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ITC Recommendations for Transporter Kinetic Parameter Estimation and Translational Modeling of Transport-Mediated PK and DDIs in Humans

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Abstract

This white paper provides a critical analysis of methods for estimating transporter kinetics and recommendations on proper parameter calculation in various experimental systems. Rational interpretation of transporter-knockout animal findings and application of static and dynamic physiologically based modeling approaches for prediction of human transporter-mediated pharmacokinetics and drug–drug interactions (DDIs) are presented. The objective is to provide

appropriate guidance for the use of *in vitro*, *in vivo*, and modeling tools in translational transporter science.

IN VITRO TRANSPORTER KINETICS

The importance of active uptake and efflux in the intestine, liver, kidney, and brain to drug pharmacokinetics has become increasingly more appreciated.^{1,2} Understanding pharmacokinetic (PK) and drug–drug interaction (DDI) implications of transporter involvement in absorption, distribution, and excretion requires appropriate characterization of uptake and efflux kinetics *in vitro*. Two general types of *in vitro* systems are commonly used to study uptake and efflux transport kinetics: (i) expression systems, including immortalized cell lines (e.g., CHO, HEK, HeLa, LLC-PK1, and MDCKII), oocytes, and vesicles, and (ii) whole cells, such as primary or cultured cells (e.g., hepatocytes) and derived cell lines (e.g., Caco-2, HepG2). Expression systems can be directly used to estimate kinetic/inhibition parameters for the overexpressed transporter. By contrast, cellular systems can be optimized to estimate kinetic parameters specific to uptake, metabolism, or efflux, as well as the interplay of multiple processes.

Estimation of uptake transport kinetics

Conventional two-step method—This approach is based on the extended Michaelis–Menten model (Supplementary Table S1 online, equation 1) and can be applied to evaluate uptake of drugs into whole cells or expression systems. Characterization of concentration-dependent uptake is performed under initial rate conditions and in the time-linear range. Parallel experiments are performed at 37 °C and 4 °C, and saturable active uptake in intact cells is estimated by subtracting uptake at 4 °C (nonsaturable passive transport) from total cellular uptake at 37 °C.³ Altered membrane fluidity at 4 °C represents one of the limitations of this method. Alternatively, coincubation with specific transport inhibitors may be used, an approach limited by the lack of truly specific inhibitors, and potential for organic anion-transporting polypeptide (OATP) substrate-dependent inhibition.^{4,5} In expression systems, saturable active uptake by the overexpressed transporter is obtained by subtracting uptake into vector-control cells from the transfected cells. This static model considers uptake to be an isolated process, relies on data transformation for parameter estimation, and does not account for the bidirectional nature of passive diffusion, intracellular binding, metabolism, or active efflux.

Mechanistic compartmental uptake model—To overcome the issues listed above, mechanistic compartmental models have been developed.^{3,5} These models include media and cellular compartments for dynamic evaluation of the changes in drug concentration due to active transport, passive diffusion, and intracellular/extracellular binding processes occurring during uptake into cells (e.g., plated or suspended hepatocytes) (Table 1). Unlike the static model, multiple time and drug concentration data points are fitted simultaneously to estimate the appropriate parameters (see Supplementary Table S1 online, equation 2a,b). Passive diffusion clearance is estimated solely from 37 °C data and is assessed in a more mechanistic manner because the possibility of bidirectional transport is considered in the model. In addition, time points beyond the time-linear range of uptake may be used to attain steady-state intracellular conditions, enabling more accurate estimation of intracellular binding. The mechanistic model assumes that the intracellular binding is not saturated at the conditions studied, which may lead to overestimation of the fraction unbound in cell ($f_{u,cell}$). For drugs that do not undergo metabolism, uptake parameters (Michaelis–Menten constant, K_m and maximum transport rate, V_{max}) were reported to be comparable between shorter (2 min) and extended (45–90 min) incubation times.⁵

Assessment of active uptake, passive diffusion, and intracellular binding may be biased if potential loss of drug due to metabolism is not considered. In the case of P450 metabolism, this issue can be overcome by performing the uptake experiment in the presence of either a nonspecific P450 inhibitor (e.g., 1-aminobenzotriazole) or enzyme selective inhibitors if metabolism of a drug is characterized. However, in the situation of phase II enzymes (e.g., for uridine 5'-diphospho-glucuronosyltransferases (UGT)), nonselective inhibitors are not available. Uptake kinetic parameter estimates obtained using whole cells may represent the composite of several individual transporters with distinct K_m and V_{max} values, resulting in potentially complex nonlinear pharmacokinetics (e.g., multiple hepatic OATPs or renal OATs).

Mechanistic compartmental uptake, efflux, and metabolism model—The mechanistic uptake model can be extended to account for metabolism occurring during uptake studies (Supplementary Table S1 online, equation 3; see Translational Modeling of Transporter-Mediated PK and Drug Interactions for application of such data).^{5–7} This is of particular relevance if the studies are performed over extended incubation times and for drugs subject to glucuronidation due to the lack of nonselective inhibitors.⁵ Use of this mechanistic approach reduced the error in parameter estimates and enabled simultaneous characterization of uptake, metabolism, and intracellular binding in plated hepatocytes for repaglinide and telmisartan.⁵ Although increasing the complexity of the data analysis enables a more mechanistic description of *in vitro* processes, it also increases the number of fitted parameters and data requirements because both parent drug and metabolite(s) are measured (Table 1). The mechanistic compartmental modeling approach can also account for canalicular efflux in sandwich-cultured hepatocytes.⁸ Multiple processes occurring in this cellular system are generally characterized by the clearance terms (rather than V_{max} and K_m) because a single low-substrate concentration is often studied over multiple time points (0.5–30 min). Recently, immortalized hepatic cell lines and human hepatocytes cultured in three-dimensional bioreactor systems have become available; however, their utility as *in vitro* transporter tools and in *in vivo* translation remains to be established.

In addition to *in vitro* systems, rodent liver perfusions may be used to assess the role of active uptake relative to metabolism and biliary secretion,^{9,10} with the caveat of potential species differences. These studies can improve our qualitative understanding of the relevance of uptake in overall hepatobiliary disposition, as well as provide quantitative whole-organ uptake K_m and passive diffusion unbound clearance estimates, if performed at different dose levels.

Practical considerations for active uptake assessment—Mechanistic compartmental modeling is the tool of choice to investigate the interplay between multiple processes occurring in hepatocytes. This dynamic approach is crucial for identifying the rate-limiting step in cases of complex hepatic drug disposition, as seen for repaglinide. Criteria for the model selection will depend on the stage of drug development and compound-specific issues. Despite limitations, data generated by empirical/static approaches can represent useful prior information to guide the experimental design for mechanistic modeling and simulation (see Translational Modeling of Transporter-Mediated PK and Drug Interactions). Determination of uptake K_m and V_{max} (rather than clearance parameter based on a single concentration) is preferable for subsequent *in vitro*–*in vivo* extrapolation (IVIVE) because it considers potential saturation of the transporter(s).

Estimation of efflux transport kinetics

Efflux transport in inside-out plasma membrane vesicles prepared from overexpressing cells follows principles of enzyme kinetics because the transporter interacts directly with the

substrate/inhibitor in the incubation medium. Transporter activity is determined in the time-linear range of uptake by subtracting accumulation in vector-control vesicles from transporter overexpressing vesicles. Enzyme kinetic models can be applied directly to the data to estimate transporter affinity and inhibition potency. Vesicles are best suited for low-permeability substrates because of poor retention of moderate- to high-permeability compounds. As such, vesicles are commonly used to study efflux transporters for polar substrates (e.g., multidrug resistance proteins (MRPs), multidrug and toxin extrusion proteins (MATEs), and the less lipophilic substrates of breast cancer resistance protein (BCRP)), as well as inhibition of bile acid transport by bile salt export pump (BSEP). The utility of vesicles may be limited for more lipophilic BCRP and especially P-glycoprotein (P-gp) substrates, which can exhibit greater permeability. Therefore, efflux by BCRP and P-gp is often studied in confluent cell monolayers.

