of breastfed and formula-fed infants, and determine whether energy utilization was different between the two feeding groups.	Sixty-five healthy term infants whose mothers were recruited from a milk bank program were observed at 1 and 4 months of age. Of the participating infants, 32 were exclusively breastfeeding (44% male).	EBF volume
Cabrera Lafuente, Montes Bueno (143)	Longitudinal, observational	Spain	To describe the intake of mothers' own milk and its composition according to gestational age and postnatal age in preterm infants and to	One-hundred-and-seventy-six preterm infants (born <32 weeks GA; 52% male) were included, where the majority of infants received partial feeding of mothers' own milk.	PBF volume

Study	Design	Country	Main study aim	Population	Feeding parameter(s)
			correlate them with neonatal weight, length and morbidities.	The infants were followed at 72 hr, 15 and 30 days and monthly until discharge (90 days).	
Carnielli, Pederzini (144)	Longitudinal, observational	Italy	To determine whether feeding very low birth weight infants exclusively with preterm human milk can ensure constant plasma and red blood cell long chain polyunsaturated fatty acids levels during the first month of life.	Twenty-two preterm infants (born 750–1,750 g), of which 14 infants received mother's own milk and eight received preterm human milk, were followed for 4 weeks.	EBF volume
Casey, Neifert (145)	Longitudinal, observational	U.S.	To report the intakes of milk, energy and some selected nutrients to estimate nutrient intake in the young neonate.	Eleven healthy term infants from mothers with uncomplicated pregnancies, who were put to the breast within two hours of birth and were followed for 5 days. The infants were exclusively breastfed, except for two who were given water or glucose water 3–6 times after breast feeds.	EBF volume
Cohen, Brown (146)	Longitudinal, randomized controlled	Honduras	To examine breast milk intake, total energy intake and infant growth among breastfed infants randomly assigned to receive nutritionally adequate, hygienically prepared complementary foods beginning at 16 weeks or to be exclusively breastfed until 26 weeks.	Fifty healthy term infants whose mothers were recruited from two public hospitals in Honduras. Infants were exclusively breastfed and at 16 weeks were randomly assigned to continue exclusively breastfeeding (control group), to introduce solid foods with ad libitum breastfeeding,	EBF frequency

Study	Design	Country	Main study aim	Population	Feeding parameter(s)
				or to introduce solid foods with maintenance of preintervention breastfeeding frequency. The infants were followed until 26 weeks of age.	
de Carvalho, Robertson (147)	Longitudinal, observational	U.S.	To provide normative data for true demand breastfeeding mothers during the first 14 days postpartum, and to determine whether the frequency and duration of breastfeeding affect milk production at 1 month.	Forty-six term infants (59% male) whose mothers were encouraged to nurse on true demand, were observed during the first 14 days after delivery.	EBF frequency
Dewey, Heinig (148), Dewey, Heinig (149)	Longitudinal, observational	U.S.	To present data on indexes of functional outcomes to judge whether a particular pattern of intake or growth, particularly for breastfeeding infants, is advantageous in a given environment.	Ninety-two healthy term infants and their mothers as part of the Davis Area Research on Lactation, Infant Nutrition and Growth (DARLING) study who were followed until 12 months of age.	EBF volume, PBF volume
English (150)	Longitudinal, observational	Australia	To report the milk production of a mother post-partum, while considering the observed production against weight changes and activity of the mother, and breast milk intake of the infant.	One fully breastfed term infant followed for 13 weeks since birth.	EBF volume
Ettyang, van Marken Lichtenbelt (151)	Cross-sectional, observational	Kenya	To evaluate the association between maternal body composition and intake of breast milk in infants in Kenya.	Ten exclusively breastfed infants aged 2–4 months, from a pastoral community living in West Pokot, Kenya. The infants were a	EBF volume

Study	Design	Country	Main study aim	Population	Feeding parameter(s)
				randomly selected subset of a larger longitudinal study to determine the prevalence of undernutrition, low iron stores and vitamin A deficiency during the third trimester of pregnancy and at 4 months after birth.	
Evans, Evans (152)	Longitudinal, observational	Australia	To determine the effect of caesarean section on breast milk transfer to the normal term infant over the first week of life.	Of the 185 mother–infant pairs, 88 term infants (50% male) from mothers who had a normal vaginal delivery were exclusively breastfed.	EBF volume
Ferris, Neubauer (153), Neubauer, Ferris (154)	Longitudinal, observational	U.S.	Ferris, Neubauer (153): To examine whether differences in prenatal care and maternal health, perinatal management of lactation and infant birth outcome among women with IDDM compared with control and reference women explain the duration of human lactation and breastfeeding pattern in women with IDDM. Neubauer, Ferris (154): To report the composition of breast milk from mothers with IDDM and their intake from their infants longitudinally.	Thirty-three infants with mothers with IDDM, 33 control infants from mothers without IDDM and matched by demographic characteristics to the IDDM group, and 11 healthy reference infants followed until 84 days postpartum. Of the 11 term infants with volume of intake data, 10 infants at 7 days and nine infants at 14 days, were exclusively breastfeeding.	EBF volume, EBF frequency

Study	Design	Country	Main study aim	Population	Feeding parameter(s)
Forsum and Sadurskis (155)	Longitudinal, observational	Sweden	To examine the interactions between growth and body composition of infants, and the amount of breast milk these infants consume during the first 8–10 weeks of life.	Twenty-two healthy breastfed infants (59% male) during the first 10 weeks of life.	EBF volume
Hendrickse, Spencer (156)	Longitudinal, observational	U.K.	To describe the weight gain and calorie intake of LBW infants who were either fed predominantly on fresh raw breast milk or commercially available LBW formula.	Of the 24 preterm infants (born <33 weeks GA, <1500 g), 10 were fed predominately their own mother's fresh unpasteurized expressed breast milk and followed for their first 6 weeks of life at the NICU.	EBF volume
Hofvander, Hagman (157)	Cross-sectional, observational	Sweden	To compare the amount of breast milk and breast milk substitutes consumed by 1–3 month-old infants fed ad libitum, and living under similar conditions in their homes.	Of the 150 singleborn, healthy, term infants 75 were exclusively breastfed infants (25 infants studied per age group: at approximately 1, 2 and 3 months of age) were recruited following discharge from randomly sampling mothers from records at a maternity ward.	EBF volume
Hörnell, Aarts (158)	Longitudinal, observational	Sweden	To elucidate the variations in three components of breastfeeding pattern (frequency of feeds, suckling duration and longest interval between two consecutive feeds) in exclusively breastfed	Five-hundred-and-six infants were followed up from the first week after delivery until their mother's second menstruation postpartum or until a new pregnancy. At 26 weeks of follow up, seven of the original 506 infants remained exclusively	EBF frequency

Study	Design	Country	Main study aim	Population	Feeding parameter(s)
			infants, while analyzing factors influencing the duration of exclusive breastfeeding and total duration of breastfeeding.	breastfed. The study was part of a larger, multicentre study of duration of lactational amenorrhea in relation to breastfeeding practices in seven countries.	
Houston, Howie (159)	Longitudinal, observational	U.K.	To examine whether minimal intrusion by one, or at the most two, test-weighs using an electronic balance in 24 hr would give assessments of 24-hr feed volumes for sufficient accuracy.	Eighteen term infants (born >38 weeks GA, >2,500 g) of breastfeeding mothers were followed for the first 6 postpartum days of life.	EBF frequency
Howie, McNeilly (160)	Longitudinal, observational	U.K.	To report on the effect that the introduction of supplementary food may have on the frequency and duration of suckling and the resumption of ovarian activity after childbirth.	Twenty-seven term infants who were exclusively breastfed, and had supplementary food first introduced between 3 and 24 weeks postpartum, were followed up until 40 weeks after delivery.	EBF frequency, PBF frequency
Itabashi, Miura (161)	Longitudinal, observational	Japan	To investigate the nutritional intake of ELBW infants to propose an advisable and practically feasible nutritional intake from Japanese infants with smaller average gains in body weight, head circumference and length than infants from Western countries.	Sixteen ELBW preterm infants (born 26–33 weeks GA) who were admitted to the NICU and were mainly fed preterm milk during the first 4 weeks, gradually reducing to 60% of total milk intake by the 13th week, were followed until 12 weeks of postnatal age.	PBF volume
Janas, Picciano (162)	Longitudinal, observational	U.S.	To determine the relationships among specific parameters of protein, nitrogen and amino acids	Thirty-seven term infants whose mothers were recruited at prenatal classes or immediately	EBF volume

Study	Design	Country	Main study aim	Population	Feeding parameter(s)
			of term infants fed either human milk, a whey predominant formula, or a cow's milk formula.	following their infants' birth in the hospital, were followed until 16 weeks of age. Eleven of these infants (27% male) received human milk and were introduced supplementary food after 8 weeks of age.	
Jia, Gu (163)	Longitudinal, observational	China	To conduct a longitudinal study on tracing the growth of exclusively breastfed infants and combining human milk analysis to support breastfeeding and to achieve optimal development of infants.	Of the 130 term infants who were enrolled from seven cities in China, 59 infants (51% male) were exclusively breastfed and followed up until 6 months of age.	EBF frequency
Kent, Hepworth (164)	Longitudinal, observational	Australia	To assess the reproducibility of measures of breastfeeding behaviour and breast milk intake, and to provide clinicians with evidence-based information to inform parents' expectations of their infants' breastfeeding behaviour and breast milk intake from 1 to 6 months of exclusive breastfeeding.	Fifty-two healthy term infants who were exclusively breastfed on demand, where mother–infant pairs participated in one of four larger longitudinal studies on energy balance of lactating women, the effect of a progesterone-only contraceptive pill on lactation, prolactin concentrations in milk and blood and the rate of milk synthesis, and breast volume and milk production during exclusive breastfeeding.	EBF frequency
Krebs, Reidinger (165)	Longitudinal, observational	U.S.	To determine the growth pattern of normal infants who were fed	Seventy-one healthy term infants who were exclusively human	EBF volume, PBF

Study	Design	Country	Main study aim	Population	Feeding parameter(s)
			human milk exclusively for at least 5 months and investigate the relationship of growth to milk intake and whether the growth was related to zinc intake from human milk.	milk fed for at least 4.5 months and were randomly assigned to receive 15-mg zinc supplementation or placebo at 2 weeks of age (milk output between the groups were not significantly different). Of the infants, 43 continued in the study through 9 months of age.	volume
Martinez and Chavez (166)	Longitudinal, observational	Mexico	To obtain longitudinal data on breast milk yields of mothers from a poor rural community of the Mexican plateau under conditions of strict control and standardization.	Seventeen full term infants who were not breastfed in the first 48–72 hr of life, and were instead fed sugarless leaf infusions or donor milk. Infants were allowed to be fed on demand, including water and other supplementary foods. The mother–infant pairs were part of a larger project to understand the relationship between infant nutrition and its physical, mental and social development.	PBF volume, PBF frequency
Matheny and Picciano (167)	Longitudinal and cross-sectional, observational	U.S.	To determine the existence, extent and nature of relationships among nursing frequency, quantity of milk consumed and growth characteristics of exclusively breastfed infants.	Fifty healthy term infants with anthropometric measurements, milk intake and nursing frequency data acquired from three different conducted studies. Milk intake and nursing frequency data were obtained for up to 16 weeks of age.	EBF frequency

Study	Design	Country	Main study aim	Population	Feeding parameter(s)
Michaelsen, Larsen (168)	Prospective, observational	Denmark	To describe the nutritional role of breastfeeding and provide a detailed description of the intake, protein, fat, carbohydrate, and energy of human milk, and potential influencing factors.	Ninety-one infants (46% male) were followed until 12 months of age and were grouped as EBF or PBF. The infants were part of a larger study, The Copenhagen Cohort study on Infant Nutrition and Growth.	EBF volume, EBF frequency, PBF volume, PBF frequency
Motil, Sheng (169)	Longitudinal, observational	U.S.	To investigate the differences in nitrogen and energy utilization between breast- and formula-fed infants by measuring longitudinally the differences in body composition and protein and energy intakes who were fed either human milk or a commercial formula.	Twenty term infants, of which ten were breastfed, were followed until 24 weeks postnatal age.	EBF volume
Neville, Keller (68)	Longitudinal, observational	U.S.	To perform a longitudinal study in highly motivated lactating women, focusing particularly on the first 14 days postpartum to gain a better understanding of the relationship between milk transfer during the initiation of lactation and later lactational performance.	Thirteen term infants who were breastfed, with solids introduced between 4 and 9 months of age and with formula used occasionally after 4 months in three mother–infant pairs.	EBF frequency
Nielsen, Reilly (170)	Longitudinal, observational	Scotland	To test whether and how human lactation and breastfeeding practices can adapt to fulfil infant energy requirements during	Of the 47 infants, 36 infants provided data at 15.4 ± 1.3 weeks, and 38 infants at 24.5 ± 1.3 weeks. At the second time point, six infants were reported to	EBF volume, EBF frequency

Study	Design	Country	Main study aim	Population	Feeding parameter(s)
			exclusive breastfeeding for 6 months.	have received complementary foods, however, there were no statistically significant differences in milk intake between these infants and those who were exclusively breastfed.	
Nommsen, Lovelady (171)	Longitudinal, observational	U.S.	To examine factors, including maternal anthropometric indicators, dietary intake data from a subgroup of the participants, and mother–infant variables such as nursing frequency, feed duration and milk volume, that are potentially related to interindividual differences in the amount of protein, lactose, lipid and energy in milk.	Ninety-two mother–infant pairs were initially recruited, of which 73, 60, 50 and 46 term infants provided data at 3, 6, 9 and 12 months postpartum, respectively. Mothers of the infant had planned not to introduce solid foods before 4 months of age, or to feed >120 ml/day of other milk or formula throughout the first 12 months. The mother–infant pairs were part of the larger Davis Area Research on Lactation, Infant Nutrition and Growth (DARLING) study, designed to document total nutrient intakes and growth patterns of breastfed and formula-fed infants during the first 18 months of life.	EBF frequency, PBF frequency
Novotny and Mata (172)	Cross-sectional, observational	Costa Rica	To examine the breast milk consumption and anthropometric status of rural Costa Rican infants.	Twenty term breastfeeding infants, of which ten infants	EBF volume, EBF frequency

Study	Design	Country	Main study aim	Population	Feeding parameter(s)
				were fully breastfed and the remaining ten infants were partially breastfed. Data from the infants were collected at a range of ages, including 2 to 103 days and 26 to 184 days, for the fully breastfed and partially breastfed infants, respectively.	
O'Connor, Khan (130)	Longitudinal, randomized controlled	Canada	To determine whether mixing a multinutrient fortifier to approximately one half of the human milk fed each day for a finite period after discharge improves the nutrient intake and growth of predominately human milk-fed LBW infants through a pilot study.	Thirty-nine preterm infants (born <33 weeks GA, 750–1,800 g; control group: 47% male, intervention group: 74% male) randomly assigned to the control group where infants were discharged from the NICU on unfortified human milk (n = 20), or to the intervention group receiving half their volume of human milk as nutrient enriched feedings after hospital discharge (n = 19). Infants were followed on study day 1, and 4, 8 and 12 weeks after discharge; and 6 and 12 months CA in a follow-up study by Aimone, Rovet (129).	PBF volume
Oras, Blomqvist (173)	Longitudinal, observational	Sweden	To describe breastfeeding patterns in preterm infants up to 1 year of CA.	Eighty-three exclusively breastfed preterm infants (born 28–33 weeks GA, 740–2,920 g; 63% male) who along with their mothers, were part of a larger	EBF frequency, PBF frequency

Study	Design	Country	Main study aim	Population	Feeding parameter(s)
				study on kangaroo mother care, where a breastfeeding diary was sent home after discharge from hospital, and at 2, 6 and 12 months of the infant's CA.	
Pao, Himes (174)	Longitudinal, observational	U.S.	To describe milk consumption and total dietary intake of completely and partially breast-fed infants and identify factors related to these patterns.	Of the 22 term infants studied at 1, 3, 6 and 9 months, seven infants were completely breastfed at 1 month, one infant was completely breastfed at 3 months, and three infants were partially breastfed at 9 months.	EBF frequency, PBF frequency
Paul, Black (175)	Longitudinal, observational	U.K.	To measure the growth, energy and nutrient intake longitudinally throughout infancy and to investigate the factors influencing breast milk intake. Additionally, to explore the relationship between breast milk intake and growth and provide fuller details of breast milk intake and anthropometry from 2 to 10 months of age.	Forty-eight term infants (58% male) who received breast milk up until at least 4 months of age. At approximately 7, 8 and 10 months of age, all infants were no longer exclusively breastfed and were partially feeding.	EBF frequency, PBF frequency
Quandt (176)	Longitudinal, observational	U.S.	To determine the range of variation in individual breastfeeding behaviours known to have biological and cultural significance, and whether these behaviours are patterned; if patterns exist, whether individual	Sixty-two term infants who showed evidence of well-established exclusive breastfeeding were selected, who were followed at 4 and 8 weeks of age.	EBF frequency

Study	Design	Country	Main study aim	Population	Feeding parameter(s)
			mothers maintain a similar pattern through the early lactation period; and whether patterns of breastfeeding behaviours are associated with outcomes such as duration of exclusive breastfeeding and time of weaning.		
Salmenperä, Perheentupa (177)†	Longitudinal, observational	Finland	To evaluate the growth of infants exclusively breastfed and compare the growth with infants who weaned early and/or were given complementary foods.	One-hundred-and-ninety-eight term infants (born 37–42 weeks GA; 53% male) were followed up to 12 months of age.	EBF volume
Sievers, Oldigs (178)	Longitudinal, observational	Germany	To examine whether breastfed infants are able to adapt to zinc intakes that are lower than the recommended dietary allowance; what differences may exist between zinc intake, excretion and retention in breastfed infants and formula-fed infants, and to compare zinc balances from term breastfed infants with those of term and preterm infants who were formula-fed.	Of the breastfed and formula-fed infants enrolled in the study, 7, 10, 9, 9 and 10 infants were of term birth and breastfed at 17, 35, 57, 85 and 113 days, respectively.	EBF volume
Stuff and Nichols (179)†	Longitudinal, observational	U.S.	To determine whether the ad libitum addition of solid foods to the diet of exclusively human milk-fed infants will	Forty-five healthy term infants who were exclusively breastfed for at least 16 weeks, where three groups	EBF volume, PBF volume

Study	Design	Country	Main study aim	Population	Feeding parameter(s)
			increase energy intake and reverse the decline in weight-for-age percentiles observed during the exclusive breastfeeding period.	emerged during the transition to monthly mixed feedings of human milk and solid foods. The groups were categorized according to introduction of mixed feeding at Group 1, 20 weeks; Group 2, 24 weeks; and Group 3, 28 weeks.	
van Steenbergen, Kusin (180)	Cross-sectional, observational	Indonesia	To describe the feeding practices, breast milk intake and the consumption of additional food during infancy.	Seventy-seven PBF infants providing cross-sectional data at 37–56 weeks were studied from three villages along the island of Madura in East Java, Indonesia. Infants were given supplementary foods, such as mashed banana, and solid foods such as egg and fish, as the infants aged.	PBF frequency
Van Steenbergen, Kusin (181)[†]	Cross-sectional, observational	Kenya	To report on breastfeeding behaviour, breast milk yield and breast milk composition from mothers living in a rural highland area.	Eighty-five infants with mothers at different stages of lactation who were examined according to season. The participants of this study were part of two cross-sectional studies on food intake of infants and toddlers by the Joint Project Machakos. Most infants began to be supplementally fed with some cow's milk at 3 months of age.	EBF volume, PBF volume

Study	Design	Country	Main study aim	Population	Feeding parameter(s)
Yamauchi and Yamanouchi (182)	Longitudinal, observational	Japan	To investigate the factors contributing to the frequency of breastfeeding during the first 24 hours after birth and the neonatal response to breastfeeding frequency.	Two-hundred-and-ten healthy term (born 37–44 weeks GA, 2,525–4,030 g; 44% male) breastfed newborns who were observed during the first 24 hr after birth at their hospital stay.	EBF frequency

Abbreviations: CA: corrected age; EBF: exclusively breastfeeding; ELBW: extremely low birth weight; GA: gestational age; IDDM: insulin-dependent diabetes mellitus; LBW: low birthweight; NICU: neonatal intensive care unit; PBF: partially breastfeeding. [†]Data not originally available from the studies were supplemented with data presented in the review by Arcus-Arth, Krowech (128).

Table 2-2. Daily weight-normalized human milk intake for preterm infants

Study	Setting and population	Feeding protocol	Volume of intake[†]
Aimone, Rovet (129)	Setting: Post-discharge GA at birth (weeks): Control group: 29.8 ± 1.7 Intervention group: 28.9 ± 1.2 Birth weight (g): Control group: 1322 ± 332 Intervention group: 1253 ± 242	One day prior to discharge, infants were randomized to either an intervention or control group. The control group was discharged home on unfortified human milk, whereas the intervention group received nutrient enrichment of human milk. A detailed feeding protocol can be found in the description for O'Connor, Khan (130).	Control group, receiving unfortified milk: At 6 months CA (n = 17) Enteral human milk intake (mL/kg/day): 70.6 ± 43.6 Proportion of all milk feeds (%): 69 ± 38 At 12 months CA (n = 16) Enteral human milk intake (mL/kg/day): 15.1 ± 23.9 Proportion of all milk feeds (%): 31 ± 46 Intervention group, receiving fortified milk: At 6 months CA (n = 17) Enteral human milk intake (mL/kg/day): 32.8 ± 15.6 Proportion of all milk feeds (%): 43 ± 46 At 12 months CA (n = 14) Enteral human milk intake (mL/kg/day): 9.1 ± 21.4 Proportion of all milk feeds (%): 22 ± 39

Study	Setting and population	Feeding protocol	Volume of intake[†]
Atkinson, Bryan (132)	Setting: NICU GA at birth (weeks): 28.3 Birth weight (g): 970	Available feeding regimens were pooled breast milk, mother's own milk, and formula. Infants received a daily multivitamin. Infants started on oral feedings within the first 48 hours of life, having received only dextrose and electrolytes by IV prior to entry into the study. Milk intakes were increased as tolerated to a maximum of 180-200 mL/kg/day and fed by intermittent nasogastric gavage every 2 hours. Infants received formula if mothers' own milk supply diminished.	Pooled breast milk group: At 1 week PNA (n = 8) Human milk intake (mL/kg/day): 151 ± 15 Total fluid intake (mL/kg/day): 210 ± 10 At 2 weeks PNA (n = 8) Human milk intake (mL/kg/day): 202 ± 9 Total fluid intake (mL/kg/day): 211 ± 8 Preterm mother's milk group: At 1 week PNA (n = 8) Human milk intake (mL/kg/day): 159 ± 10 Total fluid intake (mL/kg/day): 184 ± 11 At 2 weeks PNA (n = 8) Human milk intake (mL/kg/day): 182 ± 6 Total fluid intake (mL/kg/day): 182 ± 6 Total fluid intake include fluid of milk, IV dextrose, water, and formula if the mothers' milk supply was diminished.
Cabrera Lafuente, Montes Bueno (143)	Setting: NICU GA at birth (weeks): ≤28 weeks GA group: 26.5 ± 1.4 28 to 32 weeks GA group: 30 ± 1 Birth weight (g): ≤28 weeks GA	Minimal enteral feeding (20 mL/kg) with mother's own milk, or preterm infant formula if mother's own milk was not available, begun if the infant was stable on day 2 of life. Advancement pace of enteral feeding was 10-20 mL/kg/day, as tolerated, according to the unit's feeding protocol. Standardized human milk fortifiers were added when oral feeding reached 100 mL/kg/day. PN was stopped when enteral feeding reached 120 mL/kg/day.	≤28 weeks GA group (n = 81) At 3 days PNA Mother's own milk intake (mL/kg/day): 7.9 ± 0.1 Proportion of enteral intake (%): 68.7 ± 2.3 At 15 days PNA Mother's own milk intake (mL/kg/day): 53.9 ± 5.6 Proportion of enteral intake (%): 82.2 ± 2.1 At 30 days PNA Mother's own milk intake (mL/kg/day): 76.8 ± 6.9 Proportion of enteral intake (%): 76.2 ± 3.8 At 60 days PNA Mother's own milk intake (mL/kg/day): 66.8 ± NR

Study	Setting and population	Feeding protocol	Volume of intake[†]
	group: 905 ± 235 28 to 32 weeks GA group: 1331 ± 292		Proportion of enteral intake (%): 62 ± 3.6 At 90 days PNA Mother's own milk intake (mL/kg/day): 61.8 ± 8.6 Proportion of enteral intake (%): 51.4 ± 4.5 28 to 32 weeks GA group (n = 95) At 3 days PNA Mother's own milk intake (mL/kg/day): 15.8 ± 1.7 Proportion of enteral intake (%): 47.1 ± 3.1 At 15 days PNA Mother's own milk intake (mL/kg/day): 66.3 ± 5.0 Proportion of enteral intake (%): 68.0 ± 3.3 At 30 days PNA Mother's own milk intake (mL/kg/day): 71.9 ± 5.2 Proportion of enteral intake (%): 59.0 ± 3.8 At 60 days PNA Mother's own milk intake (mL/kg/day): 59.7 ± 8.8 Proportion of enteral intake (%): 50.9 ± 3.9 At 90 days PNA Mother's own milk intake (mL/kg/day): 28.7 ± 11.5 Proportion of enteral intake (%): 35.8 ± 6.1
Carnielli, Pederzini (144)	Setting: NICU GA at birth (weeks): 29.8 ± 2.4 Birth weight (g): 1180 ± 290	IV fluids (5% dextrose in water) were started if the gastrointestinal tolerance of infant did not allow a sufficient fluid intake or if blood glucose <2.5 mmol/L. Infants did not receive PN.	At 6-7 days PNA (n = 22) Milk intake (mL/kg/day): 113.0 ± 27.6 At 13-14 days PNA (n = 22) Milk intake (mL/kg/day): 155.4 ± 17.7 At 20-21 days PNA (n = 22) Milk intake (mL/kg/day): 173.8 ± 9.2 At 27-28 days PNA (n = 22) Milk intake (mL/kg/day): 177.0 ± 9.7

Study	Setting and population	Feeding protocol	Volume of intake[†]
			Milk intake included mother's own milk and/or donor milk when mother's milk was insufficient.
Hendrickse, Spencer (156)	Setting: NICU GA age at birth (weeks): 30 Birth weight (g): NR, however, infants were LBW and <1500 g at birth.	Infants fed nasogastrically or nasojejunally as much milk as they would tolerate. Maximum volume offered on the first day was 90 mL/kg and increased by 30 mL/kg/day in a stepwise fashion. Infants in the own mother's expressed breast milk group fed predominantly breast milk. These infants received additional breast milk from donor milk, where necessary. In the event that donor milk was unavailable, formula was provided. Infants ceased to receive IV dextrose after the third week.	Own mother's expressed breast milk group (n = 10): At 1 week PNA Total volume taken orally (mL/kg/day): 67.3 At 2 weeks PNA Total volume taken orally (mL/kg/day): 182.5 At 3 weeks PNA Total volume taken orally (mL/kg/day): 193.1 At 4 weeks PNA Total volume taken orally (mL/kg/day): 194.4 At 5 weeks PNA Total volume taken orally (mL/kg/day): 194.0 At 6 weeks PNA Total volume taken orally (mL/kg/day): 187.0
Itabashi, Miura (183)	Setting: NICU GA at birth (weeks): 26.7 ± 1.4 Birth weight (g): 879.6 ± 108.2	IV fluids with glucose and electrolytes started immediately at birth and continued until day 3. On day 4, peripheral PN intake with amino acids and lipids are started and PN intake was gradually increased. Mother's own milk was fed enterally as soon as possible if the infant was stable. Formula was used if mother's milk could not be given. Phosphorus was added to the mother's own milk until fortified human milk was started. PN was discontinued when the infant tolerated 100-120 mL/kg/day. Human milk fortifier supplemented mother's milk if amount of preterm milk was >50% of enteral	At 1 week PNA (n = 6) PN (mL/kg/day): 84.4 ± 13.2 Enteral human milk (mL/kg/day): 3.7 ± 3.6 Total enteral intake (mL/kg/day): 4.2 ± 3.6 At 2 weeks PNA (n = 13) PN (mL/kg/day): 84 ± 25.7 Enteral human milk (mL/kg/day): 35.7 ± 22.7 Total enteral intake (mL/kg/day): 39.1 ± 23.1 At 3 weeks PNA (n = 15) PN (mL/kg/day): 34.4 ± 28.1 Enteral human milk (mL/kg/day): 75.9 ± 36.1 Total enteral intake (mL/kg/day): 87.7 ± 34.1 At 4 weeks PNA (n = 15) PN (mL/kg/day): 9.4 ± 12.2 Enteral human milk (mL/kg/day): 103.1 ± 39.9

Study	Setting and population	Feeding protocol	Volume of intake[†]
		feeding. Vitamin D metabolites supplemented milk when the milk intake >50 mL/kg/day.	Total enteral intake (mL/kg/day): 117.4 ± 26.5 At 5 weeks PNA (n = 15) PN (mL/kg/day): 11.1 ± 16.9 Enteral human milk (mL/kg/day): 107.6 ± 42.3 Total enteral intake (mL/kg/day): 127.8 ± 27 At 6 weeks PNA (n = 15) PN (mL/kg/day): 7.7 ± 14 Enteral human milk (mL/kg/day): 86.4 ± 59.9 Total enteral intake (mL/kg/day): 134.5 ± 21.5 At 7 weeks PNA (n = 12) PN (mL/kg/day): 0.6 ± 1.4 Enteral human milk (mL/kg/day): 91.2 ± 65.1 Total enteral intake (mL/kg/day): 145.7 ± 6.9 At 8 weeks PNA (n = 12) PN (mL/kg/day): 3.1 ± 6.7 Enteral human milk (mL/kg/day): 97.4 ± 60.4 Total enteral intake (mL/kg/day): 139.8 ± 14.7 At 9 weeks PNA (n = 14) PN (mL/kg/day): 3.2 ± 8.9 Enteral human milk (mL/kg/day): 96.4 ± 65.4 Total enteral intake (mL/kg/day): 140.9 ± 21.1 At 10 weeks PNA (n = 14) PN (mL/kg/day): 0 Enteral human milk (mL/kg/day): 101.8 ± 60.2 Total enteral intake (mL/kg/day): 145.5 ± 9.6 At 11 weeks PNA (n = 14) PN (mL/kg/day): 0 Enteral human milk (mL/kg/day): 87.4 ± 63.8 Total enteral intake (mL/kg/day): 146.8 ± 11.2 At 12 weeks PNA (n = 14)

Study	Setting and population	Feeding protocol	Volume of intake[†]
			PN (mL/kg/day): 0 Enteral human milk (mL/kg/day): 77.3 ± 65.8 Total enteral intake (mL/kg/day): 148.9 ± 9.8
O'Connor, Khan (130)	Setting: Post-discharge GA at birth (weeks): Control group: 29.8 ± 1.7 Intervention group: 28.9 ± 1.2 Birth weight (g): Control group: 1322 ± 332 Intervention group: 1253 ± 242	Daily iron supplement and vitamin drops after discharge; however, those in the intervention group did not receive vitamins A and C. One day prior to discharge, infants who were randomly assigned to the control group were discharged from the hospital on unfortified human milk. Infants randomly assigned to the intervention group, had half the volume of human milk enriched with a powdered multinutrient human milk fortifier. Remaining feedings were provided as unfortified milk. Families choose when during the day they wished to provide the nutrient-enriched feedings and use of a bottle or supplemental nursing system. Infants in the control group who demonstrated poor intake and growth received nutrient enrichment under the discretion of the infants' pediatrician, (e.g., powdered postdischarge formula added to human milk).	Control group, receiving unfortified milk: At 4 weeks post-discharge (n = 16) Total human milk (mL/kg/day): 145 ± 46 Total intake (mL/kg/day): 155.9 ± 28.3 At 8 weeks post-discharge (n = 17) Total human milk (mL/kg/day): 123 ± 45 Total intake (mL/kg/day): 146.2 ± 24.3 At 12 weeks post-discharge (n = 17) Total human milk (mL/kg/day): 102 ± 46 Total intake (mL/kg/day): 134.1 ± 32.7 Intervention group, receiving fortified milk: At 4 weeks post-discharge (n = 17) Total human milk (mL/kg/day): 130 ± 45 Total intake (mL/kg/day): 146.7 ± 34.3 At 8 weeks post-discharge (n = 15) Total human milk (mL/kg/day): 114 ± 26 Total intake (mL/kg/day): 121.7 ± 25.8 At 12 weeks post-discharge (n = 15) Total human milk (mL/kg/day): 99 ± 24 Total intake (mL/kg/day): 110.6 ± 20.3 Total human milk includes milk at the breast, and unfortified and fortified milk. Total intake includes nutrients from all sources, including human milk and formula.

[†]Presented as mean ± SD, or only as mean if SD was not available. CA: corrected age; GA: gestational age; IV: intravenous; NICU: neonatal intensive care unit; NR: not reported; PN: parenteral nutrition; PNA: postnatal age; SD: standard deviation.

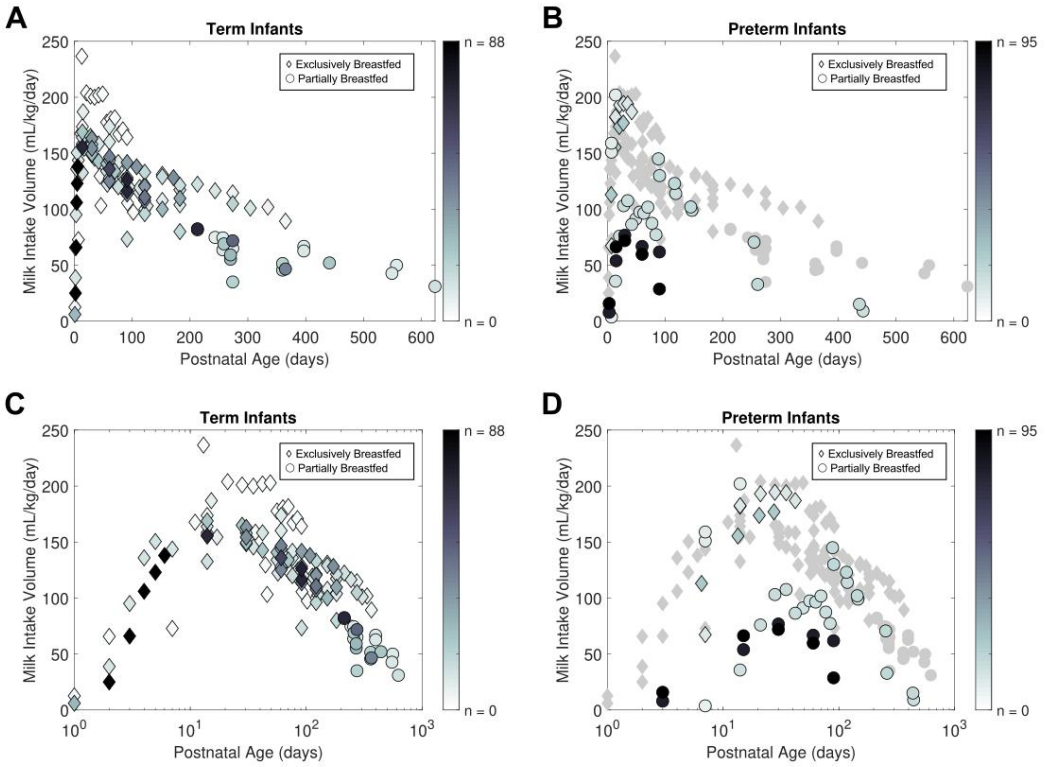

Figure 2-2. Daily weight-normalized human milk intake (WHMI) of term and preterm infants by age. Each data point represents the mean WHMI of the study's reported age group. Intensity of marker shade corresponds to the sample size of the mean data point. (a) WHMI of exclusively breastfeeding (of all ages) and partially breastfeeding term infants (>6 months of age). (b) Exclusively breastfeeding and partially breastfeeding preterm infants with mean WHMI across postnatal ages superimposed on term infant data. (c) WHMI of term infants across log transformed postnatal age. (d) Preterm WHMI over log transformed postnatal age superimposed on term infant data.

Across all studies, mean WHMI increases from birth until reaching a maximum of 152.6 ml/kg/day at 19.7 days of age, and then declines thereafter (**Figure 2-2a**). **Figure 2-3** presents the results of nonlinear regression modelling on the WHMI for EBF term infants over PNA (**Figure 2-3a**) and log-transformed PNA (**Figure 2-3b**). The modelling resulted in the following optimized parameter values, with no evidence of correlations between the parameters: $\theta_1 = 160.39$, $\theta_2 = 0.232$ and $\theta_3 = 0.00252$. The equation describing the mean WHMI of EBF term infants is as follows:

$$WHMI\ (ml/kg/day) = \ 160.39 \cdot \frac{0.232}{0.232-0.00252} \cdot (e^{-0.00252 \cdot t} - e^{-0.232 \cdot t})$$

The linear regression equations using individual subject data from five studies acquired by Arcus-Arth, Krowech (128) and a single study by Daniels, Gibson (184) are shown in **Figure 2-3a** and **Figure 2-3b** for comparison.

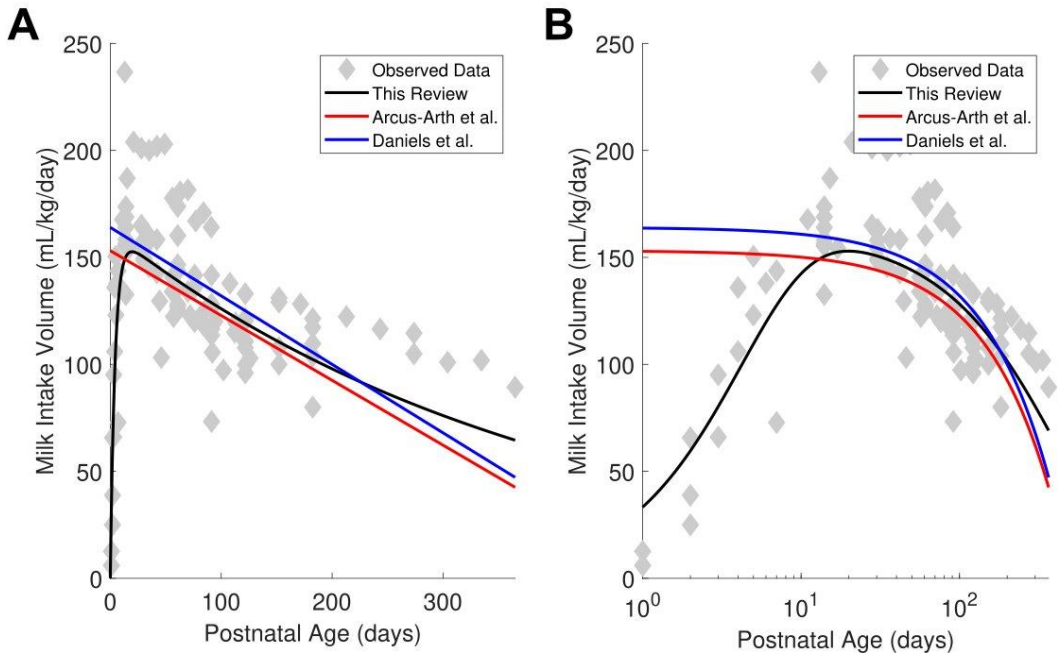

Figure 2-3. Regression equations describing the mean weight-normalized human milk intake (WHMI) of exclusively breastfeeding term infants across postnatal age, with postnatal age untransformed (a) and log-transformed (b). Data are presented from this review, Arcus-Arth, Krowech (128) and Daniels, Gibson (184).

In comparing the WHMI between preterm and term infants, **Figure 2-2b** and **Figure 2-2d** show similar WHMI among EBF preterm and EBF term infants from 7 to 28 days PNA; PBF preterm and EBF term infants from 7 to 14 days PNA, and 88 to 146 days PNA; and PBF preterm and PBF term infants from 254 to 443 days PNA. Of the studies reporting on the milk intake of preterm infants described in **Table 2-2**, two provided WHMI volumes from EBF preterm infants in the neonatal intensive care unit (NICU) setting (144, 156). The remaining studies with milk intake at the NICU presented preterm infants PBF with mother's own milk or donor human milk and preterm formula (132, 143, 161). Two studies included infants who were also parenterally fed, which is reflected in their substantially lower WHMI as compared with those of term infants (143, 161) (**Figure 2-2d)**. No studies informed EBF preterm infants after NICU discharge. O'Connor, Khan (130) and Aimone,

Rovet (129) reported intake volume data on PBF preterm infants after discharge, where the latter followed infants from the randomized controlled trial by O'Connor, Khan (130) up to 12 months CA.

2.3.2 Frequency of Human Milk Feeding

Twenty-four studies and one study reported on the frequency of human milk intake throughout the day for term and preterm infants, respectively. The mean feeding frequencies of term and preterm infants EBF and preterm infants PBF according to PNA in days are presented in **Appendix A2: Supplementary Table 2**. There were no studies reporting on the feeding frequencies of term infants past 6 months of age. **Figure 4a** presents the daily feeding frequencies of term infants plotted against PNA. Feeding frequency in term infants increased until approximately 10 days of age and was relatively stable thereafter. The average feeding frequency across all ages of EBF term infants, weighted by sample size in each dataset, was 7.7 feeds/day (range: 4.3 to 13.8 feeds/day). The mean feeding frequencies at 16, 21, and 26 weeks PNA from a single study (146) were much greater than values of other EBF term infants. PBF term infants tended to either feed at a high (>9 feedings/day) or low frequency (<6 feedings/day). **Figure 4b** shows the feeding frequency of preterm infants from a single study by Oras, Blomqvist (173). The study reported the frequency of EBF preterm infants postdischarge from the NICU directly feeding at the breast, or at the breast and expressed milk (from the tube, cup and/or bottle feeding), were 12.5 and 14 feeds/day, respectively. At 2 months CA, the infants were feeding at the breast 9 feeds/day and at the breast with expressed milk 10 feeds/day. At 6 months CA, infants who were consuming expressed milk were fed 11.5 feeds/day.

As shown in **Figure 2-4**, EBF preterm infants tend to feed at a greater frequency than preterm infants who were PBF (**Figure 2-4a**) and the average feeding frequency of term infants (**Figure 2-4b**). Conversely, PBF preterm infants were feeding at a lower frequency than the average for term infants, except at post-discharge (**Figure 2-4b**).

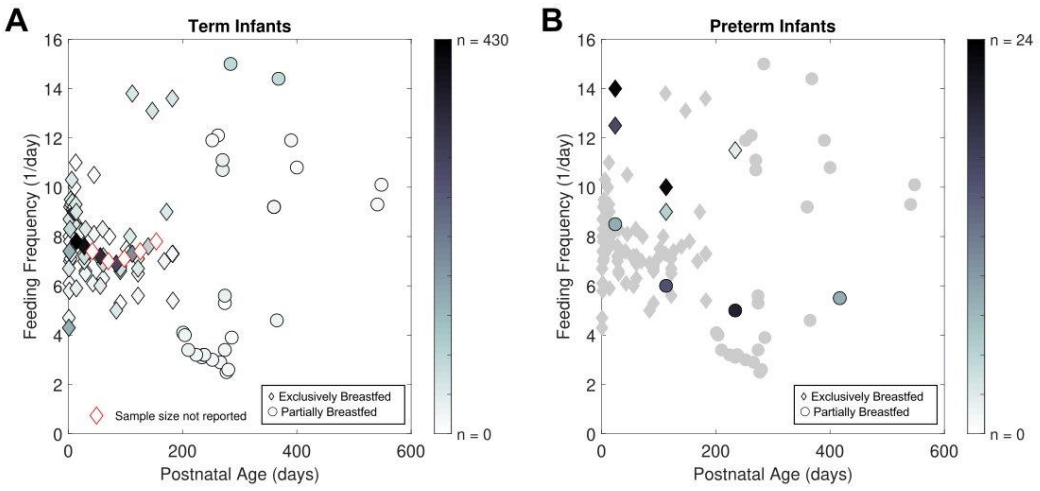

Figure 2-4. Daily frequency of human milk feeding of term and preterm infants by age. (a) Human milk feeding frequency of term infants by the mean number of feeds per day of each study's reported age group. (b) Exclusively and partially breastfed preterm infants with human milk feeding frequency across postnatal ages superimposed on term infant data. Data are presented from a single study by Oras, Blomqvist (173) and as median number of feeds per day.

2.4 Discussion

In this review, human milk feeding parameters of daily WHMI and frequency of feeds as a function of age were quantified for term and preterm infants with literature data for inputs into PBPK models designed for drug-in-milk risk assessment. This review is the first to perform a literature search to report on daily WHMI and feeding frequencies for preterm infants, and feeding frequencies for term infants. Through the literature search process, weight-adjusted intake values were obtained and reported for term and preterm infants. For term infants, a regression model described average volume intake levels in those exclusively breastfed from birth to 1 year of age. Frequency of feeding was described for both term and preterm infants across PNAs.

The rate of human milk consumption by term infants from approximately 20 days to 6 months PNA was consistent with published analyses that also examined WHMI (**Figure 2-3**) (128, 184). Linear regression models by Arcus-Arth, Krowech (128) and Daniels, Gibson (184) appeared to predict similar rates of WHMI as the nonlinear regression model of this review until 6 months of age. Data past 6 months of age were not comparable due to the inclusion of only EBF infants >6 months of age in this review, whereas Arcus-Arth, Krowech (128) included PBF infants >6 months of age and

Daniels, Gibson (184) modelled intake from a cross-sectional study of 113 infants with data only until 5–6 months of age. As a strength of this review, WHMI of EBF term infants were captured by the regression model for >6 months of age and will therefore provide conservative values for subsequent risk assessments. A maximum mean intake of 152.6 ml/kg/day at 19.7 days of age was identified. This suggests that EBF term infants are at greatest risk of drug exposure at 2–4 weeks of age. These results are consistent with a review by Anderson and Valdés (185), reporting a maximum average volume of intake between 170 and 184 ml/kg/day at 4 to 35 days of age from a longitudinal study in 13 lactating women (68). In a review by McNamara and Abbassi (186), the reported peak intake volume was approximately 173.8 ml/kg/day at 1 month of age from four studies (139, 187-189). Furthermore, the increased risk at 2–4 weeks of age is reflected in a review of case reports and studies of adverse reactions in breastfed infants of mothers taking medication. In their review, approximately two thirds of reported adverse reactions occurred during the first month PNA, and more than three quarters occurred in the first 2 months PNA (67).

WHMI of EBF preterm infants (7–28 days PNA) and PBF preterm infants (7–14 days, 88–146 days PNA and 254–443 days PNA), who did not receive parenteral nutrition, were comparable to that of term infants (**Figure 2-2b** and **Figure 2-2d**). The proportion of human milk consumption from all enteral intake appeared to dictate whether EBF and PBF preterm infants, who did not receive parenteral nutrition, approximated the WHMI of EBF or PBF term infants. However, more data regarding the proportion of breast milk intake by PBF term infants would be necessary to confirm these findings. Nevertheless, the results suggest that preterm infants do not present a substantial difference in weight-normalized feeding volume trajectory across ages as compared with term infants, as one might expect from their late development of suck-swallow-breath coordination and mother's delayed onset of lactogenesis. Controlled enteral feeds (e.g., NG tubes) dictated by hospital protocols to reach target volumes and introduction of donor human milk when mother's own milk is unavailable may contribute to the strong observed WHMI. The observation that preterm infants are able to feed at similar WHMI as term infants has important implications. With similar weight-normalized doses and lower clearance in preterm infants relative to term infants, this group is at risk for higher exposure and toxic effects of the drug.

Human milk feeding frequency of EBF term infants slightly increased in the first 10 days of life and subsequently declined and stabilized (**Figure 2-3**). Daily frequency of feeds as a function of age shown in **Figure 2-3** suggests that feeding frequency was fairly constant over 6 months of age. In

contrast, frequencies were either high (>9 feeds/day) or low (<6 feeds/day) across ages past 6 months for PBF term infants. This stark contrast between feeding frequencies could be due to differences in PBF patterns of developed and developing countries, and rural and urban communities. Studies reporting higher frequencies were conducted in developing countries or rural communities (131, 166, 180), whereas the lower frequencies were reported in studies conducted in developed countries and urban communities (160, 168, 171, 174, 175). Similarly, the relatively high mean feeding frequencies of 13.1 to 13.8 feeds/day in those EBF were from term infants of a rural community (146). More research may uncover the influence of cultural practices on feeding frequency, such as time spent at home, support from family members and willingness to breastfeed.

A single study by Oras, Blomqvist (173) reported on the frequency of human milk feeding in the preterm population. EBF preterm infants tended to feed at a greater frequency than PBF preterm infants and the average feeding frequency of term infants (**Figure 2-4b**). Conversely, PBF preterm infants tended to feed less frequently than the term infant average (**Figure 2-4b**).

This review is not the first to identify quantified milk feeding parameters as inputs into PBPK models for predicting infant drug exposures through breast milk. In fact, existing literature shows that published PBPK models have used different values of milk intake feeding parameters as inputs. Schreiber (190) used PBPK modelling to predict infant exposure to perchloroethylene, where an infant weighing 7.2 kg was assumed to ingest 700 ml/day of breast milk for 12 months postpartum. Equivalent to an average WHMI of 97.2 ml/kg/day, infant daily dose would be largely underpredicted at 2–4 weeks to 6 months PNA according to the WHMI findings in this review (**Figure 2-3**). In one case report, obstructive jaundice and hepatomegaly were observed in an infant receiving 1.4 mg/kg/day of tetrachloroethylene (191). Updating the calculations for the infant dose through breast milk with the WHMI from this review yields a daily intake of 1.3 mg/kg/day, which was previously calculated as 0.82 mg/kg/day (190). This demonstrates the potential influence of different intake values and the importance of identifying an appropriate milk volume in such risk assessments. In another study by Delaney, Malik (101), the authors used values reported by Kent, Mitoulas (80) to simulate out the variability in feeding parameters, employing a mean ± SD of 76 ± 12.6 ml/feed and 11 ± 3 feeds/day. Interestingly, the calculated mean of these parameters across all age groups evaluates to 153 ml/kg/day. While generous, this metric overestimates doses for children >2 months of age. For this reason, milk intake feeding parameters that capture changes across PNA, such as the

regression equation as derived from the volume of intake literature values in this review, would be an improvement.

Although mean WHMI and frequency of feeds were obtained through the literature, variation around these values was not explored in this review. Future research focusing on the variability of milk feeding parameters as inputs into the PBPK models will be important to subsequent drug-in-milk risk assessments. Particularly, these efforts can help identify infants who are outliers and may be at highest risk for receiving toxic effects of the drug.

2.5 Conclusion

In summary, the volume and frequency of human milk intake in term and preterm infants were quantified to provide dose information for paediatric PBPK models that will be used to inform infant exposure and subsequent risk assessment. The derived nonlinear regression equation of WHMI can be used to describe the volume of intake for EBF term infants. Because the WHMI of preterm infants were consistent with the observed WHMI of EBF term infants, the nonlinear regression equation may be applicable to preterm infants. For daily frequency of feeds, a weighted mean of 7.7 feeds/day can be used for EBF term infants across all ages. The data from Oras, Blomqvist (173) provided context in preterm infant milk intake feeding frequencies, however, more data are needed to inform the frequency of feeds in this population.

Chapter 3
Incorporating breastfeeding-related variability with physiologically based pharmacokinetic modeling to predict infant exposure to maternal medication through breast milk: A workflow applied to lamotrigine

This chapter is reflective of an original manuscript published by the PhD candidate (Cindy Hoi Ting Yeung) in *The AAPS Journal*. All pertinent dialogue in this chapter was written by the PhD candidate.

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3.1 Introduction

In the past decade, the U.S. Food and Drug Administration (FDA) has been increasingly seeing the value of evidence-based decisions for lactating women and their health care providers on drug treatment and breastfeeding during therapy. As part of the FDA's initiative to provide recommendations and guidance on conducting clinical lactation studies to inform breastfeeding with medication use, a draft industry guidance was published in May 2019 (115). The draft guidance outlined key considerations, including those involved in study design and measurement of infant milk intake. Several of these considerations emphasized the need to collect and understand the variability that exists for the mother and breastfed infant (115). First, maternal and infant factors (weight, age, ethnicity, race, etc.) should be accounted for. Second, pharmacokinetic (PK) variability and the variability in lactation physiology should be considered. Finally, depending on the design and primary objective of the study, inter- and intra-subject variability for the mother and breastfed infant would also be of interest. The role of variability in the measurement of infant milk intake volume was highlighted. The typically applied 150 mL/kg/day milk intake by the infant was acknowledged as a reasonable assumption, however, estimates based on a 200 mL/kg/day milk intake were encouraged. This recommendation reflects the idea that, although an intake of 150 mL/kg/day is often applied in measures of drug exposure risk to the infant, such as the relative infant dose (RID), feeding volumes are not constant across postnatal ages and between infants (68, 79). RID as an example, is measured by infant dose of drug in milk per body weight divided by weight-adjusted maternal dose, expressed as a percentage. As a result, if only a standard milk volume intake (together with maternal milk

concentrations) is used to determine the daily dose of drug in milk to an infant, the variability in infant exposure to the drug will not be accounted for. Consequently, drug exposure through breast milk might be under- or over-estimated. Without accounting for this variability, the infants who are at the highest risk for dose-dependent toxic effects of the drug (e.g., outliers consuming >150 mg/kg/day), are not considered in the risk assessment. There is a clear importance of incorporating variability of both daily infant milk intake volume and drug in maternal milk levels in this assessment.

PK variability can be accounted for using physiologically-based pharmacokinetic (PBPK) models. These are mechanistic representations of drug disposition in the body with the ability to provide *in silico* estimates of drug exposure given the proper parameterization of host physiology and drug properties (192). Current metrics such as the RID, focus on assessing risk directly from maternal and infant dose. These metrics do not specifically account for how infants handle drugs in milk, age dependent factors, nor the variability in infant exposure that would identify outliers who may be most vulnerable to drug toxicity. In contrast, integrating the infant dose with a PBPK model can lead to a metric of exposure that, when linked to a measure of safety, can serve as the basis for risk assessment. Thus, PBPK models are able to account for how an infant uniquely handles the drug. With a study framework that incorporates variability in milk intake volume and drug in milk concentration, these models can improve risk assessment.

To date, few PBPK models have been developed to simulate breast milk exposure to maternal drug therapy. Cibert, Gouraud (100) and Guedat, Gouraud (103) report in published abstracts, PBPK models developed to predict breastfeeding exposure of lamotrigine and clonidine, respectively. Both studies used workflows involving a mammary gland compartment and parameters to describe kinetics into the milk and infant (e.g., milk fat fraction and sucked milk flow) with resulting infant plasma concentrations simulated. Cibert, Gouraud (100) predicted median (90^{th} and 10^{th} percentiles) lamotrigine infant plasma levels of 1 µg/mL (0.75 and 1.2 µg/mL; 200 mg maternal daily dose). This narrow band is however unlikely with one, a case report showing lamotrigine plasma concentrations >1.5 µg/mL in the infant breastfed by a mother taking the same daily dose (193). Cibert, Gouraud (100) and Guedat, Gouraud (103) used predicted rather than observed drug in milk concentrations to estimate infant exposure. Using measured drug concentrations obtained from this easily accessible media would have greatly reduced uncertainty in the models. Garessus, Mielke (102) and Willmann, Edginton (104) used pediatric PBPK models to predict infant exposure to breastfeeding mothers

taking isoniazid and codeine, respectively. The strength of their workflows included accounting for metabolizing enzyme polymorphisms. Olagunju, Rajoli (194) report on a PBPK model to predict breastfed infant exposure to efarvirenz. Simulated infant exposure showed good agreement with observed values for mothers taking a standard daily dose of 600 mg, although the lower end of infant plasma concentrations tended to be underestimated (194). The last study of interest is a pediatric PBPK model for escitalopram published by our group (101). A mean daily milk intake of 153 mL/kg/day based on measured values by Kent, Mitoulas (80) was employed across all age groups. Additionally, our group recently quantified the mean weight-normalized milk infant intake across postnatal age for full-term exclusively breastfeeding infants from a comprehensive literature review (195). This current study uses the variability of daily infant breast milk intake quantified by our literature review (195) to expand on our work with escitalopram (101). The feasibility and utility of incorporating variability from anatomy and physiology of breastfed infants, volume of milk intake, and maternal milk concentrations, was assessed with lamotrigine. Finally, a novel drug in milk risk assessment metric incorporating breastfeeding-related variability to account for potential infant outliers who may be at-risk of adverse reactions was developed.

3.2 Materials and Methods

3.2.1 Modeling Strategy

A PBPK model was first developed to describe the disposition of lamotrigine in adults. The adult PBPK model was then scaled to simulate lamotrigine exposure in a population of virtual infants. The adult and pediatric PBPK models were validated using multiple dose administration datasets and PK parameters of children directly administered lamotrigine, respectively. Model evaluation was quantitatively assessed by calculating the average fold error (AFE; bias) and absolute AFE (AAFE; precision) of drug plasma concentrations. Two-fold error was deemed reasonable. The mean and variability in volume of milk intake and maternal milk lamotrigine concentrations were obtained from the literature and used to calculate daily infant dose. Daily infant dose was used as an input to the virtual infants to provide predicted lamotrigine exposure levels.

Evaluation of the simulated infant exposures were performed with two types of datasets from the literature: 1) lamotrigine concentrations measured in maternal milk and infant plasma at similar time points (paired mother-infants), and 2) lamotrigine concentrations measured in infant plasma only (unpaired infants).

3.2.2 Software Used

PBPK modeling was performed using the open-source PBPK modeling platform, PK-Sim version 8 (Open Systems Pharmacology Suite). Published PK profiles were digitized with Plot Digitizer (v2.6.8 by Joseph Huwaldt) to obtain concentration-time data. Model fitting and simulation to define ontogeny profiles, variability assessment in milk volume intake and milk lamotrigine concentrations, simulation of infant daily doses, calculation of exposure metrics, predictive performance evaluation, and creation all graphical plots were performed with R (R Core Team, 2019, Vienna, Austria).

3.2.3 Development and Evaluation of Adult and Pediatric PBPK Models

The workflow of Maharaj, Barrett (99) was followed for development of the pediatric PBPK model. Briefly, a PBPK model in adults following intravenous (IV) administration was constructed based on lamotrigine physicochemical properties, knowledge of distribution, metabolism, and excretion, and IV PK datasets for model building. The study by Yuen and Peck (196) was used in the IV model construction. Lipophilicity and non-specific enzyme clearance were optimized to the IV dataset. The Rodgers and Rowland (197, 198) and PK-Sim standard methods were used to predict partition coefficient and cellular permeability, respectively. The glomerular filtration rate fraction was optimized using the fraction excreted unchanged in urine with the resulting value of 0.05. Two main lamotrigine metabolizing enzymes, UGT1A4 and UGT1A3, were incorporated and their relative contributions to clearance were allocated accordingly. Following optimization of the IV model, a PBPK model using 15 studies with lamotrigine single-dose oral administration ranging from 25-300 mg in adults (196, 199-212) was built. Optimization of absorption-specific parameters (specific intestinal permeability, dissolution half-time) provided a final model.

The adult population PBPK model was evaluated with PK data following multiple-dose administration of 50-200 mg. The appropriateness of PK variability following single dose oral administration was assessed by creating 100 virtual adults with an age, weight and height range similar to the participant pool of the respective observed study used for evaluation (n=4). **Appendix B1** provides a complete description of the model development and evaluation process.

The adult oral PBPK model was scaled to simulate drug exposure in virtual breastfeeding infants. The anatomy and physiology were scaled to that of infants at different ages. Growth and maturation of relevant processes (metabolic capacity, glomerular filtration rate, protein binding, and body composition) were accounted for (213, 214), and realistic variability around anatomy and physiology

were applied (215) to produce a virtual infant population. The ontogeny of the two main enzymes involved in lamotrigine metabolism, UGT1A4 and UGT1A3, were based on *in vitro* studies by Badée, Qiu (216) and Miyagi and Collier (217). **Appendix B1** outlines the maturation functions used for each enzyme. After normalizing enzyme activity levels to adult activity (between 0 and 1), a Hill and linear function were fitted for UGT1A4 and UGT1A3, respectively and included in the model. The pediatric PBPK models were evaluated against observed PK studies in children who were directly administered lamotrigine in oral dosage form.

3.2.4 Quantifying Infant Milk Intake and Selection of Drug in Milk Concentrations

Mean weight-normalized human milk intake (WHMI) increases from birth until reaching a maximum of 152.6 ml/kg/day at 19.7 days of age, and then declines thereafter (195) (equation 1).

$$WHMI = 160.39 \times \frac{0.232}{0.232-0.00252} \times (e^{-0.00252t} - e^{-0.232t}) \quad [1]$$

where WHMI is in mL/kg/day and t is infant age in days. The variability in milk intake by breastfeeding infants was obtained by averaging the coefficient of variation (CV) using study data obtained from the literature by Yeung, Fong (195). Studies with reported mean and standard deviation (SD) in weight-normalized human milk intake were grouped by age: by day from >0 to 7 days old, by week from >1 to 4 weeks, and by month from >1 to 12 months. If an age group consisted of one study, that study's CV was used to represent the age group. If an age group consisted of multiple studies, an average of the study CVs weighted by study sample size was used to represent the age group. Since the resulting CVs for each of the age groups did not differ greatly across all groups (i.e., 1-2 weeks: 20.5%, 2-3 weeks: 15.5%, and 3-4 weeks: 17.1%), the age groups were further simplified. The >0 to 1 days old group CV was first compared with the next age group of >1 to 2 days old. If the percent change in CV was <30%, the age group CVs were averaged and classified under a single age bin. This procedure was repeated until a change in CV was ≥30% as compared to the previous age group, leading to the start of a new age bin. The mean WHMI equation together with the created bin CVs were referred to as the "milk intake model".

