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Utility of Minimal Physiologically-Based Pharmacokinetic (mPBPK) Models for Assessing Fractional Distribution, Oral Absorption and Series-Compartment Models of Hepatic Clearance

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Running Title: Minimal physiologically-based pharmacokinetic models

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Number of text pages: 51

Number of tables: 5

Number of figures: 14

Number of references: 115

Number of words in Abstract: 248

Number of words in Introduction: 739

Number of words in Discussion: 1737

ABBREVIATIONS

$AUC_{\text{inf}}$, area under the curve from time 0 to infinity; $B_{\text{max}}$, maximum binding capacity; $CL_b$, total blood clearance; $CL_h$, hepatic clearance; $CL_{\text{int}}$, hepatic intrinsic clearance; $C_{\text{max}}$, maximum concentration; CQ, chloroquine; CyA, cyclosporine A; DLZ, diltiazem; $D_N$, dispersion number; DM, dispersion model; DPH, phenytoin; DZP, diazepam; EB, ethoxybenzamide; $ER$, extraction ratio; $F$, oral bioavailability; $F_g$, pre-hepatic bioavailability; FTY720, fingolimod; $f_{ub}$, unbound fraction in blood; $f_{up}$, unbound fraction in plasma; GI, gastrointestinal; HB, hexobarbital; IP, intraperitoneal; IV, intravenous; IVIVE, in vitro-in vivo extrapolation; $K_d$, equilibrium dissociation constant; $K_m$, Michaelis-Menten constant; $K_{ph}$, liver-to-plasma partition coefficient;
$n$, number of liver sub-compartments; NIC, nicotine; $P$, permeability coefficient; PAMPA, parallel artificial membrane permeability assay; PO, oral; PBPK, physiologically-based pharmacokinetic; PD, pharmacodynamic; PK, pharmacokinetic; PPN, propranolol; PTM, parallel tube model; PTZ, pentazocine; QD, quinidine; $R_b$, blood-to-plasma ratio; SCM, series-compartment model; SS, steady state; TB: tolbutamide; $T_{\text{max}}$, time to reach $C_{\text{max}}$; VEM, verapamil; $V_{\text{max}}$, maximum rate of metabolism; WSM, well-stirred model.
Abstract

Minimal physiologically-based pharmacokinetic (mPBPK) models are physiologically relevant, require less information than full PBPK models, and offer flexibility in pharmacokinetics (PK). The well-stirred hepatic model (WSM) is commonly used in PBPK, while the more plausible dispersion model (DM) poses computational complexities. The series-compartment model (SCM) mimics the DM but is easier to operate. This work implements the SCM and mPBPK models for assessing fractional tissue distribution, oral absorption and hepatic clearance using literature-reported blood and liver concentration-time data in rats for compounds mainly cleared by the liver. Further handled were various complexities including nonlinear hepatic binding and metabolism, differing absorption kinetics, and sites of administration. The SCM containing 1 to 5 (n) liver sub-compartments yield similar fittings and provide comparable estimates for hepatic extraction ratio (ER), pre-hepatic availability (Fg), and first-order absorption rate constants (ka). However, they produce decreased intrinsic clearances (CLint) and liver-to-plasma partition coefficients (Kph) with increasing n as expected. Model simulations demonstrated changes in IV and oral PK profiles with alterations in Kph and ka and with hepatic metabolic zonation. The permeability (PAMPA P) of the various compounds well explained the fitted fractional distribution (fd) parameters. The SCM and mPBPK models offer advantages in distinguishing systemic, extrahepatic, and hepatic clearances. The SCM allows for incorporation of liver zonation and is useful in assessing changes in internal concentration gradients potentially masked by similar blood PK profiles. Improved assessment of intra-organ drug concentrations may offer insights into active moieties driving metabolism, biliary excretion, pharmacodynamics and hepatic toxicity.
Keywords Minimal physiologically based pharmacokinetic modeling; Series-compartment models; Hepatic clearance; Intrinsic clearance; Liver-to-plasma partition coefficient; Oral absorption; Liver zonation; Intrahepatic concentration; Permeability.
Significance Statement

The mPBPK and SCM models are useful in assessing oral absorption and hepatic clearance. They add flexibility in accounting for various drug- or system-specific complexities including fractional distribution, nonlinear binding and saturable hepatic metabolism, and hepatic zonation. These models can offer improved insights into the intra-organ concentrations that reflect physiologically active moieties often driving disposition, pharmacodynamics and toxicity.
Introduction

The liver is a pivotal eliminating organ for numerous endogenous substances and therapeutic drugs from the body. The PK field has sought to assess and predict hepatic clearances ($CL_h$) utilizing in vitro-in vivo extrapolation (IVIVE) (Jones et al., 2022; Tess et al., 2022) and physiologically based pharmacokinetic (PBPK) modeling approaches (Taskar et al., 2021; Lin et al., 2022), where an assumed structural liver model is required. Among the well-stirred model (WSM), the dispersion model (DM), and the parallel-tube model (PTM) (Bischoff and Dedrick, 1970; Rowland et al., 1973; Pang and Rowland, 1977; Roberts and Rowland, 1986a; Roberts and Rowland, 1986b; Li and Jusko, 2022), the DM is considered most compatible with the known architecture and physiology of the liver. Nevertheless, its implementation in PBPK has been hindered due to the complicated secondary partial differential equations required (Roberts and Rowland, 1986a; Roberts and Rowland, 1986b; Oliver et al., 2001), while the WSM is most adopted given its mathematical simplicity. The series-compartment model (SCM) that treats the liver as a cascade of identical well-stirred segments connected by hepatic blood flow ($Q_h$) reasonably approximates the DM (Roberts and Rowland, 1986a; Gray and Tam, 1987; Anissimov and Roberts, 2002). Subsequently, variants of the SCM were applied to mimic the DM for characterizing the hepatic disposition of transporter substrates (Watanabe et al., 2009; Jones et al., 2012; Li et al., 2014; Morse et al., 2017) and predicting the clinical drug-drug interactions (DDIs) involving hepatic transporters/enzymes (Asaumi et al., 2019; Zhang et al., 2022). The quantitative correlation of the SCM and DM was not elucidated until our recent work (Li and Jusko, 2023), where we demonstrated how the number of liver sub-compartments ($n$) in the SCM correlated with the dispersion number ($DN$) of the DM that describes the relative spread of solute on passage through the liver. We also showed that the SCM closely resembles the DM
in PBPK with easier operative features and offers many flexibilities such as intra-organ concentration gradients along the sinusoidal path of the liver and zonal heterogeneity of enzymes and transporters.

Minimal physiologically-based pharmacokinetic (mPBPK) models, introduced for characterizing and predicting the pharmacokinetics (PK) of both small (Cao and Jusko, 2012) and large molecules (Cao et al., 2013), inherit and lump major physiologic attributes from the whole-body PBPK models yielding physiologically relevant PK parameters (Jeong et al., 2022a; Jeong et al., 2022b), and thereby provide more practical assessments than do mammillary models. Owing to their physiological relevance, flexibilities in handling different mechanisms pertaining to the absorption, distribution, metabolism, and excretion processes, easier operative features and less information required than full PBPK models, mPBPK models have been progressively applied in both preclinical and clinical settings. These include: target-mediated drug disposition (Cao and Jusko, 2014; Chen et al., 2016), the neonatal Fc receptor recycling mechanism (Hardiansyah and Ng, 2022) of large molecules, interspecies PK scaling of small (Cao and Jusko, 2012; Jeong and Jusko, 2021; Song and Jusko, 2021) and large molecules (Zhao et al., 2015; Chen et al., 2017), the nonlinear binding PK of naproxen (Li et al., 2017), sex differences in hepatic metabolism of methylprednisolone (Ayyar et al., 2019), drug-drug-disease interactions of rivaroxaban and verapamil in subjects with varying degrees of renal impairment (Ismail et al., 2018), anti-inflammatory effects of dexamethasone (Swierczek and Jusko, 2023), predicting lung exposure of ivermectin for COVID-19 drug repurposing (Jermain et al., 2020), brain distribution of the antibiotic metronidazole (Chauzy et al., 2022), antibody therapies (Bloomingdale et al., 2021) targeting central nervous system (CNS) disorders, the kinetics of novel treatment modalities including small interfering RNA (siRNA) (Ayyar et al., 2021) and chimeric antigen receptor
(CAR) T cells (Tsai et al., 2022) and the PK of antenatal betamethasone and dexamethasone in parturient women and their fetuses (Krzyzanski et al., 2023).

Considering the advantages of mPBPK models and evidence that the SCM closely approximates the DM in PBPK, this work demonstrates applications of the SCM in mPBPK and further expands the utility of mPBPK models for: 1) assessing hepatic distribution and clearance, 2) characterizing the oral absorption kinetics and first-pass effects (pre-hepatic and hepatic extraction), utilizing literature-reported single intravenous (IV) and oral blood, plasma, and liver concentration-time data in rats for several flow-limited compounds that are primarily eliminated through the liver, and 3) relating the fractional distribution parameter \( f_d \) to tissue permeability.

The changes in the blood and liver PK after IV or oral dosing due to alterations in drug- or biological system-specific factors were also explored.
Methods

Data Sources

The observed blood or plasma and liver concentration-time data in rats for 14 compounds, viz, tolbamamide (TB), chloroquine (CQ), cyclosporine A (CyA), ethoxybenzamide (EB), fingolimod (FTY720), nicotine (NIC), pentazocine (PTZ), hexobarbital (HB), phenytoin (DPH), quinidine (QD), propranolol (PPN), diazepam (DZP), verapamil (VEM), and diltiazem (DLZ) were digitized from the references listed in Table 1 using GetData Graph Digitizer version 2.26 (http://getdata-graph-digitizer.com/). For the selected compounds: 1) liver is the major eliminating organ, and the extrahepatic clearances are known or assumed to be negligible; 2) distribution into the liver and access to the hepatic enzymes are flow-limited (high permeability) with minor or negligible transporter involvements; 3) hepatic extraction ratios ($ER_i$) range from low to high. The reported plasma concentrations ($C_p$) were first converted to the blood concentrations ($C_b$) by multiplying the blood-to-plasma ratio ($R_b$) (Supplementary Table S3) before fitting.