Monolayer flux entails diffusion/transport across two permeability barriers with the efflux transporter interacting with intracellular and/or membrane concentrations. Conceptually and kinetically, monolayer flux does not follow the principles of simple enzyme kinetics. Traditionally, efflux transport in monolayers is assessed by measuring transcellular flux at different initial donor concentrations and described with the maximal achievable transport rate ($J_{\max}$) and the donor chamber concentration associated with half-maximal transport rate (EC_{50}).¹¹ The $J_{\max}$ and EC_{50} values are often misinterpreted as the efflux transport $V_{\max}$ and K_m , respectively, and are misleading because maximal achievable transport rate is not proportional to changes in active efflux activity.¹² Application of enzyme kinetics directly to monolayer flux data created the perception that efflux transport parameters cannot be consistently estimated. For example, P-gp K_m estimates varied up to 27-fold when estimated by applying Michaelis–Menten kinetics directly to flux data in five different monolayers,¹³ but were within ~2-fold when this data set was analyzed with a compartmental model estimating intracellular concentrations.¹⁴

Three different compartmental approaches have emerged to allow determination of kinetic parameters for efflux transporters in monolayers (Figure 1); because the models differ in the level of complexity, they provide different levels of mechanistic detail. Briefly, Model I (three-compartment model) accounts for the presence of two diffusional barriers and can be solved by simple algebraic equations. Model II (five-compartment model) and Model III (structural model) are similar to Model I but include membrane compartments with different configurations and require multiple differential equations to estimate efflux kinetic parameters (Table 2).

Model I—The simplest compartmental model for efflux transport in polarized cell monolayers is a three-compartment model comprised of apical, basolateral, and cellular compartments.¹² In this model, three different efflux ratios (ERs) directly describe monolayer efflux transport activity: basolateral ER_B (transcellular passive permeability/ $P_{app,A \rightarrow B}$), asymmetry ER_A ($P_{app,B \rightarrow A}/P_{app,A \rightarrow B}$), and cellular ER_C (cellular concentration in absence of efflux/cellular concentration in presence of efflux). These ER values can be used to calculate efflux transport kinetic parameters (K_m , $V_{\max}$, and concentration of inhibitor to achieve 50% inhibition (IC_{50})) with basic enzyme relationships under assumptions of a minimal lag time in flux and similar passive permeability across the apical and basolateral membranes.¹² An apparent K_m based on extracellular concentrations is estimated with this approach, and it must be converted to the true intracellular K_m (Supplementary Table S1 online, equation 4a). The three ER values are proportional to efflux activity even in the presence of metabolism.¹⁵ The fourth apical ER_A ($P_{app,B \rightarrow A}/$ transcellular passive permeability) is minimally sensitive to efflux activity because $B \rightarrow A$ flux is limited by the basolateral diffusional barrier; thus for passive diffusion followed by efflux, $B \rightarrow A$ flux by itself is not helpful in determining apical efflux activity. When

basolateral uptake is present (e.g., digoxin), ER_A reflects both uptake and efflux processes that comprise $B \rightarrow A$ flux.¹⁶ If the assumptions of the “simplified” three-compartment model are violated, such as appreciable flux lag time or involvement of additional transporters, then the simple explicit mathematical relationship is no longer valid, and the compartmental modeling approach below should be used instead.

Model II—As a result of observed lag times in the monolayer flux of certain molecules, a five-compartment model (with additional membrane compartments) has been developed. In this model, drug partitions into membranes that serve as “storage” compartments when the cellular concentration does not achieve rapid equilibrium, resulting in flux lag time.¹⁷ The five-compartment model includes a membrane-to-water partition coefficient (K_p) estimated in human liver microsomes. The model can also be used to describe substrate access to the P-gp binding site via the membrane compartment rather than from the cytosol. Hence, the five-compartment model describes the K_m and IC_{50} in terms of the unbound drug concentration in membrane and cytosol, respectively. After correcting for K_p estimates, the five- and three-compartment models estimate comparable K_m and IC_{50} values. Although the three- and five-compartment models predict similar intracellular concentrations following apical addition of substrate, they differ in their prediction of intracellular concentrations for flux in the $B \rightarrow A$ direction. The five-compartment model should be used when appreciable lag time in monolayer flux exists, and it may be more appropriate than the three-compartment model for prediction of tissues with basolateral exposure to the drug (e.g., liver or kidney). Metabolism can also be incorporated into the five-compartment model.¹⁵

Model III—The structural model considers binding of substrate from within the inner leaflet of the apical membrane, with on [k_{on} ($M^{-1}s^{-1}$)] and off [k_{off} (s^{-1})] rate constants and an efflux rate constant from P-gp into the apical chamber [k_{out} (M)].¹⁸ The model also explicitly estimates the P-gp active protein level, known as P-gp efflux active surface density, $T(0)$. These parameters can then be used to obtain K_m and V_{max} values (Supplementary Table S1 online, equation 4b).¹⁸ For each new drug, fitting these parameters requires use of multiple initial drug concentrations and time points until steady state is reached between P-gp-mediated efflux into the apical chamber and passive permeability from the apical chamber back into the cytosol.¹⁹ Although model III requires a large data set for analysis, it provides estimates of the on, off, and efflux rate constants, as well as active surface P-gp concentration. This more detailed description of efflux enabled elucidation of the relationship between P-gp IC_{50} and inhibition constant (K_i) values (Supplementary Table S1 online, equation 4c), as well as provided kinetic support for the presence of basolateral uptake transporters for drugs such as digoxin.¹⁹

Practical considerations for *in vitro* estimation of uptake and efflux parameters

Direct application of enzyme kinetic principles to the analysis of monolayer flux data should not be performed. Instead, these data should be analyzed by compartmental models that provide more realistic and consistent kinetic constants because they account for flux through two diffusional barriers with the efflux transporter interacting with intracellular and membrane concentrations. The J_{max}/EC_{50} approach of fitting enzyme kinetics directly to monolayer flux data is fundamentally incompatible with these complexities and resulted in incorrect parameter estimates. Currently, the algebraic relationships of the three-compartment model can be used to describe efflux transport in the absence of basolateral uptake transport and appreciable flux lag time. The more complex models can accommodate the involvement of additional transporters and flux lag time, and can estimate fundamental rate constants (Table 2). Application of compartmental analysis to monolayer efflux transport should advance the transport field by enabling correct and consistent estimation of kinetic parameters.

An increasing number of DDIs and safety concerns have been associated with inhibition of uptake (e.g., OATP inhibition leading to statin-induced muscle toxicity) and efflux transporters (e.g., digoxin toxicity). Overexpressed systems are often used to assess the inhibition potential *in vitro*^{20–22} by generating an IC₅₀ (rather than a K_i) value with a single-probe substrate concentration. To allow unbiased parameter estimation regardless of the transporter inhibition mechanism (competitive or noncompetitive), this probe concentration needs to be significantly below its K_m for the transporter of interest because under these conditions the IC₅₀ estimate would be equivalent to K_i for uptake transporters and for efflux transport experiments conducted with membrane vesicles. For efflux transporter inhibition assessment in polarized cell lines, there is not yet an agreed method for obtaining a K_i value, nor is there evidence that this extra effort is justified over simply determining an IC₅₀ value. For substrates such as digoxin that require two active processes, basolateral uptake and apical efflux, a change in digoxin B→A flux could come from inhibition of either apical efflux or basolateral uptake in which the measured IC₅₀ is a net value representing multiple transporters.