The literature was searched for studies where milk concentrations of lamotrigine were obtained from mothers. Daily dose of the mother and resulting milk concentrations were recorded. Lamotrigine transfer into milk was assumed to be a passive process. Concentrations of lamotrigine in plasma have also been reported to be proportional to dose when administered over 50-400 mg as single doses

(218). Therefore, a linear function was used to describe the relationship of mean drug in milk concentrations and maternal doses. Variability in drug in milk concentrations was determined by taking the average and SD of dose-normalized drug concentrations to derive the CV across all samples. The mean drug in milk concentration equation together with the overall CV were referred to as the "milk concentration model".

3.2.5 Evaluation of Models Incorporating Variability on Predicting Breastfed Infant Exposure

Evaluation of the ability of the model to accurately recapitulate infant exposure was completed by comparing simulated exposure in virtual infants to observed infant plasma lamotrigine concentrations following exposure through breastfeeding. The following paragraphs describe the evaluation steps.

Data from mother-infant pairs with recorded maternal milk concentrations (i.e., before a feed) and infant plasma levels (i.e., after a feed), hereafter referred to as "paired data", were used to evaluate the milk intake model. These samples were taken 2-15 hours after a maternal dose (193, 219, 220), however, most of the studies did not report time sampled after dose. Infant plasma lamotrigine values from the literature were excluded if infant age was not reported and if there were clear indications of partial breastfeeding.

For evaluation using the paired data, infant populations of 100 individuals were simulated from the evaluated PBPK model for each age bin in days: >0 to 7, >7 to 14, >14 to 30, >30 to 60, and >60 to 84. The virtual infant populations used the International Commission on Radiological Protection (ICRP) population (221) (50% female) available in PK-Sim. Each virtual infant was assigned a single oral dose of 1 mg/kg lamotrigine as a solution (i.e., dissolved in breast milk) to obtain the area under the plasma concentration vs time curve from time zero to infinity (AUC_{∞}). The average concentration at steady state ($C_{avg,ss}$) over 24 hours was then calculated. Next, the weight-normalized dose received by each infant was calculated by multiplying daily milk intake volume (mL/kg) by the observed drug in milk concentration (µg/mL). The milk intake volume was either informed by the typical 150 mL/kg (no variability applied) or through use of the milk intake model. For the milk intake model, a WHMI was drawn from a normal distribution with mean derived from equation 1 and SD specific to the age bin of each infant.

Predictions of infant plasma lamotrigine concentrations were considered successful if observed data were within 90% prediction interval (PI) of simulated $C_{avg,ss}$ plasma levels. Sensitivity analyses

were conducted in the instance where the model consistently failed to correctly predict infant plasma levels at certain age bins to assess those model parameters most likely to be influential.

A similar method was applied to the evaluation using "unpaired data", where only infant plasma concentrations after breastfeeding were reported. Virtual infant populations of 100 individuals were created for each age bin in days: >0 to 7, >7 to 14, >14 to 30, >30 to 60, and >60 to 213. The paired maternal drug in milk concentrations were not known for these infants. Therefore, the milk concentration model built from literature lamotrigine in milk values was used to give a concentration for each virtual infant. Evaluation used the same method as for the paired data.

3.2.6 Determining Measures of Exposure: RID, Predicted AUC_{∞}, and Novel Metric

The RID was calculated by taking the mean of lamotrigine in milk concentrations from the literature multiplied by the standard 150 mL/kg/day, divided by maternal dose. A dose of 200 mg was selected as it is the recommended maximum dose in the manufacturer's label for bipolar disorder for maintenance treatment (labeled use), and acute bipolar major depression (off label) (218). The mean maternal dose on a per body weight basis was estimated by using a value of 70 kg for the mother. Equation 2 provides a single value for lamotrigine risk assessment in breastfeeding infants.

$$RID\ (\%) = \frac{Milk\ Concentration\ \frac{mg}{mL} \times 150 \frac{mL}{kg \cdot day}}{Maternal\ Dose\ \frac{mg}{kg \cdot day}} \times 100\% \qquad [2]$$

To account for variability in the risk assessment process, the predicted infant plasma AUC_{∞} of lamotrigine across the five age bins were calculated by creating 100 infants per age bin. The virtual infants were provided doses from the milk intake volume and milk concentration models and assuming a maternal dose of 200 mg. The resulting AUC_{∞} distribution was used to calculate an upper AUC ratio (UAR) defined as the 95th percentile of simulated pediatric AUC_{∞} divided by the median adult therapeutic AUC_{∞} (equation 3):

$$UAR = \frac{95th\ percentile\ simulated\ pediatric\ AUC_{\infty}}{Median\ adult\ therapeutic\ AUC_{\infty}} \qquad [3]$$

The median adult therapeutic AUC_{∞} was calculated by using the adult oral PBPK model to simulate concentration-time profiles of 100 women (25-34 years old, using the ICRP population) administered 200 mg lamotrigine.

3.3 Results

The development and evaluation of the adult and pediatric PBPK models are reported in **Appendix B1**. The adult IV and oral datasets used in model optimization produced an average fold error (AFE) of 0.95 and absolute AFE (AAFE) of 1.27. Model performance was successfully evaluated in three pharmacokinetic datasets with adult subjects administered multiple doses of lamotrigine (222-224). The evaluation produced acceptable AFE and AAFE values, 1.04 and 1.13, respectively. Adult virtual populations of 100 individuals were created in PK-Sim using four studies (200, 203, 209, 210) and PK variability was successfully captured. The oral adult models were scaled to children and predicted PK parameters were comparable to two studies where a single dose of 2 mg/kg lamotrigine was administered to infants and children (225, 226).

The results of capturing variability in infant WHMI using the CV method are presented in **Table 3-1**. The final age bins in days were >0 to 1, >1 to 2, >2 to 3, >3 to 182.4, >182.4 to 212.8, and >212.8 to 365. SDs were calculated at every infant age using the relevant bin CV and mean WHMI equation, resulting in the plot depicted in **Figure 3-1**.

Table 3-1. Coefficient of variation values of WHMI applied to each infant age bin

Age bin (days)	Coefficient of variation (%)
>0 to 1	119.4
>1 to 2	80.0
>2 to 3	50.8
>3 to 182.4	21.4
>182.4 to 212.8	23.5
>212.8 to 365	17.0

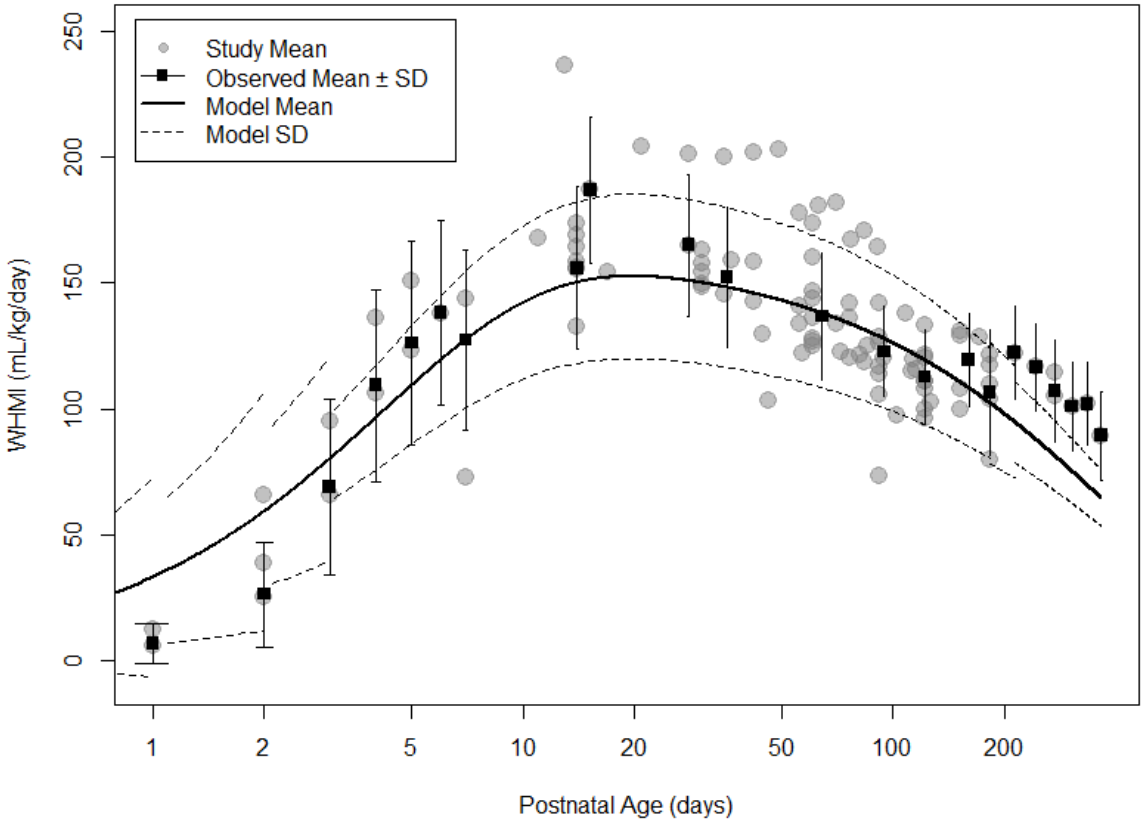

Figure 3-1. Mean and variability of WHMI up to 1-year postnatal age of exclusively breastfed term infants. The solid line represents the mean WHMI derived by fitting a nonlinear regression to the mean study WHMI (grey circles) weighted by sample size. The dashed line represents +SD and -SD from the fitted mean WHMI line as determined by the age bin CV (**Table 3-1**). Sample-size weighted mean ± SD for each age bin (by day up to 7 days old, by week up to 4 weeks old, and by month up to 12 months old) are described by the black squares and associated error bars.

Next, the workflow incorporating variability in infant anatomy and physiology, maternal milk lamotrigine levels, and infant WHMI was evaluated. **Figure 3-2** shows the lamotrigine in milk levels retrieved from the literature. Most of the studies did not report the timing of sample collection, however, when reported, these were 2-15 hours after maternal dose (193, 219, 220). Paired data collected from the literature are presented in **Table 3-2**. The results of the evaluation in predicting the observed infant plasma levels are shown in **Table 3-3**. The milk intake model predicted only 20-30%

of the samples outside of the 90% PI for infants 7 to 60 days old. However, the model underpredicted in the youngest (n=2) and oldest (n=4) age groups, which showed 100% of the observed plasma levels outside of the 90% PIs. The lack of predictive ability at these ages were shared with using the standard 150 mL/kg/day milk intake value (**Table 3-3**). A sensitivity analysis in PK-Sim revealed that UGT1A4 activity level was one of the most important parameters of the model (**Appendix B2: Supplementary Figure 1**). Over all ages, the model incorporating variability in milk intake performed slightly better or similar compared to without, where 44% and 48% of the paired samples fell outside the 90% PI across infants, respectively (**Table 3-3**).

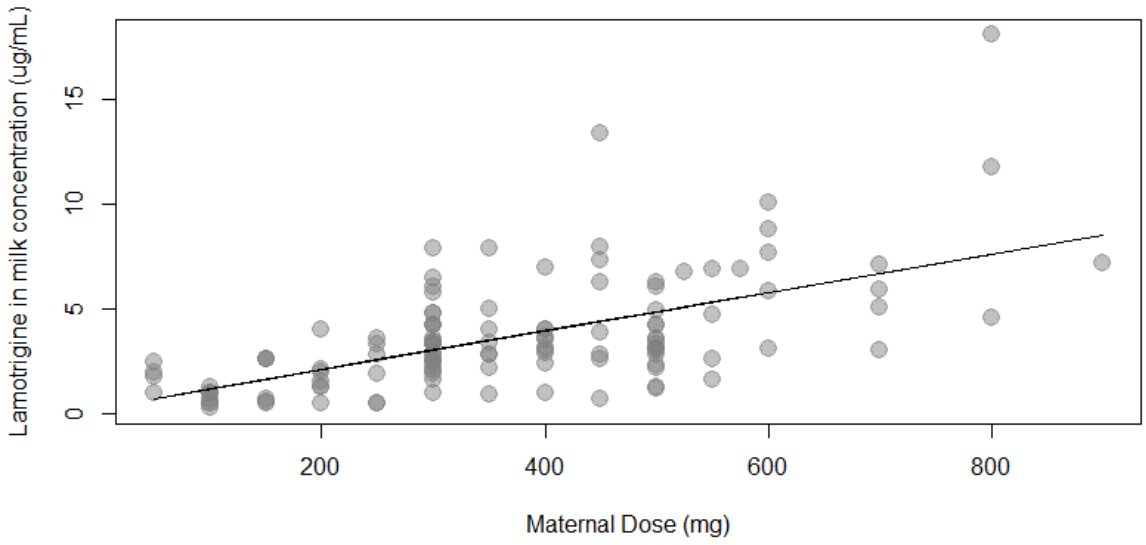

Figure 3-2. Maternal milk lamotrigine concentrations across different maternal doses from multiple studies (193, 219, 220, 227-230). Solid line represents the mean fitted line across dose and milk lamotrigine concentrations.

Table 3-2. Mother-infant pairs with measured maternal milk and infant plasma levels

Study	Maternal-infant pair[a]	Infant age (days)	Maternal dose (mg)	Nursing (%)	Maternal milk level (ug/mL)[b]	Infant plasma level (ug/mL)[b]
Fotopoulou, Kretz (227)	FC2_1	14	300	NR	6.1	2.2
Fotopoulou, Kretz (227)	FC2_2	84	250	NR	3.6	1.7

Fotopoulou, Kretz (227)	FC4_1	17.5	450	NR	8	2.1
Fotopoulou, Kretz (227)	FC4_2	84	400	NR	3.7	2.7
Fotopoulou, Kretz (227)	FC6_1	7	900	NR	7.2	3.3
Fotopoulou, Kretz (227)	FC7_1	31.5	300	NR	4.2	2.3
Fotopoulou, Kretz (227)	FC7_2	73.5	300	NR	2.8	2
Newport, Pennell (228)	R	21	500	100	1.3	0.5
Nordmo, Aronsen (219)	NE_4	22	600	100	7.68	1.33
Nordmo, Aronsen (219)	NE_5	25	600	100	10.06	0.51
Ohman, Vitols (220)	OI5_1	15	250	NR	0.51	<0.51
Ohman, Vitols (220)	OI6a_1[c]	14	300	NR	3.59	1.54
Ohman, Vitols (220)	OI6b_1[c]	14	300	NR	3.33	1.79
Ohman, Vitols (220)	OI7_1	18	100	NR	1.02	<0.51
Ohman, Vitols (220)	OI8_1	15	500	NR	3.33	2.05
Ohman, Vitols (220)	OI9_1	17	250	NR	3.33	1.54
Rambeck, Kurlemann (193)	RB_1	2	300	100	2.52	2.79
Rambeck, Kurlemann (193)	RB_2	11	300	100	2.4	1.69
Rambeck, Kurlemann (193)	RB_3	22	300	100	6.51	2.25
Rambeck, Kurlemann (193)	RB_4	29	300	100	4.25	2.68
Rambeck, Kurlemann (193)	RB_5	36	300	100	3.35	2.13
Rambeck, Kurlemann (193)	RB_6	43	300	100	4.81	2.33
Rambeck, Kurlemann (193)	RB_7	49	200	100	4.04	2.1
Rambeck, Kurlemann (193)	RB_8	64	200	100	1.95	1.54
Tomson, Ohman (230)	TT_3	14	300	NR	3.48	1.43

NR: Not reported. [a]Subjects coded by those provided in the original study, or defined by author initials, followed by "_n", where "n" is the occasion representing the same mother-infant pair sampled more than once. [b]Maternal milk and infant plasma levels are presented according to the time point the samples were collected (e.g., pre-feed level in breast milk and post-feed level in infant plasma). [c]Mother participated twice in the study with different infants.

Table 3-3. Evaluation of mother-infant pair samples with infant PBPK generated predictions

Age bin (days)	Number of infants	Number of samples	Number (%) of samples outside the 90% PI using 150 mL/kg/day	Number (%) of samples outside the 90% PI using the milk intake model
>0 to 7	2	2	1 (50%)	2 (100%)

>7 to 14	5	5	1 (20%)	1 (20%)
>14 to 30	8	10	4 (40%)	3 (30%)
>30 to 60	2	4	2 (50%)	1 (25%)
>60 to 84	4	4	4 (100%)	4 (100%)

PI: prediction interval

The studies retrieved from the literature and used in the unpaired dataset evaluation are shown in **Table 3-4**. Mean drug in milk concentrations were determined by using the known lamotrigine dose the mother took into the linear function describing their relationship in **Figure 3-2** (equation 4).

$$lamotrigine\ in\ milk\ concentration = \beta_0 + (\beta_1 \times\ maternal\ dose) \qquad [4]$$

where lamotrigine in milk concentration is in μg/mL, β_0 is 0.274 μg/mL, β_1 is 0.00921 μg/mL/mg, and maternal dose is in mg. A drug in milk concentration was then drawn from a normal distribution with the lamotrigine in milk concentration mean and SD calculated from the dose-normalized drug concentration CV of 69.3%. Both workflows incorporating the milk intake standard and milk intake model were comparable and performed well, with 0-75% of samples outside the 90% PI (**Table 3-5**). Over all ages, the standard and milk intake model resulted in 28% and 11% of the unpaired samples falling outside the 90% PI, respectively. As with the paired evaluation, the youngest age group had lower predictability. The single unpaired infant in the oldest age group was successfully captured within the 90% PI (**Table 3-5**).

Table 3-4. Infants with measured infant plasma levels only

Study	Infant ID*	Infant age (days)	Maternal dose (mg)	Nursing (%)	Infant plasma level (ug/mL)
Bedussi, Relli (231)	BF	40	150	100	1.4
Liporace, Kao (232)	LJ1	10	400	NR	1.8
Liporace, Kao (232)	LJ2	10	800	NR	1.3
Liporace, Kao (232)	LJ3	10	750	NR	2
Liporace, Kao (232)	LJ4	10	200	NR	<1

Newport, Pennell (228)	AA	11.9	400	100	1
Newport, Pennell (228)	AB	25.9	125	100	1
Newport, Pennell (228)	AC	41.3	350	100	1.3
Newport, Pennell (228)	AE	212.8	100	100	0.6
Newport, Pennell (228)	B_2	56	100	100	1.2
Newport, Pennell (228)	I_3	51.8	300	100	3.9
Newport, Pennell (228)	K_2	21	400	100	2
Newport, Pennell (228)	W_2	20.3	550	100	0.5
Nordmo, Aronsen (219)	NE_1	0.52	875	100	7.71
Nordmo, Aronsen (219)	NE_2	3	875	100	5.81
Nordmo, Aronsen (219)	NE_3	16	850	100	4.87
Tomson, Ohman (230)	TT_1	1	300	NR	1.23
Tomson, Ohman (230)	TT_2	2	300	NR	0.95

NR: Not reported. *Subjects coded by those provided in the original study, or defined by author initials, followed by "_n", where "n" is the occasion representing the same mother-infant pair sampled more than once.

Table 3-5. Evaluation of infant plasma samples with infant PBPK generated predictions

Age bin (days)	Number of infants	Number of samples	Number (%) of samples outside the 90% PI using 150 mL/kg/day	Number (%) of samples outside the 90% PI using the milk intake model
>0 to 7	2	4	1 (25%)	1 (25%)
>7 to 14	5	5	0 (0%)	0 (0%)
>14 to 30	4	4	1 (25%)	0 (0%)
>30 to 60	4	4	3 (75%)	1 (25%)
>60 to 213	1	1	0 (0%)	0 (0%)

PI: prediction interval

For determining RID, the mean lamotrigine in milk concentration from studies in **Figure 3-2** was 3.67 µg/mL. The estimated RID was determined as 19.3%. Predicted AUC_{∞} of infant lamotrigine

plasma levels using the variability-incorporated workflow are presented in **Figure 3-3**. Infant plasma AUC_{∞} did not appear to widely differ across age bins, however, the youngest and oldest age group had lower median AUC_{∞} as compared to 7 to 60 day olds (**Figure 3-3**). Several outliers with relatively high predicted plasma AUC_{∞} are evident across all age groups. **Table 3-6** presents the 95th percentile of simulated pediatric AUC_{∞} across the five age groups that was divided by the median adult therapeutic AUC_{∞} to produce age-dependent UAR values. The median simulated maternal AUC_{∞} of women taking 200 mg was 109 µg·h/mL, which is similar to values observed in PK studies (209-211).

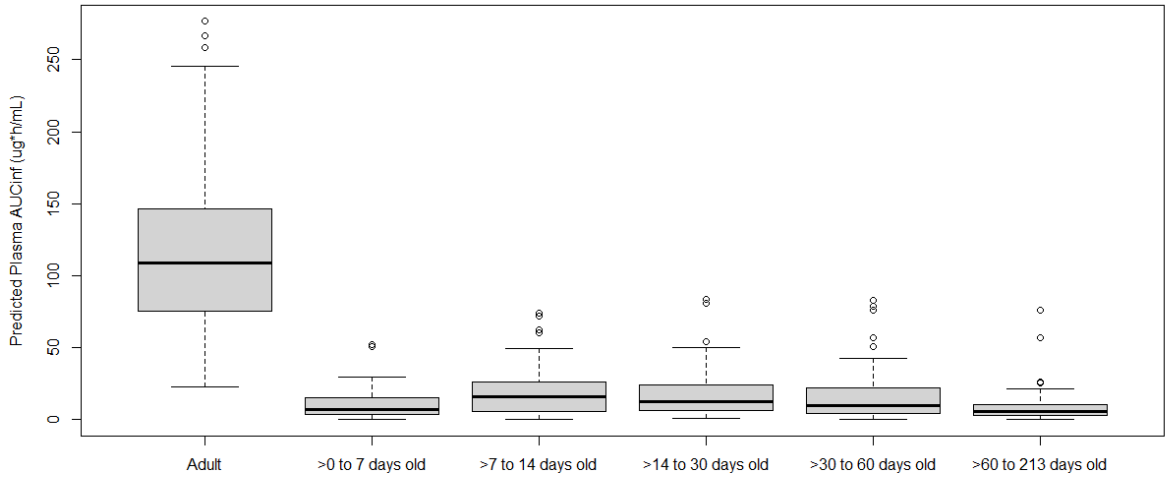

Figure 3-3. Predicted exposure of lamotrigine to adults taking 200 mg oral single dose and of infants breastfed by mothers taking 200 mg oral single dose. Each age group consists of 100 virtual subjects with simulated plasma AUC_{∞}. The population of breastfed infants was derived from the unpaired dataset.

Table 3-6. Risk ratio of infants breastfed by mothers taking 200 mg lamotrigine calculated at each age bin

Age bin (days)	95th percentile of simulated pediatric AUC_{∞} (µg·h/mL)	Upper AUC ratio
>0 to 7	26.7	0.24
>7 to 14	46.0	0.42
>14 to 30	48.3	0.44
>30 to 60	43.4	0.40
>60 to 213	20.1	0.18

AUC: area under the curve

3.4 Discussion

This study demonstrated the feasibility of incorporating breastfeeding-related variability into a workflow to predict infant exposure to maternal medications through breast milk. The model using variability from infant anatomy and physiology, daily WHMI, and lamotrigine in milk concentrations performed slightly better than using the standard 150 mL/kg/day in exclusively breastfed infants up to approximately 7 months of age. However, the improvement offered by the milk intake model did not appear significantly greater than the standard intake in our workflow example with lamotrigine. The addition of milk concentration variability allowed for the capture of more observed infant plasma levels indicating the sensitivity of the milk concentration to overall exposure. This becomes important when capturing outliers is a goal of the risk assessment.

Although the model was able to predict most observed breastfed infant plasma levels, the concentrations were generally underpredicted. The ontogeny of UGT1A4 in young children was an important model parameter as defined using sensitivity analysis and this was deemed a likely reason for the underprediction. UGT1A4 accounts for about 90% of lamotrigine liver metabolism (233, 234). The UGT1A4 ontogeny model was informed with 17 data points below 1 year of age with the lowest age being 12 days old. There was also high uncertainty with some ages showing in vitro UGT1A4 activity ranging from almost no activity to activity similar to the adult value (**Appendix B1**). Setting a lower activity would have fixed some underprediction however this would not have been evidence-based. While this is clearly a limitation caused by uncertain model inputs, the UAR outcome still suggests that outlier infants would reach exposures similar to those of adults taking lamotrigine. It would be of interest to test this workflow with additional lamotrigine sample data as there were very few samples to test, and in drugs where ontogeny profiles are well established to confirm this workflows' efficacy.

The model tended to largely underpredict in the youngest age group, >0 to 7 day olds, however, likely due to an alternative rationale. These infants were breastfed by mothers who were taking lamotrigine to treat their condition while pregnant (193, 219, 227, 230). As a result, the higher than expected concentrations observed in infant plasma may be due to placental transfer that was not accounted for in the breastfeeding-variability incorporated model. Three samples of lamotrigine concentrations in neonatal plasma, 7.71 ug/mL (unpaired), 3.30 µg/mL (paired), and 2.79 µg/mL

(paired), were relatively high and unable to be captured by the 90% PI windows with the milk intake model (193, 219, 227). Future studies should explore including this placental transfer for neonatal predictions in the first week of life. Understanding its influence can aid in the prediction of different risk scenarios. For instance, mothers taking lamotrigine during pregnancy and breastfeeding versus only during breastfeeding.

To apply the workflow in this study, an assumption on dose was made. In recognizing the complications of assessing infant exposure during multiple daily feeds, the total dose per day was used with no attempt to split the dose by number of feeds. By calculating the estimated average concentration at steady state and comparing to infant plasma level, we assume that the observed plasma levels approximated average steady state concentrations. Timing of the infant plasma level as related to maternal dose timing or timing of feed was largely unknown and this added a layer of uncertainty that was not accounted in the simulations. Additionally, assay development for drugs in this matrix has been known to be an analytical challenge due to the high protein and fat content of breast milk (235). Not all the studies reporting lamotrigine in milk concentrations provided information on the validity of the assays used, thus limiting further insight into this source of variability.

Another source of uncertainty in the workflow was the inclusion of plasma concentrations from infants who were breastfed at an unknown extent (220, 227, 230, 232). Therefore, if these infants were partially rather than exclusively breastfed, the observed infant lamotrigine plasma levels would be lower than expected. It is possible that these observed plasma concentrations would no longer be captured in the 90% PI, thus affecting model performance. However, since infant plasma levels from exclusively and unreported nursing extent were similar, the latter were treated as exclusively breastfed values. Nonetheless, future lactation studies should report breastfeeding extent to reduce potential uncertainties.

Three exposure metrics were examined in this study. Using maternal milk lamotrigine level and dose data reported in the literature, the average RID was calculated as 19.3%. The workflow in this study produced predicted infant plasma AUC_{∞} across the five unpaired age groups. Relative to each other, the median predicted infant plasma AUC_{∞} was low in the first age group, rose in line with increased feeding rate across the next age groups and was lower in the highest age group where clearance was greatest. The higher plasma AUC_{∞} in the very young infants was expected due to low

CL capacity and high WHMI in the second week of life. The predicted 95th percentile varied at different ages. Compared to the median predicted maternal AUC_{∞}, infants appear to be at high risk in the first month of age where there is a high likelihood for outliers. This finding is consistent with a review of case reports and studies indicating that breastfed infants of mothers taking medications had most adverse effects reported in the first month of postnatal age (67).

There are several advantages of the UAR over currently used risk metrics. The UAR serves as a metric to identify outlying infants with exposure as demonstrated using the infant predicted 95th percentile in AUC_{∞}. To date, adverse effects were observed in three breastfed infants (12, 16, and 40 day olds) from mothers who were taking lamotrigine (219, 231, 236). In this study, the presented UARs demonstrated that lamotrigine exposure through breastfeeding can reach levels similar to those in mothers taking 200 mg, although the probability is likely to be low. Furthermore, the metric can determine infants at-risk breastfed from mothers taking other doses. For example, lamotrigine is also used to treat focal (partial) onset seizures and generalized onset seizures. Typically, a maintenance dose of 225-375 mg/day is suggested and thus the UAR can be calculated with this range of doses (218). Finally, the UAR is based on a simulated population of mothers and infants whereas metrics such as the RID are unable to account for variation. For example, the RID is limited to use of a single maternal weight value to calculate maternal dose (e.g., 70 kg).

Future studies should focus on determining the UAR on further medications commonly taken by lactating mothers. Obtaining a range of UAR values would help identify high-risk medications and safety thresholds. This will help to facilitate evidence-based recommendations for breastfeeding when mothers are taking medications. It is possible to produce the UAR for multiple drugs since the workflow relies on easily accessible breast milk sample data. Essentially, application of the workflow to other medications would simply follow a similar process as the example with lamotrigine. First, a developed model describing the drug in milk concentration would be used with the milk volume intake model to calculate daily infant doses. The daily infant doses would serve as inputs to a pediatric PBPK model developed and validated for the drug. Outputs of plasma AUC_{∞} of infant virtual populations would be used to calculate the 95th percentiles per age bin. These values together with the maternal median plasma AUC_{∞} would derive the UAR for the drug.

This study showed the feasibility of incorporating variability into a novel drug in milk risk assessment metric and builds the foundation for future studies in this area. This includes examining

variability in the maternal population, where drug in milk concentrations can be used to develop population milk-PK models to uncover covariates that contribute to inter-individual variability (e.g., pharmacogenotypes, maternal body weight, maternal age, dose). The covariates will be used to identify mothers in the general population who are at high risk for achieving potentially dangerous drug concentrations in milk. Furthermore, specific groups of infants who would most benefit from use of the UAR to identify outliers would be of interest to study. One such group would be preterm infants, where clearance tends to be relatively low and thus these infants may be highly vulnerable to high drug exposure through breast milk.

3.5 Conclusion

This study applied a workflow incorporating variability in infant anatomy and physiology, milk intake volume, and milk concentration to predict breastfed infant exposure to lamotrigine as a case example. Pediatric PBPK modeling, and milk lamotrigine concentration and infant intake volume data from the literature, were used to capture sources of variability. The workflow produced a model that showed some improvement over the use of the standard 150 mg/kg/day milk intake volume. Only 11% as compared to 44% of the samples fell outside of a 90% prediction interval for the unpaired (infant plasma samples only; variability in milk lamotrigine concentrations applied) and paired datasets (infant plasma and maternal milk samples available; variability in milk lamotrigine concentrations not applied), respectively. These results demonstrated the importance of incorporating milk concentration variability into the workflow. From the workflow, a novel upper AUC ratio (UAR) metric to identify outlying infants at-risk of high drug exposure through breast milk was applied. The UAR across the examined infant ages ranged from 0.18-0.44 for those breastfed by mothers receiving a single 200 mg dose of lamotrigine.

Chapter 4

Verifying *in vitro*-determined enzyme contributions to cannabidiol clearance for exposure predictions in human through physiologically-based pharmacokinetic modeling

This chapter is reflective of an original manuscript published by the PhD candidate (Cindy Hoi Ting Yeung) in *Clinical Pharmacology & Therapeutics: Pharmacometrics & Systems Pharmacology*. All pertinent dialogue in this chapter was written by the PhD candidate.

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4.1 Introduction

The Cannabis sativa plant, commonly known as cannabis, is widely recognized for its pharmaceutical effects in humans. These effects have been mainly studied in a group of cannabis extracted chemicals referred to as cannabinoids (237). The most widely known cannabinoid is delta-9-tetrahydrocannabinol (THC) which is mainly responsible for the psychoactive effects of cannabis. Cannabidiol (CBD), a cannabinoid that is an isomer of THC, is less known but has been gaining attention for its therapeutic potential without having any psychotoxic effects. In 2018, the Food and Drug Administration (FDA) approved an oral solution of CBD, Epidiolex®, to treat Lennox-Gastaut and Dravet syndrome. These forms of epilepsy manifest in infancy and early childhood.

A study in subjects given an IV administration of CBD characterized its pharmacokinetics (PK) with an average half-life of 24 hours, a volume of distribution of 32.7 ± 8.6 L/kg, and a clearance of 74 ± 14 L/h (238, 239). Additionally, 16% and 33% of the total dose were found in urine (fraction unchanged not reported) and feces (12% unchanged), respectively (239). The plasma concentration

vs. time profiles of these subjects also suggested no significant enterohepatic circulation nor reabsorption once CBD is cleared through the biliary route (239). Although a significant presence of CBD in feces revealed the occurrence of biliary clearance, *in vitro* studies to date have not uncovered the mechanism of transport into the bile. These studies reported CBD unlikely to be a substrate of P-glycoprotein and breast cancer resistance protein (240). Furthermore, CBD was not a substrate for a number of human renal and hepatic uptake transporters (240). Following single oral administration doses of 1500-6000 mg in fasted adults, CBD exhibits nonlinear kinetics with less than a proportional increase in exposure with dose (241).

CBD is a BCS Class II drug with high permeability and low solubility. It is highly lipophilic with an *in silico* predicted logP of approximately 6 log units (242). CBD has a low absolute oral bioavailability of 6% in humans (243) and it increases to 14-25% with the administration of food (244). The low bioavailability of CBD is likely due to incomplete absorption and significant presystemic elimination and 70-75% of an oral dose is estimated to be removed by hepatic metabolism before reaching systemic circulation (243). The extensive metabolism of CBD by cytochrome P450 (CYP) and uridine 5´-diphospho-glucuronosyltransferase (UGT) enzymes appear to support this estimation. Yet to receive consensus in the scientific community is the identity of CYP and UGT enzymes responsible for CBD metabolism and their extent of contribution to CBD clearance. In a drug-drug interaction (DDI) study where subjects were given a THC/CBD oromucosal spray, metabolism of CBD was attributed to CYP3A4 and not CYP2C19 (245). Conversely, a DDI study with subjects administered an oral solution of CBD demonstrated the significance of CYP2C19 rather than CYP3A4 in CBD clearance (246). Neither of these studies assessed the potential contribution of other CYP enzymes on CBD metabolism. However, *in vitro* studies have shown no significant contribution by CYP2C9 (247) and potential contributions by UGT1A7, UGT1A9, and UGT2B7 to phase II metabolism of CBD (248). Most recently, Beers, Fu (249) performed a thorough *in vitro* investigation of enzyme contributions to CBD clearance. The authors showed a larger influence of CYP enzymes compared to UGT enzymes, and contributions from three CYP enzymes, CYP3A4, CYP2C19, and CYP2C9.

In consideration of the conflicting results from these existing *in vivo* and *in vitro* studies, further investigation of CBD metabolism pathways is warranted. Appropriately characterizing the role of metabolism in CBD clearance is especially important in the pediatric context. Prediction of CBD exposure in pediatrics relies on an understanding of accurate relative enzyme contributions to account

for the maturation of these metabolic enzyme pathways. The active metabolite of CBD formation and exposure in pediatrics are also of interest. Over 30 CBD metabolites have been identified by Harvey and Mechoulam (250). Of the known metabolites, 7-hydroxy-CBD (7-OH-CBD) has similar activity as CBD and exhibits a little more than half of its exposure (244). Prediction of 7-OH-CBD exposure in pediatrics also requires properly partitioned enzyme contributions to CBD clearance.

To address these gaps, this current study applies physiologically based pharmacokinetic (PBPK) modelling. PBPK models can provide *in silico* estimates of drug exposure given the proper parameterization with host physiology and drug properties (99). These models can be used to leverage existing *in vitro* data to confirm enzyme contributions to CBD clearance in humans. Thus, the first objective was to develop and validate an adult oral CBD PBPK model that incorporates *in vitro*-determined enzyme contributions to CBD clearance. The second objective was to assess whether the *in vitro* estimates accurately predict enzyme contributions observed in CBD DDI studies in human. Appropriately partitioned CBD clearance by enzyme metabolism pathways demonstrated by this study would give confidence to scale the adult PBPK model for pediatric use. As a result, predicted CBD and 7-OH-CBD exposures in infants and children using the scaled PBPK model can address a gap where PK data are currently limited in these populations.

4.2 Methods

4.2.1 Software

PBPK modelling was performed using the open-source PBPK modelling platform, PK-Sim version 9.1 (Open Systems Pharmacology Suite). Published PK profiles were digitized with Plot Digitizer version 2.6.8 (by Joseph Huwaldt) to obtain concentration-time data. Analyses of the clinical DDI study simulations, including linear mixed effect modelling, were conducted using R (R Core Team, 2019, Vienna, Austria).

4.2.2 IV PBPK Model Construction and Evaluation

An IV model was constructed using CBD physicochemical properties and knowledge of absorption, distribution, metabolism, and excretion (ADME), and an IV dataset. Prediction methods for cellular permeability (PK-Sim Standard) and partition coefficients (Rodgers & Rowland (197, 198, 251), Schmitt (252), Berezkhovskiy (253), and PK-Sim Standard) were evaluated. Local optimization was carried out in PK-Sim using a Monte Carlo approach for exploring the parameter space of influential

model variables. The optimized model was evaluated using a different IV dataset than used for optimization. Model evaluation was quantitatively assessed by calculating the average fold error (AFE; bias) and absolute AFE (AAFE; precision) of drug plasma concentrations. Two-fold error was deemed reasonable.

4.2.3 Oral PBPK Model Construction and Evaluation

Leveraging knowledge of the systemic disposition from the IV model, an oral PBPK model was built for CBD administered in the fasted and fed states. Absorption-specific parameters (specific intestinal permeability and dissolution model) were adjusted to account for absorption-related PK nonlinearity (254). Specific intestinal permeability was set based on the BCS Class II drug status of CBD. Model evaluation was quantitatively assessed by calculating AFE and AAFE as described for the IV PBPK model evaluation. Additionally, the oral model predicted $AUC_{0-\tau}$ were compared to observed values (development and evaluation datasets) by calculating the percent difference, attained by (observed – predicted) / observed × 100%.

4.2.4 Population Models Construction and Evaluation

To assess the ability of the models to reproduce PK variability following their respective IV and oral administrations, adult virtual populations of 100 individuals were created. Virtual populations in PK-Sim are created based on the methods described by Willmann, Höhn (215). Briefly, virtual populations were built based on sex, age, and weight distributions of clinical studies used to evaluate PK variability where PK variability was therefore a function of anatomical and physiological interindividual variability for relevant model parameters.

4.2.5 Metabolite Model Construction and Evaluation

An initial evaluation of the *in vitro* study-informed clearance partitioning was performed using knowledge that CBD to 7-OH-CBD metabolite formation is mainly attributed to CYP2C19 and CYP2C9 metabolism (249). An oral model was constructed using 7-OH-CBD physicochemical properties and its known ADME. The metabolite model was evaluated by assessing AFE and AAFE with published observed 7-OH-CBD plasma concentration-time profiles.

4.2.6 Clinical DDI Studies Simulation

In order to evaluate clearance partitioning, completed using *in vitro* information, clinical DDI studies reported in Patsalos, Szaflarski (246) were simulated. CBD was the victim drug and modelled using the fed state oral CBD PBPK model. In this model, metabolic clearance was partitioned according to the relative contributions of CBD metabolizing enzymes as defined in *in vitro* studies (248, 249). Three perpetrator drugs were used in the clinical DDI studies of Patsalos, Szaflarski (246). The inhibitor itraconazole and inducer rifampicin, and inhibitor fluconazole, were included for their strong effects on CYP3A4 and CYP2C19, respectively. Compound properties of these perpetrator drugs were extracted from existing PBPK models (255-257). These models were modified to incorporate the main inhibition or induction processes affecting CBD clearance and relevant formulations of the perpetrator drug. Although rifampicin and fluconazole additionally induces CYP2C19 and CYP2C9 (258), and inhibits CYP3A4 and CYP2C9 (259, 260), respectively, only CYP3A4 for both perpetrators and CYP2C19 for fluconazole were included in their PBPK models to reflect dominant metabolism pathways.

To create a population of subjects who were administered CBD in the fed state, 100 individuals were built based on the sex, age, and weight distributions reported by Patsalos, Szaflarski (246). The virtual subjects were given a single dose of 750 mg CBD oral solution after 30 minutes of a high-fat breakfast for the CBD alone arm. These subjects were subsequently administered the perpetrator drug according to their respective protocol (246). On the last day of perpetrator drug treatment, 750 mg CBD oral solution was co-administered 1 hour after itraconazole or fluconazole and within 30 minutes of starting a high-fat breakfast. The rifampicin group received 750 mg CBD concomitantly and within 30 minutes of starting a high-fat breakfast on day 16 of treatment. The predicted CBD $AUC_{0-\infty}$ of each subject from CBD alone and CBD co-administered with perpetrator were exported from PK-Sim and into R.

For each perpetrator, a linear mixed effect model was estimated with log-transformed $AUC_{0-\infty}$ values with treatment as fixed effect and subject as random effect. The estimated treatment effect was obtained, representing the log-mean difference of $AUC_{0-\infty}$ of CBD and co-administration of the perpetrator, and CBD alone. The estimate was then back-transformed to obtain the ratio of treatment medians, also known as the $AUC_{0-\infty}$ geometric mean treatment ratio. The difference between the predicted and observed treatment median ratios were quantified by percent error. The percent errors

were calculated by taking the absolute of the observed value subtracted by the predicted value divided by the observed value and multiplying by 100%.

4.3 Results

4.3.1 IV PBPK Model

The drug specific parameters of CBD and the values used for the IV model prior to optimization, termed the naïve IV model, are presented in **Table 4-1. Table 4-2** presents the CBD dataset used to construct the IV model.

Table 4-1. Physicochemical properties and ADME of CBD for IV model construction

	Used in naïve model	Used in optimized model
Physicochemical properties		
Lipophilicity (logP)	6.1 log units (ALOGPS), 6.3 log units (ChemAxon) (242)	2.43 log units
Fraction unbound in plasma (f_u)	0.06-0.07 (261, 262), 0.18 (263)	0.18
Fraction excreted in urine, feces	0.16, 0.12 (unchanged) (239)	0.16, 0.12 (unchanged)
Molecular weight	314.5 g/mol (244)	314.5 g/mol
pK_a	9.7 (acid) (264)	9.7 (acid)
Solubility	0.0126 mg/mL (water, ALOGPS) (242), 34 ± 7.5 µM (FaSSIF buffer) (265), 40 ± 2.5 µM (FeSSIF buffer) (265)	1.2×10^{-6} mg/mL (water, fasted state), 1.88 mg/mL (water, fed state)
ADME		
Partition coefficient	Rodgers and Rowland, Schmitt, Berezkhovskiy, PK-Sim Standard	Schmitt
Cellular permeability	PK-Sim Standard	PK-Sim Standard
CYP3A4 reference concentration[a], CL_{spec}, CL contribution	4.32 µM, 0 1/min, 38% (249)	4.32 µM, 0.34 1/min, 38%
CYP2C19 reference concentration[a], CL_{spec}, CL contribution	0.76 µM, 0 1/min, 21% (249)	0.76 µM, 1.06 1/min, 21%
CYP2C9 reference concentration[a], CL_{spec}, CL contribution	3.84 µM, 0 1/min, 11% (249)	3.84 µM, 0.10 1/min, 11%

UGT1A7 reference concentration[a], CL_{spec}, CL contribution	1 µM, 0 1/min, 4% (248)	1 µM, 0.21 1/min, 4%
UGT1A9 reference concentration[a], CL_{spec}, CL contribution	1 µM, 0 1/min, 16% (248)	1 µM, 5.3 1/min, 16%
UGT2B7 reference concentration[a], CL_{spec}, CL contribution	1 µM, 0 1/min, 10% (248)	1 µM, 0.37 1/min, 10%
Efflux biliary K_m, V_{max}	0 µM, 0 µM/L/min	2000 µM, 1742 µM/L/min
GFR fraction[b]	1.0	1.0

FaSSIF: fasted state simulated intestinal fluid; FeSSIF: fed state simulated intestinal fluid; CL_{spec}: specific clearance; GFR: glomerular filtration rate. [a]Reference concentrations are 100% for the organ with the most abundant enzyme (e.g., liver); it is a fraction thereof for all other relevant organs. [b]GFR fraction of 1.0 indicates renal clearance calculated as GFR*fu with no reabsorption or tubular secretion.

Table 4-2. Pharmacokinetic datasets for IV and oral model construction and evaluation

Study	Dose and administration	Cohort	N	Age (years)[a]	Weight (kg)[a]
IV PBPK model construction					
Ohlsson, Lindgren (266)[c]	20 mg IV infusion over 2 min	European males	5	26.4 ± 5.7	78.6 ± 10.9
IV PBPK model evaluation					
Wall, Brine (239)	20 mg IV bolus	White American males	5	25.0[b]	76.9[b]
Oral PBPK model construction – fasted state					
Tayo, Taylor (267)[d]	200 mg oral solution	European males (63%) and females	8	60.4 ± 11.5	75.0[b]
Taylor, Crockett (262)[d]	200 mg oral solution	European males (50%) and females	8	55.0 ± 10.0	89.4 ± 11.6
Crockett, Critchley (268)[c]	750 mg oral solution	European males (41%) and females	29	36.6 ± 14.3	74.7 ± 11.0
Schoedel, Szeto (269)[c]	750 mg oral solution	American males (72%) and females	41	37.7 ± 8.9	81.0[b]
Center for Drug Evaluation	750 mg oral solution	American males (44%) and females	49	33.0	66.0[b]

and Research (270)[d]					
Schoedel, Szeto (269)[c]	1500 mg oral solution	American males (72%) and females	41	37.7 ± 8.9	81.0[b]
Taylor, Gidal (241)[d]	1500 mg oral solution	European males (17%) and females	6	26.0 ± 3.2	62.0[b]
Taylor, Gidal (241)[d]	3000 mg oral solution	European males (50%) and females	6	25.0 ± 4.7	70.0[b]
Schoedel, Szeto (269)[c]	4500 mg oral solution	American males (72%) and females	41	37.7 ± 8.9	81.0[b]
Taylor, Gidal (241)[d]	4500 mg oral solution	European females	6	25.8 ± 7.9	57.0[b]
Center for Drug Evaluation and Research (270)[d]	4500 mg oral solution	American males (44%) and females	48	33	66.0[b]
Taylor, Gidal (241)[d]	6000 mg oral solution	European males (33%) and females	6	22.8 ± 3.2	62.0[b]
Oral PBPK model construction – fed state					
Crockett, Critchley (268)[c]	750 mg oral solution with high-fat meal	European males (60%) and females	15	41.1 ± 12.4	71.6 ± 13.0
Taylor, Gidal (241)	1500 mg oral solution with high-fat meal	European males (33%) and females	12	25.1 ± 6.2	59.0[b]
Oral PBPK model evaluation – fasted and fed state					
Morrison, Crockett (271)[e]	750 mg oral solution bid fed state	European males (60%) and females	15	27.7 ± 8.2	74.8 ± 13.0
Morrison, Crockett (271)[e]	750 mg oral solution bid fed state	European males (67%) and females	12	35.1 ± 12.9	81.1 ± 14.4
Morrison, Crockett (271)[e]	750 mg oral solution bid fed state	European males (64%) and females	14	29.9 ± 10.5	74.5 ± 12.3
Taylor, Gidal (241)	750 mg oral solution bid fasted and fed states	European males (22%) and females	9	28.6 ± 8.5	59.0[b]

Taylor, Gidal (241)	1500 mg oral solution bid fasted and fed states	European males (56%) and females	9	25.1 ± 4.8	69.0[b]

bid: twice a day; [a]Mean and standard deviation (if reported); [b]Estimated values are presented since demographics were not reported by the study; [c]Studies were used population models development; [d]Studies were also used for 7-OH-CBD oral model evaluation; [e]Data are from separate clobazam, stiripentol, and valproate DDI studies

A naïve model was set up for a mean male individual weighing 78.6 kg. Of the four partition coefficient calculation methods (**Table 4-1**), Schmitt was selected based on visual model performance for curve shape. Lipophilicity, as a scalar for tissue-to-plasma partition coefficients (K_p), was set 2.5-fold lower than predicted *in silico* to be in alignment with experimental values. The experimental values were attained from a study by Gronewold and Skopp (272), which measured CBD concentrations in body fluids and tissues from human cadavers. Gronewold and Skopp (272) performed single dose rather than continuous infusion studies, thus potentially underestimating actual steady state K_p values. Nevertheless, the study by Gronewold and Skopp (272) justified that volume of distribution was well captured with a lipophilicity of 2.43 resulting in predicted K_p values (median: 3.7; range: 0.18, 43.4), which were similar to K_p values measured in humans (median: 3.3; range: 1.8, 21.3) (272).

Clearance was partitioned as biliary and metabolic. IV plasma profiles from the study by Wall, Brine (239) did not present a distinct second peak. Therefore, absence of significant enterohepatic circulation was suggested and therefore not modelled. The study also suggested significant biliary clearance occurring. To model this process, a transporter was added to the apical side of the liver and its properties optimized to reach a fraction excreted unchanged in feces of 12%. Glomerular filtration rate times fraction unbound in plasma accounted for renal clearance.

CYPs and UGTs contributed 70% and 30% of total metabolic clearance, respectively (249). Clearance was further partitioned according to the relative contributions of metabolizing enzymes, CYP3A4, CYP2C19, CYP2C9, UGT1A7, UGT1A9, and UGT2B7, based on *in vitro* studies by Beers, Fu (249) and Mazur, Lichti (248) (**Table 4-1**). The organ-specific expression of UGT1A7 was informed by the Human Protein Atlas (https://www.proteinatlas.org/) and Strassburg, Manns (273). The remaining enzymes were populated by the PK-Sim expression database reverse transcription polymerase chain reaction (RT-PCR) profiles (274-276).

The optimized values for the IV PBPK model are presented in **Table 4-1**. The outcomes of IV model optimization and evaluation are presented in **Appendix C: Supplementary Figure 2** and **Figure 4-1**, respectively.

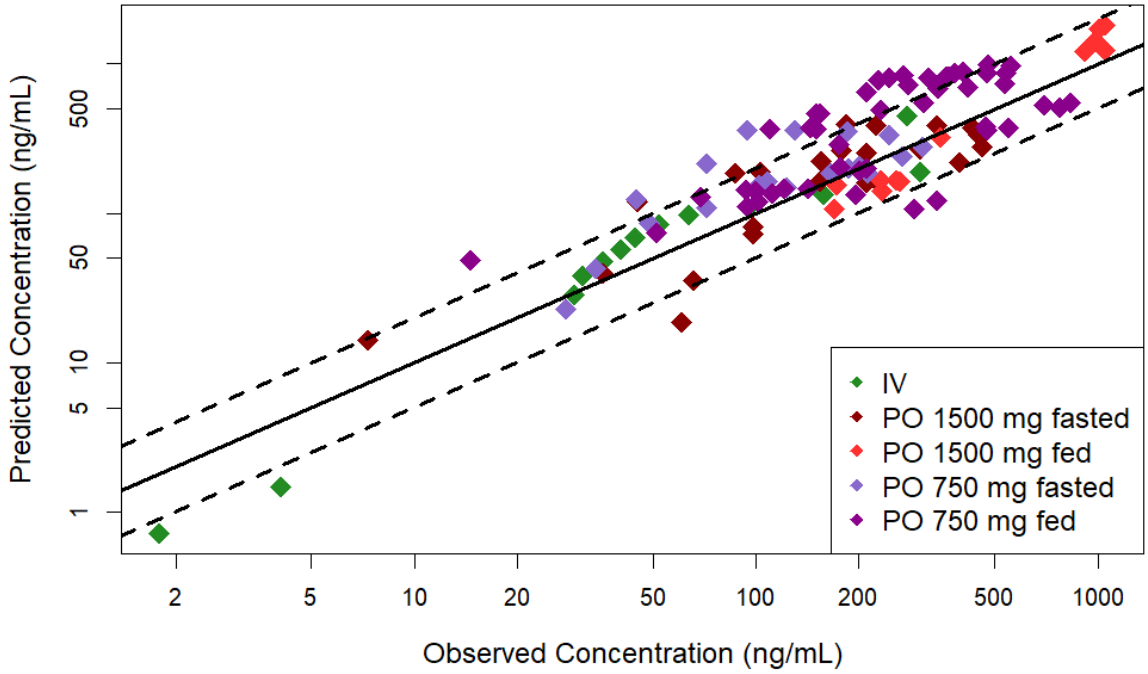


Figure 4-1. The IV and oral model predicted vs observed mean CBD plasma concentrations for evaluation (R^2 = 0.68). The solid and dashed lines represent the identity line and two-fold differences, respectively. CBD, cannabidiol.

4.3.2 Oral PBPK Models

The systemic parameters developed for the mean male IV PBPK model were used for the model defining oral administration. Drug/formulation-specific parameters that were defined in the oral model included CBD solubility, formulation dissolution, and specific intestinal permeability. The single dose administration PK datasets used for model building are shown in **Table 4-2**. CBD as an oral solution is virtually insoluble in water (277). As a result, solubility was expected to rate limit absorption and therefore specific intestinal permeability was set to a high and non rate-limiting value. The expected dissolution-precipitation-dissolution cycle was modelled assuming overall dissolution followed a Weibull function. To account for absorption-related PK non-linearity (254), dissolution

shape and half-time were optimized for each dose, and solubility was optimized globally. To account for the significant change in CBD bioavailability due to the food effect (241, 268), dissolution parameters and solubility were optimized using studies with fed state subjects (**Table 4-2**). The results of the optimization of oral absorption parameters in both the fasted and fed states are shown in **Table 4-3**.

Table 4-3. Oral absorption parameters for fasted and fed state oral models construction

	Used in naïve model		**Used in optimized model**	
	Fasted state	**Fed state**	**Fasted state**	**Fed state**
Dissolution half-time, shape				
200 mg	10 min, 0.92	NA	790 min, 1.0	NA
750 mg	10 min, 0.92	NA	460 min, 2.2	NA
1500 mg	10 min, 0.92	10 min, 0.92	350 min, 3.3	95 min, 1.6
3000 mg	10 min, 0.92	10 min, 0.92	650 min, 2.4	120 min, 2.0
4500 mg	10 min, 0.92	NA	350 min, 3.3	NA
6000 mg	10 min, 0.92	NA	840 min, 2.4	NA
Water solubility	0.0107 mg/mL (242)	1.2×10^{-6} mg/mL	1.20×10^{-6} mg/mL	1.88 mg/mL
Specific intestinal permeability	2.47×10^{-5} cm/min	2.47×10^{-5} cm/min	1 cm/min	1 cm/min

NA: no available fed state data to inform this parameter

The developed oral PBPK models in the fasted and fed states were evaluated in five datasets with multiple dosing presented in **Table 4-2**. The study by Morrison, Crockett (271) was used to evaluate the fed state model with 750 mg oral solution administered twice a day. In the studies by Taylor, Gidal (241), an oral solution dose was administered in the morning after 10 hours of fasting and administered again in the evening 2 hours after the end of a meal. This regimen was repeated for a total of 7 days. Model performance for the evaluation is presented in **Figure 4-1** and **Appendix C: Supplementary Figure 3**, **Supplementary Figure 4**, and **Supplementary Figure 5**. The IV and oral evaluations produced acceptable AFE and AAFE values of 1.28 and 1.65, respectively. The oral model predicted $AUC_{0-\tau}$ compared to those observed were well captured, resulting in a mean [range] percent difference of -8.54% [-90 to 28%].

4.3.3 Metabolite Model

The oral metabolite model was parameterized with 7-OH-CBD physicochemical and ADME properties presented in **Table 4-4**. Uncertain parameters, such as fraction unbound in plasma and partition coefficient prediction method, were informed by the optimized CBD model. As with CBD, lipophilicity was set 2.5-fold lower than predicted *in silico*. CBD single dose administration studies with measured 7-OH-CBD plasma concentrations used to evaluate the metabolite model are presented in **Table 4-2**. The results of metabolite model evaluation are shown in **Figure 4-2**. The sum of CBD intrinsic clearance by CYP2C19 and CYP2C9 was used to define 7-OH-CBD intrinsic clearance. Further increases in 7-OH-CBD clearance retained the parallel elimination rates of 7-OH-CBD and CBD while reducing the first phase of the former compound. Therefore, the set 7-OH-CBD intrinsic clearance value supported a formation rate limited process and led to a reasonable representation of the observed data. Acceptable AFE and AAFE values of 1.09 and 1.71, respectively, were produced.

Table 4-4. Physicochemical properties and ADME of 7-OH-CBD for model construction

	Used in naïve model	**Used in optimized model**
Physicochemical properties		
Lipophilicity (logP)	5.3 log units (XLogP3 3.0) (278), 5.0 log units (ChemAxon)	1.94 log units[a]
Fraction unbound in plasma (f_u)	0.06-0.07 (261, 262), 0.18 (263)	0.18[a]
Molecular weight	330.5 g/mol (278)	330.5 g/mol
pK_a	9.7 (acid) (264)	9.7 (acid)[a]
Solubility	4×10^{-3} mg/mL (water, ChemAxon), 0.26 mg/mL (water, ChemSpider) (278)	4×10^{-3} mg/mL
ADME		
Partition coefficient	Schmitt	Schmitt[a]
Cellular permeability	PK-Sim Standard	PK-Sim Standard[a]
P450 concentration, CL_{spec}[b]	1 µM, 0 1/min	1 µM, 1.16 1/min
Efflux biliary K_m, V_{max}	0 µM, 0 µM/L/min	2000 µM, 1742 µM/L/min[a]
GFR fraction[c]	1.0	1.0[a]

FaSSIF: fasted state simulated intestinal fluid; FeSSIF: fed state simulated intestinal fluid; CL_{spec}: specific clearance; GFR: glomerular filtration rate. [a]Uncertain parameters were assumed from CBD physicochemical properties and ADME processes. [b]The identity of enzymes involved in 7-OH-CBD metabolism are yet to be elucidated. [c]GFR fraction of 1.0 indicates renal clearance calculated as GFR*fu with no reabsorption or tubular secretion

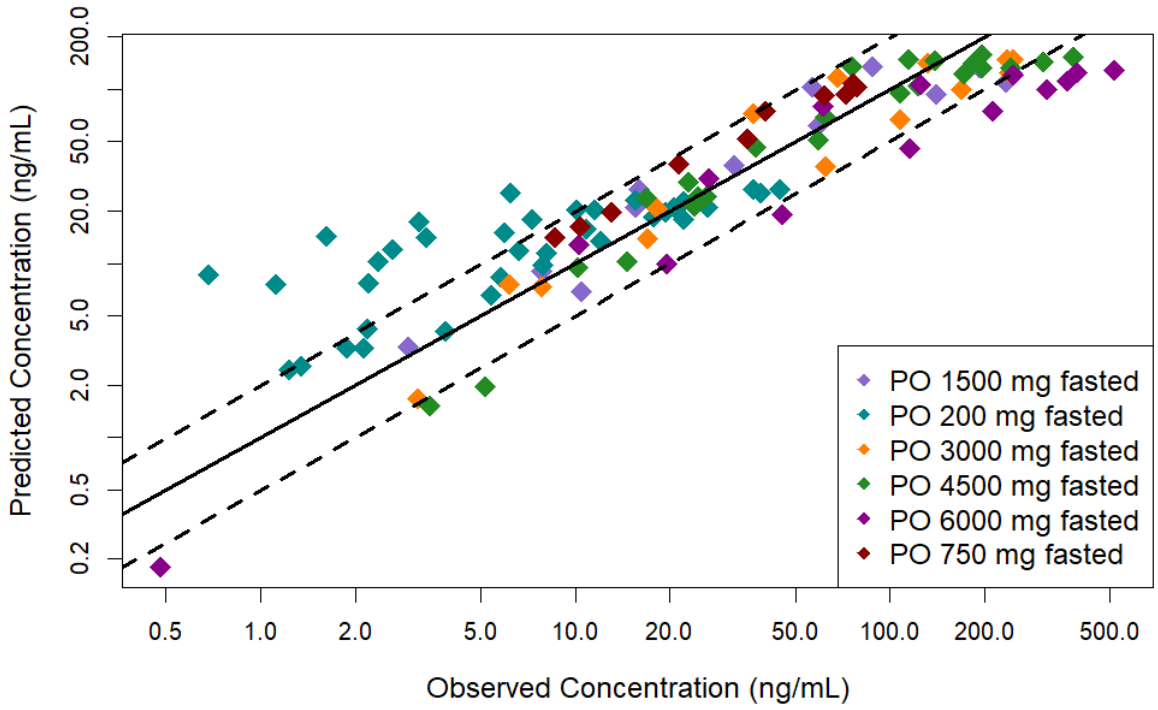


Figure 4-2. Oral model predicted vs observed mean 7-OH-CBD plasma concentration for evaluation (R^2 = 0.65). The solid and dashed lines represent the identity line and two-fold differences, respectively. CBD, cannabidiol.

4.3.4 Population Models

To assess the accuracy of predicted PK variability, virtual populations were constructed as in the respective clinical study presented in **Table 4-2**. While anatomy and physiology variability are captured in PK-Sim, the interindividual variability in enzyme concentration is not included for every enzyme and must therefore be user-defined. User-defined proteins included UGT1A7 and UGT1A9. The reference concentrations of these enzymes were previously defined in **Table 4-1** and represent the most abundant organ concentration of the enzyme with all other relevant organs as a fraction of the concentration. For UGT1A7 and UGT1A9, a geometric standard deviation of 1.5 was applied, based on the following assessment.

The variability of UGT1A9 concentration was based on the *in vitro* studies by Badée, Qiu (216) and Miyagi, Milne (279). In these studies, enzyme activity was attained by measuring UGT1A9 glucuronidation in human liver microsomes of 0-78 year olds. Activity was not found to be age

dependent, and thus a linear function with a geometric mean of 1 and geometric standard deviation of 1.5 was used to describe UGT1A9 activity. To address the lack of variability information on UGT1A7, variability was assumed to be similar to UGT1A9.