Model Structures

The minimal-PBPK model with one or two tissue compartments extended with the SCM of hepatic elimination is shown in Fig 1. The SCM has been described in detail (Li and Jusko, 2023). Briefly, the liver is divided into $n$ well-stirred sub-compartments connected by hepatic blood flow ($Q_h$), and the tissue volume ($V_{hi}$), availability ($F_i$), extraction ratio ($ER_i$), and intrinsic clearance ($CL_{int,i}$) for each sub-compartment $i$ ($i=1, 2, 3...n$) are initially assumed to be identical:

$$V_{hi} = \frac{V_h}{n}$$ (1)

$$F_i = \sqrt{n/1 - ER}$$ (2)

$$ER_i = 1 - F_i$$ (3)
\[ f_{ub}CL_{inti} = \frac{Q_h^{ER_i}}{1-ER_i} \quad (4) \]

\[ f_{ub}CL_{int} = n f_{ub}CL_{inti} \quad (5) \]

where \(Q_h\) is hepatic blood flow; \(f_{ub}\) is the unbound fraction in blood.

According to Eqs. 2-5, the total hepatic \(ER\) for the SCM with \(n\) liver sub-compartments is expressed as:

\[ ER = 1 - \left( \frac{Q_h}{Q_h + f_{ub}CL_{inti}} \right)^n \quad (6) \]

The outflow blood concentration from the liver sub-compartment \(i\) (\(C_{outi}, i=1, 2, 3…n\)) is the input concentration for the subsequent sub-compartment \(i+1\) and is assumed to be in equilibrium with the liver tissue concentration throughout the \(i^{th}\) compartment (\(C_{hi}, i=1, 2, 3…n\)):

\[ K_{phi} = \frac{R_b C_{hi}}{C_{outi}} \quad (7) \]

where \(K_{phi}\) is the liver-to-plasma concentration ratio for liver sub-compartment \(i\), and \(R_b\) is the blood-to-plasma ratio.

It is assumed that each liver sub-compartment shares the same \(K_{phi}\) for all model compounds except CQ and QD for which nonlinear binding in the liver has been reported (Harashima et al., 1985; Liu and Jusko, 2021), thus resulting in a concentration-dependent \(K_{phi}\). For the \(i^{th}\) (\(i=1, 2, 3…n\)) sub-compartment, the mathematical relationship between the bound (\(C_{bhi}\)) and free (\(C_{ubi}\)) hepatic concentration of CQ or QD is expressed by:

\[ C_{bhi} = \frac{B_{max} C_{ubi}}{K_d + C_{ubi}} \quad (8) \]

where \(B_{max}\) and \(K_d\) are the binding capacity of the liver and the equilibrium dissociation constant that were assumed to be the same for each of the sub-compartments.

The \(C_{hi}\) and \(C_{ubi}\) of CQ or QD can be calculated from:
\[ C_{hi} = \frac{B_{\text{max}} C_{uhi}}{K_d + C_{uhi}} + C_{uhi} \]  

(9)

and

\[ C_{uhi} = f_{uh} C_{hi} = f_{up} \frac{C_{hi}}{K_{phi}} \]  

(10)

where \( f_{uh} \) and \( f_{up} \) are the unbound fractions of CQ or QD in the liver and plasma.

Combining Eqs. 9 and 10 yields the nonlinear \( K_{phi} \) for CQ and QD in the \( i^{th} \) \((i=1, 2, 3...n)\) sub-compartment:

\[ K_{phi} = f_{up} \frac{-(C_{hi} - B_{\text{max}} - K_d) + \sqrt{(C_{hi} - B_{\text{max}} - K_d)^2 + 4K_d C_{hi}}}{2K_d} \]  

(11)

For the SCM shown in Fig 1, the concentration changes in the 1st and \( i^{th} \) \((i=2, 3...n)\) well-stirred liver compartment are described by:

\[ V_{h1} \frac{dC_{h1}}{dt} = \text{Input} + Q_h \left( C_b - \frac{R_b C_{h1}}{K_{phi}} \right) - f_{ub} C L_{int1} \frac{R_b C_{h1}}{K_{phi}} \quad C_{h1}(0) = 0 \]  

(12)

and

\[ V_{hi} \frac{dC_{hi}}{dt} = Q_h \left( \frac{R_b C_{h(i-1)}}{K_{phi(i-1)}} - \frac{R_b C_{hi}}{K_{phi}} \right) - f_{ub} C L_{inti} \frac{R_b C_{hi}}{K_{phi}} \quad C_{hi}(0) = 0 \]  

(13)

where \( C_b \) is the total concentration in blood; \( \text{Input} \) is the dosing input for the oral \( (\text{Input}_{po}) \) or intraportal \( (\text{Input}_{intraportal}) \) administration that directly enters the 1st hepatic compartment \( (V_{h1}) \):

\[ \text{Input}_{po} = k_a F_g A_a \]

\[ \text{Input}_{intraportal} = Dose_{intraportal} \]

where \( k_a \) is the first-order absorption rate constant; \( F_g \) is the pre-hepatic bioavailability; \( Dose_{intraportal} \) is the intraportal dose (DZP only); \( A_a \) is the amount of drug in the assumed absorption compartment for all compounds except CyA and PTZ:

\[ \frac{dA_a}{dt} = -k_a A_a \quad A_a(0) = Dose_{po} \]  

(14)

where \( Dose_{po} \) is the oral dose.
Delays in oral absorption were observed for both CyA and PTZ, which were best described by a 3-transit compartment model (Fig 1) where the oral dose enters the 1st transit compartment instead of the absorption compartment. The changes in drug amounts in the transit and absorption compartments are:

\[
\frac{da_1}{dt} = -k_{tr}a_1 \quad a_1(0) = Dose_{po} \quad (15)
\]

\[
\frac{da_2}{dt} = k_{tr}(a_1 - a_2) \quad a_2(0) = 0 \quad (16)
\]

\[
\frac{da_3}{dt} = k_{tr}(a_2 - a_3) \quad a_3(0) = 0 \quad (17)
\]

\[
\frac{dA_a}{dt} = k_{tr}a_3 - k_aA_a \quad A_a(0) = 0 \quad (18)
\]

where \(a_1\), \(a_2\) and \(a_3\) are drug amounts in the 1st, 2nd, and 3rd transit compartments; \(k_{tr}\) is the first-order transit rate constant.

The total liver concentration is assumed to be the average of \(C_{hi}\) in each of the sub-compartment:

\[
C_h = \frac{1}{n} \sum_{i=1}^{n} C_{hi} \quad (19)
\]

The concentration changes in blood \((V_b)\) and two tissue compartments \((V_{t1}\) and \(V_{t2}\)) are described by (Cao and Jusko, 2012):

\[
V_b \frac{dc_b}{dt} = f_d1(Q_{co} - Q_h) \left( \frac{R_bC_{t1}}{K_{pt}} - C_b \right) + f_d2(Q_{co} - Q_h) \left( \frac{R_bC_{t2}}{K_{pt}} - C_b \right) + Q_h \left( \frac{R_bC_{hn}}{K_{phn}} - C_b \right)
\]

\[
C_b(0) = \frac{Dose_{iv}}{V_b} \quad (20)
\]

and

\[
V_{t1} \frac{dc_{t1}}{dt} = f_d1(Q_{co} - Q_h) \left( C_b - \frac{R_bC_{t1}}{K_{pt}} \right) \quad C_{t1}(0) = 0 \quad (21)
\]

and

\[
V_{t2} \frac{dc_{t2}}{dt} = f_d2(Q_{co} - Q_h) \left( C_b - \frac{R_bC_{t2}}{K_{pt}} \right) \quad C_{t2}(0) = 0 \quad (22)
\]
where $V_b$ is blood volume; $V_{t1}$ and $V_{t2}$ are the volumes, and $C_{t1}$ and $C_{t2}$ are the concentrations for tissue compartments 1 and 2; $K_{pt}$ is the shared partition coefficient for the tissue compartments; $Q_{co}$ is cardiac output; $f_{d1}$ and $f_{d2}$ are the distribution fractions of $(Q_{co} - Q_h)$ for $V_{t1}$ and $V_{t2}$; $C_{hn}$ and $K_{phn}$ are the concentration and partition coefficient for the last liver sub-compartment; and $D_{ose_{iv}}$ is the IV dose. Given the nonlinear tissue binding of CQ and QD, the $K_{phi}(i=1, 2, 3…n)$ terms in Eqs. 12, 13 and 20 were replaced by Eq. 11.

Physiological model parameters were fixed to the literature values listed in Table S1 with the following restrictions:

$$f_{d1} + f_{d2} \leq 1 \text{ and } V_b + V_h + V_{t1} + V_{t2} = BW \text{ (Body Weight)}$$

The above model equations with or without the components colored in red apply to the two-tissue or one-tissue mPBPK model extended with the SCM.

**Data Analysis**

The IV blood/plasma and liver concentration-time data in rats for all 14 compounds (Table 1) have been assessed previously (Li and Jusko, 2022) using a piece-wise open-loop approach where the blood PK data were fitted first and then fixed as the forcing input function to model the liver PK data. The major limitation of such an approach is that $CL_{int}$ and $K_{ph}$ are highly correlated resulting in extremely large CV% values when estimated simultaneously. Therefore, it was necessary to back calculate the $CL_{int}$ from the hepatic ER that was then fixed in the model to only estimate $K_{ph}$. In this work, the same sets of IV PK data were revisited and jointly fitted applying the one-tissue mPBPK model extended with the hepatic SCM (i.e., Eqs. 12-13 and 19-21 without the components in red). As was done in our most recent work (Li and Jusko, 2023), the SCM containing 1, 2, and 5 liver sub-compartments were selected herein to mathematically mimic the WSM, and the DM with $D_N = 0.6$ and 0.1, the upper and lower boundaries of the
commonly reported $D_N$ range for the liver (Diaz-Garcia et al., 1992; Chou et al., 1993; Evans et al., 1993; Oliver et al., 2001). The $CL_{int}$ and $K_{ph}$ were simultaneously estimated with the hepatic $ER$ being obtained as a secondary parameter using Eq. 6. The apparent $K_{ph}$ values ($K_{ph,AR}$) representing the ratio of model-fitted area under the curves of liver and blood concentration-time data (Gallo et al., 1987) were also calculated. The $ER$, $K_{ph,AR}$, $CL_{int}$ and $K_{ph}$ estimated in the current modeling analysis were compared with those obtained by the open-loop approach (Li and Jusko, 2022).