Both uptake and efflux transporters have displayed substrate-dependent inhibition,^{4,23} suggesting potential for multiple binding sites. Therefore, inhibition potency should be investigated for relevant substrate comedications in addition to prototypical probes. In addition, increased inhibitory potency of cyclosporine and its main metabolite toward OATP1B1/1B3 (5- to 12-fold) has been reported following preincubation of 30 min.²² Current understanding of the transporter-inhibition mechanism is limited, and the reasons for these time-dependent IC₅₀ shifts need to be investigated further. Therefore, it is recommended that both conditions are evaluated for a potential inhibitor. Literature reports have also highlighted the importance of *in vitro* studies at pH relevant to the physiological site of transporter interaction, as well as the potential impact of pharmaceutical excipients on the inhibition potency of intestinal transporters.^{21,24}

METABOLITE DRUG TRANSPORT ASSESSMENT

The regulatory expectation is to understand the DDI potential and pharmacokinetics of parent drug, as well as the major (>25% of parent exposure) circulating metabolites (<http://www.fda.gov>, <http://www.ema.europa.eu>). Ideally, the major metabolites should be synthesized to enable generation of a drug disposition and DDI data package analogous to parent; however, in practice this data set may not be available.²⁵ As such, *in silico* prediction of metabolite–transporter interactions has emerged as an area of interest to assist with DDI predictions and rational prioritization of metabolites for synthesis and appropriate *in vitro* testing during drug development, but at present, robust *in silico* models for polar metabolites are lacking.

Quantitative structure–activity relationship (QSAR) analysis provides a relationship between physicochemical/structural features and biological activity such as transporter interactions.²⁶ The quality and success of QSAR modeling depends on the accuracy of the input data, selection of appropriate descriptors, and statistical tools. Confidence in QSAR predictions is strongest within the chemical space of the training compound library. These libraries are generally constructed with parent compounds, which tend to be more lipophilic than metabolites, resulting in differences in potency, as well as clearance, distributional, and drug-interaction properties. Thus, if the initial data set is not broad enough, metabolites are likely to fall outside of the QSAR model training space, reducing the predictive power of the model. A training set of drug metabolites will need to be systematically tested *in vitro* and validated appropriately to enable QSAR model development for metabolite–transporter interactions; however, such data are currently limited. Broad substrate specificity of many

transporters and potentially complex substrate and/or inhibitor interactions represent additional confounding factors.

Despite the promise of QSAR models to assist with assessment of metabolite DDI potential in late-stage clinical development, this proposed approach may ultimately prove less practical than systematic synthesis and direct testing of human circulating metabolites.

PRECLINICAL *IN VIVO* KINETICS

Transporter gene–knockout models can provide useful information toward understanding transporter-limited or transporter-mediated drug absorption, distribution, and excretion.¹ Data obtained from studies with knockout animals, when contextualized with *in vitro* and clinical pharmacokinetic data, can assist in determining the impact of transporters on the fraction absorbed after oral administration and the fraction excreted (f_e) in excretory clearance, as well as distribution to tissues of interest (e.g., brain and liver).

Knockout animal models

Knockout models for all transporters described as important in the International Transporter Consortium white papers^{1,2} have been generated and characterized, and most are commercially available, with the exception of *Oat* and *Mate* knockouts (Table 3). *Bsep*–deficient animals are also not commercialized, but BSEP inhibition is investigated *in vitro* and followed up preclinically and clinically *in vivo*;² knockout animals completely lack the transporter and are not helpful for inhibition studies. Although custom gene-knockout rodents can now be generated rapidly, differences in diets, housing conditions, and genetic drift between small inbred populations may lead to marked interlaboratory phenotypic differences.¹ In this respect, commercialized knockout rodents (e.g., SAGE Mdr1a-knockout rats) hold key advantages by allowing independent research groups to thoroughly characterize these models within the same colony, as well as enabling the standardization of knockout data between groups.²⁷ Examples of appropriate use of knockout models to assess transporter-mediated pharmacokinetics and drug interactions are outlined below.

Efflux-limited absorption—Knockout models have proven invaluable for *in vivo* investigation of efflux-limited drug absorption,²⁸ specifically in interrogating whether *in vitro* P-gp and/or BCRP efflux is an *in vivo* impediment to intestinal absorption, and its quantitative impact on the fraction absorbed. P-gp and BCRP *in vitro* transport assays are commonly used to identify the substrates and help determine whether clinical victim drug–interaction studies may be warranted.¹ However, based on *in vitro* data alone, it is difficult to conclude that intestinal efflux limits the extent of *in vivo* drug absorption, especially for moderate- to high-permeability drugs. As such, data obtained in preclinical and knockout models may contribute toward predicting the rate and extent of drug absorption in a clinical setting. For instance, if nearly complete absorption with minimal *in vivo* efflux can be demonstrated in a preclinical species with adequate data to support the clinical translation, it may not be necessary to conduct a clinical study to investigate the DDI potential of intestinal efflux.

Fraction excreted—The f_e method (Supplementary Table S1 online, equation 5) is the appropriate approach to interpret drug-exposure changes in knockout animals, including systemic and tissue exposure, as well as drug recovery in excreta.^{29,30} The change in exposure is a nonlinear function of the f_e (Figure 2). When parallel transport pathways are involved, the f_e can be used as an additive parameter in predicting the contribution of multiple transporters to the pharmacokinetics of overlapping substrates; however, exposure changes in single knockouts of different transporters should never be added together to predict modulation of multiple transport pathways.

Brain distribution—Although P-gp effectively attenuates brain exposure of substrate drugs, Bcrp by itself does not similarly limit central nervous system (CNS) penetration *in vivo*.^{31,32} However, the impact of Bcrp on CNS penetration of dual Bcrp/P-gp substrates appears large when both blood–brain barrier efflux pumps are ablated.³² Kinetically, the f_e values for these efflux pathways describe the impact of removing one or both transporters in knockout animals. As shown in Figure 2, the exposure increases asymptotically as the f_e value increases from 0 to 1.³⁰ For example, lapatinib CNS exposure was increased 1.3- and 3.5-fold in *Bcrp*- and *Mdr1a/1b*-knockout mice, respectively, but surprisingly was increased 40-fold in mice lacking both Bcrp and P-gp.³² This 40-fold increase cannot be predicted by adding together the increase in the individual *Bcrp*- and *Mdr1a/1b*-knockout mice. However, by using equation 5a (Supplementary Table S1 online), blood–brain barrier Bcrp f_e is 0.23 (1.3-fold increase) and f_e for P-gp is 0.74 (3.5-fold increase); thus, by addition, the f_e value for both transporters is $0.23 + 0.74 = 0.97$, which predicts the observed increase in CNS exposure.^{30,32} A more complex f_e model has also been proposed that considers both passive and active permeability, but the key underlying principles and conclusions are the same.²⁹

Hepatic distribution—The use of knockout models in the study of hepatic OATP function was initially limited by the presence of multiple isoforms and the lack of direct orthologs between humans and rodents.³³ Nevertheless, the overall role of OATPs in hepatic uptake is similar between species.³⁴ Recently, an *Oatp1a/1b* pan-knockout mouse has been developed that lacks all major rodent hepatic Oatps.³³ Using this knockout mouse, the overall role of OATP-mediated hepatic uptake can now be effectively studied *in vivo*.³⁵ Furthermore, OATP1B1 and OATP1B3 humanized mice have been developed in the *Oatp1a/1b*-knockout background,³⁶ which may prove useful for prediction of human OATP-mediated drug interactions. Knockout models also have been important in understanding metformin liver exposure during organic cation transporter (OCT)/MATE inhibition (OCT1 and MATE1 in humans; Oct1 and Mate1 in mice), which is not readily predicted by systemic exposure of this drug.³⁷

Excretory clearance—Of the drug-disposition processes that can potentially be influenced by active transport, the contribution of secretion to overall clearance is the most prone to interspecies differences.^{1,38,39} When transport-mediated excretion contributes to total clearance, these findings are only applicable to the relevant preclinical species until translation to human is supported by both *in vitro* and clinical data. For example, in both mice and humans, metformin is entirely cleared by urinary excretion of parent, which is mediated 20% by glomerular filtration and 80% by active tubular secretion via OCT/MATE transport (OCT2 and MATE1/2/2-K in humans; Oct1/2 and Mate1 in mice). Therefore, in this case *Oct1/2*- and *Mate1*-knockout mice are representative of human pharmacokinetics and OCT/MATE clinical drug-interaction potential.^{37,40} By contrast, pemetrexed is also cleared by urinary excretion of parent in humans, with OAT3/4-mediated active secretion contributing 80% to this process. However, in mice, clearance of pemetrexed is predominantly metabolic, with only 32% of the intravenous dose recovered as parent in urine via glomerular filtration.³⁸ Therefore, pemetrexed is an example in which renal clearance in mice is not representative of humans, and for which murine knockout models would provide no value in understanding the clinical mechanism(s) of active tubular secretion and their DDI potential.