Observed variability was well captured with the IV population model (**Appendix C: Supplementary Figure 6**) but tended to be underestimated following oral administration. This finding reflected the status of CBD as a poorly soluble and highly permeable molecule with low bioavailability. Therefore, a standard deviation of 50% of the mean dissolution half-time was added per dose and the final oral population models are presented in **Appendix C: Supplementary Figure 7**, **Supplementary Figure 8**, **Supplementary Figure 9**, **Supplementary Figure 10**, and **Supplementary Figure 11**.

4.3.5 DDI Simulations

The PBPK model for fluconazole (256) did not include inhibition on CYP2C19, a main effect of the perpetrator drug (258). A competitive inhibition process (K_i = 2.1 µM) (280) was added and the model was qualified in humans (281) with results presented in **Appendix C: Supplementary Figure 12**. Furthermore, the rifampicin PBPK model (257) was updated to include a capsule formulation using typical BCS Class II properties of 200 min dissolution half-time for a Weibull function. The model was evaluated using data from subjects administered 600 mg of rifampicin capsule daily (**Appendix C: Supplementary Figure 13**) (282). The fed state oral CBD, itraconazole, and updated fluconazole and rifampicin PBPK models produced treatment ratios presented in **Table 4-5**. The calculated percent error comparing the predicted and observed $AUC_{0-\infty}$ geometric mean treatment ratios were 16%, 19%, and 29% for itraconazole, fluconazole, and rifampicin, respectively.

Table 4-5. Model-predicted and observed $AUC_{0-\infty}$ geometric mean treatment ratios

Study	Itraconazole	Fluconazole	Rifampicin
Patsalos, Szaflarski (246)	1.07	1.22	0.69
This study	1.24	1.45	0.49

Note: $AUC_{0-\infty}$, area under the concentration-time curve from zero to infinity.

4.4 Discussion

This study assessed the appropriateness of using *in vitro* estimates of enzyme contributions to CBD clearance for predicting exposures in humans. The developed IV and oral CBD PBPK models demonstrated acceptable AFE and AAFE values in model evaluation. Although clinical data on CBD metabolism pathways are sparse, we were able to propose the enzymes involved and their contributions to CBD clearance through *in vitro* studies. According to our assessment of the *in vitro* data, CYPs provided a 2.3-fold greater contribution than UGTs, and the individual enzymes involved by order of decreasing contributions included: CYP3A4, CYP2C19, UGT1A9, CYP2C9, UGT2B7, and UGT1A7. Finally, model-predicted treatment median ratios were reasonable with a percent error ranging from 16-30%. Thus, the partitioning of clearance was deemed reasonable, reflecting the observed degree of influence of the perpetrator drugs on CBD clearance.

The methods used in this study present several advantages. To our knowledge, only two other groups have reported developed CBD PBPK models to date (240, 283). The first model was constructed by the sponsors for the main purposes of predicting DDIs with CBD as a perpetrator in adult and pediatric populations (≥2 years old). However, a limitation to the model was that the major metabolic enzyme of CBD, CYP2C19, was not incorporated (240). The second model was developed by Qian and Markowitz (283), with one of the aims to study interactions between CBD and methylphenidate. The model mainly differs from the model in this current study by incorporating a lower fraction unbound value and only the CYP class of enzymes. Our study provides an improved adult PBPK model that incorporates all potentially clinically important metabolic pathways of CBD via both CYP and UGT enzyme classes. Additionally, our work uses an updated fraction unbound value determined by the 3-solvent extraction technique, which provides higher cannabinoid recovery and assay reproducibility (263). Moreover, our study provides a robust evaluation step by validating the models developed with single dose administration data in subjects who were administered multiple dosing. The limited number of published CBD PBPK models may be related to the difficulty in modelling this BCS Class II drug. Our modelling methods addressed a highly permeable and poorly soluble compound through increasing permeability to a non rate-limiting value, setting timing of dissolution changes as a function of dose to capture nonlinear absorption, and modelling the fed state to confirm the sensitivity of dissolution half-time and solubility in affecting CBD bioavailability. Although we employed these methods, the variability in absorption was still underestimated in most model predictions. With the inability for all oral PK variability to be adequately captured, variability

of treatment effect ratios (i.e., as 90% confidence intervals) were not examined in this study. Nevertheless, the assessment with mean treatment effect ratios was sufficient to verify the appropriateness of *in vitro*-determined metabolic enzyme contributions to CBD clearance. Furthermore, based on the use cases of the adult CBD PBPK model to predict exposures and subsequently scale to pediatric populations for exposure estimations, the model was deemed appropriate with $AUC_{0-\infty}$ being similar to observed. Ultimately, with absorption, distribution, and excretion parameters solidified, we had increased confidence that most of the model uncertainty belonged to the partitioning of clearance through metabolism.

In relation to previous work, Jiang, Yamaori (247) also attempted to classify the relative CBD clearance contributions by CYP enzymes. In their studies, the effect of anti-CYP3A4 antibody on 6α-OH-CBD, 6β-OH-CBD, and 4´´-OH-CBD metabolite hydrolase activities were examined in human liver microsomes. Results showed that all activities measured were inhibited to approximately 50% of the control level when the antibody was added. Due to the potential activity of CYP3A4 and CYP2C19 in forming these metabolites, it may be inferred that each enzyme produced even contributions of CBD clearance in the liver. Additionally, chemical inhibition studies and correlation analysis by Jiang, Yamaori (247) suggested CYP2C19 to play a role in 7-OH-CBD metabolite formation. These results by Jiang, Yamaori (247) provided an alternative to the percent contributions determined through the studies by Beers, Fu (249) and Mazur, Lichti (248). Our study used the results of the latter publications to explore the contributions of a greater range of CYP and UGT enzymes.

In vitro studies were used to determine the relative enzyme CBD clearance contributions. There are limitations to use of these data particularly for the partitioning of clearance by CYP and UGT enzymes, and among UGT enzymes. For distinguishing the contributions provided by each enzyme superfamily, we used results from substrate-depletion studies in human liver microsomes (249). Specifically, we based the relative contributions from their depletion rate constants. To further support this decision, an *in vitro* study measuring total metabolite formation by each superfamily to derive intrinsic clearance values and their estimated percent contributions would be necessary. Moreover, specific UGT enzyme contributions were derived from an *in vitro* study that measured UGT isoform activity from incubated microsomal protein that contained recombinant UGT and human liver microsomes (248). Activity towards CBD was limited and UGT1A9, UGT2B7, and UGT2B17 only formed minimal amounts of glucuronidated CBD product (248). Further substrate depletion and metabolite formation studies would be needed to confirm the relative contributions of

UGT enzymes using human liver microsomes. These two main sources of *in vitro*-derived enzyme partitioning of clearance uncertainties may have affected the 16-30% percent difference seen across the three perpetrator drugs. A sensitivity analysis revealed that the CYP versus UGT enzymes relative contributions to total CBD clearance would be influential to the $AUC_{0-\infty}$ geometric mean treatment ratio predictions. As examples, when CYPs provide 9-fold and 0.43-fold difference in contribution compared to UGTs, predicted treatment median $AUC_{0-\infty}$ ratios are 1.36 and 1.08 with itraconazole, 1.74 and 1.13 with fluconazole, and 0.44 and 0.69 with rifampicin, respectively. Consequently, our study may have incorporated an overestimation of activity from CYP enzymes and thus greater magnitudes of treatment median ratios than expected. It may also be possible that the contribution by CYP3A4 was overestimated. The potential overpartitioning of CYP3A4 is relevant for fluconazole and rifampicin since they are known to act on several enzymes that also metabolize CBD, whereas itraconazole is thought to mainly inhibit CYP3A4. Therefore, decreasing the relative contribution of CYP3A4 by increasing the contributions of one or more of CYP2C19, CYP2C9, or UGT enzymes may result in a decreased percent error. Due to the large uncertainty on the exact source of percent error deviation, we based our study on *in vitro* data. Further exploration of potential clinical DDIs in CYP2C9, UGT1A7, UGT1A9, and UGT2B7 would assist in assessing their roles on CBD exposure in human participants.

We attempted to simulate the clinical DDI study by Stott, White (245), where THC/CBD was administered as an oromucosal spray and subsequently with ketoconazole or omeprazole. Since ketoconazole and omeprazole as perpetrator drugs mainly target CYP3A4 and CYP2C19, respectively, uncovering similar model-predicted and observed treatment effect ratios may reinforce our study findings. However, there was substantial uncertainty in the dose directly absorbed by the nasal versus oral passages, which was an essential input for a drug with very low bioavailability (F = 6%). As a result, we were unable to draw conclusions from the simulation exercise using the study by Stott, White (245). Despite this limitation, contributions to CBD clearance by CYP2C19 and CYP2C9 were adequately supported by our developed metabolite model presented in this study.

Finally, a limitation of the model is use of describing the dissolution-precipitation process with Weibull equations that were dose-specific. Therefore, the absorption model was fit to describe the data and we were able to meet the study objective to verify *in vitro*-derived enzyme contributions to CBD clearance for exposure predictions in adults. However, as an outcome of fitting our absorption model, extrapolating to a new population such as pediatrics will require careful consideration.

Our study provides a basis for an understanding of the metabolizing enzymes involved and their potential relative contributions to CBD clearance. The resulting increased confidence in the relative enzyme contributions to CBD clearance allows for the investigation of PK predictions in the pediatric population. Currently, CBD and active metabolite PK data are limited in pediatrics and thus exposure predictions in this populations would be valuable.

Chapter 5
Cannabidiol exposure through maternal marijuana use predictions in breastfed infants

This chapter is reflective of an original manuscript in preparation by the PhD candidate (Cindy Hoi Ting Yeung) for peer-reviewed journal publication. All pertinent dialogue in this chapter was written by the PhD candidate.

5.1 Introduction

The American College of Obstetrics and Gynecologists (ACOG) and the American Academy of Pediatrics (AAP) recommend avoiding cannabidiol (CBD) and CBD-containing products during breastfeeding due to potential neurodevelopmental risks to the infant (2, 284). CBD use is widespread and increasing among adults, especially for medical purposes (285, 286). However, information on CBD risk to the breastfed infant is largely unknown due to limited and variable existing evidence (287). To support the strength of recommendations, more knowledge is required on the dose-exposure-response relationship of CBD in breastfed infants. Such information would lead to better understanding of whether observed CBD concentrations in milk consumed by infants (dose) lead to relevant systemic concentrations in breastfed infants (exposure) associated with neurodevelopmental delays (response).

Beginning in 2014, Mommy's Milk Human Milk Biorepository (HMB) investigators (CDC and KAB) sought to improve understanding of maternal exposure to various agents, including marijuana and its metabolites, during breastfeeding and the potential for infant exposure to specific agents and subsequent adverse infant outcomes. The HMB is a US and Canada-wide study that collects human milk samples from mothers who were or were not taking medications and recreational drugs, including marijuana, CBD and CBD-containing products (116). The HMB investigators continue to study breastfeeding exposures and potential infant outcomes through administration of neurodevelopmental questionnaires and face-to-face testing. In the present secondary data analysis, we seek to fill a gap by further defining CBD exposures to breastfed infants. In this work, we leverage real-world CBD concentrations in breastmilk from the HMB, knowledge of breastmilk intake as a function of infant age (288), dose and route of administration, and physiologically-based pharmacokinetic (PBPK) models to translate CBD dose through breastfeeding into neonatal exposures. PBPK modeling is a mathematical tool used to predict drug exposures based on the physicochemical properties of a compound, and the anatomy and

physiology of organisms. We sought to answer, among breastfeeding mothers taking CBD based on real-world use, what is the predicted exposure and its associated variability in breastfed infants?

5.2 Methods

5.2.1 Software Used and Data Source

The open-source PBPK modeling platform, PK-Sim version 11 (Open Systems Pharmacology Suite), was used to perform PBPK modeling. Plot Digitizer version 2.6.8 (by Joseph Huwaldt) was used to digitize published pharmacokinetic (PK) profiles to obtain concentration-time data. R (R Core Team, 2019, Vienna, Austria) was used to curate the HMB dataset, analyze subgroups, and simulate infant daily doses.

The HMB was established in 2014 at the University of California San Diego for research purposes. The HMB collects voluntary human milk samples from lactating women who are or are not exposed to any medication, recreational drug, or environmental chemical primarily in the two weeks prior to sample collection. Detailed information on recruitment, data collection, and sample preparation and analysis methods have been presented previously (116). Participants complete an interview to provide their demographics, maternal and child health history, breastfeeding habits, and all exposures focused in the previous two weeks prior to sample collection. Exposure information from women who reported marijuana use at any time since giving birth included route of administration, frequency of use, dose, and time since last use before milk sample collection. Milk samples were previously measured for metabolites, including CBD concentrations and the date and time of the milk collection were ascertained. This present study received ethics clearance from the parent study through the UC San Diego Human Research Protections Program, and for secondary data analysis through the University of Waterloo Research Ethics Board (REB# 42860).

5.2.2 Dose Determination

5.2.2.1 CBD Concentrations in Milk

Information on maternal exposures and measured CBD concentrations in milk collected and assayed by the parent study between 2015 and 2021 were extracted from the existing HMB dataset. The dataset was organized to describe: all concentrations in milk (Dataset 1); and concentrations by self-reported maternal frequency, dose, and type of administration (Dataset 2). From the existing data for the sample on quantification of CBD, three methods were assessed to account for below limit of quantification (BLQ) values: (1) BLQ = lower limit of quantification (LLOQ)/2, (2) BLQ are drawn from uniform distributions of 0 to LLOQ, and (3) BLQ = LLOQ.

For concentrations from samples with maternal reported type of administration (Dataset 2), only concentrations with one type of maternal administration were retained. Missing end time of exposure was replaced with the time of concentration sample collection and vice versa. The effects of administration type, time after last dose (TAD), and dose-frequency on concentration were assessed. Administration type was a categorical variable defined as: edible, joint, oil, pipe, or other (vaporizer, topical, etc.), and N/A (not reported) categories. As a continuous variable, TAD was described as time in hours elapsed from the end of maternal administration to milk sample collection for concentration measurement. TAD was calculated by subtracting the date and time of sample collection by date and time of the last reported date of maternal administration. To account for the varying ways in which dose and frequency of CBD and CBD-containing products were consumed (e.g., number of puffs per day versus mg per week), dose-frequency was categorized as low, medium, and high based on the data of each week-normalized dose type. To compare these subgroups, the exposure-concentration subset (Dataset 2) was considered with and without BLQ values. A linear regression model to predict log-concentrations was obtained after testing the significance of subgroups on CBD in milk concentrations including TAD, administration type, and interactions between TAD and administration type, and administration type and dose-frequency. Model goodness of fit was evaluated through standard residual analysis. Post-hoc pairwise comparisons of estimated marginal means of the significant subgroups were performed using various p-value adjustment methods (from most to least conservative: Bonferroni, Holm, and Tukey) due to lack of a gold standard method.

5.2.2.2 Volume of Milk Intake

The volume of milk intake that an infant typically receives on a weight-normalized basis and as a function of postnatal age, was drawn from a literature review-derived milk intake model described in our previous work (288, 289).

5.2.2.3 Dose Simulation

To simulate weight-normalized doses received by each virtual breastfed infant, daily milk intake volume (mL/kg) was multiplied with an observed or simulated CBD in milk concentration (ng/mL). For all concentrations, random sampling with replacement was performed on the full dataset (Dataset 1). For the significant subgroups (Dataset 2), above LLOQ concentrations were simulated from a log-normal distribution using the mean and variance from the subgroup log-concentrations. Concentrations that were BLQ were simulated based on the estimated probabilities obtained from a logistic regression model. The Hosmer-Lemeshow goodness-of-fit test was used for model assessment (290). Milk intake volumes were selected from a normal distribution with a mean (288)

obtained from a non-linear age-dependent equation and standard deviation (289) specific to the age group of the infant.

5.2.3 Model Development and Evaluation

The pediatric PBPK model was developed according to the workflow of Maharaj, Barrett (99). An adult oral CBD PBPK model established from our previous work (291) was scaled to simulate CBD exposure in virtual breastfeeding infants. Briefly, anatomy and physiology were scaled for different infant ages, and growth and maturation of relevant processes including metabolic capacity, glomerular filtration rate, protein binding, and body composition, were adjusted for. Variability was applied to the anatomy and physiology to produce a virtual infant population. For user defined proteins, UGT1A7 and UGT1A9, activity was found not to be age dependent, and thus ontogeny was described with a linear function and geometric standard deviation of 1.5 (291).

Two studies reported on the PK of CBD administered in children, however the experimental data were not consistent (292, 293). Particularly, the $AUC_{0-\tau}$ on day 1 presented by Wheless, Dlugos (292) vastly differed from the $AUC_{0-\tau}$ reported in adults (241, 262, 267-270) and 4-11 year olds reported by Devinsky, Patel (293) with similar weight-normalized doses. Thus, evaluation of the pediatric PBPK model was performed with Devinsky, Patel (293), where children 4-11 years old were randomized to receive one of three doses of CBD oral solution daily (5, 10, or 20 mg/kg).

5.2.4 Exposure Predictions

Using the developed pediatric PBPK model, infant populations of 200 individuals using the National Health and Nutrition Examination Survey (NHANES) population (294, 295) (50% female) were simulated per age group in days: >0 to 7, >7 to 14, >14 to 30, >30 to 60, and >60 to 365. Administration of CBD to these virtual breastfed infants differed from that given to adults (291). In the adult oral model, CBD solution was described as a dissolution-precipitation process that was dose-specific and fit to describe the data. For extrapolation to pediatric populations, CBD was assumed to remain as a solution due to the small doses that breastfed infants receive. Since CBD exhibits non-linear kinetics, each infant was assigned a daily dose of CBD solution until steady state was reached and $AUC_{0-\tau}$, where $\tau = 24$ hours, was taken. This process to simulate doses was performed with all CBD concentrations in milk and for each of the subgroups.

Simulated $AUC_{0-\tau}$ was determined for 200 virtual breastfed infants per age group and 100 virtual children administered the lowest therapeutic dose of 5 mg/kg/day [8] for approved indications as comparison. The upper area under the curve ratio (UAR) was calculated for each breastfed infant age group using the following equation (289):

$$UAR = \frac{95th\ percentile\ simulated\ breastfeeding\ infants\ AUC_{0-\tau}}{Median\ therapeutic\ AUC_{0-\tau}\ for\ approved\ indications}$$

The median therapeutic $AUC_{0\text{-}\tau}$ was calculated from the 100 virtual children administered 5 mg/kg/day based on observed data in Devinsky, Patel (293).

5.3 Results

The HMB dataset contained 200 breast milk samples of CBD concentrations (42% BLQ) obtained from 181 unique breastfeeding mothers. Of these samples, 124 (45% BLQ) from 118 participants had only one maternal type of administration. The three methods to account for BLQ values produced similar results. Thus LLOQ/2 with LLOQ as 0.1 ng/mL, was applied. Only concentrations above LLOQ were used in the subgroup analyses since BLQ values tended to produce unsatisfactory residual distributions when incorporated into the log-linear regression models. The proportion of BLQ values were similar across subgroups (33-54%). Descriptive plots of each assessed subgroup using the exposure-concentration subset (Dataset 2) while accounting for BLQ values are presented in **Figure 5-1**.

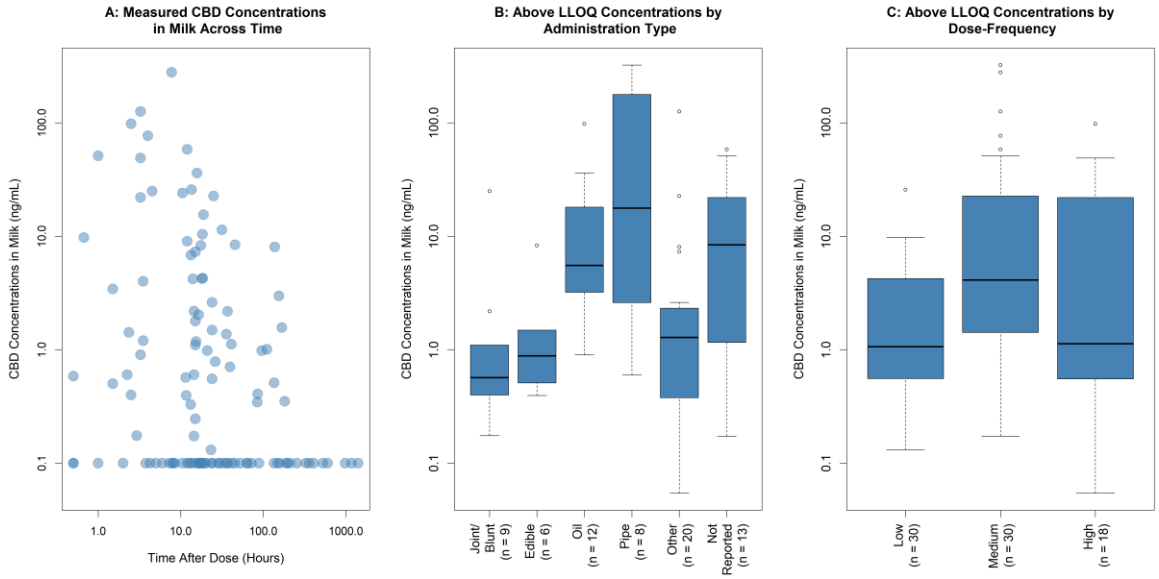


Figure 5-1. Descriptive plots to assess potential subgroups from the exposure-concentration subset (Dataset 2). A: BLQ values reported as 0.1 ng/mL. Five concentrations (0.055 ng/mL, 1.16 ng/mL, 325 ng/mL, BLQ, and BLQ) at TAD = 0 hours not shown. B and C: BLQ values were not included. Number of samples in each subgroup are presented in brackets.

A backward step-wise elimination procedure was performed involving TAD, administration type, and their interactions. In interaction with administration type, dose-frequency was not feasible for

model testing due to low sample size. The final model included administration type which exhibited satisfactory residual behavior. *Post-hoc* pairwise comparisons between administration types across the three p-value adjustment methods suggested that oil versus joint/blunt, joint/blunt versus pipe, and edible versus pipe had significantly different estimated marginal means. Therefore, administration type was grouped into two contrasting subgroups, oil or pipe and joint/blunt or edible, for subsequent dose simulations. Goodness-of-fit plots, estimated marginal means, their 95% confidence intervals, and model estimates are presented in **Appendix D**: **Supplementary Figure 14**, **Supplementary Figure 15,** and **Supplementary Table 3**.

The logistic regression model used to simulate the probabilities of BLQ concentrations found that TAD was significant. Administration type was not found to be significant after controlling for TAD, and thus BLQ values had the same chance of occurring for all administration types. The model performed well with the Hosmer-Lemeshow test resulting in a p-value of 0.768.

The distributions of CBD in milk concentrations and administered doses to virtual breastfed infants are presented in **Table 5-1**. The developed pediatric PBPK model evaluation with Devinsky, Patel (293) results are shown in **Table 5-2**. Predicted $AUC_{0-\tau}$ were comparable to observed which provided confidence in the ability of the model to accurately predict exposures in pediatrics.

Table 5-1. Characteristics of CBD in milk concentrations and doses distributions

Dataset	Distribution	Geometric mean	Geometric SD
Concentrations for sampling			
Full dataset	Resampling from 200 concentrations	N/A	N/A
Joint/Blunt or Edible only	Log-normal	0.94 ng/mL	3.67
Oil or Pipe only	Log-normal	9.74 ng/mL	6.31
Doses administered to virtual breastfed infants			
Full dataset	Log-normal	0.48 ng/kg	2.03
Joint/Blunt or Edible only	Log-normal	0.075 ng/kg	0.27
Oil or Pipe Only	Log-normal	1.65 ng/kg	5.93

SD: standard deviation.

Table 5-2. Pediatric PBPK model-predicted versus observed $AUC_{0-\tau}$*

Study	5 mg/kg/day target dose	10 mg/kg/day target dose	20 mg/kg/day target dose
Day 1 at 1.25 mg/kg			
Devinsky, Patel (293)	70.6 (20.4) (N = 10)	66.4 (121) (N = 8)	73.7 (96.6) (N = 9)
This study	80.6 (55.9)	80.6 (55.9)	80.6 (55.9)
Day 22 at target dose via BID			

Devinsky, Patel (293)	241 (101) (N = 10)	722 (79.9) (N = 8)	963 (93.4) (N = 9)
This study	221.5 (61.8)	428.9 (61.6)	800 (57.1)

*τ: 5 hours, $AUC_{0-\tau}$ presented as geometric mean in ng·h/mL (% coefficient of variation). N: Number of patients. BID: two times a day.

Pediatric PBPK model-predicted daily steady state $AUC_{0-\tau}$ of breastfed infants across the age groups for all CBD concentrations, joint/blunt or edible exposure only, and oil or pipe exposure only compared to children administered CBD therapeutic dose are presented in **Figure 5-2**. Calculated UAR for each age group are shown in **Table 5-3**.

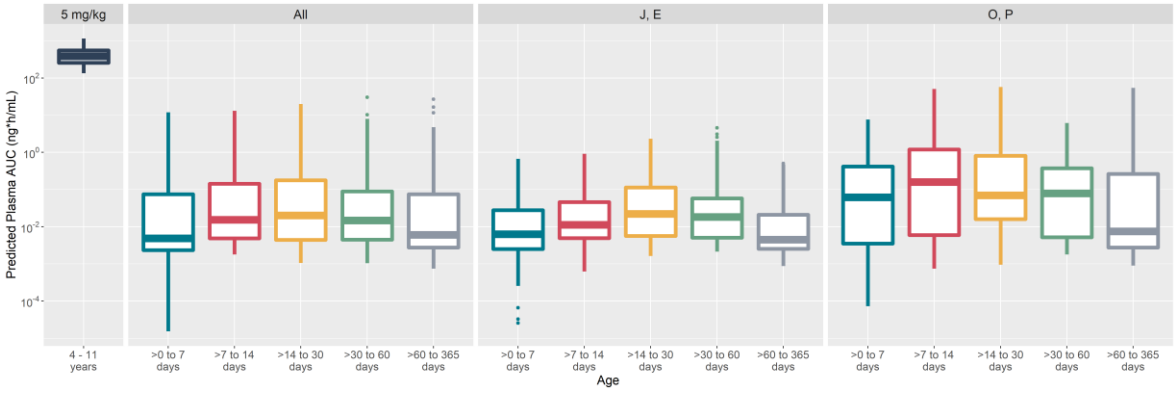


Figure 5-2. CBD PBPK model-predicted daily steady state AUC of children (N = 100, receiving 5 mg/kg/day) compared to breastfed infants across age groups (N = 200 per group, receiving CBD in milk doses). All: CBD in breast milk concentrations from the full HMB dataset; J, E: CBD in breast milk concentrations from joint/blunt or edible exposures; O, P: CBD in breast milk concentrations from oil or pipe exposures.

Table 5-3. UAR of infants (N = 200 per age group) breastfed by mothers during real-world use of CBD and CBD-containing products

Parameter	>0 to 7 days old	>7 to 14 days old	>14 to 30 days old	>30 to 60 days old	>60 to 365 days old
All concentrations					
95th percentile of simulated breastfeeding infants $AUC_{0-\tau}$ (ng·h/mL)	1.37	2.33	3.42	2.84	0.86
UAR*	0.0018	0.0030	0.0044	0.0037	0.0011
Joint/blunt or edible exposure					
95th percentile of simulated breastfeeding infants $AUC_{0-\tau}$ (ng·h/mL)	0.17	0.25	0.51	0.30	0.18

UAR*	0.00022	0.00033	0.00066	0.00039	0.00023
Oil or pipe exposure					
95th percentile of simulated breastfeeding infants $AUC_{0-\tau}$ (ng·h/mL)	2.36	25.9	12.8	3.39	3.06
UAR*	0.0031	0.034	0.017	0.0044	0.0040

AUC: area under the curve; UAR: upper area under the curve ratio. *UAR denominator consists of the simulated median $AUC_{0-\tau}$ based on the 4-11 year olds from Devinsky, Patel (293) receiving therapeutic doses for approved indications.

5.4 Discussion

Through use of real-world CBD concentrations in breastmilk, this study provided additional information on potential levels of CBD exposure in breastfed infants. By examining the relationship between maternal type of administration and concentrations in breast milk, it was determined that oil or pipe tended to result in higher predicted concentrations as compared to joint/blunt or edible forms. Additionally, this work found that the longer the TAD, the greater the presence of BLQ concentrations were in breast milk. Moreover, BLQ values had the same chance of occurring for all administration types. Knowledge about the impact of TAD on BLQ concentrations across administration types could have clinical advising implications, such as the existence of optimal breastfeeding times when taking CBD and CBD-containing products.

A strength of this study was based on the ability of the PBPK model to predict $AUC_{0-\tau}$ reasonably in adults.(291) This increased our confidence especially in the $AUC_{0-\tau}$ predictions in children 4-11 years old for model evaluation. Although geometric mean $AUC_{0-\tau}$ was predicted 1.7-fold less than observed in Devinsky, Patel (293) for 10 mg/kg/day dosing, our findings were in-line with Wheless, Dlugos (292) (494.5 ng·h/mL).

Beyond the ability to predict exposures, the UAR accounts for the anatomy and physiology of breastfeeding infants; age dependent factors, such as milk intake volumes as a function of age; and variability in the infant and maternal population, such as maternal pharmacogenotypes that could lead to an increased presence of medication in breast milk. The UAR was calculated using the pediatric PBPK model-predicted exposures in virtual breastfed infants. This novel metric offers an improvement over current metrics which focus solely on the potential dose received by the breastfed infant, without accounting for exposure (i.e., infant plasma concentrations). The UAR calculated for CBD revealed that even the exposures of the most vulnerable breastfed infant (95th percentile on the higher exposure end) are well below the exposures of 4-11 year olds receiving the lowest approved dose for approved indications. This finding serves as additional exposure

information to healthcare providers to consider when discussing CBD use by mothers in relation to their breastfeeding infants.

For context, our group has simulated breastfeeding exposures for lamotrigine (289) and escitalopram (101) in previous work. Predicted breastfeeding infant exposures tended to reach levels of exposure from adults taking therapeutic doses for lamotrigine, but not for escitalopram. The UAR was also calculated for lamotrigine and was determined to be relatively high for some age groups. These observations were in line with adverse reactions reported for lamotrigine and escitalopram, with more observed in the former than the latter. Thus, the UAR serves as a useful tool to anticipate potential responses in breastfeeding infants. In regards to CBD, it would be of interest to follow-up in future studies assessing breastfeeding infant adverse reactions and effects on neurodevelopment to understand the relationship between the UAR results of this study with response information.

This work recognizes the great uncertainty of CBD bioavailability in breastfed infants. In adults, bioavailability is low and greatly impacted by food. To address this issue, we used the idea that breastfed infants receive small doses of CBD and thus the precipitation-dissolution-precipitation cycle experienced in adults was not expected. Therefore, CBD was given as an oral solution without the dissolution complexities. Moreover, since a solution is already dissolved, the food effect was not relevant in our virtual breastfed infants. As a result, our work was conservative with the pediatric PBPK model predicted 0 to 1 year old infant bioavailability being 0.51-0.59, as compared to 0.23 in adults. Even with this larger infant bioavailability, the UAR was still very low.

The low sample sizes per subgroup serve as a limitation to this study. Although type of administration type was found to be a significant subgroup, further data to support this finding are warranted. Likewise, larger sample sizes are needed to assess other potential subgroups, such as those given by dose-frequency. It is possible that oil or pipe maternal type of administration tended to have higher dose-frequencies. Similarly, the relationship between TAD and BLQ concentrations in milk could be confounded by dose-frequency. However, analyses with the few dose-frequency information we had suggest this not to be the case.

A limitation of the parent study is that maternal exposure information on dose, timing, and route of administration relied on maternal report and may therefore be inaccurate. Furthermore, maternal administration information was typically measured in the previous two weeks prior to milk sample collection. As a result, less data were acquired on long-term frequency of use which may contribute to infant dose.

A further limitation to this study relates to the inability to validate our workflow with CBD concentrations measured in breastfed infant plasma. Since these data have not been reported in the literature, we were not able to check whether the pediatric PBPK model-predicted infant plasma concentrations were in-line with observed. Future studies should focus on collecting and analyzing plasma levels from infants breastfed by mothers taking CBD or CBD-containing products to confirm our results.

Since the study of CBD in milk concentrations was based on highly-dispersed observational data, it can only shed light on the potential association between concentrations and administration types and any statement on causality should be avoided. Nevertheless, this study was able to draw conclusions on infant exposures from real-world maternal use of CBD and CBD-containing products which can be insightful to healthcare providers in advising breastfeeding mothers taking CBD and CBD-containing products. A future direction to study further cannabinoids, such as tetrahydrocannabinol which is observed to have magnitudes greater concentrations in milk (116), can provide a fuller perspective on cannabis use during breastfeeding.

5.5 Conclusion

Predicted CBD exposures in breastfed infants being magnitudes lower than exposures based on observed children (4-11 years old) administered the lowest approved CBD dose. This finding allows healthcare providers to be better informed to discuss CBD and CBD-product use with breastfeeding mothers. This study combined with future work studying infant response to CBD exposure via breast milk, can lead to a better understanding of the entire dose-exposure-response pathway for improved breastfeeding advising.

Chapter 6

Maternal ezetimibe exposure predictions in breastfed infants: Applying the physiologically based pharmacokinetic-derived upper area under the curve ratio workflow

This chapter is reflective of an original manuscript in preparation by the PhD candidate (Cindy Hoi Ting Yeung) for peer-reviewed journal publication. All pertinent dialogue in this chapter was written by the PhD candidate, with the exception of sections 6.2.1, 6.2.2, and 6.2.4 which include major writing contributions from collaborators, SickKids (conducted collection of patient data) and CHU Ste-Justine (conducted assay development and validation).

6.1 Introduction

Breastfeeding is known to benefit maternal and infant health. Examples of benefits include lowered risks of postpartum depression, type 2 diabetes mellitus, breast cancer, and ovarian cancer in the mother; and reduced incidence and severity of respiratory tract infections and otitis media in the newborn and protection against allergic disease states in the infant (118-121, 296, 297). Although the advantages to breastfeeding are clear, the decision to breastfeed becomes uncertain when there is maternal medication use. Contributors to this uncertainty are a lack of pharmacokinetic (PK; what the body does to the drug) and pharmacodynamic (PD; what the drug does to the body) information. In other words, there is a paucity of knowledge in dose to exposure (PK) and exposure to response (PD) in the breastfed infant. Lack of information in drug PK and PD in breastfed infants is a result of limited studies conducted in lactating mothers and their infants in the drug development process. Consequently, a review of drugs approved in the US between 2003-2012 found that nearly half of the labels (47.9%) had no data on breastfeeding, and only 4.7% presented human data (84).

Physiologically based pharmacokinetic (PBPK) modelling is a promising tool for improving our understanding of maternal medication dose to exposure relationships in breastfeeding infants. PBPK models use a mechanistic understanding of drug behaviour in virtual organisms in order to predict drug PK. Thus, with knowledge about the drug properties combined with knowledge about the anatomy and physiology of the organism, drug exposures can be predicted. The main utility of PBPK modeling in maternal medication use during breastfeeding is the ability to extrapolate from adult to infants. Essentially, PBPK models can be built and evaluated with rich adult data and subsequently

scaled to infants. Given doses the infants would receive through breast milk, their exposures can be calculated.

Leveraging the utility of pediatric PBPK modeling, our group has developed the upper area under the curve ratio (UAR) (289). To arrive at the UAR, a workflow involving typical weight-normalized milk intake volume (288) and measured drug in milk concentrations to simulate doses in breastfed infants as an input into a developed pediatric PBPK model for exposure predictions. The UAR is proposed as an improvement over current metrics since it incorporates the anatomy and physiology of the infant, age-dependent factors (e.g., milk intake volume is a function of age), and variability in infant and maternal populations (e.g., maternal pharmacogenotypes resulting in varying drug in milk concentrations). Moreover, the UAR emphasizes exposure, whereas existing metrics such as the relative infant dose, are limited to dose information (298).

To date, our group has developed the UAR workflow using lamotrigine as an example (289) and performed an application to cannabidiol [manuscript in preparation]. These two study drugs provided unique opportunities to examine UAR use and potential. For lamotrigine, rich published data that included breastfed infant plasma concentrations, were used to evaluate the performance of a developed pediatric PBPK model to predict exposure levels (289). With the workflow solidified, we had confidence in predicting CBD exposures in breastfed infants without infant plasma data in the literature [manuscript in preparation]. Lamotrigine served as a medication typically difficult to discontinue for mothers treating their epilepsy, whereas CBD provided an example of a highly searched drug in databases such as LactMed [personal communication], which curates information on drugs and substances during lactation. Additionally, the application of UAR for CBD involved the use of real-world CBD concentrations in breast milk. Finally, the UAR was greater for lamotrigine as compared to CBD, suggesting cases with differing degrees of exercising caution.

A medication that would offer further insight into use of the UAR is ezetimibe. Ezetimibe is an antihyperlipidemic agent that inhibits cholesterol absorption. It is classified as BCS II, which defines the drug with low solubility and high permeability. Ezetimibe does not follow linear kinetics (299). Absolute bioavailability has not been determined for ezetimibe since it is virtually insoluble in aqueous media, however, studies in dogs report a low bioavailability (F = 0.58-1.1%). Ezetimibe and its primary metabolite, phenolic ezetimibe-glucuronide (EZE-glucuronide) undergo extensive enterohepatic circulation as demonstrated through multiple animal studies (300, 301). The multiple

plasma concentration-time profile peaks can be observed in humans with oral administration (302). Various factors contribute to ezetimibe and EZE-glucuronide clearance, including metabolism by UGT2B15 (~90% activity in human liver microsomes), UGT1A1, and UGT1A3 primarily in the liver. Additionally, several transporters are proposed to act on ezetimibe and EZE-glucuronide, including P-glycoprotein (MDR1), and OATP1B1, MRP2, and MRP3, respectively (303-305). Both ezetimibe and EZE-glucuronide are highly protein bound (>90%) (306), exhibit slow elimination (terminal half-life of 22 hours), and result in a 2-fold accumulation after multiple administration. Measured radiolabelled ezetimibe following oral administration in humans was excreted as 0.69 unchanged in feces at 96 hours (302). The fraction excreted was likely a combination of unmodified ezetimibe and de-glucuronidated EZE-glucuronide entering the large intestine.

Breastfeeding women tend to be excluded in clinical trials regarding ezetimibe use (307). As a result, scant information exists regarding maternal ezetimibe use during breastfeeding. Only animal studies have reported ezetimibe to pass into breast milk (308). Thus, the presence and concentration of ezetimibe in human breast milk are not known. Furthermore, there are no published pediatric PBPK models on ezetimibe nor its primary active metabolite, EZE-glucuronide. Information on ezetimibe concentrations in milk and its PK through PBPK modeling are needed to improve our understanding of exposure to infant through breast milk. This knowledge is especially important since ezetimibe is typically taken with statins (rosuvastatin and simvastatin). In 2021, the FDA provided communication stating that breastfeeding is not recommended in patients who require statins (309). However, this recommendation was thought to be based on limited evidence (310). Applying the UAR workflow to ezetimibe would help start the conversation to better understand the exposure of cholesterol lowering medications from breast milk and whether the addition of ezetimibe has the potential to exacerbate the proposed statin concerns.

The purpose of this work was to collect ezetimibe and EZE-glucuronide in breast milk samples from real world maternal use, develop and validate an assay to measure drug and metabolite in breast milk concentrations, and perform the UAR workflow for the prediction of ezetimibe and EZE-glucuronide exposures in breastfed infants. Comparisons between predicted exposures in breastfeeding infants and therapeutic exposures would aid in our assessment of potential risk to the breastfeeding infant population.

6.2 Materials and Methods

6.2.1 Study Population

Breast milk samples and study data (demographics, and breastfeeding- and sample-related information) were collected from two women recruited through the "Transfer of Ezetimibe into Breast Milk" study (Research Ethics Board (REB) #HS19991) at the University of Manitoba (PI: Dr. Pamela Katz), in collaboration with the "Drugs in Lactation" Analysis Consortium (DLAC) at the Hospital for Sick Children in Toronto, Canada (PI: Dr. Shinya Ito). Inclusion criteria included women on chronic therapy with ezetimibe at steady state, lactating more than 1 week postpartum, and able to communicate in English.

6.2.2 Data Collection and Management

Breastfeeding mothers at steady state, taking a 10 mg daily dose of ezetimibe were asked to provide 10 mL of milk at two time points to collect hind-milk and fore-milk. The hind-milk and fore-milk samples were collected immediately before taking medication and up to four samples at any time after taking the medication, respectively. The milk samples were collected using an electric breast pump (a gift from Medela Canada Inc.). Milk samples were frozen until analysis.

A questionnaire was used to collect: (1) Demographic data for the maternal-infant pair including date of birth, ethnicity, body measurements (body weight and height/length), pregnancy history (length, type of delivery (vaginal or cesarean section), pregnancy-related complications, medical conditions, and medications during pregnancy), past medical history (any long-term illness or health condition and its treatment). (2) Additional information on the infant's general health condition, including symptoms requiring medical attention. (3) Specific information on breastfeeding type (exclusively breastfed, predominantly breastfed, or breastfed <80% of the infant's nutrition) and milk sample collection such as time and type of milk samples. Study data were collected and managed using REDCap electronic data capture tools hosted at the Hospital for Sick Children.

6.2.3 Modeling Software

PK-Sim version 11.1 (Open Systems Pharmacology Suite) was used as the PBPK modeling platform. Plot Digitizer version 2.6.8 (by Joseph Huwaldt) was used to digitize published PK and ontogeny profiles to obtain plasma concentration and enzyme activity across time data, respectively. R (R Core Team, 2019, Vienna, Austria) was used to model the ontogeny profiles, calculate measures of bias,

and perform dose and exposure simulations. Ethics clearance for analysis of breast milk samples and study data was received through the University of Waterloo (REB # 41155).

6.2.4 Analytical Methods

Ezetimibe and EZE-glucuronide concentrations in the breast milk samples were analyzed at the Centre Hospitalier Universitaire Sainte-Justine Pharmacology Research Unit.

6.2.4.1 Materials

Ezetimibe, ezetimibe phenoxy B-D glucuronide (EZE-glucuronide), ezetimibe-D4, and ezetimibe-D4 B-D glucuronide were purchased from Toronto Research Chemicals. Ezetimibe-D4 and ezetimibe-D4 B-D glucuronide are deuterated ezetimibe and EZE-glucuronide compounds, respectively, which were used to develop internal standards. Methanol, acetonitrile, acetic acid, and ammonium hydroxide were purchased from Fisher Scientific. Ammonium acetate and formic acid were purchased from Sigma Aldrich. Water was purified by a Milli-Q water system. Donated breast milk samples from Hema-Québec were provided from multiple women to have a variety of matrices for the calibration curve and for quality control. All solvents and reagents were analytical or mass spectrometry grade. Qualitative and quantitative results were acquired by the Masshunter Acquisition and Masshunter Quantitative software (Agilent Technologies Inc.), respectively.

6.2.4.2 Assay Development

A high performance liquid chromatographic method was coupled with a tandem mass spectrometer to separate and quantify ezetimibe and EZE-glucuronide. Liquid chromatography-tandem mass spectrometry (LCMS/MS) Agilent Triple-Quad LCMS 6460c in negative mode with Eclipse XDB-C8, 4.6X150mm, 3.5µ column were used. The mobile phase consisted of 0.08% formic acid in water (solvent A) and acetonitrile (solvent B) for a total of 5-minutes sample run time at 0.8 mL/min in an isocratic mode. The expected transition ions were 408.1→271.1, 412.1→271.1, 584.1→271.1 and 588.1→271.1 for ezetimibe, ezetimibe-D4, ezetimibe phenoxy B-D glucuronide, and ezetimibe-D4 B-D glucuronide, respectively (311).

6.2.4.3 Assay Validation

Assay performance characteristics of selectivity, accuracy, precision, linearity and lower limit of quantification (LLOQ), matrix effect, carry-over, stability post-extraction, and recovery, were

conducted using spiked breast milk controls from donated samples and standards. All performance studies were evaluated in accordance with Clinical and Laboratory Standards Institute guidelines. To be deemed acceptable, the performance metrics for linearity (standard concentrations), accuracy (mean accuracy), and precision (coefficient of variation (%CV)) had to deviate less than ±15%, and the LLOQ had to deviate less than ±20%.

Selectivity of the assay was assessed in six breast milk samples from different blank samples of donated breast milk. Selectivity was ensured at the LLOQ for each compound and LLOQ was required to be larger than the background noise (signal-to-noise ratio of 5:1). Linearity and the LLOQ were evaluated by analyzing five calibration curves of eight (one curve per day) prepared standards by half-dilution and blank breast milk from a stock solution containing known amounts of certified ezetimibe and EZE-glucuronide (standards). The calibration curve was deemed reasonable if it consisted of six to eight points including the LLOQ.

Accuracy was verified by analyzing three levels of controls (low, medium, and high) spiked from analyte powder and the LLOQ. Five samples were assessed per level per day. Accuracy was evaluated by calculating the percent deviation between the mean concentrations of the unknown samples, and true concentration of known samples. Over five days, within- and between-day precision was assessed by running five replicates of each quality-control level that included the LLOQ. The %CVs were calculated intra- and inter-day.

A matrix effect was assessed by comparing the analysis of ten different matrices spiked at a low concentration in duplicate. Carryover was checked by preparing one high (H) and one blank (B) sample. Each sample was dosed in triplicate (H1-3 and B1-3) and repeated five times. The percentage of carry-over was calculated according to Broughton (312):

$$Carry\ over\ (\%) = \frac{B1-B3}{H3-B3} \times 100\% \quad (1)$$

Stability at post-extraction was verified with three levels of controls (low, medium, and high) that were spiked from powder and LLOQ at 4 hours and 24 hours. The concentration of each reinjection was compared with the initial injection to assess stability. Samples remained in the sampler that was either at a controlled temperature (10°C) or at room temperature. Percent extraction recovery was assessed by comparing the response of controls at three levels (low, medium, and high) and the LLOQ spiked in a breast milk matrix. The response consisted of four solutions of ezetimibe/EZE-

glucuronide in the mobile phase (pure) which represented the amount of ng injected into the matrix of the three levels and the LLOQ.

6.2.4.4 Sample Preparation

An internal standard mixed working solution (ezetimibe D-4, ezetimibe D-4 B-D glucuronide, and acetonitrile) of 20 µL was created to correct for fluctuations present during the solid phase extraction (SPE). The internal standard mixed working solution was added to 400 µL of standard or patient breast milk. After the samples were vortexed for 5 seconds, 600 µL of 0.2M ammonium acetate buffer (pH 6) was added. The samples were then vortexed for an additional 30 seconds and then set aside. SPE was performed on the matrices for drug recovery. The SPE cartridges were sequentially conditioned with 3 mL of methanol, 3 mL of nanopure water, and 0.2M ammonium acetate buffer (pH 6). Following the conditioning, the cartridges were washed with 1 mL of 5% methanol and 2% ammonia washing solution followed by 3 mL of nanopure water. Methanol (1 mL) and then acetonitrile (1 mL) were used for elution. The eluate was evaporated with nitrogen steam and reconstituted with 100 µL of mobile phase 40% A and 60% B. The column was injected with 20 µL of the sample.

6.2.5 Dose Simulations

Random sampling was performed to select measured ezetimibe and EZE-glucuronide concentrations (ng/mL) from the DLAC milk samples. Daily weight-normalized volume of milk intake as a function of postnatal age (mL/kg) were sampled from a literature review-derived milk intake model as previously published (288). Infant daily doses were simulated by multiplying an ezetimibe and EZE-glucuronide in milk concentration with a weight-normalized milk volume of intake that would be received by each virtual breastfed infant.

6.2.6 PBPK Model Development and Evaluation

6.2.6.1 Adult PBPK Model

A whole-body PBPK model was first established in adults for subsequent extrapolation to children and breastfed infants. Pediatric PBPK model development followed the workflow described by Maharaj, Barrett (99). First, the disposition of ezetimibe was parameterized by data from dogs administered 5 mg/kg IV bolus (300). In the dog study, plasma concentrations were 7,723 ng/mL at 5

minutes post-dose which then declined biphasically (300). C_{max} provided a reasonable indication of volume of distribution and a logP was selected to match half-life (mean of 4.3 hours).

Next, a naïve oral model was set up for a mean male individual weighing 71 kg. Rodgers and Rowland (197, 198, 251) and Schmitt (252) prediction methods for partition coefficients were evaluated based on visual model performance for curve shape. Since the fraction excreted in urine from oral administration studies in humans was low (0.05-0.1% (303)), renal clearance was negligible and glomerular filtration rate was set to 0. Clearance was partitioned as hepatic and biliary. To reduce the uncertainty in ezetimibe clearance mechanisms, the contribution by UGT2B15 was ascertained from Ghosal, Hapangama (313). Although UGT1A1 and UGT1A3 are also proposed to contribute to ezetimibe clearance, UGT2B15 was shown to produce nearly 90% of EZE-glucuronide through *in vitro* inhibition studies (313). The following equation was used to scale the intrinsic clearance of UGT2B15 in human microsomes (CL_{int}) to hepatic intrinsic clearance ($CL_{H,int}$) (314):

$$CL_{H,int} = CL_{int}(UGT2B15) \times MPPGL \times liver\ weight \quad (2)$$

where MPPGL is the amount of microsomal protein per gram of liver. An MPPGL of 45 mg of protein/g of liver was used (315). EZE-glucuronide served as the sole metabolite formed by UGT2B15 (313). The remaining clearance of ezetimibe was attributed to MDR1 which transports compounds from the hepatocyte into bile (305). MDR1 V_{max} was optimized using a Monte Carlo approach to explore the parameter space. All parameter optimizations were conducted with oral single dose datasets. The organ specific expressions of UGT2B15 and MDR1 were informed by reverse transcription polymerase chain reaction profiles (RT-PCR) in the PK-Sim expression database (274-276). Reference concentrations (µM) of UGT2B15 and MDR1 were calculated from knowledge that on average, 30.5 pmol UGT2B15/mg protein (316) and 2.1 pmol MDR1/mg total membrane protein (317) are present.

Absorption parameters of the adult PBPK model consisted of a dissolution profile described by a Weibull distribution with dissolution half-time and shape indicative of an immediate release formulation. Specific intestinal solubility was manually optimized. To capture enterohepatic circulation and the emptying of the gallbladder, a 750 calorie meal was given at study-specified times. Meal times were assumed based on visually inspecting the plasma concentration-time profile when the study did not provide information.

With the ezetimibe adult oral PBPK model established, EZE-glucuronide was modelled. The logP of EZE-glucuronide was modified by a factor of 1.37, which was the factor by which the ezetimibe *in silico* (ALOGPS) predicted value differed from the logP optimized in dog. The partition coefficient calculation algorithm for EZE-glucuronide followed that of ezetimibe. Clearance was described by MRP2 to transport EZE-glucuronide into bile (305). MRP2 was optimized to the EZE-glucuronide plasma concentration-time profile. The expression and reference concentration of MRP2 was populated by the RT-PCR profiles and an average abundance of 0.3 pmol MRP2/million hepatocytes (318), respectively. Although *in vitro* studies have suggested OATP1B1 to transport EZE-glucuronide from the portal vein into hepatocytes, pharmacogenotype studies in humans appeared to show no significant differences in plasma concentration-time profiles between genotypes (304). Therefore, OATP1B1 was not included in the adult PBPK model. Finally, EZE-glucuronide specific intestinal absorption was set magnitudes lower than ezetimibe to reflect the general properties of a glucuronide (319).

Studies reporting plasma concentration-time data with variability were used to develop an adult population model. Anatomy and physiology variability are captured in PK-Sim (215) while interindividual variability in enzyme concentration for UGT2B15, MDR1, and MRP2 were user-defined and described in section 6.2.6.2.

The optimized adult oral PBPK model was evaluated for predicting multiple dose and steady state kinetics using observed data. Model performance for bias and precision were calculated with average fold error (AFE) and absolute AFE (AAFE), respectively (320, 321). Generally, an AFE and AAFE of 0.7-1.3 and <2, respectively, for plasma concentration-time simulated versus observed data are indicative of almost no bias and good precision.

6.2.6.2 Pediatric PBPK Model

The adult oral PBPK model was scaled to a virtual infant population for simulating drug exposure through breast milk. Anatomy and physiology of adult were scaled to infants at different ages. Relevant growth and maturation processes, including metabolic capacity, glomerular filtration rate, protein binding, and body composition, were adjusted for (213, 214). Variability was then applied to create the population of infants. The ontogeny profiles of UGT2B15, MDR1, and MRP2 were user-defined. UGT2B15 ontogeny was modeled using human liver microsomes protein content from 8 weeks gestation to 18 years (N = 236) (322) and glucuronidation activity from 0 to 69 years of

postnatal age (N = 237) (216). MDR1 and MRP2 ontogeny were modeled using protein content of 0 to 71 years of postnatal age (N = 110) (323). Enzyme content or activity were normalized to adult activity and used to fit functions to describe the ontogeny profile. For all function fitting, an L1 metric, also known as the sum of absolute deviations, was used to estimate the geometric mean adult-normalized levels across postmenstrual ages (PMA).

To capture the rich UGT2B15 data at 0 to 1 years PMA reported by Divakaran, et al. [42], a polynomial function was used:

$$Normalized\ Content\ or\ Activity = B1 \times PMA + B2 \times PMA^2 + B3 \times PMA^3 + B4 \times PMA^4 + B5 \times PMA^5 \quad (3)$$

where B1-B5 represents coefficients determined through fitting the model. A virtual population (N = 6,000) was created across the PMA (0-1 years old) to assess variability in content or activity. Geometric standard deviations were applied on the determined geometric means of B1-B5 to capture the observed variability. Past 1 year PMA, content nor activity were age-dependent and thus 5,000 virtual individuals were created with a geometric mean of 1 and a uniform geometric standard deviation. For MDR1, a Hill function described by:

$$Normalized\ Content = \frac{kPMA^n}{PMA^n + A_{0.5}{}^n} \quad (4)$$

where k is the vertical transformation factor, n is the Hill coefficient, and $A_{0.5}$ is PMA at 50% activity. Variability in content was assessed with 6,000 virtual individuals across 0 to 71 years PMA. Following a geometric mean calculation of normalized content from the Hill function, a geometric standard deviation was applied to capture the observed variability. Finally, normalized MRP2 content was not age-dependent and thus a population size of 5,000 was created a geometric mean of 1 was used with a uniform geometric standard deviation.

Evaluation of the pediatric oral PBPK model was performed by simulating children 6-11 years old administered 10 mg tablet daily. The mean $AUC_{0\text{-}24h}$ were compared between model-predicted and observed data from Kusters, Caceres (324).

6.2.7 Exposure Estimations: RID, Predicted AUC_{∞}, and the UAR

To attain the relative infant dose (RID), the mean DLAC milk concentrations of ezetimibe was multiplied by the standard 150 mL/kg/day intake volume, which were then divided by a weight-normalized therapeutic dose of 10 mg daily in adult (70 kg).

To predict ezetimibe and EZE-glucuronide AUC_{∞}, infant populations of 100 individuals were simulated from the pediatric PBPK model for each age bin in days: >0 to 7, >7 to 14, >14 to 30, >30 to 60, and >60 to 365. The infant populations were based on the National Health and Nutrition Examination Survey (NHANES) population (50% female) (294, 295). Due to the large uncertainty in ezetimibe absorption experienced in the adult models, administration to breastfed infants was through the portal vein. The infants were each assigned 1 mg/kg to attain a simulated AUC_{∞}. Average concentration at steady state ($C_{avg,ss}$) over 24 h was calculated. Simulated infant daily dose from the selected weight-normalized milk intake volume and ezetimibe or EZE-glucuronide concentration in milk was then provided to each infant to attain AUC_{∞}. Ezetimibe and EZE-glucuronide AUC_{∞} were converted into molar equivalents and summed together to represent the complete breastfed exposure to the active components of the medication. The UAR at each age group was then calculated by dividing the 95^{th} percentile of simulated breastfed infant AUC_{∞} by the median adult therapeutic AUC_{∞}. Adult therapeutic AUC_{∞} were simulated from 100 women of 25-34 years of age using the NHANES population taking 10 mg of ezetimibe daily. For sensitivity analysis, the exposure simulation process described was repeated for the virtual infant population receiving ezetimibe as an oral solution.

6.3 Results

6.3.1 Characteristics of the Maternal-Infant Pair, Breastfeed, and Breast Milk Samples

Two maternal-infant pairs were included in the study. Both mothers completed the study questionnaire and provided breast milk samples. A summary of their demographic and breastfeeding characteristics is provided in **Table 6-1**. The maternal-infant pairs contributed to a total of 15 breast milk samples. The time elapsed from the last ezetimibe dose to sample attainment, sample type (pre-feed, between feeds, or post-feeds), and ezetimibe and EZE-glucuronide measured concentrations from the developed and validated assay (section 6.2.4) are shown in **Table 6-2**.

Table 6-1. Maternal-infant pair demographics and breastfeeding characteristics

Characteristic	Maternal-infant pair 1		Maternal-infant pair 2	
	Mother	Infant	Mother	Infant
Age	35 years	21.8 weeks	42.5 years	20.4 weeks
Ethnicity	Caucasian	NR	Caucasian	Caucasian
Weight	56.7 kg	5.44 kg	NR	5.44 kg
Height/Length	167.6 cm	63.5 cm	NR	NR
Past medical history	Heart condition	None	None	None
Pregnancy history	NR		Length of pregnancy: 37 weeks Type of delivery: Cesarean section Complication: Placenta previa	
Type of breastfeed	NR		Exclusively breastfeeding	

NR: not reported.

Table 6-2. Breast milk sample data from mothers taking ezetimibe 10 mg tablets daily

Maternal-infant pair, sample num.	Time since last dose (h)	Sample type	Ezetimibe concentration (ng/mL)	EZE-glucuronide concentration (ng/mL)
1, 1	-0.25	Pre-feed	0.17	0.45
1, 2	1.75	Pre-feed	0.91	1.2
1, 3	2.08	Post-feed	0.95	1.39
1, 4	3.75	Pre-feed	0.41	0.87
1, 5	4.08	Post-feed	0.42	1.2
1, 6	5.75	Pre-feed	0.37	0.78
1, 7	8.75	Pre-feed	0.33	0.73
1, 8	9.75	Post-feed	0.35	0.68
1, 9	NR	NR	0.28	0.42
1, 10	NR	Post-feed	0.47	1
2, 1	-0.12	Pre-feed	0.45	1.19
2, 2	1.47	Post-feed	1.02	1.16
2, 3	5.22	Between feeds	0.98	1.61
2, 4	8.47	Post-feed	0.87	2.65
2, 5	10.88	Post-feed	0.94	2.2

NR: not reported.

6.3.2 LC-MS/MS Assay Validity

Selectivity and LLOQ:LLOQ response for ezetimibe was 40 times higher than the response of breast milk without ezetimibe. LLOQ response for EZE-glucuronide was 250 times higher than response of breast milk with no EZE-glucuronide.

Based on the plotted expected versus observed concentrations, the assay method was linear over the range of 0.039-5 ng/mL for ezetimibe and 0.39-50 ng/mL for EZE-glucuronide (R^2 = 0.99, quadratic, 1/x weight).

Within- and between-day accuracy and precision using LLOQ and quality controls are presented in **Table 6-3** and **Table 6-4**. The table results show accuracy, intra-day precision, and between-day precision met the criteria for less than ±15% deviation. LLOQ also met the criteria of less than ±20% deviation.

Post-extraction stability at both room temperature and at 10°C exhibited no significant difference for both ezetimibe and EZE-glucuronide. Extraction recovery was between 65% and 91% for ezetimibe, and between 60% and 86% for EZE-glucuronide which included LLOQ and quality controls. No significant carry-over nor matrix effects were observed for ezetimibe and EZE-glucuronide.

Table 6-3. Accuracy (% deviation from the mean) and precision (%CV) of the LC-MS/MS assay method for ezetimibe concentrations.

Level	Accuracy	Within-day precision	Between-day precision
LLOQ (0.039 ng/mL)	15.9	3.32	4.74
Low (0.156 ng/mL)	6.56	1.57	2.21
Medium (0.625 ng/mL)	6.23	1.77	3.17
High (2.5 ng/mL)	5.09	0.77	2.19

LLOQ: lower limit of quantification.

Table 6-4. Accuracy (% deviation from the mean) and precision (%CV) of the LC-MS/MS assay method for EZE-glucuronide concentrations.

Level	Accuracy	Within-day precision	Between-day precision
LLOQ (0.39 ng/mL)	13.42	2.06	3.37

Low (1.56 ng/mL)	3.4	1.62	1.73
Medium (6.25 ng/mL)	2.12	2.05	2.63
High (25 ng/mL)	1.28	1.31	1.68

LLOQ: lower limit of quantification.

6.3.3 Dose Simulations

Across all age groups, simulated weight-normalized milk intake volumes resulted in a normal distribution with mean of 120.5 mL/kg and standard deviation of 45.7 mL/kg. The simulated doses produced a lognormal distribution with a median of 60.1 ng/kg and interquartile range of 71.8 ng/kg.

