Biomedicine & Pharmacotherapy 136 (2021) 111038

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Review

A comprehensive review on non-clinical methods to study transfer of medication into breast milk – A contribution from the ConcePTION project

Nina Nauwelaerts [a], Neel Deferm [a], Anne Smits [b,c], Chiara Bernardini [d], Bart Lammens [e], Peggy Gandia [f], Alice Panchaud [g,h], Hedvig Nordeng [i], Maria Laura Bacci [d], Monica Forni [d], Domenico Ventrella [d], Kristel Van Calsteren [j], Anthony DeLise [k], Isabelle Huys [l], Michele Bouisset-Leonard [m], Karel Allegaert [c,l,n], Pieter Annaert [a,*]

[a] KU Leuven Drug Delivery and Disposition Lab, Department of Pharmaceutical and Pharmacological Sciences, O&N II Herestraat, 49 3000, Leuven, Belgium
[b] Neonatal Intensive Care Unit, University Hospitals Leuven, UZ Leuven, Neonatology, Herestraat 49, 3000, Leuven, Belgium
[c] Department of Development and Regeneration, KU Leuven, Belgium
[d] Department of Veterinary Medical Sciences, University of Bologna, 40064, Ozzano dell'Emilia, BO, Italy
[e] BioNotus, Galileilaan 15, 2845, Niel, Belgium
[f] Laboratoire de Pharmacocinétique et Toxicologie, Centre Hospitalier Universitaire de Toulouse, France
[g] Service of Pharmacy Service, Lausanne University Hospital and University of Lausanne, Switzerland
[h] Institute of Primary Health Care (BIHAM), University of Bern, Switzerland
[i] PharmacoEpidemiology and Drug Safety Research Group, Department of Pharmacy, University of Oslo, PB. 1068 Blindern, 0316, Oslo, Norway
[j] UZ Leuven, Gynaecology and Obstetrics, Herestraat 49, 3000, Leuven, Belgium
[k] Novartis Pharmaceuticals Corporation, Novartis Institutes for BioMedical Research, One Health Plaza, East Hanover, NJ, 07936, USA
[l] KU Leuven, Department of Clinical Pharmacology and Pharmacotherapy, ON II Herestraat 49 – bus, 521 3000, Leuven, Belgium
[m] Novartis Pharma AG, Novartis Institutes for BioMedical Research, Werk Klybeck Postfach, Basel, CH-4002, Switzerland
[n] Department of Clinical Pharmacy, Erasmus MC, Rotterdam, the Netherlands

ARTICLE INFO

Keywords:
In vitro
In vivo
PBPK
Maternal medication
Breastfeeding
Lactation
Pharmacokinetics
Neonates
Infants
Medication exposure

ABSTRACT

Breastfeeding plays a major role in the health and wellbeing of mother and infant. However, information on the safety of maternal medication during breastfeeding is lacking for most medications. This leads to discontinuation of either breastfeeding or maternal therapy, although many medications are likely to be safe. Since human lactation studies are costly and challenging, validated non-clinical methods would offer an attractive alternative. This review gives an extensive overview of the non-clinical methods (*in vitro, in vivo* and *in silico)* to study the transfer of maternal medication into the human breast milk, and subsequent neonatal systemic exposure. Several *in vitro* models are available, but model characterization, including quantitative medication transport data across the *in vitro* blood-milk barrier, remains rather limited. Furthermore, animal *in vivo* models have been used successfully in the past. However, these models don't always mimic human physiology due to species-specific differences. Several efforts have been made to predict medication transfer into the milk based on physico-chemical characteristics. However, the role of transporter proteins and several physiological factors (*e.g.*,

Abbreviations: 3R, Refine, Reduce & Replace; ADME, Absorption, Distribution, Metabolism & Excretion; AUC, Area Under The Curve; BCRP, Breast Cancer Resistance Protein; BMAA, beta-N-methylamino-alanine; BME-UV, Bovine Mammary Epithelial (cell line); BMI, Body Mass Index; Caco-2, Cancer colon-2 (cell line); EGF, Epidermal Growth Factor; HMECs, Human Mammary Epithelial Cells; HIV, Human Immunodeficiency Virus; IVIVE, *In vitro* to *In vivo* Extrapolation; MATE, Multidrug And Toxin Extrusion protein; MDCK II, Madin-Darby canine kidney II; MEBM, Mammary Epithelial cell Basal Medium; M/P, Milk-to-Plasma; MRP, Multidrug Resistance-associated Protein; MCF-7, Michigan Cancer Foundation-7; OAT, Organic Anion Transporter; OATP, Organic Anion Transporting Polypeptide; OCT, Organic Cation Transporter; P, preterm; PAH, p-aminohippurate; PBPK, Physiologically-Based Pharmacokinetic; pMECs, porcine Mammary Epithelial Cells; PEPT, PEPtide Transporter; pgMECs, primary goat Mammary Epithelial Cells; P-gp, P-glycoprotein; popPK, population pharmacokinetic; RME cells, Rat Mammary Epithelial cells; T, term; TEA, tetraethylammonium; VP, very preterm.

* Corresponding author at: O&N II Herestraat 49-box 921, 3000, Leuven, Belgium.
E-mail addresses: nina.nauwelaerts@kuleuven.be (N. Nauwelaerts), neel.deferm@kuleuven.be (N. Deferm), anne.smits@uzleuven.be (A. Smits), chiara.bernardini5@unibo.it (C. Bernardini), bart.lammens@bionotus.com (B. Lammens), gandia_chu@yahoo.fr (P. Gandia), h.m.e.nordeng@farmasi.uio.no (H. Nordeng), marialaura.bacci@unibo.it (M.L. Bacci), monica.forni@unibo.it (M. Forni), domenico.ventrella2@unibo.it (D. Ventrella), kristel.vancalsteren@uzleuven.be (K. Van Calsteren), anthony.delise@novartis.com (A. DeLise), isabelle.huys@kuleuven.be (I. Huys), michele.bouisset-leonard@novartis.com (M. Bouisset-Leonard), karel.allegaert@uzleuven.be (K. Allegaert), pieter.annaert@kuleuven.be (P. Annaert).

https://doi.org/10.1016/j.biopha.2020.111038
Received 24 August 2020; Received in revised form 3 November 2020; Accepted 16 November 2020
Available online 30 January 2021

variable milk lipid content) are not accounted for by these methods. Physiologically-based pharmacokinetic (PBPK) modelling offers a mechanism-oriented strategy with bio-relevance. Recently, lactation PBPK models have been reported for some medications, showing at least the feasibility and value of PBPK modelling to predict transfer of medication into the human milk. However, reliable data as input for PBPK models is often missing.

The iterative development of *in vitro*, animal *in vivo* and PBPK modelling methods seems to be a promising approach. Human *in vitro* models will deliver essential data on the transepithelial transport of medication, whereas the combination of animal *in vitro* and *in vivo* methods will deliver information to establish accurate *in vitro/in vivo* extrapolation (IVIVE) algorithms and mechanistic insights. Such a non-clinical platform will be developed and thoroughly evaluated by the Innovative Medicines Initiative ConcePTION.

1. Introduction

About 50 % of postpartum women need medication, and the evidence on the beneficial effects of breastfeeding is growing [1]. Yet, data on the safe use of medication during breastfeeding is lacking for a large share of medicines [2]. This knowledge gap often forces women either to postpone a much-needed treatment or to discontinue breastfeeding at the advice of their healthcare professionals. Strong scientific evidence could prevent this unnecessary choice. In fact, many medications are likely to be safe as the transfer into the breast milk and/or neonatal gastrointestinal absorption are expected to be low for a majority of medications. However, clinical lactation studies are costly, time-consuming and encounter many practical and ethical limitations. Therefore, validated non-clinical research methods could play a crucial role in filling the knowledge gap in this field.

This comprehensive review aims to provide a state-of-the art of non-clinical (*in vitro, in vivo* and *in silico*) methods to determine transfer of medication during lactation.

2. Methods

A comprehensive ("non-systematic") review was performed. Five distinct literature searches (see Supplemental Data S1: Search Terms) for *in vitro* models, *in vivo* animal models, empirical and semi-mechanistic models, PBPK models and the effect of maternal conditions on the macro-nutrient composition of breast milk were performed searching PubMED between September 2019 and December 2019. In addition, Embase was searched for the *in vitro* and *in vivo* models. Articles were excluded if no full text was available or if they were not written in English. The selection of the articles for the *in vitro* models and PBPK models was performed using Rayyan [3].

2.1. In vitro animal models

Articles regarding *in vitro* models for the blood milk epithelial barrier, using cell lines or primary cells, were included.

2.2. In vivo animal models

Articles on animal models to predict human breast milk exposure or human neonatal systemic exposure to maternal medications *via* breastfeeding were included.

2.3. Empirical and semi-mechanistic models (human)

Articles on empirical or semi-mechanistic models to predict human breast milk exposure were included.

2.4. Physiologically-based pharmacokinetic (PBPK) models

Articles about PBPK models to predict human breast milk exposure or neonatal systemic exposure to maternal medication *via* breastfeeding were included. A review on the use of PBPK modelling regarding lactation was conducted previously [4]. However, at that time (in 2003) PBPK models were only available for chemical substances or for the dairy industry.

2.5. The effect of maternal conditions on macro-nutrient composition of breast milk

Articles about the effect of maternal conditions on the macro-nutrient composition of breast milk were included.

3. Results

3.1. In vitro models

3.1.1. Available in vitro models for the mammary epithelium

In vitro cell culture models for the mammary epithelium have been established to predict medication partitioning into the breast milk, based on the assumption that the mammary epithelium (Fig. 1) is the main barrier between the systemic (maternal) circulation and the milk. Many cell culture models have been established based on human or animal mammary epithelial cell cultures, including both primary cells and cell lines (Table 1*)*. These cell culture models can be used to study not only passive, but also active transport of medication across the blood milk barrier. In 2006, the first human model to predict medication transfer into the human breast milk was developed by Kimura et al. [6]. They used the method from Schmidhauser et al. [7] to obtain trypsin-resistant cells, which have the ability to differentiate into the lactating state. More recently, Andersson et al. [8] developed a model to evaluate the transfer of the neurotoxic amino acid beta-*N*-methylamino-alanine (BMAA) into the breast milk based on its uptake in the human mammary MCF-7 cell line. Other mammary cell lines have been used to investigate the mammary gland (*e.g.*, PMC42-LA [9]) and in the field of breast cancer (*e.g.*, R5, MCF-7, MDA-MB-231-LUC, MCF10A and primary epithelial cells [10]). In addition, MDCK-II cells transfected with human Breast Cancer Resistance Protein (BCRP) have also been used to optimize predictions of milk-to-plasma (M/P) ratios for medications [11]. Many *in vitro* models relying on mammary epithelial cells have been established based on cells obtained from animal tissue.

3.1.2. Culture conditions

Mammary epithelial cells are either obtained *via* isolation from normal breast tissue [25], tumor breast tissue [25] or breast milk [26]. Currently, some cell lines can also be obtained from commercial cell suppliers. Mammary epithelial cell basal medium (MEBM) (Table S1*)*, with addition of several supplements (Table S2), is recommended for the growth of HMEC [6]. RPMI 1640, DMEM and DMEM:F12 (50:50) have been used for human mammary epithelial cell lines and cells from animal origin [8]. Basal RPMI 1640 and DMEM:F12 (50:50) are not specific for epithelial cells. However, the addition of particular supplements makes them suitable for the growth of mammary epithelial cells. Different supplements concentrations (Table S2) are recommended by the suppliers and in previously mentioned *in vitro* culture models. The choice of supplements and in particular, prolactin, is strategic when working with a model of secreting cells. For instance, Freestone et al. [9] were able to make a model for the resting, lactating and suckled

mammary epithelium, by adding either no, 200 ng/ml, or 800 ng/ml prolactin, respectively, to the PMC42-LA cell line.

3.1.3. Characterization

Characterization of the *in vitro* models, especially of the transporters (Fig. 2), is important to ensure that the model is applicable to the *in vivo* situation for a specific lactation period and a given compound. An overview of the available information regarding transporters has recently been given by Ventrella et al. [38].

3.1.4. Human in vitro models to predict medication transfer into the breast milk

Two human models have currently been reported for the prediction of medication transfer into the breast milk. Kimura et al. [6] used HMEC: they were able to obtain a monolayer with a transepithelial electrical resistance of 227 +/- 11 Ohm. cm^2 after three trypsin treatments, indicating a tight monolayer. They detected beta-casein mRNA, which demonstrates that the monolayer is differentiated into the lactating state. Shipman et al. [39] showed that beta-casein is only expressed in the lactating state. Besides beta-casein, Kimura et al. [6] also detected mRNA of organic cation transporter (OCT)1 and OCT3. Interestingly, they found that OCT1 mRNA increased, whereas OCT3 mRNA decreased with increasing number of trypsin treatments. This observation was consistent with the observations of Alcorn et al. [29], who observed that OCT1 mRNA levels are higher, while OCT3 mRNA levels are lower *in vivo* in the lactating state compared to the non-lactating state. Kimura et al. [6] also investigated the function of OCT and organic anion transporter (OAT) with the substrates tetraethylammonium (TEA) and p-aminohippurate (PAH), respectively. A clear directionality was observed for TEA, indicating that functional OCT is present. However, no directionality was observed for PAH. Other transporters that have not been investigated in this study, for instance BCRP, play an important role *in vivo*. Therefore, further characterization of this *in vitro* model is required in order to conclude whether this is a good model to evaluate medication transfer into the human breast milk.

Andersson et al. [8] developed a human *in vitro* model to investigate the transfer of D-BMAA and L-BMAA into the human breast milk based on their uptake in MCF-7 cells. MCF-7 (Fig. 3) is a cell line derived from a metastatic site of an adenocarcinoma, *via* pleural effusion [40]. MCF7 expresses estrogen and progesterone receptors and is known to have some characteristics of the differentiated mammary epithelium [40]. Andersson et al. [8] found that uptake was higher in the differentiated model. Furthermore, *via* inhibition studies with natural amino acids, they concluded that several amino acid transporters might be involved in the uptake. Additionally, they found some differences in mRNA levels of orthologous transporters in the MCF-7 cell line compared to the mouse HC11 cell line. Finally, they compared mRNA expression in undifferentiated and differentiated HC11 cells and found that mRNA increased for some transporters, whereas mRNA decreased for others.

Table 1
Cell culture models of the mammary epithelium.

Cell culture model	Species	References
Primary culture of Human Mammary Epithelial Cells (HMEC)	Human	[6]
Michigan Cancer Foundation-7 (MCF-7) cells	Human	[8]
Madin-Darby canine kidney (MDCK) II cells transfected with BCRP	Dog	[11]
HC11 mouse mammary epithelial cells	Mouse	[8]
CIT3 mouse mammary epithelial cells	Mouse	[12,13,14]
Rat Mammary Epithelial (RME) cells	Rat	[15]
Primary culture of porcine Mammary Epithelial Cells (pMECs)	Porcine	[16,17,18,19]
BME-UV Immortalized Bovine Mammary Epithelial cells	Bovine	[20,21,22,23]
Primary goat Mammary Epithelial Cells (pgMECs)	Goat	[24]

Besides MCF-7, many other human cell lines have been used to investigate the mammary gland. For example, MCF-10A has been used commonly as a model to investigate normal breast cells. MCF-10A is an immortalized, non-tumorigenic cell line obtained from benign proliferative breast tissue [41]. MCF-10A does not express estrogen or progesterone receptors. Furthermore, no beta-casein or alfa-lactalbumin were detected [41]. In addition, Ying Qu et al. [41] questioned whether MCF-10A cells are a good model for normal breast cells. They conclude that further investigations are required.