Biliary excretion of parent is a much less common clearance pathway than metabolism or urinary excretion.⁴¹ The extent of biliary excretion in bile-cannulation studies should not be interpreted as the true *in vivo* clearance because bile is removed during collection and is not made available for intestinal reabsorption.⁴² For example, although the f_e approach predicted the increase in systemic exposure in Mrp2-deficient rats for pravastatin,³⁹ clinical translation

of these data was poor. This is probably due to enterohepatic cycling of pravastatin, as well as interspecies differences in hepatic Mrp2/MRP2 expression and pravastatin metabolism.

Practical considerations for transporter-knockout animals—When designing and interpreting pharmacokinetic studies in transporter-knockout models, it is helpful to consider the following points. First, it is critical to confirm that the compound exhibits similar behavior in the aspect of pharmacokinetics under investigation between humans and the knockout species. Second, species differences may exist in the expression, affinity, and inhibitory potency of transporters between preclinical species and humans, and these potential differences should be assessed *in vitro* to support the translation. Third, a general concern in the use of transporter-knockout animals for pharmacokinetic studies is the potential up- or downregulation of other transporters and drug-metabolizing enzymes, which may complicate the interpretation of data obtained with knockout models. Therefore, it is critical to understand such compensatory mechanisms, and assess whether drug exposure could be affected. In most cases, it is not possible to predict human PK based solely on data obtained from rodent studies, and preclinical-to-clinical translation must be established on a case-by-case basis with supporting *in vitro* and human pharmacokinetic data.

TRANSLATIONAL MODELING OF TRANSPORTER-MEDIATED PK AND DRUG INTERACTIONS: APPLICATION OF PBPK MODELS

Similar to drug-metabolizing enzymes, IVIVE of transporter-mediated processes can apply either a static or a dynamic physiologically based pharmacokinetic (PBPK) modeling approach. Static models are based on clearance concepts, whereas PBPK models integrate drug-dependent parameters (including those generated *in vitro*, see *In Vitro* Transporter Kinetics) with physiological and systems parameters to account for simultaneous absorption, distribution, metabolism, and excretion processes in predicting *in vivo* drug disposition.⁴³ PBPK as a decision-making tool is increasingly used at all stages of drug development to address a wide range of PK issues.^{25,44} These models may be applied to predict the impact of complex drug interactions, disease states, and physiological conditions on drug pharmacokinetics,^{22,25} scenarios that are not readily addressed by static models. Furthermore, PBPK models can simulate not only systemic, but also tissue concentration–time profiles at pharmacologically and toxicologically relevant sites. The following sections will critically assess different static and PBPK modeling approaches currently used for the prediction of transporter-mediated pharmacokinetics and DDIs.

Static models for transporter *in vitro*–*in vivo* extrapolation

Intestinal bioavailability—The Q_{Gut} static model predicts the intestinal availability (F_G) from *in vitro* metabolic clearance and permeability data.^{45,46} The model incorporates blood flow as a nominal hybrid parameter of physiological flow (i.e., enterocytic blood flow) and drug permeability (Supplementary Table S1 online, equations 6a and 6b, respectively), enabling description of both permeability or perfusion-rate limitations. Analysis conducted on a diverse set of 25 CYP3A substrates highlighted a decreased F_G prediction accuracy for drugs with moderate to high intestinal extraction *in vivo* ($F_G < 0.5$); whereas compounds with $F_G > 0.5$ were predicted accurately.⁴⁶ The Q_{Gut} model overestimated the extent of intestinal first-pass metabolism for a number of drugs (e.g., saquinavir) by not accounting for potential nonlinearity/saturation of intestinal first-pass clearance or drug reabsorption.⁴⁷ In addition, victim drugs with very low F_G (e.g., lovastatin) are common outliers when intestinal interactions are considered in the static inhibition/induction DDI models,⁴⁶ emphasizing the need for more dynamic F_G prediction approaches for these drugs.

Hepatic clearance—The hepatic clearance concept described by the well-stirred model (Supplementary Table S1 online, equation 7a) provides the basis for both perfusion- and permeability-limited scenarios. For drugs with high passive permeability, hepatic blood flow becomes the rate-limiting factor for drug entry into hepatocytes, and overall hepatic intrinsic clearance ($CL_{int,h}$) is approximated by the sum of unbound metabolic and biliary clearances (equation 7b). The opposite is the case for drugs with low passive permeability, for which sinusoidal uptake transporters (e.g., OATPs) represent the rate-limiting step in hepatic disposition and elimination. The extended clearance model described in equation 7c illustrates the critical importance of considering more than one transport/metabolism mechanism when assessing potential DDIs. Transporter and metabolic kinetic data obtained in different *in vitro* systems (e.g., uptake in plated/suspended hepatocytes, biliary excretion in sandwich cultured hepatocytes and metabolism in microsomes) can be integrated into a static model to predict hepatic clearance.^{6,9,48}

Renal clearance—Three major processes contribute to renal clearance (Supplementary Table S1 online, equation 8), each occurring at a distinct location along the nephron. The anatomy and physiology of the nephron are difficult to reproduce *in vitro*, leading to problems in assessing and predicting renal drug clearance as a result of active secretion and/or reabsorption. As a consequence, prediction of renal clearance has been mostly empirical, using glomerular filtration and scale-up of animal measurements.⁴⁹ An IVIVE approach to renal clearance of drugs has not been established to date with primary culture of proximal tubular cells. Application of kidney slices is more advanced, and despite clear limitations, uptake in this system correlates with *in vivo* clearance for actively secreted drugs with moderate to low renal clearance.⁵⁰ Two recent studies illustrated IVIVE for renal tubular secretion clearance based on rat and human kidney slices; however, ESFs ranging from 10 to 100 were applied.^{51,52}

CNS distribution—Brain distribution is physically restricted by the tightness of the blood–brain barrier; active efflux by P-gp, BCRP, and MRP4 further limits CNS penetration. Human CNS concentration data are relatively sparse. Preclinical species are widely used to predict human brain concentrations based on the preclinical unbound brain:unbound plasma concentration ratio.⁵³ Recently, noninvasive and quantitative positron-emission tomography methods have been increasingly used to support preclinical-to-clinical translation of CNS distribution, but these methods are limited by detection of total drug-related material, unless a metabolically stable probe is used.

Static models for transporter drug interactions

P-gp inhibition—The static model for P-gp inhibition is used to identify the risk of clinical drug interactions (<http://www.fda.gov>, <http://www.ema.europa.eu>). This model is based on the ratio of perpetrator drug concentration and the inhibition potency; following the assumption of a single clearance route, the fold increase in the area under the curve in the presence of an inhibitor is calculated as $(1 + [I]/K_i)$. The 2012 draft US Food and Drug Administration guidance (<http://www.fda.gov>) recommends two cutoff values, $[I_1]/IC_{50}$, where I_1 is the total maximum plasma concentration at steady state, and $[I_2]/IC_{50}$, where I_2 is the gut concentration defined as the highest oral dose diluted in 250 ml. On the basis of retrospective IVIVE analysis, the FDA recently concluded that 0.1 and 10 represent reasonable cutoffs for $[I_1]/IC_{50}$ and $[I_2]/IC_{50}$, respectively to minimize false-negative rates.⁵⁴ However, this assessment is based on IC_{50} values from different laboratories and cellular systems without consideration of laboratory variability/inconsistency in data analysis discussed previously and predominantly using digoxin as the probe substrate, whose inhibition of monolayer flux represents combined inhibition of uptake and efflux. More recently, the IC_{50} variability determined for digoxin and 15 inhibitors has been used to

propose new thresholds for $[I_1]/IC_{50}$ and $[I_2]/IC_{50}$ of 0.03 and 45, respectively, which were determined on the basis of maximum accuracy and minimization of false-negative and false-positive rates (C. Lee *et al.*, IC_{50} working group recommendations, unpublished data). Individual companies may prefer to conduct their own IC_{50} assessment in which at least 25 compounds should be included. Of note, digoxin transport is mediated by P-gp as well as by uptake transporters (refs. 16,19 and references therein), so this analysis represents variability in inhibition of overall digoxin transport; therefore, digoxin $[I]/IC_{50}$ cutoffs should not be directly extended to other P-gp substrates.