6.3.4 PBPK Model and Evaluation

6.3.4.1 Adult PBPK Model

Table 6-5 presents the naïve and optimized ezetimibe and EZE-glucuronide models. **Table 6-6** shows the datasets used for development (single dose administration) and evaluation (multiple dose administration). Plasma concentration-time profiles of predicted versus observed for studies used in model optimization are presented in **Figure 6-1**. Ezetimibe and EZE-glucuronide model evaluation produced AFE of 0.98 and AAFE of 2.06, and AFE of 0.46 and AAFE of 3.26. Thus, the model for ezetimibe was reasonably non-biased and showed precision. Conversely, the EZE-glucuronide model performed sub-optimally in terms of bias and precision. However, a comparison of predicted and observed $AUC_{0\text{-}\tau}$ in **Table 6-7** suggest that the EZE-glucuronide performed adequately in exposure predictions. Comparisons of predicted and observed plasma concentrations over time for ezetimibe and EZE-glucuronide are presented in **Figure 6-2** and **Figure 6-3**, respectively. Results of the adult population models are shown in **Figure 6-1**.

Table 6-5. Physicochemical properties and ADME of ezetimibe for adult PBPK model construction

Parameter	Used in naïve model		Used in optimized model	
	Ezetimibe	EZE-glucuronide	Ezetimibe	EZE-glucuronide
Physicochemical properties				
Lipophilicity (logP)	4.14 log units (ALOGPS), 4.56 log units (ChemAxon)	2.48 log units (ALOGPS), 2.61 (ChemAxon)	3.02 log units	1.81 log units

Fraction unbound to plasma (fu)	*In vivo*: 0.055-0.061 (325), *in vitro*: 0.002-0.005 (306)	0.095 (306)	0.06	0.095
Molecular weight	409.4 g/mol	585.5 g/mol	409.4 g/mol	585.5 g/mol
pKa	9.75 (acid)	3.23 (acid)	9.75 (acid)	3.23 (acid)
Solubility	0.00846 mg/mL (ALOGPS)	0.0847 mg/mL (ALOGPS)	0.00846 mg/mL (ALOGPS)	0.0847 mg/mL (ALOGPS)
ADME				
Partition coefficient	Rodgers and Rowland, Schmitt	Rodgers and Rowland, Schmitt	Schmitt	Schmitt
Cellular permeability	PK-Sim Standard	PK-Sim Standard	PK-Sim Standard	PK-Sim Standard
UGT2B15 reference concentration [1]	1.22 µM (316) [2]	NA	1.22 µM	NA
UGT2B15 Km	21.2 µM (313)	NA	21.2 µM	NA
UGT2B15 Vmax	126.48 µM/min (313)	NA	126.48 µM/min	NA
MDR1 reference concentration [1]	1 µM	NA	0.077 µM (317) [3]	NA
MDR1 Km	0 µM	NA	1 µM	NA
MDR1 Vmax	0 µM/min	NA	820.72 µM/min	NA
MRP2 reference concentration [1]	NA	1 µM	NA	0.04 µM (318) [4]
MRP2 Km	NA	0 µM	NA	1 µM
MRP2 Vmax	NA	0 µM/min	NA	27.19 µM/min
GFR fraction	1	1	0	0
Intestinal permeability	4.34E-5 cm/min	4.34E-5 cm/min	1E-4 cm/min	1E-6 cm/min

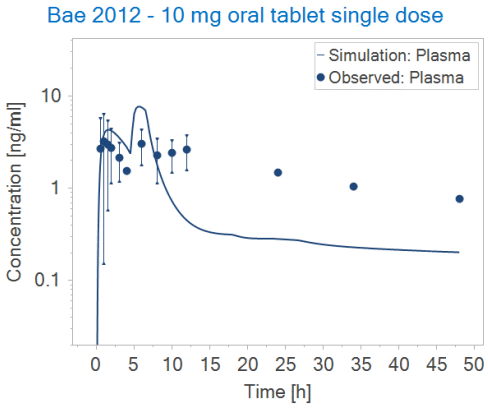
[1] Reference concentrations represent the most abundant organ concentration of the enzyme or transporter. Concentrations of all other relevant organs are a fraction of the reference concentration. [2] Reference concentration = 30.5 pmol UGT2B15/mg protein * 40 mg protein/g liver, where the density of liver is approximately 1 g/mL. [3] Reference concentration = 2.1 pmol MDR1/mg membrane protein * 26.2 mg protein/g kidney, where the density of kidney is approximately 1 g/mL. [4] Reference concentration = 0.3 pmol MRP2/million hepatocytes * 130 million hepatocytes/g of liver, where the density of liver is approximately 1 g/mL. NA: not applicable.

Table 6-6. Pharmacokinetic datasets for ezetimibe adult PBPK model construction and evaluation

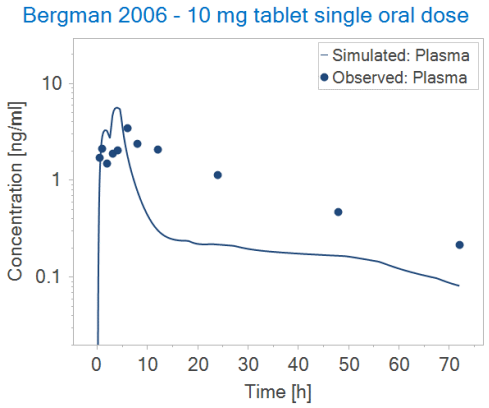
Study	Dose and Administration	Cohort	N	Age (years) [1]	Weight (kg) [1]
IV Distribution					
Schering-Plough (300)	5 mg/kg IV bolus	Male and female dogs	NR	NR	NR
Oral PBPK Model					
Bae, Choi (326)	10 mg tablet	Korean males	11	NR	NR
Bergman, Burke (327)	10 mg tablet	New Zealand males (88%) and females	17	52 [33-67]	86 [54-113]
Gustavson, Schweitzer (328) [2,3]	10 mg tablet	American males (67%) and females	18	43.4 [27-55]	78.7 [60-98]
Gustavson, Schweitzer (328)	10 mg tablet qd for 10 days	American males (67%) and females	18	43.4 [27-55]	78.7 [60-98]
Jackson, D'Avolio (329)	10 mg tablet qd for 10 days	European males (50%) and females	20	37 [21-62]	80 [50-115]
Kim, An (330)	10 mg tablet qd for 7 days	Korean males	25	27 ± 7	69 ± 8.3
Kim, Choi (331)	10 mg tablet qd for 10 days	Korean males	20	24.7 ± 3.5	70.5 ± 8.2
Kosoglou, Statkevich (332)	10 mg tablet for 14 days	European males and females	8	40 [25-53]	NR
Kosoglou, Statkevich (333)	10 mg tablet for 14 days	European males (38%) and females	8	37.9 [19-50]	73.6 [54-89]
Oswald, Haenisch (303) [2,3]	2x 10 mg tablets	European males (91.7%) and females	12	[21-31]	NR; BMI: [19.2-26.4 kg/m^2]
Oswald, König (304) [2,3]	2x 10 mg tablets	European males (63%) and females	35	[20-36]	NR; BMI: [19.1-27 kg/m^2]
Oswald, Meyer zu Schwabedissen (334)	10 mg tablet qd for 10 days	European males	12	[20-36]	NR; BMI: [19.9-27.2 kg/m^2]

Reyderman, Kosoglou (335)	10 mg tablet qd for 7 days	European males (74%) and females	18	29.3 [18-41]	73.6 [49-95]
Reyderman, Kosoglou (336)	10 mg tablet qd for 7 days	White American males	12	36.8 [25-45]	74.9 [61.8-102.7]
Reyderman, Kosoglou (337)	10 mg tablet qd for 14 days	White American males	8	34.3 [22-44]	80.9 ± 9.8

NR: not reported. qd: once daily. [1] Presented as mean, standard deviation, and/or with minimum and maximum in square brackets. [2] Studies used to construct the population model. [3] Studies reported both ezetimibe and ezetimibe-glucuronide plasma-concentration time profiles.



(A)



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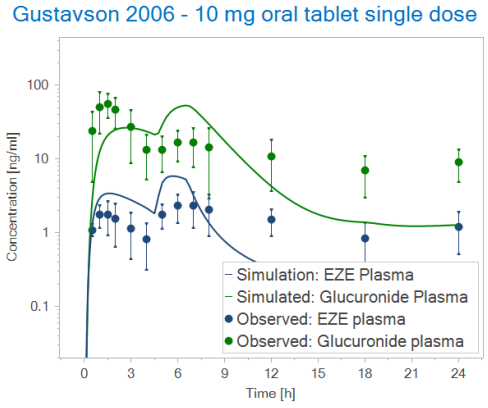

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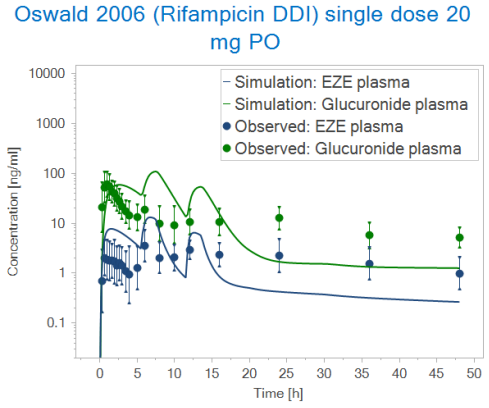


(D)

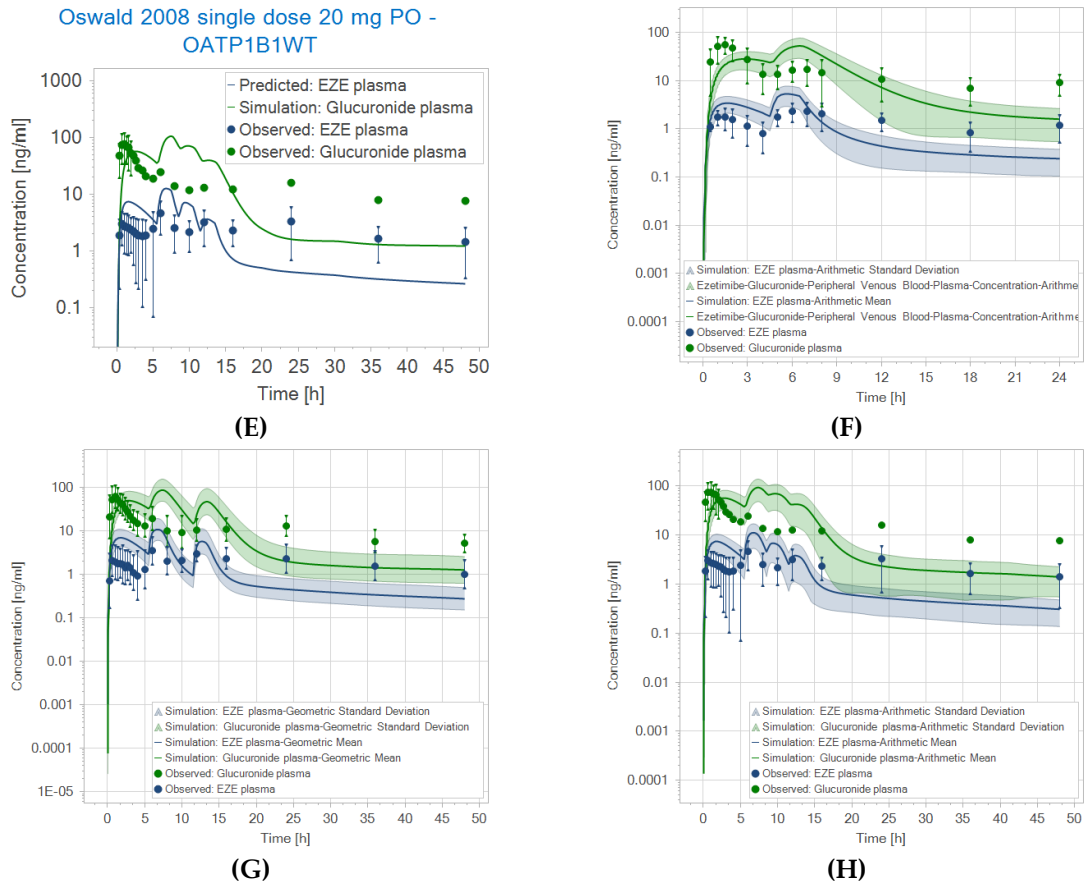


Figure 6-1. Ezetimibe oral model optimization using the single dose datasets: (A) Bae, Choi (326), (B) Bergman, Burke (327), (C) Gustavson, Schweitzer (328), (D) Oswald, Haenisch (303), and (E) Oswald, König (304) wild-type patients. Population models are presented with single dose datasets: (F) Gustavson, Schweitzer (328), (G) Oswald, Haenisch (303), and Oswald, König (304) wild-type patients. PO: taken by mouth.

Table 6-7. Observed optimization dataset and ezetimibe adult PBPK model predicted $AUC_{0-\tau}$ (ng·h/mL)

Study	Observed Ezetimibe	Predicted Ezetimibe	Observed EZE-glucuronide	Predicted EZE-glucuronide
Bae, Choi (326)	75.7 ± 39.8 [1]	48.05	NR	NA
Bergman, Burke (327)	66.8 ± 36.3 [2]	36.89	NR	NA
Gustavson, Schweitzer (328)	33.2 ± 11.8 [3]	32.28	352 ± 128.2 [3]	314.54
Oswald, Haenisch (303)	116 ± 78.1 [1]	95.68	635 ± 302 [1]	846.32
Oswald, König (304)	112 ± 66.4 [1]	97.33	704 ± 296 [1]	881.02

NR: not reported. NA: not applicable. [1] τ: 48 hours. [2] τ: 72 hours. [3] τ: 72 hours.

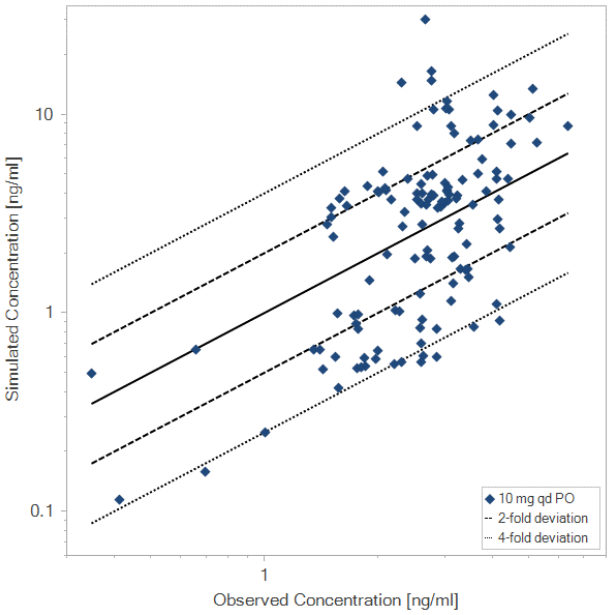


Figure 6-2. Oral predicted versus observed mean ezetimibe plasma concentrations for evaluation (R^2 = 0.14). The solid line represents the line of identity. qd: once a day. PO: taken by mouth.

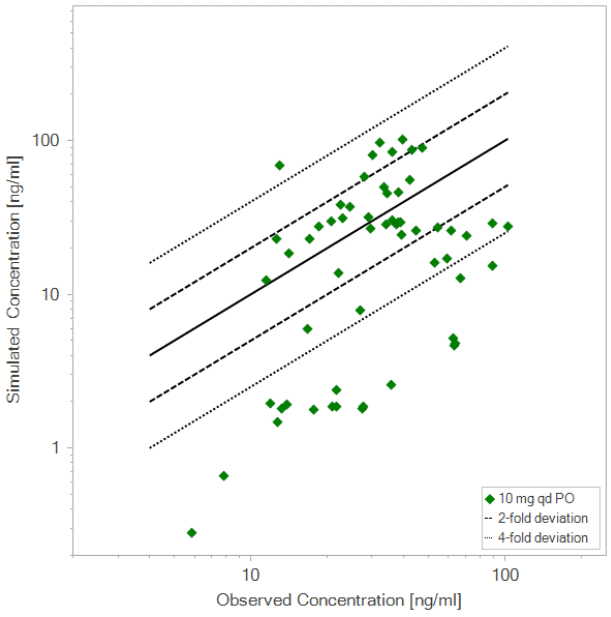


Figure 6-3. Oral predicted versus observed mean ezetimibe-glucuronide plasma concentrations for evaluation (R^2 = 0.016). The solid line represents the line of identity. qd: once a day. PO: taken by mouth.

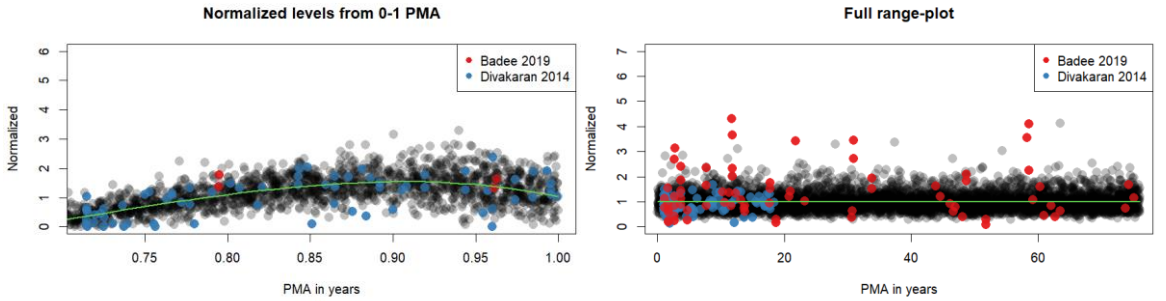
6.3.4.2 Oral Pediatric PBPK Model

Results of population simulations to capture UGT2B15, MDR1, and MRP2 ontogeny variability are shown in **Figure 6-4**. Demographic information from the 6-11 year olds studied in Kusters, Caceres (324) is presented in **Table 6-8**. Model-predicted $AUC_{0\text{-}24h}$ for ezetimibe and total ezetimibe (ezetimibe plus EZE-glucuronide) appeared to resemble observed values from Kusters, Caceres (324) (**Figure 6-5**).

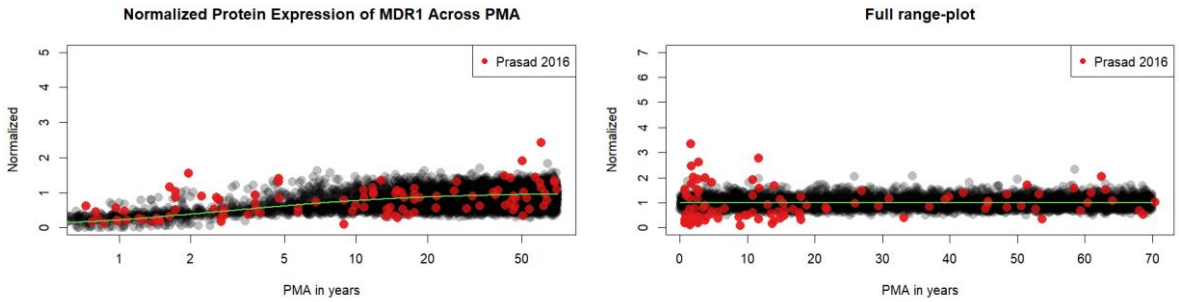
Table 6-8. Pharmacokinetic dataset for ezetimibe pediatric PBPK model evaluation

Study	Dose and Administration	Cohort	N	Age (years) [1]	Weight (kg) [1]
Kusters, Caceres (324)	10 mg tablet qd	American males (44%) and females	12	8.2 ± 1.7	NR

NR: not reported. qd: once daily. [1] Presented as mean, standard deviation, and/or with minimum and maximum in square brackets.



(A)



(B) **(C)**

Figure 6-4. Ontogeny profiles for (A) UGT2B15 content and activity normalized to the adult value described by a polynomial function from 0 to 1 years PMA with the following parameters, B1 = -9.25 ± 0.01, B2 = 6.17 ± 0.5, B3 = 13.69 ± 0.2, B4 = 8.3 ± 0.2, and B5 = -17.87 ± 0.01 (B1-5 lognormally distributed); and by a non age-dependent linear function beyond 1 year PMA with a geometric standard deviation of 1.41; (B) MDR1 content normalized to the adult value described by a Hill function from 0 to 71 years PMA with the following parameters, k = 1 ± 0.16 (lognormal), n = 1.04 ± 1 (lognormal), and $A_{0.5}$ = 3.06 ± 1.5 (normal); and (C) MRP2 content normalized to the adult value described by a linear function from 0 to 71 years PMA with a geometric standard deviation of 1.22. Grey points represent simulated individuals. Green line represent the geometric mean. PMA: postmenstrual age in years.

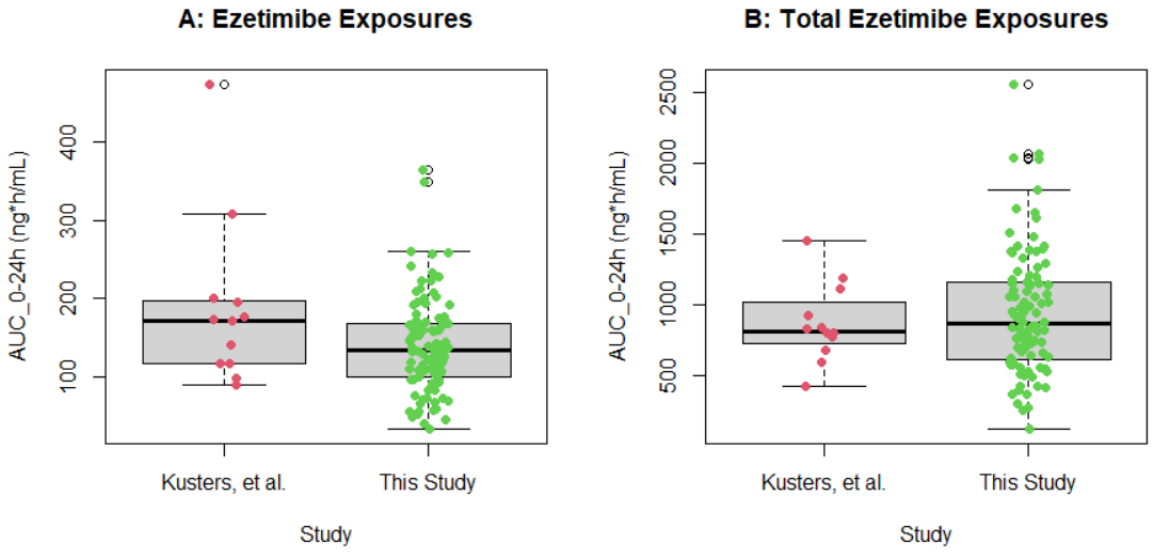


Figure 6-5. Comparison of (A) ezetimibe and (B) total ezetimibe (ezetimibe + EZE-glucuronide) AUC from 0-24 hours on day 14 of 10 mg daily administered to children 6-11 years old from Kusters, Caceres (324) compared to pediatric PBPK-model simulated ("This Study"). The coloured points represent AUC_{0-24} from each individual.

6.3.5 RID, Predicted Exposures, and the UAR

The calculated RID for ezetimibe was 0.062%. Predicted AUC from 0-24 hours of simulated adults administered a therapeutic dose compared to virtual breastfed infants across the five age groups are depicted in **Figure 6-6**. Finally, the calculated UARs per age group are presented in **Table 6-8**. Results of the sensitivity analysis revealed that when administered as an oral solution, the 95th

percentile $AUC_{0\text{-}24h}$ (1.32 to 3.02 nmol·h/L) and UARs (0.0012 to 0.0027) across age groups were generally lower than direct administration into the portal vein.

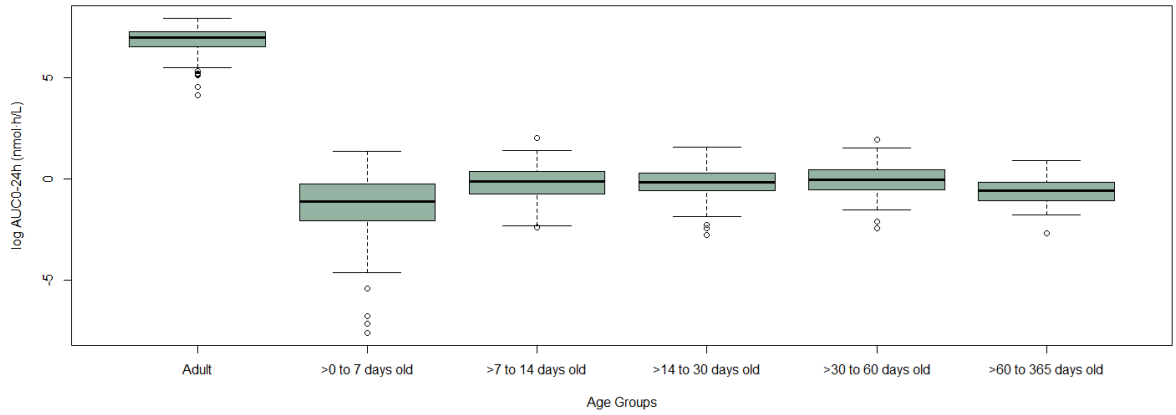


Figure 6-6. Simulated $AUC_{0\text{-}24h}$ in molar equivalents of ezetimibe and EZE-glucuronide of adults administered 10 mg daily compared to breastfed infants across five age groups.

Table 6-9. Calculated UAR for each age groups as compared to adults taking 10 mg orally once daily.

Age Groups	Infant 95th percentile $AUC_{0\text{-}24h}$ (nmol·h/L)	Adult Median $AUC_{0\text{-}24h}$ (nmol·h/L)	UAR
>0 to 7 days	1.88	1110.75	0.00169
>7 to 14 days	2.50	1110.75	0.00225
>14 to 30 days	2.88	1110.75	0.00260
>30 to 60 days	2.84	1110.75	0.00255
>60 to 365 days	1.62	1110.75	0.00146

AUC: area under the curve. UAR: upper area under the curve ratio.

6.4 Discussion

This work details the first instance ezetimibe and EZE-glucuronide from maternal breast milk samples has been successfully measured and reported. Through the collection of voluntary samples where mothers demonstrated real world use, a LCMS/MS method of assay for the complex milk matrix was developed and validated. Moreover, the measured concentrations were used to perform the UAR workflow and predict ezetimibe and EZE-glucuronide exposures in breastfeeding infants. Attainment of the UAR demonstrated minimal overlap between infants at the highest risk of exposures and adults administered a therapeutic dose. This work demonstrated the first time the UAR

was applied to both parent and active metabolite. The flexibility of the UAR to portray potential risk in multiple compounds is an improvement over the RID. For instance, the difficulty in ascertaining the maternal dose for the metabolite serves as a hinderance to calculating the RID.

The oral ezetimibe pediatric PBPK model was evaluated in children 6-11 years old administered 10 mg tablet daily. Both ezetimibe and total ezetimibe $AUC_{0\text{-}24h}$ were relatively well-predicted by the model which gives confidence to our ability to predict exposures in infants. Notwithstanding, an important limitation of this study considers the general underprediction of both ezetimibe and EZE-glucuronide models for adult plasma concentrations and AUC, and existing bias and imprecision of the EZE-glucuronide model. Essentially, the ezetimibe and EZE-glucuronide enterohepatic circulation process was non-identifiable. Several influx and efflux transporters are proposed to be involved between the portal vein and hepatocyte, hepatocyte and bile, and hepatocyte to systemic circulation. Moreover, transporters on the enterocyte may also play a role in ezetimibe and EZE-glucuronide clearance (313). This work performed a large reduction in uncertainty in ezetimibe clearance by establishing UGT2B15 clearance from *in vitro* studies. However, the mechanism by which EZE-glucuronide is rapidly absorbed during the first few hours, and the repeated conversion of ezetimibe to glucuronide and vice versa were not captured, thus resulting in lower than expected plasma concentration predictions. This was particularly evident in the elimination phase where observed concentrations of both compounds showed slow elimination. In light of the developed model underpredictions, the possibility for the simulated ezetimibe and EZE-glucuronide exposures in breastfeeding infants could be higher and thereby increase the UAR. This has potential to be achieved with appropriately modeling the accumulation of ezetimibe and EZE-glucuronide observed with multiple dosing in adults (2-fold increase) (338).

As a future direction, the incorporation of a population pharmacokinetic (PopPK) model to adequately describe the ADME processes empirically would be advantageous since it involves less assumptions. The PopPK model would be scaled down to virtual breastfeeding infants with allometry for growth and volume of distribution, and ontogeny factors for clearance. Uncertainties with ezetimibe and EZE-glucuronide absorption would be reduced with the idea that K_a and bioavailability of adult would be similar in infants. Two PopPK models exist for ezetimibe and EZE-glucuronide (339-341). It would be of interest to apply these models and compare results with our pediatric PBPK modeling efforts

Chapter 7

Addressing maternal medication use during breastfeeding using clinical resources and a novel physiologically based pharmacokinetic model-derived metric: A qualitative study

This chapter is reflective of an original manuscript published by the PhD candidate (Cindy Hoi Ting Yeung) in *Frontiers in Pediatrics*. All pertinent dialogue in this chapter was written by the PhD candidate.

Published Paper, Yeung CHT, Houle SKD, Anderson PO, Best BM, Dubinsky S, Edginton AN. (2023). Addressing maternal medication use during breastfeeding using clinical resources and a novel physiologically based pharmacokinetic model-derived metric: A qualitative study. Front Pediatr. 11:1147566. doi: 10.3389/fped.2023.1147566.

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7.1 Introduction

Breastfeeding is known to be beneficial for both the mother and infant, but its practice is often questioned during maternal medication use. According to several guidelines, only a small percentage of medications are contraindicated while breastfeeding (2, 298, 342, 343). Yet, healthcare providers have a tendency towards advising mothers not to breastfeed during medication use (344, 345). Several factors contribute to overly cautious recommendations. First, data on the use of medication during lactation are limited. In fact, almost half of drugs approved in the U.S. between 2003 and 2012 (47.9%) had labels with no data on breastfeeding and only 4.7% contained human data (84). More recently, a review of 1,408 medications reported in LactMed revealed that only 2% had strong data with information in four categories (maternal drug levels, infant drug levels, effects on infants, and effects on lactation) from research studies (346). Second, even if data from studies of medications in lactation do exist and are increasing in number, disseminating this information to healthcare providers is a challenge. A cross-sectional study conducted in 2021 showed that knowledge of the new Food and Drug Administration (FDA) Pregnancy and Lactation Labeling Rule (PLLR) by pharmacists and physicians was generally low (347). Third, resources developed to improve the knowledge translation

of existing drugs in lactation data have variable reliability. An evaluation of lactation recommendations of 19 medications from ten drug information resources were highly variable (348). Specifically, the number of medications recognized as low risk were different among the resources. For instance, at one extreme LactMed and Hale's Medications and Mothers' Milk (MMM) stated that 71% of the medications were compatible with breastfeeding, whereas at the other extreme the Physicians' Desk Reference (PDR) cited only 5% as compatible (348).

The consequence of limited, unfamiliar, and unreliable information is reflected in healthcare providers feeling inadequately knowledgeable on maternal medication use in lactation. A review of the literature by Hussainy and Dermele (349) reported that most studies found healthcare providers to have poor knowledge and variable practices mostly guided by personal experience. These themes are further exemplified in studies by Schrempp, Ryan-Haddad (350), Lee, Moretti (351), Long and Montouris (44), Maher and Hughes (352), and McAuley, Casey (353).

To improve healthcare provider knowledge and advising, we developed a novel risk assessment metric, the Upper Area Under the Curve Ratio (UAR) (289). The UAR is defined by dividing the 95th percentile of simulated pediatric area under the curve (AUC) by the median adult therapeutic AUC (**Appendix E1**). The simulated AUCs are produced by leveraging physiologically based pharmacokinetic (PBPK) modeling. PBPK modeling is a computational tool that uses a mathematical description of drug pharmacokinetics (PK) in the body to predict its exposure. The approach is mechanistic and "bottom up", with physicochemical properties of the compound and system parameters (anatomy and physiology) being the two main inputs. At minimum, a daily bodyweight-normalized infant volume of milk intake model (288) and information about drug concentration in breast milk, together with the drug's pediatric PBPK model that translates dose via breast milk into exposure in neonates, are needed to produce the UAR.

The UAR aims to improve the reliability of current resources and address the sparse data that exist on drugs in lactation. Current metrics do not account for important considerations when predicting breastfed infant risk to maternal medications. These factors are the anatomy and physiology of the infant, age-dependent factors (e.g., milk intake volumes and elimination rate), and variability in the infant and maternal populations. The UAR adequately addresses these components, for instance, by incorporating variability to capture breastfed infants who may be at most risk of high drug exposure from mothers with a pharmacogenotype resulting in the excretion of potentially dangerous levels of

drug in milk. Further, the UAR does not depend on data that are typically unavailable, such as breastfed infant plasma drug concentrations. Thus, the UAR can be calculated for drugs where only sparse data are available. If data are available (e.g., a few infant plasma drug concentrations), they are used only for confirmatory rather than exploratory purposes. Increasing work that validates pediatric PBPK models to accurately predict breastfeeding infant exposures gives confidence in our workflow and UAR determination (289). With more drugs assessed with our workflow, eventually we can rank the drugs according to their potential risk and focus resources on those with significant risk (i.e., highest UAR).

Although the UAR was created in an effort to improve available clinical resources, how it is perceived and potentially used in practice by healthcare providers has not been formally assessed. To further understand healthcare provider perspectives, it is important to gather information on how resources are currently being used, whether there is a need for the UAR in addition to current resources, how the UAR could be used in current practice, whether the UAR would confer benefits, which healthcare providers would particularly benefit from use of the UAR, and how the UAR could be further improved for clinical practice. Thus, the objective for this study was to understand existing resource use and UAR use in practice, their advantages and disadvantages, and areas of improvement for the UAR through one-on-one semi-structured interviews with healthcare providers. We hypothesized that the novel risk metric will confer multiple benefits over existing resources, and improvements in the metric and how it is described to healthcare providers will be ascertained.

7.2 Methods

7.2.1 Sampling and Recruitment

Stratified purposeful sampling was employed to recruit healthcare providers from a variety of backgrounds (teratogen/lactation information specialists, nurses, pharmacists, and physicians) and multiple disciplines (midwifery, neonatology, obstetrics, pediatrics, and lactation consultants). To ensure we attained perspectives from a range of experiences, we also specifically recruited from settings where providers may have less exposure to this type of advising, including emergency medicine and community pharmacies. Healthcare providers who were eligible to participate must have met the following criteria: able to communicate in English, experienced in providing or advising care for mothers taking medications while breastfeeding, and familiarity with drugs and breastfeeding clinical resources used to advise clinicians or patients. Breastfeeding clinical resources included both

informational online or book resources (LactMed, Hale's MMM, Briggs' Drugs in Pregnancy and Lactation, etc.) and metrics (Relative Infant Dose (RID), Milk-to-Plasma (M/P) ratio, Hale's Lactation Risk Categories (L1-5), etc.).

Recruitment was conducted through several strategies. Emails were sent to mailing lists and website listings of University of California San Diego (UC San Diego) Faculty from School of Medicine Departments of Family Medicine and Public Health; Obstetrics, Gynecology, and Reproductive Sciences (Nurse Midwifery Program); Pediatrics (Divisions of Gastroenterology, Hepatology, and Nutrition; and Neonatology), and Skaggs School of Pharmacy and Pharmaceutical Sciences (Division of Clinical Pharmacy; and Affiliate Faculty Community Pharmacists). Lactation and teratogen services, hospital perinatal units, and hospital neonatal intensive care units (NICUs) staff were also contacted through mailing lists. Snowball sampling and personal connections were also used to enhance recruitment. Recruitment was primarily performed in San Diego, California because of the high breastfeeding rates and to present perspectives with some similarities to attain saturation. Saturation occurred when no new information appeared to emerge during data analysis.

Individuals interested in participating contacted the study coordinator, provided consent, and scheduled an interview. Written informed consent was obtained from all participants prior to their interview. Participants received a US $50 gift card for appreciation of their time and possible travel costs.

This study received ethics clearance through the UC San Diego Institutional Review Board (IRB #803063) and the University of Waterloo Research Ethics Board (REB #43702). NVivo software (QSR International Pty Ltd., released in March 2020) was used for qualitative data analysis.

7.2.2 Data Collection

Participant demographics on gender identity, race/ethnicity, practicing discipline, primary occupation and specialty, and measures of experience providing or advising care for patients breastfeeding or considering breastfeeding were attained through a written questionnaire. Measures of experience included number of years of experience, International Board of Lactation Consultation Examiners certification, Academy of Breastfeeding Medicine membership, and frequency of inquiries about medication use during breastfeeding from patients and other providers.

Semi-structured interviews of 25-60 minutes were conducted between June and September 2022 by the study coordinator (PhD candidate who developed the UAR, with a life sciences and health research methods background) either in-person or through video call. The interview guide received feedback from healthcare providers within the study team and colleagues. The final version of the guide is presented in **Appendix E2** and included questions to generate discussion on the provider's current practices when advising on medication use in breastfeeding and, given a scenario, how they would proceed in practice currently and with information about the UAR metric. Provided materials on the scenario, and the introduction to and application of the UAR are shown in **Appendix E1**. Interviews were audio recorded and subsequently transcribed.

7.2.3 Data Analysis

The Framework Method, as described by Gale, Heath (354), was applied as the overarching analysis method to guide the thematic analysis of textual data. This method is commonly used to create a new structure for summarizing textual data to answering research questions. Briefly, descriptive or conceptual labels were assigned to excerpts of the interview transcripts and referred to as "codes". Two members of the study team (CHTY and SD) independently coded the interview transcripts. Applied codes were compared and reviewed and disagreements were discussed and resolved. After coding the first few transcripts, an agreed set of codes to apply to all subsequent transcripts, also known as an analytical framework, was developed and presented in a code book.

To assess the extent of agreement between the coders, inter-rater agreement determined from Cohen's Kappa statistic was calculated using a coding comparison query. Interviews with a Kappa statistic less than 80% were reviewed, and coding strategies and descriptions were clarified. To analyze the codes and identify themes that grouped the codes by similarities and interrelated ideas or concepts, data were charted into a framework matrix. The framework matrix provided a summary table depicting the codes as columns and participant quotations as rows to visualize themes and patterns. Illustrative quotations were selected to represent the resulting themes and codes.

7.3 Results

7.3.1 Participant Demographics

Twenty-eight participants were interviewed and their demographics are presented in **Table 7-1**. Of the participants, five had International Board of Lactation Consultation Examiners certification and one had Academy of Breastfeeding Medicine membership.

Table 7-1. Study participant characteristics

Characteristic[a]	Number of individuals
Gender identity	
Man	4
Non-binary	0
Woman	24
Race/ethnicity	
Aboriginal/American Indian/Alaska Native	0
Asian	7
Asian-White	1
Black or African American	0
Hispanic, Latino, or Spanish origin	0
Middle Eastern or North African	2
Native Hawaiian or Other Pacific Islander	0
White	18
Primary practice setting and role	
Midwifery	
Nurse Midwife	3
Neonatology	
Neonatologist	2
Pharmacist	2
Registered Nurse	1
Obstetrics	
Obstetrician	1
Pharmacist	1
Registered Nurse	1
Pediatrics	
Nurse Practitioner	1
Pediatrician	3
Pharmacist	1
Teratogen/Lactation Information Specialist[b]	4
Adult Critical Care	
Pharmacist	1
Community Pharmacy	
Pharmacist	4

Emergency Medicine Pharmacist	1
Family Medicine Physician	2
Experience providing or advising care for lactating breastfeeding	
<1 year	0
1-3 years	2
4-6 years	2
>6 years	24
Frequency of patient or other healthcare provider inquiries on medication risk while breastfeeding	
Daily	4
Weekly	10
Monthly	12
Less than Monthly	2

[a]For demographics questions regarding identity, participants had the options of selecting "prefer not to disclose" and "prefer not to say". [b]Participants' primary role was defined as Teratogen/Lactation Information Specialist and their healthcare provider roles included Genetic Counsellors, Nurse Practitioners, and Registered Nurses.

7.3.2 Themes and Codes

The Framework Method produced several themes and codes. Six broad themes emerged: (1) Current Practice Approaches, (2) Advantages of Existing Resources, (3) Disadvantages of Existing Resources, (4) Advantages of the UAR, (5) Disadvantages of the UAR, and (6) Strategies to Improve the UAR. **Figure 7-1** depicts Current Practice Approach as the connecting theme to all others by outlining an opportunity when the UAR could be applied in practice, a reflection of the disadvantages of existing resources and how they could be addressed by advantages of existing resources and the UAR (**Table 7-2**), and the disadvantages of the UAR with strategies for improvement (**Table 7-3**). For each theme, their representative codes are defined in **Appendix E3: Supplementary Table 4** and their selected illustrative quotations are shown in **Appendix E4**. In the following sections, overarching themes and their descriptive codes will be presented. Themes and codes will be labelled with T# and C#, respectively. Participants who contributed to each code will be referred to by their study identifier, BFR# (**Appendix E3: Supplementary Table 4**).

7.3.2.1 Current Practice Approaches

Current Practice Approaches [T3] encompassed healthcare provider use of resources that are considered when addressing medication use in breastfeeding, and how these resources are applied

given a scenario (**Appendix E1**). Providers described a workflow that they typically employed when presented with a case (**Figure 7-1**).

7.3.2.1.1 Three main approaches: Informational Resource Use, Clinical Experience, and Identify Need for Referral or Consultation

When provided with a scenario of a mother who has epilepsy and is taking lamotrigine, providers gravitated towards one of three initial actions: to use informational resources [Resource as a First Go-to; C58], clinical experience, or involve additional sources of expertise.

Application of clinical experience mainly focused on advising the patient to Continue Medication as a First Go-to [C28], with knowledge that lamotrigine was taken during pregnancy and thus breastfeeding resulting in less exposure than *in utero*. The immediate recommendation to continue the medication appeared to be based on clinical experience. For example, knowledge that discontinuing anti-seizure drugs taken during pregnancy is generally not advised, thus breastfeeding while on the medication may be most reasonable (BFR03). As another example of using experience, a provider expressed that Continue Medication as a First Go-to [C28] is highly relevant for healthy term infants and thus safety during pregnancy should reflect safety during breastfeeding (BFR15).

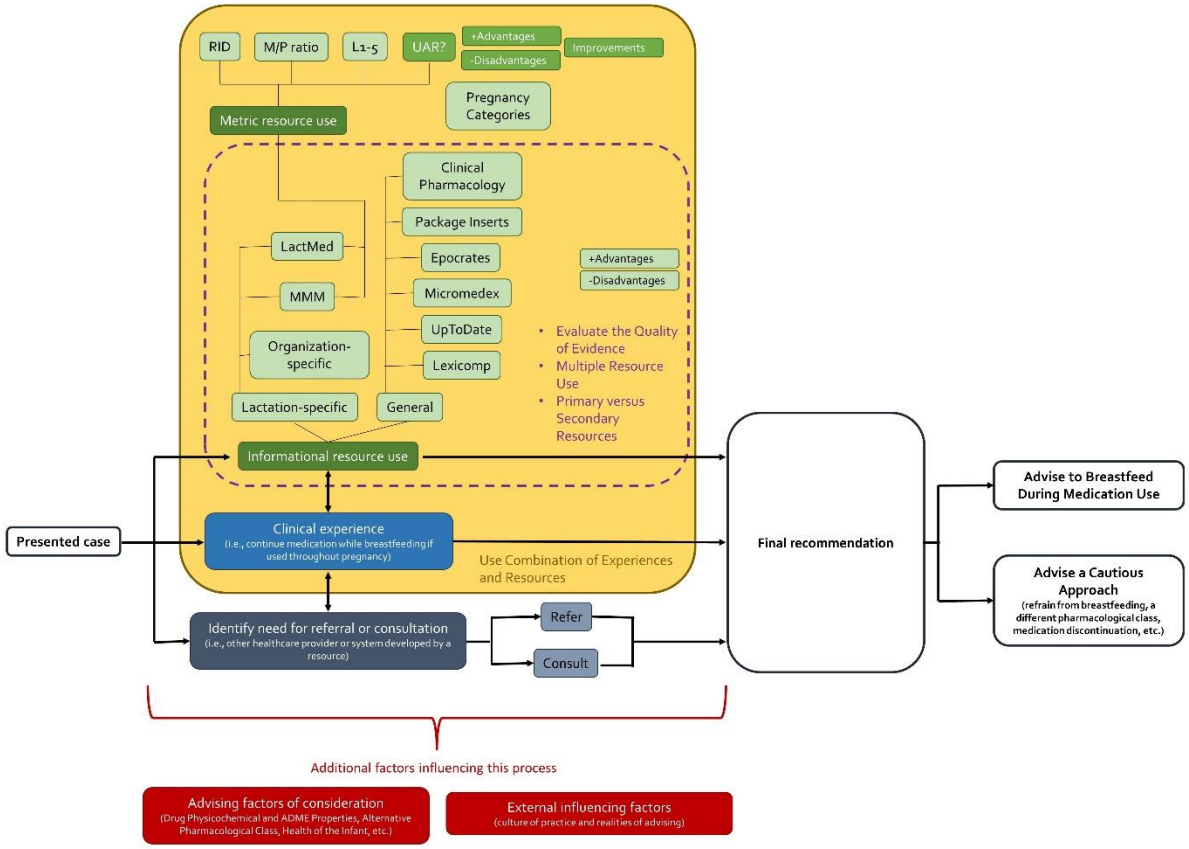

Figure 7-1. A flow diagram of summarized healthcare provider current practice approaches when advising mothers taking medication during breastfeeding. RID: relative infant dose. M/P ratio: milk-to-plasma ratio. L1-5: Hale's Lactation Risk Categories, 1-5. UAR: upper area under the curve ratio. MMM: Medications and Mothers' Milk. ADME: absorption, distribution, metabolism, and excretion.

Alternatively, providers responded to the presented case with identified need for referral [Refer to Other Provider; C56] or consultation [Reliance on Other Provider or Resource; C57]. Referrals described a preference of the provider to pass decision-making in patient advising to another provider. The referral was often in the form of sharing some information to the patient paired with advising them to contact their primary care physician (BFR20) or directly contacting the patient's provider for their recommendation or decision (BFR06, BFR27, and BFR28). Among all providers, the act of referring was rare, but were more likely to be practiced by community pharmacists. These participants tended to highlight the realities of advising, including a lack of awareness of and access to lactation-specific resources [Inaccessible; C71] (BFR06, BFR11, and BFR20) which can be due to

hurdles in their institution to attain such materials [Institution Needs Resource Justification; C51] (BFR06 and BFR20) and patient information, such as electronic medical records for diagnosis codes [Lack of Information About the Patients; C52] (BFR06 and BFR16) to perform more informed advising.

As another form of identify need for referral or consultation use, healthcare providers would rely on the assessment or advice of another provider (NICU pharmacist, lactation consultant, etc.) or from a resource (LactMed summary statement, Hale's L1-5, etc.). In contrast to referrals, these consultations were not made for the necessity of decision-making nor judgments due to the realities of advising for some healthcare providers. Consultations were mainly to lactation consultants and health system pharmacists such as those specializing in the NICU or pediatrics. Participants voiced their appreciation for consulting providers with experience and training (e.g., knowledge in pharmacokinetics and interpreting metrics) in advising maternal medication use during breastfeeding (BFR01, BFR02, BFR03, BFR14, BFR18, and BFR28). An example of consulting a resource with reliance on a metric or appraisal conducted by the author, providers would cite a high level of dependency on Hale's L1-5 categories as a quick method of assessment of risk (BFR25 and BFR27). One provider noted not delving deeper into the information on, nor Hale's risk assessment of, the medication if it was categorized as L5 (BFR25).

The majority of healthcare providers practiced Resource Use as a First Go-to [C58]. Accessed informational resources were separated into lactation-specific or general. Lactation-specific resources included LactMed, Hale's MMM, and organizational-specific resources such as MotherToBaby information sheets commonly accessed by their teratogen specialists. General resources consisted typically of pregnancy and lactation sections of UpToDate, Lexicomp, Clinical Pharmacology, and package inserts. Regardless of which informational resources were employed, providers applied three practices: Evaluate the Quality of Evidence [C31], Multiple Resource Use [C46], and Primary versus Secondary Resources [C48]. In reviewing the existing published studies provided by their accessed resources, providers commonly made assessments on the quality of available evidence. Quality assessments consisted of considering the study designs (case report, case study, extensive PK study, etc.), study population (age of infant, maternal dose received, etc.), and dose to response data availability and type (drug levels measured in milk and infant plasma, reported adverse effects in infant, etc.). Nearly all participants cited Multiple Resource Use [C46] and many used Primary versus Secondary Resources [C48]. This practice consisted of referring to a tertiary resource that was

typically their first go-to and frequently accessed, while other informational resources were examined afterwards or only as needed. Some providers noted using general informational resources as primary resources since they provided concise background information and later accessed lactation-specific resources such as LactMed or MMM for more depth (BFR04). Conversely, other providers accessed lactation-specific resources first for full information, followed by a general resource if they found the former to be insufficient for the medication (BFR017).

Beyond informational resources, healthcare providers would access metric resources often reported in lactation-specific resources (LactMed and MMM). Metric resources included the RID, M/P ratio, Hale's L1-5, and FDA Pregnancy Categories. A little over half of providers reported a Lack of Existing Metric Use [C45]. Many of these providers expressed being unfamiliar with metrics due to a lack of exposure to them during their education and training. Nearly all physicians across disciplines lacked metric use and preferred to rely on their team's dedicated pharmacist to account for the metrics because of their training. Almost half of the interviewed health system pharmacists cited a dearth of metric use, with one provider explaining that their application did not align with their practice approach. In referencing the RID, the health system pharmacist mentions not using the RID threshold and instead focusing on each maternal-infant pair's uniqueness and overall risk versus benefit (BFR05). The remaining health system pharmacists who did apply the RID in practice applied the metric in specific scenarios, including a Mother on Co-Medications [C63], a Mother with Conditions [C64], Comparing within Drug Class [C61], to Explain a Range of Outcomes in Infants [C62], for a New Medication [C65], and for Reassurance Along with Other Resources [C66].

While the FDA Pregnancy Categories were not intended as an example of a lactation metric resource, interviews revealed that this system was considered in current practice [Pregnancy Categories (Using or Avoiding Them); C47]. Community pharmacists tended to apply the Pregnancy Categories, which are available through general information resources such as Clinical Pharmacology. For example, one community pharmacist noted that a medication classified as category C would prompt physician referral (BFR06). In contrast to this approach, another provider expressed trying to avoid using these reproductive categories since they were intended for pregnancy and deemed inadequate for use in both pregnant and lactating populations (BFR05).

Once a healthcare provider had taken a first go-to approach, it was common practice to employ one of the other two strategies thereafter (**Figure 7-1**). More often, resource use and clinical experience

were applied together and thus coded as, Use Combination of Experiences and Resources [C59]. Several providers stated using the RID as a metric resource and screening tool, but also applied the metric in context with other information and clinical experience such as how much actually gets into breast milk, options to try other medications, knowledge of the baby exposed to the medication *in utero*, and variation of exposures across infants.

7.3.2.1.2 Considered Factors in Advising

Several factors were considered by healthcare providers along the advising process when presented with the case scenario (**Figure 7-1**). These factors included components related to the drug, infant, mother, breastfeed, and the provider's general advising approach. The factors were not applied in any specific order during the advising process, nor were they prescribed to a single approach (e.g., only when informational resource use took place). For instance, some providers discussed considering maternal health early on their advising (BFR07 and BFR12), while other providers may acknowledge this factor later in their process.

For drug-related factors, providers would seek an understanding of Drug Physicochemical and Absorption, Distribution, Metabolism, and Excretion (ADME) Properties [C33]. For example, needing to be aware of the medication's absorption properties such as steroids not passing well into breast milk especially via nasal administration (BFR07). Factors related to the infant and mother include their health [Health of the Infant; C35 and Health of the Mother; C36], whether there was Drug Use in Pregnancy [C34], Information on Drug Used in Infants [C37], Maternal Co-medications [C38], Maternal Dose Taken [C39], and Alternative Pharmacological Class [C32]. Breastfeeding factors were Type of Breastfeeding (Exclusive or Partial) [C44] and Time of Breastfeed Relative to Dose [C43].

Neonatologists and health system pharmacists in neonatology, and teratogen/lactation information specialists were more likely to consider the entirety of the mentioned factors. For example, one neonatologist reflected on safe maternal medication alternatives, lowered risk of medication to infant via breast milk compared to pregnancy, preterm versus term status of the infant (i.e., thoroughly explaining the benefits of breast milk since preterm parents tend to be more cautious), the condition of the mother and need for the medication, and possibility to discard pumped milk to get the medication out of the maternal system for a breastfeed (BFR15).

Additionally, healthcare provider general advising approaches were factors to account for in their current practice methods. Four such factors were examining Risks and Benefits (Analysis) [C40], a Team Approach (Present or Absent) [C42], Select Drug Cases for Non-Resource and Resource Use [C41], and Approach for Lack of Evidence [C27]. The majority of providers performed a Risks and Benefits (Analysis) [C40] as part of their advising by making a thoughtful assessment to weigh the risks and benefits of breastfeeding during medication use. The Team Approach (Present or Absent) [C42] described whether healthcare providers experienced multiple providers in the patient's care being involved in the advising process. A far greater number of providers stated a presence rather than an absence of a team approach.

It was evident that some healthcare providers had Select Drug Cases for Non-Resource and Resource Use [C41]. Within disciplines, common medications were prescribed, and thus clinical experience and knowledge were applied over the need to seek additional resources. Providers would discuss distinct situations in which informational resources were and were not needed. An example of all three general advising approaches in practice comes from an emergency department health system pharmacist (BFR09). The provider mentioned being asked by other emergency providers (physicians and nurses) for consultation on a newly started patient medication that caused the emergency department visit (i.e., an adverse drug reaction). These medications were typically pain medications and antibiotics and the provider felt that their current approach to advising breastfeeding while on these drugs determined from prior use of informational resources and clinical experience, was sufficient. In contrast, if a less familiar medication was introduced, such as an antidepressant, the provider would use informational resources in the advising process. Throughout the advising, the provider described accounting for the risks of an untreated condition (i.e., mother not taking their medication) to both the mother and infant (e.g., can affect infant development if mother has depressive symptoms).

Lastly, when certain drugs did not have enough information in existing resources, some providers had a specific Approach for Lack of Evidence [C27]. Providers tended to cite a manual search for studies through the internet, and use of metrics including the M/P ratio and Hale's L1-5 when there is not a lot of evidence available (BFR02, BFR07, and BFR10).

7.3.2.1.3 General Outcomes and External Influencing Factors

Following healthcare provider descriptions of their Current Practice Approaches [T3] to address the case scenario, a recommendation was made that broadly followed two categories: (1) Advise to Breastfeed During Medication Use [C26] and (2) Advise a Cautious Approach [C25] (**Figure 7-1**). For the former approach, providers would work towards having the infant breastfeed rather than defaulting to a simpler recommendation to not breastfeed. Providers who practiced this approach tended to reflect on multiple advising factors, practiced either Continue Medication as First Go-to [C28] or Resource as First Go-to [C58], and exemplified Use a Combination of Experiences and Resources [C59]. On the other hand, the latter approach led to recommendations such as refraining from breastfeeding, using a different pharmacological class, and discontinuing medication. Providers would suggest alternatives such as pumping and dumping, withholding breast milk until a later infant age, and any indication of potential exposure to infants would lead them to be more cautious (BFR06, BFR11, and BFR12).

To arrive at these two main recommendation pathways, external factors could influence decision-making (**Figure 7-1**). Culture of practice acted as an external impact where providers would acknowledge the Pro-breastfeeding Culture of California [C30], which provided an abundance of breastfeeding supports (lactation consultants, teratogen information specialists, breast milk donor banks, etc.) and the benefits of breastfeeding were widely known and advertised (BFR02, BFR06, BFR15, and BFR24). Conversely, a Culture of Leaning Towards Caution [C29] was noted to be prevalent. Healthcare providers would remark that other providers advise not to breastfeed even though the medication is known not to enter breast milk, over-recommend pumping and dumping, and have a lack of awareness that most medications are compatible with breastfeeding (BFR02, BFR05, BFR08, and BFR27). The interviewed providers cited adult primary care providers as mainly adopting this culture of advising. Much of this perspective could be due to the realities of advising. Several realities were faced by providers that could influence advising, including Concern for Liability [C49], Concerns Relaying Evidence-based Decisions [C50], Institution Needs Resource Justification [C51], Lack of Information About the Patients [C52], Minimal Time for Clinical Decision Making [C53], Variable Patient Health Literacy [C55], and Motives of Manufacturers [C54].

7.3.2.2 Disadvantages of Existing Resources

Disadvantages of Existing Resources [T4] outline healthcare provider perceived drawbacks to currently used resources to address medication use while breastfeeding. These disadvantages were encountered during informational and metric resource use (**Figure 7-1**) and could lead providers to be selective in their use of materials. Many of the identified shortcomings of existing resources can be addressed by the Advantages of Existing Resources [T1] and Advantages of the UAR [T2] (**Table 7-2**) discussed in section 7.3.2.3 of this thesis.

7.3.2.2.1 Areas of Subjectivity

Several healthcare providers thought that existing resources had Areas of Subjectivity [C67] (**Table 7-2**). Many of the comments on this disadvantage were universal across resources, in recognition that searching on medication use during breastfeeding can lead to a plethora of results with many of the resource authors providing their opinions that can be based on a selected study to create their own conclusions (BFR09). One provider voiced the disadvantage of making quick decisions based on another individual's evaluation, specifically, authors and developers of informational and metric resources (BFR12). This method of resource use may lead to less critical thinking in clinical practice. A resource metric thought to be subjective was described as being "soft", not applied evenly, based on small study sample sizes, opinionated, and potentially adversely impacting drug policies (BFR02 and BFR09).

7.3.2.2.2 Several Factors Not Considered

Healthcare providers cited numerous factors that are important for advising that are not considered in most current resources. These factors were Non-average Cases Not Considered [C74], Co-medications Not Considered [C68], Effect on Milk Not Considered [C70], Infant Age Not Considered [C72], and Maternal Dose Not Considered [C73] (**Table 7-2**). First, for non-average cases were not considered, providers noted the lack of information on the upper and lower percentiles of exposed breastfeeding infants to maternal medications (BFR06). One neonatologist elaborated on the paucity of data in preterms with unique considerations such as different renal clearances, neurodevelopmental stages, and bodyweights from reported term infants (BFR15). Second, co-medications are not addressed by existing resources. Providers felt it was not possible with current resources to assess the risk of multiple medications a hypothetical mother would be taking, and on supplements containing multiple ingredients (BFR01 and BFR17). Third, one provider described a resource lacking

information on drug effect on milk supply which can influence advising practices (BFR01). Fourth, providers commented the lack of infant age taken into consideration (BFR15 and BFR16). Fifth, providers noted that resources generally do not include the doses and specific drug formulations breastfeeding mothers used in studies (BFR07 and BFR28).

7.3.2.2.3 Inaccessible

Inaccessible [C71] resources was described as a disadvantage by several of healthcare providers (**Table 7-2**). Frequently, providers pointed out resources that needed to be purchased, and in some cases, only a physical copy format was available. An accessibility example with an online resource, such as LactMed, includes the idea that high literacy levels would be needed for families to understand the material (BFR01). Additionally, some medications were difficult to find in current resources, especially when other countries and jurisdictions use alternative drug names (BFR23).

7.3.2.2.4 Unclear Conclusions

Although not a common concern, some healthcare providers did note that some resources had Unclear Conclusions [C80] due to lack of summary statements which would be useful in making recommendations to their patients (BFR04 and BFR25) (**Table 7-2**).

7.3.2.2.5 Easily Outdated

Many healthcare providers identified that existing resources were Easily Outdated [C69] (**Table 7-2**). Most providers referenced physical resources that were not up to date since they required at least a year to produce a revised publication. It was noted that an annual update was not enough to keep up with rapidly changing information on drugs in lactation risk. Online resources were not exempt from concerns of outdatedness. Providers gave examples of drugs that they had inquired about but could not be found in online resources (BFR07 and BFR18).

7.3.2.2.6 Overreliance on Case Reports and Published Data

Several healthcare providers recognized the universal problem of existing resources solely relying on scarce published data on drugs in lactation (**Table 7-2**). The data are typically in the form of case reports and studies with small sample sizes thereby resulting in limited certainty in study conclusions and generalizability to their patients. Providers noticed the impact of Overreliance on Case Reports and Published Data [C76], especially when recommendations are forced to conclude that there is

insufficient information to advise for or against medication use during breastfeeding (BFR14 and BFR16). Because of insufficient published data, providers were aware that often the adverse effects of a drug to a breastfed infant through maternal medication use are unknown (BFR05, BFR22, and BFR24). Without informing mothers on expected drug side effects to the breastfed infant, monitoring for effects of concern and general risk-benefit analyses become difficult to conduct.

7.3.2.2.7 Too Broad

Multiple healthcare providers have classified existing resources as Too Broad [C78] (**Table 7-2**). Providers specifically identified general resources as having broad and limited information as compared to lactation-specific resources (BFR05). The lack of more detailed information, such as bioavailability and drug clearance in an infant, was also recognized as missing in current resources (BFR15).

7.3.2.2.8 Overreliance on a Single Resource

Healthcare providers highlighted the negative consequences of relying too heavily upon specific resources (**Table 7-2**). For example, providers explained the impact of package inserts and the PDR which typically specify that the medication should not be taken while breastfeeding, thus at times unnecessarily leading patients to be overly cautious (BFR01, BFR03, BFR10, and BFR27). Another example of Overreliance of a Single Resource [C75], was with a provider noting that metric resources intended to be a screening tool are being used for definitive decision-making, thereby bypassing a proper risk-benefit analysis (BFR03). Furthermore, the RID was frequently overgeneralized by other providers applying the arbitrarily proposed 10% cut-off definitively. One provider explained that although a drug has an RID >10%, the drug is not necessarily high risk to the breastfeeding infant, especially when the medication has been directly administered to pediatric populations (BFR07). As an additional example, one provider explained that certain benzodiazepines having a low RID may mislead providers into thinking that the medication is a low risk to the infant when that is not always the case (BFR10).