Another frequently used cell line is the MDA-MB-231, which was obtained *via* pleural effusion of a patient with metastatic mammary adenocarcinoma [42]. The MDA-MB-231 cell line does not express an estrogen or progesterone receptor. Moreover, MDA-MB-231 might not be suitable as a model for normal lactating mammary epithelial cells, as it is a highly aggressive, invasive and poorly differentiated cell line that is mainly used to investigate triple-negative breast cancer.

Finally, PMC42-LA is an epithelial cell line derived from PMC42, a mesenchymal breast carcinoma cell line that has been obtained from a pleural effusion. Although PMC42-LA has not been used as frequently as the previously mentioned cell lines, it might be a good model for the lactating mammary gland. In fact, Freestone et al. [9] were able to develop a resting, lactating and suckling *in vitro* model with this cell line by using different concentrations of prolactin. They indicated that the capacity to differentiate into a lactating state is a major advantage of this cell line compared to many other cell lines. It has also been shown that PMC42-LA cells express beta-casein after stimulation with lactation hormones [9].

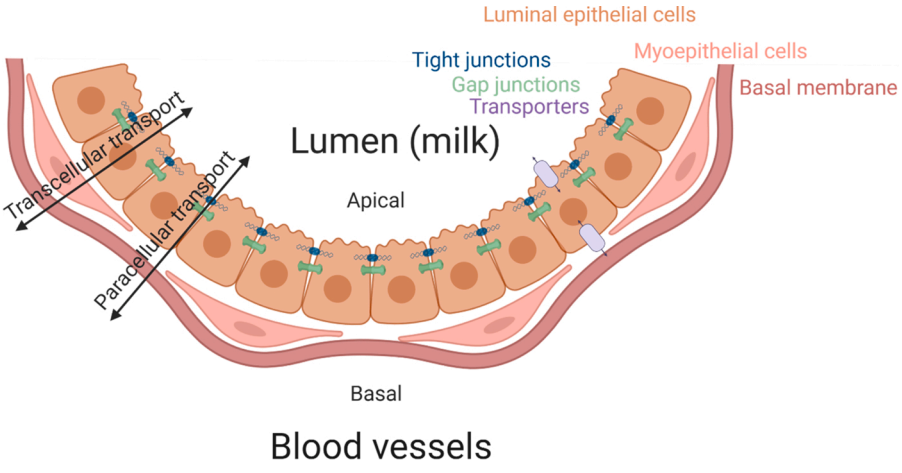

Fig. 1. The mammary epithelium consists of lobes, containing alveoli and milk ducts. The luminal epithelial cells or acini cells secrete milk into the lumen of the alveolus. The surrounding myoepithelial cells contract under the stimulation of oxytocin and cause excretion of the milk into the ducts. Transport of endogenous compounds as well as medication is possible *via* passive or active transport mechanisms. Transcellular transport implies crossing of cell membranes, whereas paracellular transport occurrs via the confined spaces between cells.

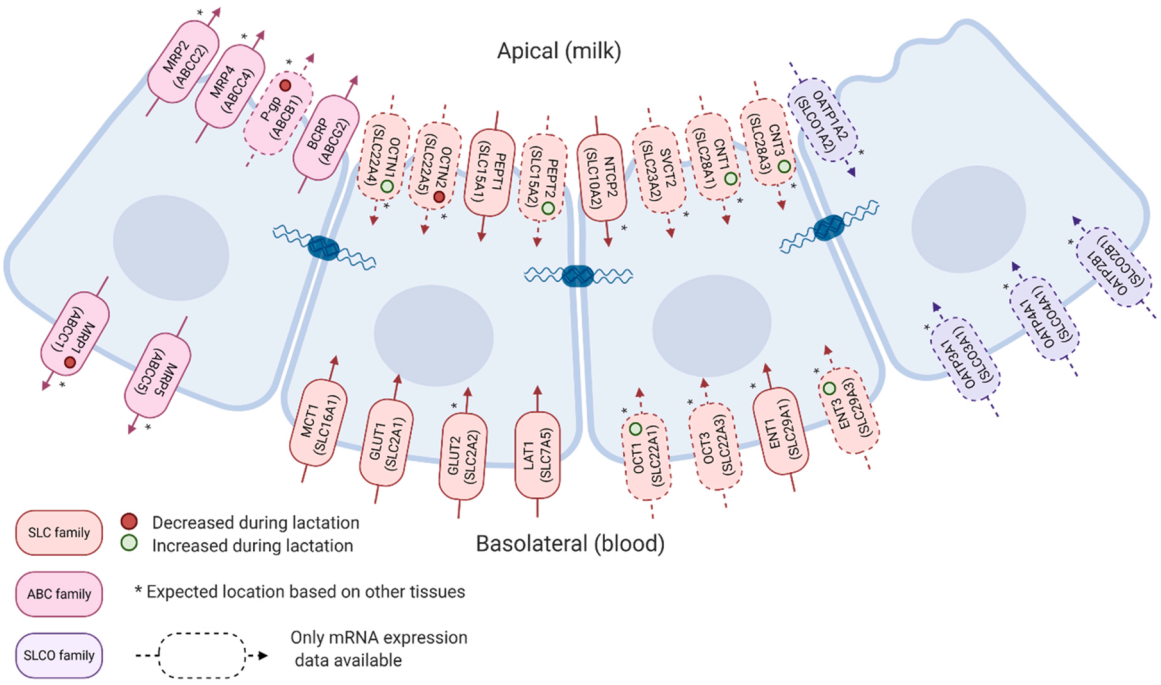

Fig. 2. Transporter expression in human mammary epithelial cells [6,27,36,37,28–35].
Transporters detected either in primary human mammary epithelial cells from commercial suppliers and/or in mammary epithelial cells isolated from milk or breast tissue. The arrows indicate the direction of the transport. If no evidence was found for the location of the transporter in the mammary gland, the transporter was placed on the expected location based on locations in other tissues (indicated with asterisk). Furthermore, transport protein expression was also found for ABCA7, ABCA13, ABCF2, ABCF3, SLC1A4, SLC1A5, SLC3A1, SLC4A2, SLC4A7, SLC5A3, SLC7A1, SLC7A2, SLC9A1, SLC9A3R1, SLC9A3R2, SLC12A1, SLC12A4, SLC12A9, SLC15A4, SLC16A2, SLC16A3, SLC20A2, SLC26A2, SLC27A1, SLC27A2, SLC30A1, SLC31A1, SLC35E1, SLC39A14, SLC44A2 & SLC52A2. However, these transporters were not included in the figure since they are currently not known to play an important role in the transport of medication.

3.1.5. Animal in vitro models to predict medication transfer into the breast milk

Prediction of medication transfer into the human breast milk based on animal *in vitro* models might be difficult due to species-specific differences (*e.g.* differences in enzyme and transporter expression and activity). However, these *in vitro* models, in combination with animal *in vivo* models, can play a pivotal role in the *in vitro to in vivo* extrapolation of human *in vitro* data, as they can generate fundamental and mechanistic knowledge.

Two main categories can be distinguished: rodent and non-rodent models. Among the rodent cell lines: Rat Mammary Epithelial (RME) cells are derived from normal mammary glands of 50−60-day-old virgin female Lewis rats [15], while both mouse cell lines (HC11 and CIT3 cells) are derived from COMMA-1D cells, obtained from mammary tissue of BALB/c mice in the middle of pregnancy. CIT3 are selected for resistance to triple trypsinization, while HC11 have been immortalized. Both cell lines have been used for active transport studies of medications such as nitrofurantoin.

Among non-rodent cell lines, Bovine Mammary Epithelial cell line BME-UV is a clonal cell line established from primary epithelial cells from a lactating Holstein cow. This cell line expresses functional markers such as microvilli and desmosomes and secreting properties [43], and expresses functional organic anion and cation transporters [21,22]. Primary goat mammary epithelial cells (pgMECs) derived both from mammary tissue of lactating or non-lactating juvenile goats grow *in vitro* for several passages and remain hormone and immune responsive with a secreting phenotype (55). Porcine mammary epithelial cells (pMECs) can be derived from non-pregnant and non-lactating gilt [17]. These primary cells can be maintained in culture for at least 15 passages and could represent a good model for studying molecular regulation and synthesis of milk (excretion). Alternatively, porcine mammary epithelial cells can be obtained from mammary gland after parturition [18].

3.2. In vivo animal models

Different aspects need to be considered when selecting an animal species for modelling the lactational transfer of xenobiotics in human [44]. The main parameters related to lactation (anatomy of the udder, amount of milk production, composition of milk, duration of lactation and hormone responsiveness) and medication disposition (transporters and enzymes) vary across different animal species. [23,45,54,55,46–53].

Rodents are usually considered an excellent model in many fields of research, but their metabolic and digestive patterns and milk composition are quite different from humans, with significant differences in medication levels reached in blood and the potential lactational transfer [56–58]. Indeed, in such species, the general aspects of reproduction (age of sexual maturation, hormone sensitivity, reproductive lifespan, litter size) are very different when compared to humans [49]. Nevertheless, most studies clarifying the development of mammary cancer have been performed in rodents due to their relatively easy manipulation and housing requirements [59,60]. An additional issue with working with rodents relates to the body dimension. More specifically, rodents allow only for small volume milk sampling limiting the possibility of end point analysis.

Medication transfer from blood to milk has been extensively studied in ruminants [46,61], mainly for human safety reasons, due to their role as food producing animals (in particular milk and dairy products). Nevertheless, these animals differ significantly from human from an anatomical point of view and mainly, in absorption and metabolic capacity due to their peculiar gastrointestinal physiology.

Swine offer a generally accepted model in translational medicine based on their anatomical and physiological similarities with humans. Its use as a model for nutritional physiology, medication testing and metabolism has been generally acknowledged [45,52,62–65]. Mammary gland anatomy shows macroscopic differences but, at a molecular

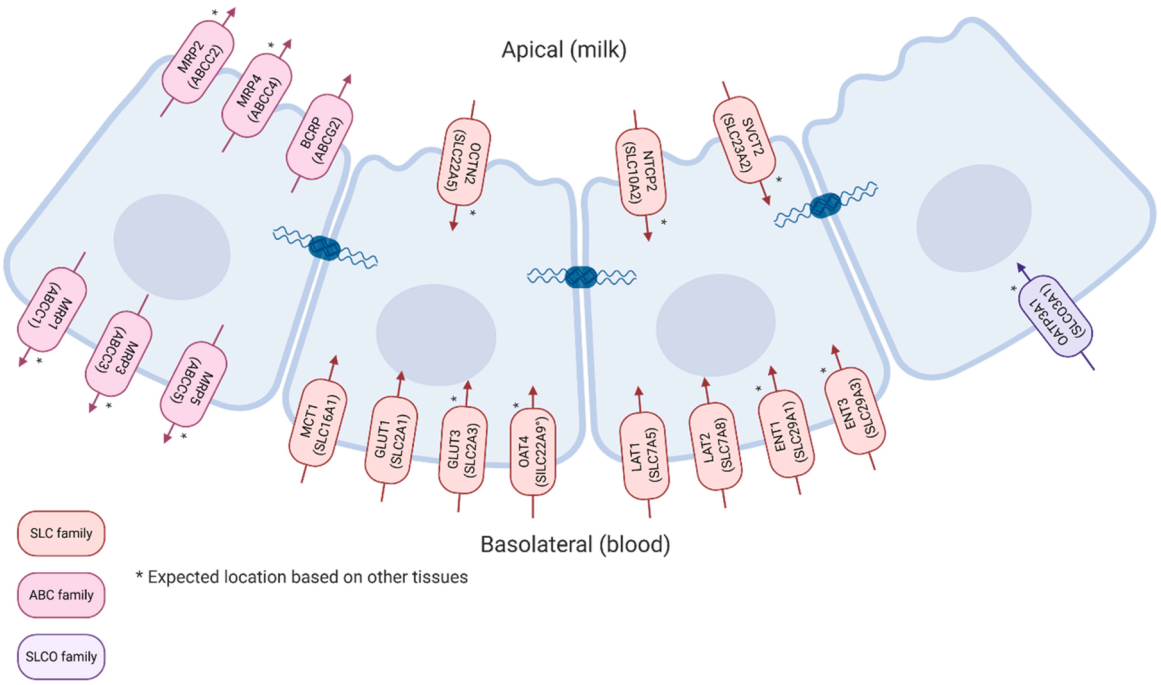

Fig. 3. Transporter expression in the MCF-7 cell line [73,74,83,84,75–82].
The arrows indicate the direction of the transport. When no evidence was found for the membrane localization of the transporter in the mammary epithelial gland cells, the transporter was placed on the expected location based on other tissues. Furthermore, transporter protein expression was also found for ABCA1, ABCA3, ABCA7, ABCA11, ABCA12, ABCA13, ABCB5, ABCB6, ABCF2, ABCF3, ABCG1, SLC1A3, SLC1A4, SLC1A5, SLC1A6, SLC3A1, SLC3A2, SLC4A1, SLC4A2, SLC4A7, SLC4A8, SLC4A11, SLC5A3, SLC5A6, SLC6A6, SLC6A8, SLC6A15, SLC6A18, SLC7A1, SLC7A2, SLC7A6, SLC7A10, SLC8A3, SLC9A1, SLC9A3R1, SLC9A3R2, SLC9A7, SLC10A7,vSLC11A2, SLC12A2, SLC12A4, SLC12A6, SLC12A7, SLC12A9, SLC15A4, SLC16A2, SLC16A4, SLC16Z6, SLC16A12, SLC17A9, SLC19A1, SLC19A2, SLC20A1, SLC20A2, SLC22A18, SLC22A23, SLC24A1, SLC26A2, SLC26A3, SLC26A4, SLC26A6, SLC26A8, SLC26A11, SLC27A1, SLC27A2, SLC27A5, SLC27A6, SLC30A1, SLC30A9, SLC31A1, SLC34A3, SLC35C2, SLC35E1, SLC35F2, SLC35F3, SLC36A, SLC36A4, SLC38A1, SLC38A2, SLC38A6, SLC39A1, SLC39A3, SLC39A6, SLC39A8, SLC39A9, SLC39A10, SLC39A14, SLC41A3, SLC43A2, SLC43A3, SLC44A1, SLC44A2 SLC44A3 & SLC52A2. However, these transporters were not included in the figure since they are currently not known to play an important role in the transport of medication.

level, the presence of medication transporters similar to human has been reported (*See* Table 2 *from Ventrella* et al. [38]). However, the lactation duration is shorter, compared to humans. These points have to be considered when studying the lactational transfer in the different phases of lactation, especially for colostrum composition [55]. The litter size allows for an easy milk collection without interfering with the lactation process and piglet's growth; piglets can be analyzed individually and have been already utilized as animal model for pediatric PK/PD [66]. In recent years, the minipig, and in particular Göttingen Minipigs, has been proposed as a more relevant animal model in translational medicine in particular for toxicological studies [67,68]. They show, together with all the positive characteristics described for domestic swine breeds, a reduced growth rate that makes them easier to use. Moreover, since some minipig breeds, such as the Göttingen one, are specifically bred and produced for experimental purposes, they allow for a much more precise genetic and microbiologic standardization, as well as a standardization of the interactions between animals and the housing context, including operators, leading to a significant reduction in the number of animals needed for experimental trials [69,70]. The fact that Göttingen Minipigs are available in a constant and uniform quality worldwide forms the basis of their recognition by regulatory bodies as a suitable non-rodent species for toxicology studies. However, data regarding qualitative and quantitative composition of the milk, as for the domestic pig, are lacking.

The most relevant papers on animal models of lactational transfer are listed in Table 2with indication of the relevant applications and the species studied.