Dose-dependent PK was reported for DPH due to the saturable aromatic hydroxylation by CYP2C11 in rat and CYP2C9 in man (Gerber et al., 1971; Doecke et al., 1991; Soucek and Gut, 1992; Ashforth et al., 1995). Accordingly, the blood PK data for DPH after 3 different IV doses (10, 25, 40 mg/kg) from (Gerber et al., 1971) along with the blood and liver PK data after a 10 mg/kg IV dose from (Itoh et al., 1988) were simultaneously fitted by the extended mPBPK model with the incorporation of saturable metabolism in the liver to quantitatively describe the nonlinear PK characteristics of DPH. The concentration-dependent $CL_{int}$ for DPH is described by the Michaelis-Menten equation:

$$CL_{int} = \frac{V_{max} f_{up} C_h}{K_m + f_{up} K_{ph}}$$

(23)

where $V_{max}$ is the maximal rate of metabolism, $K_m$ is the Michaelis-Menten constant, and $f_{up} C_h / K_{ph}$ is the unbound hepatic concentration of DPH at the site of metabolism assuming an instant equilibrium existing between the plasma and liver tissue. Therefore, the concentration changes of DPH in the liver with the consideration of nonlinear hepatic metabolism is expressed as:

$$V_h \frac{dC_h}{dt} = Q_h \left(C_b - \frac{R_h C_h}{K_{ph}}\right) - \frac{V_{max}}{K_m + f_{up} K_{ph}} f_{up} \frac{C_h}{K_{ph}} C_h$$

(24)
The extended mPBPK model with one hepatic compartment (Fig 1) was also applied to assess the oral absorption and first-pass effects by simultaneously characterizing the available IV and oral blood and/or liver PK data in rats for all model compounds except EB and DPH (Table 1). Oral PK data for EB could not be found in the literature. Although oral PK data for DPH was available, they were excluded due to data inconsistency. Therefore, the IV and oral PK data for 12 out of 14 compounds were included in this part of the analysis (Table 1).

The impacts of \( n \) (\( n=1, 2, 5 \)) on the model fittings and parameter estimates were also explored using the data for NIC, DZP, VEM, and DLZ. The oral bioavailability \( (F_{po}) \) for all compounds were obtained using two methods:

\[
F_{po} = \left\{ \frac{AUC_{po}}{Dose_{po}} \right\} \frac{AUC_{iv}}{Dose_{iv}} \left( F_g (1 - ER) \right)
\]  

(25)

where \( AUC_{iv} \) and \( AUC_{po} \) are the model-fitted AUC values for the blood concentration-time profile after IV and oral dosing.

To further explore the impacts of changing \( K_{ph} \) or \( k_a \), and metabolic enzyme zonation on IV and oral PK profiles in the blood, liver, and each of the liver sub-compartments, model simulations were performed for NIC, DLZ, and DPH using the mPBPK model extended with the SCM under the following conditions: 1) different \( K_{ph} \) values (i.e., \( K_{ph}, \pm 2\)-fold \( K_{ph} \), and \( \pm 5\)-fold \( K_{ph} \)); 2) different \( k_a \) values (i.e., \( k_a, \pm 2\)-fold \( k_a \), and \( \pm 5\)-fold \( k_a \)); 3) different patterns of hepatic enzyme zonation as were assumed previously (Li and Jusko, 2023), i.e., either lower metabolic activity at the periportal (PP) region assuming \( F_{i+1} = F_i^2 \) (\( i=1, 2...4 \)):

\[
F_1 = \sqrt[1+2+4+8+16]{(1 - ER)}
\]  

(26)

\[
F_{i+1} = F_i^2, \quad i = 1, 2...4
\]  

(27)

Or lower metabolic activity at the perivenous (PV) region assuming \( F_{i+1} = F_i^{1/2} \) (\( i=1, 2...4 \)).
With the assumed $F_i$ values, the corresponding $f_{ubCL_{inti}}$ were obtained using Eqs. 3 and 4 for the subsequent model simulations. The PK parameters including $C_{max}$, $T_{max}$ and $AUC$ of the simulated data were obtained by non-compartmental analysis (NCA) performed using Phoenix® WinNonlin® version 6.4 (Certara USA, Inc., Princeton, NJ), and the fold-changes in these parameters relative to the nominal values obtained at 1x $K_{ph}$, 1x $k_a$ or even enzyme distribution were assessed.

The model fitted fractional distribution parameter $f_d$ for all the compounds was examined in relation to apparent parallel artificial membrane permeability assay (PAMPA) permeability coefficient ($P$) values (i.e., $f_{ub}P/R_b$). Compound-specific physicochemical properties and blood partitioning parameters used for calculating the apparent PAMPA $P$ were listed in Supplementary Table S3.

**Model Fitting**

The model fittings of blood and liver concentration-time data were performed by nonlinear regression using the maximum likelihood algorithm in ADAPT 5 (Biomedical Simulations Resources, Los Angeles, CA) (D'Argenio et al., 2009). The variance model was:

$$V_i = (\sigma_{inter} + \sigma_{slope}Y_i)^2$$

(30)

where $V_i$ is the variance of the $i^{th}$ data point; $Y_i$ is the $i^{th}$ model-predicted concentration; $\sigma_{inter}$ and $\sigma_{slope}$ are the variance model parameters. Model selection was based on the goodness-of-fit criteria, which included the Akaike Information Criterion (AIC), visual inspection of the fitted profiles, and CV% of the parameter estimates.
The ADAPT code for 1) one-tissue mPBPK extended with the SCM (n=5) and nonlinear tissue binding for the IV PK of QD, 2) two-tissue mPBPK extended with the SCM (n=5) for the IV, oral and intraportal PK of DZP, 3) one-tissue mPBPK with absorption delays for the IV and oral PK of CyA, 4) one-tissue mPBPK with nonlinear hepatic metabolism for the IV PK of DPH are provided in the Supplemental Materials.
Results

Assessing Hepatic Distribution and Clearance by the SCM: mPBPK Model vs Open-Loop Approach

The reported blood and liver concentration-time data for each of the 14 compounds after IV administration (Table 1) were simultaneously described by the one-tissue mPBPK model extended with the hepatic SCM \((n=1, 2, \text{ and } 5)\) shown in Fig 1. The model fittings are presented in Fig 2 and the final parameter estimates are listed in Table 2. As can be seen, the observed data for all compounds were well fitted with low CV\% values for most parameter estimates except for the \(K_{ph}\) and \(CL_{int}\) values of PPN and DZP when fitted by the 1-compartment SCM (i.e., WSM), indicating that these parameters were not well estimated by the WSM given the currently available PK data. The nonlinear \(K_d\) values for CQ and QD tend to have higher CV\%, which may be attributed to the limited range of concentrations producing some difficulty in fitting a nonlinear function. The early phases of the liver profiles for CyA and EB that were previously underpredicted by the open-loop approach (Li and Jusko, 2022) were nicely captured by the extended mPBPK model. For CQ and QD, the nonparallel terminal phases of blood and liver PK profiles were reasonably explained by incorporating the nonlinear tissue binding in the hepatic compartments. The reported IV PK profiles of CQ were obtained after an intraperitoneal (IP) dose but were better fitted without an absorption phase as presented given the very fast absorption and low first-pass extraction of CQ (Al Shoyaib et al., 2019). The \(B_{max}\) and \(K_d\) estimates for CQ are close to those obtained in our previous analysis \((B_{max} =12000 \text{ ng/mL}; \ K_d =8 \text{ ng/mL})\) (Li and Jusko, 2022).

As was shown, the one-tissue mPBPK model with the SCM containing different \(n\) yielded mostly comparable values of the hepatic \(ER\) and \(K_{ph,AR}\) for each of the compounds. They
exhibited similar model performance as indicated by the AIC values (Table 2) and almost overlapping model fittings with slight differences in the early phases (Fig 2), however, there is a tendency that the CV% of $CL_{int}$ and $K_{ph}$ for some of the compounds are larger with $n=1$, suggesting that the fittings are more reliable with a larger $n$. The $CL_{int}$ and $K_{ph}$ estimates for each of the compounds decrease with increasing $n$ and such dependency becomes more remarkable as $ER$ increases consistent with previous assessments applying the open-loop approach (Li and Jusko, 2022).

As indicated in Fig 3, the estimated $ER$ and $K_{ph,AR}$ by the mPBPK model with the SCM are independent of the number of liver sub-compartments and correlate well with those obtained by the open-loop approach within a 2-fold error (Figs 3A and 3B). According to the quantitative correlations between the SCM and the basic hepatic clearance models demonstrated previously (Li and Jusko, 2023), the comparisons of $CL_{int}$ and $K_{ph}$ were made between the SCM with $n=1$ (i.e., mPBPK SCM-1) and the WSM, and between the SCM with $n=2$ (i.e., mPBPK SCM-2) and the DM with $D_N=0.6$ (Figs 3C-3F). As shown, the two different modeling approaches gave comparable values of $CL_{int}$ and $K_{ph}$ for most of the compounds except for PPN and DZP. Comparing to the open-loop approach, the mPBPK model slightly overestimated the $CL_{int}$ of PPN by 2.26-fold with SCM-1 (Fig 3C); nonetheless, the resulting $ER$ (0.76) is comparable to the reported value (0.77) (Li and Jusko, 2022). In addition, the $CL_{int}$ and $K_{ph}$ of DZP were overestimated by the one-tissue mPBPK model extended with the SCM by 2.48~3.59-fold (Figs 3C and 3E) and 2.85~4.05-fold (Figs 3D and 3F) with a larger $n$ yielding a smaller deviation from the open-loop approach. Such overestimation may be related to the overprediction at the terminal phase of the liver PK profile for DZP. Therefore, a two-tissue mPBPK model with the SCM was further applied to the PK data for DZP and the fitting results are indicated as DZP.
(Two-Tissue) in Fig 2. As can be seen, the IV liver PK for DZP was better captured by the two-tissue model and the estimated parameters are more consistent with those from the open-loop approach (Fig 3).