7.3.2.2.9 Perceived Lack of Reported Information Due to Resource

In contrast to Overreliance on Case Reports and Published Data [C76] as an underlying disadvantage among all existing resources, Perceived Lack of Reported Information Due to a Resource [C77] describes current resources that tend to not include available published evidence (**Table 7-2**).

Providers observed that some informational resources would state there were not enough studies when in fact studies exist in the literature (BFR16). Moreover, supplements, bioactives, new medications, and medications to treat rare conditions were thought to be missing from existing resources (BFR17 and BFR18).

7.3.2.2.10 Too Much Information or Text-Heavy

Healthcare providers identified a disadvantage in informational resources that were labelled as Too Much Information or Text-heavy [C79] (**Table 7-2**). A provider explained that listing study after study and going through their summaries could get one lost in the content (BFR03). Particularly, for emergency department health system pharmacists, going through each study could be anxiety-inducing and suboptimal for making quick decisions with high-risk patients (BFR09).

Table 7-2. Codes describing disadvantages of existing resources with potential to be addressed by advantages of existing resources and the UAR

Disadvantages of existing resources	Advantages of existing resources and the UAR
• Areas of Subjectivity [C67]	Existing resources: • Evidence to Support Use [C4] • Trusted Authors [C10] • Summarizes and References Evidence [C8] UAR: • Numerical Metric [C20] • Objective [C21]
• Non-average Cases Not Considered [C74] • Co-medications Not Considered [C68] • Effect on Milk Not Considered [C70] • Infant Age Not Considered [C72] • Maternal Dose Not Considered [C73]	Existing resources: • Distinguishes and Provides Various Types of Data [C3] UAR: • Addresses Clearance Differences [C12] • Addresses Multiple Considerations [C14] • Addresses the Worst Case Scenario [C18] • Addresses the Age of the Infant [C16]
• Inaccessible [C71]	Existing resources: • Accessible Through the Institution [C1] • Generally Accessible [C6] • Patient-friendly [C7] UAR: • Visual Representation [C24] • Can Share with Other Providers and Patients [C19]

• Unclear Conclusions [C80]	Existing resources: • Summary Statements [C9]
• Easily Outdated [C69]	Existing resources: • Up to Date [C11]
• Overreliance on Case Reports and Published Data [C76]	UAR: • Addresses Scarcity of Published Information [C15]
• Too Broad [C78]	Existing resources: • Comprehensive [C2]

UAR: upper area under the curve ratio

7.3.2.3 Advantages of Existing Resources and the UAR

Advantages of Existing Resources [T1] and Advantages of the UAR [T2] outline healthcare provider perceived benefits of currently used resources and the novel UAR metric, respectively. These advantages were considered at the informational and metric resource use stage (**Figure 7-1**). Similar to Disadvantages of Existing Resources [T4], the advantages could persuade some providers to use some existing materials over others. In this section, areas where the Disadvantages of Existing Resources [T4] have potential to be addressed by the advantages of existing resources and the UAR are outlined (**Table 7-2**). Current resource disadvantages of Overreliance of a Single Resource [C75], Perceived Lack of Reported Information Due to a Resource [C77], and Too Much Information or Text-heavy [C79], were unable to be addressed by existing resources nor the UAR. The remainder of this section consists of Advantages of Existing Resources [T1] [Familiarity; C5] and Advantages of the UAR [T2] [Addresses Exposures (AUC); C13, Addresses the Maternal-infant Pair; C17, Opens Up the Thought Process; C22, and Understand Existing Observations, Evidence, and Recommendations; C23] that do not necessarily combat Disadvantages of Existing Resources but could be seen as an added value to the current advising landscape.

7.3.2.3.1 Strategies to Reduce Areas of Subjectivity

Several advantages of resources considered to be less subjective were discussed. Strategies that these resources employ include Evidence to Support Use [C4], Trusted Authors [C10], and Summarizes and References Evidence [C8] (**Table 7-2**). For Evidence to Support Use [C4], one healthcare provider noted an improvement of a general informational resource over the years where there was a published study showing its developments over the past few decades (BFR03). Trusted Authors [C10]

was a key advantage for most providers, especially when they were aware of the authors' academic and practice background (BFR01, BFR04, and BFR25). In referencing the improvement of a general informational resource over the years, a provider commended the addition of editors with appropriate skillsets in the lactation population (BFR03). Experience with resources and trusting the authors' process also created a perception of Trusted Authors [C10]. For example, providers would confirm that a resource author had gone through all available studies and that the presented evidence was accurate (BFR17, BFR22, and BFR26).

To further reduce the potential for subjective resources, the UAR was considered an advantage by serving as an Objective [C21] and a Numerical Metric [C20] (**Table 7-2**). Providers recognized the strength of having a numerical objective metric free from author personal interpretations of existing study data (BFR04). Additionally, the idea that the UAR is derived from data and not from subjective interpretation was thought to be a positive (BFR10). One provider recognized that because the UAR is developed from data, its results are reproducible and thus Objective [C21] (BFR06). The concept of a Numerical Metric [C20] was thought to give a more concise judgement for medication use while breastfeeding compared with existing resources that were vague and left to provider interpretation (BFR08). Some providers preferred the numeric format of the UAR which was easier to interpret and could be easily added to their existing resources (BFR09 and BFR12). One neonatologist explained that a numerical metric would especially be useful in the NICU since providers tend to be number-focused (BFR14).

7.3.2.3.2 Ability to Consider Several Factors

Healthcare providers valued that most informational resources Distinguishes and Provides Various Types of Data [C3] to address issues from other resources neglecting non-average cases, co-medications, effect on milk, infant age, and maternal dose (**Table 7-2**). The fact that resources divide their information by study types (animal versus human studies and case reports versus large clinical trials), maternal components (measured breast milk and plasma drug concentrations), infant components (measured plasma drug concentrations, adverse reactions, and whether the medication has been used in pediatrics), and potential alternative medications was helpful to consider different aspects of existing evidence (BFR05, BFR07, BFR09, BFR10, BFR12, BFR14, BFR21, BFR25, BFR27, and BFR28). An advantage to these existing resources is that information about missing factors such as effect on milk could easily be added to the existing categorized framework.

More concretely, the UAR already offers opportunities to overcome the typically neglected factors as the novel metric Addresses Clearance Differences [C12], Addresses the Worst Case Scenario [C18], Addresses the Age of the Infant [C16], and Addresses Multiple Considerations [C14] (**Table 7-2**).

Clearance differences were considered in the UAR, with one neonatologist impressed that renal clearance was accounted for, especially in the NICU setting (BFR15). Another provider found that the ability of the UAR to be used in different metabolizer statuses was an asset (BFR21).

Using the UAR to identify the worst case scenario rather than the average case was thought to be valuable. One pediatric health systems pharmacist described the UAR as being able to provide the worst case scenario because the comparison is with the 95th percentile exposure in infants compared to the median exposure in adults (BFR03). Another provider recognized the importance of the UAR in a scenario where existing resources may deem a medication to be mostly low risk, but the UAR would be able to demonstrate a point of risk (BFR09). In line with another provider's observation, identifying a point of risk would be an advantage of the UAR to show which drugs might be of higher risk in terms of outliers (BFR19).

Many providers recognized the significance of the UAR to account for infant ages. It was helpful to understand that risk of drug exposure to infant varies across ages and could dictate periods of time for the presence or absence of caution. Providers explained the specific value to their advising in understanding risk from early infant ages (i.e., exposures peaking at the first two weeks of life) when infants are most vulnerable and in cases of highly lipophilic drugs, receiving high fat colostrum (BFR24 and BFR27).

Finally, the UAR has the ability to address multiple considerations. Providers would reference the study material depiction comparing the UAR with existing metrics (**Appendix E1)** and appreciated that the UAR could address multiple factors at once (BFR01, BFR06, BFR09, BFR10, and BFR22). One teratogen/lactation information specialist understood that the UAR considered vulnerable children, metabolites, systemic exposure, pediatric concerns, and development of the gastrointestinal tract as a function of age (BFR10). Providers also noted that the ability to address multiple considerations would deem the UAR to be more individualized and specific to the situation rather than a one-size-fits-all approach (BFR13, BFR21, and BFR26). Again, neonatal perspectives reflected on the utility of the UAR to account for multiple considerations in the NICU where age, renal

clearance, protein binding levels, bioavailability, maternal pharmacogenotypes, and other factors are particularly influential to preterm exposures (BFR15 and BFR26).

7.3.2.3.3 Improved Accessibility

Healthcare providers identified several current resources that were Generally Accessible [C6] and Accessible Through the Institution [C1] to overcome accessibility shortcomings of physical copy and paid resources (**Table 7-2**). General accessibility of resources was a common advantage expressed by providers. Providers identified informational resources as Generally Accessible [C6] when they were readily available at any electronic device, convenient to access without needing extra steps to view the resource, free-of-charge, and simple to read (e.g., summary table of ADME and physicochemical properties). Resources were also found to be accessible through the provider's institution. In inquiring about the resources providers used in current practice, it appeared that the frequently accessed general informational resources were those available through their institution. One provider noted this observation by stating their preference to a general resource first because it is readily available at their institution (BFR03). For community pharmacists, there was a strong tendency for Use of Package Inserts [C60] and Clinical Pharmacology due to their work settings and organizational subscriptions (BFR6, BFR11, and BFR16). Teratology/lactation information specialists were able to access their own unique institutional databases entered by other specialists in their institution (BFR10).

Accessibility to existing resources also benefit from being Patient-friendly [C7] (**Table 7-2**). One provider described an informational resource as quick to access and in plain English to print out for patients for knowledge empowerment and improved decision-making (BFR06). Similarly, the UAR was thought to be a metric resource that providers Can Share with Other Providers and Patients [C19] (**Table 7-2**). For instance, a neonatal health systems pharmacist explained that it would be useful to share the UAR with the patient's neonatologist and primary care physician, especially for unusual medications since the UAR provides more information (BFR12).

To further improve accessibility, the UAR offers Visual Representation [C24] of potential exposure risk to the breastfeeding infant via maternal medications (**Table 7-2**). Multiple providers described the benefit of having graphical and concise representations (i.e., exposure across age groups boxplots and exposure table) which helps to visually interpret data and show patients in their advising process.

7.3.2.3.4 Summary Statements for Clearer Conclusions

Providers found that informational resources such as LactMed included short, quick, and useful Summary Statements [C9] (**Table 7-2**). These statements were thought to pull all available data together and synthesize a clear and concise recommendation based on the information (BFR02). One provider described the advantage of acknowledging all published information, regardless of strong or weak evidence, and providing a consensus on risk with breastfeeding with alternatives (BFR09).

7.3.2.3.5 Up To Date to Overcome Outdatedness

The faster updates of online resources as compared to physical published copies was acknowledged as an advantage of existing resources [Up to Date; C11] (**Table 7-2**). Providers commended resources such as LactMed that provide a monthly update with an exact timestamp of the update (BFR02).

7.3.2.3.6 Avoids Overreliance on Case Studies and Published Data

Only one healthcare provider noted that the UAR Addresses Scarcity of Published Information [C15], thereby removing the necessity to rely on case studies and published data (**Table 7-2**). The provider mentioned the UAR being more data-driven without relying on single case study reported results, and that there is evidence to support the risk estimate it produces (BFR18).

7.3.2.3.7 Comprehensive to Overcome Reporting Too Broadly

Healthcare providers identified resources such as LactMed, as a Comprehensive [C2] resource that has considered the entirety of available information (BFR04 and BFR17) (**Table 7-2**). When providers were satisfied with their Comprehensive [C2] resource, they tended to forego using further resources for their advising (BFR07, BFR12, and BFR21). Other resources such as Reprotox were considered Comprehensive [C2] in describing agents which can be particularly helpful when the product is a less known herbal (BFR07).

7.3.2.3.8 Familiarity

Although the high level of Familiarity [C5] of existing resources would not necessarily overcome a specific disadvantage of current resources, it was brought up frequently by providers as an advantage. When sharing a recommended course of action to other providers, one provider explained bypassing a buy-in by using a well-known and accepted resource in their institution (BFR03). Other providers noted their inclination to use certain general informational resources because they are familiar with

them overall (i.e., in their daily care of patients) (BFR04 and BFR22). Additionally, when resources became familiar, they were deemed easy to access and simple to use (BFR09, BFR17, and BFR26).

7.3.2.3.9 Addresses Exposures (AUC)

A unique benefit of the UAR is that it Addresses Exposures (AUC) [C13]. Providers recognized the ability of the UAR to provide exposure assumptions as an improvement over current dose-based metric resources such as the RID (BFR03 and BFR04). One neonatologist summarized the strength of providing exposure estimates by explaining that the UAR ratio provided information from the predicted dose in milk, to the bioavailability to the infant, to the infant's clearance ability from the bloodstream, and how long the medication remains in the infant (BFR15). One pediatrician noted the UAR going beyond the M/P ratio and RID by incorporating the entire process from the dose administered to the mother, how much gets into breast milk, how much the infant gets exposed to, and the infant's biology (BFR02). Providers also used the UAR to frame their advising in terms of level of exposure. For example, by reviewing the UAR for a drug, the provider could make a quick observation that the medication results in a tiny exposure and thus is not too concerning to the maternal-infant pair (BFR22).

In using exposures to define risk, one provider described calculating an infant's theoretical PK as less subjective for decision-making (BFR14). Another provider described how they would use the exposure estimates by giving an example of infants potentially reaching adult therapeutic levels and having elevated transaminases (BFR15).

7.3.2.3.10 Addresses the Maternal-Infant Pair

Having a metric resource that Addresses the Maternal-infant Pair [C17] was seen as an advantage to several healthcare providers. Providers found the UAR to be beneficial in performing a relative comparison with mother and infant exposures (BFR04 and BFR05). Especially when viewing the predicted adult and infant exposure boxplots across age groups, one provider appeared to account for the maternal-infant pair by voicing a thought process that reassured to continue the medication and to breastfeed during the first week of life and be more vigilant after two months of postnatal age (BFR09). Another provider perceived the UAR to be advantageous for considering the maternal-infant pair more broadly, which would benefit neurologists to simultaneously account for the mother and infant (BFR17). Another provider identified a further way the UAR Addresses the Maternal-

infant Pair [C17] by concluding that risk to the infant based on maternal exposures would most likely be accurate (BFR25).

7.3.2.3.11 Opens Up the Thought Process

Healthcare providers often demonstrated a detailed thought process initiated after being introduced to the UAR and how it could be used in practice. The UAR Opens Up the Thought Process [C22] by prompting providers to consider factors that they may have not considered with existing resources. Mainly, providers went beyond dose considerations and reflected more deeply in the components and implications of current metric resources as referenced in **Appendix E1**. A health system pharmacist specializing in neonatology described how providers might see a low RID and consider the medication to be low risk to the infant, however, seeing the UAR might prompt retrieving cord blood levels in the first few days postpartum, a deeper thought into whether the infant is truly at the 95th percentile, and developing strategies for a monitoring plan (BFR03). In a similar comment, a registered nurse in obstetrics described moving from the RID for a yes or no type of answer, to the UAR which forces considerations on the age of the infant, dose, exposure boxplots across ages, and exposure percentiles to aid in counselling (BFR13).

Another provider commented on how each presented case is individualized because the UAR guides providers to consider factors they may have not accounted for (BFR07). For most providers, seeing the exposures and UAR metric across infant age groups helped reflect on level of caution throughout breastfeeding (BFR12, BFR17, BFR22, and BFR23). In visualizing the lamotrigine exposure histograms across different age groups, one provider explained that the plots might prompt providers to see potential risk and encourage the patient to speak with their neurologist if there is sub-optimal seizure control, and on the other hand to remain on the medication if seizure control has been attained (BFR12).

7.3.2.3.12 Understand Existing Observations, Evidence, and Recommendations

In addressing the case scenario, the UAR helped to Understand Existing Observations, Evidence, and Recommendations [C23]. Looking at infant exposure predictions across age groups allowed one provider to reflect on an observation that infants tend to have a small portion of their glomeruli at birth and yet the UAR demonstrated it is possible to have lower risk at early age as compared to later age because other factors were at play (BFR02). Another provider introduced a way they might use

the UAR, which would be to see whether predicted exposure levels would match their observations in clinical practice (BFR03). For other providers, seeing the UAR for lamotrigine was reassuring as it reinforced their expectations from clinical experience (BFR02, BFR03, BFR05, and BFR09). For instance, an emergency department health systems pharmacist explained the UAR providing reassurance to continue breastfeeding especially during the first week of life and identify areas of potential high exposures later in life (BFR09).

7.3.2.4 UAR Disadvantages and Strategies for Improvement

Table 3 presents codes describing the Disadvantages of the UAR [T5] matched with identified Strategies to Improve the UAR [T6] that have potential to overcome current shortcomings. This section starts with codes describing the path to understanding the UAR which were captured when healthcare providers were first introduced to the novel metric and asked questions or commented on the UAR to develop their understanding. Information from this code identified where the UAR could improve to better describe the metric to providers. The remaining codes described in this section outline Disadvantages of the UAR [T5] and Strategies to Improve the UAR [T6] deliberately discussed by the participants. In **Figure 7-1**, disadvantages and areas for improvement are presented alongside its advantages as they would likely be considered altogether in deciding resource use during the advising process.

7.3.2.4.1 Path to Understanding the UAR

The path to understanding the UAR involved inquiries and comments about exposure comparisons between adults and infants, and between infants across age groups [Exposure Comparisons; C89]; how to interpret the exposure estimates [Interpreting the Exposure Estimates; C90]; and how to interpret the UAR [Interpreting the UAR; C91].

In studying the relative exposure estimates between adult and infant from the provided lamotrigine case scenario, healthcare providers voiced their interpretations. Some providers viewed minimal crossover in the boxplots of adult and infant lamotrigine exposures, with infants only receiving miniscule exposures compared to adult (BFR01 and BFR28). Other providers also recognized the potentially low risk to infants due to minimal exposure overlap, but acknowledged that some infants above the 95th percentile could reach adult levels (BFR15).

There were several inquiries on interpreting the exposures, in the form of $AUC_{0-\infty}$, from the illustrated histograms and boxplots. Providers asked for assistance to interpret the histogram y- and x-axes and whether milk or plasma concentrations were shown, how the infant $AUC_{0-\infty}$ was derived, whether the PBPK model used to produce the simulated infant $AUC_{0-\infty}$ was validated, and clarity on the inputs into the PBPK models (e.g., adults received a single versus multiple dose administration) (BFR02-04, BFR06, BFR14, BFR19, BFR22, and BFR26).

As with the simulated exposure depictions, providers inquired about interpreting the UAR metric. Providers asked for confirmation on their interpretation of the relationship between 95th percentile infant exposures and median adult exposures (BFR08, BFR10-12, BFR18, and BFR28). Additionally, reaffirming to themselves or with the interviewer about the magnitude of the UAR value, for instance, whether a higher UAR implies a larger risk (BFR01, BFR05, BFR11, BFR17, BFR23, and BFR24). A pediatric health systems pharmacist appeared to have a firm grasp on the UAR, explaining their understanding that a UAR of 0.44 represented the 95th percentile of pediatric $AUC_{0-\infty}$ being 44% of the median adult $AUC_{0-\infty}$, and compared the value to an RID of 15% to realize that the UAR has a larger emphasis on outlier infants (BFR03).

7.3.2.4.2 Several Factors Not Apparent: Specify Inclusion of Factors in Further Cases and Scenarios

Several factors that can be accounted for in the UAR were frequently requested by the healthcare providers, likely because the provided case scenario did not demonstrate the UAR's ability to include various circumstances. Discussed factors included Co-medications Not Apparent [C81], *In utero* Exposures Not Apparent [C83], Metabolites Not Apparent [C86], Multiple Administrations to the Mother Not Apparent [C87], and Prematurity Not Apparent [C93] (**Table 7-3**).

First, there was a request to account for a combination of medications a mother might be taking, for example, three co-medications affecting essential nervous systems (BFR01). Second, in recognizing that infants after birth may have significant exposure to both the medication through breast milk and passed i*n utero*, it was essential the latter to be accounted for (BFR03). Third, when important, drug metabolites were suggested to be incorporated into the UAR (BFR03). Fourth, one provider noted the high likelihood that mothers would be taking medications regularly and thus multiple dose regimens should be addressed (BFR22). Fifth, the need to consider prematurity and increased vulnerability at different gestational ages was expressed (BFR01).

To overcome these apparent disadvantages, a fuller explanation of the different possible scenarios the UAR can cover would be necessary. Moreover, a range of case scenarios with each of the mentioned factors [Separate by Specific Cases and Scenarios; C108] could be provided to show the UAR's capabilities (**Table 7-3**). In creating distinct scenarios for each factor, providers suggested various scenarios including infants of different gestational ages and with specific vulnerable conditions (renal and liver disease); mothers with single versus multiple administrations; the presence and absence of transplacental passages; and metabolite exposures. Having the UAR metric calculated for additional variables, such as breastfeeding infant ages beyond 12 months, different maternal drug doses, and a relative comparison of several different drugs (e.g., psychiatric drugs or anticonvulsants) would further the understanding of potential variables the UAR could incorporate (BFR07 and BFR10).

7.3.2.4.3 Potential to Appear Subjective or Misinterpreted: Improve Explanations on Metric Development and its Advantages

Some healthcare providers had concerns that the UAR has Potential to Appear Subjective or Misinterpreted [C92] (**Table 7-3**). In terms of subjectivity, one provider was concerned that the predicted exposures across infant age groups may encourage delaying breastfeeding until exposures reach a level deemed safe which was thought to be impractical (BFR05). Another concern came from a teratogen/lactation information specialist who noted the issue of not realizing the UAR already accounts for multiple elements (e.g., infant age and drug bioavailability), and thus factoring them in again can make the medication artificially appear riskier to use (BFR07). A neonatologist who grasped the benefits of breast milk had apprehensions that the results of the UAR would immediately prompt a provider to advise withholding breastfeeding without further considerations (BFR14).

As measures to reduce potential subjectivity and misinterpretation that lead to negative outcomes, providers suggested to Explain More About How the Model was Made (Inputs and Assessments) [C97] and Explain More About UAR Advantages [C98] (**Table 7-3**). One suggestion was to present a deeper explanation about how each factor was weighted into the UAR, for instance, the importance of infant age playing a role in influencing the UAR (BFR05). Additionally, providing information (i.e., in the form of a table) that showed variables the UAR includes and excludes would portray which factors have been already accounted for and how they make the UAR advantageous (BFR01, BFR07,

BFR10, and BFR27). Breaking down the UAR value into an understandable format by showing how each piece was determined was also thought to be helpful (BFR25).

7.3.2.4.4 Difficult to Understand or Too Complex: Provide Guidance and Rationale for Using the Metric

At times, healthcare providers found the UAR Difficult to Understand or Too Complex [C82] (**Table 7-3**). This difficulty was commonly exhibited in the pathway to understanding the UAR. Although expressed across professions and specialties, physicians appeared more likely to express this disadvantage. Providers tended to note the complexity and complicatedness of the UAR and its potential to overwhelm and confuse others with too much information (BFR02, BFR05, BFR07, BFR18, BFR26, and BFR27).

Providing guidance and clear rationales for using the UAR would be an effective method to overcome the lack of understanding and overwhelming complexity of the novel metric. Providers postulated several strategies which were to Provide Guidance to Interpret the UAR [C106], Make Visual Representation Essential [C102], make the metric and path to its use audience-dependent, and Provide a Definitive Bottom Line [C104] (**Table 7-3**).

A guide to interpret the current presentation of the UAR was often requested by providers. The guidance would be on what each UAR value may imply, for example, if it were 0.44. Providers gave a variation of ideas to approach guidance including informing values when they would be problematic, displaying a colour-coded scheme from dangerous to minimal concern, constituting values to interpret as high versus low exposure, and giving cut-off values with recommendations of action (e.g., through a well-devised algorithm system). Several providers valued the visual aspect of the UAR and reinforced the colour-coding concept to define potential risk.

A dichotomy became apparent in the way the UAR was preferred to be presented to healthcare providers [User Friendly for Non-Pharmacists; C100 and User Friendly for Pharmacists; C101]. Non-pharmacist providers were more inclined to only have a basic understanding of the UAR and have it presented in a simpler format that would require minimal time to provide a binary, yes or no, recommendation for the maternal-infant dyad (BFR01, BFR02, and BFR23). In contrast, it was suggested that pharmacist providers receive more detail about the UAR for a deeper understanding (BFR01 and BFR22). There were also notable nuances to the two distinct suggested approaches. Some providers suggested that regardless of the profession, having a shorter and longer form version

of the UAR could be tailored to those who want a quick answer and those who tend to be more inquisitive, respectively (BFR02 and BFR09). Variation also existed within the pharmacy practice. One health systems pharmacist trained in pediatrics thought the current presentation of the UAR was appropriate (BFR22). However, another pharmacist specializing in the emergency department preferred the learning component to be thorough and once trained and familiar, an easily accessible quick version would be welcome (BFR09). One community pharmacist felt that the distinction between a less and more complex version of the UAR depended on the busyness of their practice (BFR16).

Having a definitive bottom line was a suggestion divided among providers. On one hand, providers wanted a format akin to the outdated FDA Pregnancy Categories or Hale's L1-5, a numbering system from 1-10 with 10 being high risk to the infant, or an ultimate thumbs up or down (BFR06, BFR11, BFR15, BFR17, BFR18, BFR25, and BFR28). On the other hand, providers recognized the downside to providing a definitive bottom line. One pediatrician explained that categorization would make advising easier, however, there was utility in moving towards an approach to presenting the information and having the provider make the decision (BFR20). Other issues to definitive bottom lines more generally were examined in section 7.3.2.2.8.

7.3.2.4.5 Lack of Maternal Perspective: Provide a Greater Maternal Emphasis

Although only one provider perceived the UAR to have a Lack of Maternal Perspective [C84] (**Table 7-3**), this viewpoint warranted a closer examination. For the provider, the metric seemed to focus only from the infant perspective without weighing the maternal perspective (BFR05). Therefore, it was suggested to Provide a Greater Maternal Emphasis [C105] to ensure that maternal health was also an important factor in the advising process (BFR05).

7.3.2.4.6 Limited Information on Adverse Effects (Exposure-Response Relationship): Provide Prospective Predictive Evidence

A commonly cited disadvantage of the UAR by healthcare providers was Limited Information on Adverse Effects (Exposure-Response Relationship) [C85] (**Table 7-3**). Essentially, information on potential effects on the infant were described as limited with the UAR. Observations by providers included not knowing if the exposure of the medication to the infant would be harmful, having a lack of toxicity information, and the need for a clinical correlate with the UAR values. To supplement the

UAR and its prediction of the dose-exposure relationship, providers suggested to Provide Prospective Predictive Evidence [C107] (**Table 7-3**). For instance, conducting prospective studies to see if the UAR would be predictive of any effects in infants (BFR03 and BFR14). Another provider explained that buy-in in their department would consist of showing that basing decisions off the novel metric would alter patient outcomes (BFR09).

7.3.2.4.7 Not Enough for Clinical Decision-Making: Combine the Metric with Another Resource

Another commonly coded UAR disadvantage was Not Enough for Clinical Decision Making [C88] (**Table 7-3**). For many providers, the UAR alone would not convince them to immediately change practice. Instead, the need to compare results of the UAR with other resources was necessary (BFR01). Providers also had concerns that the difficulty of explaining the UAR to the family would be an obstacle to incorporate the metric into practice (BFR18). One family medicine physician clearly voiced they would not use the metric alone to make a medical decision and valued existing resources that compiled evidence from all existing studies to provide guidance (BFR19).

The proposal to Combine the UAR with Another Resource [C96] was mentioned by multiple providers (**Table 7-3**). In their view, adding the UAR to an existing informational resource such as LactMed or MMM would be beneficial and having both the novel metric and summary of the existing scope of evidence would give confidence to use the UAR (BFR01, BFR02, BFR04, BFR05, BFR07, BFR10, BFR11, and BFR27). Incorporating the UAR to an existing informational resource could also assist with access to the novel metric (BFR08). Alternatively, one provider suggested incorporating the most useful sections of existing informational resources into the UAR (BFR17). Nevertheless, results from this code suggest that the UAR is presented as a complementary piece within commonly used resources as illustrated in **Figure 7-1**.

7.3.2.4.8 Unusable in its Current Form (Too Novel): Simplify, Train, and Educate to Reduce Effects of Novelty

Healthcare providers frequently voiced that the UAR was Unusable in its Current Form (Too Novel) [C94] (**Table 7-3**). Generally, providers felt that the period of time they were exposed to learn about and use the novel metric was too short (BFR06, BFR07, BFR10, BFR13, BFR14). More assistance would be needed to interpret the UAR to feel comfortable with its use (BFR18). The UAR was also

too novel for immediate uptake and providers needed more experience with it (BFR10, BFR20, BFR21, BFR25, and BFR27).

Provider suggested strategies to Add a Summary Statement [C95], Give Specific Training [C99], and Overcome Simulation Skepticism [C103] for alleviating concerns about the current form and novelty of the UAR (**Table 7-3**). A summary statement for the UAR was imagined as a common sense recommendation to translate the UAR results so that they are practical and understandable (BFR02, BFR15, BFR19, and BFR23).

Giving specific training about the UAR was a widely discussed strategy to improve the UAR. Providers had different suggested methods for training including providing course lectures and presentations, targeting training to departments for improved uptake, incorporating the metric into educational programming (i.e., pharmacy education), and conference talks and seminars. Related to training was a suggestion to overcome provider simulation skepticism. One provider described that the simulation component could be difficult to trust and understand and thus giving more education on this topic would help with UAR uptake (BFR01). Another provider suggested educating others on the idea that PBPK modeling is not a novel approach and is in fact a method commonly used in drug development and FDA approvals (BFR03).

Table 7-3. Codes describing the disadvantages of the UAR and strategies for improvement

Disadvantages of the UAR	Strategies to improve the UAR
• Co-medications Not Apparent [C81] • *In utero* Exposure Not Apparent [C83] • Metabolites Not Apparent [C86] • Multiple Administrations to Mother Not Apparent [C87] • Prematurity Not Apparent [C93]	• Separate by Specific Cases and Scenarios [C108]
• Potential to Appear Subjective or Misinterpreted [C92]	• Explain More About How the Model was Made (Inputs and Assessments) [C97] • Explain More About UAR Advantages [C98]
• Difficult to Understand or Too Complex [C82]	• Provide Guidance to Interpret the UAR [C106] • Make Visual Representation Essential [C102] • User Friendly for Non-pharmacists [C100]

	• User Friendly for Pharmacists [C101] • Provide a Definitive Bottom Line [C104]
• Lack of Maternal Perspective [C84]	• Provide a Greater Maternal Emphasis [C105]
• Limited Information on Adverse Effects (Exposure-Response Relationship) [C85]	• Provide Prospective Predictive Evidence [C107]
• Not Enough for Clinical Decision-making [C88]	• Combine the UAR with Another Resource [C96]
• Unusable in Current Form (Too Novel) [C94]	• Add a Summary Statement [C95] • Give Specific Training [C99] • Overcome Simulation Skepticism [C103]

UAR: upper area under the curve ratio.

7.4 Discussion

Our paper sought to address the question, among healthcare providers advising mothers taking medications while breastfeeding, whether the UAR will confer benefits over existing resources and whether improvements for optimal uptake could be attained. We were interested in how resources are currently being used, whether there is a need for the UAR in addition to current resources, how the UAR could be used in practice, whether the UAR would confer benefits, which healthcare providers would particularly benefit from use of the UAR, and how the UAR could be further improved for clinical practice. To investigate these questions, we used one-on-one semi-structured interviews with healthcare providers followed by the Framework Method strategy of analysis. Results of our work are highlighted in the following main findings. First, informational and metric resources are used as one of three tactics in current advising practices, with two other methods being clinical experience and identifying a need for referral or consultation. Second, based on the number of disadvantages of existing resources that can be addressed and supplemented by the UAR, we have deemed there to be a need for improvement of current resources and that the UAR would confer benefits. Third, the UAR in its current state would most benefit from use as a complementary piece within commonly used resources, such as LactMed and MMM. Fourth, although providers valued the format of the UAR to be dependent on profession, results suggest that providers across professions and disciplines would benefit from UAR use. Fifth, through the interviews, we were able to identify multiple strategies to improve the UAR for clinical practice.

Through examining current practice approaches, a workflow that healthcare providers typically followed in their practice was identified (**Figure 7-1**). This workflow served as a backbone that related all other aspects and themes of advising. Three main approaches were discovered as informational resource use, clinical experience, and identify need for referral or consultation. The most exercised approach by providers was Resource Use as a First Go-to [C58]. The use of informational resources appeared valuable, including its application to evaluate the quality of evidence and conducting risk-benefit analyses. Our study expands on a pilot study by Byerley, Dykhuizen (355), which indicated that pharmacists reported use of a wide range of resources such as UpToDate, LactMed, and MMM. We confirm this finding and categorize the resources as general and lactation-specific.

Three findings regarding current practice approaches were found to be unexpected. First, the act of referrals occurred rather frequently with community pharmacists. These providers felt that when presented with a case for which they did not feel able to adequately provide a recommendation, other providers would be consulted. Community pharmacists are encouraged to play a greater role in maternal health services, including providing breastfeeding guidance, however, their extent of practice in this area needs to be strengthened (352, 356). Our results indicated that community pharmacists would be better equipped to advise breastfeeding patients if some of the hurdles of advising were overcome, including access to lactation-specific resources. Second, our study was the first to examine resource metric use and found that there was a universal lack of overall application among interviewed healthcare providers. When asked about common metrics such as the RID and Hale's L1-5 categories, most providers were unfamiliar with them or not sufficiently confident to use them in their practice. Third, there was a prevalent use of FDA Pregnancy Categories by interviewed healthcare providers. As Burkey and Holmes (357) explain, these categories are often confusing and misleading, and moreover, not intended for use in lactation.

Exploring the disadvantages of existing resources uncovered several shortcomings. Accessibility was identified as the most cited disadvantage to some resources and affected healthcare provider perception and use of the resource. Through the interview process, it became clear that many advantages of existing resources and the UAR have potential to overcome disadvantages of current resources. Although not an intended outcome from this study, asking questions regarding existing resources and subsequently the UAR assisted in the comparison of existing resources with the UAR. Providers were able to critically identify specific disadvantages that the UAR may address and vice

versa. For example, reflecting on an advantage of the UAR reminded the provider that current resources are unable to address this advising need and thus deemed it as a disadvantage to existing resources.

Several disadvantages of the UAR were revealed and healthcare providers identified strategies to overcome its limitations. An important finding from the interviews was that the UAR in its current state would not be used alone for clinical practice. The complexity of the UAR was a main barrier to use. Two additional notable influences include Potential to Appear Subjective or Misinterpreted [C92] and Limited Information on Adverse Effects (Exposure-Response Relationship) [C85]. These codes were likely acknowledged since the interviewed providers were generally well-versed in resource use and had many years of advising experience. Additionally, due to almost all providers belonging to a Pro-breastfeeding Culture of California [C30], and San Diego in particular, it would be fitting that there are concerns about the UAR in increasing the likelihood of inappropriately advising against breastfeeding. Nonetheless, these shortcomings signal the importance of considering the culture and environment of practice, and the importance of the educational and training aspects of the UAR. Thus, developed training of the UAR should account for the environment of practice and speak to issues regarding the exposure-response relationship. An example of addressing the latter would be explaining that although the UAR does not directly assess drug response, it does account for the idea that some breastfed infants may get to adult therapeutic, and potentially supratherapeutic, exposures. With this understanding combined with knowledge of the mechanism of action and toxicity in adults, clinicians would be better poised to make more informed assessments. Improved training on resource use generally should improve practice, especially since previous work has found that provider knowledge and training can influence their interpretation of drug risk (358).

This study is a prime example of gathering information from potential end-users on a novel tool in order to identify targeted areas of improvement to ensure future optimal use. Our work was the first to compile rich information on advantages and disadvantages of currently used resources from end-users. The gathered information was insightful and could be directly applied to identify gaps for UAR improvement. As another strength of the study, we recruited and interviewed a broad range of professions and specializations. Therefore, our interview findings were from a diverse range of role and discipline perspectives that could be compared. However, other than profession and specialization, our participants tended to be uniform in other demographic areas. Consequently, we could not discern meaningful patterns across other variables, such as gender identity and level of

advising experience. Additionally, it should be noted that the use of snowball sampling led to providers recruited from similar institutions with comparable practices and perspectives (e.g., pro-breastfeeding and advanced users of lactation-specific resources). Accordingly, we were unable to receive a direct understanding from providers who practiced a Culture of Leaning Towards Caution [C29] to gain more insight on their current practice approaches and viewpoints on existing resources and the UAR.

To further our understanding and improve the uptake of the UAR, future studies are suggested. First, a study to understand how to optimally provide training to providers in each profession and discipline for both existing resources and the UAR would be valuable. Second, research into the use of various existing resources such as the package insert and general drug information databases (e.g., Micromedex, Lexicomp) would help clarify their potential on decision making in order to improve provider knowledge and confidence in advising breastfeeding mothers during medication use. Finally, it would be of interest to improve the UAR based on this study's findings (e.g., Combine the UAR with Another Resource [C96] and Provide Guidance to Interpret the UAR [C106]) and perform another qualitative study to assess whether the needed improvements were adequately addressed to ensure optimal use.

Chapter 8
Discussion, Conclusions, and Future Directions

8.1 Discussion

This thesis aims to advance the knowledge of breastfeeding infant exposure to maternal medications through the creation of a novel risk metric, termed the UAR. Each chapter of this thesis describes the concerted efforts towards this advancement in the context of assessing maternal medication risk to the breastfeeding infant.

First, the components needed to capture the nuances and realities of medication use during breastfeeding were identified. Standard weight-normalized milk intake volume (150 mL/kg/day) commonly applied to ascertain doses infants that would receive through breast milk was recognized as not reflecting reality. Thus, Chapter 2 and Chapter 3 define a new milk intake model with captured variability, respectively. In Chapter 2, a comprehensive review was conducted using systematic methods, including a defined search strategy, developed eligibility criteria, screening of 2,274 articles, and extraction of 56 articles. The review resulted in 52 studies containing volume and frequency of breast milk intake that were used to develop a descriptive weight-normalized non-linear regression equation and a weighted mean, respectively. Weight-normalized milk intake reached a maximum of 152.6 mg/kg/day at 19.7 days and the weighted mean frequency of intake was 7.7 feeds/day. The trend for weight-normalized milk intake portrayed the greatest risk for breastfeeding infant exposure to maternal medications during the 2-4 week postnatal age window. This conclusion coincided with a review of case reports and studies by Anderson, Manoguerra (67), which cited the most reported adverse reactions occurring during the first and second months postnatal age. Besides identifying an at-risk period through breast milk intake volumes, Chapter 2 also addresses nuances that reflect actual practices. These include defining preterm infant patterns in weight-normalized milk intake volumes and frequencies, and separating exclusive and partial breastfeeding types. Overall, with the volume and frequency of milk intake across postnatal ages understood, the next step to incorporate the milk intake model to acquire infant daily doses for the prediction of exposures to maternal medications could be commenced.

In Chapter 3, lamotrigine was used as a test drug to undergo a workflow that incorporated the developed milk intake model, drug concentrations in milk, and pediatric PBPK modeling. Through lamotrigine, there was an opportunity to validate the workflow to predict breastfeeding infant

exposures with an accurate PBPK model and rich literature data consisting of knowledge about maternal doses and their associated drug in milk concentrations, and breastfed infant plasma concentrations. Undergoing the workflow validation process uncovered several findings. First, as a follow up to Chapter 2, weight-normalized milk intake variability was described with coefficient of variation ranging from 17 to 119.4% across different postnatal ages. Second, maternal doses appeared to be correlated with lamotrigine concentrations in milk. Together, the weight-normalized milk intake and the concentration in milk models with their associated variability, were used to determine doses for virtual breastfeeding infants created by the evaluated lamotrigine pediatric PBPK model. Third, when compared with the standard 150 mg/kg/day milk intake volume, the workflow with the incorporated variability did not provide a significant improvement. The lack of improvement could be due to a low sample size of paired samples (milk concentrations and resulting infant plasma concentrations reported) within each studied age group. Nonetheless, the milk intake volume model appeared to play a role in influencing predicted infant exposures, with 7 to 30 day olds presenting higher relative exposures. Fourth, the addition of the milk concentration model allowed for capture of more observed infant plasma levels which suggests the sensitivity of milk concentration to overall infant exposure. In all, the work demonstrated an example of potential exposure overlap with maternal therapeutic dose exposures and introduced the UAR for the first time.

With the workflow and UAR established, Chapter 4 and Chapter 5 were centered on CBD as the next molecule of interest. Chapter 4 focused on conflicting and general lack of information on the identity and contribution of metabolizing enzymes to CBD clearance. The study aimed to elucidate these mechanisms to solidify a CBD adult PBPK model prior to scaling to breastfeeding infants. As a BCS Class II drug, CBD is a mechanistically complex drug to model. However, using available IV data in humans and various single and dose oral studies to verify CBD distribution and clearance, respectively, were essential. *In vitro* values were used to inform the partitioning of clearance by six metabolizing enzymes, CYP3A4, CYP2C19, CYP2C9, UGT1A7, UGT1A9, and UGT2B7. When incorporated into the developed CBD oral PBPK model, these *in vitro*-determined clearance were deemed reasonable when comparing model-predicted and observed $AUC_{0\text{-}\tau}$ of clinical drug-drug interaction studies with CBD (percent error ranging from 16-30%).

Following the consolidation of the adult CBD model, Chapter 5 described scaling the model to breastfeeding infants for exposure predictions through the established workflow in Chapter 3. Although no CBD concentrations in breastfed infant plasma concentrations are available for

evaluation, confidence on the results of the exposure predictions was made possible by leveraging the fact that the workflow was validated through lamotrigine. Moreover, the presented work provided a use case of the UAR, showing that a drug does not need to have rich information to apply the workflow and attain the UAR. Rather, CBD was selected based on its large interest from the breastfeeding community. Interest in maternal use of recreational drugs had led the University of California San Diego investigators to collect administration information and milk samples from mothers taking marijuana, CBD, and CBD-containing products. Information on CBD concentrations in milk were used with the milk intake volume model to determine infant daily doses. Furthermore, the influence of maternal administrations on affecting drug concentrations in milk and subsequent infant exposures were evaluated. Mothers with a joint/blunt or edible administration tended to have lower infant exposures as compared to those taking oil or pipe. Lastly, results of the work in CBD demonstrates an example of the sensitivity of the workflow to predict minimal exposure overlap between breastfed infants and children receiving therapeutic doses, in contrast to lamotrigine.

Chapter 6 provides another use case of the UAR where ezetimibe was selected as a drug with no information on breastfeeding risk to the infant. Furthermore, applying the workflow on ezetimibe contributed to a growing body of evidence in showing the application of the UAR. As another BCS Class II drug, ezetimibe and its glucuronide were difficult to model. Although simulated plasma concentration-time profiles were suboptimal (e.g., AFE of 0.98 and AAFE of 2.06 for ezetimibe), adult and children exposures were reasonably predicted. Thus, the model was deemed appropriate to predict exposures in breastfeeding infants. Ezetimibe and glucuronide milk concentration samples collected by SickKids and were used to inform infant daily doses. Similar to CBD, ezetimibe was a drug with an absence of overlap in breastfed infant and therapeutic exposures. However, further studies to improve the accuracy of the model through population pharmacokinetic models are warranted for confirmation.

The final chapter of this thesis describes a culmination of the modeling efforts to develop and theoretically assess the UAR in previous chapters. Chapter 7 provides an opportunity to move from bench-side to clinical use where perspectives on the end-users provided insight on the benefits of the UAR and strategies for further improvement in practice. The work recognizes that a novel tool is only useful once deemed advantageous and feasible by the end-users. To acquire end-user perspectives, 28 interviews were conducted with healthcare providers mainly practicing in San Diego, California. Through the Framework Method, six main themes emerged that addressed current advising

approaches, including existing resource use; the advantages and disadvantages of existing resources and the newly introduced UAR; and strategies to improve the UAR. With an improved understanding of the UAR in the context of current practice, future efforts can work towards advancing the metric for eventual clinical use.

8.2 Conclusions

This thesis leverages a network of collaborators to further existing knowledge of infant exposure to maternal medications through breastfeeding. The chapters reflect efforts beginning with the identification and improvement of existing methods to predict breastfeeding infant exposures to develop a novel metric, and concludes with assessing the utility of the metric in practice. Each aspect of this thesis strives to improve the confidence of healthcare providers and mothers in their decision-making when considering medication use during breastfeeding.

In 2016, a two-day public workshop sponsored by the U.S. Food and Drug Administration (FDA) was held to discuss the safety of drugs and biological products used during lactation (85). Two of the primary discussion topics during the workshop were to consider future approaches to design and guide clinical lactation studies; and to consider novel approaches to improve the quality and quantity of data to assess the safety of medications used during lactation, and inform the public of potential risks. This thesis is well-positioned to address these gaps in medication use during lactation.

The workshop highlighted the large number of drugs and biological products used by lactating women and the necessity of prioritizing products for clinical studies. Immense resources would be needed to evaluate the exposure for all drugs in breastfeeding. The newly derived metric has the potential to impact the risk assessment process by streamlining focus to high-risk drugs and redirect resources. Using the 1677 drugs in LactMed, an online resource for clinicians providing recommendations to breastfeeding mothers taking medications, drugs can be selected for prioritization. Drugs with a high priority can include those that mothers typically cannot discontinue, are known to enter breast milk, taken during the “higher risk” period of 2-4 weeks, and have notable potential effects in breastfed infants. After the UAR is applied to these drugs they can be ranked with respect to risk categorization; for example: <0.1 corresponds to low risk of unwanted effects, 0.1-0.5 corresponds to moderate risk, and >0.5 corresponds to significant risk. Researchers designing clinical trials would only need to focus on this subset of high-risk drugs and requires the recruitment of only a few infants for confirmatory rather than exploratory purposes. Essentially, the samples would be used

to evaluate whether the confirmatory samples are in line with the predicted infant PBPK model exposures.

The workshop also called for healthcare providers to be supported by trusted, accurate, comprehensive, and consistent information about maternal medication and breastfeeding management to inform clinical recommendations. Results of the thesis work can be directly translated for clinical recommendation. Specifically, the identification of high-risk mothers through pharmacogenotyping will provide more insight into this rarely studied area. This information along with the new drug in milk safety metric for each of the study drugs, can be published in existing resources for clinicians to confirm or change current recommendations to mothers. Mothers and healthcare providers will be empowered by the UAR and information on how time after dose and administration types can impact their breastfeeding infant to help make more informed decision making.

8.3 Future Directions

Interest in the breastfeeding risk-benefit analysis from a pharmacometrics perspective has been building in the past few years. The significant growth in this area is evident from reviews and workshops acknowledging the value of PopPK and PBPK modelling methods in improving our understanding of drug in milk concentrations and infant exposures (85, 359-361), to investigators beginning to incorporate postpartum/lactation maternal anatomy and physiology for whole-body PBPK model predictions (112). Moreover, there is recognition from the FDA that assistance for healthcare providers in assessing the risk-benefit in nursing mothers taking medications is needed. This demand is evidenced by the 2014 shift from the FDA to improve prescription drug labels through the Pregnancy and Lactation Labeling Rule (PLLR). In light of the growing desire to advance our knowledge about breastfeeding infant risk to maternal medications, this section provides recommendations on future directions that can build upon the work of this thesis.

As discussed throughout this thesis, preterm infants are particularly vulnerable due to having reduced clearance and thus higher likelihood for dangerous levels of drug exposure as compared to term infants. Studies have also demonstrated breastfeeding benefits to the preterm population, such as improved neurodevelopmental outcomes compared to their counterparts (11). Additionally, it was evident from the interviews of Chapter 7 that healthcare providers practicing in the NICU would benefit from a more evidence-informed risk-benefit analyses in preterm infants.

In Chapter 2, it was determined that preterm infants had a similar pattern of weight-normalized milk volume intake as term infants. However, studies informing the preterm infant volumes of intake (7 studies) were lacking as compared to term infants (28 studies), especially past 3 months of age. Further studies measuring preterm infant intake volumes that extend beyond their stay at the NICU and capture months of typical at-home breastfeeding practices. The proposed work would better inform dose estimates that preterm infants would receive for input into pediatric PBPK models for exposure predictions. Moreover, this thesis developed the workflow and the UAR in the context of term infants. Currently, there is difficulty in performing exposure predictions in preterm infants with oral administration due to the lack of a well evaluated preterm oral model. Therefore, a next step would consist of establishing an adequate oral preterm model and applying the workflow and UAR.

In this thesis, the influence of maternal dose (Chapter 3) and administration type (Chapter 5) on drug in milk concentrations and breastfed infant exposures were explored. Future work should study further factors influencing infant exposures. Of particular interest would be pharmacogenotypes, where the effect of maternal and infant polymorphisms on relevant enzymes and transporters can be significant. For example, studies suggest that UGT1A4 and UGT2B7 polymorphisms may have an effect on lamotrigine plasma concentrations (362). With more data collected on maternal and infant factors, it would be possible to produce PopPK models to improve our understanding of influences on increased or decreased drug in milk concentrations. The results of the PopPK models could then be paired with the pediatric PBPK model to predict exposures and the UAR based on identified subgroups in a similar process as Chapter 5.

A topic that was frequently discussed in the healthcare provider interviews in Chapter 7 was the limited information on the adverse effects for many of the drugs used during lactation. As a future study, the added utility of the UAR in understanding the exposure-response relationship could be conducted. Future work would include attaining the UAR on drugs in which the mechanism of action is well established and conducting a prospective studies to establish an improved understanding of the exposure-response relationship of these drugs. Moreover, a study applying the UAR on a percentage of a large group of drugs labelled as “low risk” to “high risk” and vice versa, and relating their risk outcome on reported side effects would be of interest.

Finally, to meet the future objective to use a streamlining system to identify those that might be high-risk to infants (e.g., UAR >0.5), the workflow established in this thesis should be repeated on

multiple drugs to attain UARs. This work can be made possible through collaborations with additional groups such as Pediatric Trials Network (PTN) which conducts the Pharmacokinetics and Safety of Commonly Used Drugs in Lactating Women and Breastfed Infants (CUDDLE) study (ClinicalTrials.gov Identifier: NCT03511118). CUDDLE aims to enroll 50 lactating women for each of the following study drugs: azithromycin, clindamycin, escitalopram, labetalol, metformin, nifedipine, ondansetron, oxycodone, sertraline, tranexamic acid, ciprofloxacin, doxycycline, levofloxacin, methylphenidate, sumatriptan. In tandem, drugs for further study can be selected through reviewing informational lactation resources such as LactMed, where trends that drive interest towards certain medications can be examined.

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Appendix A

Supplementary Material for Chapter 2

1. Search Strategy

Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R) 1946 to July 02, 2019

1. exp Infant, Low Birth Weight/
2. exp Infant, Premature/
3. Premature Birth/
4. ((premature or preterm or pre-term or full-term) adj (infant* or neonate* or newborn* or birth*)).tw.
5. ((breastfeed* or breast-feed* or breastfed or breast-fed or feeding or breastmilk or breast-milk or milk) adj3 (infant* or neonate* or newborn*)).tw.
6. 1 or 2 or 3 or 4 or 5
7. Breast Feeding/
8. Milk, Human/
9. Lactation/
10. 7 or 8 or 9
11. ((breastfeed* or breast-feed* or breastfed or breast-fed or feeding or breastmilk or breast-milk or milk) adj3 (intake* or volume* or consum* or frequency or frequencies or pattern*)).tw.
12. 6 and 10 and 11
13. limit 12 to (english language and humans)

Embase 1974 to 2019 July 02

1. prematurity/
2. ((premature or preterm or pre-term or full-term) adj (infant* or neonate* or newborn* or birth*)).ti,ab.
3. ((breastfeed* or breast-feed* or breastfed or breast-fed or feeding or breastmilk or breast-milk or milk) adj3 (infant* or neonate* or newborn*)).ti,ab.
4. 1 or 2 or 3
5. breast feeding/
6. breast milk/
7. lactation/
8. 5 or 6 or 7
9. ((breastfeed* or breast-feed* or breastfed or breast-fed or feeding or breastmilk or breast-milk or milk) adj3 (intake* or volume* or consum* or frequency or frequencies or pattern*)).ti,ab.
10. 4 and 8 and 9
11. limit 10 to (human and english language)

2. Daily weight-normalized human milk intakes and milk intake frequencies by study

Supplementary Table 1. Daily weight-normalized human milk intakes for term infants exclusively breastfed of all ages and partially breastfed at >6 months of age

Age (days) †	Study	No. of infants	WHMI volume (mL/kg/day)	SD
Exclusively breastfed				
1	Casey et al., 1986	3	12.6	15.5
	Evans et al., 2003	26	6	7.1
2	Casey et al., 1986	10	38.8	22.3
	Evans et al., 2003	88	25	20.6
	Novotny & Mata, 1983	1	65.7	NR
3	Casey et al., 1986	10	95.1	45.6
	Evans et al., 2003	88	66	33.8
4	Casey et al., 1986	11	135.9	49.5
	Evans et al., 2003	88	106	36.6
5	Casey et al., 1986	11	150.5	28.2
	Evans et al., 2003	88	123	42.2
6	Evans et al., 2003	88	138	36.6
7	English, 1985	1	72.8	NR
	Ferris et al., 1993; Neubauer et al., 1993	10	143.7	36.9
11	Novotny & Mata, 1983	1	167.7	NR
13	Novotny & Mata, 1983	1	236.6	NR
14	Bhutta et al., 2004	12	132.6	27.7
	English, 1985	1	158.3	NR
	Ferris et al., 1993; Neubauer et al., 1993	9	156.3	40.8
	Forsum & Sadurskis, 1986	22	168.9	38.8
	Janas, Picciano, & Hatch, 1985	11	164	NR
	Krebs et al., 1994	71	155.3	29.1
	Novotny & Mata, 1983	2	173.7	49.9
15	van Steenbergen, Kusin, & van Rens, 1981	7	187	29
17	Sievers et al., 1992; Sievers et al., 2002‡	7	154.4	NR
21	English, 1985	1	203.9	NR
28	English, 1985	1	201.0	NR
	Forsum & Sadurskis, 1986	22	165.0	28.2
30	Borschel, Kirskey, & Hannemann, 1986	15	158	46.5
	Brown, Robertson, & Akhtar, 1986	36	163.1	29.1
	Butte et al., 1984a, b	37	154.4	23.3
	Butte, Smith & Garza, 1990	17	148.5	25.2
	Hofvander et al., 1982	25	149.5	24.3
35	English, 1985	1	200.0	NR
	Sievers et al., 1992; Sievers et al., 2002‡	10	145.6	NR
37	Butte et al., 1983	2	158.8	13.2
42	English, 1985	1	201.9	NR

Age (days) †	Study	No. of infants	WHMI volume (mL/kg/day)	SD
	Forsum & Sadurskis, 1986	22	142.7	37.9
	Motil et al., 1997	10	158.3	17.5
45	Novotny & Mata, 1983	2	129.6	3.5
46	Butte et al., 1988	1	103.2	NR
49	English, 1985	1	202.9	NR
56	English, 1985	1	177.7	NR
	Forsum & Sadurskis, 1986	22	140.8	23.3
	Janas, Picciano, & Hatch, 1985	11	134	NR
57	Sievers et al., 1992; Sievers et al., 2002‡	9	122.3	NR
61	Butte et al., 1983	4	126.9	35
	Bandara et al., 2015	8	160.2	29.1
	Bandara et al., 2015	8	173.8	21.4
	Borschel, Kirskey, & Hannemann, 1986	15	128	27.1
	Brown, Robertson, & Akhtar, 1986	42	146.6	31.1
	Butte et al., 1984a, b	40	125.2	18.4
	Hofvander et al., 1982	25	143.7	22.3
	Michaelsen et al., 1994	60	135.9	23.3
63	English, 1985	1	180.6	NR
70	English, 1985	1	181.6	NR
	Forsum & Sadurskis, 1986	22	134.0	21.4
72	Novotny & Mata, 1983	1	122.8	NR
76	Bandara et al., 2015	8	135.9	45.6
	Bandara et al., 2015	8	141.7	16.5
	van Steenbergen, Kusin & van Rens, 1981	13	120	41
77	English, 1985	1	167.0	NR
81	Butte et al., 1988	1	121.7	NR
84	English, 1985	1	170.9	NR
	Motil et al., 1997	10	118.4	14.6
85	Sievers et al., 1992; Sievers et al., 2002‡	9	125	NR
91	English, 1985	1	164.1	NR
	Bhutta et al., 2004	12	73.3	10.2
	Brown, Robertson, & Akhtar, 1986	33	141.7	24.3
	Butte et al., 1984a, b	37	113.6	19.4
	Dewey et al., 1991a, b	73	126.2	17.5
	Hofvander et al., 1982	25	128.2	17.5
	Krebs et al., 1994	71	116.5	15.5
92	Butte et al., 1988	1	105.6	NR
94	Butte et al., 1983	11	120	24
102	Novotny & Mata, 1983	2	97.3	22.4
108	Nielsen et al., 2011	36	137.9	16.5
112	Ettyang et al., 2005	10	115	15.8
113	Sievers et al., 1992; Sievers et al., 2002‡	10	119.4	NR

Age (days) †	Study	No. of infants	WHMI volume (mL/kg/day)	SD
116	Butte et al., 1983	7	115.6	19.8
122	Borschel, Kirskey, & Hannemann, 1986	15	96	27.1
	Brown, Robertson, & Akhtar, 1986	15	133	18.4
	Butte et al., 1984a, b	41	107.8	16.5
	Butte, Smith, & Garza, 1990	15	110.7	19.4
	Michaelsen et al., 1994	36	120.4	16.5
	Salmenpera, Perheentupa, & Siimes, 1985	12	121.4	20.4
	Stuff & Nichols, 1989	45	110.7	17.5
	Butte et al., 1988	2	99.9	17.3
126	Motil et al., 1997	10	102.9	26.2
152	Bandara et al., 2015	9	131.1	16.5
	Bandara et al., 2015	7	107.8	28.2
	Brown, Robertson, & Akhtar, 1986	14	129.1	19.4
	Stuff & Nichols, 1989	26	100.0	21.4
172	Nielsen et al., 2011	38	128.2	14.6
183	Bhutta et al., 2004	12	117.3	49.2
	Borschel, Kirskey, & Hannemann, 1986	15	80	23.2
	Brown, Robertson, & Akhtar, 1986	13	121.4	23.3
	Salmenpera, Perheentupa, & Siimes, 1985	31	109.7	16.5
	Stuff & Nichols, 1989	8	103.9	26.2
213	Brown, Robertson, & Akhtar, 1986	12	122.3	18.4
243	Brown, Robertson, & Akhtar, 1986	10	116.5	17.5
274	Brown, Robertson, & Akhtar, 1986	5	114.6	23.3
	Salmenpera, Perheentupa, & Siimes, 1985	16	104.9	19.4
304	Salmenpera, Perheentupa, & Siimes, 1985	10	101.0	17.5
335	Salmenpera, Perheentupa, & Siimes, 1985	5	101.9	16.5
365	Salmenpera, Perheentupa, & Siimes, 1985	4	89.3	17.5
Partially breastfed				
213	Stuff & Nichols, 1989	8	81.6	26.2
	Krebs et al., 1994	71	82.5	16.5
243	Stuff & Nichols, 1989	7	74.8	25.2
257	Martinez & Chavez, 1971	9	63.7	10.2
	Martinez & Chavez, 1971	8	74.4	16.7
259	van Steenbergen, Kusin, & van Rens, 1981	22	69	25.0
270	Amatayakul et al., 1999	26	55.3	20.4
	Amatayakul et al., 1999	26	59.2	17.5
274	Dewey et al., 1991a, b	50	71.8	23.3
	Stuff & Nichols, 1989	7	65.0	18.4
	Michaelsen et al., 1994	18	35	21.4
360	Amatayakul et al., 1999	16	45.6	21.4
	Amatayakul et al., 1999	18	51.5	25.2
365	Dewey et al., 1991a, b	42	46.6	26.2

Age (days) †	Study	No. of infants	WHMI volume (mL/kg/day)	SD
397	Martinez & Chavez, 1971	9	62.7	9.6
	Martinez & Chavez, 1971	8	66.8	6.1
441	van Steenbergen, Kusin, & van Rens, 1981	22	52	17.0
549	Martinez & Chavez, 1971	9	42.7	7.2
557	Martinez & Chavez, 1971	8	49.9	7.2
624	van Steenbergen, Kusin, & van Rens, 1981	12	31	15.0

†Age reported as weeks or months were converted into an approximate age in days (e.g., 2 weeks × 7 days/week = 14 days, 5 months × (365 days/12 months) = 152 days). ‡Data is reported as median WHMI. All other data is presented as mean WHMI. NR: not reported; WHMI: weight-normalized human milk intake.