3.3. *Empirical and semi-mechanistic models (human)*

Purely empirical models describe the correlation between data, while mechanistic models (including PBPK models) also account for the underlying physiological processes. Semi-mechanistic models lay between the empirical models and the mechanistic models. For some aspects they rely on physiologically relevant mechanisms, whereas other aspects of the model are not physiologically relevant.

Several attempts have been made to predict the transfer of medication into human milk using different physicochemical parameters. In 1959, Rasmussen et al. [71] first assumed pH-dependent diffusion of medication, while Notarianni et al. [72] developed an equilibrium dialysis model to test the partitioning of medication between freeze dried plasma and baby formula powder over a dialysis membrane. Other diffusion models have been developed by Atkinson et al. [73] and Fleishaker et al. [74]. Meskin and Lien [75] each developed an *in silico* model based on the relation between physicochemical properties (the molecular weight, partition coefficient and degree of dissociation) and the transfer of medication into the milk. This model was later extended with an artificial neural network by Agatonovic-Kustrin [76]. Quantitative structure activity (or property) relationship tools have been explored as well [77]. Many others have tried to predict milk transfer using similar methods. However, the major limitation with all of these methods was that they do not take transporter-mediated processes into account.

In 2011, a semi-mechanistic model was developed by Koshimichi et al. [78] (*See Fig (1) from Ventrella* et al. [38]) to predict medication transfer into the milk [78]. Milk secretion and reuptake clearance values were estimated by curve fitting against observed milk and plasma concentration-time profiles. Next, the fraction of unbound medication in

Table 2
Animal models of lactational transfer.

Main aspect of model discussed	Species	References
Animal models in evaluation of the safety of medication used during lactation	General aspects not species-related	[44]
Biomarkers	Swine	[63,64]
Colostrum	Swine	[55]
Comparative pathology of tumors	Mouse	[59]
Digestive system	Swine	[52]
Drug development	Rodents	[65]
Drug metabolism	Rodents, guinea pig, rabbit	[47]
	Rodents	[56]
Medication milk transfer	Sheep	[61]
	Rat	[58]
Efflux Transporter	Rodents, swine, bovine	[23,50,51]
	Bovine	[46]
	Swine	[53]
Comparison of human and mouse metabolism and reproductive lifespan	Mouse	[49]
Hormonal sensitivity	Mouse	[60]
Metabolism	Rodents	[57]
Metformin treatment during pregnancy	Swine	[62]
Nutritional aspects	Swine	[45]
Non-clinical models of Lactational transfer	Rodents, swine, rabbit, sheep, bovine	[38]
Pharmacokinetics	Swine (minipig)	[68]
Pharmacokinetics and pharmacodynamics	Swine	[66]
Placentation	Rodents, guinea pig, hamster, rabbit, cat, swine, sheep, bovine, horse	[54]
Sexual maturation	Swine (minipig)	[69]
Toxicology	Swine (minipig)	[67,70]

the milk was compared to the fraction of unbound medication in the plasma for each medication to determine whether passive diffusion or transporter-mediated transfer is the most likely route for medication transfer into the human breast milk. For the medications with passive diffusion as main pathway, an equation describing the relation between the physicochemical properties and the secretion and reuptake clearance values was determined by multiple linear regression. This semi-mechanistic model was applied to determine M/P Area Under the Curve (AUC) ratios for 71.9 % of the 49 medications, be it within a 3-fold error compared to observed values [78].

3.4. Physiologically-based pharmacokinetic (PBPK) models

3.4.1. Physiologically-based pharmacokinetic (PBPK) modelling

A PBPK model is defined by the European Medicines Agency as "a mathematical model that simulates the concentration of a medication over time in tissue(s) and blood, by taking into account the rate of medication Absorption into the body, Distribution in tissues, Metabolism and Excretion (ADME) on the basis of interplay between physiological, physicochemical and biochemical determinants" [81].

PBPK modelling is for instance often applied to predict drug-drug interactions and to select an initial dose for pediatrics and first-in-human trials [81]. Furthermore, PBPK modelling can be used to predict transfer of compounds into the breast milk, and subsequent neonatal systemic exposure. Research in this field has focused on long lasting, bio-accumulative substances (*e.g.* trichloroethylene) in the field of toxicology, and milk transfer in animals providing milk for human consumption [4].. More recently, some PBPK models for the prediction of transfer of medication into the human breast milk and subsequent neonatal systemic exposure, further referred to as lactation PBPK models, have been reported (Table 3). Five articles and four conference abstracts about lactation PBPK models were retrieved, all within the last decade. The reported lactation PBPK models consist of a maternal PBPK model coupled to a neonatal PBPK model (Fig. 4) allowing them to predict milk transfer and neonatal systemic exposure *via* breastfeeding. The PBPK models for alprazolam, caffeine and tramadol only consist of a maternal PBPK model, and can thus only predict milk transfer [82,83]. The model for escitalopram is a combination of population pharmacokinetics (popPK) to analyze human breast milk data and PBPK modelling to predict infant exposure [84]. These five cases will be discussed in the next sections.

The goal of the lactation PBPK models was to predict the exposure of neonates to maternal medication *via* breastfeeding. In the case of codeine, the focus was on differences in exposure due to maternal and neonatal differences in CYP2D6 genotype and therefore, morphine formation [86]. The PBPK model for isoniazid also aimed to investigate the impact of the polymorphic N-acetyltransferase-2 [89]. No genotype specific simulation was performed for escitalopram, rifampicin or ethambutol [84,88]. In the case of efavirenz, CYP2B6 polymorphism is known to have an impact on the metabolism. Olagunju et al. did not perform genotype specific simulations but used reported data for

Table 3
PBPK models for transfer of medication into the human breast milk and subsequent neonatal systemic exposure.

Compound (indication)	Dose	Administration route	Software	References
Alprazolam (Anxiety disorder)	0.5 mg single dose	Oral	SimCyp Simulator V16	Abstract [82]
Caffeine (mental alertness)	200 mg single dose	Oral	SimCYP Simulator V16	Abstract [82]
Clonidine (hypertension)	150 μg twice a day	Oral	ADAPT II Software	Abstract [85]
Codeine (post-labor pain)	2.5 mg/kg/day (twice a day administration)	Oral	PK-Sim version 4.0 MoBi version 2.0 MATLAB version 7 MoBi Toolbox for Matlab version 2.0	[86]
Efavirenz (human immunodeficiency virus)	400 mg/day or 600 mg/day	Intramuscular	SimBiology version 5.1 MATLAB 2014b	[87]
Escitalopram (depression, including postpartum)	20 mg/day	Oral	PK-SIM version 6.3 MATLAB	[84]
Ethambutol (Mycobacterium tuberculosis infection)	25.4 mg/kg	Oral	MATLAB version 8.0	[88]
Isoniazid (Mycobacterium tuberculosis infection)	300 or 900 mg/3days	Oral	R version 3.4.1. Packages: deSolve ggplot2 zoo	[89]
Lamotrigine (anti-epileptic)	200 mg/day		ADAPT II Software PK Sim	Abstract [90]
Rifampicin (Mycobacterium tuberculosis infection)	10.9 mg/kg	Oral	MATLAB version 8.0	[88]
Tramadol (Opioid Analgesic)	100 mg twice a day	Oral	SimCYP Simulator V16	Abstract [83]

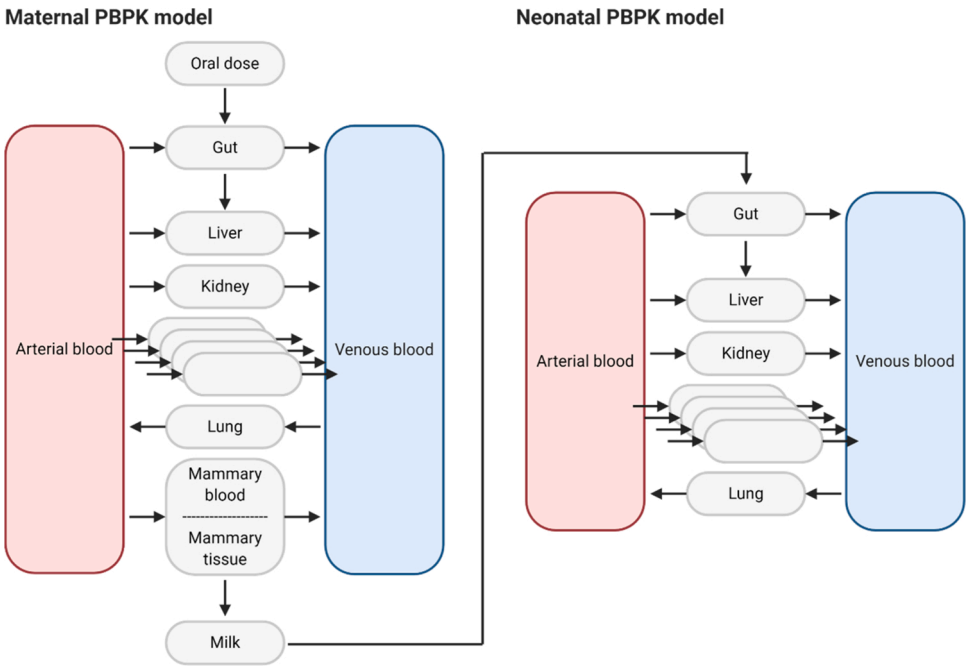

Fig. 4. Structure of lactation physiologically-based pharmacokinetic (PBPK) models for medication milk transfer and neonatal exposure of the the breastfeeding infant to maternal medication.
The lactation PBPK models are built with a maternal PBPK model coupled to a neonatal PBPK model with oral administration.

pediatric CYP2B6 protein expression in which they induced variability.

3.4.2. *Physiologically-based pharmacokinetic (PBPK) model structure*

Transfer of medication into the human breast milk can be modelled in several ways [89]:

(i) direct transfer of the medication from the blood into the human breast milk [91];
(ii) *via* uptake into the breast adipose tissue [92]; or
(iii) a combination of both routes [93].

All three approaches have been used for chemical substances. The currently available lactation PBPK models for medication all used the first approach as described below. Distinct software packages have been used to develop the existing models.

The lactation PBPK models used a whole-body PBPK modelling approach. They combine a maternal PBPK model including a breast compartment with a neonatal PBPK model. The model for escitalopram combines popPK on maternal monitoring data with a neonatal PBPK model [84]. Willmann et al. [86] combined four PBPK models: maternal PBPK models for codeine and its main active metabolite morphine, and neonatal PBPK models for codeine and morphine.

Different approaches are used to couple the maternal and neonatal models. Delaney et al. [84] first analyzed the escitalopram concentrations in the breast milk using popPK. In a next step, they calculated daily infant doses using a random combination of the predicted milk escitalopram concentrations, milk volumes per feed and frequencies of feeding. The calculated daily infant doses are then administered to the neonatal PBPK model as a single dose. Willmann et al. [86] used a similar approach, but they administered the medication to the neonatal PBPK models as multiple doses. They assumed breastfeeding, and thus dosing the neonate to take place each 3 h. The doses were calculated using the medication concentrations at the time of breastfeeding predicted by their maternal PBPK model and the breast milk volume. Willmann et al. assumed that the absorption of the medication in the neonatal model is fast and complete. Furthermore, Olagunju et al. [87] calculated the milk concentration by multiplying the M/P ratio with the simulated plasma concentration. Subsequently, they calculated the infant dose per breastfeeding session by multiplying the milk volume with the milk concentration.

Garessus et al. [89] used another approach, assuming that the breast is completely emptied during each feed. This means that the dose given to the neonatal PBPK model is equal to the amount of isoniazid in the breast milk at the time of breastfeeding. Dosing of the neonatal model is repeated every two hours. Partosch et al. [88] developed a PBPK model for ethambutol and a PBPK model for rifampicin. The PBPK models for both medications have a similar structure. They included the breast compartment in the maternal PBPK model as a reservoir. Excretion into the reservoir can be calculated by multiplying the milk volume with the milk concentration. The milk concentration is calculated by multiplying the plasma concentration with the M/P ratio. Every 4 h, the reservoir is opened for 30 min, allowing the medication to transfer to the neonate *via* a milk dose compartment.

Table 4 gives an overview of the different breastfeeding parameters that have been used in the lactation PBPK models. A review aiming to

Table 4
Breastfeeding parameters used in the lactation PBPK models.

Infant weight	Milk intake	Frequency of feeds	Duration of breastfeeding	References
5.43 kg SD: 1.3	76.0 ml/feed SD: 12.6 150 ml/kg/day	11 feeds/day SD: 3	N/A	[83]
4 kg	0.1134 l/feed	Every 2h	N/A	[84]
N/A	13 g/kg/day (d1) 40 g/kg/day (d2) 98 g/kg/day (d3) 140 g/kg/day (d4) 155 g/kg/day (d5)	Every 3h	N/A	[85]
3.5 kg	0.185 l/kg/day (8d – 4 months)	Every 4h	30 min	[86]
N/A	Milk volume controlled by infant suckling rates from literature	Every 2h	N/A	[87]

Abbreviations: SD: standard deviation.

quantify breast milk intake feeding parameters for input into PBPK models has recently been published by Yeung et al. [94]. They developed a nonlinear regression equation on the weight-normalized human milk intake. Their results show a maximum intake of 152.6 ml/kg/day and a weighted mean feeding frequency of 7.7 feeds/day for exclusively breastfed term infants.

PBPK modelling aims to predict *in vivo* concentration-time profiles based on:

(i) medication-specific parameters; and
(ii) physiological parameters.

The medication-specific data required for the development a PBPK model using Simcyp™ (Certara, UK) are summarized in the manuscript of Zuhang et al. [95]. Several sources of input data can be used. Delaney et al. [84] measured *in vivo* escitalopram concentrations in breast milk from 18 lactating women. They used clearance values from literature, which had been determined *in vitro*. Age-dependent algorithms were used to scale the parameters for the neonatal PBPK model. Garessus et al. [89] used *in vivo* data, including an AUC based M/P ratio, obtained from literature. AUC based M/P ratios are preferred over single M/P ratios, since single M/P ratios vary over time. Some of the *in vivo* data were re-calculated to match their population. Partition coefficients were calculated according to an algorithm from Schmitt et al. [96], which was also used by Partosch et al. [88]. Both Garessus et al. [89] and Willmann et al. [86] used adult clearance values fitted from *in vivo* data, whereas the neonatal clearances were *in vivo* values obtained from literature. Willmann et al. [86] used a range of M/P ratios based on several *in vivo* values reported in literature for both morphine and codeine. Partosch et al. [88] used physiological data from literature. *In vivo* clearance values were obtained from literature. For ethambutol, the M/P ratio was based on two *in vivo* data pairs from literature. For rifampicin, an algorithm to estimate the M/P ratio was used, because it was not clear how the measurements were done for the sparse available *in vivo* data [88]. Olagunju et al. [87] used an *in vivo* M/P_{AUC} ratio. They used anthropometric values to predict organ weight and blood flows based on a HIV positive cohort of breastfeeding women. For the maternal PBPK model, CYP450 abundances were taken from *in vivo* data. For the neonatal PBPK model, data from human liver microsomal samples were used.

3.4.3. Evaluation of the physiologically-based pharmacokinetic (PBPK) models

PBPK models should be evaluated for their ability to predict *in vivo* pharmacokinetic data. All lactation PBPK models exist as a maternal PBPK model coupled to a neonatal PBPK model [84,86–89]. The lactation PBPK models, except for codeine [86] and escitalopram [84], used a separate evaluation for the maternal PBPK models and the neonatal PBPK models. The evaluation for the maternal PBPK models was done by comparing *in vivo* plasma concentration profiles from literature with predicted plasma concentration profiles. Garessus et al. [89] used matched dosing regimens and also compared breast milk concentrations. For escitalopram, medication milk concentration data was coupled to a neonatal PBPK model. They first evaluated an adult PBPK model against *in vivo* data and then extrapolated it to neonates and again verified this with *in vivo* data [84]. A bootstrapping technique was used by Delaney et al. to evaluate the adult PBPK model, for which they pre-specified that the PBPK model would be accepted if the mean plasma AUC_{∞} of the observed data fell within a 95 % confidence interval of the mean of the predicted data. Olagunju et al. [87] mentioned an acceptance criterion of a 2-fold difference against observed data.