Inhibition of hepatic uptake—Current strategy for the prediction of uptake transporter-mediated DDIs generally focuses on OATP1B1 and relies on the static assessment of reduced transporter activity (R, Supplementary Table S1 online, equation 9) from the ratio of the estimated maximum unbound hepatic inlet concentration and the inhibition constant,¹ where an $R \geq 1.25$ represents the threshold for a follow-up clinical study with rosuvastatin, pitavastatin, or pravastatin as probes (<http://www.fda.gov>). In fact, variability in OATP IC_{50} , due to choice of substrate and experimental conditions (e.g., $\pm$ with or without a preincubation step), prevents identification of universal cutoffs; therefore, the recommendation is to determine IC_{50} with the relevant comedication substrate and inhibitor. The static R approach assumes that transport occurs exclusively via the particular OATP transporter subject to inhibition and that any potential contributing effects of passive diffusion or inhibition of efflux/metabolism are negligible.⁵⁵ This assumption may lead to overestimation of the magnitude of the *in vivo* DDI due to the absence of other processes in the model. To address the differential contribution of OATP1B1 to the victim drug disposition and reduce the occurrence of false positives, the fraction of drug transported via a particular transporter can be incorporated into the model⁵⁵ (Supplementary Table S1 online, equation 10). Even minor changes in this parameter (e.g., assuming transporter contribution of 80% rather than 100%) have a significant effect on the predicted area under the curve ratio (as illustrated in Figure 2 with the analogous f_e relationship). A current limitation to successful application of this equation is the general lack of these estimates for common OATP1B1 substrates. Relative activity factors obtained *in vitro* from transfected cell lines and hepatocytes may be used as initial estimates of the contribution of the specific transporter to total uptake,¹ an approach similar to estimation of fractional metabolism by one drug-metabolizing enzyme *in vitro*. Alternatively, comparison of reported area under the curve in subjects with *SLCO1B1* 521TT and 521CC genotype can be used.^{22,55} More recently, a database ($n = 53$ interactions) was generated to assess the static uptake transporter DDI model.⁵⁶ In addition to the assumptions stated above, the model assumed complete inhibition of either intestinal enzymes or transporters of victim drugs (inhibited fraction absorbed $\times F_G = 1$) and maximal inhibition of hepatic clearance (Supplementary Table S1 online, equation 11) resulted in decreased number of false negatives, but the predictive accuracy was poor.

Limitations of static models for transporter DDI predictions

One clear caveat of static DDI models is the assumption that a single transporter for a victim drug's absorption, distribution, and hepatic or renal clearances is the dominant process. Although these processes can in theory be accommodated with organ-specific models (e.g., Supplementary Table S1 online, equations 6–8), it is evident that assessment of multiple transporters and/or transporter-enzyme interplay in different tissues is difficult by the static approach. In addition, the dynamic nature of the processes mentioned above is not considered by assuming constant concentration of either inhibitor or a substrate during the dosing interval. In certain instances, the concentrations considered for the assessment of the DDIs in the static model (e.g., P-gp inhibition) are not adequate because plasma or luminal concentrations are used as a surrogate for the intracellular concentration, and any regional

variations in organ concentrations are not considered. This conservative approach may eliminate false-negative predictions by assessing “the worst-case scenario,” but it can result in false-positive predictions and becomes very limited for complex DDIs (combined effects on uptake, metabolism, and/or efflux). In the latter case, mechanistic description of the concentration–time course of the inhibitor and the victim drug across different tissues becomes crucial.

PBPK for prediction of transporter-mediated PK

PBPK models with tissue compartments parameterized according to the well-stirred, perfusion-limited concepts have proven successful for highly permeable compounds with negligible contribution of transporters to the overall disposition.⁴⁴ *In vitro* kinetic inputs from different cellular systems are applied in the PBPK models either as a single CL_{int} parameter or full kinetic characterization (K_m and V_{max}).^{8,52,57} To describe transporter-mediated pharmacokinetics, PBPK models need to incorporate permeability-limited tissue compartments to account for diffusional barriers at either the capillary or cell membrane^{22,52,57,58} (Figure 3). Except for the first-in-human PK prediction, PBPK models can be optimized with existing clinical data for improved predictability (“top–down” approach). In certain instances, a reduced PBPK model can be considered when the focus is on a particular organ, either due to DDI concerns (e.g., liver and intestine) or safety issues (e.g., muscle in the case of statins).

Intestine—Mechanistic intestinal absorption models currently used represent adaptations of the compartmental absorption and transit model.^{59–62} Generally, these models retain the use of *in vitro* metabolic clearance and permeability data but also account for differential intestinal transit times, drug dissolution, solubility, and active transport. Differences in expression and activity of metabolizing enzymes and efflux transporters along the small intestine need to be incorporated in the model, allowing for the prediction of drug concentration in the enterocyte in different gut segments and assessment of any potential saturation of intestinal first-pass clearance. The latter has been implicated in the underprediction of F_G observed with the use of the Q_{Gut} model.⁶² The main advantage of mechanistic intestinal models is the ability to incorporate/assess the inhibitory or induction effects on transporters and metabolizing enzymes in the intestine in a dynamic way.²² The main limitation (applicable to all mechanistic organ models) is a need for detailed kinetic characterization of drug-metabolizing enzymes and transporters, and formulation-specific data (e.g., dissolution, solubility) in conjunction with the system data (e.g., transporter abundance in different segments and associated variability).

Liver—To account for permeability limitations, a liver compartment within a PBPK model is either separated into liver tissue and blood compartment⁵⁸ or subdivided into multiple units of extracellular and intracellular compartments, connected by blood flow in tandem to create a concentration gradient^{8,52} (Table 4). Watanabe and coworkers used five equally sized liver subcompartments as an approximation of the dispersion model.⁵² This multicompartment liver model has been validated for pravastatin using rat *in vivo* data, and estimated uptake ESI was subsequently applied in the human PBPK model for this drug. Liver models can also consider blood-cell compartments (Figure 3) or nonlinear blood binding, as reported for cyclosporine.²² However, rich tissue measurements are required to develop such complex mechanistic models and to obtain precise parameter estimation.⁶³ Several recent studies^{8,52,58} have incorporated either active uptake or efflux transport activity in the liver compartment, assuming other organs in the PBPK model to be perfusion rate limited (Table 4), which may not be appropriate considering transporter expression in these organs.

Kidney—Commonly applied well-stirred assumptions may not be appropriate for the kidney considering its heterogeneity, transporter and UGT enzyme expression, and existence of distinct regions with varying blood and tubular fluid flows. Recently, a more complex kidney model has been proposed in which renal physiology has been defined by a segmental structure consisting of blood, glomerulus, and proximal and distal tubules in a PBPK framework, with incorporation of hydrodynamic balance among glomerular filtration rate, urine production rate, and renal blood flow.⁶⁴ The model can account for the renal clearance contribution of various transporters located on the basolateral (e.g., OCT2, OAT1) and apical (e.g., P-gp, MATEs) membrane of proximal tubules. The effect of concurrent active secretion, active reabsorption processes, and metabolism (glucuronidation in the proximal tubular cells) on renal clearance, and assessment of potential renal transporter-mediated DDIs can be simulated. However, as highlighted in the case of liver, mechanistic *in vitro* renal transporter kinetic data are required in conjunction with transporter/UGT absolute (or at least relative) abundance data to enable full utility of the proposed mechanistic model.