Assessing Nonlinear Metabolism

The nonlinear PK characteristics of DPH were reasonably described by incorporating saturable metabolism in the hepatic compartment of the extended mPBPK model (Fig 4), yielding parameters with low CV% values (Table 3). Previously, a two-site Michaelis-Menten model consisting of a saturable high affinity, low capacity site and a non-saturable low affinity, high capacity site was applied to the IV infusion data for DPH with a steady-state (SS) $C_p$ range of 378.4~22542 ng/mL, which generated a $V_{max}$ of 70560 ng/min/kg and a $K_m$ of 504 ng/mL based on the unbound DPH concentration (Ashforth et al., 1995). The estimated $V_{max}$ in the present analysis (86630 ng/min/kg) is comparable, while the $K_m$ (91.68 ng/mL) is lower. Due to the saturable metabolism, the $ER$ of DPH decreases with increasing hepatic concentrations and was predicted to be 0.04~0.78 within the predicted liver concentration range of 1~52433 ng/mL according to Eq. 6 and 24.

Assessing Oral Absorption and First-Pass Effects

The oral absorption and first pass of 12 compounds were assessed using the mPBPK model extended with the SCM. During the preliminary analysis, alternative structures of the mPBPK models were assessed such as using more tissue compartments, the same or different $K_{pt}$ values, and the same or different $f_d$ values for multiple tissue spaces, with or without absorption delays. Finally, the one-tissue mPBPK model extended with the SCM was sufficient for jointly fitting the oral and IV data for TB, CyA, CQ, FTY720, HB, NIC, QD, and PPN. However, after inclusion of the oral PK data, two tissue compartments ($V_1$ and $V_2$) sharing the same $K_{pt}$ in
addition to the hepatic compartment were found to be optimal for DZP, VEM, DLZ and PTZ. As shown in Figs 5-7, there is good agreement between the model predictions and observations obtained with the different dosing routes. The fitted parameters for all compounds were precisely estimated with low CV% values except that the modeling software was unable to generate CV% values for DLZ (Table 4). For most compounds, parameters related to the hepatic distribution and elimination (hepatic \( ER, CL_{int} \) and \( Kp_h \)) were comparable to those obtained without the oral PK data (Table 2). The oral absorption of all selected compounds except for CyA and PTZ was well described by a first-order process with the estimated absorption rate constant (\( ka \)) ranging from 0.0007 min\(^{-1}\) for FTY720 to 0.149 min\(^{-1}\) for HB. The oral absorption delays for CyA and PTZ were reasonably captured by incorporating the 3-compartment transit model yielding first-order transit rate constants (\( k_{tr} \)) of 0.019 and 0.089 min\(^{-1}\). The oral dosing vehicle for CyA was olive oil (Wassef et al., 1985), which might account for the delay in absorption by retarding gastric emptying (Ueda et al., 1984). PTZ was also reported to potentially delay gastric emptying time and inhibit propulsive activity in the rat (Danhof et al., 1966).

For TB, NIC, and DLZ, the pre-hepatic bioavailability (\( F_g \)) was fixed to 1 as the initial estimate exceeded 1, indicating that the pre-hepatic loss of the oral formulations for these compounds is negligible. In contrast, the oral dosage forms of the remaining compounds exhibit varying degrees of pre-hepatic loss, with the estimated \( F_g \) being the lowest for HB (~0.11) and the highest for PPN (~0.59). The \( F_{po} \) for each of the compounds calculated using Eq. 24 from the model estimated \( F_g \) and \( ER \), and from the ratio of model-fitted \( AUC \) values demonstrate good consistencies within a 2-fold range (Supplementary Fig S1) except for CyA. In the original paper where the oral PK data of CyA were obtained (Wassef et al., 1985), the influences of different dosing routes on the PK of CyA were assessed in rats and the bioavailability of the oral route at 5
and 10 mg/kg relative to the extravascular dosing routes that gave the highest $AUC$ were reported to be 10.3–13.3%. This is comparable with the $F_{po}$ values for CyA (7 and 14.5%) estimated in the current work. The differing absorption and bioavailability of QD after oral doses as a solution or powder in hydroxypropyl methylcellulose (HPMC) capsule were well discerned by the model by assigning dosage form-specific $ka$ (0.033 vs 0.023 min$^{-1}$) and $F_g$ (0.311 vs 0.405) values. The oral bioavailability ($F_{po}$) for the QD powder calculated according to Eq. 25 was 31–36% higher than that for the solution, and comparable to that reported (36%) (Mori et al., 2012).

**Assessing Fractional Distribution ($f_d$) and Partition Coefficients**

In mPBPK models, the $f_d$ values may represent either differential blood distribution to tissues/organs and/or flow/permeability limitations provided there is no transporter involvement. The ability to resolve such values offers an advantage over the use of forcing functions. The well characterized liver PK for all compounds is supportive of the assumption that distribution into the liver is flow-limited (i.e., $f_d = 1$). The high estimate of $f_{dl}$ for PPN, and $f_{dl} + f_{d2}$ for DZP and DLZ indicate that the distribution of these compounds into non-liver tissues is also primarily controlled by blood flow. For the remaining compounds, the $f_{dl}$ or $f_{dl} + f_{d2}$ were estimated to be low ranging from 0.005 for TB and 0.4 for VEM, suggesting that permeability-limited distribution may be operative for some of the non-liver tissues. Such heterogeneous tissue distribution has been reported for CQ (Liu and Jusko, 2021), HB (Igari et al., 1982a), FTY720 (Meno-Tetang et al., 2006), TB (Sugita et al., 1982), and CyA (Kawai et al., 1998). As shown by (Jeong et al., 2017), $f_d$ values can be positively correlated with the apparent PAMPA $P$ (i.e., $f_{up}P/R_b$). The relationship between the tissue $f_d$ values obtained herein and the apparent PAMPA $P$ is in line with such findings (Fig 8).
The distribution of CyA, FTY720, NIC, QD, VEM, and DLZ into the liver is more extensive than into the other tissues in the body as reflected by the appreciably larger $K_{ph}$ than the $K_{pt}$. While the accumulation of PTZ and PPN in liver is much lower than in other tissues, which is in line with previous findings (Schneck et al., 1977; Fujio et al., 1983). The estimated $K_{ph}$ and $K_{pt}$ are similar (within 2-fold) for TB, HB, and DZP, suggesting that the extent of distribution into the liver and non-liver tissues is comparable. Both the $K_{ph}$ (simulated from $B_{max}$ and $K_d$) and $K_{pt}$ of CQ were estimated as high. Such extensive tissue distribution of CQ is consistent with a previous report (Liu and Jusko, 2021) and is expected as CQ is subject to lysosomal trapping and binds appreciably to the acidic phospholipids on cell membranes (Allison and Young, 1964; Tietz et al., 1990; Zheng et al., 2011). Nonlinear tissue binding of CQ and QD was also reported for non-liver tissues (Harashima et al., 1985; Liu and Jusko, 2021), nevertheless, a constant rather than concentration-dependent $K_{pt}$ representing the time/concentration-averaged value was assigned here for simplicity given the limited data available and was shown to be sufficient for the purpose of the current analysis.

Role of Numbers of Liver Sub-Compartments

The effects of different numbers of liver sub-compartments ($n=1, 2$ and $5$) were further explored using the IV and oral PK data for NIC, DZP, VEM and DLZ, with the model fitting results presented in Supplementary Fig S2 and Table S2. All parameters were precisely estimated except that the software was not able to generate the CV% values for DZP (when $n=5$) and DLZ (all models). As expected, $CL_{int}$ and $K_p$ decrease with increasing $n$, while no major difference was observed in the model fittings and estimates for other parameters with varying values of $n$ except that the $k_a$ for VEM was estimated larger ($0.099 \text{ min}^{-1}$) when $n=5$ producing a higher peak for the fitted oral blood PK profile.
Assessing Impacts of Changing $K_{ph}$ or $k_a$ and Hepatic Enzyme Zonation on the Blood, Liver, and Intrahepatic Concentrations

To assess how the IV and oral PK profiles are affected by the changes in $K_{ph}$ or $k_a$, model simulations were performed using the mPBPK model with the SCM ($n=1$ and 5) utilizing the final parameter estimates for NIC and DLZ listed in Table 4 and varying values of $K_{ph}$ or $k_a$. The simulated PK profiles with differed $K_{ph}$ are presented in Fig 9 for DLZ and Supplementary Fig S3 for NIC, and the fold-changes in the resulting PK parameters (i.e., $C_{max}$, $T_{max}$ and $AUC_{inf}$) relative to the corresponding nominal values at 1x $K_{ph}$ are displayed in Fig 10 for DLZ and Supplementary Fig S4 for NIC. No changes were observed in the IV blood PK with changing $K_{ph}$ (data not shown), while the IV liver PK, and the PK profiles for blood and liver after oral dosing are all sensitive to the changes in $K_{ph}$ with the oral liver PK being the most affected. All the PK parameters exhibit a decreasing trend as $K_{ph}$ becomes smaller except for unchanged $AUC_{inf}$ and slightly increased $C_{max}$ for the oral blood PK profiles. Regardless of the dosing route, the fold-changes in $AUC_{inf}$ of the liver PK profiles are always the same as those in $K_{ph}$. The changes in PK parameters are independent of the number of liver sub-compartments except that the $T_{max}$ of the oral blood PK profiles are more sensitive to the change in $K_{ph}$ when $n=5$. Fig 11 displays the simulated PK profiles for DLZ in blood, liver, and each of the liver sub-compartments after oral dosing with varying $k_a$. The fold-changes in the corresponding $T_{max}$ and $C_{max}$ of the simulated profiles comparing to the nominal values obtained at 1x $k_a$ are presented in Fig 12. In line with the intrahepatic concentration gradient demonstrated previously (Li and Jusko, 2023), more delayed $T_{max}$ and smaller $C_{max}$ are observed for the later sub-compartments (Fig 11). All the PK profiles after oral administration are affected by the change of $k_a$, i.e., when $k_a$ decreases, the $T_{max}$ increases with the most remarkable changes observed for the blood PK
profiles especially with the smaller $k_a$ values, while the $C_{max}$ decreases with that for the liver PK exhibiting a steeper decline than that for the blood PK. The intrahepatic PK profiles were affected differently by the alteration of $k_a$, with the 1st sub-compartment being the most sensitive followed by each of the subsequent compartments. The $AUC_{inf}$ for all PK profiles remained unchanged with varying $k_a$ (data not shown).