Different approaches were taken to evaluate the neonatal PBPK models. Delaney et al. [84] and Olagunju et al. [87] compared predicted neonatal plasma concentrations to *in vivo* plasma concentrations obtained after exposure *via* breastfeeding, while Garessus et al. [89] and Partosch et al. [88] compared their predicted neonatal plasma concentrations with *in vivo* concentrations after direct oral or intravenous dosing of the neonates. Partosch et al. [88] only evaluated the neonatal model for rifampicin. Delaney et al. [84] also compared four age groups within the first year of life and concluded that the variation was limited for escitalopram. Olangunju et al. [87] also predicted the infant exposure for four age groups. Garessus et al. [89] also simulated a worst-case scenario by implementing breastfeeding at the time of maximal breast milk concentration and using the highest reported individual M/P ratio.

Willmann et al. [86] simulated a situation comparable to a reported fatal case of codeine use during breastfeeding and compared the simulated breast milk and plasma concentrations with the values observed in this case. Willmann et al. (132), Garessus et al. [89], and Partosch et al. [88] all performed a sensitivity analysis. Willmann et al. (132) investigated the effect of different values for maternal and neonatal morphine clearances in the sensitivity analysis. They found that the morphine concentration in the neonate was mainly dependent on morphine (as codein metabolite) clearance by the neonate and the maternal daily dose of codeine. Garessus et al. [89] found that the maternal PBPK model for isoniazid for the fast metabolizers was most sensitive to the isoniazid clearance, the partition coefficient (Kp) of isoniazid between liver tissue and plasma, the dose, the liver organ blood flow and the breast milk volume. Partosch et al. [88] found that the maternal model was most sensitive to the dose, the clearance and the partition coefficient of the liver, whereas the neonatal model was most sensitive to the M/P ratio and the bioavailability in the infant.

One of the assumptions made by the PBPK models is that a general, 'mean' milk composition exists for macronutrients. However, milk composition changes, especially during the first days after delivery, but also during the time course of each feed, differences related to either preterm or term delivery, and the duration of lactation. Milk contains carbohydrates, fat, proteins, vitamins and minerals. Delaney et al. [84] investigated the intra-feed alteration by comparing escitalopram concentrations in foremilk (refers to the milk at the start of a feed, containing less fat) with concentrations in hindmilk (refers to the milk at the end of a feed, containing more fat). They concluded that there was a significantly higher escitalopram concentration in hindmilk, but the impact of the sampling phase for monitoring of the concentration will be negligible. In addition, maternal characteristics might have an impact on the milk composition. An overview on the effect of several maternal conditions on milk composition is presented in Table 5. Overall, the differences in macro-nutrient composition are rather limited but can be considered during modelling for condition specific settings, *e.g.*, diabetes, or when antibiotic concentrations in human milk are collected in women with mastitis as an indication for these antibiotics.

3.4.4. Animal PBPK models

Lactation PBPK models have also been developed for animals. Generally, the animal PBPK models are based on the same concepts as the human PBPK models. One of the advantages of animal PBPK models, in comparison to human PBPK models, is that the systematic collection of *in vivo* data is easier. However, a limitation is that the knowledge of the animal physiology is limited compared to humans. The animal lactation PBPK models address different types of research questions. Animal PBPK lactation models have been used to address risk assessment questions in food producing animals about residual medication in edible tissues and milk for human consumption (*e.g.*, [138–140]). Other animal lactation PBPK models, typically for rodents, aim to get insight into (human) toxicology (*e.g.* [141–143],). Animal PBPK lactation models have also been used as the basis for the development of human lactation PBPK models [91]. In this case, the animal PBPK lactation model is first developed and validated for animals and thereafter extrapolated to humans by interspecies scaling of the physiological factors.

Table 5
Effect of specific maternal conditions (pre-pregnancy existing, pregnancy related or related to logistic settings, environment and lifestyle) on human milk composition for macro-nutrients.

Pre-pregnancy existing maternal conditions	Main findings as reported	References
Systematic search on maternal medical conditions (diabetes, hypertension and overweight)	Qualitative, not quantitative reporting. *Diabetes studies (n = 9):* Fat: lower concentration (n = 4); Protein: lower (n = 1); Lactose: lower concentration (n = 3); Energy value: higher (n = 1); No differences (n = 2); *Hypertensive mothers (n = 1):* Protein: higher (n = 1); *Overweight (n = 4):* Fat: higher (n = 2); Energy content: higher (n = 2); No differences (n = 2)	[97]
Overweight or obesity compared to term normal weight, colostrum	Fat: increased in colostrum (craematocrit, 5.6 to 3.3 %); Protein: higher (4.2 to 3.9 g/dl) in term colostrum, no differences from 2nd week onwards; Carbohydrates: similar or higher (3.2 to 1.9 mmol/l); Caloric content: higher (688 to 538 kcal/l)	[98,99]
Pre-pregnancy Body Mass Index (BMI), colostrum composition	Protein: positively related to pre-pregnancy BMI (normal weight vs obese, 4.23 vs 3.9 g/dl); Energy content: not affected; Macro-nutrient: not affected	[100]
Body Mass Index	Carbohydrate: 7.0 g/dl; Protein: 1.1 g/dl (IQR 1.1−1.2); Fat: 3.5 g/dl (3−4.1); Energy content: 66 (62−72.5) kcal/dl. Maternal BMI positively related to lipid (r = 0.37) and energy (r = 0.39) milk content ($p < 0.05$)	[101]
Diet and Body Mass Index	Not diet, but maternal body composition (BMI) associated with human milk composition. Milk fat content related (r = 0.33) to BMI, and between protein content and body composition (% fat mass (r = 0.60), fat-free mass/kg (r = 0.63; p = 0.001), and muscle mass (r = 0.47; p = 0.027). However, postnatal age is a relevant driver (1st, 3th and 6th month)	[102]
Fat mass	Protein: Higher with maternal % fat mass (difference 0.16, SD 0.07 g/l, p = 0.028). Limited changes over the first year of lactation as the mean concentrations were 12.94, 11.7, 10.83, 12.83 and 11.96 g/l in the 2nd, 5th, 9th and 12th month of lactation.	[103]
After bariatric surgery	Fat: higher on day 4 after delivery 3.0 ± 0.7 versus 2.2 ± 0.9 g/100 ml; Carbohydrate: slightly higher on day 4 and 6.6 ± 0.6 versus 6.3 ± 0.4 g/100 ml; Energy: higher on day 4 61.0 ± 7.2 versus 51.7 ± 9 kcal/100 ml. The nutritional value of breast milk after bariatric surgery appears to be at least as high as in non-surgical controls	[104]
Celiac disease	Protein: decrease during first 3 months; n-6 long chain polyunsaturated fatty acids: decrease during the first 3 months of lactation; No relevant effect of celiac disease	[105]
Human immunodeficiency virus (HIV) infection	Fat: higher (4.42 to 3.49 g/100 g); Protein: HIV-infected women contained higher (1.95 to 1.78 g/100 g); Carbohydrate: lower (5.37–6.67 g/100 g); Zinc: lower (5.26–5.78 mg/l) Copper: higher (0.64 to 0.56 mg/l)	[106]
Lactational mastitis	Lactational mastitis (n = 15) to controls (n = 15). Fat: lower (2.1 vs 3.6 g/dl); Protein: not different (1.8 vs 1.4 g/dl); Carbohydrates: lower (5.1–6.9 g/dl); Energy: lower (54–67 kcal/dl)	[107]
Hemodialysis	Case report; Creatinine: different; Urea: different; Sodium: different; Chloride: different; Phosphate: different; Otherwise high similarity to control breast milk	[108]
Cystic fibrosis	Milk secreted by 2 women with CF appears to be physiologically normal, including sodium. Third case report confirms these finding	[109,110]
Homogenous familial hypo-betalipoproteinemia	Lipid: lower, with another profile, based on 2 cases	[111,112]
Age, < or ≥ 35 years	Fat: higher in colostrum of mothers with advanced age elevated; Carbohydrate: higher in mature milk of mothers with advanced age; positive correlation between maternal age and carbohydrate content in mature milk	[113]
Lactating adolescents	Fat: unaffected (6th week: 41.6 ± 3.3; 10th week: 36.2 ± 3.4; 14th week: 31.5 ± 9.0 g/day); Protein: significant reduction ($p < 0.05$) during the postpartum weeks studied (6th week: 16.6 ± 1.1; 10th week 13.7 ± 1.0; 14th week: 12.3 ± 1.1 g/day); Lactose: unaffected (6th week: 60.2 ± 1.9; 10th week: 60.4 ± 2.6; 14th week: 65.1 ± 4.0 g/day)	[114]

Maternal pregnancy-related conditions	Main findings as reported	References
Preterm delivery	Systematic review and meta-analysis, 13 papers. Fat: (r2 = 0.94); Protein: decreases massively and significantly (r2 = 0.93) from day 1 to 3 to reaches 50% of the initial value at week 10−12; Lactose: (r2 = 0.80); Energy: (r2 = 0.81) (linear trends)	[115]
Nutrition and preterm delivery	367 milk samples, 81 mothers after preterm delivery. Lipids: 3.4(1.3−6.4) g/100 ml; Protein: 1.3(0.1−3.1) g/100 ml. Carbohydrates: 6.8(4.4−7.3) g/100 ml; There was a (weak) relationship between mothers' carbohydrates intake (r = 0.164; $p < 0.01$) and milk composition [lipids r2 = 0.087; protein 0.299; calories 0.101]. Postnatal age was the most relevant covariate for protein (r=-0.505) and carbohydrates (r = 0.202)	[116]
Systematic review on human milk composition after preterm delivery	Based on 24 studies, comparing lactation week 1 to lactations weeks 2−8, and in mean values. Protein: 1.9 to 1.27 g/100 ml; Lipid: 2.59–3.46 g/100 ml; Carbohydrate: 6.55 to 6.15 g/100 ml; Energy content: 57.11–65.6 kcal/100 ml	[117]
Preterm delivery	Fat: 1.9 ± 1.8 g/dl to 3.4 ± 2.1 g/dl; Protein: decline from 4.1 ± 2.1 g/dl to 2.2 ± 0.6 g/dl; Lactose: increase from 2.2 ± 0.7 g/dl to 3.0 ± 0.9 g/dl; Energy: increase from 42.3 ± 18.8 Kcal/dl to 51.9 ± 21.5 Kcal/dl (all, day 3–28)	[118]
Preterm to term delivery	No significant differences between preterm and full-term milk ($p > 0.05$). The lowest creamatocrit, calories and fat concentration was in morning preterm milk (4.86 %, 663.8 kcal/l and 33.6 g/l, respectively). The highest milk parameters were observed in the night full term samples (9.6 %, 919.7 kcal/l, and 60.7 g/l, respectively)	[119]
Preterm to term delivery	Fat: higher ($p < 0.05$) in preterm milk (2.9–6.8 and 2.9–4.9 g/dl); Protein: both preterm (2.6 to 1.9 g/dl) and term milk (2.2 to 1.1 g/dl) decreased with lactation duration, with higher values in extremely preterm (<28 weeks) than in moderately (pre)term milk ($p < 0.0001$); Carbohydrate: higher ($p < 0.05$) in preterm milk (6.3–8.5 and 5–7.4 g/dl, week 1–8, preterm versus term); Energy: higher ($p < 0.05$) in preterm milk	[120]
Preterm (<33, or 33−36 weeks) to term delivery	Human milk samples were collected from 86 mothers on days 3, 7, 14 and 28 of lactation. Day 3–28, <33, 33−36, or term: Fat: 1.2, 1.3 and 2–3.1, 3.6 and 3.11 g/dl Lower in preterm samples, post-delivery increase; Protein: (g/dl): 4.1, 4 and 1.9 to 1.6, 0.9 and 1.1 Higher in preterm samples, post-delivery decrease; Lactose: (g/dl): 3.8, 4.74 and 5.18–7, 7.5 and 7.7 Lower in preterm samples, post-deliver increase	[121]
Very preterm (VP) to preterm (P) to term (T) delivery	Fat: colostrum, transitional and mature milk was 4.05, 4.76 and 4.67 (VP), 2.58, 3.75, 2.98 (P) and 2.6, 3.11, 3.06 g/100 ml (T). Creamatocrit: 6.3, 7.1, 7 (VP), 4.2, 5.8, 5 (P) and 4, 5.1, 5 (T) %	[122]
Small-for-gestational-age to appropriate infant	Crematocrite: similar on day 3 (7.8 to 6.8), 7 (11.9 to 9.7) and 14 (9.6–10.3) %	[123]
Pre-eclampsia	Macro-nutrient: no quantitative differences; Free fatty acids: qualitative differences	[124]

Logistic settings, environment and lifestyle	Main findings as reported	References
Donor milk, compared to literature	Fat: 3.22, SD 1.00; Protein: Banked donor milk mean values (g/100 ml) were found to be 1.16, SD 0.25; Lactose: 7.80, SD 0.88; Energy: 65+/-11 kcal/d; Macronutrient: differs from the values reported in the literature for mature human milk	[125]
Manually expressed milk	Paired study in 21 women, 48−72 h after delivery; Fat: higher (2.3 to 1.84 g/100 ml) in breastmilk expressed manually	[126]
Feeding over 24 h time interval	Fat: significantly differed over 24 h ($p = 0.01$); Protein: remained the same, the mean 24 -h total protein, whey, and casein inversely ($pP0 < .01$) related to the number of feeds per day. Pre-feed samples differ from post-feed samples. Lactose: remained the same, positively ($p = 0.03$) related to the number of feeds per day	[127]
Across 9 different countries, protein content in mature human milk	Total protein: steady decline from 30 to 151 days of lactation, significantly higher in the second month of lactation compared with the following 4 months ($y = 23.251x-0.1554$ (g/l), x = lactation days); True protein: steady decline from 30 to 151 days of lactation, significantly higher in the second month of lactation compared with the following 4 months ($y = 18.86x-0.1705$ (g/l), x = lactation days); Individual amino acid: steady decline from 30 to 151 days of lactation, significantly higher in the second month of lactation compared with the following 4 months. There is a high level of consistency in the protein content and amino acid composition of human milk across geographic locations, with Chile as an outlier. Stage of lactation explained 22.9 and 16.9 % of the variation in total protein and total amino acid concentration	[128]
High-altitude adapted population (Tibet)	Fat: averaged 5.2 ± 2.0 g/100 ml; Protein: 1.26 ± 0.35 g/100 ml; Total sugar: 7.37 ± 0.49 g/100 ml; Energy density: 81.4 ± 17.4 kcal/100 ml. No associations between altitude of residence and milk composition	[129]
Vegetarian and non-vegetarian diet	Fat: no differences; Precursors of arachidonic acid: higher (n = 12)	[130]
Vegetarian *versus* omnivore diet	Fat: lower in women with a vegan (3.0), compared to vegetarian (4.0) or omnivore (4.0) g/dl diet; Qualitative differences in (un)saturated fats	[131]
Diet	Macronutrient: (fat, protein, and lactose) not affected by maternal diet; Fatty acid profile: affected by maternal diet	[132]
Active nicotine smoking	Fat: (3.47 vs. 4.34 g/dl) lower in smokers; Lipid: lower (-26%, 31.1 vs 42.4 mg/ml); Protein: lower (-12%, 13.1 vs 14.9 mg/ml) Nicotine: 3-fold higher for smoking women than in maternal plasma	[133,134]
Passive nicotine smoking	Lipids: affected (-28 % and -35 % in triglycerides at baseline and at 4 months)	[135]
Acute fasting (24−25 h)	Immediately after fast: Protein: increase; Lactose: decrease; Sodium: increase; Calcium: increase; Phosphorus: decrease; Triglycerides: unchanged 24 h after fast, parameters are no longer significantly different from baseline except for mean protein levels and lactose. No relevant changes in macronutrients	[136]
Chronic fasting (repetitive model, Ramadan)	Macronutrient: no significant effect (morning samples); Zinc: decreased; Magnesium: decreased; Potassium: decreased	[137]

4. Discussion

4.1. In vitro human and animal models

Several *in vitro* models using mammary epithelial cells are available to investigate the transfer of medications into breast milk, while other epithelial cell lines derived from different tissue (intestine, kidney) can be used as reference models for the epithelial cell barrier (Table 6). The *in vitro* model needs to be 'biorelevant', *i.e.*, representative for the *in vivo* human physiology. Therefore, HMECs are expected to be a good model for the blood milk epithelial barrier. HMECs have previously been used by Kimura et al. [6], but further characterization is required before concluding whether this is an adequate model for medication crossing over the mammary epithelium.