Brain—The number of models describing CNS pharmacokinetics is relatively limited. These models usually divide the brain into two, three, or more compartments to describe drug concentrations as intracellular or extracellular brain.^{65,66} As with the liver and intestinal transporter models, modeling requires the assumption of low permeability and relevant scaling factors for the IVIVE of transporter data. In some instances, cerebrospinal fluid was considered (three-compartmental model). A more sophisticated four-compartmental model that accounts for human cerebrospinal fluid hydrodynamic and physiological variability has recently been developed in which contribution of passive processes and active transport via P-gp, BCRP, and MRP4 are incorporated.⁶⁷

Use of clinical data to refine PBPK models

Recent years have seen an increase in the number of studies using a whole-body PBPK modeling framework for the integration of active uptake, passive permeability, intracellular binding, metabolism, and efflux *in vitro* data to predict *in vivo* pharmacokinetics. Similar to *in vitro* metabolism data, direct use of *in vitro* transporter kinetic data in PBPK models has proven challenging, as underprediction of hepatic clearance is apparent, regardless of the hepatocyte system used (Table 4). In those cases, corresponding clinical data were used to bridge the gap in transporter IVIVE by generating ESFs for either uptake clearance or uptake $V_{\max}$, assuming that remaining model assumptions and parameter estimates were correct. These efforts highlight the importance of combining “top-down” approaches with IVIVE to construct a PBPK model with improved confidence for prediction purposes. The limitation of these approaches is the reliance on the plasma rather than tissue data for parameter optimization.

Interindividual variability in uptake activity resulted in drug- and donor-specific ESFs (Table 4). In addition, cellular system differences were evident as ESFs in sandwich-cultured human hepatocytes⁸ were generally higher than the ones reported for data from plated hepatocytes for the comparable drug set.⁶ Multiple factors can contribute to this need for uptake ESFs and the large range observed. Current PBPK models generally do not account for differences in uptake transporter expression and activity between hepatocytes and liver tissue or rely on relative activity/expression factors based on limited data sets. Currently emerging proteomic data^{68,69} report only two- to threefold difference in OATP1B1/1B3 protein levels between whole-liver and sandwich-cultured human hepatocytes and cannot solely account for the observed underprediction. Potential reduction in OATP activity/expression due to isolation, cryopreservation, and culture time cannot be ignored.³⁴ In addition, potential differences in the transporter activity due to mismatch/lack of information of the allelic variants of the hepatocyte donor and the clinical data used for

validation of the PBPK model performance can lead to genotype-dependent uptake ESF estimates.

PBPK prediction of transporter-mediated DDIs

In contrast to static models, a PBPK model can simulate the concentration–time course of the DDI perpetrator at the actual site of transporter/enzyme interaction (e.g., hepatic inlet concentration for OATP1B1 inhibition, liver intracellular concentration for efflux transporters and CYP3A4, or enterocytic concentration along the small intestine for efflux transporters and CYP3A4). The differential location of transporters and potential effect on multiple transporters can be incorporated into the model, allowing assessment of complex transporter-metabolism DDIs in a mechanistic manner.^{22,70} The difference in the estimated reduction in OATP1B1 activity using the dynamic and static approaches is illustrated in Figure 4a. Assuming that transporter inhibition is a competitive process, the reduction in the transporter activity should reflect the unbound concentration–time profile of the inhibitor at the relevant site, whereas static models assume constant interaction potential throughout the dosing interval. Examples of the use of PBPK modeling for the prediction of transporter-mediated DDIs are limited; however, this approach provides a mechanistic framework for quantitative DDI prediction and guidance for the design of clinical studies.^{22,25,70}

PBPK models can be extended to investigate the contribution of the perpetrator metabolite to the DDI, in particular if its exposure exceeds 25% of the parent (<http://www.fda.gov>) or if the metabolite is also a potent inhibitor of certain transporters/metabolic enzymes, as seen in the case of the cyclosporine metabolite AM1.²² Although PBPK modeling can be a useful tool to assess theoretical interaction potential of metabolites, availability of either exposure or potency data for metabolite(s) in early stages of drug development represents a key limitation (see Metabolite Drug Transport Assessment section). As with any inhibitor combination, the extent of the overall reduction in transporter activity will depend on the relative potency, exposure, and respective inhibition mechanism(s) of parent drug and metabolite(s) (Figure 4b,c).

SUMMARY AND CURRENT CHALLENGES

In vitro to *in vivo* extrapolations require the identification of various processes that govern the disposition of a compound, including passive permeability, transporter-mediated uptake and efflux, and metabolism, as well as the differential contribution of these mechanisms in various organs. This white paper provides best practices for the estimation of transporter *in vitro* kinetic parameters and their advantages/limitations in clinical translation.

Compartmental modeling is highlighted as the approach of choice for mechanistic description of both uptake and efflux transporter kinetics *in vitro* and consideration of intracellular concentration and binding. Whenever possible, full kinetic characterization of transporters of relevance, i.e., generation of K_m and V_{max} parameters, is encouraged to be used for subsequent IVIVE of transporter-mediated pharmacokinetics. Knockout animal models are valuable for studying the impact of transporters on drug absorption, distribution, and excretion, and DDIs. Employment of these animal models, as long as their limitations are adequately appreciated, allows the generation of preclinical data that can be used to improve the understanding of human PK.

Mechanistic *in vitro* kinetic parameter inputs in PBPK models and comprehensive understanding of the expression, as well as localization of transporters, are crucial to enable simulation of drug disposition governed by multiple mechanisms (e.g., combinations of active uptake/efflux, and metabolism). Advantage of the combined use of top–down and bottom–up modeling techniques has been highlighted for the refinement of existing PBPK models. Further research is needed to derive system-dependent (i.e., drug-independent)

scaling factors for predicting transporter contribution of a new molecular entity using PBPK modeling approaches. PBPK modeling allows quantitative prediction of complex transporter–metabolism DDIs by simultaneously assessing the modulation of transporter/enzyme activity in different organs. Current PBPK DDI models assume reversible inhibition of transporter activity; further work is required to determine if this is the true transporter inhibition mechanism. In addition to inhibitors, development of mechanistic models for common victim drugs associated with transporter-mediated DDIs is crucial for the quantitative DDI framework.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Diagrammatic representations of (a, model I) the simplified three-compartment monolayer efflux model, (b, model II) the five-compartment model containing lipid partitioning compartments at the basolateral and apical membranes (it is capable of accounting for lag time in monolayer flux), and (c, model III) the structural monolayer efflux model.

$CL_{act,efflux}$, active efflux clearance; CL_{diff} , passive diffusion unbound clearance; $C_{cell,u}$, unbound cellular concentration; CL_i , diffusional unbound clearance into membrane; CL_o , diffusional unbound clearance out of membrane; $C_{membrane,u}$, unbound membrane concentration; k_1 , substrate binding on rate; k_{-1} , substrate binding off rate; k_2 , substrate efflux rate constant to apical compartment; P-gp, P-glycoprotein; TJ, tight junction.

Figure 2.

Relationship between change in exposure in a knockout animal vs. wild-type control and the fraction excreted (f_e) by the knocked-out transporter at different inhibitor concentrations: $I/K_i = 1$ (dotted line), 2 (dotted-dashed line), 5 (short-dashed line), 10 (long-dashed line), and ∞ (genetic ablation; solid line). Exposure refers to systemic or tissue exposure, as well as recovery in any particular excreta.

Figure 3.

Permeability-limited liver model depicting the existence of a diffusional barrier at the cell membrane and processes occurring in extracellular and intracellular compartments. $CL_{act,uptake}$, $CL_{act,efflux}$, and CL_{diff} represent clearances via active uptake, sinusoidal efflux, and passive diffusion, respectively. $CL_{int,met}$ and $CL_{int,bile}$ represent loss of drug due to metabolism and biliary excretion, respectively. $f_{u,p}$, $f_{u,ISF}$, and $f_{u,cell}$ represent fraction unbound in plasma, interstitial fluid, and liver cell, respectively. Q_H , C_b , and C_T represent hepatic blood flow, unbound blood, and tissue concentration, respectively.

Figure 4.