The impacts of metabolic zonation on the IV and oral PK were assessed using the SCM with 5 liver sub-compartments assuming a) lower metabolic activity at the PP region, b) uniform metabolic activity, or c) lower metabolic activity at the PV region, where each of the liver sub-compartments was assigned a segment-specific $CL_{inti}$ producing a maximum difference in the metabolic clearance from the PP to the PV region of 25.4-fold for DLZ and 18.7-fold for DPH (Table 5). The simulated blood, liver, and sub-compartment concentration-time profiles for DLZ after IV or oral dosing with different enzyme zonation patterns are displayed in Fig 13, and the resulting fold-changes in PK parameters ($T_{max}$, $AUC_{inf}$ and $C_{max}$) with hepatic zonation comparing to those obtained assuming even enzyme distribution are shown in Fig 14. As can be seen, the blood PK are not sensitive to the change in zonation patterns regardless of the dosing route. In contrast, the total liver concentration-time profiles were markedly affected, for which the $T_{max}$, $AUC_{inf}$ and $C_{max}$ are the highest when the metabolic activity is higher at the PV region and the lowest when the PP region exhibits the higher metabolic clearance. In general, the changes in $C_{max}$ after oral dosing are more noticeable than those after IV dosing when hepatic zonation is considered. The PK profile in each of the liver segments is affected differently by metabolic zonation and such differences are also dependent on the dosing route. Among all the sub-compartments, the last one is the least affected. For the remaining sub-compartments, lower $CL_{int}$ at the PP region yields higher $T_{max}$ with the largest increase observed in the 3rd compartment for
the IV dose and the 1st compartment for the oral dose, while lower PV \( CL_{int} \) produces lower \( T_{max} \) with the largest decrease occurring in the 1st sub-compartment for both IV and oral doses as compared to the uniformly distributed \( CL_{int} \). The \( C_{max} \) and \( AUC_{inf} \) show similar trends but more remarkable changes with differed zonation patterns than those observed for \( T_{max} \). Such impacts of metabolic zonation were found to be similar for DPH (Supplementary Figs S5 and S6).
Discussion

The mPBPK models serve as a semi-mechanistic top-down approach between full PBPK models and empirical compartment models when only the blood/plasma PK data are available (the most common situation in human studies). These models have demonstrated broad applications in both preclinical and clinical research owing to the flexibility of handling different mechanisms in PK and offer reduced complexity compared with full models. Thus far, the WSM has been the most applied liver structural model in both mPBPK and full-PBPK models despite that it is the least physiologically relevant. In contrast, the application of the more physiologically plausible DM has been limited by its computational complexities. As shown previously, the SCM closely approximated the DM in PBPK as it yields comparable values and consistent model dependencies of $CL_{int}$ and $K_{ph}$ with those obtained by the DM (Li and Jusko, 2022; Li and Jusko, 2023). More importantly, it offers insights regarding the sequential intrahepatic concentration gradients and metabolic heterogeneity. Therefore, the current work demonstrates the implementation of the SCM in mPBPK models as an alternative to the DM, and further expands the utility of mPBPK models for assessing hepatic distribution and clearance, oral absorption and first-pass effects using the literature-reported blood and liver PK data for several substances mainly cleared by the liver after single IV and oral dosing.

The previous assessments of different hepatic models in PBPK (Li and Jusko, 2022; Li and Jusko, 2023) adopted a stepwise open-loop approach (Ebling et al., 1994; Cheung et al., 2018), where the blood PK was fitted first using empirical exponential functions to obtain the hepatic $ER$ and model-specific $CL_{int}$ which were then fixed for fitting the liver PK to only estimate $K_{ph}$. Here, operating the SCM in mPBPK allows joint fitting of the blood and liver PK data and simultaneous estimation of the hepatic $ER$, $CL_{int}$ and $K_{ph}$, and generates comparable results with
those obtained by the open-loop approach (Fig 3). Additionally, the blood and liver PK data for all 14 model compounds after IV dosing were more naturally captured and the previous underpredictions for the liver PK of CyA and EB by the DM applying the open-loop approach were significantly improved (Fig 2). Early time oscillations particularly seen in the mixed venous and arterial PK profiles following IV bolus administration is one of the distinct features of the whole-body PBPK models incorporated with the DM, with the lower $D_N$ yielding more significant divergence from that obtained by the WSM (Oliver et al., 2001). Interestingly, the early phases of the SCM-fitted blood PK profiles for some of the compounds also exhibited similar oscillatory features and diverged from that predicted by the WSM (i.e., when $n=1$ for the SCM), and such a divergence becomes more significant as $n$ increases (Supplementary Fig S7). This was not observed in the previous assessments of the SCM as the blood PK were fixed (Li and Jusko, 2023). Such findings are consistent with the expected quantitative relationships between the SCM and DM, i.e., the SCM with increasing $n$ quantitatively correlates with the DM with decreasing $D_N$ (Li and Jusko, 2023).

The utility of the extended mPBPK models in assessing the oral absorption kinetics and first-pass effects was demonstrated by jointly fitting the blood and liver PK data for 12 model compounds after IV and oral dosing, with all the PK data well captured and parameters precisely estimated. As was shown, the extended mPBPK models not only allow for characterization of nonlinear binding and metabolism in the liver but are also flexible in handling different sites of administration as well as the oral absorption kinetics either simply as a first-order process or applying the transit compartment models to account for absorption delays. Given that mPBPK models retain key physiological components of the full PBPK models, it is readily feasible to incorporate more mechanistic absorption models as can be done with full models when relevant.
information is available (Huang et al., 2009; Cheng and Wong, 2020). The pre-hepatic availability ($F_g$) (i.e., the fraction of oral dose escaping pre-hepatic loss) was nicely informed and precisely estimated after the inclusion of oral data, with the estimated hepatic $ER$ being comparable to those obtained by fitting the IV data alone. The $F_g$ is a hybrid parameter representing the product of the fraction of dose absorbed and the fraction escaping GI extraction [metabolism and/or P-glycoprotein (P-gp)], the exact value of which can be further identified with available information pertaining to the PK processes happening before the orally dosed drug reaches the liver. Even though the selected compounds are mainly cleared by the liver, some of them showed noticeable pre-hepatic loss as reflected by low $F_g$ values. This could be explained by poor absorption due to the solubility/dissolution issues if the GI extraction is negligible. This may not be true for CyA (Ducharme et al., 1995), QD (Darbar et al., 1997) and VEM (Darbar et al., 1998) as their intestinal metabolism was reported to be appreciable after oral doses. QD and VEM are known substrates of P-gp, nonetheless, the contribution of P-gp to the absorption and overall oral bioavailability of these compounds were considered limited given their high permeability and the easily saturable feature of intestinal P-gp especially at the doses assessed herein (Varma and Panchagnula, 2005; Mori et al., 2012). It is expected that absorption and bioavailability would differ with different oral formulations and this work demonstrated how such formulation-specific PK characteristics can be easily differentiated by the extended mPBPK models utilizing the oral PK data for QD in two different dosage forms (i.e., solution and powder in HPMC capsules).

In recent years, commercial software such as GastroPlus PBPK Simulator (Simulation Plus Inc., Lancaster, CA), PKSim (Open Systems Pharmacology Suite, Bayer Technology Services, Leverkusen, Germany), and Simcyp (Certara UK Ltd., Simcyp Division, Sheffield, UK) have
made the implementation of PBPK modeling and simulation easier utilizing input parameter values obtained from various sources (Sager et al., 2015; Lin et al., 2022). Specifically, the drug distribution parameters (e.g., $K_{ph}$) are often predicted by in silico approaches (Poulin and Theil, 2002; Berezhkovskiy, 2004; Rodgers and Rowland, 2006; Lukacova et al., 2008; Asaumi et al., 2019) based on the physicochemical properties of the drug substance and tissue compositions. However, abundant evidence has shown that such in silico predicted $K_{ph}$ values can differ appreciably from known in vivo values (Li et al., 2017; Song et al., 2020; Liu and Jusko, 2021; Jeong and Jusko, 2022; Li and Jusko, 2022). Here, we demonstrated that different $K_{ph}$ values can result in markedly different predictions for the liver PK even though the IV blood PK are well matched (Figs 9 and 10).

Both the extent and rate of absorption play critical roles in determining the bioavailability of orally administered drugs and are affected by many factors, e.g., the system physiological parameters, the physicochemical properties and formulation characteristics of the drug, and concomitant consumption of food or other medications (Lennernas, 1998; Martinez and Amidon, 2002; Cheng and Wong, 2020). The present work further explored the impact of altering $k_a$ on the oral PK characteristics. Not surprisingly, all oral PK profiles were sensitive to the changes in $k_a$ (Figs 11 and 12) with more remarkable increases in $C_{max}$ for the 1st liver sub-compartment as $k_a$ increases. This is in line with predictions (Li and Jusko, 2023) that oral dosing with rapid absorption may exacerbate the differences in early model predictions and may be relevant when a drug shows hepatic toxicity.

It has been a common simplification in PBPK that metabolizing enzymes and transporters are uniformly distributed across the liver despite the recognition otherwise (Jungermann, 1986; Meyer-Wentrup et al., 1998; Oinonen and Lindros, 1998; Reichel et al., 1999; Tachikawa et al.,
2018). Moreover, some hepatic enzymes are also regulated in a zonal pattern (Buhler et al., 1992; Lindros, 1997; Donner et al., 2004), which is considered to be associated with the zonal patterns of certain liver injuries (Cunningham and Porat-Shliom, 2021). For example, the regiospecific induction of CYP2E1 was reported to be the major reason for the centrilobular damage related to acetaminophen overdose (Anundi et al., 1993). The SCM offers needed flexibility in modeling liver zonation and regulation; however, related applications have been limited (Tirona and Pang, 1996; Abu-Zahra and Pang, 2000; Liu and Pang, 2006; Schenk et al., 2017; Li et al., 2019). Applying the extended mPBPK models with the SCM demonstrates incorporation of hepatic metabolic zonation and showed that differed zonation patterns can predict significantly different liver concentrations and intrahepatic concentration gradients even though the blood PK profiles are almost identical (Figs 13 and 14).