Several supplements should be added to the basal media. Some types of supplements, such as glutamine [24,144,145] and foetal bovine serum [8,13,146,14,16–20,23,24], are used in most cell cultures to ensure proper energy metabolism and appropriate growth. Bovine pituitary extract is commonly used in the culture of HMECs when working serum-free [6,13,145–149]. The Guidance Document on Good *in vitro* Method Practices guideline [150] recommends to work serum-free where possible. The guideline further advises to minimize the use of antibiotics. Hormones, such as insulin [6,8,146,148,149,151,13–17,23,24,145] and hydrocortisone [6,13,146,148,149,152,15–19,23,24,145], are added to stimulate correct proliferation and differentiation in epithelial cells. Furthermore, the specific Epidermal Growth Factor (EGF) is almost always added to stimulate growth [6,8,148,149,153,13–15,17,16–19,23,146]. In order to develop a differentiated model of the lactating mammary epithelium, EGF is removed and prolactin is added to the medium. Several other supplements could be added to cell culture models but did not seem to be critical.

Human cell lines are an alternative for HMEC. The advantage is that cell lines are generally easier to culture and have a longer life span. The cell lines should be a surrogate for the *in vivo* mammary barrier physiology. Therefore, MCF-7, MCF-10A or PMC42-LA could be good options. In addition, animal cells and/or cell lines can be explored. This might especially be useful to obtain mechanistic insights and scaling information for *in vitro to in vivo* extrapolation (IVIVE) and the development of PBPK models. Even if rodent mammary epithelial cells are the most widely studied and provided many biological insights, it is evident that the rodent mammary gland is not fully representative of the human setting. Therefore, other *in vitro* animal models have been explored, including bovine, goat and porcine. From a physiological, anatomical and metabolic point of view, ruminants provide a model very far from humans, whereas the porcine species is recognized as an excellent model for translational purposes [155–158]. Among the different *in vitro* models of mammary epithelial cells available, primary cell cultures offer the opportunity to study the factors that regulate physiologically relevant development of normal mammary epithelial cells under defined conditions.

Overall, it can be concluded that several *in vitro* models are available which allow for determination of the partitioning of medications over the epithelial blood milk barrier. However, characterization of the cell culture models for this application remains rather limited.

4.2. In vivo animal models

Medication excretion in milk during lactation can be successfully investigated utilizing *in vivo* studies in lactating animals [38,44]. The principal benefits of *in vivo* animal studies in this field are:

(i) the possibility to clarify also the mechanistic aspect of milk/blood barrier;
(ii) the possibility to evaluate the influence of various parameters on the rate of medication excretion in milk (milk composition, timing of milking, drug-drug interaction, and different models of excretion even at molecular level); and
(iii) the possibility to evaluate the effects of excreted medication or metabolites on the offspring.

The combination of the animal model with an *in vitro*-based preliminary screening phase may reduce the number of animals needed and

Table 6
Overview of selected epithelial cell culture models that may be useful when mimicking the blood-milk barrier..

In vitro model	Species	Tissue	Cell type	Characteristics	References
HMECs	Human	Breast	Primary epithelial cells, normal	Differentiation into lactating state after stimulation with lactogenic hormones Used to investigate active transport (e.g. TEA or PAH)	[6]
MCF-7	Human	Breast	Cancer epithelial cell line	Several characteristics of differentiated mammary epithelium retained (e.g. formation of domes) Estrogen and progesterone receptor Used to investigate active transport (e.g. BMAA)	[8,40]
MCF-10A	Human	Breast	Non-tumorigenic epithelial cell line	Suitability as a model for normal breast cells questioned No estrogen and progesterone receptor	[41]
MDA-MB-231	Human	Breast	Cancer epithelial cell line	Highly aggressive, invasive and poorly differentiated Not suitable as a model for normal breast cells	[42]
PMC42-LA	Human	Breast	Cancer epithelial cell line	Differentiation into lactating state after stimulation with lactogenic hormones Suitable as a model for normal breast cells	[9]
Caco-2	Human	Colon	Cancer epithelial cell line	Frequently used as a model to determine permeability of medication	[154]
MDCK II	Dog	Kidney	Epithelial-like cell line, normal	Transfection with BCRP possible	[11]
IPEC-J2	Porcine	Jejunum	Primary epithelial cell	Suitable as a model of normal epithelial barrier to determine permeability of medication	[80]
HC11	Mouse	Breast	Immortalized and non-transformed epithelial cell line derived from Comma 1D cells (normal mammary cell line)	Differentiation into lactating state after stimulation with lactogenic hormones Used to investigate active transport (e.g. mitoxantrone)	[8]
CIT3	Mouse	Breast	Epithelial cell line derived from Comma 1D cells (normal mammary cell line) *via* triple trypsinization	Differentiation into lactating state after stimulation with lactogenic hormones Used to investigate active transport (e.g. nitrofurantoin)	[12,13,14]
RME	Rat	Breast	Primary epithelial cells, normal	Differentiation into lactating state after stimulation with lactogenic hormones	[15]
pMECs	Porcine	Breast	Primary epithelial cells, normal	Differentiation into lactating state after stimulation with lactogenic hormones	[16,17,18,19]
BME-UV	Bovine	Breast	Immortalized epithelial cell line	Differentiation into lactating state after stimulation with lactogenic hormones Used to investigate active transport (e.g. [14C]-tetraethylammonium bromide and [3 H]-estrone sulphate)	[20,21,22,23]
pgMECs	Goat	Breast	Primary epithelial cells, normal	Differentiation into lactating state after stimulation with lactogenic hormones	[24]

Abbreviations: HMECs: human mammary epithelial cells; TEA: tetraethylammonium; PAH: p-aminohippurate; MCF-7: Michigan Cancer Foundation 7; BMAA: beta-N-methylamino-alanine; caco-2: cancer colon 2; MDCK II: Madin-Darby canine kidney II; BCRP: breast cancer resistance protein; HC11: HC11 mouse mammary epithelial cells; CIT3: CIT3 mouse mammary epithelial cells; RME: rat mammary epithelial cells; pgMECs: primary goat mammary epithelial cells.

mitigate ethical concerns.

4.3. Empirical and semi-mechanistic models (human)

Koshimichi et al. [78] showed that semi-mechanistic models can be used to predict the transfer of compounds into human breast milk. The main advantage of this semi-mechanistic model over other reported methods to predict medication transfer into the human milk is that the model of Koshimichi did consider that milk and plasma concentration-time profiles do not change in parallel. Koshimichi et al. found that secretion and reuptake values are similar for most compounds, suggesting mainly passive diffusion. However, for some compounds, transporter-mediated secretion or reuptake plays an important role. The model developed by Koshimichi et al. thus allows to distinguish between compounds that undergo passive diffusion into the milk and compounds that undergo transporter-mediated partitioning. However, with a 3-fold error tolerance, there is still room for improvement.

The main advantage of PBPK models over empirical and semi-mechanistic models is that PBPK models are based on the underlying *in vivo* physiological mechanisms. This will allow inclusion of transporter-mediated milk secretion or reuptake in medication-specific PBPK models. Therefore, it can be expected that more reliable predictions can be obtained with PBPK modelling.

4.4. Physiologically-based pharmacokinetic (PBPK) models

4.4.1. Human lactation PBPK models

Recently, some PBPK models became available for the prediction of breast milk exposure, and neonatal systemic exposure to maternal medication *via* breastfeeding [84,86–89]. The available models, despite some limitations, show the value of PBPK modelling in this research field. The models illustrate that PBPK modelling can be used to handle several research questions, including breast milk exposure and neonatal systemic exposure *via* breastfeeding. A major advantage of PBPK modelling is that non-clinical data can be used to predict *in vivo* PK behavior of medication. This is especially important, given that clinical studies in a vulnerable population like lactating women and their neonates are time-consuming, and give rise to ethical and practical issues.

One of the main challenges is that there is currently no breast milk compartment available in either Simcyp™ (Certara, UK) or PK-Sim® (Open Systems Pharmacology). Another important challenge is the need for high quality input data. The knowledge regarding the physiology of lactating women and nursing neonates is growing, but further research in this field is required to optimize existing, and develop new PBPK models. Furthermore, an immense information gap exists regarding the excretion of medication into the human breast milk and subsequent neonatal gastrointestinal absorption. However, information will become available within the course of ConcePTION. ConcePTION is a project funded by the Innovative Medicines Initiative, which aims to reduce uncertainty about the use of medication during pregnancy and lactation [159]. The quality of the input data is critical for the quality of the final PBPK model. The lack of robust data is illustrated by the hurdles that some of the articles had for obtaining the M/P ratio for the respective model medication. Furthermore, AUC-based M/P ratios, which are more reliable than single M/P ratios, are not always available. In the absence of (high quality) clinical data, some of the M/P ratios were estimated

using the Schmitt et al. algorithm [96]. However, a major limitation of this algorithm is that it does not account for transporter involvement.

The guideline on the reporting of PBPK modelling and simulation of European Medicines Agency [81] and the Physiologically Based Pharmacokinetic Analyses – Format and Content Guidance for Industry from Food and Drug Administration [160] should be followed. This will assure the quality of the developed PBPK models and also their suitability for registration purposes. Multiple aspects are important to consider during the development of the PBPK models, as indicated by the available models. For example, some of the lactation PBPK models performed a genotype-specific simulation, whereas others did not take genotype into account. However, the example of codeine shows the importance of genotype specific simulation for certain medications. Codeine has long been considered safe until the death of a neonate was attributed to it [161]. This event has led to caution using codeine while breastfeeding, although it has to be noted that there is some doubt in the literature about whether neonates can develop opioid toxicity from breastfeeding [162]. The PBPK model of Willmann et al. [86] suggests that a combination of worst case factors, including the genotype of mother and infant, could have led to the death of the neonate. Neglecting the genotype might lead to the conclusion that a medication is safe, while this is not the case for all genotypes. Especially since the prevalence of polymorphisms in the infant are not disconnected from the mother and the limited metabolic (glucuronidation) capacity of neonates.

Another important aspect is how the transfer of medication into the breast milk and breastfeeding of the neonate is implemented in the PBPK models. The transfer of medication has been modelled as either direct transfer from the blood to the breast milk or as transfer *via* uptake into the breast tissue. Furthermore, there was some variation in the parameters that have been used to implement breastfeeding in the PBPK models (*e.g.*, duration of breastfeeding, frequency of breastfeeding and daily milk consumption). Recently, Yeung et al. quantified breast milk intake feeding parameters in infants for input into PBPK models [94]. Even though literature shows that the milk composition is relatively constant in several maternal conditions, possible effects of the specific disease population on the milk composition should be kept in mind. Also, Delaney et al. [84] showed that there was a significant difference in medication concentrations between foremilk and hindmilk. The composition of the milk might thus play a role in the exposure of infants to maternal medication *via* breastfeeding. It is therefore important to understand the factors that influence the milk composition. Delaney et al. also investigated the variation between different age groups within the first year of life. Although the conclusion was that the variation is limited between the age groups for escitalopram, the age might have an important effect on the exposure to some medications, as it has been shown that clearance is dependent on the age of the neonate [163]. Olugunja et al. [87] did take this into account by doing separate predictions for different age groups. All of these factors can also be used to simulate worst-case scenarios.

PBPK models should be evaluated for their ability to predict the *in vivo* exposure. Delaney et al. [84] and Olagunju et al. [87] were the only ones to use pre-specified acceptance criteria. There is no consensus on which criteria should be used for acceptance of PBPK models as this depends on the purpose, but a 2-fold deviation is often used as default in literature. The guideline for PBPK models [81] indicates that a comparison of the simulated and observed individual plasma concentration-time profiles should be presented as plots and tabulations. Matched predicted and *in vivo* data should be used.

4.4.2. Animal lactation PBPK models

Besides human lactation PBPK models, several animal PBPK lactation models have been reported. Typically, these animal lactation PBPK models have been developed for dairy animals or rodents. The goal can either be to gain information for the modelled animal species or to translate the information to humans. Translation of animal PBPK information to humans is especially valuable in case *in vivo* human data are lacking. However, species differences, for example in transporter expression, complicate the direct translation of data from animal PBPK models to humans. Nevertheless, lessons learned during IVIVE-PBPK modelling while relying on animal *in vitro* data for the blood-milk barrier, followed by comparison with corresponding *in vivo* data will be instrumental for improving human PBPK lactation models. A similar approach was taken by Parrott et al. to predict oseltamivir disposition in neonates and infants [164]. At least initial estimates for scaling factors for the IVIVE step can be derived in this way. Simcyp™ v18 allows to build PBPK models in the rat, dog, mouse and monkey, while PK-Sim® also supports mini-pig.

5. Conclusion

The iterative development of a non-clinical platform should allow to predict breast milk transfer, and subsequent neonatal systemic exposure to maternal medication *via* breastfeeding. First, a human-relevant *in vitro* cell culture model, representative for the *in vivo* physiology should be developed and characterized. Transepithelial permeability data, generated with the *in vitro* model can then be subjected to IVIVE followed by PBPK modelling. Essential scaling information for IVIVE can be generated by the paired interpretation of animal *in vitro* and *in vivo* models. Furthermore, the *in vivo* animal models can deliver key mechanistic insights to support the physiological plausibility of the PBPK models. The non-clinical tools should be evaluated for their predictive performance using diverse model compounds for which *in vivo* data are available. The iterative development of several non-clinical tools will ultimately lead to robust predictions of breast milk transfer and neonatal systemic exposure to maternal medication, for which data are currently lacking, ultimately driving a paradigm shift in the domain of pharmacotherapy during lactation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The research leading to these Results was conducted as part of the ConcePTION consortium. This paper only reflects the personal views of the stated authors.

The ConcePTION project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 821520. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA. Figures were created with BioRender.com.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.biopha.2020.111038.

References

[1] M.R. Saha, K. Ryan, L.H. Amir, Postpartum women's use of medicines and breastfeeding practices: a systematic review, Int. Breastfeed. J. 10 (2015), https://doi.org/10.1186/s13006-015-0053-6.

[2] Breastfeeding (n.d.). https://www.who.int/health-topics/breastfeeding#tab=tab_1 (Accessed 2 June 2020).

[3] M. Ouzzani, H. Hammady, Z. Fedorowicz, A. Elmagarmid, Rayyan-a web and mobile app for systematic reviews, Syst. Rev. 5 (2016) 1–10, https://doi.org/10.1186/s13643-016-0384-4.