PBPK prediction of transporter-mediated DDIs. **(a)** PBPK-model simulated dynamic reduction of hepatic uptake transporter activity (solid line) and constant interaction potential based on static calculation (dashed line) by cyclosporine over time.²² **(b)** PBPK-simulated dynamic reduction of hepatic OATP1B1 activity by the parent (solid line) and when metabolite is also included in the assessment (dashed/dotted lines). Metabolite exposure is either 25% (dashed lines) or 100% (dotted lines) of the parent exposure. Assumptions: equipotent OATP1B1 inhibition by parent and metabolite, the same plasma f_u for parent and metabolite, and competitive OATP1B1 inhibition. **(c)** All conditions as described in **b**, except metabolite is assumed to be a 10-fold more potent OATP1B1 inhibitor than the parent. OATP, organic anion-transporting polypeptide; PBPK, physiologically based pharmacokinetics.

Table 1

Advantages and limitations of modeling approaches used to assess uptake transport

Type of model	Output parameters	Comments	Ref.
Uptake			
Conventional two-step approach	$CL_{act,uptake}$	• Single low substrate concentration and incubation time point • Valid for low-permeability compounds • Simplicity in the experimental setup and data analysis • Parameter estimation based on prior data transformation is a limitation • Other processes apart from active uptake not integrated	20,48,51
	K_m , V_{max} , CL_{diff} , $f_{u,cell}$ for a chemically diverse set (16 drugs)	• Range of substrate concentrations and multiple time points required • $f_{u,cell}$ vs. $\log D_{7.4}$ relationship established for the data set of acidic and neutral drugs	71
Mechanistic compartmental uptake model	K_m , V_{max} , CL_{diff} , $f_{u,cell}$	• Applicable to suspended or plated hepatocytes • Simultaneous fitting of uptake, passive diffusion, and intracellular or membrane binding • Active efflux not considered; also internalization of efflux transporters • Metabolism not incorporated; uptake data obtained in the presence of ABT should be used for P450 substrates • Applicable for compounds with minimal metabolism	3,5
Uptake/metabolism/efflux			
Mechanistic compartmental model uptake and metabolism model	K_m , V_{max} , CL_{diff} , $f_{u,cell}$, $CL_{int,met,n}$	• Applicable to suspended or plated hepatocytes • Extended (45–90 min) time points to attain steady state between cell and media and to reduce the error in $f_{u,cell}$ estimate • No requirement to inhibit metabolism, as parent drug and metabolite(s) are measured in the cell • Large number of data points/cells required • Active efflux not considered; also internalization of efflux transporters • Provides more mechanistic <i>in vitro</i> parameter estimates for subsequent PBPK modeling	5–7
Mechanistic compartmental model—uptake, metabolism, and efflux	$CL_{act,uptake}$, CL_{diff} , $f_{u,cell}^*$, $CL_{int,bile}$	• Applicable to sandwich-cultured hepatocytes • Use of a single low substrate concentration—kinetic profile would be more informative • $CL_{int,met}$ from another <i>in vitro</i> system fixed in the model • $*f_{u,cell}$ predicted from $f_{u,p}$ • Potential upregulation of efflux transporters	8
Multiple indicator dilution	$CL_{act,uptake}$	• Rat perfused liver • Assessment of active uptake relative to <i>in vivo</i> clearance and hepatocyte data	9

Type of model	Output parameters	Comments	Ref.
		Allows elucidation of the rate-determining process in hepatic drug disposition	
Barrier-limited, variable capillary transit-time model	K_m , V_{max} , CL_{diff} , $CL_{int,met,n}$	- Rat perfused liver - Investigation of vascular protein binding, uptake, metabolism, tissue binding, and efflux - Enzymatic processes are lumped, unless metabolites are monitored. Transport processes are lumped - Tissue binding space is revealed	10

ABT, 1-aminobenzotriazole; $CL_{act,uptake}$, clearance via active uptake; CL_{diff} , passive diffusion unbound clearance; $CL_{int,bile}$, loss of drug due to biliary excretion; $CL_{int,met,n}$, loss of drug due to metabolism; $f_{u,cell}$, fraction unbound in liver cell; $f_{u,p}$, fraction unbound in plasma; K_m , Michaelis–Menten constant; PBPK, physiologically based pharmacokinetic; V_{max} , maximum transport rate.

Table 2

Advantages and limitations of modeling approaches used to assess efflux transport

Type of model	Output parameters	Comments	Ref.
$J_{\max}$ model	EC_{50} and $J_{\max}$; Michaelis–Menten kinetic model used with initial donor concentration (C_0)	• Not appropriate because C_0 does not represent concentration at efflux transporter • $J_{\max}$ model incorrectly assumes the efflux activity is proportional to net change in flux or permeability • EC_{50} determined from $J_{\max}$ model instead of K_m	11,13
Model I: three-compartment model	K_m , $V_{\max}$: relate to $C_{\text{cell,u}}$	• Does not require compartmental modeling and equations are simple to use • Can use standard linear regression software • Capable of interrelating EC_{50} from $J_{\max}$ model to IC_{50} or K_m	12
Model I: three-compartment (catenary) model ($\pm$ metabolism)	K_m : relates to $C_{\text{cell,u}}$; determined from fitting timed donor, receiver, and cell concentration ($C_{\text{cell,u}}$) data after correction for binding $V_{\max}$: determined from fitting timed data; can be normalized to P-gp concentration	• Reproduces tissue concentration differences between basolateral exposure and apical exposure • Does not account for lag time • Quantification of efflux transporter activity • Expressed ER is not influenced by metabolism • Fitting of data in the presence of metabolism, reveals apical transport	13,15,72
Model II: five-compartment model	K_m : relates to concentration at active site (membrane); derived value differs from K_m from three-compartment model unless corrected for K_p $V_{\max}$: relates to concentration at active site (membrane)	• Reproduces tissue-concentration differences between basolateral exposure and apical exposure • Accounts for lag time and drug partitioning into membrane • Cell membrane volume of 40% is assumed to fit the data • Quantification of efflux transporter activity	17
Model III structural model	K_m : molecular K_m , defined by the elementary on /off/out rate constants for binding to P-gp and efflux by P-gp $V_{\max}$: molecular $V_{\max}$	• Efflux active P-gp surface density fitted explicitly • Intracellular unbound concentrations are obtained as a function of time • Assumes competitive inhibition • Requires extensive data set for analysis	18 – 19

C_0 , initial donor concentration; $C_{\text{cell,u}}$, unbound cellular concentration; EC_{50} , concentration associated with half-maximal transport rate; ER, efflux ratio; IC_{50} , concentration of inhibitor to achieve 50% inhibition; $J_{\max}$, maximal achievable transport rate; K_m , Michaelis–Menten constant; K_p , membrane-to-water partition coefficient; P-gp, P-glycoprotein; $V_{\max}$, maximum transport rate.

Table 3 Available knockout models for drug transporters recommended for evaluation by the ITC^{1,2}