The applications of the SCM are not only limited to the liver; for example, mechanistic absorption models such as the advanced compartmental absorption and transit (ACAT) model and the advanced dissolution, absorption, and metabolism (ADAM) model treat the GI tract as a series of well-mixed compartments with linear transfer kinetics and uniform concentrations (Huang et al., 2009). Recently, (semi)-mechanistic kidney models have been developed to assess renal clearances where the kidney was divided into various nephron segments (Dave and Morris, 2015; Huang and Isoherranen, 2018). All these models encompass the basic structure of the SCM. One limitation of this work is that the IV and extravascular PK data were not available from the same literature sources except those for FTY720, and thus the parameter estimates are approximate due to potential data heterogeneity. Nevertheless, the results are deemed sufficient for demonstrating the expanded utilities of the extended mPBPK models.
In conclusion, the present work showcases the application of the SCM in mPBPK models and demonstrates that the extended mPBPK models are highly flexible in handling hepatic distribution and clearance, nonlinear tissue binding and metabolism, different oral absorption kinetics and the relative contribution of pre-hepatic loss and hepatic extraction to the first-pass effects without the need for extensive PK data and tissue predictions such as required in full-PBPK models. The extended mPBPK models with the SCM allow for the consideration of liver zonation and are particularly useful in assessing changes in the internal concentration gradients potentially masked by the similar or identical blood PK profiles due to alterations in drug- or system-specific factors. This is meaningful as intra-organ concentrations are considered the physiologically active moiety often driving metabolism, transport, PD effects and toxicity. The mPBPK models extended with the SCM may be further appreciated with the technological advances in the study of liver zonation (Cunningham and Porat-Shliom, 2021; Paris and Henderson, 2022) and other aspects of intrahepatic functioning.
Acknowledgments

We appreciate the review and insights provided by Dr. Yoo-Seong Jeong.
Data Availability Statement

The authors declare that all the data supporting the findings of this study are contained within the paper.
Authorship Contributions

Participated in research design: Li and Jusko.

Performed data analysis: Li.

Wrote or contributed to the writing of the manuscript: Li and Jusko.
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Footnotes

This work was supported by NIH Grant R35 GM131800. No author has an actual or perceived conflict of interest with the contents of this article.
Figure Legends

Fig 1. Minimal-PBPK model extended with the hepatic series-compartment model (SCM) containing \( n \) liver sub-compartments. The model components shown with broken lines are applied as needed.

Fig 2. Blood and liver concentration-time profiles of 14 model compounds after IV dosing. Measured concentrations in blood and liver are indicated by solid symbols in red and black. Solid and dashed lines represent model fittings of blood and liver data by one-tissue mPBPK extended with the SCM containing 1 (purple), 2 (orange) and 5 (green) liver sub-compartments. The estimated \( ER \), \( f_{uCLint} \) and \( K_{ph} \) (\( B_{max} \) and \( K_d \) for CQ and QD) values for each of the compounds are listed with the same color coding as those for the model fittings.

Fig 3. Comparisons of the estimated \( ER \) (A), \( K_{ph,AR} \) (B), \( f_{uCLint} \) (C and E), and \( K_{ph} \) (D and F) by the one-tissue mPBPK model with the SCM \((n=1, 2 \text{ and } 5)\) and those obtained by the open-loop approach (Li and Jusko, 2022). The \( K_{ph} \) values for CQ and QD shown were calculated from \( B_{max}/K_d \). The parameter values for DZP estimated by the two-tissue mPBPK model with the SCM are also plotted for comparisons (open triangles). The solid line represents unity, and the dashed lines indicate two-fold range from unity. The color coding is the same as used in Fig 2.

Fig 4. Blood and liver concentration-time profiles of DPH after iv bolus administration of 10 (red), 25 (green), or 40 (orange) mg/kg doses. Closed circles and solid lines represent the observations and model fittings of blood concentrations. Open circles and dashed lines indicate the observed and model-fitted liver concentrations. The measured data in panel (A) were obtained from (Gerber et al., 1971) and those in panel (B) were from (Itoh et al., 1988).

Fig 5. Blood and liver concentration-time profiles of TB, CyA, CQ, and FTY720 after oral, IV or IP (CQ only) dosing. Measured concentrations in blood and liver are indicated by solid and open symbols. Solid and dashed purple lines represent model fittings of blood and liver data by the
SCM with one liver sub-compartment (=WSM). The estimated $ER$ value for each of the compounds is listed.

Fig 6. Blood and liver concentration-time profiles of HB, PTZ, NIC, and QD after oral or IV dosing. Symbols, lines, and color coding are the same as used in Fig 5.

Fig 7. Blood and liver concentration-time profiles of PPN, DZP, VEM, and DLZ after oral, IV or intraportal (DZP only) dosing. Symbols, lines, and color coding are the same as used in Fig 5.

Fig 8. Relationship between the model estimated tissue $f_d$ and the apparent PAMPA $P$ (i.e., $f_{up}P/R_b$).

Fig 9. Effects of changing $K_{ph}$ on predicting the blood and liver concentration-time profiles for DLZ after IV or oral dosing by the mPBPK model extended with the SCM ($n=1$ and 5). Color coded lines represent model simulations with $K_{ph}$ (17.87 for SCM1 and 8.09 for SCM5, black), -2-fold $K_{ph}$ (green), +2-fold $K_{ph}$ (orange), -5-fold $K_{ph}$ (red), and +5-fold $K_{ph}$ (purple).

Fig 10. Fold-changes in the PK parameters calculated based on the PK profiles for DLZ simulated with varying values of $K_{ph}$ displayed in Fig 9. Different colors represent the results obtained by the mPBPK model extended with SCM-1 (purple) or SCM-5 (green). The nominal PK parameter values at 1x $K_{ph}$ are listed with the same color coding as that for the indicated model.

Fig 11. Effects of changing $k_a$ on predicting the blood, liver, and hepatic sub-compartment concentration-time profiles for DLZ after oral dosing by the mPBPK model extended with the SCM ($n=5$). Color coded lines indicate model simulations with $k_a$ (0.125 min$^{-1}$, black), -2-fold $k_a$ (green), +2-fold $k_a$ (orange), -5-fold $k_a$ (red), and +5-fold $k_a$ (blue).

Fig 12. Fold-changes in the PK parameters calculated based on the PK profiles for DLZ simulated with varying $k_a$ values shown in Fig 11. Different compartments are color coded as...
indicated. The nominal parameter values at 1x $k_d$ are listed with the same color coding as that used for the corresponding compartments.

Fig 13. Simulated concentration-time profiles for DLZ in blood, liver, and each of the liver sub-compartments in rats after bolus administration of a 5 mg/kg IV dose (solid lines) or 20 mg/kg PO dose (dashed lines). Different hepatic zonation patterns are color coded as follows: lower PP $CL_{int}$ (green), uniform $CL_{int}$ (black), and lower PV $CL_{int}$ (orange).

Fig 14. Fold-changes in $T_{max}$, $AUC_{inf}$ and $C_{max}$ computed from the simulated PK profiles for DLZ in blood ($C_b$), liver ($C_h$) and each of the liver sub-compartments ($C_{h1}$ through $C_{h5}$) after IV (solid bars) and oral (open bars) administration with hepatic enzyme zonation [lower PP $CL_{int}$ (green) or lower PV $CL_{int}$ (orange)] compared to those obtained with uniform enzyme distribution (black).
Table 1. PK data included in the current analysis.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dosing Route</th>
<th>Dose (mg/kg)</th>
<th>PK Sample</th>
<th>Literature Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tolbutamide (TB)</td>
<td>IV</td>
<td>80</td>
<td>Plasma, liver</td>
<td>(Sugita et al., 1982)</td>
</tr>
<tr>
<td></td>
<td>PO</td>
<td>50</td>
<td>Plasma</td>
<td>(Nishimura et al., 1998)</td>
</tr>
<tr>
<td>Chloroquine (CQ)</td>
<td>IP</td>
<td>10</td>
<td>Plasma, liver</td>
<td>(Adelusi and Salako, 1982)</td>
</tr>
<tr>
<td></td>
<td>PO</td>
<td>10</td>
<td>Plasma</td>
<td>(Parson et al., 2012)</td>
</tr>
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<td>Cyclosporine A (CyA)</td>
<td>IV infusion</td>
<td>5.9 (duration: 2 min)</td>
<td>Blood, liver</td>
<td>(Kawai et al., 1998)</td>
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<tr>
<td></td>
<td>PO</td>
<td>5, 10</td>
<td>Serum</td>
<td>(Wassef et al., 1985)</td>
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<tr>
<td>Ethoxybenzamide (EB)</td>
<td>IV</td>
<td>20, 50, 100</td>
<td>Plasma, liver</td>
<td>(Lin et al., 1978)</td>
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<td>Fingolimod (FTY720)</td>
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<td>0.3, 2,</td>
<td>Blood, liver</td>
<td>(Meno-Tetang et al., 2006)</td>
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<td>PO</td>
<td>2.8, 7.5</td>
<td>Plasma, liver</td>
<td>(Ribas et al., 2009)</td>
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<td>0.1</td>
<td>Plasma, liver</td>
<td>(Plowchalk et al., 1992)</td>
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<td></td>
<td>IV &amp; PO</td>
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<td>Plasma</td>
<td>(Jung et al., 1999)</td>
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<td>Pentazocine (PTZ)</td>
<td>IV infusion</td>
<td>2 (duration: 2 min)</td>
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<td>(Fujio et al., 1983)</td>
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<td>PO</td>
<td>5</td>
<td>Plasma</td>
<td>(Khan et al., 2022)</td>
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<td>Hexobarbital (HB)</td>
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<td>(Igari et al., 1982a)</td>
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<td>PO</td>
<td>25</td>
<td>Blood</td>
<td>(Van der Graaff et al., 1985)</td>
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<td>10</td>
<td>Serum, liver</td>
<td>(Itoh et al., 1988)</td>
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<td>Blood</td>
<td>(Gerber et al., 1971)</td>
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<td>(Harashima et al., 1985)</td>
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<td>PO</td>
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<td>Plasma</td>
<td>(Shibasaki et al., 1989)</td>
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<td>Diazepam (DZP)</td>
<td>IV</td>
<td>1.2</td>
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<td>(Igari et al., 1982b)</td>
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<td>Plasma</td>
<td>(Zhou et al., 2020)</td>
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<tr>
<td>Verapamil (VEM)</td>
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<td>Plasma, liver</td>
<td>(Yamano et al., 1984)</td>
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<td>PO</td>
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<tr>
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<td>IV</td>
<td>10</td>
<td>Plasma</td>
<td>(Yamano et al., 2000)</td>
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<td>Diltiazem (DLZ)</td>
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<td>5</td>
<td>Plasma, liver</td>
<td>(Lee et al., 2012)</td>
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<tr>
<td></td>
<td>PO</td>
<td>20</td>
<td>Plasma</td>
<td>(Tsui et al., 1994)</td>
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Table 2. Final parameter estimates for 14 model compounds obtained by jointly fitting the blood and liver concentration-time data after single IV dosing by the mPBPK model extended with the hepatic SCM ($n=1, 2, \text{and } 5$).