Supplementary Table 2. **Daily frequency of human milk feeds for term and preterm infants**

PNA (days) †	Study	No. of infants	Feeds per day	SD
Exclusively breastfed term infants				
1	Yamauchi & Yamanouchi, 1990	140	4.3	2.5
	de Carvalho et al., 1982	46	6.7	2.8
	Houston, Howie, & McNeilly, 1983	18	4.7	1.2
2	Ferris et al., 1993; Neubauer et al., 1993	11	6.1	1.4
	Yamauchi & Yamanouchi, 1990	140	7.4	3.9
	de Carvalho et al., 1982	46	8.7	3.1
	Novotny & Mata, 1983	1	8	NR
	Houston, Howie & McNeilly, 1983	18	5.8	1.2
3	Ferris et al., 1993; Neubauer et al., 1993	11	7.8	1.9
	Jia et al., 2018	71	8.3	2.1
	Houston, Howie, & McNeilly, 1983	18	7	0.9
	de Carvalho et al., 1982	46	9.2	2.7
4	Houston, Howie, & McNeilly, 1983	18	7.1	0.9
	de Carvalho et al., 1982	46	9.5	3.3
5	Houston, Howie, & McNeilly, 1983	18	7.3	1.4
	de Carvalho et al., 1982	46	9.3	2.7
6	de Carvalho et al., 1982	46	10.3	3.4
7	Ferris et al., 1993; Neubauer et al., 1993	11	8.2	1.4
	de Carvalho et al., 1982	46	9.4	3.0
8	de Carvalho et al., 1982	46	9.1	2.5
9	de Carvalho et al., 1982	46	9.0	3.1
10	Jia et al., 2018	45	8.5	1.2
	de Carvalho et al., 1982	46	9.2	2.9
11	Neville et al., 1988	12	7.5	3.8
	Novotny & Mata, 1983	1	10	NR
	de Carvalho et al., 1982	46	9.0	3.0
12	de Carvalho et al., 1982	46	9.0	3.1
13	Novotny & Mata, 1983	1	11	NR

PNA (days) †	Study	No. of infants	Feeds per day	SD
	de Carvalho et al., 1982	46	9.3	3.0
14	Ferris et al., 1993; Neubauer et al., 1993	11	8.4	2.6
	Hornell et al., 1999‡	430	7.8	NR
	Howie et al., 1981	27	5.9	1.0
	Novotny & Mata, 1983	2	8.5	0.7
	de Carvalho et al., 1982	46	9.0	2.6
22	Neville et al., 1988	12	8.2	2.8
28	Hornell et al., 1999‡	395	7.6	NR
	Matheny & Picciano, 1986	37	7	1.6
	Kent et al., 2013	52	7.6	NR
	Quandt, 1986	62	7.2	1.7
30	Borschel, Kirskey, & Hannemann, 1986	15	6.5	1.2
	Butte et al., 1990	10	7.2	1.6
	Butte et al., 1984a,b	37	8.3	1.9
	Pao, Himes & Roche, 1980	7	6.6	1.1
42	Hornell et al., 1999‡	NR	7.4	NR
43	Paul et al., 1988	20	6.1	1.0
45	Novotny & Mata, 1983	2	10.5	3.5
	Neville et al., 1988	13	8.1	2.2
56	Hornell et al., 1999‡	337	7.2	NR
	Matheny & Picciano, 1986	37	6	1.6
	Quandt, 1986	48	7.1	1.6
60	Jia et al., 2018	25	8.3	1.0
61	Borschel, Kirskey, & Hannemann, 1986	15	6.3	0.8
	Michaelsen et al., 1994	60	6.9	1.8
	Butte et al., 1984a,b	40	7.2	1.9
70	Hornell et al., 1999‡	NR	7	NR
72	Novotny & Mata, 1983	1	8	NR
84	Hornell et al., 1999‡	290	6.9	NR
	Matheny & Picciano, 1986	47	5	1.7
91	Kent et al., 2013	52	6.6	NR
	Nommsen et al., 1991	58	6.7	1.5
	Butte et al., 1984a,b	37	6.8	1.9
	Pao, Himes, & Roche, 1980	1	5.3	NR
98	Hornell et al., 1999‡	NR	7.1	NR
102	Novotny & Mata, 1983	2	7.5	2.1
105	Neville et al., 1988	13	7.3	1.8
108	Nielsen et al., 2011‡	43	8	NR
112	Hornell et al., 1999‡	189	7.3	NR
	Cohen et al., 1994	50	13.8	3
122	Borschel, Kirskey, & Hannemann, 1986	15	5.6	1.5
	Michaelsen et al., 1994	36	7.1	1.9
	Butte et al., 1990	10	6.5	1.1
	Butte et al., 1984a,b	41	6.7	1.8

PNA (days) †	Study	No. of infants	Feeds per day	SD
126	Hornell et al., 1999‡	NR	7.4	NR
140	Hornell et al., 1999‡	79	7.6	NR
147	Cohen et al., 1994	50	13.1	2.7
154	Hornell et al., 1999‡	NR	7.8	NR
168	Hornell et al., 1999‡	20	7	NR
172	Nielsen et al., 2011‡	41	9	NR
182	Hornell et al., 1999‡	7	7.3	NR
	Cohen et al., 1994	50	13.6	1.8
183	Borschel, Kirskey, & Hannemann, 1986	15	5.4	1.2
	Pao, Himes, & Roche, 1980	1	7.3	NR
Partially breastfed term infants				
201	Paul et al., 1988	16	4.1	2.0
204	Paul et al., 1988	21	4.0	1.7
210	Howie et al., 1981	22	3.4	1.4
224	Howie et al., 1981	21	3.2	1.3
234	Paul et al., 1988	12	3.1	1.7
237	Paul et al., 1988	18	3.2	2.1
238	Howie et al., 1981	19	3.2	1.2
252	Howie et al., 1981	17	3.0	1.3
	Martinez & Chavez, 1971	9	11.9	1.2
262	Martinez & Chavez, 1971	8	12.1	1.7
266	Howie et al., 1981	15	2.9	1.5
270	Amatayakul et al., 1999	26	10.7	3.9
	Amatayakul et al., 1999	26	11.1	2.8
274	Michaelsen et al., 1994	18	3.4	1.7
	Pao, Himes, & Roche, 1980	3	5.3	0.9
	Nommsen et al., 1991	28	5.6	1.7
277	Paul et al, 1988	12	2.5	2.5
280	Howie et al, 1981	15	2.6	0.6
284	van Steenbergen et al., 1991	77	15.0	1.6
286	Paul et al., 1988	7	3.9	2.9
360	Amatayakul et al., 1999	16	9.2	4.8
	Amatayakul et al., 1999	18	9.2	2.8
365	Nommsen et al., 1991	21	4.6	2.1
368	van Steenbergen et al., 1991	77	14.4	2
390	Martinez & Chavez, 1971	9	11.9	1.4
400	Martinez & Chavez, 1971	8	10.8	1.9
541	Martinez & Chavez, 1971	9	9.3	0.9
548	Martinez & Chavez, 1971	8	10.1	0.8
Exclusively and partially breastfed preterm infants				
24	Oras et al., 2015‡	24	14	NR
24	Oras et al., 2015‡	16	12.5	NR
24	Oras et al., 2015‡§	8	8.5	NR
113	Oras et al., 2015‡	23	10	NR

PNA (days) [†]	Study	No. of infants	Feeds per day	SD
113	Oras et al., 2015[‡]	5	9	NR
113	Oras et al., 2015[‡§]	15	6	NR
234	Oras et al., 2015[‡]	2	11.5	NR
234	Oras et al., 2015[‡§]	20	5	NR
417	Oras et al., 2015[‡§]	8	5.5	NR

[†]Age reported as weeks or months were converted into an approximate age in days (e.g., 2 weeks × 7 days/week = 14 days, 5 months × (365 days/12 months) = 152 days). [‡]Median number of feeds per day. All other studies present mean number of feeds per day. [§]PBF data from preterm infants. All other feeding frequency data for preterm infants is presented for EBF. NR: not reported; PNA: postnatal age.

Appendix B
Supplementary Material for Chapter 3

1. Building of a PBPK model for lamotrigine in breastfeeding infants

(1) Introduction

Lamotrigine is a phenyltriazine anticonvulsant indicated for epilepsy as adjunctive therapy in those 2 years and older, and monotherapy in those 16 years and older (218). It is also indicated for bipolar disorder in patients 18 years and older as maintenance treatment to delay the time of occurrence of mood episodes for those taking standard therapy and experiencing acute mood episodes. In adults, lamotrigine has a plasma half-life ranging from 22.8 to 59 hours depending on co-medications with peak plasma concentrations occurring between 1.4 to 4.8 hours following oral administration (363, 364).

Lamotrigine is available as compressed tablets (25 mg, 100 mg, 150 mg, and 200 mg), chewable dispersible tablets (2 mg, 5 mg, and 25 mg), and orally disintegrating tablets (25 mg, 50 mg, 100 mg, and 200 mg). The tablet is available in immediate release (IR), sustained release (SR), and extended release (ER) formulations. Classified as a BCS Class 2 drug, lamotrigine has high permeability and low solubility. Its oral bioavailability is high at 98 ± 0.05% (196, 365).

The predominate route of lamotrigine elimination is through hepatic metabolism, with renal excretion accounting for <10% (366). The enzyme mainly responsible for its liver metabolism is UGT1A4, however, the role of further enzymes, UGT2B7 and UGT1A3, is less clear (233, 367). Total oral clearance (CL/F) was 0.44 mL/min/kg (range: 0.12-1.10 mL/min/kg) from healthy adults taking a single dose of lamotrigine (368). Following oral administration of 240 mg radiolabeled lamotrigine to 6 healthy volunteers, 94% of the drug and its metabolites were recovered in urine and 2% in the feces. The majority of radioactivity consisted of unchanged lamotrigine (7-10%) and its inactive metabolite, 2-N-glucuronide (76%) (196, 368, 369).

Studies in patients with epilepsy have shown a linear relationship between dose and lamotrigine plasma concentration at steady state, following doses of 50 to 350 mg twice daily (368). For ER formulations, an increase in systemic exposure to lamotrigine in healthy volunteers was dose proportional between 50 and 200 mg; however, at doses between 25 and 50 mg, the increase in exposure was less than dose proportional (1.6-fold increase in exposure due to a 2-fold increase in dose) (370).

This appendix reports the building of a pediatric physiologically-based pharmacokinetic (PBPK) model for lamotrigine.

(2) Methods and Results

(2.1) Modeling Software and Strategy

PBPK modelling and simulation were performed using PK-Sim (version 8; Open Systems Pharmacology). The small molecule PBPK model structure includes fifteen organs connected through venous and arterial blood pools with each organ compartment divided into 4 sub-compartments (red blood cells, plasma, interstitial space, intracellular space).

Pediatric PBPK model development followed a typical method as described in Maharaj, Barrett (12). First, model parameters were optimized to describe systemic disposition in an adult based on the PK following IV administration. Once solidified, PK data following single dose oral administration was used for optimization of model parameters specific to oral absorption in an adult. Model evaluation was completed using PK data following multiple administration regimens. An adult population was then used to assess the appropriateness of the virtual individuals in capturing PK variability as compared to observed PK data. Extrapolation to the pediatric age range took into account changes in anatomy and physiology relevant to describe PK of the medication while leaving all drug specific parameter as used in the adult model. Evaluation of the pediatric model was completed using PK data from children directly administered lamotrigine. The final pediatric model was used to simulate the dose exposure relationship in breastfeeding infants.

(2.2) Adult IV Model

(2.2.1) IV Model Parameterization

Table 1 presents the drug specific parameters of lamotrigine and the values used for the naïve model.

Table 1. Physicochemical properties and ADME of lamotrigine for IV model construction

	Used in naïve model	Used in optimized model
Physicochemical properties		
Lipophilicity (logP)	1.19 (371, 372) 1.87 (ALOGPS) (373) 1.98 (ADMET Predictor)	1.81

Fraction unbound in plasma (fu)	0.45 (368)	0.45
Molecular weight	256.09 g/mol (363)	256.09 g/mol
pKa	5.7 (base) (368) 5.5 (base) (363)	5.7
Water solubility	0.17 mg/mL (368)	0.17 mg/mL
ADME		
Partition coefficient	Rodgers and Rowland Schmitt Berezkhovskiy PK-Sim Standard	Rodgers and Rowland
Cell permeability	PK-Sim Standard	PK-Sim Standard
Total clearance (CL/F)	0.44 (0.12 – 1.10) mL/min/kg (368)	0.44 (0.12 – 1.10) mL/min/kg
Renal clearance (CL/F)	0.043 ± 0.012 mL/min/kg (211)	0.043 ± 0.012 mL/min/kg
UGT1A4 concentration	1.0 µM	1.0 µM
UGT1A4 specific clearance	0 1/min	0.029 1/min
UGT1A3 concentration	1.0 µM	1.0 µM
UGT1A3 specific clearance	0 1/min	0.0032 1/min
GFR fraction	1.0	0.05

Table 2 presents the lamotrigine dataset used for building the IV model. Local optimization was carried out in PK-Sim using a Monte Carlo approach for exploring the parameter space.

Table 2. Pharmacokinetic dataset for lamotrigine IV model construction

Study	Dose and administration	Cohort	N	Age (years)[a]	Weight (kg)[a]
Yuen & Peck 1988	67.82 mg IV infusion over 30 min	European males (75%) and females	8	27.5 [20-35][b]	71 [59-83][b]

[a]Mean ± SD reported, or range in square brackets if SD not reported. [b]Mean not reported in study, therefore the median, an average of the range, or BMI of approximately 23 kg/m^2 was used instead.

First, a naïve model was set up for a mean male individual weighing 71 kg. Clearance was partitioned as renal and hepatic. GFR fraction was fixed to 0.05 to account for glomerular reabsorption (GFR <1) to reach a fraction excreted unchanged in urine of 7.33% (369). Each of four partition coefficient calculation methods (**Table 1**) were evaluated with optimization of logP and non-specific hepatic enzymatic clearance using the IV dataset. The Rodgers and Rowland method for

predicting partition coefficients and logP was selected on the basis of visual model performance for curve shape (**Table 1**). The optimized logP was similar to published values (**Table 1**).

The optimized non-specific enzyme clearance was 0.033 1/min as is a function of more than one enzyme. In a study with 240 mg administered orally to man, 94% of the dose was found in urine with 10% excreted unchanged (374). The study proposed the following metabolites and their abundance in urine: 2-N-glucuronide (76%), 5-N-glucuronide (10%), 2-N-methyl glucuronide (0.14%), and other minor metabolites (4%) (374). However, a more recent study by Beck, Ohman (375) found 2-N-glucuronide as the main metabolite, noting the weak evidence supporting the presence of the further metabolites. Based on Beck, Ohman (375), 2-N-glucuronide was considered the sole metabolite by UGT1A4 and UGT1A3 (233). Although previous in vitro studies in human liver microsomes have determined the involvement of UGT2B7 in lamotrigine to 2-N-glucuronide metabolism (367), these results could not be replicated by Argikar and Remmel (233), suggesting that further studies are required to assess the involvement of UGT2B7. Clearance was partitioned according to 2-N-glucuronide formation by UGT1A4 (90%) and UGT1A3 (10%). These relative contributions were determined from in vitro studies (233) with appropriate scaling as performed by Ladumor, Thakur (372). The organ-specific expressions of UGT1A4 and UGT1A3 were informed by the Human Protein Atlas (https://www.proteinatlas.org/) and specifically Kaivosaari, Toivonen (376) and Nakamura, Nakajima (377), and Strassburg, Oldhafer (378), respectively.

The optimized values for the adult IV PBPK model are presented in **Table 1**. **Figure 1** presents the outcome of the IV model optimization using the Yuen and Peck (196) dataset.

(2.3) Adult Oral Model

(2.3.1) Oral Model Parameterization

The same systemic parameters as developed for the mean male IV PBPK model were used for the model defining oral administration. Those drug/formulation-specific parameters needing definition included lamotrigine solubility, formulation dissolution and intestinal permeability. The oral PBPK model for each lamotrigine dose for which observed PK data was available was created using the same mean male as in the IV model. Lamotrigine water solubility was defined at 0.17 mg/mL at a reference pH of 7 and solubility gain per charge (factor by which the solubility increases with each ionization step) of 10 to describe pH-dependent solubility (379). Dissolution was defined based on a Weibull function (inputs of curve shape and dissolution half-time). Dissolution half-time was an

optimized parameter for each study of the same dose whereas intestinal permeability was a globally optimized parameter and therefore the same for each oral PK study.

Table 3. Oral absorption parameters for lamotrigine oral model construction

	Used in naïve model	Used in optimized model
Dissolution half-life IR 25 mg	10 min	11.39 min
Dissolution half-life IR 75 mg	10 min	30.93 min
Dissolution half-life IR 100 mg	10 min	2.95 min
Dissolution half-life IR 200 mg	10 min	43.97 min
Dissolution half-life IR 300 mg	10 min	10.36 min
Dissolution profile shape	0.92	0.92
Water solubility	0.17 mg/mL (368)	0.17 mg/mL
Specific intestinal permeability	1.503E-5 cm/min	2.269 cm/min

Table 4 shows the PK datasets used for oral model building. All are single dose administrations. The datasets used for optimization of dissolution half-time and specific intestinal permeability included the drug in a compressed tablet, capsule form, and chewable/dispersible tablet. Lamotrigine chewable/dispersible tablets, whether administered as dispersed in water, chewed, or swallowed whole, were found to be equivalent to the compressed tablet form in terms of rate and extent of absorption (368). Therefore, the datasets from one dose, regardless of formulation type, were used together for optimization purposes.

Table 4. Pharmacokinetic dataset for lamotrigine oral model construction

Study	Dose and administration	Cohort	N	Age (years)[a]	Weight (kg)[a]
Berg 2017	25 mg PO IR tablet	White (86%) American males (57%) and females	49	46 ± 16	80 ± 18
Ebert 2000	25 mg PO IR capsule	European males	10	25 ± 4	74.4 [63-100][b]
Gidal 2003	25 mg PO IR tablet	American males (19%) and females	28	34 ± 13	78 ± 23
Yuen & Peck 1988	75 mg PO capsule	European males (75%) and females	8	27.5 [20-35][b]	70.2 [59-83][b]
Birnbaum 2000	100 mg PO IR tablet	White American males	12	40.8 ± 11.5	83.6[b]

Birnbaum 2001	100 mg PO IR chewable/dispersible tablet	White American males (92%) and a female	12	32.1 ± 7.1	81.0[b]
Burger 2008	100 mg PO capsule	European males	17	35 [19-54]	77 [65-92]
Fillastre 1993	100 mg PO tablet	European males	6	27 ± 9	69 ± 5
Marcellin 2001	100 mg PO solution	European males (33%) and females	12	50 ± 8.9	71.2 ± 9.9
Srichaiya 2008	100 mg PO IR tablet	Southeast Asian males	24	20.5 ± 1.3	62.5 ± 7.4
van Luin 2009	100 mg PO IR tablet	European males	24	34 [20-52]	79 [63-94]
Hermann 2003	200 mg PO IR tablet	White (60%) American males	15	28 ± 8	72.7 ± 9
Incecayir 2007	200 mg IR chewable/dispersible tablet	European males (64%) and females	14	23 ± 2	65.1[b]
Wootton 1997	200 mg PO IR tablet	European males (55%) and females	11	46 [35-57][b]	73.6[b]
Depot 1990	300 mg PO capsule	White American males	8	28.5 [20-37][b]	77.7 ± 9.7

The adult population model was derived from the green-shaded studies. [a]Mean ± SD reported, or range in square brackets if SD not reported. [b]Mean not reported in study, therefore the median, an average of the range, or BMI of approximately 23 kg/m^2 was used instead.

In vitro dissolution studies with lamotrigine IR tablets provided different dissolution half-times, ranging from 0.7-6.6 minutes when described with a Weibull function (380-382). Initially, dissolution half-time was set to 10 minutes. Given the variability in the observed Tmax likely due to the dissolution of the drug limited by low solubility, half-times were optimized individually per dosage. Optimization of specific intestinal permeability and the individual dissolution half-times was carried out using a Monte Carlo approach to explore the parameter space. The results are shown in **Table 3**. Dissolution half-time was not a value of interest for pediatric extrapolation since lamotrigine in breastmilk is in solution. This exercise was primarily a means to estimate intestinal permeability which is important in this pediatric context. Intestinal permeability was optimized to be very high and therefore is not rate limiting absorption. This is in line with its BCS II status.

A comparison of the observed PK from each study and the estimated plasma concentration vs. time profile following optimization are presented for each PK study in **Figures 2–16**. The aggregated results of the fits using the oral datasets are depicted in **Figure 17** as model-fitted concentrations compared to observed concentrations. Calculated average fold error (AFE) was 0.95 and absolute AFE (AAFE) was 1.27 demonstrating almost no bias and good precision.

(2.4) Oral Model Evaluation

The optimized oral model was then evaluated for predicting multiple-dose and steady state kinetics using the observed data from the studies presented in **Table 5**. Model performance for the evaluation is presented in **Figures 18-20**. The evaluation produced acceptable AFE and AAFE values of 1.04 and 1.13, respectively.

Table 5. Pharmacokinetic datasets for lamotrigine oral model validation

Study	Dose and administration	Cohort	N	Age (years)[a]	Weight (kg)[a]
Jann 2006	50 mg PO daily for 10 days	American males (86%) and females	14	24.4 ± 2.4	78.9 ± 11.1
Gastrup 2016	100 mg PO daily for 8 days	European males	10	25[b] [22-32]	NR
Theis 2005	200 mg PO daily for 18 days	White (87%) European males	13	[19-54]	[80.2-83.2]

[a]Mean ± SD reported, or range in square brackets if SD not reported. [b]Reported as a median.

(2.5) Population Model

To assess the ability of the model to reproduce PK variability following oral administration, adult virtual populations (n=100) were created. These virtual populations were built based on the sex, age and weight distributions of each clinical study used to evaluate PK variability as presented in **Table 4**. The studies were selected due to an adequate reporting of PK variability in plasma concentration-time profiles. Variability was incorporated based on anatomical and physiological differences between people for relevant model parameters in the software. The exception was any user-defined proteins including UGT1A4 and UGT1A3. The reference concentration of these enzymes was modelled as a log normal distribution with mean of 1 and a standard distribution of 1.6, based on assessment outlined below. The results of the population simulations are shown in **Figures 21–24** and demonstrate that overall, variability was well captured.

(2.6) Scale Adult Model to Infants

The oral adult model was scaled to children to predict breastfed infant exposure to lamotrigine from mothers taking the medication at steady state. All drug-specific inputs were kept the same as in the adult model. The anatomy and physiology were scaled to that of neonates at different ages. Growth and maturation of different processes (metabolic capacity, glomerular filtration rate, protein binding, body composition, and transporter expression) were accounted for, and realistic variability around anatomy and physiology were applied to give a virtual infant population.

The ontogeny profiles of UGT1A4 and UGT1A3 were modeled after in vitro studies by Badée, Qiu (216) and Miyagi and Collier (217). Enzyme activity levels were normalized to the adult activity and used to fit a Hill and linear function, for UGT1A4 and UGT1A3, respectively.

For UGT1A4, the Hill function was described by the following parameters: $A = kPMA^n/(PMA^n + A_{0.5}^n)$, where A is normalized enzyme activity, PMA is postmenstrual age, k is the vertical transformation factor, n is the Hill coefficient, and $A_{0.5}$ is postmenstrual age at 50% activity. To perform the Hill function fitting, an L1 regression method was used to minimize the sum of absolute error. To assess variability in activity, a virtual population (n=5,000) was created across postmenstrual ages (>0 to 77 years old) and following a mean calculation of A from the Hill function, a geometric standard deviation was applied to capture the observed variability. Variability was not found to be age dependent and was set at a standard deviation of 1.6. For UGT1A3, activity was not-age dependent. A population of size 5,000 was similarly created and A was given a geometric mean of 1 and geometric SD of 1.6. The final ontogeny profiles used and their associate variability along with the observed data are presented in **Figures 25 and 26**.

To verify the infant PBPK models, a population of children 1-6 years old were simulated with a single oral administration of 2 mg/kg. The results were compared with observed data from Vauzelle-KervroËDan, Rey (225) (capsules) and Chen, Casale (226) (chewable tablets) and are shown in **Table 6** and **Figure 27**. Half-life was well predicted suggesting that the clearance to volume of distribution ratio was reasonable. AUC_{0-48} predicted by the pediatric PBPK model (38.5 ± 11 µg·h/mL) was greater than the two studies (25.4 ± 6.8 and 27.4 ± 7.2 µg·h/mL). Based on a T_{max} that may have been underpredicted, although not consistently, the absorption profile of the formulation seemed not to follow that in adults. The dose in breast milk is fully dissolved and therefore formulation effects are inconsequential. Based on a reasonable clearance to volume ratio and thus systemic PK, the pediatric PBPK model was deemed reasonable for use in the breastfeeding workflow.

Table 6. Infant PBPK model evaluation with mean infant data

Study	N	Age (years)	T_{max} (hrs)	C_{max} (µg/mL)	AUC_{0-48} (µg·h/mL)	$T_{1/2}$ (hrs)
Vauzelle-Kervroeden 1996	10	2.5 ± 1.4	6*	1.11 ± 0.29	25.4 ± 6.8	21.9 ± 6.8
Patient 28	1	1.17	1.55	1.55	30.4	36.5
Patient 31	1	6	0.93	0.93	20.9	20.3
Chen 1999	4	[3.8-5.9]	4.5 ± 5.1	1.1 ± 0.37	27.4 ± 7.2	30.5 ± 5.6
This study	100	3.4 ± 1.5	1.35 ± 0.47	1.91 ± 0.36	38.5 ± 11	21.6 ± 8.7

(3) Discussion and Conclusion

The final lamotrigine PBPK model adequately describes the PK of lamotrigine in adults and children. The optimized IV and oral adult models produced AFE and AAFE values of 0.95 and 1.27, respectively. The oral models sufficiently predicted three pharmacokinetic datasets with adults administered multiple doses of lamotrigine. The evaluation produced acceptable AFE and AAFE values, 1.04 and 1.13, respectively. An evaluation of the pediatric PBPK model in children was supported by two small studies. Although there were no published studies in infants <1 years old, the results from this evaluation in 1-6 year olds provided insight on the model's performance. Essentially, while the absorption kinetics appeared to be a function of the formulations used, the systemic PK appeared well estimated. As such, the pediatric model was deemed reasonable for use in the breastfeeding workflow.

(4) Figures

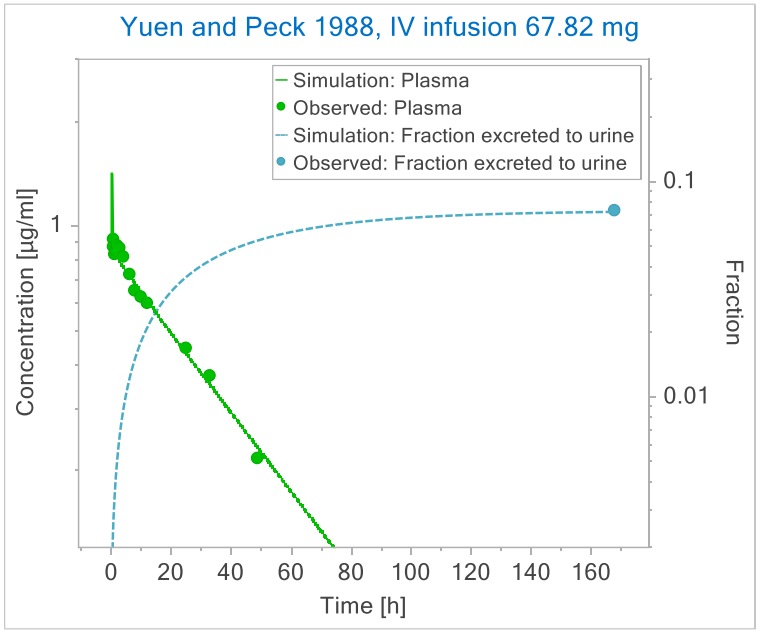


Figure 1. IV model optimization using Yuen & Peck 1988, 67.82 mg infusion dataset.

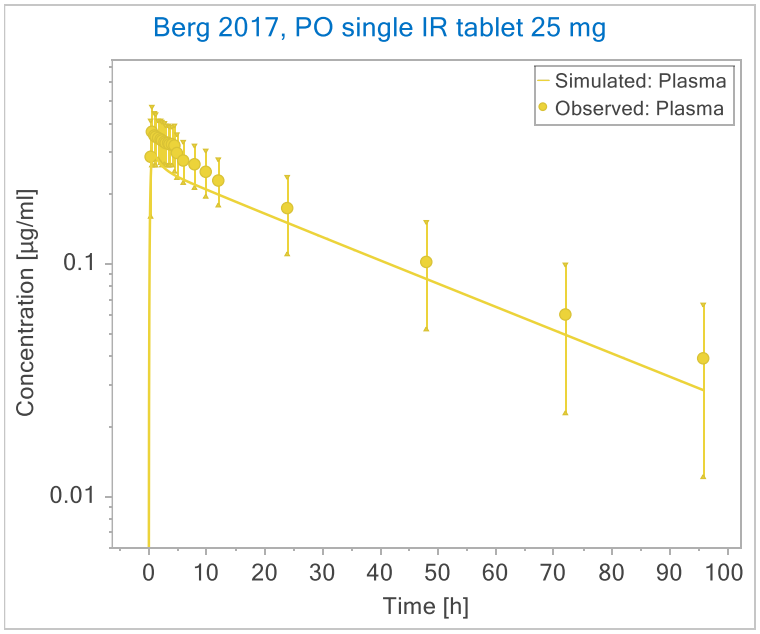


Figure 2. Oral model optimization using Berg 2017, 25 mg IR formulation single dose dataset.

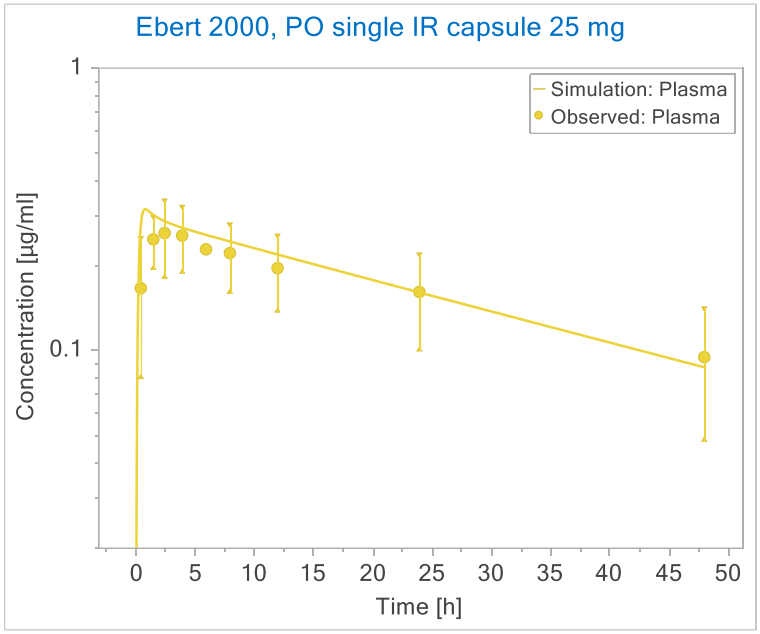


Figure 3. Oral model optimization using Ebert 2000, 25 mg IR formulation single dose dataset.

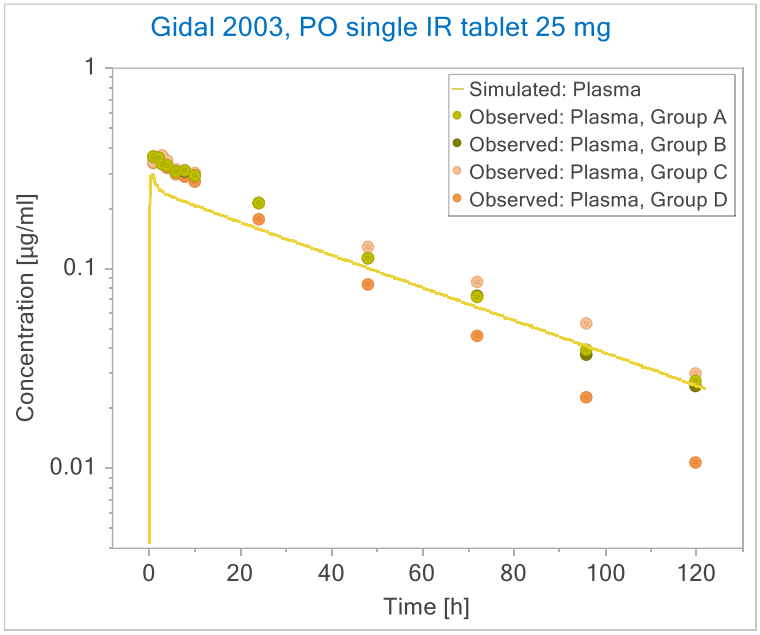


Figure 4. Oral model optimization using Gidal 2003, 25 mg IR formulation single dose dataset.

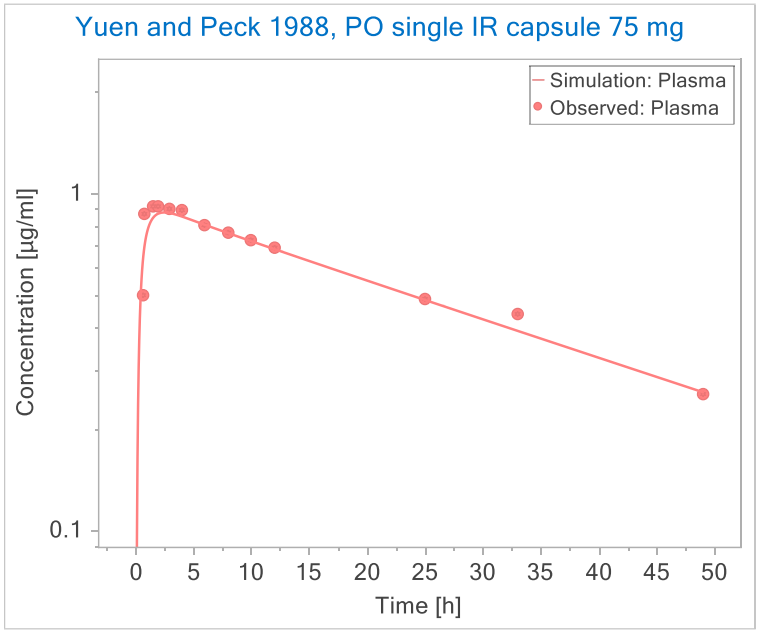


Figure 5. Oral model optimization using Yuen and Peck 1988, 75 mg IR formulation single dose dataset.

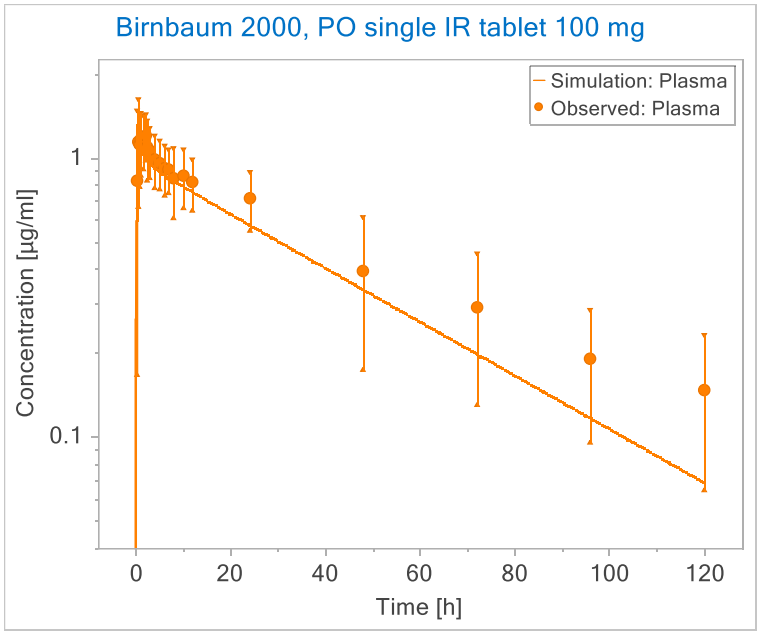


Figure 6. Oral model optimization using Birnbaum 2000, 100 mg IR formulation single dose dataset.

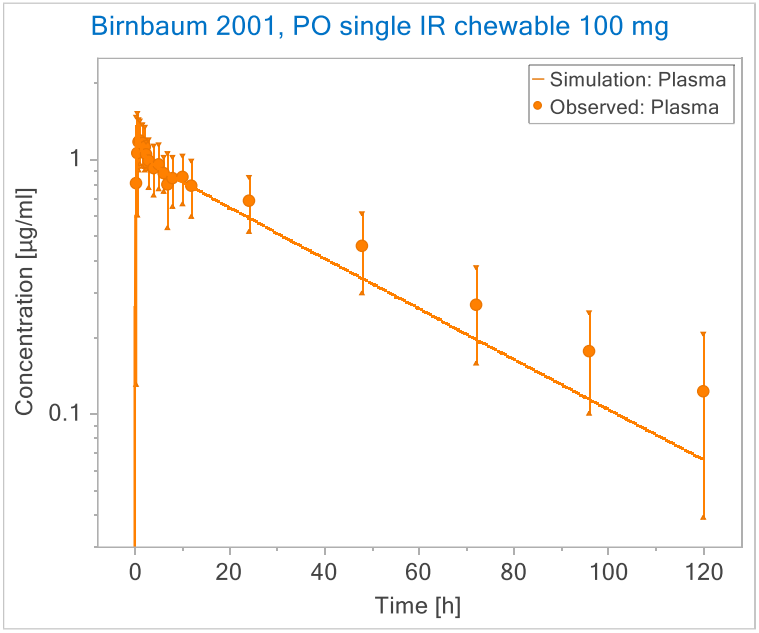


Figure 7. Oral model optimization using Birnbaum 2001, 100 mg IR formulation single dose dataset.

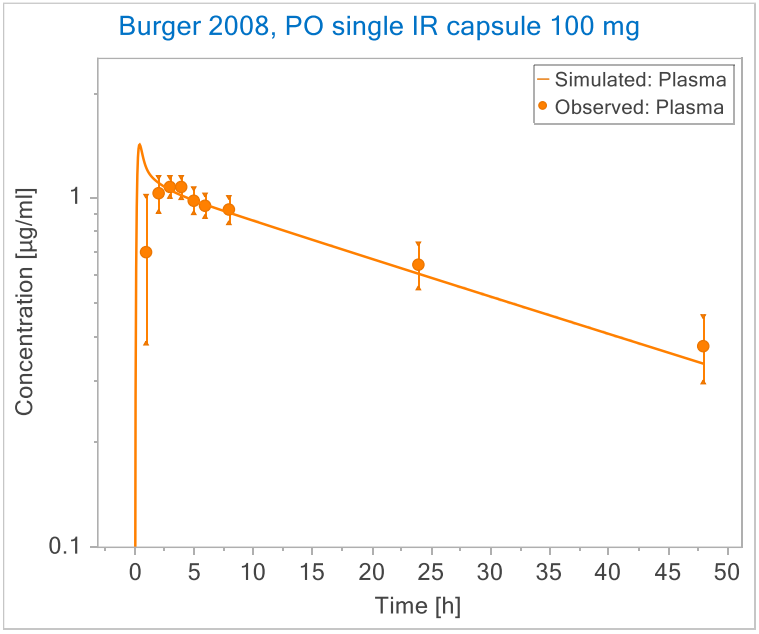


Figure 8. Oral model optimization using Burger 2008, 100 mg IR formulation single dose dataset.

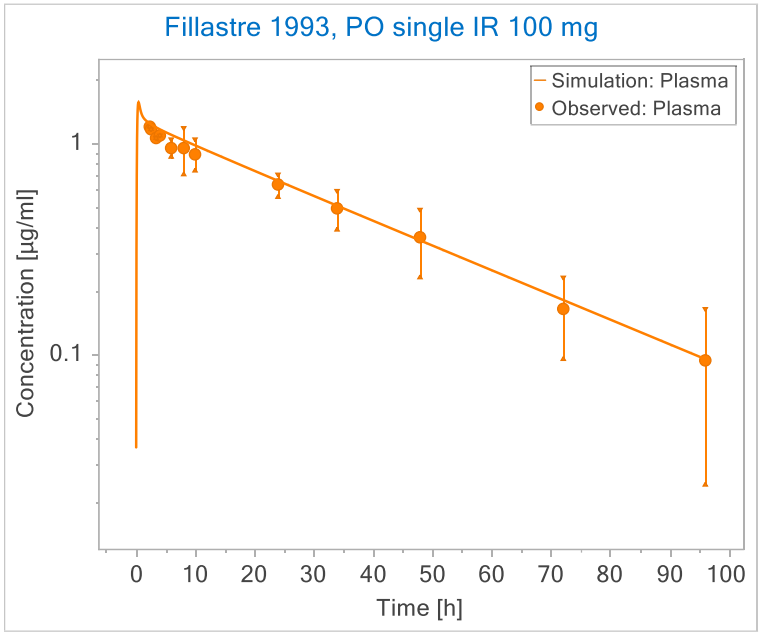


Figure 9. Oral model optimization using Fillastre 1993, 100 mg IR formulation single dose dataset.

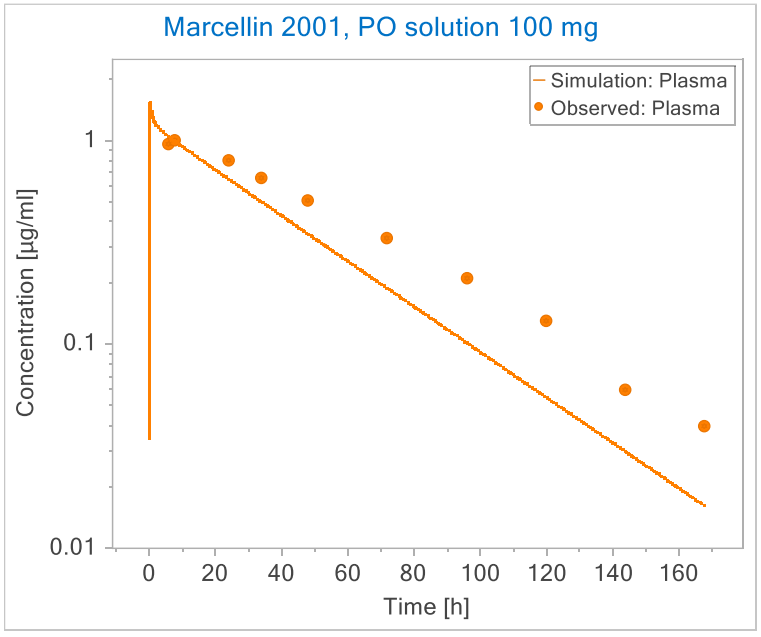


Figure 10. Oral model optimization using Marcellin 2001, 100 mg IR formulation single dose dataset.

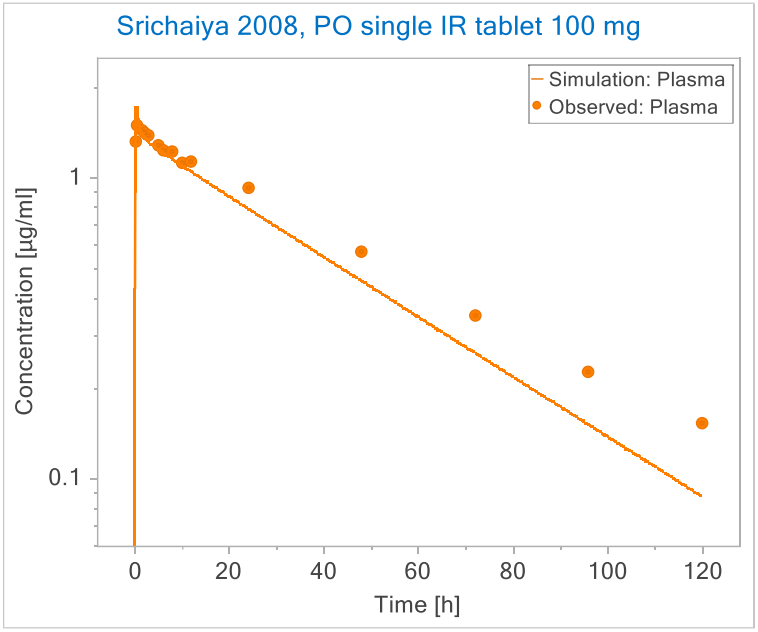


Figure 11. Oral model optimization using Srichaiya 2008, 100 mg IR formulation single dose dataset.

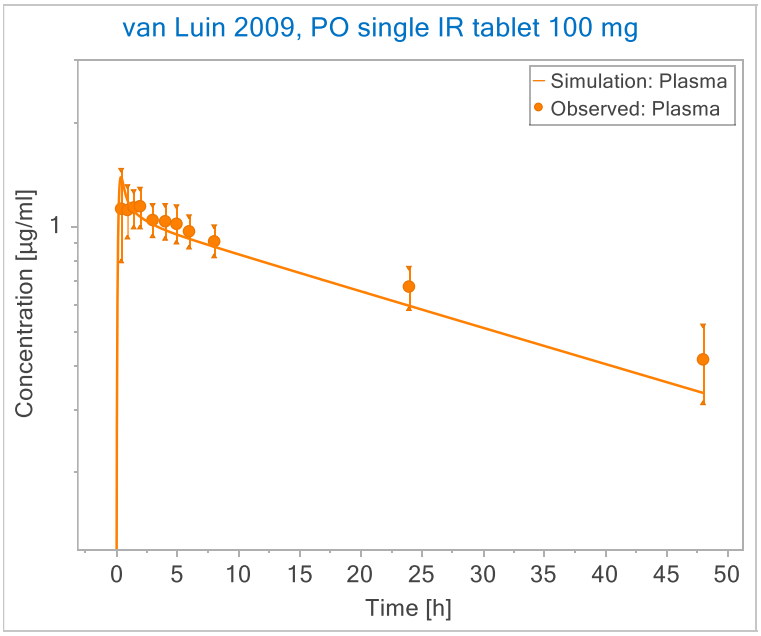


Figure 12. Oral model optimization using van Luin 2009, 100 mg IR formulation single dose dataset.

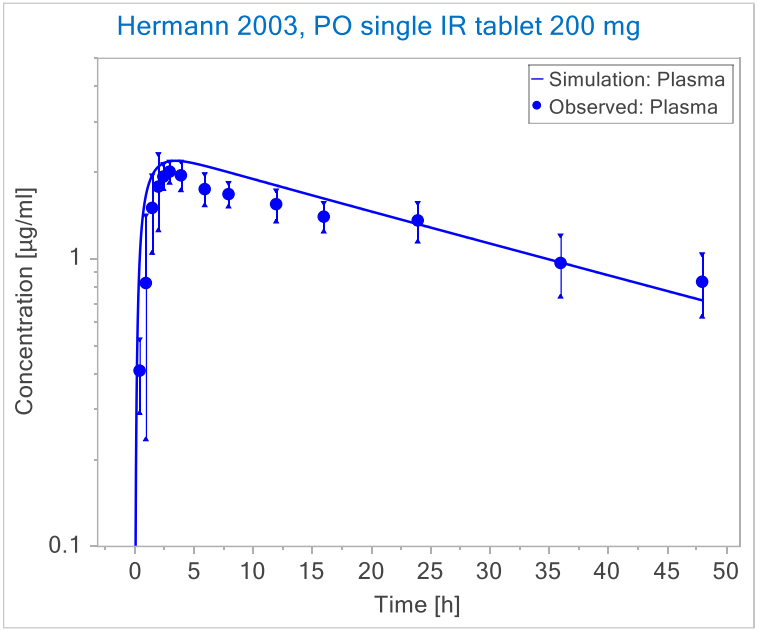


Figure 13. Oral model optimization using Hermann 2003, 200 mg IR formulation single dose dataset.

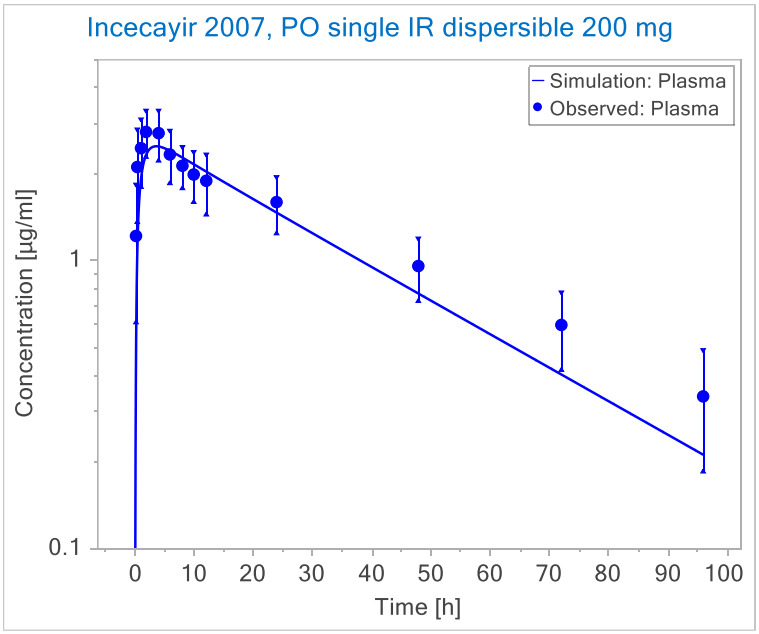


Figure 14. Oral model optimization using Incecayir 2007, 200 mg IR formulation single dose dataset.

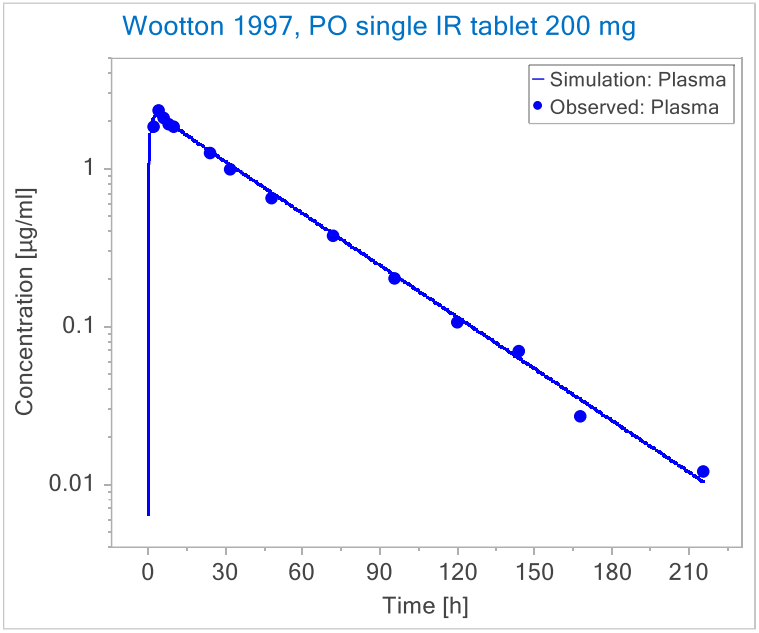


Figure 15. Oral model optimization using Wootton 1997, 200 mg IR formulation single dose dataset.

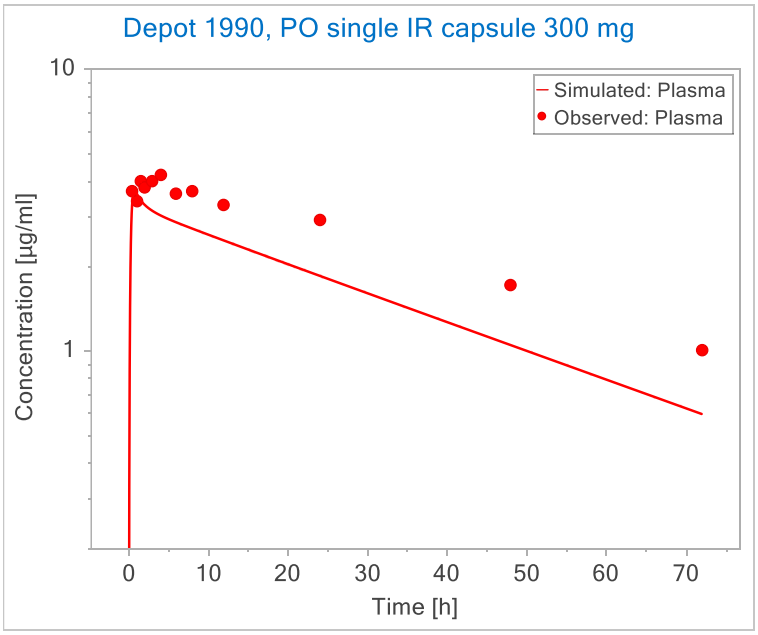


Figure 16. Oral model optimization using Depot 1990, 300 mg IR formulation single dose dataset.

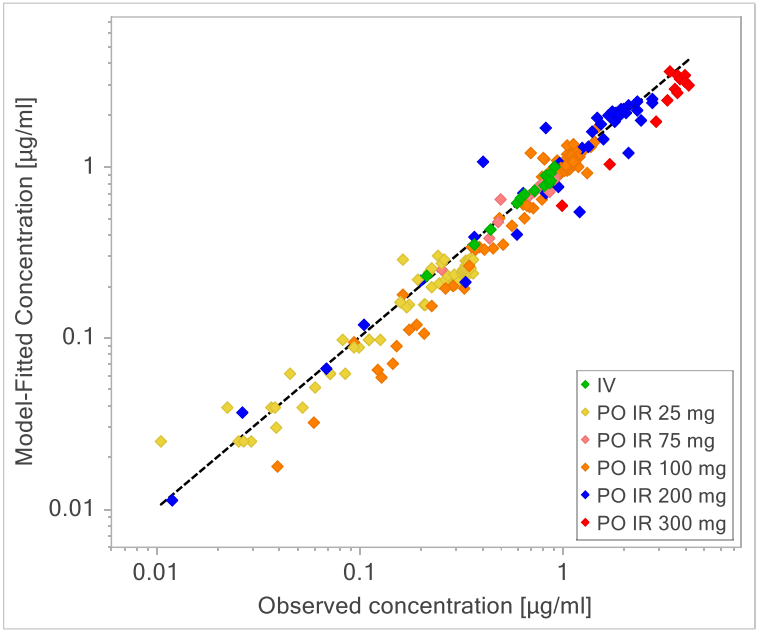


Figure 17. Model-fitted vs observed concentrations of all model-building PO datasets. Dashed line represents the line of identity. Calculated average fold error (AFE) was 0.95 and absolute AFE was 1.27.

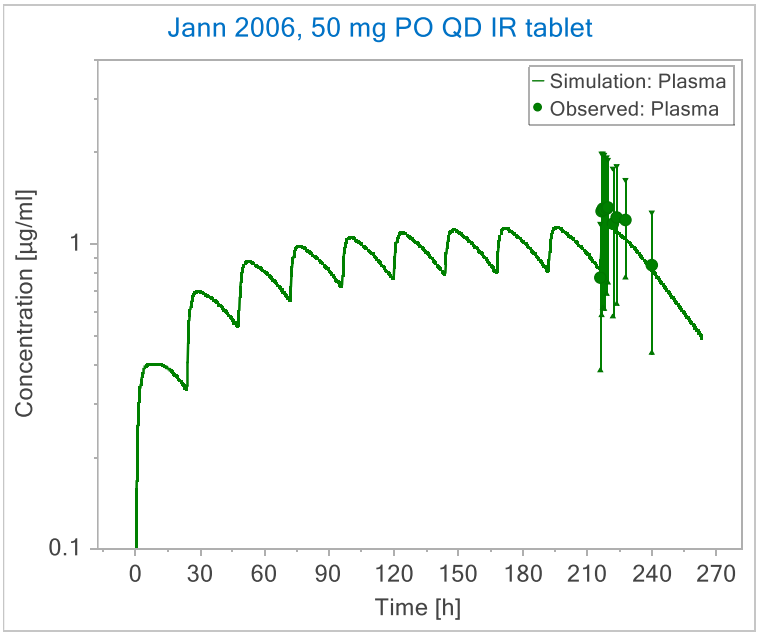


Figure 18. Simulation for model verification. Observed data reported as mean (circles) with standard deviation (error bars).

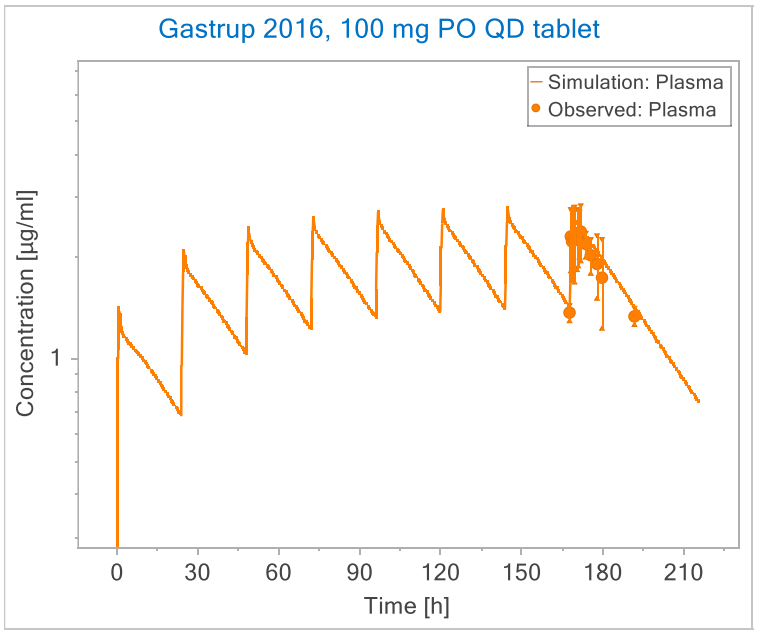


Figure 19. Simulation for model verification. Observed data reported as mean (circles) with standard deviation (error bars).

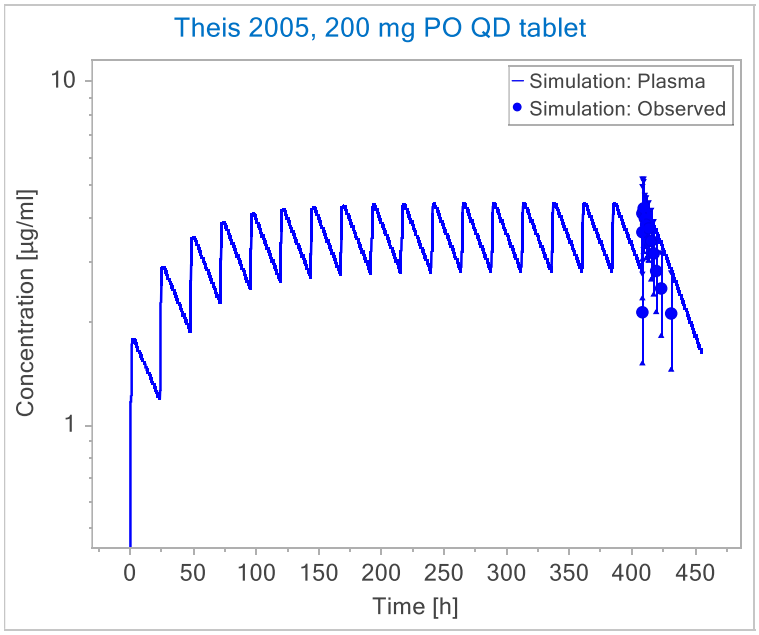


Figure 20. Simulation for model verification. Observed data reported as mean (circles) with standard deviation (error bars).

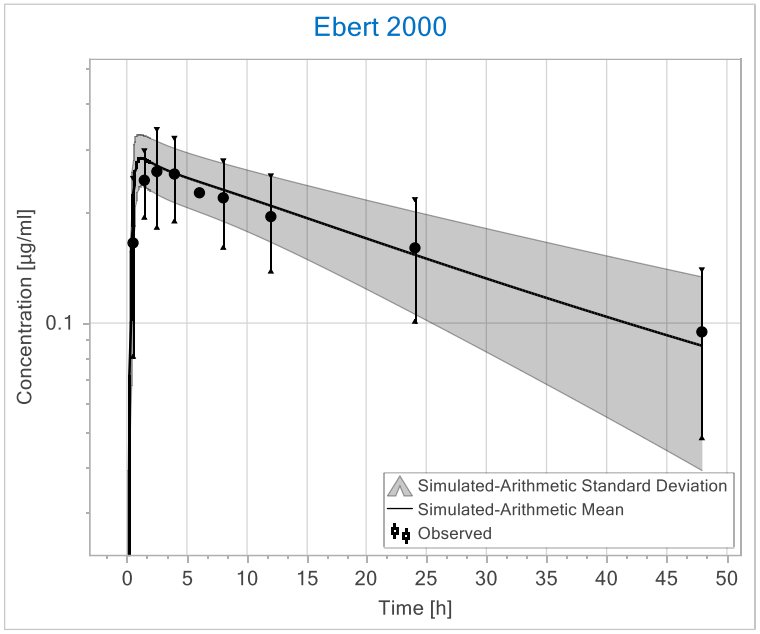


Figure 21. Adult population PBPK simulation (line = mean; gray shaded area = 90th prediction interval) compared to observed data from Ebert 2000.

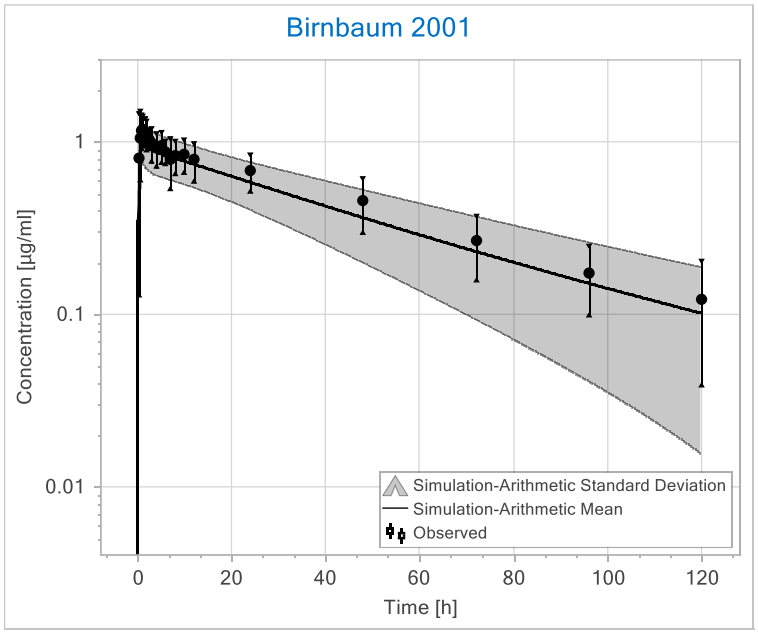


Figure 22. Adult population PBPK simulation (line = mean; gray shaded area = 90th prediction interval) compared to observed data from Birnbaum 2001.