Transporter	Knockout model	Comments	Major species differences (rodent vs. human)
Commercialized (as of 2012)			
P-glycoprotein (P-gp) (ABCB1, commonly MDR1)	<i>Mdr1a</i> ^{-/-} mice ⁷³	• Single Mdr1a KO is not a limitation for PK studies • Mdr1b contributes little to functional P-gp expression	• In rodents both Mdr1a and Mdr1b encode P-gp vs. a single <i>MDR1</i> gene in humans
	<i>Mdr1a</i> ^{-/-} rats ²⁷	• More convenient than mice for PK/excretion studies	• Mdr1a mRNA is predominately expressed in gut and brain, Mdr1b mostly in kidney, both in liver
	<i>Mdr1a/1b</i> ^{-/-} mice ⁷³	• Lacks both rodent <i>Mdr1</i> genes • Preferred for renal clearance and biliary excretion • Used in support of studies in <i>Mdr1a/1b/Bcrp</i>^{-/-} mice	• PK studies support Mdr1a accounting for majority of functional P-gp expression in organs key to drug disposition • Mdr1a protein in mouse brain microvessels is ~2-fold higher than in human^a
BCRP (ABCG2)	<i>Bcrp</i> ^{-/-} mice ⁷³	• No advantage over KO rats, except when used in support of studies in <i>Mdr1a/1b/Bcrp</i>^{-/-} mice	• Murine BBB Bcrp protein expression was ~2-fold lower than in human^a
	<i>Bcrp</i> ^{-/-} rats ²⁷	• More convenient than mice for PK/excretion studies	• In the liver, BCRP/Bcrp protein expression was mouse (1.2 fmol/μg protein) > rat (0.28 fmol/μg protein) > human (0.15 fmol/μg protein)^d
P-gp + BCRP (MDR1 + ABCG2)	<i>Mdr1a/1b/Bcrp</i> ^{-/-} mice ^{29,32}	• Utility for dual substrates, but exposure increase is not additive and requires <i>f_c</i> analysis approach^{29,30}	• See P-gp and BCRP above
MRP2 (ABCC2)	<i>Mrp2</i> ^{-/-} mice ⁷³	• Compensatory hepatic Mrp3 induction lower than in rats	• Rat hepatic Mrp2 protein expression (5.45 fmol/μg protein) was ~9-fold higher than in human (0.63 fmol/μg protein)^d
	<i>Mrp2</i> ^{-/-} rats ²⁷	• Hepatic Mrp2 importance exaggerated relative to other species and considerable hepatic Mrp3 upregulation • More convenient than mice for PK/excretion studies	
OATP1B1, 1B3 (SLCO1B1, 1B3)	<i>Oatp1a/1b</i> ^{-/-} mice ³³	• Lack majority of murine hepatic Oatp function, except Oatp2b1 • Lacks CNS uptake by Oatp1a4	• In rodents, no direct ortholog of human OATP1B1 and OATP1B3 • Rodents express Oatp1b2, 1a1, 1a4, and 2b1 in the liver
	OATP1B1 or 1B3 humanized	• Little PK characterization	
	<i>Oatp1a/1b</i> ^{-/-} mice ³⁶	• May enable estimation of OATP1B1 vs. 1B3 hepatic uptake	

Transporter	Knockout model	Comments	Major species differences (rodent vs. human)
OCT2 (SLC22A2)	<i>Oct1/2</i> ^{-/-} mice ^{33,37}	• Ablation of both Oct1 and 2 necessary to represent absence of human renal OCT2, but model also lacks hepatic Oct1	• Mice express renal Oct1 and 2 vs. human OCT2
Developed but not yet commercialized (as of 2012)			
MATE1, 2-K, 2 (SLC47A1-2)	<i>Mate1</i> ^{-/-} mice ³³	• MATE1 is sole murine renal isoform; potentially useful model for study of MATE renal clearance, as well as role of MATE in hepatic distribution	• The tissue distribution of MATE1 in mice is generally consistent with that in human • Murine MATE1 expressed in kidney vs. human renal MATE1, 2-K, and 2
OAT1, 3 (SLC22A6, 8)	<i>Oat1</i> ^{-/-} and <i>Oat3</i> ^{-/-} mice ^{33,73}	• Potentially useful model, especially as double knockout, for study of OAT renal distribution/clearance	• N/A
BSEP (ABCB11)	<i>Bsep</i> ^{-/-} mice ⁷³	• Not helpful in determining if drug inhibits BSEP bile acid transport due to complete knockout of transporter	• N/A

BBB, blood-brain-barrier; BCRP, breast cancer resistance protein; BSEP, bile salt export pump; CNS, central nervous system; f_e , fraction excreted; ITC, International Transporter Consortium; KO, knockout; MDR, multidrug resistance; N/A, insufficient data on rodent vs. human species differences; OATP, organic anion-transporting polypeptide; OCT, organic cation transporter; PK, pharmacokinetics.

^a Measured by liquid chromatography–tandem mass spectrometry.

Table 4

PBPK models incorporating transporter kinetics and DDIs

Model complexity	Drugs	Specificity of the model and <i>in vitro</i> input/system	Key application/findings	Assumptions	Ref.
Focus on one or two organs					
Intestine and liver	$n = 12$, Common victim drugs in DDIs, $F_G < 0.5$	Mechanistic description of liver and intestine	Prediction of F_G , intravenous and oral clearance	$f_{u,gut} = 1$	62
		Metabolism, permeability, and solubility data implemented	Capable of describing saturable intestinal metabolism		
Reduced PBPK					
Rat and dog	Ibuprofen, indomethacin, losartan, ramatroban, sulfisoxazole, telmisartan, tolmetin	Blood, liver, and rest of the body compartments Fresh hepatocytes in suspension ^{a,b}	Use of rat plasma, liver, and muscle concentration–time data and the PBPK model to refine predicted uptake clearance from <i>in vitro</i>	The same as discussed in the whole-body PBPK modeling examples	74
Whole-body PBPK					
Mouse	Digoxin	Western blotting of P-gp in tissues in $\pm 1,25$ -dihydroxyvitamin D ₃	The vitamin D-receptor increased P-gp in brain and kidney and not intestine or liver Predicted by changes in expression levels	Linear conditions throughout	75
Rat	Napsagatran ^c , fexofenadine	Liver model—intracellular and extracellular compartments Fresh-plated hepatocytes ^c	Use of rat <i>in vivo</i> data to refine predicted uptake clearance from <i>in vitro</i>	Application of drug-specific ESF from preclinical species to improve the scale-up to human	58
Rat and human	Cyclosporine	Kinetic parameters estimated by fitting local PBPK models to the individual organ tissue concentration–time data	Translation from rat to human PK in the mechanistic manner	Availability of tissue-concentration data in rat and complexity of the model	63
	Pravastatin	Five-compartment liver model	Sensitivity analysis on the impact of uptake and efflux parameters on plasma and liver profile	Does not account for potential species differences in activity and uptake ESF	52
		Cryopreserved hepatocytes in suspension ^a	Estimation of uptake ESF in rat (3.7) and application to human	Drug and donor-specific uptake ESF values	
	Valsartan	Liver model—intracellular and extracellular compartments Fresh-plated hepatocytes ^c	Use of rat and human plasma and rat bile concentration–time data and the PBPK model to	Perfusion-limited nonhepatic tissues	57

Model complexity	Drugs	Specificity of the model and <i>in vitro</i> input/system	Key application/findings	Assumptions	Ref.
			refine predicted uptake clearance from <i>in vitro</i>		
Human	Valsartan, pravastatin, repaglinide, cerivastatin, rosuvastatin, fluvastatin, bosentan	Five-compartment liver model	Use of human plasma profiles and the PBPK model to optimize <i>in vitro</i> uptake clearance (ESF = 58)	Does not account for <i>in vitro</i> and <i>in vivo</i> differences in transporter abundance	8
		Cryopreserved sandwich-cultured hepatocytes ^a	ESF required also for biliary efflux		
DDIs					
Human	Cyclosporine/gemfibrozil/rifampin-pravastatin	Transporter-mediated DDI	Use of human plasma profiles and the PBPK model to optimize <i>in vitro</i> uptake clearance	Drug-specific uptake ESF values	70
		Liver model—intracellular and extracellular compartments			
		Cryopreserved sandwich-cultured hepatocytes ^a	Transporter-mediated hepatobiliary disposition does not fully explain cyclosporine DDI	Use of <i>in vivo</i> K_i values for OATP1B1 inhibition	
	Cyclosporine–repaglinide	Transporter- and metabolism-mediated DDI in the liver and intestine	Use of human plasma profiles and the PBPK model to optimize <i>in vitro</i> uptake clearance	Drug-specific uptake ESF value	22
		Liver model—intracellular and extracellular compartments	Quantitative prediction of transporter DDI with simultaneous consideration of P450 inhibition		
		Cryopreserved plated hepatocytes ^c	Perpetrator metabolite also considered in the DDI prediction		

DDI, drug–drug interaction; ESF, empirical scaling factor for hepatic uptake clearance; FG , intestinal availability; K_i , inhibition constant; K_m , Michaelis–Menten constant; OATP, organic anion-transporting polypeptide; PBPK, physiologically based pharmacokinetics; P-gp, P-glycoprotein; V_{max} , maximum transport rate.

^aSingle clearance parameter for active uptake.

^bUptake characterized using media-loss assay.

^cPotential saturation of active uptake considered (K_m and V_{max})