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<tr>
<th>Compound</th>
<th>SCM</th>
<th>$ER$ (CV%)</th>
<th>$f_d$ (CV%)</th>
<th>$K_{pt}$ (CV%)</th>
<th>$K_{ph}$ (CV%)</th>
<th>$f_d\text{CL}_{int}$ (mL/min/kg)</th>
<th>$K_{ph, AR}$</th>
<th>AIC</th>
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<tr>
<td>Tolbutamide</td>
<td>$n=1$</td>
<td>0.027 (4.27)</td>
<td>0.045 (38.90)</td>
<td>0.183 (8.73)</td>
<td>0.156 (7.63)</td>
<td>1.72 (4.39)</td>
<td>0.152</td>
<td>256</td>
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<td></td>
<td>$n=2$</td>
<td>0.027 (4.27)</td>
<td>0.045 (38.76)</td>
<td>0.183 (8.72)</td>
<td>0.155 (7.61)</td>
<td>1.71 (4.36)</td>
<td>0.152</td>
<td>256</td>
</tr>
<tr>
<td></td>
<td>$n=5$</td>
<td>0.027 (4.28)</td>
<td>0.045 (39.12)</td>
<td>0.183 (8.74)</td>
<td>0.154 (7.62)</td>
<td>1.70 (4.35)</td>
<td>0.152</td>
<td>256</td>
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<tr>
<td>Chloroquine</td>
<td>$n=1$</td>
<td>0.027 (4.28)</td>
<td>0.045 (39.12)</td>
<td>0.183 (8.74)</td>
<td>0.154 (7.62)</td>
<td>1.70 (4.35)</td>
<td>0.152</td>
<td>256</td>
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<tr>
<td></td>
<td>$n=2$</td>
<td>0.065 (7.51)</td>
<td>0.021 (27.53)</td>
<td>38.14 (16.10)</td>
<td>149.6</td>
<td>345</td>
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<td>0.021 (27.66)</td>
<td>38.19 (16.16)</td>
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<td>345</td>
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<td>0.211 (14.07)</td>
<td>5.70 (10.81)</td>
<td>15.00 (9.91)</td>
<td>5.37 (8.56)</td>
<td>13.77</td>
<td>409</td>
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<td>$n=2$</td>
<td>0.081 (7.81)</td>
<td>0.204 (14.12)</td>
<td>5.69 (10.84)</td>
<td>14.56 (9.39)</td>
<td>5.23 (8.32)</td>
<td>13.66</td>
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<td>$n=5$</td>
<td>0.079 (8.57)</td>
<td>0.168 (14.72)</td>
<td>5.66 (12.06)</td>
<td>13.63 (9.69)</td>
<td>5.03 (9.00)</td>
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<td>0.624 (4.04)</td>
<td>1.32 (6.05)</td>
<td>5.55 (4.99)</td>
<td>1.21</td>
<td>283</td>
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<td>0.082 (4.45)</td>
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<td>0.622 (4.01)</td>
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<td>0.427 (14.07)</td>
<td>0.622 (4.00)</td>
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<td>Fingolimod</td>
<td>$n=1$</td>
<td>0.155 (8.62)</td>
<td>0.161 (18.47)</td>
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<td>11.18 (10.20)</td>
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<td>$n=2$</td>
<td>0.145 (8.38)</td>
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<td>$n=5$</td>
<td>0.134 (7.41)</td>
<td>0.167 (18.66)</td>
<td>6.76 (12.67)</td>
<td>48.35 (8.66)</td>
<td>9.42 (8.56)</td>
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<td>Pentazocine</td>
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<td>0.304 (15.85)</td>
<td>1.29 (12.74)</td>
<td>0.381 (15.40)</td>
<td>18.77 (8.40)</td>
<td>0.291</td>
<td>169</td>
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<td>$n=2$</td>
<td>0.236 (6.44)</td>
<td>0.304 (15.85)</td>
<td>1.30 (12.74)</td>
<td>0.356 (15.07)</td>
<td>17.53 (7.89)</td>
<td>0.291</td>
<td>169</td>
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<td>$n=5$</td>
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<td>0.304 (15.86)</td>
<td>1.30 (12.75)</td>
<td>0.342 (14.90)</td>
<td>16.84 (7.60)</td>
<td>0.292</td>
<td>169</td>
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<td>Hexobarbital</td>
<td>$n=1$</td>
<td>0.399 (9.87)</td>
<td>0.475 (23.44)</td>
<td>0.827 (14.04)</td>
<td>1.14 (18.65)</td>
<td>40.37 (16.45)</td>
<td>0.686</td>
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<td>$n=2$</td>
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<td>408</td>
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<td>$n=5$</td>
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<td>0.655</td>
<td>408</td>
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<tr>
<td>Phenytoin</td>
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<td>Compound</td>
<td>SCM</td>
<td>Estimates (CV%)</td>
<td>$K_{pt}$</td>
<td>$K_{ph}$</td>
<td>$f_{d}CL_{int}$ (mL/min/kg)</td>
<td>$K_{ph,AR}$</td>
<td>AIC</td>
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<td>0.562 (3.00)</td>
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<td>11.16 (96.03)</td>
<td>57.45 &lt;br&gt; 207</td>
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<td>11.16 (96.03)</td>
<td>65.22 &lt;br&gt; 207</td>
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<td>0.58 &lt;br&gt; 178</td>
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<td>Propranolol</td>
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<td>13.70</td>
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<td>1.97</td>
<td>10.12</td>
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<td></td>
<td>n=5</td>
<td>0.837 (5.97)</td>
<td>0.322</td>
<td>1.90</td>
<td>10.09</td>
<td>9.29</td>
<td>20.21</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>133</td>
<td>3.55 &lt;br&gt; 295</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n=1</td>
<td>0.875 (9.86)</td>
<td>0.183</td>
<td>3.13</td>
<td>15.25</td>
<td>38.60</td>
<td>76.97</td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>424</td>
<td>4.84 &lt;br&gt; 195</td>
<td></td>
</tr>
<tr>
<td>Diazepam</td>
<td>n=2</td>
<td>0.875 (9.59)</td>
<td>0.176</td>
<td>3.08</td>
<td>15.66</td>
<td>20.63</td>
<td>49.86</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>223</td>
<td>4.93 &lt;br&gt; 195</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n=5</td>
<td>0.830 (9.71)</td>
<td>0.151</td>
<td>2.76</td>
<td>16.50</td>
<td>12.40</td>
<td>31.97</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>129</td>
<td>4.84 &lt;br&gt; 195</td>
<td></td>
</tr>
<tr>
<td>Diazepam c</td>
<td>n=1</td>
<td>0.689</td>
<td>0.999</td>
<td>2.23</td>
<td></td>
<td>6.76</td>
<td>135</td>
<td></td>
</tr>
<tr>
<td>(Two-Tissue)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.10</td>
<td>167</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n=2</td>
<td>0.610</td>
<td>1.000</td>
<td>2.20</td>
<td></td>
<td>4.80</td>
<td>95.19</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td>2.10</td>
<td>168</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n=5</td>
<td>0.563</td>
<td>0.999</td>
<td>2.17</td>
<td></td>
<td>3.97</td>
<td>78.26</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.10</td>
<td>168</td>
<td></td>
</tr>
</tbody>
</table>

a The $K_{ph}$ ranges of CQ and QD were obtained from the estimated $B_{max}$ and $K_d$ with the $C_h$ range of 2.98–14.64 µg/mL for CQ and 30–35 µg/mL for QD that covers the measured liver concentrations in the source datasets.

b $K_{ph,AR}$ is the apparent $K_p$ calculated by the area ratio (AR) method (Gallo et al., 1987).

c The $f_d$ values for DZP estimated by the two-tissue mPBPK model were calculated as the sum of $f_{d1}$ and $f_{d2}$. 

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Table 3. Final parameter estimates for DPH obtained by fitting the IV blood (10, 25, 40 mg/kg) and liver (10 mg/kg) PK data using the mPBPK model extended with a liver compartment and saturable hepatic metabolism.

<table>
<thead>
<tr>
<th>Parameter (Unit)</th>
<th>Estimates</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>$f_d$</td>
<td>0.351</td>
<td>21.95</td>
</tr>
<tr>
<td>$K_{pt}$</td>
<td>1.460</td>
<td>8.52</td>
</tr>
<tr>
<td>$K_{ph}$</td>
<td>1.462</td>
<td>21.13</td>
</tr>
<tr>
<td>$V_{max}$ (ng/min/kg)</td>
<td>86630</td>
<td>7.84</td>
</tr>
<tr>
<td>$K_m$ (ng/mL)</td>
<td>91.68</td>
<td>37.72</td>
</tr>
</tbody>
</table>
Table 4. Final parameter estimates for 12 model compounds obtained by jointly fitting the blood and liver concentration-time data after single IV, IP (CQ only), intraportal (DZP only), and oral dosing with one/two-tissue mPBPK extended with the hepatic compartment and compound-specific complexities shown in Fig 1.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Parameter Estimates (CV%)</th>
<th>Parameter Estimates (CV%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ER</td>
<td>$f_{ab}CL_{int}$ mL/min/kg</td>
</tr>
<tr>
<td>TB</td>
<td>0.017 (4.95)</td>
<td>1.06 (5.04)</td>
</tr>
<tr>
<td>CQ</td>
<td>0.075 (11.59)</td>
<td>4.90 (12.52)</td>
</tr>
<tr>
<td>CyA</td>
<td>0.086 (8.47)</td>
<td>5.74 (9.27)</td>
</tr>
<tr>
<td>FTY720</td>
<td>0.143 (7.05)</td>
<td>10.12 (8.22)</td>
</tr>
<tr>
<td>NIC</td>
<td>0.442 (4.42)</td>
<td>48.11 (7.92)</td>
</tr>
<tr>
<td>HB</td>
<td>0.400 (7.54)</td>
<td>40.46 (12.55)</td>
</tr>
<tr>
<td>PTZ</td>
<td>0.522 (5.36)</td>
<td>66.47 (11.21)</td>
</tr>
<tr>
<td>QD</td>
<td>0.585 (3.79)</td>
<td>85.78 (9.14)</td>
</tr>
<tr>
<td>PPN</td>
<td>0.651 (10.19)</td>
<td>113.7 (29.23)</td>
</tr>
<tr>
<td>DZP</td>
<td>0.732 (3.43)</td>
<td>166.4 (12.82)</td>
</tr>
<tr>
<td>VEM</td>
<td>0.753 (5.01)</td>
<td>185 (20.28)</td>
</tr>
<tr>
<td>DLZ</td>
<td>0.825</td>
<td>286</td>
</tr>
</tbody>
</table>

$a$ The $K_{ph}$ ranges of CQ and QD were obtained as described in Table 2. The estimated $K_d$ of QD after including the oral data is about 5-fold smaller than that obtained with only IV data (Table 2), resulting in a much larger $K_{ph}$ range.