[4] R.A. Corley, T.J. Mast, E.W. Carney, J.M. Rogers, G.P. Daston, Evaluation of physiologically based models of pregnancy and lactation for their application in

children's health risk assessments, Crit. Rev. Toxicol. 33 (2003) 137–211, https://doi.org/10.1080/713611035.
[6] S. Kimura, K. Morimoto, H. Okamoto, H. Ueda, D. Kobayashi, J. Kobayashi, Y. Morimoto, S. Kimura, K. Morimoto, H. Okamoto, H. Ueda, D. Kobayashi, J. Kobayashi, Y. Morimoto, Development of a human mammary epithelial cell culture model for evaluation of drug transfer into milk, Arch. Pharm. Res. 29 (2006) 424–429, https://doi.org/10.1007/BF02968594.
[7] C. Schmidhauser, M.J. Bissell, C.A. Myers, G.F. Casperson, Extracellular matrix and hormones transcriptionally regulate bovine beta-casein 5' sequences in stably transfected mouse mammary cells, Proc. Natl. Acad. Sci. 87 (1990) 9118–9122, https://doi.org/10.1073/pnas.87.23.9118.
[8] M. Andersson, L. Ersson, I. Brandt, U. Bergstrom, U. Bergström, U. Bergstrom, Potential transfer of neurotoxic amino acid β-N-methylamino-alanine (BMAA) from mother to infant during breast-feeding: predictions from human cell lines, Toxicol. Appl. Pharmacol. 320 (2017) 40–50, https://doi.org/10.1016/j.taap.2017.02.004.
[9] D. Freestone, M.A. Cater, M.L. Ackland, D. Paterson, D.L. Howard, M.D. de Jonge, A. Michalczyk, Copper and lactational hormones influence the CTR1 copper transporter in PMC42-LA mammary epithelial cell culture models, J. Nutr. Biochem. 25 (2014) 377–387, https://doi.org/10.1016/j.jnutbio.2013.11.011.
[10] W. W, D. Z, H. H, M. L, D. X, T. L, D.J.R.B, T. S, T. F, IRP2 regulates tumor growth in breast cancer, Am. J. Hematol. 88 (2013) E15. http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L71431415.
[11] N. Ito, K. Ito, Y. Ikebuchi, Y. Toyoda, T. Takada, A. Hisaka, A. Oka, H. Suzuki, Prediction of drug transfer into milk considering breast cancer resistance protein (BCRP)-mediated transport, Pharm. Res. 32 (2015) 2527–2537, https://doi.org/10.1007/s11095-015-1641-2.
[12] F.W. Kari, R. Weaver, M.C. Neville, Active transport of nitrofurantoin across the mammary epithelium in vivo, J. Pharmacol. Exp. Ther. 280 (1997) 664–668 (Accessed 6 September 2019), http://www.ncbi.nlm.nih.gov/pubmed/9023278.
[13] P.M. Gerk, J.A. Moscow, P.J. McNamara, Basolateral active uptake of nitrofurantoin in the CIT3 cell culture model of lactation, Drug Metab. Dispos. 31 (2003) 691–693, https://doi.org/10.1124/dmd.31.6.691.
[14] A. Maitra, S. Patel, V.R. Bhate, V.S. Toddywalla, M.A. Athavale, Development of an in vitro cell culture model to study milk to plasma ratios of therapeutic drugs, Indian J. Pharmacol. 45 (2013) 325, https://doi.org/10.4103/0253-7613.114994.
[15] S. Deeks, J. Richards, S. Nandi, Maintenance of normal rat mammary epithelial cells by insulin and insulin-like growth factor 1, Exp. Cell Res. 174 (1988) 448–460, https://doi.org/10.1016/0014-4827(88)90314-X.
[16] A. Jaeger, F. Hadlich, N. Kemper, A. Lübke-Becker, E. Muráni, K. Wimmers, S. Ponsuksili, MicroRNA expression profiling of porcine mammary epithelial cells after challenge with Escherichia coli in vitro, BMC Genomics 18 (2017) 1–14, https://doi.org/10.1186/s12864-017-4070-2.
[17] S. Dahanayaka, R. Rezaei, W.W. Porter, G.A. Johnson, R.C. Burghardt, F. W. Bazer, Y.Q. Hou, Z.L. Wu, G. Wu, Technical note: isolation and characterization of porcine mammary epithelial cells, J. Anim. Sci. 93 (2015) 5186–5193, https://doi.org/10.2527/jas2015-9250.
[18] Y.M. Zheng, X.Y. He, Characteristics and EGFP expression of porcine mammary gland epithelial cells, Res. Vet. Sci. 89 (2010) 383–390, https://doi.org/10.1016/j.rvsc.2010.03.023.
[19] Y. Lv, S. Zhang, W. Guan, F. Chen, Y. Zhang, J. Chen, Y. Liu, Metabolic transition of milk triacylglycerol synthesis in response to varying levels of palmitate in porcine mammary epithelial cells, Genes Nutr. 13 (2018) 18, https://doi.org/10.1186/s12263-018-0606-6.
[20] B. Zavizion, M. van Duffelen, W. Schaeffer, I. Politis, Establishment and characterization of a bovine mammary myoepithelial cell line, Vitr. Cell. Dev. Biol. Anim. 32 (1996) 149–158, https://doi.org/10.1007/BF02723680.
[21] M.M. Al-Bataineh, D. Van Der Merwe, B.D. Schultz, R. Gehring, Cultured mammary epithelial monolayers (BME-UV) express functional organic anion and cation transporters, J. Vet. Pharmacol. Ther. 32 (2009) 422–428, https://doi.org/10.1111/j.1365-2885.2009.01057.x.
[22] M.M. Al-Bataineh, D. Van Der Merwe, B.D. Schultz, R. Gehring, Molecular and functional identification of organic anion transporter isoforms in cultured bovine mammary epithelial cells (BME-UV), J. Vet. Pharmacol. Ther. 35 (2012) 209–215, https://doi.org/10.1111/j.1365-2885.2011.01309.x.
[23] Y. Yagdiran, A. Oskarsson, C.H. Knight, J. Tallkvist, ABC- and SLC-Transporters in murine and bovine mammary epithelium–effects of prochloraz, PLoS One 11 (2016), e0151904, https://doi.org/10.1371/journal.pone.0151904.
[24] J. Ogorevc, M. Zorc, P. Dovč, Development of an in vitro goat mammary gland model: establishment, characterization, and applications of primary goat mammary cell cultures. Goat Sci., InTech, 2018, p. 13, https://doi.org/10.5772/intechopen.71853.
[25] N.S. Yang, D. Kube, C. Park, P. Furmanski, Growth of human mammary epithelial cells on collagen gel surfaces, Cancer Res. 41 (1981) 4093–4100. http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L11011201.
[26] W.L. Kirkland, N.S. Yang, T. Jorgensen, C. Longley, P. Furmanski, Growth of normal and malignant human mammary epithelial cells in culture, JNCI J. Natl. Cancer Inst. 63 (1979) 29–41, https://doi.org/10.1093/jnci/63.1.29.
[27] K. Takebe, J. Nio-Kobayashi, H. Takahashi-Iwanaga, T. Yajima, T. Iwanaga, Cellular expression of a monocarboxylate transporter (MCT1) in the mammary gland and sebaceous gland of mice, Histochem. Cell Biol. 131 (2009) 401–409, https://doi.org/10.1007/s00418-008-0543-3.
[28] W. Obuchi, S. Ohtsuki, Y. Uchida, K. Ohmine, T. Yamori, T. Terasaki, Identification of transporters associated with etoposide sensitivity of stomach cancer cell lines and methotrexate sensitivity of breast cancer cell lines by quantitative targeted absolute proteomics s, Mol. Pharmacol. Mol Pharmacol. 83 (2013) 490–500, https://doi.org/10.1124/mol.112.081083.
[29] J. Alcorn, X. Lu, J.A. Moscow, P.J. McNamara, Transporter gene expression in lactating and nonlactating human mammary epithelial cells using real-time reverse transcription-polymerase chain reaction, J. Pharmacol. Exp. Ther. 303 (2002) 487–496, https://doi.org/10.1124/jpet.102.038315.
[30] H. Ahmadzai, Pharmacological role of efflux transporters: clinical implications for medication use during breastfeeding, World J. Pharmacol. 3 (2014) 153, https://doi.org/10.5497/wjp.v3.i4.153.
[31] D.A. Groneberg, F. Döring, S. Theis, M. Nickolaus, A. Fischer, H. Daniel, Peptide transport in the mammary gland: expression and distribution of PEPT2 mRNA and protein, Am. J. Physiol. Endocrinol. Metab. 282 (2002), https://doi.org/10.1152/ajpendo.00381.2001.
[32] J.W. Jonker, G. Merino, S. Musters, A.E. Van Herwaarden, E. Bolscher, E. Wagenaar, E. Mesman, T.C. Dale, A.H. Schinkel, The breast cancer resistance protein BCRP (ABCG2) concentrates drugs and carcinogenic xenotoxins into milk, Nat. Med. 11 (2005) 127–129, https://doi.org/10.1038/nm1186.
[33] Álvarez-Fernández García-Lino, Merino Blanco-Paniagua, A.M. Álvarez, Transporters in the mammary gland—contribution to presence of nutrients and drugs into milk, Nutrients 11 (2019) 2372, https://doi.org/10.3390/nu11102372.
[34] K.M. Morrissey, C.C. Wen, S.J. Johns, L. Zhang, S.-M.M. Huang, K.M. Giacomini, The UCSF-FDA transportal: a public drug transporter database, Clin. Pharmacol. Ther. 92 (2012) 545–546, https://doi.org/10.1038/clpt.2012.44.
[35] UniProt consortium, Universal Protein Resource Knowledgebase, (n.d.). https://www.uniprot.org/ (Accessed 14 May 2020).
[36] Technical University of Munich, Proteomics D.B., (n.d.). https://www.proteomicsD.B.org/ (Accessed 14 May 2020).
[37] B.H. Solvo, Biotechnilogy. The Transporter Book, 3th ed., 2017.
[38] D. Ventrella, M. Forni, M.L. Bacci, P. Annaert, Non-clinical models to determine drug passage into human breast milk, Curr. Pharm. Des. 25 (2019) 534–548, https://doi.org/10.2174/1381612825666190320165904.
[39] L.J. Shipman, A.H. Docherty, C.H. Knight, C.J. Wilde, Metabolic adaptations in mouse mammary gland during a normal lactation cycle and in extended lactation, Q. J. Exp. Physiol. 72 (1987) 303–311, https://doi.org/10.1113/expphysiol.1987.sp003076.
[40] Ş. Comşa, A.M. Cîmpean, M. Raica, The story of MCF-7: anticancer research, Anticancer Res. 35 (1981) 3147–3154. http://ar.iiarjournals.org/content/35/6/3147.long.
[41] Y. Qu, B. Han, Y. Yu, W. Yao, S. Bose, B.Y. Karlan, A.E. Giuliano, X. Cui, Evaluation of MCF10A as a reliable model for normal human mammary epithelial cells, PLoS One 10 (2015) 1–16, https://doi.org/10.1371/journal.pone.0131285.
[42] E. Cruz, Cell line profile MDA-MB-231 (ECACC catalogue no. 92020424), Eur. Collect. Authenticated Cell Cult. 2780 (2017) 87092802 (Accessed 3 January 2020), https://www.phe-culturecollections.org.uk/products/celllines/generalcell/detail.jsp?refId=85120602&collection=ecacc_gc.
[43] B. Zavizion, Establishment and characterization of a bovine mammary epithelial cell line with unique properties, Vitr. Cell. Dev. Biol. Anim. 32 (1996) 138–148, https://doi.org/10.1007/BF02723679.
[44] J. Wang, T. Johnson, L. Sahin, M.S. Tassinari, P.O. Anderson, T.E. Baker, C. Bucci-Rechtweg, G.J. Burckart, C.D. Chambers, T.W. Hale, D. Johnson-Lyles, R. M. Nelson, C. Nguyen, D. Pica-Branco, Z. Ren, H. Sachs, J. Sauberan, A. Zajicek, S. Ito, L.P. Yao, Evaluation of the safety of drugs and biological products used during lactation: workshop summary, Clin. Pharmacol. Ther. 101 (2017) 736–744, https://doi.org/10.1002/cpt.676.
[45] E. Roura, S.J. Koopmans, J.P. Lallès, I. Le Huerou-Luron, N. De Jager, T. Schuurman, D. Val-Laillet, Critical review evaluating the pig as a model for human nutritional physiology, Nutr. Res. Rev. 29 (2016) 60–90, https://doi.org/10.1017/S0954422416000020.
[46] H. Mahnke, M. Ballent, S. Baumann, F. Imperiale, M. Von Bergen, C. Lanusse, A. L. Lifschitz, W. Honscha, S. Halwachs, The ABCG2 efflux transporter in the mammary gland mediates veterinary drug secretion across the blood-milk barrier into milk of dairy cows, Drug Metab. Dispos. 44 (2016) 700–708, https://doi.org/10.1124/dmd.115.068940.
[47] G. Allegri, A. Bertazzo, M. Biasiolo, C.V.L. Costa, E. Ragazzi, Kynurenine pathway enzymes in different species of animals, Adv. Exp. Med. Biol. 527 (2003) 455–463, https://doi.org/10.1007/978-1-4615-0135-0_53.
[48] E. Capuano, G. van der Veer, R. Boerrigter-Eenling, A. Elgersma, J. Rademaker, A. Sterian, S.M. van Ruth, Verification of fresh grass feeding, pasture grazing and organic farming by cows farm milk fatty acid profile, Food Chem. 164 (2014) 234–241, https://doi.org/10.1016/j.foodchem.2014.05.011.
[49] L. Demetrius, Of mice and men, EMBO Rep. 6 (2005) S39–S44, https://doi.org/10.1038/sj.embor.7400422.
[50] J.A. Schrickx, J. Fink-Gremmels, Implications of ABC transporters on the disposition of typical veterinary medicinal products, Eur. J. Pharmacol. 585 (2008) 510–519, https://doi.org/10.1016/j.ejphar.2008.03.014.
[51] M.S. Warren, N. Zerangue, K. Woodford, L.M. Roberts, E.H. Tate, B. Feng, C. Li, T. J. Feuerstein, J. Gibbs, B. Smith, S.M. de Morais, W.J. Dower, K.J. Koller, Comparative gene expression profiles of ABC transporters in brain microvessel endothelial cells and brain in five species including human, Pharmacol. Res. 59 (2009) 404–413, https://doi.org/10.1016/j.phrs.2009.02.007.
[52] L.M. Gonzalez, A.J. Moeser, A.T. Blikslager Raleigh, N. Carolina, A.T. Blikslager, A.T. Blikslager Raleigh, N. Carolina, Porcine models of digestive disease: the future of large animal translational research, Transl. Res. 166 (2015) 12–27, https://doi.org/10.1016/j.trsl.2015.01.004.