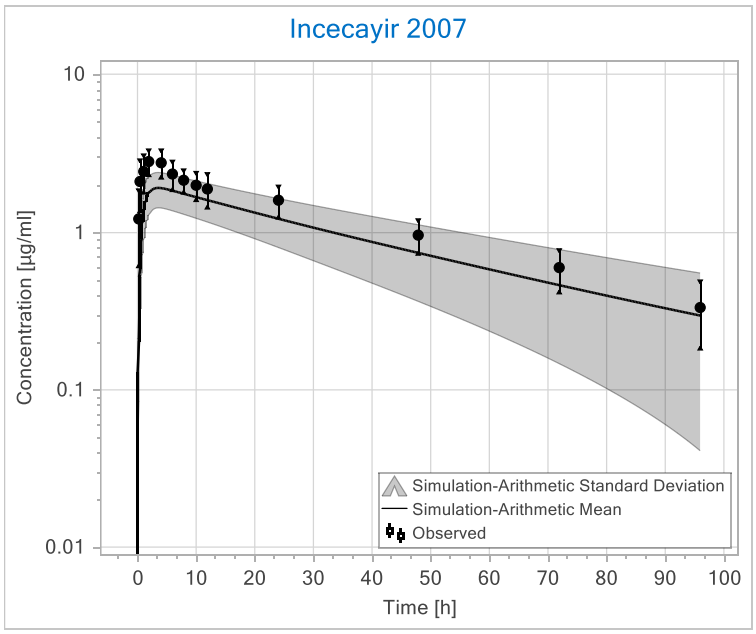


Figure 23. Adult population PBPK simulation (line = mean; gray shaded area = 90th prediction interval) compared to observed data from Incecayir 2007.

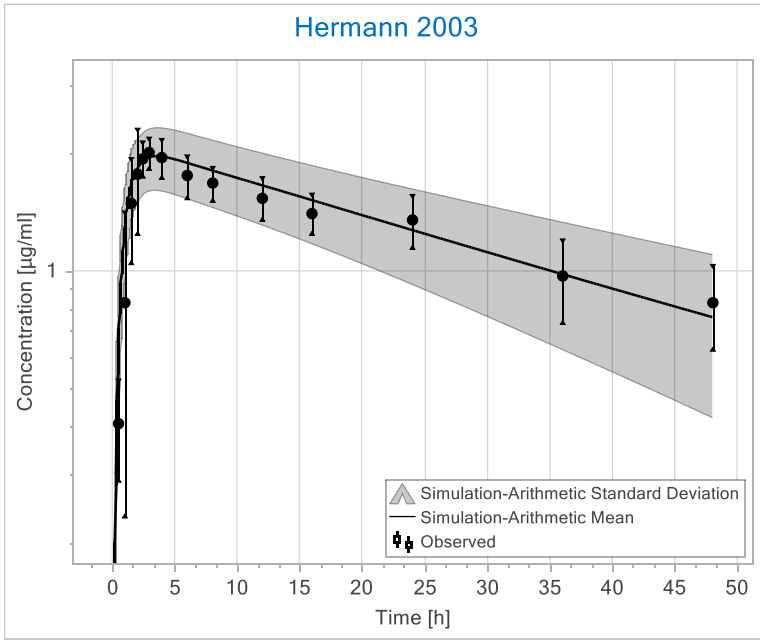


Figure 24. Adult population PBPK simulation (line = mean; gray shaded area = 90th prediction interval) compared to observed data from Hermann 2003.

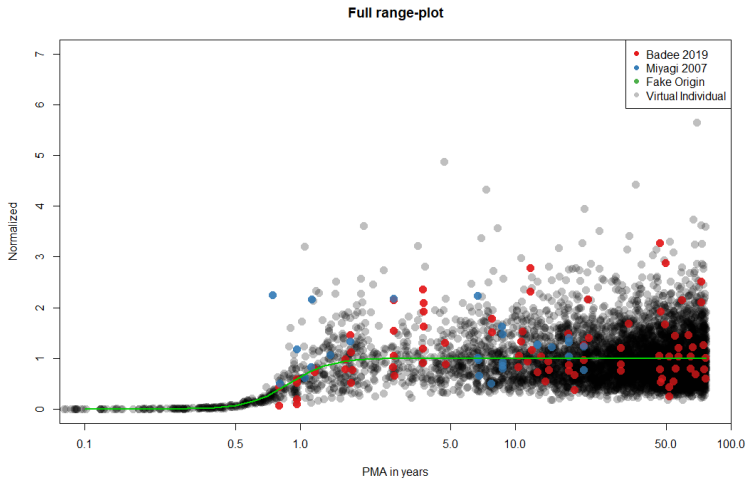


Figure 25. Ontogeny profile for UGT1A4 activity normalized to the adult value and described by a Hill function with the following parameters, mean ± SD: k = 1 ± 0.5 (lognormal), n = 4.54 ± 1.2 (lognormal), and A0.5 = 0.89 ± 0.05 (normal). PMA: postmenstrual age in years.

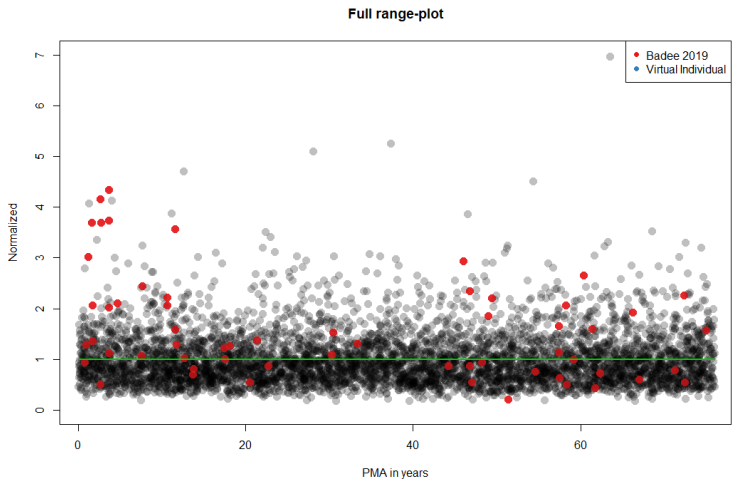


Figure 26. Ontogeny profile for UGT1A3 activity described by a linear function and not age-dependent. PMA: postmenstrual age in years.

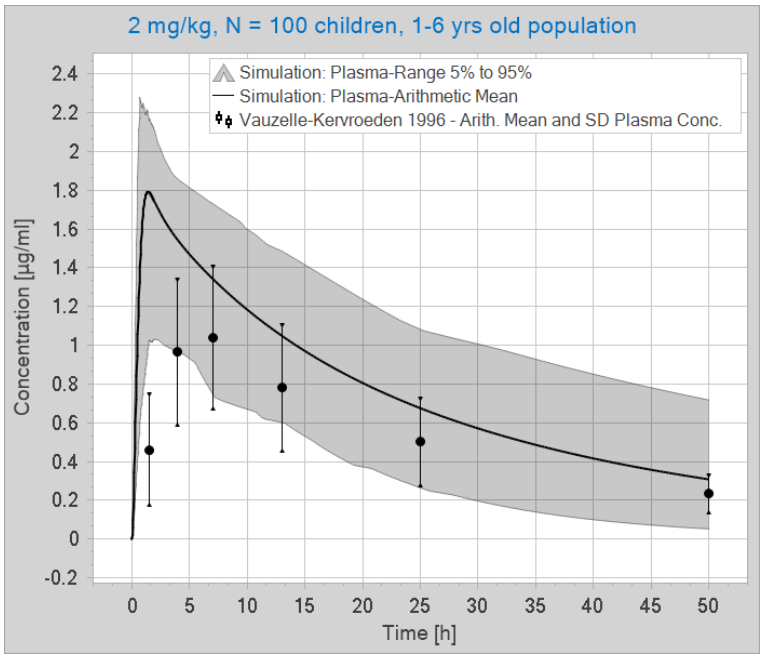
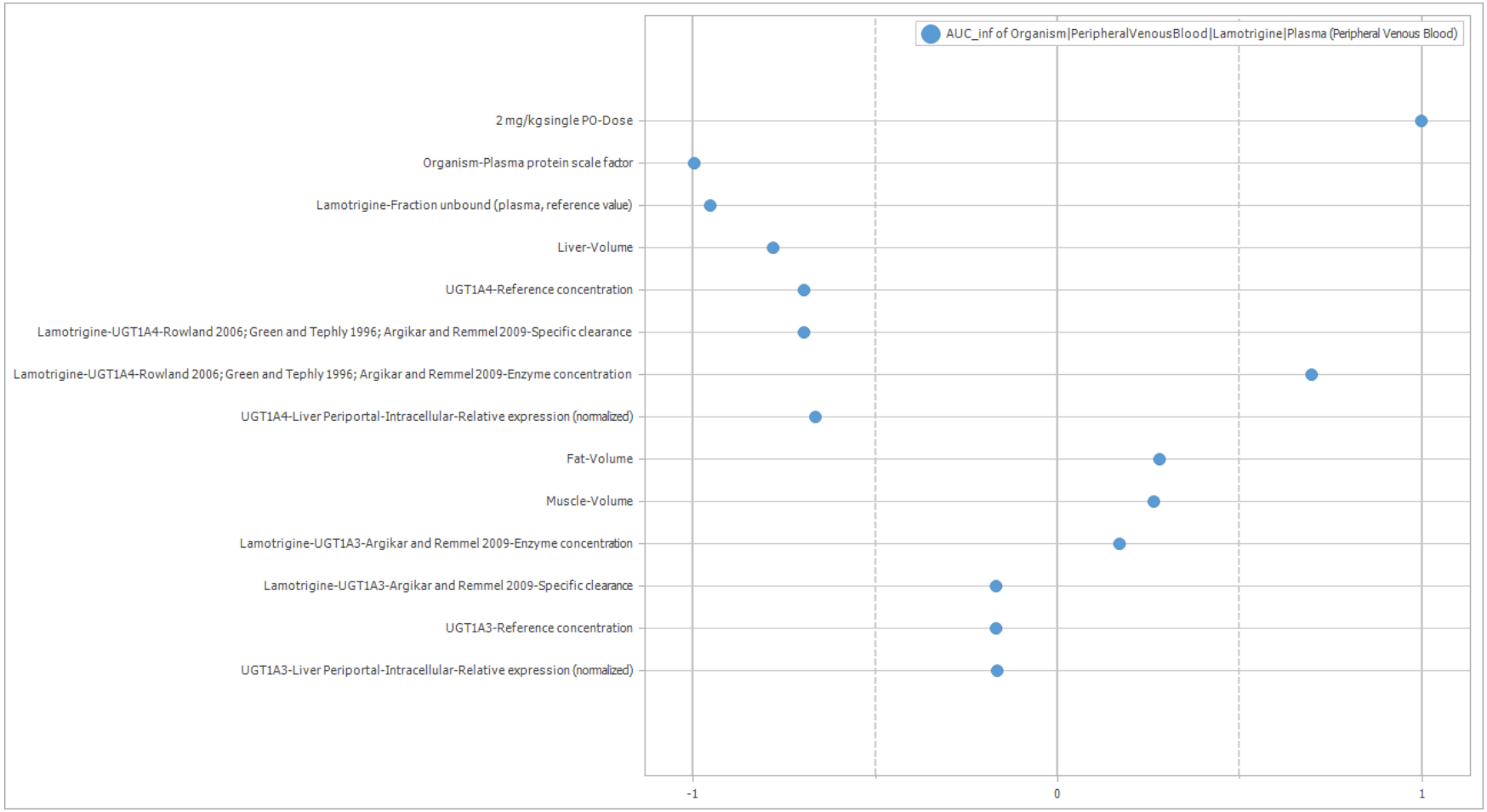


Figure 27. Child population PBPK simulation (line = mean; gray shaded area = 90th prediction interval) compared with the Vauzelle-Kervroeden 1996 dataset (2 mg/kg, N = 10, 2.5 ± 1.4 yrs old) for model verification.

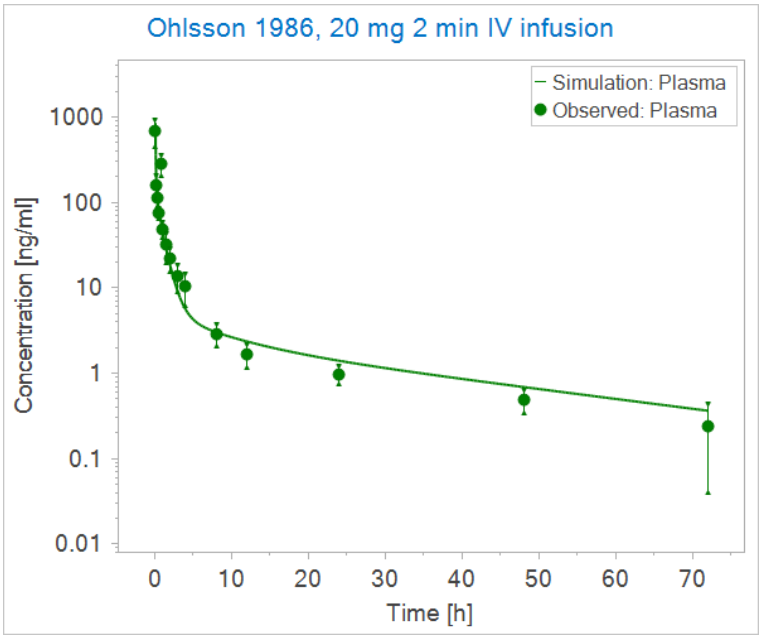
2. Sensitivity Analysis of Lamotrigine PBPK Model Predicted AUC_{∞}



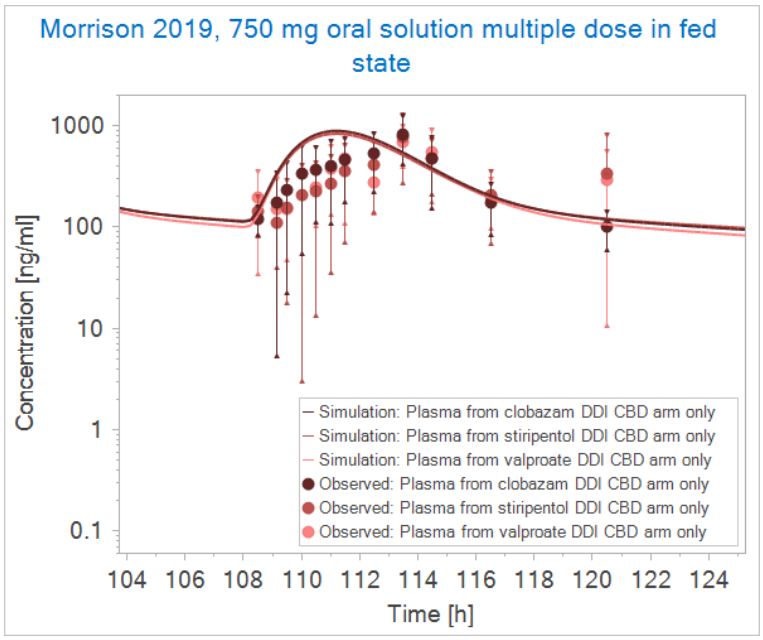
Supplementary Figure 1. Presented are results of the sensitivity analysis in 1 month old infant. Similar results were found in 3 day old, 7 day old, and 2 month olds infants.

Appendix C

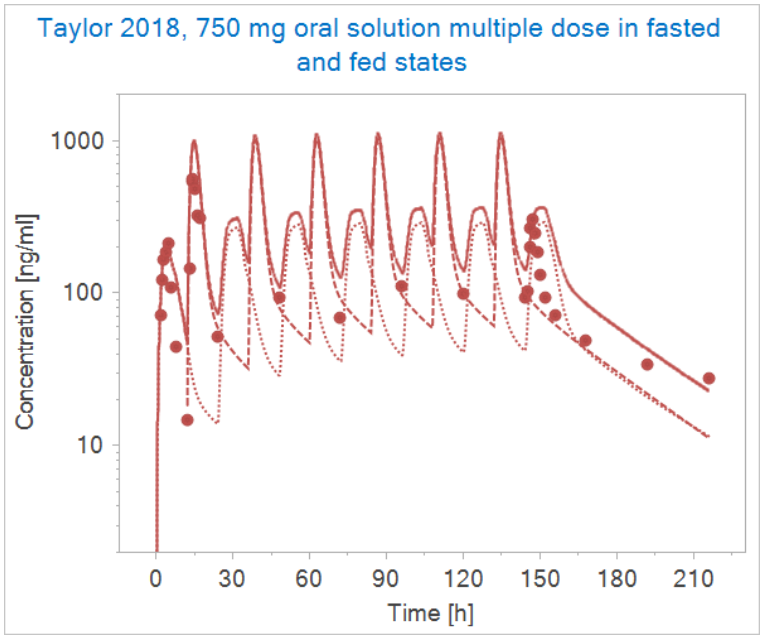
Supplementary Material for Chapter 4



Supplementary Figure 2. IV model optimization using Ohlsson, Lindgren (266), 20 mg IV infusion dataset.

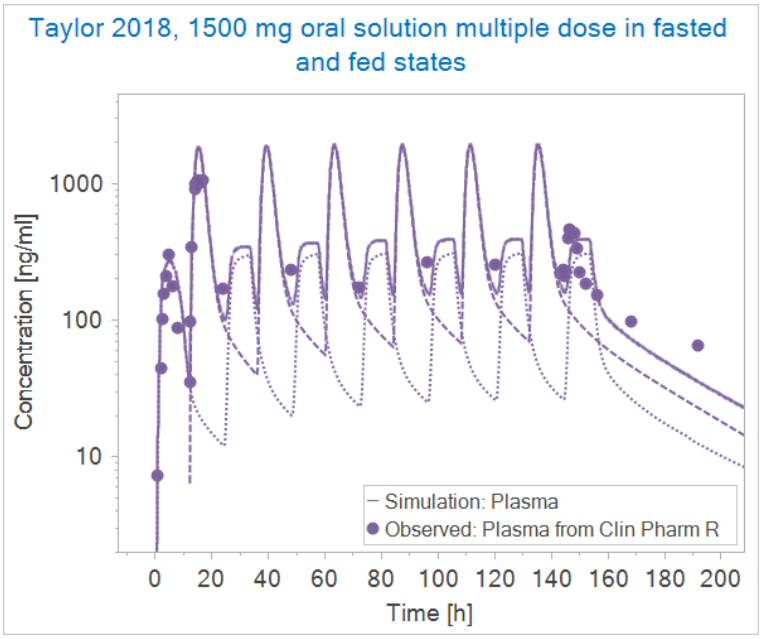


Supplementary Figure 3. Oral fed state model evaluation using Morrison, Crockett (271), 750 mg multiple dose datasets.

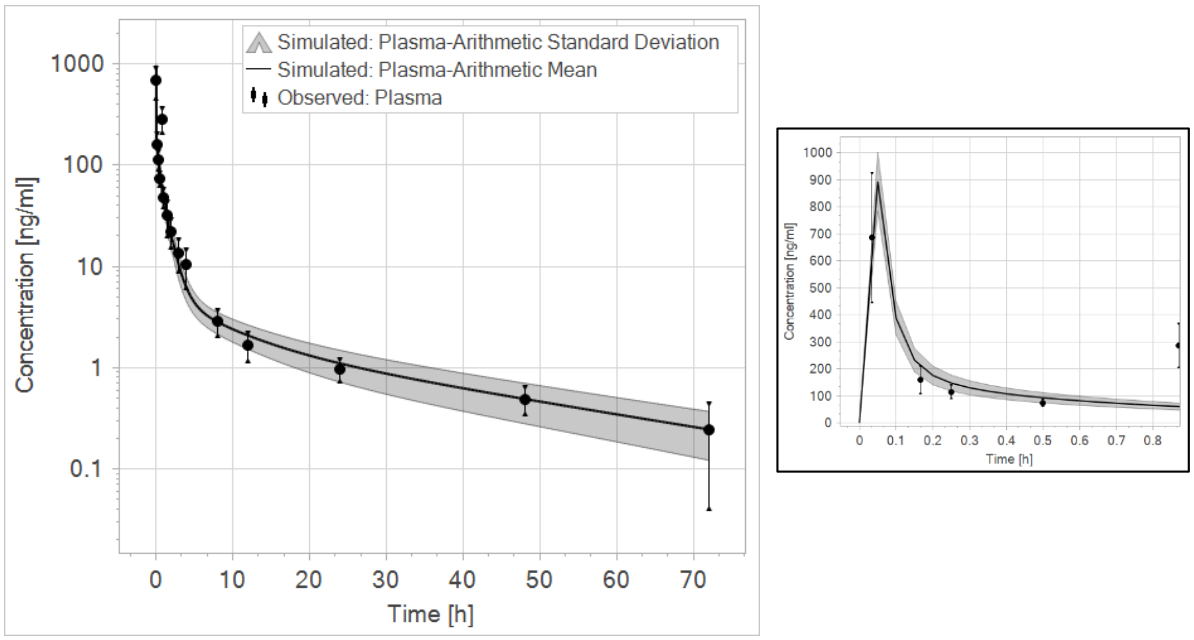


Supplementary Figure 4. Oral fasted and fed state models evaluation using Taylor, Gidal (241), 750 mg multiple dose dataset. The solid line represents simulated CBD plasma concentrations from administration in the fasted (morning) and fed (evening) states which reflect the dosing regimen in the

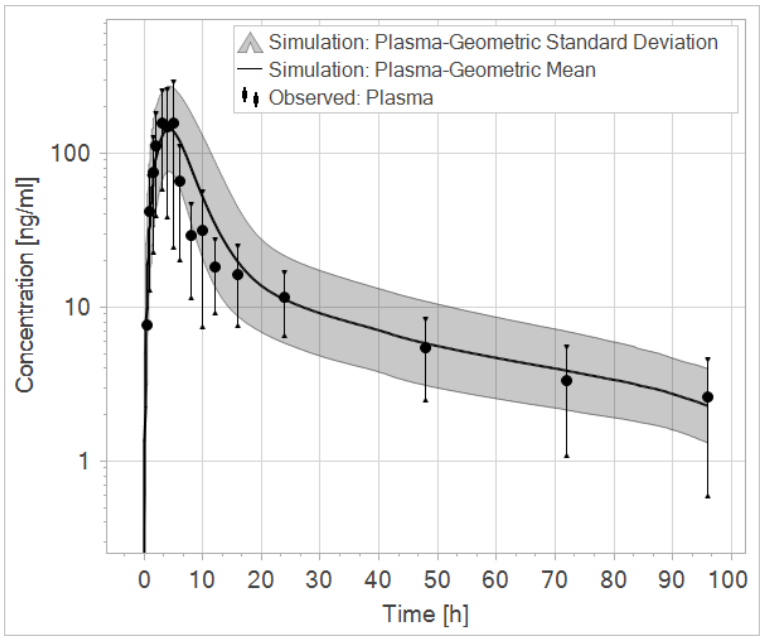
study. The dotted and dashed lines represent administration in only the fasted and fed states, respectively.



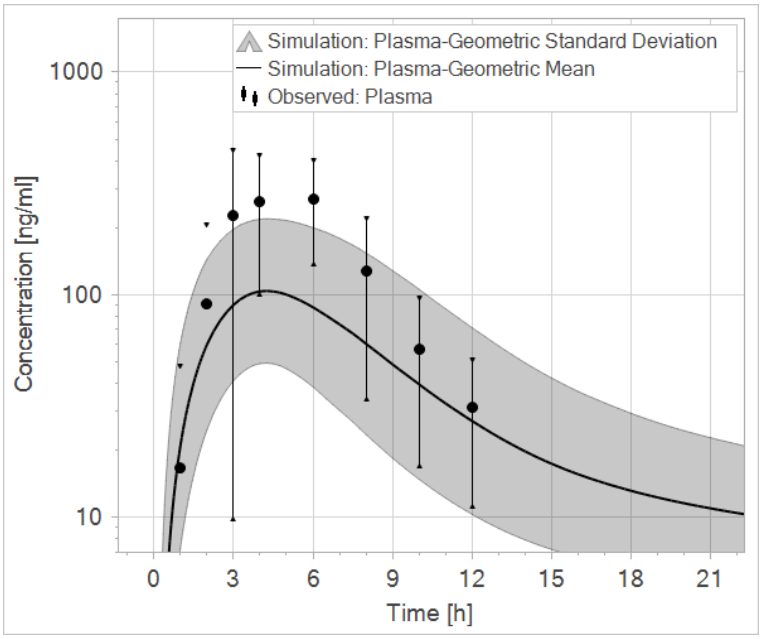
Supplementary Figure 5. Oral fasted and fed state models evaluation using Taylor, Gidal (241), 1500 mg multiple dose dataset. The solid line represents simulated CBD plasma concentrations from administration in the fasted (morning) and fed (evening) states which reflect the dosing regimen in the study. The dotted and dashed lines represent administration in only the fasted and fed states, respectively.



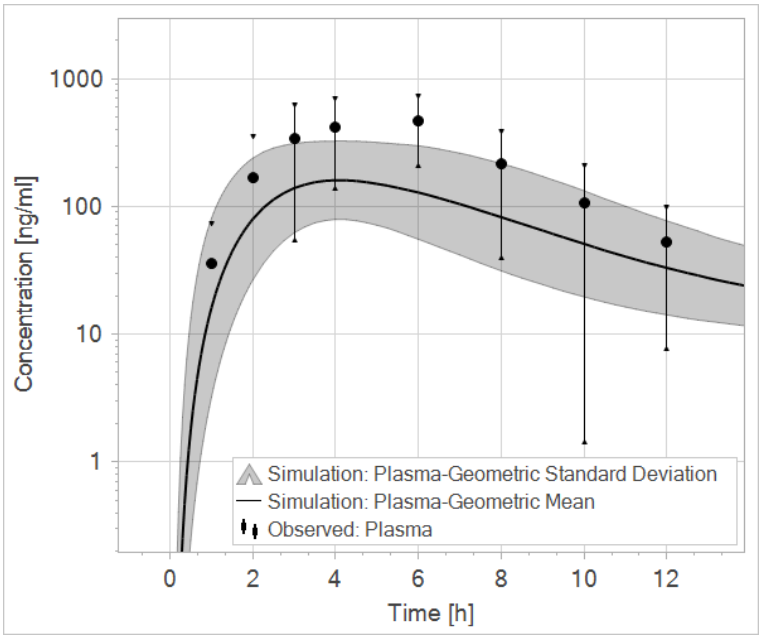
Supplementary Figure 6. Population simulation compared to observed data from Ohlsson, Lindgren (266), 20 mg IV infusion. Box shows close up of profile between 0 and 0.9 hours.



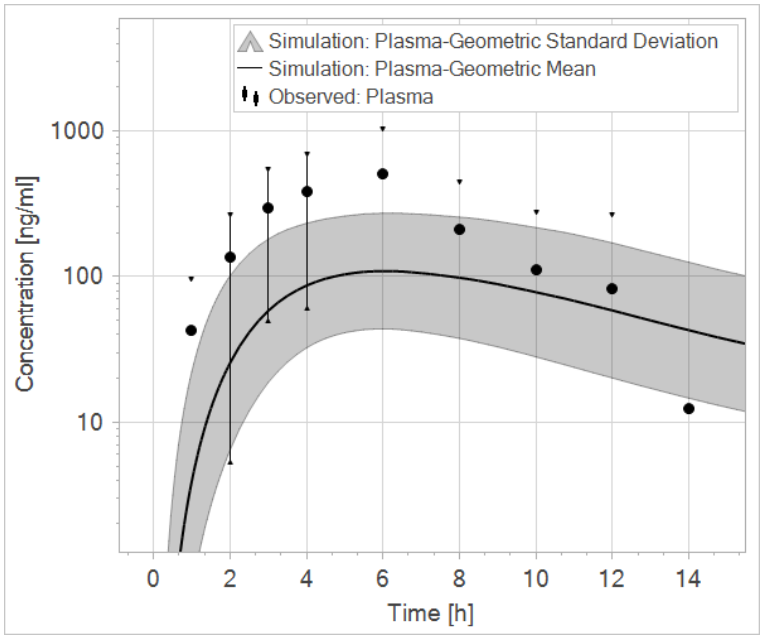
Supplementary Figure 7. Population simulation compared to observed data from Crockett, Critchley (268), 750 mg oral solution dataset.



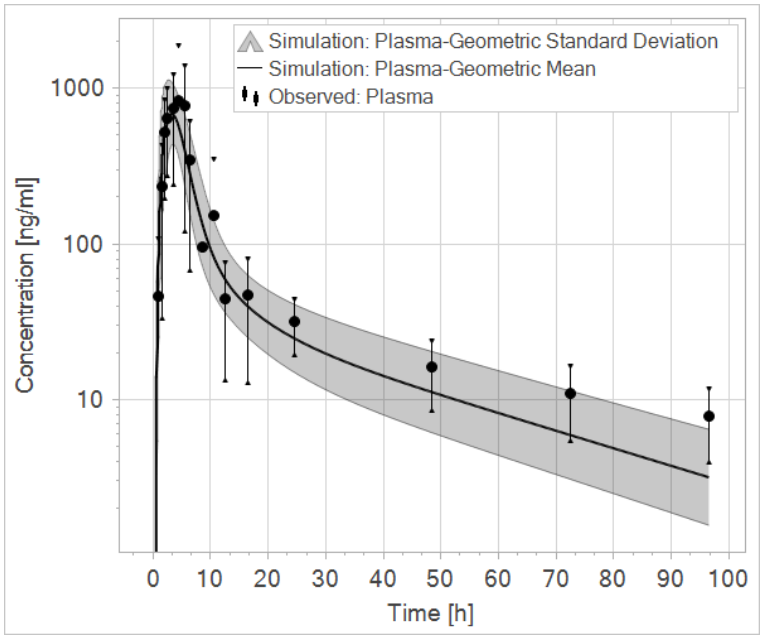
Supplementary Figure 8. Population simulation compared to observed data from Schoedel, Szeto (269), 750 mg oral solution dataset.



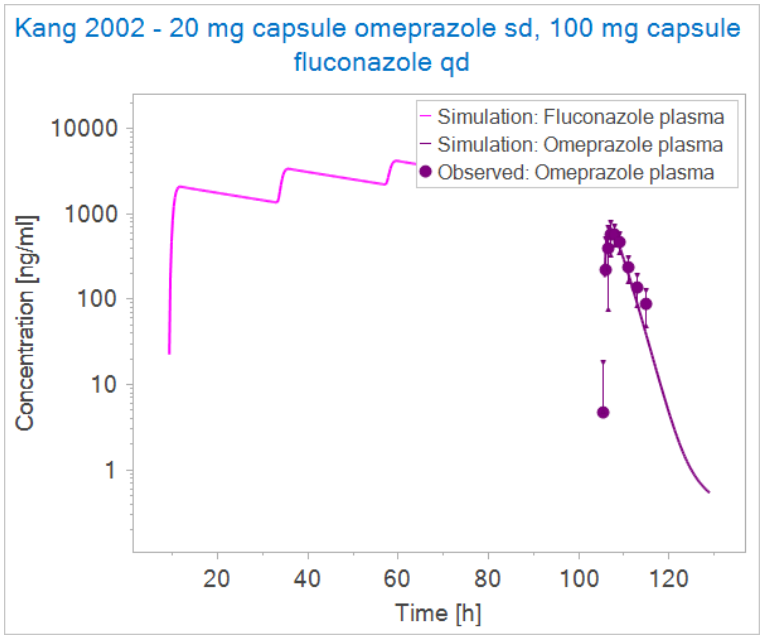
Supplementary Figure 9. Population simulation compared to observed data from Schoedel, Szeto (269), 1500 mg oral solution dataset.



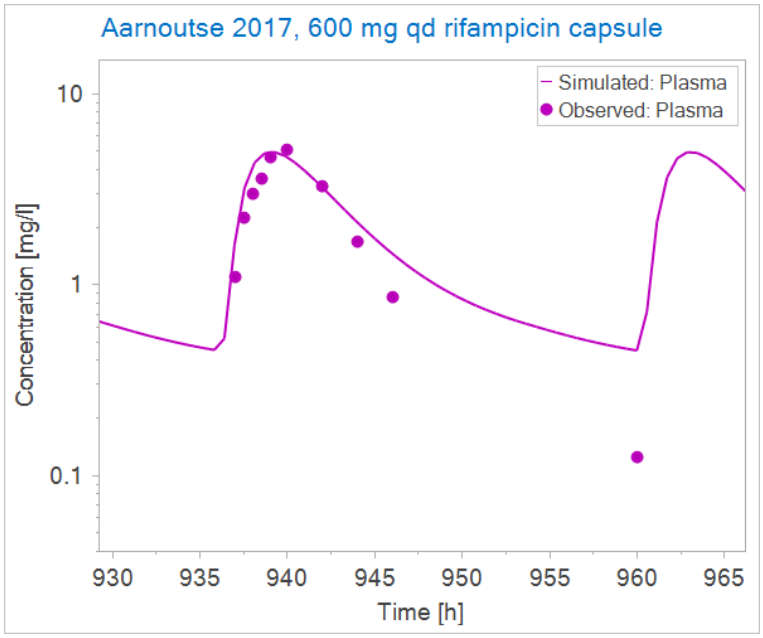
Supplementary Figure 10. Population simulation compared to observed data from Schoedel, Szeto (269), 4500 mg oral solution dataset.



Supplementary Figure 11. Population simulation compared to observed data from Crockett, Critchley (268), 750 mg oral solution in the fed state.



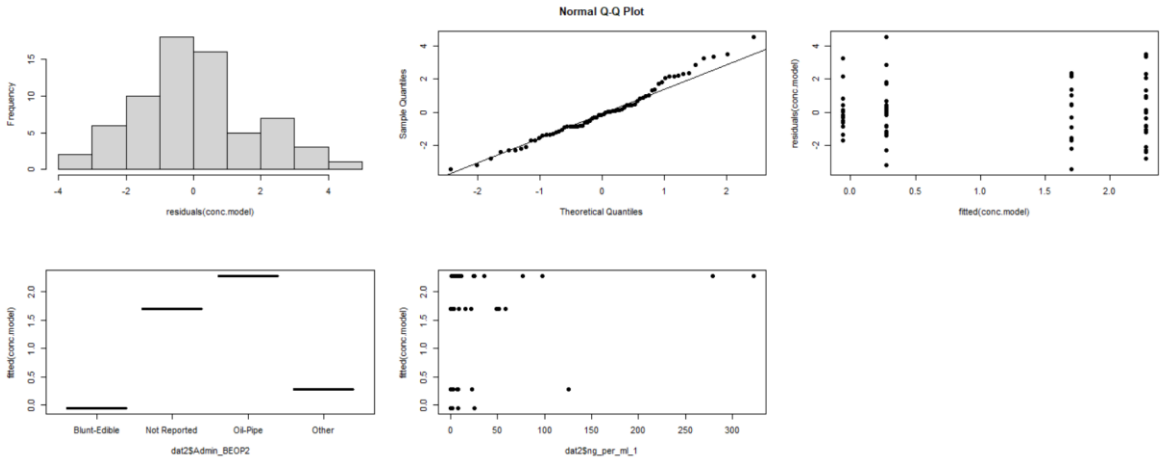
Supplementary Figure 12. Qualification of CYP2C19 inhibition effect of fluconazole in human using Kang, Yang (281). Simulated omeprazole plasma concentration-time profile resulting from the addition of CYP2C19 inhibition by co-administered fluconazole.



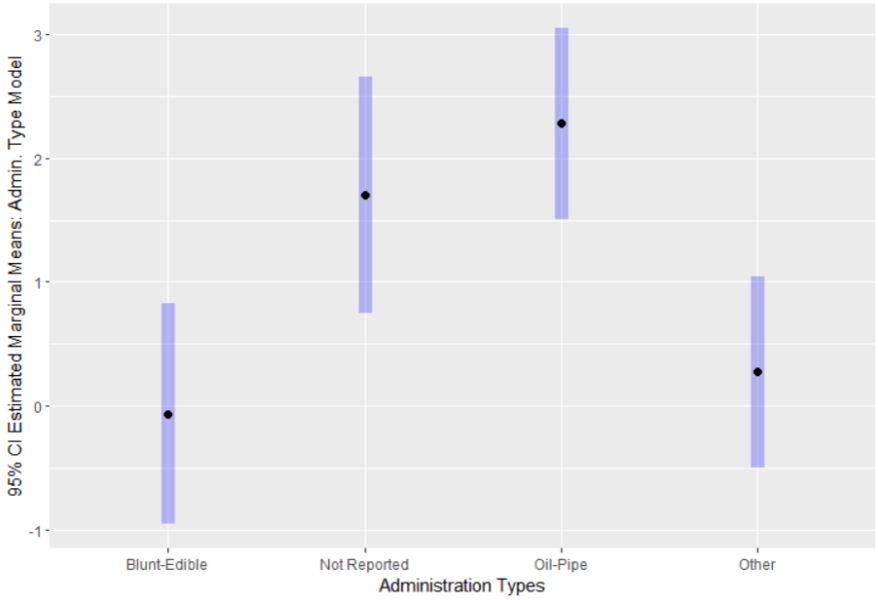
Supplementary Figure 13. Rifampicin PBPK model (257) capsule formulation evaluated using Aarnoutse, Kibiki (282) dataset.

Appendix D

Supplementary Material for Chapter 5



Supplementary Figure 14. Goodness-of-fit plots of the final fitted model with administration type for concentrations above the lower limit of quantification.



Supplementary Figure 15. Estimated marginal means and 95% confidence interval (CI) of log-concentration across administration type subgroups for concentrations above the lower limit of quantification.

Supplementary Table 3. Summary of the final fitted model with administration type for concentrations above the lower limit of quantification

Coefficient*	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-0.061	0.44	-0.137	0.89
Not Reported	1.76	0.65	2.70	0.0088
Oil or Pipe	2.34	0.59	3.97	0.00018
Other	0.34	0.59	0.57	0.57

*Reference category: Joint/Blunt or Edible

Appendix E

Supplementary Material for Chapter 7

1. Interview Guide

Upper Area Under the Curve Ratio (UAR) Guidance

There are several resources that healthcare providers such as yourself can use when addressing medication use in breastfeeding. These resources can include metrics: relative infant dose M/P ratio, relative infant dose (RID), Hale's L1-5 ratings, etc.; and online/book resources: LactMed, Hale's Medications and Mother's Milk, Briggs' Drugs in Pregnancy and Lactation, Clinical Pharmacology, etc.

We, at the Edginton Lab from the University of Waterloo (Ontario, Canada), have developed a novel metric to overcome some of the shortcomings of current resources. This metric is called the "Upper AUC Ratio" or the "UAR".

How does the UAR compare to existing metrics?

Accounts for...	M/P Ratio	RID	UAR
Dose to infant via milk	No	Yes	Yes
Comparison to maternal therapeutic dose	No	Yes	Yes
Oral bioavailability in infants	No	No	Yes
Age of infant	No	No	Yes
Most vulnerable children with lowest clearance	No	No	Yes
Most vulnerable children receiving highest dose	No	No	Yes
Active metabolites	No	No	Yes
Systemic exposure of infants	No	No	Yes
Factors that may lead to high dose to infant (e.g., maternal pharmacogenotype)	No	No	Yes

What does the UAR look like?

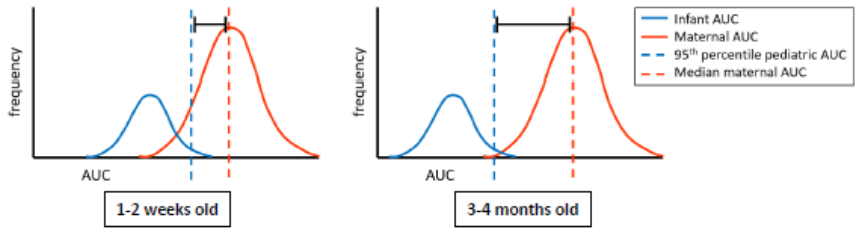
To compare the predicted breastfed infant exposure estimates with adult therapeutic exposures AND take the most conservative approach to consider infants highest at risk, we do the following calculation:

$$Upper\ AUC\ Ratio\ (UAR) = \frac{95th\ percentile\ simulated\ pediatric\ AUC}{Median\ adult\ therapeutic\ AUC}$$

How do I interpret the UAR metric?

The plots below show simulated infant and maternal AUCs at different infant age groups. The blue dashed line represents the numerator and the red dashed line represents the denominator of the UAR. We can see that:

- the closer the dashed lines are, the **higher** the UAR, and the **higher** the exposure of the infant (i.e., 1-2 weeks);
- and the **further** the dashed lines are, the **lower** the UAR, and **decreased** exposure of the infant (i.e., 3-4 months).



APPENDIX

Why was the upper area under the curve ratio (UAR) metric developed?

We at the Edginton Lab from the University of Waterloo (Ontario, Canada) have developed a novel metric to overcome some of the shortcomings of current resources. This metric is called the "Upper AUC Ratio" or the "UAR". The UAR can account for:

1) the anatomy and physiology of the breastfeeding neonate;
2) age dependent factors (i.e., milk intake volumes); and
3) variability in the neonate and maternal population.

Take the RID as an example. It is calculated by the equation:

$$\frac{Milk\ Concentration\ \frac{mg}{mL} \times 150\ \frac{mL}{kg \cdot day}}{Maternal\ Dose\ \frac{mg}{kg \cdot day}} \times 100\%$$

This metric hardly considers the breastfeeding infant, the safety of whom we are concerned about.

- The numerator focuses on the milk concentration (mother's value) and the denominator focuses on the maternal dose (mother's value).
- Only the daily volume of intake, 150 mL/kg, relates to the infant. This value was chosen as standard, but based on no empirical evidence.
- Thus, this metric does not speak to pharmacokinetic differences of and between infants (e.g., lower GFR the first few weeks after birth, pharmacogenotype variability), the differences in volume of milk intake as a function of age, etc.
- The RID cut-offs for clinical unimportance were originally proposed to be <10% for most medications, while others have proposed <5% for psychotropic medications. These cut-offs are arbitrary, but are often used to to determine drug safety.

The RID and other existing metrics are based on the infant dose. However, the UAR goes a step further to characterize the dose-exposure relationship, which brings us closer to infant response predictions (dose → exposure → response). How do we arrive at this UAR metric that predicts breastfeeding infant exposures to maternal medications?

To derive the UAR for a drug, we develop a **pediatric physiologically-based pharmacokinetic (PBPK) model**. PBPK models use mathematical representations of drug disposition in the body according to the actual mechanisms responsible. There are two main inputs into the PBPK model: physicochemical properties of the compound, and system parameters (anatomy and physiology). To build virtual breastfeeding infants we get an understanding of the disposition of the drug in adults from a rich data environment, then we change the anatomy and physiology to that of an infant (Figure 1) at different ages to account for growth and maturation of different processes, and include variability around anatomy and physiology to give a realistic virtual population. This entire workflow is depicted in Figure 1 below.

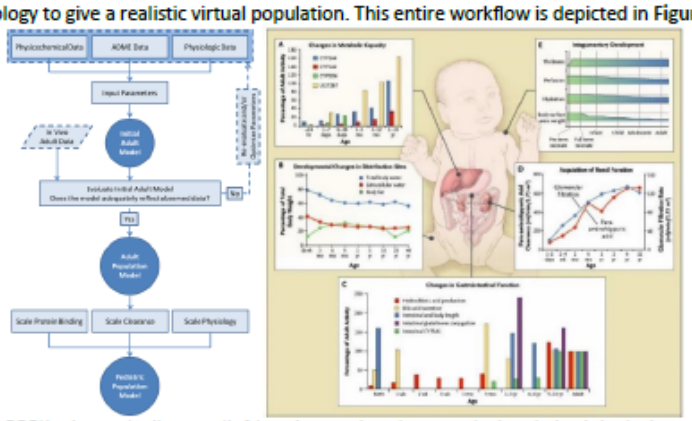


Figure 1. Pediatric PBPK schematic diagram (left) and age-related anatomical and physiological considerations (right).

What goes into the UAR metric to develop it?

At the bare minimum, there are three main components to calculate the UAR for a drug:

1) a weight-normalized daily volume of milk intake across infant ages model;
2) a drug concentration in milk model;
3) and the pediatric PBPK

Variability can be incorporated into each of these components. For example, a model can be used to predict the mean and coefficient of variation (%CV) of drug concentrations measured in milk samples as a function of maternal dose. These three components come together by multiplying (#1) weight-normalized daily volume of milk intake (i.e., mg/L/day) with (#2) drug concentrations in milk (i.e., mg/L). The product of #1 and #2 result in a distribution of daily doses that breastfeeding infants may receive (i.e., mg/kg/day). The infant daily doses are inputs into the (#3) pediatric PBPK model which is then used to simulate virtual infants given the doses to predict their exposures in different age groups. This entire schematic diagram shown in **Figure 2**.

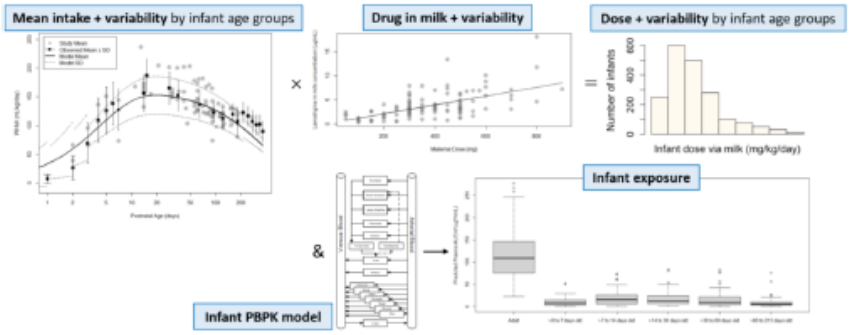


Figure 2. Deriving the UAR metric for a drug involves three main components to output predicted infant exposures.

How does the predicted exposures compare to what's observed?

We compared the results of our exposure simulations with a study by Birnbaum *et al*. 2020. The findings are in **Figure 3**.

We simulated infant and maternal lamotrigine plasma levels from a population similar to Birnbaum *et al*. 2020 (n = 73 infants) with (median [range]):

- Maternal dose (mg): 400 [100-1000]
- Infant age (weeks): 13 [5-20]

The Birnbaum *et al*. 2020 infant plasma samples had a median lamotrigine concentration of 1.6 µg/mL [range: 0.05-8.5 µg/mL).

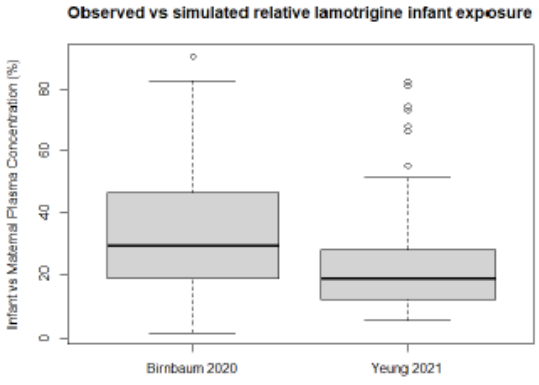


Figure 3. Our model simulated infant to maternal plasma concentrations (right) and observed values from the study by Birnbaum et al 2020 (left). Give median concentration like above.

Note: The caption for **Figure 3** was presented to participants as shown above. However, for more clarity, it should be read as: "**Figure 3.** On the right are the simulated infant to maternal plasma concentration ratios from our model, and on the left are the observed ratios from the study by Birnbaum *et al.* 2020."

References:

1. Yeung CHT, Ito S, Autmizguine J, Edginton AN. Incorporating breastfeeding-related variability with physiologically based pharmacokinetic modeling to predict infant exposure to maternal medication through breast milk: A workflow applied to lamotrigine. The AAPS Journal. 2021;23(4):70.
2. Birnbaum AK, Meador KJ, Karanam A, Brown C, May RC, Gerard EE, et al. Antiepileptic drug exposure in infants of breastfeeding mothers with epilepsy. JAMA neurology. 2020;77(4):441-50.
3. Delaney SR, Malik PRV, Stefan C, Edginton AN, Colantonio DA, Ito S. Predicting escitalopram exposure to breastfeeding infants: Integrating analytical and in silico techniques. Clinical pharmacokinetics. 2018;57(12):1603-11.

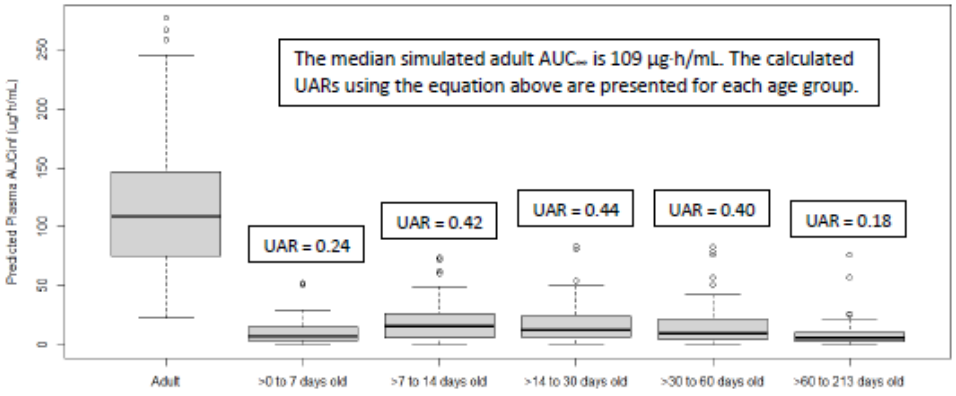
Patient Case

Y.C. is a 31 year-old woman (weight 78 kg) who has epilepsy. She has been on lamotrigine 200 mg once daily during her pregnancy. Y.C. has decided to breastfeed and she asks if she can continue taking lamotrigine while breastfeeding. She has concerns that the medication will affect her daughter (2 weeks old, 3.6 kg).

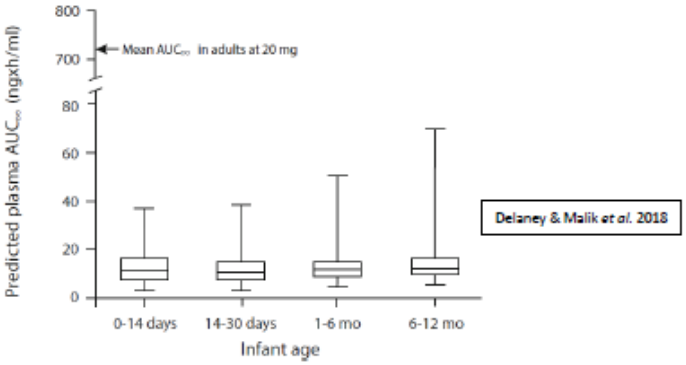
Upper AUC Ratio (UAR) Metric on Lamotrigine

$$UAR = \frac{95th\ percentile\ simulated\ pediatric\ AUC}{Median\ adult\ therapeutic\ AUC}$$

The figure below shows predicted exposures of lamotrigine of adults taking 200 mg oral single dose and of infants breastfed by these mothers. Each age group consists of 100 virtual subjects with simulated plasma AUC_{∞}.



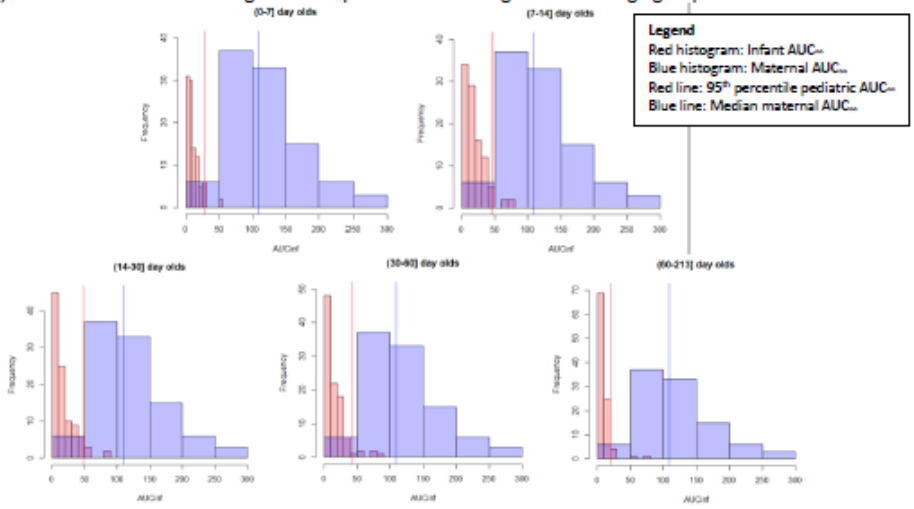
Note: There is overlap between predicted adult and infant exposures, but this isn't always the case. E.g., See escitalopram using a similar workflow.



The information on lamotrigine presented in the boxplots above are also shown in the table below.

Age bin (days)	95th percentile of simulated pediatric AUC_{∞} (µg·h/mL)	Median simulated adult AUC_{∞} (µg·h/mL)	UAR
>0 to 7	26.7	109	0.24
>7 to 14	46.0	109	0.42
>14 to 30	48.3	109	0.44
>30 to 60	43.4	109	0.40
>60 to 213	20.1	109	0.18

(OPTIONAL:) The information on lamotrigine is also presented as histograms across age groups.



2. Interview Materials: New Metric Guidance and Presented Scenario

The one-on-one semi-structured interviews will cover the following broad components:

1) Discuss current practices regarding which resources are considered when addressing drug use in breastfeeding.

   Question: When addressing drug use in breastfeeding, which resources (metrics and online/book resources) do you currently access?

   Question: Can you walk me through how you use these resources when addressing drug use in breastfeeding?

2) Provide scenarios and existing resources, asking participants how they would proceed in practice.

   Question: Given the following scenario and the resources (metrics and online/book resources) that you are currently using, can you outline how you would proceed in practice?

3) Present the same scenarios, but provide the UAR and ask if it impacts how the participants would proceed in practice.

   Present material to the participant that explains the novel metric, the upper area under the curve ratio (UAR).

   Question: With new information about the UAR and given the same scenario as before, how would you now proceed in practice?

4) Discuss the advantages and disadvantages of each method used in steps 2 and 3.

   Question: Can you describe some advantages of each current resource (metrics and online/book resources) that you used in the scenario? What about some disadvantages?

   Question: Can you describe some advantages of the UAR for use in your current practice? What about some disadvantages?

5) Seek suggestions on how the UAR can be improved or described to clinicians to ensure it is most useful to them as end-users.

   Question: Given the disadvantages of the UAR that you mentioned, what are some actionable suggestions to improve or better describe the metric to other clinicians?

3. Code Book

Supplementary Table 4. NVivo Code Book Output of Themes and Codes

Name	Description
T1: Advantages of Existing Resources	
C1: Accessible Through the Institution	Resource is readily 3. and cost is not a barrier due to the institution the provider is working at.
C2: Comprehensive	Resource is thought to contain a wide range of information which may include reporting of risk metrics, drug physicochemical properties and ADME, and multiple referenced studies. Healthcare provider may consider the resource to be sufficient.
C3: Distinguishes and Provides Various Types of Data	Resource includes and differentiates between data types which can include animal versus human, case studies versus larger cohort, maternal milk levels versus infant plasma and/or adverse reactions, etc. These data types may reflect distinguishing "strong versus weak" evidence.
C4: Evidence to Support Use	Studies have been conducted on the resource to support its use in practice.
C5: Familiarity	Resource is recognizable by the provider, colleagues, and/or the field.
C6: Generally Accessible	Resource is readily available, easy to use, and factors such as cost, cell phone compatibility, and physical copy versions are generally not a barrier to use.
C7: Patient-friendly	Resource can be easily explained or directly shown to patients.
C8: Summarizes and References Evidence	Resource incorporates and may reference available evidence in their contents.
C9: Summary Statements	Resource provides a summary statement which may summarize available evidence in a few sentences.
C10: Trusted Authors	Authors and/or administration/board overseeing the resource is trustworthy.
C11: Up to Date	Resource is updated and disseminated regularly.
T2: Advantages of the UAR	
C12: Addresses Clearance Differences	Metric is able to differentiate between individuals with differences in clearance, for instance, preterm vs term infants, hepatically impaired infants, etc.
C13: Addresses Exposures (AUC)	Incorporates a consideration of infant (and/or maternal) exposures to the drug (AUC).
C14: Addresses Multiple Considerations	Metric considers several factors, such as listing the following as advantages: infant age, maternal variables (e.g., pharmacogenotype), and infant exposure. As a result, the metric can be thought of more individualized and specific to situations.
C15: Addresses Scarcity of Published Information	Metric has potential to use the existing paucity of data to help providers make more data-informed decisions (i.e., through PBPK modeling, only requiring confirmatory rather than exploratory samples), whereas, current resources are unable to

Name	Description
	extrapolate and rely on direct conclusions of few published literature.
C16: Addresses the Age of the Infant	Incorporates a consideration of infant age into the metric. Can include considerations on volume of intake, colostrum levels, etc. as a function of infant age.
C17: Addresses the Maternal-infant Pair	Incorporates a consideration of both the mother and infant into the metric.
C18: Addresses the Worst Case Scenario	Incorporates a consideration of the worst case scenario (i.e., outliers highest at risk) for breastfeeding infants.
C19: Can Share with Other Providers and Patients	Metric can be shared with other healthcare providers and/or patients for their understanding.
C20: Numerical Metric	Metric is presented as a number (i.e., a ratio) that is useful in advising.
C21: Objective	Metric is not subjectively derived.
C22: Opens Up the Thought Process	Healthcare provider uses the metric to consider factors they may have not considered with current resources. As examples: going beyond dose considerations, thinking through a process involving exposures and risk to the infant, and reflecting on the contents of the Table that compares the UAR with RID and M/P ratio.
C23: Understand Existing Observations, Evidence, and Recommendations	Metric can help elucidate observations (i.e., breastfed infants typically do not have adverse reactions with their patients taking lamotrigine), evidence (i.e., literature describes volume of intake and thus risk to be higher at 2 weeks), and recommendations (i.e., typically leaning towards advisign to breastfeed) used by the healthcare providers in their current practice.
C24: Visual Representation	Metric is presented visually in a pictorial, flowchart, and/or graph (histogram or boxplot) that is helpful.
T3: Current Practice Approaches	
C25: Advise a Cautious Approach	A personal approach to be more cautious when advising to breastfeed (i.e., recommending to stop/refrain/"pump and dump") by the interviewed healthcare provider.
C26: Advise to Breastfeed During Medication Use	A personal approach that tends to recommend breastfeeding by the interviewed healthcare provider.
C27: Approach for Lack of Evidence	Healthcare provider has additional resources they may use when the drug has sparse information.
C28: Continue Medication as a First Go-to	Main first step of provider is to see if patient was taking medication during pregnancy.
Culture of Practice	
C29: Culture of Leaning Towards Caution	A remark regarding a tendency to recommend stopping/refraining/"pumping and dumping" at the institutional/state level which can involve a broad reference to other practitioners.

Name	Description
C30: Pro-breastfeeding Culture of California	A remark regarding a tendency to recommend breastfeeding at the institutional/state level which can involve a broad reference to other practitioners.
C31: Evaluate the Quality of Evidence	Healthcare provider typically reviews and considers the existing published evidence which can be as provided by the resource.
Factors to Consider	
C32: Alternative Pharmacological Class	In the healthcare provider's advising process, considering another drug with a similar effect (i.e., with less known risks to the infant).
C33: Drug Physicochemical and ADME Properties	Healthcare provider uses the physicochemical properties (i.e., molecular weight, lipophilicity) and ADME (i.e., Tmax, bioavailability) characteristics of the drug in their advising.
C34: Drug Use in Pregnancy	Healthcare provider uses the fact that the patient was taking the drug during pregnancy in their advising (beyond a check as the first step in their advising).
C35: Health of the Infant	Needing more information on, or giving consideration to, the health state of the infant (preterm, co-morbidities, monitoring for adverse reactions, etc.).
C36: Health of the Mother	Needing more information on, or giving consideration to, the health state of the mother (has an underlying condition, etc.). For instance, asking whether the mother needs the medication if they are not doing well health-wise.
C37: Information on the Drug Used in Infants	Whether the drug has reports of being directly administered in infants.
C38: Maternal Co-medications	Whether the mother is currently taking additional medications.
C39: Maternal Dose Taken	Dose of the drug of interest that the mother is taking.
C40: Risks and Benefits (Analysis)	Healthcare provider goes into depth (making a thoughtful assessment) about weighing the risks and benefits. For example, giving an antibiotic to the mother if the risk to the infant is getting diarrhea versus benefits of breastfeeding.
C41: Select Drug Cases for Non-Resource and Resource Use	Healthcare provider is selective on using resources because their cases can be on specific reoccurring drugs where they use experience and previous knowledge. They discuss distinct situations about making decisions without needing resources and situations where resources are needed.
C42: Team Approach (Present or Absent)	The presence or absence of a team approach to advising is taken, which may reference situations where multiple healthcare providers are or are not involved.