[53] U. Utrecht, A. Schrickx, ABC-transporters in the Pig, 2006.
[54] P. Chavatte-Palmer, A. Tarrade, Placentation in different mammalian species, Ann. Endocrinol. (Paris) 77 (2016) 67–74, https://doi.org/10.1016/j.ando.2016.04.006.
[55] M. De Vos, V. Huygelen, G. Van Raemdonck, S. Willemen, E. Fransen, X. Van Ostade, C. Casteleyn, S. Van Cruchten, C. Van Ginneken, Supplementing formula-fed piglets with a low molecular weight fraction of bovine colostrum whey results in an improved intestinal barrier, J. Anim. Sci. 92 (2014) 3491–3501, https://doi.org/10.2527/jas.2013-6437.
[56] J. Lorenz, H.R. Glatt, R. Fleischmann, R. Ferlinz, F. Oesch, Drug metabolism in man and its relationship to that in three rodent species: monooxygenase, epoxide hydrolase, and glutathione S-transferase activities in subcellular fractions of lung and liver, Biochem. Med. 32 (1984) 43–56, https://doi.org/10.1016/0006-2944(84)90007-3.
[57] P. Radermacher, P. Haouzi, A mouse is not a rat is not a man: species-specific metabolic responses to sepsis – a nail in the coffin of murine models for critical care research? Intensive Care Med. Exp. 1 (2013) 1–5, https://doi.org/10.1186/2197-425x-1-7.
[58] P.J. McNamara, D. Burgio, S.D. Yoo, Pharmacokinetics of cimetidine during lactation: species differences in cimetidine transport into rat and rabbit milk, J. Pharmacol. Exp. Ther. 261 (1992) 918–923.
[59] R.D. Cardiff, Validity of mouse mammary tumour models for human breast cancer: comparative pathology, Microsc. Res. Tech. 52 (2001) 224–230, https://doi.org/10.1002/1097-0029(20010115)52:2<224::AID-JEMT1007>3.0.CO;2-A.
[60] J.S. Carroll, T.E. Hickey, G.A. Tarulli, M. Williams, W.D. Tilley, Deciphering the divergent roles of progestogens in breast cancer, Nat. Rev. Cancer 17 (2017) 54–64, https://doi.org/10.1038/nrc.2016.116.
[61] B.E. Ősz, A. Tero-Vescan, M. Dogaru, S. Vancea, S. Imre, P. Bosa, C.E. Vari, Olanzapine transfer into sheep's milk. An animal model, Farmacia. 65 (2017) 677–682.
[62] C. Garcia-Contreras, M. Vazquez-Gomez, J.L. Pesantez-Pacheco, L. Torres-Rovira, A. Heras-Molina, T. Encinas, S. Astiz, A. Gonzalez-Bulnes, Maternal metformin treatment improves developmental and metabolic traits of IUGR fetuses, Biomolecules 9 (2019) 1–10, https://doi.org/10.3390/biom9050166.
[63] A. Bassols, C. Costa, P.D. Eckersall, J. Osada, J. Sabrià, J. Tibau, The pig as an animal model for human pathologies: a proteomics perspective, Proteomics Clin. Appl. 8 (2014) 715–731, https://doi.org/10.1002/prca.201300099.
[64] D. Ventrella, F. Dondi, F. Barone, F. Serafini, A. Elmi, M. Giunti, N. Romagnoli, M. Forni, M.L. Bacci, The biomedical piglet: establishing reference intervals for haematology and clinical chemistry parameters of two age groups with and without iron supplementation, BMC Vet. Res. 13 (2017) 1–8, https://doi.org/10.1186/s12917-017-0946-2.
[65] W. Löscher, Critical review of current animal models of seizures and epilepsy used in the discovery and development of new antiepileptic drugs, Seizure 20 (2011) 359–368, https://doi.org/10.1016/j.seizure.2011.01.003.
[66] E. Gasthuys, S. Schauvliege, T. van Bergen, J. Millecam, I. Cerasoli, A. Martens, F. Gasthuys, T. Vandecasteele, P. Cornillie, W. Van den Broeck, F. Boyen, S. Croubels, M. Devreese, Repetitive urine and blood sampling in neonatal and weaned piglets for pharmacokinetic and pharmacodynamic modelling in drug discovery: a pilot study, Lab. Anim. 51 (2017) 498–508, https://doi.org/10.1177/0023677217692372.
[67] R. Forster, P. Ancian, M. Fredholm, H. Simianer, B. Whitelaw, The minipig as a platform for new technologies in toxicology, J. Pharmacol. Toxicol. Methods 62 (2010) 227–235, https://doi.org/10.1016/j.vascn.2010.05.007.
[68] C. Suenderhauf, N. Parrott, A physiologically based pharmacokinetic model of the minipig: data compilation and model implementation, Pharm. Res. 30 (2013) 1–15, https://doi.org/10.1007/s11095-012-0911-5.
[69] B. Peter, E.P.C.T. De Rijk, A. Zeltner, H.H. Emmen, Sexual maturation in the female göttingen minipig, Toxicol. Pathol. 44 (2016) 482–485, https://doi.org/10.1177/0192623315621413.
[70] S. Beken, P. Kasper, J.W. van Der Laan, Regulatory Acceptance of Alternative Methods in the Development and Approval of Pharmaceuticals, 2016, https://doi.org/10.1007/978-3-319-33826-2_3.
[71] F. Rasmussen, Mammary excretion of benzylpenicillin, erythromycin, and penethamate hydroiodide, Acta Pharmacol. Toxicol. (Copenh.) 16 (1959) 194–200, https://doi.org/10.1111/j.1600-0773.1959.tb01201.x.
[72] L.J. Notarianni, D. Belk, S.A. Aird, P.N. Bennett, An in vitro technique for the rapid determination of drug entry into breast milk, Br. J. Clin. Pharmacol. 40 (1995) 333–337, https://doi.org/10.1111/j.1365-2125.1995.tb04555.x.
[73] U.C. Atkinson, E.J.E.J. Begg, H.C. Atkinson, E.J.E.J. Begg, Prediction of drug distribution into human milk from physicochemical characteristics, Clin. Pharmacokinet. 18 (1990) 151–167, https://doi.org/10.2165/00003088-199018020-00005.
[74] J.C. Fleishaker, N. Desai, P.J. McNamara, Factors affecting the milk-to-plasma drug concentration ratio in lactating women: physical interactions with protein and fat, J. Pharm. Sci. 76 (1987) 189–193, https://doi.org/10.1002/jps.2600760302.
[75] M.S. Meskin, E.J. Lien, QSAR analysis of drug excretion into human breast milk, J. Clin. Hosp. Pharm. 10 (1985) 269–278, https://doi.org/10.1111/j.1365-2710.1985.tb00924.x.
[76] S. Agatonovic-Kustrin, L.H. Ling, S.Y. Tham, R.G. Alany, Molecular descriptors that influence the amount of drugs transfer into human breast milk, J. Pharm. Biomed. Anal. 29 (2002) 103–119, https://doi.org/10.1016/S0731-7085(02)00037-7.
[77] A.R. Katritzky, D.A. Dobchev, E. Hür, D.C. Fara, M. Karelson, QSAR treatment of drugs transfer into human breast milk, Bioorganic Med. Chem. 13 (2005) 1623–1632, https://doi.org/10.1016/j.bmc.2004.12.015.
[78] H. Koshimichi, K. Ito, A. Hisaka, M. Honma, H. Suzuki, Analysis and prediction of drug transfer into human milk taking into consideration secretion and reuptake clearances across the mammary epithelia, Drug Metab. Dispos. 39 (2011) 2370–2380, https://doi.org/10.1124/dmd.111.040972.
[79] H. Atkinson, E. Begg, Prediction of drug concentrations in human skim milk from plasma protein binding and acid-base characteristics, Br. J. Clin. Pharmacol. 25 (1988) 495–503, https://doi.org/10.1111/j.1365-2125.1988.tb03334.x.
[80] T.W. Hale, Pharmacology review: drug therapy and breastfeeding: pharmacokinetics, risk factors, and effects on milk production, Neoreviews 5 (2004) e164–e172, https://doi.org/10.1542/neo.5-4-e164.
[81] E. Medicines Agency, European Medicines Agency, E. Medicines Agency, Committee for Medicinal Products for Human Use (CHMP) Guideline on the Reporting of Physiologically Based Pharmacokinetic (PBPK) Modelling and Simulation, 44, 2018. www.ema.europa.eu/contact.
[82] K. Abduljalil, T.N. Johnson, M. Jamei, Development and Integration of a Dynamic Lactation Functionality within a Full PBPK Model, 12, 2017, p. 7087.
[83] K. Abduljalil, T.N. Johnson, M. Jamei, Application of Physiologically-Based Pharmacokinetic Model to Predict Tramadol Concentration in Human Milk, 76, 2017, p. 7087.
[84] S.R. Delaney, P.R.V. Malik, C. Stefan, A.N. Edginton, D.A. Colantonio, S. Ito, Predicting escitalopram exposure to breastfeeding infants: integrating analytical and in silico techniques, Clin. Pharmacokinet. 57 (2018) 1603–1611, https://doi.org/10.1007/s40262-018-0657-2.
[85] 39 th ESCP European Symposium on Clinical Pharmacy & 13th SFPC Congress: Clinical Pharmacy at The Front Line of Innovations, 21–23 October 2010, Lyon, France, Int. J. Clin. Pharm, 2011, pp. 285–467, https://doi.org/10.1007/s11096-011-9481-6.
[86] S. Willmann, A.N. Edginton, K. Coboeken, G. Ahr, J. Lippert, Risk to the breast-fed neonate from codeine treatment to the mother: a quantitative mechanistic modeling study, Clin. Pharmacol. Ther. 86 (2009) 634–643, https://doi.org/10.1038/clpt.2009.151.
[87] A. Olagunju, R.K.R. Rajoli, S.A. Atoyebi, S. Khoo, A. Owen, M. Siccardi, Physiologically-based pharmacokinetic modelling of infant exposure to efavirenz through breastfeeding, AAS Open Res 1 (2018) 16, https://doi.org/10.12688/aasopenres.12860.1.
[88] F. Partosch, H. Mielke, R. Stahlmann, U. Gundert-Remy, Exposure of Nursed Infants to Maternal Treatment with Ethambutol and Rifampicin, Basic Clin. Pharmacol. Toxicol. 123 (2018) 213–220, https://doi.org/10.1111/bcpt.12995.
[89] E.D.G. Garessus, H. Mielke, U. Gundert-Remy, Exposure of infants to isoniazid via breast milk after maternal drug intake of recommended doses is clinically insignificant irrespective of metaboliser status. A physiologically-based pharmacokinetic (pbpk) modelling approach to estimate drug exposure of infants via breast-feeding, Front. Pharmacol. 10 (2019) 5, https://doi.org/10.3389/fphar.2019.00005.
[90] M. Cibert, A. Gouraud, T. Vial, M. Tod, A physiologically-based pharmacokinetic model to predict neonate exposure to drugs during breast-feeding: application to lamotrigine, Fundam. Clin. Pharmacol. 24 (Suppl) (2010). Abstract 246.
[91] J.Z. Byczkowski, J.W. Fisher, A computer program linking physiologically based pharmacokinetic model with cancer risk assessment for breast-fed infants, Comput. Methods Programs Biomed. 46 (1995) 155–163, https://doi.org/10.1016/0169-2607(94)01616-N.
[92] M.-A. Verner, P. Ayotte, G. Muckle, M. Charbonneau, S. Haddad, A physiologically based pharmacokinetic model for the assessment of infant exposure to persistent organic pollutants in epidemiologic studies, Environ. Health Perspect. 117 (2009) 481–487, https://doi.org/10.1289/ehp.0800047.
[93] R.A. Clewell, J.M. Gearhart, Pharmacokinetics of toxic chemicals in breast milk: use of PBPK models to predict infant exposure, Environ. Health Perspect. 110 (2002) 333–337, https://doi.org/10.1289/ehp.021100333.
[94] C.H.T. Yeung, S. Fong, P.R.V. Malik, A.N. Edginton, Quantifying breast milk intake by term and preterm infants for input into paediatric physiologically based pharmacokinetic models, Matern. Child Nutr. (2020), e12938, https://doi.org/10.1111/mcn.12938 n/a.
[95] X. Zhuang, C. Lu, PBPK modeling and simulation in drug research and development, Acta Pharm. Sin. B 6 (2016) 430–440, https://doi.org/10.1016/j.apsb.2016.04.004.
[96] W. Schmitt, General approach for the calculation of tissue to plasma partition coefficients, Toxicol. In Vitro 22 (2008) 457–467, https://doi.org/10.1016/j.tiv.2007.09.010.
[97] Y.N. di V. do Amaral, D.M. Rocha, L.M.L. da Silva, F.V.M. Soares, M.E.L. Moreira, Morbidades maternas modificam a composição nutricional do leite humano? uma revisão sistemática, Cien. Saude Colet. 24 (2019) 2491–2498, https://doi.org/10.1590/1413-81232018247.18972017.
[98] D.T. Robinson, J. Josefson, L. Van Horn, L.A. Parker, Considerations for preterm human milk feedings when caring for mothers who are overweight or obese, Adv. Neonatal Care 19 (2019) 361–370, https://doi.org/10.1097/ANC.0000000000000650.
[99] M. Fujimori, E.L. França, T.C. Morais, V. Fiorin, L.C. de Abreu, A.C. Honório-França, Cytokine and adipokine are biofactors can act in blood and colostrum of obese mothers, BioFactors 43 (2017) 243–250, https://doi.org/10.1002/biof.1339.
[100] L. Mangel, F.B. Mimouni, N. Feinstein-Goren, R. Lubetzky, D. Mandel, R. Marom, The effect of maternal habitus on macronutrient content of human