Name	Description
C43: Time of Breastfeed Relative to Dose	Healthcare provider considers timing of when breastfeeding occurs along in relation to dose administration in their advising.
C44: Type of Breastfeeding (Exclusive vs Partial)	Knowledge of the mother's state of breastfeeding as exclusive or partial.
C45: Lack of Existing Metric Use	Healthcare provider notes lack of metric use (Hale's L1-5, RID, M/P ratio, etc.) in their advising.
C46: Multiple Resource Use	Mentioning multiple resources that are accessed (i.e., in addition to naming a primary resource). Includes metrics (e.g., RID) as well, for instance, mentioning use of the RID and another resource such as LactMed.
C47: Pregnancy Categories (Using or Avoiding Them)	Mention of pregnancy categories applied in practice. Either the interviewed healthcare provider uses them or avoids them as a personal approach.
C48: Primary versus Secondary Resources	Healthcare provider appears to use a distinct primary resource and more secondary resources. Identifying the primary and secondary resources may suggest multiple resource use.
Realities of Advising	
C49: Concern for Liability	Healthcare provider liability mentioned as a factor to consider when advising patients.
C50: Concerns Relaying Evidence-based Decisions	Concern that an evidence-based decision (i.e., patient recommended to breastfeed) is not relayed to other healthcare providers in the patient's care.
C51: Institution Needs Resource Justification	Healthcare provider can only attain a resource if provided enough justification to their employed institution.
C52: Lack of Information About the Patients	Healthcare provider has limited access to information about the mother and infant (i.e., their patient records).
C53: Minimal Time for Clinical Decision Making	Limited time to advise a patient is remarked as a reality in practice and potentially a barrier to resource use and thorough decision-making.
C54: Motives of Manufacturers	Drug manufacturer motives are a factor in advising, such as, the source of minimal drug in lactation studies and studied adverse effects in infants.
C55: Variable Patient Health Literacy	Patient health literacy is noted as a barrier or simply a factor to advising (e.g., in explaining the recommendations of a resource that is above the Grade 6 reading level).

Name	Description
C56: Refer to Other Provider	Preference of the interviewed healthcare provider to refer to another provider (e.g., physician) typically for decision-making and risk assessment.
C57: Reliance on Other Provider or Resource	Healthcare provider tends to rely on assessment or advice of another provider (e.g., lactation consultant) or via resource (e.g., LactMed due to trusted authors, Hale's due to author already calculated or assessed metrics). Excludes referrals to another healthcare provider (i.e., physician) where limited decision-making and assessment is made by the interviewed provider.
C58: Resource as a First Go-to	Main first step of provider is to access a resource.
C59: Use Combination of Experiences and Resources	Healthcare provider uses their experience with advising (e.g., familiarity with drug) and additional resources such as the RID or Hale's.
C60: Use of Package Inserts	Mention of product monograph package insert information on the drug in lactation use in advising.
Use of RID in Specific Cases	
C61: Comparing within Drug Class	RID is specifically used when comparing drugs within a class. For example, recommending a drug in the same class with a lower RID value.
C62: Explain a Range of Outcomes in Infants	RID is used to explain why infants experience a range of outcomes, from no side effects to visible adverse events. Mentions how much of the dose an infant might receive.
C63: Mother on Co-Medications	RID is considered when the mother is on co-medications. For instance, a mother taking multiple medications and health is at risk, and drug of interest has high RID may lead to a risk vs benefit assessment.
C64: Mother with Conditions	RID is considered when the mother has comorbidities. For instance, a mother taking has multiple conditions and health is at risk, and drug of interest has high RID may lead to a risk vs benefit assessment.
C65: New Medication	RID is used when a new medication is presented with sparse information or healthcare provider has minimal familiarity.
C66: Reassurance Along with Other Resources	RID is used in combination with other resources (e.g., LactMed) to reassure the healthcare provider's assessment and recommendation.
T4: Disadvantages of Existing Resources	
C67: Areas of Subjectivity	Resource is noted to lack objectivity in their contents and conclusions.
C68: Co-medications Not Considered	Resource does not consider cases where mother and/or infant are exposed to multiple medications.

Name	Description
C69: Easily Outdated	Resource can easily be out-dated and does not necessarily present the most up-to-date information.
C70: Effect on Milk Not Considered	Resource does not consider that the medication has an effect on milk (composition, production, etc.).
C71: Inaccessible	Resource is not easily accessed generally or through the healthcare provider's institution. Barriers to use can include cost and difficulty attaining the resource (i.e., physical book copy or needing to go through several webpages).
C72: Infant Age Not Considered	Resource does not consider the age of the infant.
C73: Maternal Dose Not Considered	Resource does not consider the dose administered to breastfeeding mother.
C74: Non-average Cases Not Considered	Resource does not consider outliers, or maternal-infant pairs who are not the average case.
C75: Overreliance on a Single Resource	Concerns that when healthcare providers over rely on certain resources (Physicians Desk Reference, existing metrics, etc.), they can lead to negative consequences (i.e., recommending not to breastfeed when the drug is actually of low risk according to other resources).
C76: Overreliance on Case Reports and Published Data	Relying on scantly presented information of a single case report or minimal published data (whether due to universal lack of information on the drug, or the resource does not include all published data).
C77: Perceived Lack of Reported Information Due to a Resource	As a result of the resource not including the full available information for advising. Not necessarily a direct result of no data or information collected on the drug (i.e., lack of studies conducted), nor are specific areas specified to be lacking (e.g., lack of co-medication considerations, effect on milk considerations) as the reference is more generally missing information (i.e., the reference is generally lacking information).
C78: Too Broad	Presented information in the resource is not specific and does not provide useful information for conclusions. This can include the perception of healthcare provider that a concrete/specific bottom line is not provided.
C79: Too Much Information or Text-heavy	Resource provides too much information that may overwhelm the healthcare provider in their advising.
C80: Unclear Conclusions	Reference lacks a statement that summarizes the information or existing evidence.
T5: Disadvantages of the UAR	
C81: Co-medications Not Apparent	In the provided example scenario, co-medications of the maternal-infant pair were not considered, thus appearing as a disadvantage of the metric.

Name	Description
C82: Difficult to Understand or Too Complex	Metric appears to be difficult to understand or too complex for either the interviewed healthcare provider, or in their perspective, a wider audience.
C83: In utero Exposures Not Apparent	In the provided example scenario, in utero exposure to the infant was not considered, thus appearing as a disadvantage of the metric.
C84: Lack of Maternal Perspective	Healthcare provider felt that the maternal perspective was under considered in the presentation of the metric.
C85: Limited Information on Adverse Effects (Exposure-Response Relationship)	Information on the effect on the infant, including adverse effects from the exposure-response relationship were limited.
C86: Metabolites Not Apparent	In the provided example scenario, drug metabolites were not considered, thus appearing as a disadvantage of the metric.
C87: Multiple Administrations to the Mother Not Apparent	In the provided example scenario, multiple dosing to the mother was not considered, thus appearing as a disadvantage of the metric.
C88: Not Enough for Clinical Decision Making	Healthcare provider noted that using the metric alone (i.e., without any further resources) was insufficient to make a clinical decision.
Path to Understanding the UAR	
C89: Exposure Comparisons	When understanding the UAR and applying it to the example scenario, the healthcare provider inquired about the depictions and explanations of infant versus adult, and/or across infant ages relative exposures.
C90: Interpreting the Exposure Estimates	Interpreting the depictions and explanation of the maternal and infant exposures. Includes what was incorporated into producing the individual maternal and infant exposure estimates (versus the UAR value).
C91: Interpreting the UAR	In understanding the metric for the scenario, healthcare provider inquires on the interpretation of the UAR value (e.g., the numerical 0.44) and the use of numerator (95th percentile infant) and denominator (median maternal). It also includes inquiries on what the UAR takes into account (versus how the individual maternal and infant exposures were determined).
C92: Potential to Appear Subjective or Misinterpreted	Metric may be interpreted as subjective mainly due to lack of conclusive guidance (i.e., only based on provider's assessment of level of infant and maternal exposure overlap).
C93: Prematurity Not Apparent	In the provided example scenario, infant prematurity was not considered, thus appearing as a disadvantage of the metric.

Name	Description
C94: Unusable in Current Form (Too Novel)	More training, familiarity, and time with the metric were hinderances to its current use.
T6: Strategies to Improve the UAR	
C95: Add a Summary Statement	Provide a summary statement with how the metric is currently presented.
C96: Combine the UAR with Another Resource	Present the UAR along with other resources, such as, embedded in Hale's or LactMed.
C97: Explain More About How the Model Was Made (Inputs and Assessments)	Description of how the model was made (the inputs and assessments) could be strengthened.
C98: Explain More About UAR Advantages	Explaining more about the specific advantages of the UAR over current metrics.
C99: Give Specific Training	Provide specific training on the UAR to healthcare providers. For example, training modules on model development and how to interpret the UAR.
Make the Metric and Path to Its Use Audience-dependent	
C100: User Friendly for Non-pharmacists	Make the metric more user-friendly and path to being able to use it catered towards non-pharmacists. This may include providing a brief summary of the UAR and its conclusions, and yet include background on concepts such as AUC.
C101: User Friendly for Pharmacists	Make the metric more user-friendly and path to being able to use it catered towards pharmacists. This may include providing more detail into the development and scenarios of application.
C102: Make Visual Representation Essential	Ensure there is visual representation of the UAR when presenting the metric for drugs.
C103: Overcome Simulation Skepticism	Present the UAR in ways that reduce or prevent skepticism over developing risk estimates from simulations (i.e., virtual populations).
C104: Provide a Definitive Bottom Line	Rather than presenting the UAR value as it is, provide a strict bottom line. For example, categories that allow healthcare providers to more easily interpret the conclusion of the UAR.
C105: Provide a Greater Maternal Emphasis	In the description of the UAR, provide a larger maternal emphasis (i.e., maternal health and her needed therapy).
C106: Provide Guidance to Interpret the UAR Metric	Provide a guide to interpret the UAR as currently presented (e.g., what does 0.44 mean?).

Name	Description
C107: Provide Prospective Predictive Evidence	As a next step in UAR development, conduct and provide evidence that the UAR can prospectively predict infant exposure risk in practice.
C108: Separate by Specific Cases and Scenarios	Present the UAR according to specific cases and scenarios for the drug. For example, including in utero exposures, premature infants, different maternal doses, and in comparison with other drugs (i.e., within the same drug class).

4. Illustrative Theme and Code Quotes

Current Practice Approaches

Advise a Cautious Approach

> "… in all honesty, I, still err on the side of caution […] because there is still exposure." (BFR06)

> "… if the risks were […] high […] in the initial phase, maybe you can pump and dump until you got to […] 30 days or 60 days […] which would be difficult for a mom to do. But I mean it could be possible if she was really concerned about the exposure." (BFR12)

> "… if there is a safe alternative, is there a way we can pump and dump until we get that out of her system, and then how long do we have to pump and dump?" (BFR15)

Advise to Breastfeed During Medication Use

> "Rather than just saying, '… no you had a kidney transplant, you're on X, Y, Z, we're not gonna let you use your milk,' we try to really do everything we could to get that milk into the baby, or even suggest sometimes like a half and half diet to minimize the contact with something. We just didn't understand more, but we still wanted the baby to have the biology of the milk." (BFR02)

> "This is not a very surprising dose, with the 200 mg. I'm looking at that, once a day. That's not [a] particularly high dose, which is good. I'm glad she's decided to breastfeed. So, I would tell her that there are real advantages to herself and her baby." (BFR07)

> "I think that there's still very few medications that I'm like, 'Okay we know for sure that this is bad, and that this is not good for baby', or 'These are alternatives to this medication', and otherwise I continue to recommend breastfeeding because I feel like we know that there are benefits of breast milk and there are lots of things that we don't know about for children and on, yet, we still do them." (BFR15)

Approach for Lack of Evidence

> “But I don't know anyone that uses M/P ratio, if that's, unless it's all like got or something. I mean the problem is, the data is not there on a lot of things.” (BFR07)

> “If that feels like it's not giving me enough information, then I would probably go to InfantRisk and see if there's more information. I like InfantRisk because they also answer clinicians’ questions who submit those, so sometimes, you can kinda do a search for if somebody had a question similar to mine.” (BFR25)

Continue Medication at First Go-to

> “So this woman would've been encouraged to breastfeed before anybody looks up the medication, assuming OB had decided it was safe for the fetus, and she'd be encouraged to keep breastfeeding while we make our decision.” (BFR02)

> “That baby's already been exposed, so what's the, what's the harm of continuing some low-level exposure? If the baby hasn't been harmed already, by this point, by two weeks of age.” (BFR03)

> “If this has been a stable regimen for her throughout her pregnancy, then thus infant's already been exposed to lamotrigine which I would use maybe as a comforting measure for her to... for her to realize that this exposure's already happening and see, her baby is here and fine.” (BFR05)

Culture of Practice: Culture of Leaning Towards Caution

> “And I've had this happen with medication. Then we contact them and say the, the patient has been told that she can. A lot of patients have been inappropriately told that they cannot breastfeed with a lot of these meds that are actually okay, that's the problem. And they say, ‘Oh, I didn't. I haven't breastfeed my three other kids 'cause I've been on tegretol for the last three pregnancy.’ So de-mything that, quite honestly, and convincing them that it's safe is sometimes difficult.” (BFR08)

> “… if I have adult providers prescribing something for my moms, they often will tell them to, like, pump and dump or not use their breast milk. […] They just prescribed like an antibiotic for mastitis or […] bronchitis or something, […] so we have to do some education about that, and I can pull up LactMed to show the moms that it's safe...” (BFR15)

> “… many of them will admit, I'm just so busy, I don't have time, and it's just easier to say don't do it when we know that most medications are compatible with breastfeeding.” (BFR27)

Culture of Practice: Pro-breastfeeding Culture of California

> “I think some of the NICU providers our age tend to be like just pro breast milk period.” (BFR15)

"… some clinicians would have some caution. But, that most babies don't have side effects. That there's a known benefit to breastfeeding, and that can offer, if there's any guidance on what to look for side effects for the baby." (BFR21)

Evaluate the Quality of Evidence

"So […] these different resources will refer to other literature, but then I also wanna know is one referring to a paper of a case report of two patients versus another one is referring to a really extensive, well-designed whatever PK study in […] 30 patient[s]. Like, so I also look at the quality of evidence they're quoting and then I gear towards the ones that are higher quality." (BFR04)

"… if [information from resources are] different, but I have more evidence for this one than I do this one, then I'm gonna lean towards the one that I actually have evidence 'cause then I can personally take that evidence and make my own clinical decision." (BFR25)

Factors to Consider: Alternative Pharmacological Class

"and if there's other options, 'cause that would be a discussion with the neurologist, I think, taking care of her." (BFR22)

Factors to Consider: Drug Physicochemical and ADME Properties

"And lamotrigine is one of those agents that the amount of metabolism varies tremendously. I mean, the amount, the levels and the amount of milk and it's believed to be based on maternal pharmacogenetics." (BFR07)

"Generally, if something is over that 500 daltons, it doesn't get absorbed very well into the milk or across the placenta. So, one of the common ones that we get is the different, like medications for […] like arthritis and those different types of, those autoimmune medications. And they may be 150,000 daltons. So it's pretty easy to reassure mom that not likely that it's gonna get into the milk because it's such a huge molecular weight. […] Some of those medications are fairly new, but we know about the chemical properties." (BFR10)

"… just like as with any medication, just make sure that I have […] a good understanding of the classification of the medication, what the half life would be on that medication. What the absorption factor would be in terms of timeline for feeding to be able to counsel her […] of the amount of time that it would be, excreted into the breast milk." (BFR26)

"Some drugs, especially early on, are more available to the baby because they're fat soluble, colostrum […] it takes in more of the high fat." (BFR27)

Factors to Consider: Drug Use in Pregnancy

"But also it sounds like she's already been taking it during pregnancy so […] it's not a new exposure." (BFR21)

"… hopefully, this has all been discussed with her already during the pregnancy, that she's on this medication, and if it's a medication during pregnancy, it most likely is going to be okay for breastfeeding." (BFR27)

Factors to Consider: Health of the Infant

"I think it's a strategy patients don't always know they can use to lessen the amount to the baby. But I would also tell her that there are some things that they might, keep an eye on and the baby. And almost always, I warn them about sedation or irritability and poor feeding, because that's the thing that a patient would be most likely to be able to pick up on." (BFR07)

"… parents tend to be more scared about their preemie babies and so I find like if they have a term healthy baby, I can reassure them that way but then if they have a pre-term baby, I kind of talk to them about like the other benefits…" (BFR15)

"I would look at information like if it's negatively affecting growth, infant brain development, those types of things, rather than just minor side effects." (BFR22)

Factors to Consider: Health of the Mother

"I mean it would depend on what it is that we're treating and what the scenario to the mother's health would be. So, a good example of when we actually start a medication would be anticoagulants for postpartum DVT. So that question comes up fairly frequently, so this is not something a mother's already experiencing or already stable on." (BFR05)

"I'll also talk about the untreated condition. So a lot of times moms in pregnancy they'll stop their medications. I'm worried about this. I stop taking it. I'm not feeling very well now. Same with breastfeeding, we'll talk about her condition and the risk for the untreated condition that specifically in breastfeeding, baby has an increased chance of having, learning difficulties, developmental delays, those different types of things with the untreated condition so that we're not just looking at the risk of the medication. We're looking at the risks of the untreated condition balancing." (BFR10)

Factors to Consider: Information on the Drug Used in Infants

"Like if we were actually treating the baby, what kind of a dose would we use to give the baby. So, usually, the dose that the baby is getting through the breastmilk is like very small compared to if you were actually treating the baby, we'd be exposing them to much larger doses anyway..." (BFR04)

"Or we could have conversations about the mom needs to start, Keflex for a UTI. And we say, "Well, we routinely use this antibiotic for babies themselves if the baby has a UTI, so of course, breastfeeding is going to be okay." (BFR09)

Factors to Consider: Maternal Co-medications

"Oh, what other medications she might be taking, if she's on other things that might affect their brain? If she was still taking narcotics or if she takes an anxiolytic antidepressant or something like that." (BFR01)

Factors to Consider: Maternal Dose Taken

"Depending on mom's clinical needs, if it's desperately necessary for her clinical care, and as long as her dosing is not considered on the high end. And this is where I would involve my pharmacist, […] what range is her dosing considered? Then I would advise that it would be okay to breastfeed." (BFR14)

"I'm guessing this one can make children sleepy […] so then […] I would probably look through Lexicomp, UpToDate, to see is this safe or not. I'm guessing this is probably a dose-related thing, like how much." (BFR17)

Factors to Consider: Risk and Benefit (Analysis)

"So, I look at the risk and benefit of the mother taking the medication. So like psych medications, for example, you don't just tell a stable mom to stop taking her psych meds so that she can breastfeed. Because that destabilizes her relationships, including her ability to bond with her infant. And so that is a huge risk to the pair, as opposed to, a relative risk to the infant being exposed to a medication." (BFR05)

"… it's just more educating them on minimizing the amount of exposure to the baby while still getting the benefit of the medication for the mom." (BFR26)

Factors to Consider: Select Drug Cases for Non-Resource and Resource Use

"I would start by opening my resources, since I don't have this one memorized and I would just go through them top to bottom." (BFR09)

"Every so often we'll have a patient with autoimmune disease that might be on some stuff that I have to ask the pharmacist more specifically about the safety profile, but most of the time it's a lot of moms asking about SSRIs and on occasion pain meds, but then they fall under the Neonatal Withdrawal Symptoms category, and we usually only allow breastfeeding in the setting of that if they're in a program and they're weaning, and they're not using drugs […] for abuse as opposed to a controlled amount of methadone or buprenorphine." (BFR14)

"… my basic drugs that I already know, most of the time my patients usually are okay to breastfeed during them." (BFR23)

Factors to Consider: Team Approach (Present or Absent)

"And then we always have to then bring it back to the doctor and have a conversation and say, 'Hey, like this has never been done. Here are all the potential benefits, here all the potential harms.' And then collectively, we discuss and then make a decision together." (BFR04)

> "So then what I do is, I kind of get the pharmacy on board, the pediatricians on board, and provide the name. And then I get the information back and I say, 'Well, I communicate with so and so, and it seems that this, this is a safe medication, I'll put it on your chart.' And kind of leave a little note, 'May need help postpartum with breastfeeding.' So that the pharmacist and the pediatricians afterwards could kind of emphasize the importance of the availability." (BFR08)

> "And we, of course, being a collaborative model, consult on this type of thing. And it would be pulling in our RNs and our physician colleagues to decide if a good feeding plan and a good medical management plan for her." (BFR26)

Factors to Consider: Time of Breastfeed Relative to Dose

> "Other considerations I would have is the timing of the dose and the breastfeeding…" (BFR06)

Factors to Consider: Type of Breastfeeding (Exclusive vs Partial)

> "… my concern for breastfeeding goes way down at that point once they start supplementing with anything else. Yes, and then I would ask her, of course, if she was able to fully breastfeed or if she was having to do any kind of supplementation. Because, again, that kind of partitions out, a fraction that the baby would not be exposed to." (BFR05)

Lack of Existing Metric Use

> "… not because I believe [Resource Author] ultimate conclusions…
> […]
> I don't use the L1 through L5 at all, unless to notice if [Resource Author] for some reason slammed an L4, L5 on. I might take a look at why. But I don't use those codes.
> […]
> I don't very often use milk plasma ratio unless it's shockingly weird." (BFR07)

> "I've heard of them, but we're not currently using them." (BFR18)

> "We've never had any education about that. We've never, like... It's never been discussed." (BFR28)

Multiple Resource Use

> "Well, yeah, so I will use the RID, but I always ask myself, okay, so there's a percentage there, but how much is the baby really getting exposed to because it will be a function of a mom's dose. So, I look at the RID but […] I take it in the context with all the other information. It's a good screening tool, but it doesn't seal the deal for me." (BFR03)

> “So I go there first, and then I go to Micromedex and see what it says. Then I go to LactMed and see what it says. And like the reason that I liked to, that I go through all of this is just 'cause every resource is gonna tell us a little bit different information.” (BFR04)

> “As I work on a regular daily basis right now, I've got three different ones open […] LactMed, Reprotox doesn't have a lot, but sometimes it's useful and I have Hale's open. I've got Briggs on the bookshelf back there.” (BFR10)

> “Sometimes I refer to a textbook... herbal and naturopathic textbooks if people are asking me specifically about herbs, because those are harder to find in those other resources I was mentioning. Hale's is definitely starting to expand on that, but I have some of those textbooks that I refer to sometimes.” (BFR25)

Pregnancy Categories (Using or Avoiding Them)

> “I do try to avoid using any of the reproductive, like category X, category A, B, C, any of that information, with breastfeeding, 'cause it was really intended for pregnancy but it's also inadequate for discussing meds in pregnancy either. But I do see a lot of practitioners use those.” (BFR05)

> “… unfortunately I still use like, the pregnancy categories, ABCD. It's just because it's a quick reference and I can say, ‘Oh, well, it's category C. Let's go back to the doctor. It's category B, okay. We're good. We're okay to dispense. Category X, we're not gonna give it to you.’” (BFR06)

> “Lamotrigine is not like completely Category A, the safest drug in breastfeeding or pregnant women, but I just remember that it's considered okay […] like no serious adverse effects.” (BFR16)

Primary versus Secondary Resources

> “Currently at work, we use Clinical Pharmacology. And that, that's the main resource. If there's not enough information, I will go to the package insert.” (BFR06)

> “LactMed is my go-to, and then if it's unclear, sometimes I'll do UpToDate, and then Lexicomp through our [Centre Name] system.” (BFR17)

> “So if I'm using Hale's book, which would be the first thing I would probably grab on my bookshelf, I would look up lamotrigine…” (BFR25)

Realities of Advising: Concern for Liability

> “I'm speaking a little bit also to sort of like a liability aspect as the person giving ... Like if somebody's asking me this and I'm giving a response, there's plenty of questions that we can get asked as pharmacists that there can be like theoretical evidence for like, ‘In theory, this should be okay.’ But is it, okay, let me back up. I guess an example would be, using a dose of something that has never been studied at that dose.” (BFR04)

> "Whereas, more adult providers are just scared of the liability of it getting to the baby." (BFR15)

Realities of Advising: Concerns Relaying Evidence-based Decisions

> "Now we cut and paste it into the note and the pediatrician will do what we tell them. They just will, they wanna know that we thought that we thought it through." (BFR02)

> "… and if they've had a couple of babies […] at a different site, they're gonna trust the neurologist they've been going to since age 15, more than they're gonna trust me, who now they're only gonna see for, I don't know, 15 weeks. So kind of just revamping that whole concept might take a few visits. And even at the end of it, sometimes they feel like, 'Ah, I didn't do it the last couple times. I'm okay. I'm not gonna do it. I'm not gonna take the risk.' So it's sometimes difficult to break those barriers if they have their mindset on that, based on someone else's." (BFR08)

> "… who is managing these medications are always a difficult thing and how much of it is ... I think a lot of adult providers don't know about LactMed […] versus OB-GYN, I think they have an idea. But it ends up really falling on the pediatricians to have to be looking at it kind of thing." (BFR17)

Realities of Advising: Institution Needs Resource Justification

> "And in my current practice, there's not enough need for it, for me to justify that." (BFR06)

> "… we have to get all those approvals and things like that" (BFR10)

> "I think for the community setting, it's really helpful to have free resources, 'cause the community pharmacy I work with is an independent, so they don't subscribe. […] The chains might have resources but the independents aren't really paying for a lot of those extra resources like that, so it's great to know what's available for free." (BFR20)

Realities of Advising: Lack of Information About the Patients

> "… sometimes they don't tell us everything about the patient or the patients, in this case, we're having a baby too. So, I think for us, the team approach especially for a pharmacist's point of view, the team approach is the best way…" (BFR06)

> "In terms of prescription medications, it doesn't come up because let's say the patient picks up a medication, I'm assuming the doctor already knows or they have discussed it, and then, they wouldn't even say they're breastfeeding. So, I wouldn't know." (BFR16)

Realities of Advising: Minimal Time for Clinical Decision Making

> "I mean, I think this is great for a pharmacist who does pharmacokinetics, but I don't think this is great for a busy pediatrician in their clinic. Like they just need a yes or no most of the time, unfortunately." (BFR01)

> "People have to make these decisions in a few minutes. So if they have 30 babies to see that day, the team may be four people seeing 30 babies, 15 minutes a patient, get, it has to be pretty simple." (BFR02)

> "In the hospital […] patient room […] you may have like half an hour or 15 minutes to get back either to the doctor or a nurses." (BFR11)

> "So, often I don't have the time. I need the quick answer, and if you need to deliberate more, I fit it in in between verifying my orders, talking to physicians, rounding, helping the nurses with drug administration. So there's kind of a lot on your plate when you're in the unit." (BFR12)

Realities of Advising: Motives of Manufacturers

> "… those tertiary resources are getting their data from the manufacturers and so it's certainly not an advantage for a manufacturer to look up that information about their product. So, I can see that they spend zero resources whatsoever trying to collect or accumulate that information." (BFR05)

> "The company hasn't done the research to have in their files that says this is safe. It's just easier to say, 'Don't breastfeed.' And so I think it's from a liability standpoint because they haven't gone the extra mile to find out, is this a safe medication or not?" (BFR27)

Realities of Advising: Variable Patient Health Literacy

> "… I do that after I've gotten a sense of the health literacy of the patient, whether that will be helpful or confusing for them. Some people really like it. Some people find it overwhelming and not helpful that they prefer the summary version that I'm giving them." (BFR07)

> "I think just telling them a little bit more, 'cause all of our counseling needs to be at a sixth grade level, unless you know that the knowledge of the parent is advanced, and then of course you know you can get a little more sophisticated in your explanation." (BFR12)

Refer to Other Provider

> "Because the baby is so young, I would also have extra considerations, and probably refer back to the doctor. A lot of the resources do not go that young for me." (BFR06)

> "I would maybe just share some information with the patient and advise them to contact their provider." (BFR20)

> "I would either look at LactMed or I would ask one of the providers to come answer the question for them." (BFR28)

Reliance on Other Provider or Resource

> "And really, mostly what we do is consult our lactation consultants. So, from the nursing perspective here, that's our first go-to… is accessing our lactation consultant team and then our physician team." (BFR13)

> "We have a designated NICU pharmacist who is very readily available to us. In fact, we sit in the same writing area. We are typically in communication if we're running TNAs or TPNs for the babies. But questions about mom medications, [I] frequently […] go to them for a lot of the ones that are not as straightforward." (BFR14)

> "And then, I will call our pharmacist if we're, especially if it's a combination of medications and we're not too sure about are usually where we go for information." (BFR18)

> "… then if the information that I'm getting is not making me feel comfortable, then I also have the resource at [Centre Name]. I just call our lactation office at [Centre Name], and then I talk to one of them, and I have a couple lactation consultants…" (BFR23)

> "I feel like it's a quick way to kind of assess if something... I mean, if something is an L5, to be honest, I don't really dive much deeper to see why [Resource Author] came up with an L5. I've been using that […] rating system for long enough that I feel like if it's an L5... I'm done." (BFR25)

Use Combination of Experiences and Resources

> "The focus has to be on not just saying, 'Okay, I looked it up in Hale's and he says, no, we're not gonna allow it,' but to sit back and think, okay, wait a minute. What's the science about how much actually gets in? Is there an option to try another medication? No. Could we give the baby some of her milk?" (BFR02)

> "I'm a little bit familiar with this medication. I know that larger amounts gets into the milk can increase the risk for sedation, but I would take the time with some of these medications that I do know are more concerning.
> […]
> But then […] take a look at the half life and kind of understand the medication that those can be sedating and because of the half life, they can build up and cause that sedation. So, you have to be able to not only look at the Relative Infant Dose, but consider it within the greater context." (BFR10)

> "I know, previously the old literature stated that you should not take SSRIs while pregnant 'cause it increases risk of persistent pulmonary hypertension in the newborn. However, I think the new guidance says that mom should be more stable and be taking their SSRIs while pregnant." (BFR22)

Use of Package Inserts

> "… if some other question that [needs] more details, we can go into look at the package insert in each bottle if needed…" (BFR11)

Use of RID in Specific Cases: Comparing within Drug Class

> "I might use the metric in comparing between drugs of the same class." (BFR05)

Use of RID in Specific Cases: Explain a Range of Outcomes in Infants

> "I might give the range and the relative infant dose. I might say that there has been a general rule that […] medications used during the breastfeeding, maybe less than 10% of the mom's weighted dose. And with this one you can see a range, and so that's why some persons might have more caution, but here's what we see when actually moms have breastfed with the medication." (BFR21)

Use of RID in Specific Cases: Mother on Co-Medications

> "… if I see that it's got a higher relative infant dose and let's say it's a woman who's on multiple medications and I'm a little concerned…" (BFR02)

Use of RID in Specific Cases: Mother with Conditions

> "I'll take a look at the RID and milk/plasma ratios when we're talking. Occasionally I'll write a consult in the chart. For example, […] there was a mom a couple of years ago […] she had some comorbidities and […] the physician out in East County did prescribe codeine, and that's when we were not doing that, because we weren't looking at polymorphisms in the mom and the baby." (BFR12)

Use of RID in Specific Cases: New Medication

> "And so that's where I would use those metrics then of what's getting into breast milk, or what the infant's relative dose would be, is in a case like that where I'm choosing between drug classes for a specific new indication." (BFR05)

Use of RID in Specific Cases: Reassurance Along with Other Resources

> "And most medications have RIDs that are single digit percentages or even decimal point digit percentages. And you look at this in a really quick glance and you say, 'Ah, it's probably okay.' […] But the, the RID is your first point of reassurance for most medications, except for the few where the RID is actually higher than 10%. Which in my line of work, [of] the medications that I get asked about most often, they're few and far between." (BFR09)

Disadvantages of Existing Resources

Areas of Subjectivity

> "… it uses those codes. And, it's opinionated. It's gotten better about that. But it can be, this happened to two patients and I now think blah, blah, blah. I mean, breastfeeding research is a victim of small subjects and […] I'm like we would never, never accept it […] but that sometimes that's what you've got." (BFR07)

> "From a layperson's standpoint who is out there Googling, 'Is it okay for me to take narco while I'm breastfeeding,' they're gonna come across an ocean of stuff. And much of it opinion, much of it based on one cherry-picked, selected study or another and pulling them towards whatever conclusion it is that they're probably most likely to dive towards in the first place." (BFR09)

Co-medications Not Considered

> "So if mom's taking three medications, how can I look up if that's more concerning or not? So I just have to in my head, oh, these all three affected essential nervous systems and I have more concern would be the biggest ones. (BFR01)

Easily Outdated

> "We don't really use any books anymore, just 'cause they get outdated." (BFR13)

> "… but the problem is if you don't buy a new book every single year, who knows what information is changing as well. So that's the negative about the books. Although, I had every drug possible that you can think of in those." (BFR23)

Effect on Milk Not Considered

> "… the issue about, does it affect milk supply? I feel like that comes up a fair bit, so I dislike that Hale doesn't have that." (BFR01)

Inaccessible

> "I know there's like different, there's books, too, that we have in like, especially in our newborn office. I just haven't used them as much since they cost money." (BFR15)

Infant Age Not Considered

> "… there's not great, like specifics towards preemies. And most of the time there's not that consideration at all and they have a different renal clearance for sure. They also have a definitely different weight." (BFR15)

> "Or the infant's age is not taken into the consideration at all." (BFR16)

Maternal Dose Not Considered

> "But there's no like dosing information or anything, I will say that. This is just like general overview." (BFR28)

Non-average Cases Not Considered

> "I would say that […] it doesn't elaborate enough on, the upper and lower curve of… issues that might be coming, or other complications that might come. It just gives us the mean or medium of the information out there. And so, unfortunately not all babies fall in that middle of the bell curve." (BFR06)

Overreliance on a Single Resource

> "… there's a ton I don't use like, the […] package inserts are so problematic and that's what families sometimes have access to where it doesn't give any information about breastfeeding or just says, "Talk to your doctor." Or it says shouldn't breastfeed based on no issue. They just don't want the medical legal liability." (BFR01)

> "… even in RID, you're clinging too much to a proxy and not like looking through all the information. So, the disadvantage is you really have to look through all the information being presented and come up with a plan and apply it to your patient." (BFR03)

> "Relative infant dose? Yes, yes. Although, I think people use this 10% cutoff for relative infant dose, and it's not true. I saw somebody do that recently. And I thought, "What are they talking about?" If it's something toxic, then it's a little baby, yeah, you care, even if it's 3% or 4%, or whatever. And if it's not something you're very worried about, okay, like something like that right now, and it's 25%, but you could give it directly to the baby, well, then you might not care." (BFR07)

Overreliance on Case Reports and Published Data

> "I think the disadvantages in the literature that [Resource Author] uses to draw from it. So, again, I don't think there's really any modeling going on. It's just, what is this study shown? It's often two samples here and five samples there, but that, that's what we have. So something where there's some more scientific modeling would be fantastic…" (BFR02)

> "I guess in reality is that a lot of the times when I go to LactMed, most of the medications will say 'Insufficient Data to Advise Against or For.'" (BFR04)

> "I think the main disadvantage to everything is that because there's no human studies, we don't know exactly what the possible side effects and risks are." (BFR24)

> "I feel like a lot of that hasn't really been studied well. So that, that's the problem, right? Like we don't do a lot of studies." (BFR28)

Perceived Lack of Reported Information Due to a Resource

> "And then there are times where I'm like, "Ah, LactMed doesn't have it," […] it's not quite the prescribed medications, it's the supplements that I have a lot of issues with." (BFR17)

> "So, if you use their resource, as I have for a long time, that it's got some problems. And one of them is that they don't include the different formulations, even though there, it say Wellbutrin, bupropion. Most patients do not use the 150, just plain old non-extend. They use a 300 extended release. And so, the data in there for it is not actually correct for that particular [case]." (BFR07)

Too Broad

> "Other tertiary sources, like Micromedex and Clinical Pharmacology are easy quick grabs, but they tend to be a little pale in what quantity they provide in information." (BFR05)

> "I think a lot of them just leave it up in the air. Per provider discretion is something I see across a lot of resources, and so that's when I end up having conversations with the pharmacist reading up case reports" (BFR14)

> "It's not gonna go into kinda more the bioavailability of what's getting to the infant or the clearance. It doesn't have like those further steps of recommendations and so that is a disadvantage." (BFR15)

> "Lexicomp, for example, […] it does not have that much information about breastfeeding. And, infant exposure, it usually just has a little blurb or one sentence, so it's not very complete, or I cannot depend on that single resource. It's not all-inclusive. So if I would not use Lexicomp alone, it would just be a starting place, just to see maybe where I should branch off to look up for more information." (BFR22)

Too Much Information or Text-heavy

> "Disadvantages, sometimes they can be slow to get to the point. The more exhaustive listing of all the potential issues that have been studied can still be a little bit off-putting and can, lead you to be anxious about making a decision that ultimately is the best one for your patient." (BFR09)

> "… when I'm looking at Hale's, it's very easy to see that table. With LactMed because it's presented in a narrative structure, I have to read through that. I can still find that Relative Infant Dose, but it's harder to dig that out while I'm talking to someone on the phone." (BFR10)

Unclear Conclusions

> "I think UpToDate and Micromedex, […] they don't always give […] a clean summary statement or like a final, "At the end of the day, this is what is recommended."" (BFR04)

Advantages of Existing Resources

Accessible Through the Institution

> "And since that is a database readily available at my institution that my institution pays for anyway, I will sometimes go there first…" (BFR03)

> "… our computer system link directly to ClinPharm […] so I just click into it. This may take about five seconds so that's a number one I'm going to before Google and […] ClinPharm doesn't give me the answer first." (BFR11)

Comprehensive

> "Someone wanted to know if they could use monk fruit and breastfeed. And Reprotox is the only one that even have a monograph on it and just said nothing is known." (BFR07)

> "… at least that's very helpful, like, 'cause they have gone through everything." (BFR17)

> "So they have everything in there that I feel I need" (BFR21)

Distinguishes and Provides Various Types of Data

> "… but then also goes through and breaks down the information as to whether or not the data's coming out of animal studies, or human studies, or case reports, or if there is measured maternal milk levels or relative infant doses." (BFR05)

> "I also like the fact that it describes both the information on the exposure, the maternal side and the infant, so it separates it out in terms of if […] there's any data on levels and infants, if there's any data on infant response and adverse effects and whatever." (BFR07)

> "I do like to be able to see […] the Relative Infant Dose, some of the narrative information. For example, some of those medications we know are given directly to pediatric patients and that will be in the narrative. And that will give us a clue that yes, if it's given to a pediatric patient at birth, acyclovir, not likely to be an issue, regardless of all of those other things. So, there are a lot of those different pieces of data and narrative that help to build that risk statement that's in those different databases." (BFR10)

Evidence to Support Use

> "It works sometimes because they have gotten a lot better over the years and there's some data to back that up, at least one study I know of, showing their improvement over the last 20 or so years." (BFR03)

> "Like it would need to be validated and I, as the clinician, we need to understand where that information came from so that I trust the source." (BFR05)

Familiarity

> "Purely for, probably mostly for convenience and familiarity. So, I think the way, when I was in my training, LactMed was sort of considered the gold standard." (BFR04)

> "It's what I'm familiar with when making clinical decisions in infants, with even just medications being given to them." (BFR22)

Generally Accessible

> "It's readily available on any computer that I'm sitting next to. I don't have to log into anything since LactMed is free." (BFR01)

> "I use the app on my phone usually, and it's free. So even in the community setting where they don't have any […] institutional resources…" (BFR20)

Patient-friendly

> "… and then the MotherToBaby, the advantages, I can give it to families with lower education level and they can use it. I guess that's not beneficial in terms of my making a recommendation, but it's beneficial and, and giving that information to the family. I can copy it and […] we'll have to put it on our discharge summary so we can communicate the information to the outpatient pediatrician, why we made a decision." (BFR01)

Summarizes and References Evidence

> "I think it's more […] pre-digested. The information, the research is kind of, it's almost like a meta analysis or someone else has kind of like, looked at is, or, and also […] it's concise." (BFR19)

> "I guess my thought would be that it's laid out that there, for example, LactMed will talk about any studies that have been done […] will look at general safety profiles…" (BFR24)

Summary Statements

> "We have our kind of summary on there, for example, we'll have a summary based on the available information, there's no conclusive evidence, that type of thing. And then we'll have, we'll pull from some of those other databases like LactMed. I'll pull the summary statement and put that in our database so that as we pop those up, we can see some of those other databases compared." (BFR10)

> "A couple sentences, it's really easy to get to, and also […] understandable language." (BFR19)

> "I mean in LactMed it's pretty specific… you don't have to read too much to get the bottom line information. So I do like that about LactMed…" (BFR23)

Trusted Authors

> "And they've brought on a bunch of editors who have skill sets in those patient populations, including lactation. And so, that's the other improvement. Just their editorial board." (BFR03)

"I think LactMed also has the best description of the studies that they've mentioned. Probably 'cause it's written by pharmacists." (BFR04)

"… like with InfantRisk, I feel like that I trust as a resource because there are several pharmacists who work for... It's through the [University Name] and so the whole department is pretty much kinda looking into this and they are the ones going through all the studies, so that I don't have to kind of thing." (BFR25)

"… there's someone that's gone before and it's a standardization that you can lean in on." (BFR26)

Up to Date

"The advantage of LactMed is just that it's updated so frequently…
[…]
So I always feel like it's very well updated. You can see the date that it was updated." (BFR02)

"Yeah, it is nowadays, I don't use textbook, partly because we cover so many sites, and these books are so easily outdated that we don't go to books anymore. So these are the online resources I use." (BFR08)

Advantages of the UAR

Addresses Clearance Differences

"Okay, here's the dose in the milk then what's the bioavailability to the baby, what's the baby's clearance ability from their bloodstream? […] How much is gonna kinda stay around in the baby? So it's just a better in some cases I would use that information and be like, 'Look, baby's gonna clear at the area under the curve like this ratio it's staying pretty low for 95% of babies.' […] so having more information is always appreciated and […] helpful." (BFR15)

"… didn't it look like the UAR does like metabolism too? […] Like if it's slower and that's an area 'cause I don't know that what the person's are they fast or slow? That's helpful to have factored in." (BFR21)

Addresses Exposures (AUC)

"But this just gives you an even more exposure assumption than the RID does. So it's better." (BFR03)

"… it probably would be accurate in assessing what the risk was to the infant based on maternal plasma levels, so I would think that the value itself would probably be very accurate in terms of infant risk and that would be an advantage." (BFR25)

Addresses Multiple Considerations

> "I like that it provides another metric, another input, that appears to be relevant and take a lot of factors into consideration and present them quickly. […] I like that, if it were added to my existing resources, it wouldn't be adding a paragraph. It would be adding a number. I like that. I see it as a positive." (BFR09)

> "I do like that you guys take […] into account the clearance, […] especially with the renal and hepatic clearances and metabolites, too." (BFR14)

> "… it helps to kind of take into account all the different factors, such as the age and the clearance, like renal clearance and, like our preemies have much less renal clearance than older kids.
> […]
> So, and taking into account more than just, like, just the amount, like the dose in the milk, and then, like you're saying, so we're talking about, […] kinda all those steps." (BFR15)

> "… I like how it incorporates more aspects of, of the situation, where often […] I'm not relying on individual metrics except in a very broad approach or if it's something like biologic and oral bioavailability, or often the relative infant dose is simply […] this cutoff and here look it matches, and here oops it doesn't, but what do we know for other moms who have decided to breastfeed.
> […]
> the UAR that you'd be getting more specific to the situation." (BFR21)

Addresses Scarcity of Published Information

> "… to me looking at it, I think […] it's more data-driven. […] I mean, the majority of medications, we don't have studies on. So you're going by case studies or reporting or whatnot. So I feel like this has much more data-driven, sort of evidence to back it up, and I like that." (BFR18)

Addresses the Age of the Infant

> "… the UAR, I like that it is more specific to the, yeah age of the baby and the individual, […] because […] the way I counsel is more of broad strokes." (BFR21)

> "I think if we're really able to look at the dosing or what we think would be the probable dose, especially for those early days from like, the 0 to 14 days or the first two weeks of life where babes can be most vulnerable, I think that it would be interesting to get an idea of what we think, the concentrations are and then to really be able to talk to our patients with a better educated guess." (BFR24)

Addresses the Maternal-infant Pair

> "I mean, it's like everything in mother-baby, there's two patients involved. It's not just one patient involved. It's how much the mother takes, what gets into her milk, how much the baby takes in, what the baby's biology is. So this is actually taking that and putting them together,

which is really nice because so many times it's really just what gets into the milk and it makes people worry and not think even about the oral absorption." (BFR02)

Addresses the Worst Case Scenario

"Well, the main advantage is that it gives you almost like a worst-case scenario because you're comparing the 95th percentile to the median." (BFR03)

"And this one seems to add additional factors that might explain that it actually is even safer still. Although maybe I'm wrong. Maybe there would be a scenario where there is currently a medication that existing resources say is most likely safe, but this new number shows a point of risk, where we might want to avoid it. I mean, I suppose that's possible, and that would be interesting." (BFR09)

Can Share with Other Providers and Patients

"Actually, it's easier to tell patient, 'The number you look for is 0.7, but this drug has 0.2.' So yes, it's much, much, much lower. Kind of like a radiation dose." (BFR08)

"If something's more complicated, I could definitely see where this novel metric would be useful to help […] share with a neonatologist and talk to the mom's primary physician to have that discussion and document it." (BFR12)

Numerical Metric and Objective

"In the NICU we're very number focused, so I think it definitely helps to put a metric and a number to things." (BFR14)

"I like that it gives you a value, like if you were educated how to interpret that value […] correctly. It's, that definitely is a lot simpler." (BFR28)

Opens up the Thought Process

"But this would kind of reinforce that […] this is only in the 95th percentile. So, this isn't going to happen with every patient. But, you don't know […] if your patient is in the 95th percentile or not, so you have to plan for this. And so there needs to be kind of a monitoring plan for the mother and the baby and the physician. And if we're not able to do that maybe you should consider supplementing with breast milk to reduce the exposure." (BFR03)

"I mean the histograms are nice to look at the relative risks, but relative risk is relative risk. Some people are adverse to any risk. […] So I think it's still important to let her know that there is a risk and […] to talk to her neurologist and unless her seizure control isn't great on lamotrigine and then it would be definitely worthwhile for her to try another agent. But if she's got good seizure control and hasn't had a seizure in a while, I mean definitely now is not the time to change that." (BFR12)

> "So I would look at the age of infant, the dosage, and then be able to better […] look in the boxes, percentile, and then find a UAR and help counsel her about the safety of it.
> […]
> Whereas, given current resources, what we would do is just look at the medication itself and whether it's safe or not. So, it's more of a yes no, and this gives more of an in-depth, and personalized look at each patient scenario." (BFR13)

Understand Existing Observations, Evidence, and Recommendations

> "… it's reassuring that the infant relative exposure is low, […] I think this is really interesting. I mean, we think of baby's renal function as... borderline. They only have 10% of their glomeruli when they're born and then it gets better and better. So I'm kind of surprised to see that peak and then dropping back down, which I don't understand." (BFR02)

> "… I think that the biggest advantage I see in it is that relative comparison to maternal dosing. To use that as a graphical representation that I can show a patient and give them reassurance. I wouldn't anticipate that your general level patient would be able to interpret data out of a graph like that, but just to be able to visually say, 'Okay, here's you and here's baby and see how these bars completely don't touch each other.' […] The escitalopram graph is a very good one." (BFR05)

Visual Representation

> "… I am a very visual person, so I think that it's really great for visual people and it shows break points in ages…" (BFR12)

> "I just saw that UAR is green all down the line, which is wonderful. But I think the interpretation, like that bottom plot, is probably the most helpful part of it all." (BFR17)

> "So, I liked [that] you showed me the math where it says upper AUC ratio, and it shows me what the numerator and denominator is, and […] I understood it even better when I saw the diagrams with the dotted lines. I think that just described it really well to me 'cause I'm a very visual person." (BFR22)

Disadvantages of the UAR

Co-medications Not Apparent

> "… it doesn't combine meds and it doesn't seem like this one does either." (BFR01)

Difficult to Understand or Too Complex

> "… it's a little complex, the way this is, nobody would be able to look at this for each drug and make an assessment. You would need to put it together for us." (BFR02)

> "… it just adds more metric, to a decision making process that I wouldn't necessarily share all of that with a patient as it can be kind of overwhelming or too much information." (BFR05)

> "I think it's pretty hard to analyze unless you're really super like, into the research part of drugs, which most doctors aren't.
> […]
> But not looking at these numbers. We need it more digested, we need simpler." (BFR19)

In utero Exposures Not Apparent

> "… this doesn't account for what they already were born with. That case she was born with, right? This is assuming a birth blood level AUC of 0 in the infant." (BFR03)

Lack of Maternal Perspective Consideration

> "I think that your metric kind of just looks from the infant perspective. I don't think that it necessarily weighs in anything about the maternal perspective." (BFR05)

Limited Information on Adverse Effects (Exposure-Response Relationship)

> "… unless there's any kind of clinical coloration to that, the number isn't terrible meaningful to me. It could be a very high number, but if it's no clinical effect, then that wouldn't matter. It could be a very low number, but if it's a particularly toxic medication like an oral chemotherapy or something, then that would mean something more to me, relatively at least." (BFR05)

> "… drug doesn't [affect] the baby, then no risk, […] even though the high concentration is… and but it doesn't cause […] risk to the baby, it's still okay, you know?" (BFR11)

> "I think the question still is what does it do for baby? Like do we know if there's any adverse effects in the child in a developing brain of this small amount of exposure of lamotrigine or escitalopram or anything and we, most of the time we don't know.
> […]
> Yeah, that would be interesting like if they're up at the higher levels, what is that doing if it's able to be therapeutic for the adult, […] and then, I think taking into account like what, what we know about how it affects adults and what are the adverse effects in adults to is some useful information. Like if you know a medication causes elevated transaminases in adults, it's not like as one of their side effects or you're watching like platelet or neutrophil counts in adults because of this medication, then that's probably not great for babies if it's reaching a therapeutic level, then that's not good." (BFR15)

Metabolites Not Apparent

> "Well, we didn't talk about metabolites yet. I mean, would there be ... Are you thinking of combining the two drugs in the one giant AUC, one combined AUC? When I say two drugs, I mean the drug and its metabolites" (BFR03)

Multiple Administrations to the Mother Not Apparent

> "… at least in just the one single example that you gave me, it was just a one-time dose. This mom probably has been taking it, or will be taking it for a long time, so I don't know how that's going to change the total exposure to the infant." (BFR22)

Path to Understanding the UAR: Exposure Comparisons

> "So we're looking at the difference between the 95th percentile and mom's median, and the wider that's staying from the lower the exposure, right?" (BFR14)

Path to Understanding the UAR: Interpreting the Exposure Estimates

> "And so the closer the dash line, the higher the UAR, and the higher exposure to the infant. […] Yeah, you have higher and lower, but what's safe and not safe is what I think I'm having the hardest." (BFR18)

Path to Understanding the UAR: Interpreting the UAR

> "I don't know what the acceptable UAR is. That's the part that I didn't understand. What is the ratio supposed to be?" (BFR08)

> "I can see a UAR of 0.24 at 0 to 7 days, and then it goes up to, up 0.44 at 14 to 30 days, but […] that doesn't mean anything to me. I mean, I know it increases, but […] relative to how that affects the baby doesn't mean... I don't know how to interpret it." (BFR18)

Potential to Appear Subjective or Misinterpreted

> "I can see how people might look at that and say delay breastfeeding until it drops. But […] that impractical. If you see that curve that goes over and say, "Okay, we'll just wait till this point as some kind of arbitrary safe point." I wouldn't assess it that way, but I'd be worried that some people might look at that and interpret it in that way." (BFR05)

> "… there's always an issue when you account for all those things for them. And then, they also account for, again, on top of it. So, I guess that's the question. […] I think most of them know to be careful with newborns. So, if UAR is taking that into account, if they take into account, again, that is a newborn, is that going to make it look riskier, for example?" (BFR07)

Prematurity Not Apparent

> "And then what about prematurity? Like, is this just chronologic age? Has this taken to gestational age or is that part of the vulnerable piece? Because I think there's a lot in the NICU trying to look at, 'Is it okay for this baby to have its mother's milk or not?'" (BFR01)

Unusable in Current Form (Too Novel)

> "I think it would've, if you gave me some guidelines of how to interpret the ratio. But right now I'm still lost." (BFR08)

> "The biggest disadvantage is that those different teratogen information specialists around the world just aren't, haven't used it before. So it's a novel approach." (BFR10)

> "But for me specifically, because I'm not familiar with […] it doesn't mean as much to me, honestly." (BFR25)

Strategies to Improve the UAR

Add a Summary Statement

> "And if you can find a way to translate this into a statement, typical mother, typical baby, somehow to make it practical.
> […]
> whereas LactMed is a good balance of putting the data together and then giving you a common sense recommendation. It's really, that's part of what makes it so valuable. So trying to do something like that with this." (BFR02)

> "If you gave me the information in a form like this that was quick to take in that I'd already been trained on, if I was already familiar with this new number, this new metric, and I've already had training on it and already been convinced that, yes, this is a reliable metric, and you can present it to me in a short bullet point format, such as the RID that I already have access to in the emergency department, yes, I would absolutely look at it every time." (BFR09)

> "… so if I was reading it like online or wherever, I would have like a statement of like this as kind of a conclusive statement to make sure that it's being interpreted correctly." (BFR15)

> "I like summaries. So, like, […] 'This means this, this high number means this, this low number means this, this is what it means, okay, this is how we got to this point.'" (BFR23)

Combine the UAR with Another Resource

> "So this alone doesn't help me decide, but in parallel with the other things that I would see in a LactMed reference, which would gimme a little more information, I would feel comfortable.
> […]
> It depends how you're presenting it. So if you're melding it, let's say into LactMed or something. They trust LactMed and they're, and you don't have to tell them all that. You just have to tell them what they wanna know." (BFR02)

> "… my question is […] when these metric develop, can they […] incorporate into ClinPharm or other […] resources?" (BFR11)

Explain More About How the Model Was Made (Inputs and Assessments)

"… when you report the UAR, have it be available to know like where that, where that data came from. So like I was saying, in how many breastmilk samples from how many patients. And then with the adult AUC data, like what is this AUC represent? What dosing ranges is this AUC representative of? Like, […] we use like crazy doses that are not FDA approved. […] So I don't expect, a group like this to go and test these off label doses that we use 'cause that's you know, that's not normal. But, that could help me know whether or not I can apply the UAR to my patient better.
[…]
And, I feel reassured that you are validating with actual infant samples as well." (BFR04)

"So it's a novel approach. It would take some training to get used to understanding all of the different pieces that are in that model and how to interpret it." (BFR10)

Explain More About Its Advantages

"... most people aren't gonna understand, but if they know that it takes that into account, whereas these things don't, you'll be like, 'Oh, okay. That's great.'" (BFR01)

"to discuss the benefits that it gives more information about a drug, and based on baby's age. So I think if everybody understood it, and saw that it definitely gave them more information, it would be very usable." (BFR27)

Give Specific Training

"… disseminate the information, having practitioners get accustomed to it would be big. […] I honestly, I think this would be a one lecture or a one presentation type of topic to discuss to people. And I think, a lot of practitioners are smart enough to understand, so." (BFR06)

"Maybe it would be much easier if, if I sat through a talk and understood for different medications, what it meant." (BFR08)

"So, I think that, to be completely honest, maybe because my, like I graduated [many] years ago, I'm not used to looking at these things anymore. So, maybe some people might need some kind of, maybe just like 10 minutes of training to completely understand it." (BFR16)

"Like, breastfeeding is not talked about or thought about really in medical school at all. And so the question is can you influence pharmacological kinda things, or at least introduce the concept of […] how do you interpret these things and think about people as a whole? I mean, I think all of medical education is going towards more of a wholesome view of medical education, so I think this is sort of the time to, like, start introducing it, as well." (BFR17)

Make the Metric and Path to Its Use Audience-dependent: User Friendly for Non-Pharmacists

"I think this is great for a pharmacist who does pharmacokinetics, but I don't think this is great for a busy pediatrician in their clinic. Like they just need a yes or no most of the time, unfortunately." (BFR01)

> "Whose knowledge of pharmacology, mine included, is dated and minimal really. And we're more likely to call somebody up and ask them, call up the pharmacist and ask them what we should do. But I think understanding how the model was built and having a statement about that. 'Cause if you show them this, they're not gonna take the time, but if they know that it's a model built on X, Y, and Z, that would be another summary statement. And then this is what we found with this drug for this age, baby or whatever. So it's really how you present the educational part of it's gonna be really important." (BFR02)

> "but I also think just to help clinicians get it because this is a harder topic […] explaining that to them in a accessible way... would be one of your challenges." (BFR25)

Make the Metric and Path to Its Use Audience-dependent: User Friendly for Pharmacists

> "I think it's complex enough, it needs like I think this is probably great for like a pharmacist or somebody who's good with these sorts of ideas." (BFR01)

> "I think so. I think in pharmacy school, we're taught basic statistics, we're taught about AUC. I think […] this document is very clear and to the point, and I think pretty much any pharmacist could understand this." (BFR22)

Make Visual Representation Essential

> "I think having a picture is always helpful for people, especially, that don't read well. So if you could show something to the family... And I was thinking it might be nice like the mom could color the whole thing and then the baby could move at the different ages 'cause then they have a better idea. Like this could be a light blue and this could be a dark blue. And then it shows where they overlap. Like I think that helps people too is to kind of see that." (BFR01)

> "Yeah, I think the table is probably the most helpful, especially […] the providers are much more familiar with the box plots and things, but the bedside nurses and the lactation consultants probably aren't. So from a nursing standard the table is probably the most user friendly." (BFR13)

Overcome Simulation Skepticism

> "I think that piece is difficult for people to understand too, right? This simulated idea, like what does that mean? Sometimes people don't trust things as much if it's like from a computer. So helping people to understand that." (BFR01)

> "But, PBPK modeling has been around a long time and not a lot of clinicians know about it. But when a drug company wants to study drug and children, which they're required to do now in the US, probably in Canada, too, when the drug company brings a drug to market, they're required to study it in children and the first step is to do some PBPK modeling to see, okay, what do we think the right dose will be? And so, we're now just applying that same concept to how much, what do we think the dose is in the breast milk, and […] this might make

people more excited about it because this is just the normal way in which we figure out what the dose is.
[…]
we do it by this modeling […] which is a lot more precise. So that, that can be […] eye-opening to people. […] Every drug you prescribe to a child, it probably was studied in this way originally. Now we're just bringing that to drugs and lactation. It makes a lot more sense […] something that's normally done in pediatric drug development. […] It's something you have been applying in clinical practice and just didn't realize it." (BFR03)

Provide a Definitive Bottom Line

"So, I mean, I mean gonna reference back to an old outdated system that we really don't use anymore, with like pregnancy categories, how we had the ABCDX, and what those numbers mean. So, if there's a way for us to say like, a UAR of 0.5 is on a yellow range like, it's okay, or cautious. Whereas, a UAR below 0.25 is green, or something like that. […] While this number is below in this range, let's go." (BFR06)

"I could foresee myself really appreciating of the metrics that this rate of UARs means that there is a X percent probability that the baby could have X outcome. […] That means that baby will have QT prolongation. Or, if you accept a UAR of a […] of a higher value, then that percentage of that risk decreases. So it's almost like a probability type of scale, because at the end of the day clinicians are going to care more about that than the actual milligrams of drug circulating in the baby, because what does that even mean at the end of the day if, that X milligrams circulating in the baby doesn't end up hurting them?" (BFR14)

Provide a Greater Maternal Emphasis

"I think that'd be significantly helpful if it included the mother. […] I feel like women are sacrificed for reproduction a little too much and […] I appreciate the concern for risk to a newborn infant […] and we certainly have an instinct to protect them, but also, it shouldn't be at the risk of [the mother's] health. And so I would want something that kind of emphasized, taking good care of the mother so the mother can take good care of the infant." (BFR05)

Provide Guidance to Interpret the UAR Metric

"We would have to know how to interpret the numbers, and not just be given a number and then we just shrug our shoulders at it." (BFR14)

"But if you come up with some sort of guidelines. And maybe the guidelines are not general. Maybe you're not like, 'A UAR of less than 0.3 is okay'…" (BFR20)

"So if something like, in general, if your UAR for this medication is less than 0.3 […] we're interpreting this for term infants from age, 0 to whatever, we feel that this is safe. I would like a better range in terms of […] these are acceptable levels..." (BFR24)

Provide Prospective Predictive Evidence

> "I guess missing any prospective data that tells you which choice is the best. […] That's the next step, right? I mean, that's where we go from here is to test these prospectively to see if they're predictive. But I don't know if I would call that a disadvantage, because no one has that currently. There is no metric that currently gives us that option." (BFR03)

> "Buy-in in my department consists of showing that, if you use this metric and base your decisions on it, that patient outcomes are going to be as good or better. So in this case, do a study that shows […] if we applied this metric to breastfeeding decisions for 10,000 infants, as opposed to the current metrics, these would've been the outcomes. This would've been what you recommended, and they all would've been just as good or potentially better off." (BFR09)

> "But at the end of the day, though, this metric would still have to undergo rigorous clinical application and study to see what these values actually mean and […] what it means in the baby clinically. Because at the end of the day, a number's a number, but the baby's clinical outcome is going to be what's most important." (BFR14)

Separate by Specific Cases and Scenarios

> "It would be tremendously helpful if you took, for example, several different psychiatric drugs or several different anticonvulsants. Or, I learned thus by comparison and contrast, and I think that over the years, I mean, that is what my many years of experience provide..." (BFR07)

> "Yes. That's helpful for the specific drug. It would be helpful though to compare some of the different drugs that I'm more familiar with to see how that works and how that process happens.
> […]
> it would be helpful to have a variety of different medications with different models. So I don't want see five medications and all of the models look exactly the same. I want to see 10 different medications and every model looks different. And then a description of why that model looks different for this medication compared to this medication so that we can start to understand how to interpret the differences in that model." (BFR10)

> "Yeah, I definitely could see it being used in the NICU setting. I think you would have to like break it up by gestational age, too." (BFR15)