milk colostrum, J. Perinatol. 37 (2017) 818–821, https://doi.org/10.1038/jp.2017.51.
[101] A. Bzikowska, A. Czerwonogrodzka-Senczyna, H. Weker, A. Wesołowska, Correlation between human milk composition and maternal nutritional status, Rocz. Panstw. Zakl. Hig. 69 (2018) 363–367, https://doi.org/10.32394/rpzh.2018.0041.
[102] A. Bzikowska-Jura, A. Czerwonogrodzka-Senczyna, G. Olędzka, D. Szostak-Węgierek, H. Weker, A. Wesołowska, Maternal nutrition and body composition during breastfeeding: association with human milk composition, Nutrients 10 (2018), https://doi.org/10.3390/nu10101379.
[103] S. Kuganathan, Z. Gridneva, C.T. Lai, A.R. Hepworth, P.J. Mark, F. Kakulas, D. T. Geddes, Associations between maternal body composition and appetite hormones and macronutrients in human milk, Nutrients 9 (2017), https://doi.org/10.3390/nu9030252.
[104] G. Jans, C. Matthys, M. Lannoo, B. Van der Schueren, R. Devlieger, Breast milk macronutrient composition after bariatric surgery, Obes. Surg. 25 (2015) 938–941, https://doi.org/10.1007/s11695-015-1610-1.
[105] M. Grunewald, C. Hellmuth, F.F. Kirchberg, M.L. Mearin, R. Auricchio, G. Castillejo, I.R. Korponay-Szabo, I. Polanco, M. Roca, S.L. Vriezinga, K. Werkstetter, B. Koletzko, H. Demmelmair, Variation and interdependencies of human milk macronutrients, fatty acids, adiponectin, insulin, and IGF-II in the European PreventCD cohort, Nutrients 11 (2019), https://doi.org/10.3390/nu11092034.
[106] C. Fouché, E. Van Niekerk, L. Monica, D. Plessis, Basic Science Differences in Breast Milk Composition of HIV-Infected and HIV-Uninfected Mothers of Premature Infants, Effects of Antiretroviral Therapy, (n. d.) (2021), https://doi.org/10.1089/bfm.2016.0087.
[107] B. Say, E.A. Dizdar, H. Degirmencioglu, N. Uras, F.N. Sari, S. Oguz, F.E. Canpolat, The effect of lactational mastitis on the macronutrient content of breast milk, Early Hum. Dev. 98 (2016) 7–9, https://doi.org/10.1016/j.earlhumdev.2016.03.009.
[108] M.S. Balzer, M.M. Gross, R. Lichtinghagen, H. Haller, R. Schmitt, Got milk? Breastfeeding and milk analysis of a mother on chronic hemodialysis, PLoS One 10 (2015), e0143340, https://doi.org/10.1371/journal.pone.0143340.
[109] M.L. Shiffman, T.W. Seale, M. Flux, O.R. Rennert, P.T. Swender, Breast-milk composition in women with cystic fibrosis: report of two cases and a review of the literature, Am. J. Clin. Nutr. 49 (1989) 612–617, https://doi.org/10.1093/ajcn/49.4.612.
[110] D.J. Golembeski, M.G. Emery, Lipid composition of milk from mothers with cystic fibrosis, Pediatrics 83 (1989).
[111] C.S. Wang, D.R. Illingworth, Lipid composition and lipolytic activities in milk from a patient with homozygous familial hypobetalipoproteinemia, Am. J. Clin. Nutr. 45 (1987) 730–736, https://doi.org/10.1093/ajcn/45.4.730.
[112] G. Steiner, J.J. Myher, A. Kuksis, Milk and plasma lipid composition in a lactating patient with type I hyperlipoproteinemia, Am. J. Clin. Nutr. 41 (1985) 121–128, https://doi.org/10.1093/ajcn/41.1.121.
[113] R. Lubetzky, O. Sever, F.B. Mimouni, D. Mandel, Human Milk Macronutrients Content: Effect of Advanced Maternal Age, 10, 2015, https://doi.org/10.1089/bfm.2015.0072.
[114] K. Bolognini Pereira, V.B. de Azeredo, C. Barros da Sileira, L.M. Pedruzzi, Composition of breast milk of lactating adolescents in function of time of lactation, Nutr. Hosp. 28 (2013) 1971–1976, https://doi.org/10.3305/nutr hosp.v28in06.6909.
[115] S. Mahajan, D. Chawla, J. Kaur, S. Jain, Macronutrients in breastmilk of mothers of preterm infants, Indian Pediatr. 54 (2017) 635–637, https://doi.org/10.1007/s13312-017-1124-0.
[116] J.M. Hascoët, M. Chauvin, C. Pierret, S. Skweres, L.D. Van Egroo, C. Rougé, P. Franck, Impact of maternal nutrition and perinatal factors on breast milk composition after premature delivery, Nutrients 11 (2019), https://doi.org/10.3390/nu11020366.
[117] C. Boyce, M. Watson, G. Lazidis, S. Reeve, K. Dods, K. Simmer, G. McLeod, Preterm human milk composition: a systematic literature review, Br. J. Nutr. 116 (2016) 1033–1045, https://doi.org/10.1017/S0007114516003007.
[118] F.B. Mimouni, R. Lubetzky, S. Yochpaz, D. Mandel, Preterm human milk macronutrient and energy composition: a systematic review and meta-analysis, Clin. Perinatol. 44 (2017) 165–172, https://doi.org/10.1016/j.clp.2016.11.010.
[119] B. Kociszewska-Najman, B. Borek-Dzieciol, M. Szpotanska-Sikorska, E. Wilkos, B. Pietrzak, M. Wielgos, The creamatocrit, fat and energy concentration in human milk produced by mothers of preterm and term infants, J. Matern. Fetal. Neonatal. Med. 25 (2012) 1599–1602, https://doi.org/10.3109/14767058.2011.648239.
[120] J. Bauer, J. Gerss, Longitudinal analysis of macronutrients and minerals in human milk produced by mothers of preterm infants, Clin. Nutr. 30 (2011) 215–220, https://doi.org/10.1016/j.clnu.2010.08.003.
[121] A.P.S. Narang, H.S. Bains, S. Kansal, D. Singh, Serial composition of human milk in preterm and term mothers, Indian J. Clin. Biochem. 21 (2006) 89–94, https://doi.org/10.1007/BF02913072.
[122] C. Moltó-Puigmartí, A.I. Castellote, X. Carbonell-Estrany, M.C. López-Sabater, Differences in fat content and fatty acid proportions among colostrum, transitional, and mature milk from women delivering very preterm, preterm, and term infants, Clin. Nutr. 30 (2011) 116–123, https://doi.org/10.1016/j.clnu.2010.07.013.
[123] K.A. Domany, D. Mandel, M.H. Kedem, R. Lubetzky, Breast milk fat content of mothers to small-for-gestational-age infants, J. Perinatol. 35 (2015) 444–446, https://doi.org/10.1038/jp.2014.200.
[124] K. Dangat, D. Upadhyay, A. Kilari, U. Sharma, N. Kemse, S. Mehendale, S. Lalwani, G. Wagh, S. Joshi, N.R. Jagannathan, Altered breast milk components in preeclampsia; an in-vitro proton NMR spectroscopy study, Clin. Chim. Acta 463 (2016) 75–83, https://doi.org/10.1016/j.cca.2016.10.015.
[125] K.Y. Wojcik, D.J. Rechtman, M.L. Lee, A. Montoya, E.T. Medo, Macronutrient analysis of a nationwide sample of donor breast milk, J. Am. Diet. Assoc. 109 (2009) 137–140, https://doi.org/10.1016/j.jada.2008.10.008.
[126] L. Mangel, A. Ovental, N. Batscha, M. Arnon, I. Yarkoni, S. Dollberg, Higher fat content in breastmilk expressed manually: a randomized trial, Breastfeed. Med. 10 (2015) 352–354, https://doi.org/10.1089/bfm.2015.0058.
[127] S. Khan, A.R. Hepworth, D.K. Prime, C.T. Lai, N.J. Trengove, P.E. Hartmann, Variation in fat, lactose, and protein composition in breast milk over 24 hours: associations with infant feeding patterns, J. Hum. Lact. 29 (2013) 81–89, https://doi.org/10.1177/0890334412448841.
[128] P. Feng, M. Gao, A. Burgher, T.H. Zhou, K. Pramuk, A nine-country study of the protein content and amino acid composition of mature human milk, Food Nutr. Res. 60 (2016) 31042, https://doi.org/10.3402/fnr.v60.31042.
[129] E.A. Quinn, K. Diki Bista, G. Childs, Milk at altitude: human milk macronutrient composition in a high-altitude adapted population of tibetans, Am. J. Phys. Anthropol. 159 (2016) 233–243, https://doi.org/10.1002/ajpa.22871.
[130] B.L. Specker, H.E. Wey, D. Miller, Differences in fatty acid composition of human milk in vegetarian and nonvegetarian women: long-term effect of diet, J. Pediatr. Gastroenterol. Nutr. 6 (1987) 764–768, https://doi.org/10.1097/00005176-198709000-00018 (n.d.).
[131] M.T. Perrin, R. Pawlak, L.L. Dean, A. Christis, L. Friend, A cross-sectional study of fatty acids and brain-derived neurotrophic factor (BDNF) in human milk from lactating women following vegan, vegetarian, and omnivore diets, Eur. J. Nutr. 58 (2019) 2401–2410, https://doi.org/10.1007/s00394-018-1793-z.
[132] L. Aumeistere, I. Ciproviča, D. Zavadska, J. Andersons, V. Volkovs, K. Ceļmalniece, Impact of maternal diet on human milk composition among lactating women in Latvia, Medicina (Kaunas). 55 (2019), https://doi.org/10.3390/medicina55050173.
[133] P. Bachour, R. Yafawi, F. Jaber, E. Choueiri, Z. Abdel-Razzak, Effects of smoking, mother's age, body mass index, and parity number on lipid, protein, and secretory immunoglobulin A concentrations of human milk, Breastfeed. Med. 7 (2012) 179–188, https://doi.org/10.1089/bfm.2011.0038.
[134] M. Napierala, J. Mazela, T.A. Merritt, E. Florek, Tobacco smoking and breastfeeding: effect on the lactation process, breast milk composition and infant development. A critical review, Environ. Res. 151 (2016) 321–338, https://doi.org/10.1016/j.envres.2016.08.002.
[135] A. Baheiraei, A. Shamsi, S. Khaghani, S. Shams, M. Chamari, H. Boushehri, A. Khedri, The effects of maternal passive smoking on maternal milk lipid, Acta Med. Iran. 52 (2014) 280–285.
[136] D.R. Zimmerman, L. Goldstein, E. Lahat, R. Braunstein, D. Stahi, A. Bar-Haim, M. Berkovitch, Effect of a 24+ hour fast on breast milk composition, J. Hum. Lact. 25 (2009) 194–198, https://doi.org/10.1177/0890334409331505.
[137] N. Rakicioğlu, G. Samur, A. Topçu, A.A. Topçu, The effect of Ramadan on maternal nutrition and composition of breast milk, Pediatr. Int. 48 (2006) 278–283, https://doi.org/10.1111/j.1442-200X.2006.02204.x.
[138] D.J. MacLachlan, Influence of physiological status on residues of lipophilic xenobiotics in livestock, Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 26 (2009) 692–712, https://doi.org/10.1080/02652030802669170.
[139] T.L. Leavens, L.A. Tell, L.W. Kissell, G.W. Smith, D.J. Smith, S.A. Wagner, W. L. Shelver, H. Wu, R.E. Baynes, J.E. Riviere, Development of a physiologically based pharmacokinetic model for flunixin in cattle (Bos taurus), Food Addit. Contam. Part A Chem. Anal. Control. Expo. Risk Assess. 31 (2014) 1506–1521, https://doi.org/10.1080/19440049.2014.938363.
[140] E.D. Van Asselt, J. Kowalczyk, J.C.H.H. van Eijkeren, M.J. Zeilmaker, S. Ehlers, P. Fürst, M. Lahrssen-Wiederholt, H.J. Van Der Fels-Klerx, P. Furst, M. Lahrssen-Wiederholt, H.J. Van Der Fels-Klerx, Transfer of perfluorooctane sulfonic acid (PFOS) from contaminated feed to dairy milk, Food Chem. 141 (2013) 1489–1495, https://doi.org/10.1016/j.foodchem.2013.04.035.
[141] S.K. Lee, D. Hamer, C.L. Bedwell, M. Lohitnavy, R.S.H.H. Yang, Effect of PCBs on the lactational transfer of methyl mercury in mice: PBPK modeling, Environ. Toxicol. Pharmacol. 27 (2009) 75–83, https://doi.org/10.1016/j.etap.2008.08.014.
[142] A.E. Loccisano, J.L.J. Campbell, J.L. Butenhoff, M.E. Andersen, H.J. Clewell 3rd, Evaluation of placental and lactational pharmacokinetics of PFOA and PFOS in the pregnant, lactating, fetal and neonatal rat using a physiologically based pharmacokinetic model, Reprod. Toxicol. 33 (2012) 468–490, https://doi.org/10.1016/j.reprotox.2011.07.003.
[143] J.Z. Byczkowski, E.R. Kinkead, H.F. Leahy, G.M. Randall, J.W. Fisher, Computer simulation of the lactational transfer of tetrachloroethylene in rats using a physiologically based model, Toxicol. Appl. Pharmacol. 125 (1994) 228–236, https://doi.org/10.1006/taap.1994.1068.
[144] Glutamine Functions (n.d.). https://www.news-medical.net/health/Glutamine-Functions.aspx (Accessed 3 January 2020).
[145] ATCC: The Global Bioresource Center (n.d.). https://www.lgcstandards-atcc.org/en.aspx (Accessed 2 January 2020).
[146] V.S. Toddywalla, F.W. Kari, M.C. Neville, Active transport of nitrofurantoin across a mouse mammary epithelial monolayer, J. Pharmacol. Exp. Ther. 280 (1997) 669–676, file:///C:/Users/Nina/AppData/Local/Mendeley Ltd./Mendeley Desktop/Downloaded/Toddywalla, Kari, Neville - 1997 - Active Transport of Nitrofurantoin Across a Mouse Mammary Epithelial Monolayer 1 Materials and Met(2).pdf (accessed September 16, 2019).

[147] Extract PTM Bovine Pituitary Extract, New Zealand Origin - Lifeline Cell Technology, (n.d.). https://www.lifelinecelltech.com/shop/cell-culture-products/growth-factors/extract-p-bovine-pituitary-extract-new-zealand-origin-cm-0097/ (Accessed 3 January 2020).
[148] Lonza Bioscience | Lonza (n.d.). https://bioscience.lonza.com/lonza_bs/US/en/ (Accessed 2 January 2020).
[149] PromoCell | Human Centered Science (n.d.). https://www.promocell.com/ (Accessed 2 January 2020).
[150] O. for E.C. and Development, Guidance Document on Good In Vitro Method Practices (GIVIMP), Series on, 2018.
[151] Combining Insulin and Transferrin in Cell Culture Supplements - Cell Culture Dish, (n.d.). https://cellculturedish.com/combining-insulin-and-transferrin-in-cell-culture-supplements/ (Accessed 2 January 2020).
[152] Hydrocortisone | STEMCELL Technologies (n.d.). https://www.stemcell.com/hydrocortisone.html (Accessed 3 January 2020).
[153] D.A. Fisher, J. Lakshmanan, Metabolism and effects of epidermal growth factor and related growth factors in mammals, Endocr. Rev. 11 (1990) 418–442, https://doi.org/10.1210/edrv-11-3-418.
[154] P. Artursson, K. Palm, K. Luthman, Caco-2 monolayers in experimental and theoretical predictions of drug transport, Adv. Drug Deliv. Rev. 64 (2012) 280–289, https://doi.org/10.1016/j.addr.2012.09.005.
[155] K. Gutierrez, N. Dicks, W.G. Glanzner, L.B. Agellon, V. Bordignon, Efficacy of the porcine species in biomedical research, Front. Genet. 6 (2015), https://doi.org/10.3389/fgene.2015.00293.
[156] N. Klymiuk, F. Seeliger, M. Bohlooly-Y, A. Blutke, D.G. Rudmann, E. Wolf, Tailored Pig Models for Preclinical Efficacy and Safety Testing of Targeted Therapies, Toxicol. Pathol. 44 (2016) 346–357, https://doi.org/10.1177/0192623315609688.
[157] T.P. Sullivan, W.H. Eaglstein, S.C. Davis, P. Mertz, The pig as a model for human wound healing, Wound Repair Regen. 9 (2001) 66–76, https://doi.org/10.1046/j.1524-475x.2001.00066.x.
[158] M.M. Swindle, A. Makin, A.J. Herron, F.J. Clubb, K.S. Frazier, Swine as models in biomedical research and toxicology testing, Vet. Pathol. 49 (2012) 344–356, https://doi.org/10.1177/0300985811402846.
[159] Innovative Medicines Initiative, ConcePTION, 2019 (Accessed 29 June 2020), https://www.imi-conception.eu/.
[160] F. and D.A. Center for Drug Evaluation and Research (CDER), Green, Dionna, Physiologically Based Pharmacokinetic Analyses — Format and Content: Guidance for Industry, U.S. Dep. Heal. Hum. Serv., 2018, pp. 1–6 (Accessed 10 January 2020), https://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.
[161] G. Koren, J. Cairns, D. Chitayat, A. Gaedigk, S.J. Leeder, Pharmacogenetics of morphine poisoning in a breastfed neonate of a codeine-prescribed mother, Lancet 368 (2006) 704, https://doi.org/10.1016/S0140-6736(06)69255-6.
[162] J. Zipursky, D.N. Juurlink, The implausibility of neonatal opioid toxicity from breastfeeding, Clin. Pharmacol. Ther. (2020) 1882, https://doi.org/10.1002/cpt.1882.
[163] J. Alcorn, P.J. McNamara, Ontogeny of hepatic and renal systemic clearance pathways in infants: part I, Clin. Pharmacokinet. 41 (2002) 1077–1094, https://doi.org/10.2165/00003088-200241130-00005.
[164] N. Parrott, B. Davies, G. Hoffmann, A. Koerner, T. Lave, E. Prinssen, E. Theogaraj, T. Singer, Development of a physiologically based model for oseltamivir and simulation of pharmacokinetics in neonates and infants, Clin. Pharmacokinet. 50 (2011) 613–623, https://doi.org/10.2165/11592640-000000000-00000.