$b$ The $k_a$ and $F_g$ values are specifically for the oral powder of QD.

$c$ The $k_a$ and $F_g$ values are specifically for the oral solution of QD.
Table 5. Values of hepatic intrinsic clearance (mL/min/kg) for DPH and DLZ with different patterns of metabolic enzyme zonation in a 5-compartment liver model

<table>
<thead>
<tr>
<th>Compound</th>
<th>ER$^a$</th>
<th>Metabolism Pattern</th>
<th>$f_{ub}CL_{int_1}^b$</th>
<th>$f_{ub}CL_{int_2}^b$</th>
<th>$f_{ub}CL_{int_3}^b$</th>
<th>$f_{ub}CL_{int_4}^b$</th>
<th>$f_{ub}CL_{int_5}^b$</th>
<th>$f_{ub}CL_{int}^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLZ</td>
<td>0.83</td>
<td>Lower PP $CL_{int}$</td>
<td>3.58</td>
<td>7.37</td>
<td>15.62</td>
<td>35.26</td>
<td>90.97</td>
<td>152.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lower PV $CL_{int}$</td>
<td>90.97</td>
<td>35.26</td>
<td>15.62</td>
<td>7.37</td>
<td>3.58</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Even distribution</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25.6$^c$</td>
<td></td>
</tr>
<tr>
<td>DPH</td>
<td>0.46</td>
<td>Lower PP $CL_{int}$</td>
<td>1.22</td>
<td>2.47</td>
<td>5.03</td>
<td>10.48</td>
<td>22.77</td>
<td>41.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lower PV $CL_{int}$</td>
<td>22.77</td>
<td>10.48</td>
<td>5.03</td>
<td>2.47</td>
<td>1.22</td>
<td>39.6$^c$</td>
</tr>
</tbody>
</table>

$^a$ The ER for DPH and DLZ were from Tables 2 and 3.

$^b$ The $f_{ub}CL_{int_i}$ ($i=1, 2…5$) for uneven enzyme distribution was calculated from ER using Eq. 3-4, 20-23 and fixed in the model fitting of liver data; $f_{ub}CL_{int}$ is the sum of $f_{ub}CL_{int_i}$ of all liver sub-compartment.

$^c$ The $f_{ub}CL_{int_i}$ ($i=1, 2…5$) for even enzyme distribution is calculated from $f_{ub}CL_{int}/5$. 
Fig. 1
Fig. 2
Open-Loop Approach

(A) \( mPBPK \text{ SCM (n=1, 2, 5)} \)

(B) \( K_{ph,AR} \)

(C) \( f_{ub}CL_{int} \)

(D) \( K_{ph} \)

(E) \( f_{ub}CL_{int} \)

(F) \( K_{ph} \)

Fig. 3
Fig. 4

(A) and (B) show the DPH concentration (ng/mL) over time (min). The graphs illustrate the decline in concentration with increasing time.
Fig. 5
Fig. 6
PPN (10 mg/kg PO)  
 ER = 0.651

PPN (7.5 mg/kg IV)  

DZP (1.2 mg/kg intraportal & 10 mg/kg PO)  
 ER = 0.732

DZP (1.2 mg/kg IV)  

VEM (10 mg/kg iv & PO)  
 ER = 0.75

VEM (5 mg/kg IV)  

DLZ (20 mg/kg PO)  
 ER = 0.825

DLZ (5 mg/kg IV)  

Fig. 7
Fig. 8

Apparent PAMPA $P$ ($10^{-6}$ cm/s)

- TB
- CQ
- CyA
- EB
- FTY720
- NIC
- PTZ
- HB
- DPH
- QD
- PPN
- DZP
- VEM
- DLZ
Fig. 9
Fig. 10

**Blood (PO)**

- Fold Change in $T_{max}$
  - 7.75 min
  - 8.55 min

- Fold Change in $C_{max}$
  - 1291 ng/mL
  - 1364 ng/mL

- Fold Change in $AUC_{inf}$
  - 70274 ng·min/mL
  - 68886 ng·min/mL

---

**Liver (PO)**

- Fold Change in $T_{max}$
  - 3.74 min
  - 3.47 min

- Fold Change in $C_{max}$
  - 89696 ng/mL
  - 102799 ng/mL

- Fold Change in $AUC_{inf}$
  - 1350252 ng·min/mL
  - 1360964 ng·min/mL

---

**Liver (IV)**

- Fold Change in $T_{max}$
  - 0.53 min
  - 0.80 min

- Fold Change in $C_{max}$
  - 19450 ng/mL
  - 20315 ng/mL

- Fold Change in $AUC_{inf}$
  - 336953 ng·min/mL
  - 339647 ng·min/mL

**Partition Coefficients**
Fig. 11
Fig. 12

Blood & Liver

- $C_b$
- $C_h$

Liver Sub-Compartment

- $C_{h1}$
- $C_{h2}$
- $C_{h3}$
- $C_{h4}$
- $C_{h5}$

Fold Change in $T_{max}$

- 8.55
- 3.47

Fold Change in $C_{max}$

- 1364
- 102279

Absorption Rate Constant

- $k_a$

Fold Change in $T_{max}$

- 1.87
- 2.94
- 4.01
- 4.81
- 5.88

Fold Change in $C_{max}$

- 214741
- 141542
- 95258
- 64702
- 44209

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Fig. 13
Supplemental Materials
Manuscript number: DMD-AR-2023-001403

Title:
Utility of Minimal Physiologically Based Pharmacokinetic (mPBPK) Models for Assessing Fractional Distribution, Oral Absorption and Series-Compartment Models of Hepatic Clearance

Authors:
Xiaonan Li and William J. Jusko

Affiliation:
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Journal Title:
Drug Metabolism and Disposition
Supplemental Figures

Fig. S1

Fig S1. Comparing the oral bioavailability ($F_{po}$) calculated from $F_g (1-ER)$ and model-fitted AUC ratio.
Fig S2. Blood and liver concentration-time profiles of NIC, DZP, VEM, and DLZ. Symbols represent observations for the liver after IV dosing (black open circle) and the blood after IV (red solid circles), oral (red solid triangles) or intraportal (DZP only, red solid squares) dosing. Solid and dashed lines indicate fittings for the blood and liver data by the mPBPK model extended with the SCM containing 1 (purple), 2 (orange), and 5 (green) liver sub-compartments.
Fig S3. Blood and liver concentration-time profiles for NIC after IV or oral dosing simulated by the mPBPK model extended with the SCM (n=1 and 5) applying different values of $K_{ph}$, i.e., $K_{ph}$ (black), -2-fold (green), +2-fold $K_{ph}$ (orange), -5-fold (red), and +5-fold $K_{ph}$ (purple).
Fig S4. Fold-changes in the PK parameters at ±2-fold and ±5-fold $K_{ph}$ relative to the corresponding nominal values at 1x $K_{ph}$ for the blood and liver PK profiles of NIC simulated by the mPBPK model extended with SCM-1 (purple) or SCM-5 (green) shown in Fig. S3. The nominal PK parameter values are listed with the same color coding as that for the indicated model.
Fig S5. Simulated concentration-time profiles for DPH in blood, liver, and each of the liver sub-compartments in rats after bolus administration of 10 mg/kg IV doses. Different hepatic zonation patterns are color coded as follows: lower PP $CL_{int}$ (green), uniform $CL_{int}$ (black), and lower PV $CL_{int}$ (orange).
Fig S6. Fold changes in $T_{max}$, $AUC_{\infty}$ and $C_{max}$ computed from the simulated PK profiles for DPH in blood ($C_b$), liver ($C_h$) and each of the liver sub-compartments ($C_{h1}$ through $C_{h5}$) after 10 mg/kg IV bolus dosing with hepatic enzyme zonation (lower PP $CL_{int}$ (green) or lower PV $CL_{int}$) compared to the nominal values obtained with uniform enzyme distribution. Different compartments are color coded as indicated and the nominal parameter values are listed with the same color coding as that used for the corresponding compartments.
Fig. S7. Blood concentration-time profiles of FTY720, NIC, VEM, and CyA. Red solid symbols represent measured blood concentrations after bolus IV dosing. Solid lines indicate fittings for the blood PK data by the mPBPK model extended with the SCM containing 1 (purple), 2 (orange), and 5 (green) liver sub-compartment.
## Supplemental Tables

Table S1. Physiological parameters fixed in the models.

<table>
<thead>
<tr>
<th>Parameter (unit)</th>
<th>Definitions</th>
<th>Value $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$BW$ (mL/kg)</td>
<td>Body Weight</td>
<td>1000</td>
</tr>
<tr>
<td>$V_b$ (mL/kg)</td>
<td>Blood volume</td>
<td>78</td>
</tr>
<tr>
<td>$V_h$ (mL/kg)</td>
<td>Liver volume</td>
<td>36.6</td>
</tr>
<tr>
<td>$V_t$ (mL/kg)</td>
<td>Tissue volume</td>
<td>885.7</td>
</tr>
<tr>
<td>$Q_{co}$ (mL/min/kg)</td>
<td>Cardiac output</td>
<td>332.34</td>
</tr>
<tr>
<td>$Q_{h}$ (mL/min/kg)</td>
<td>Hepatic blood flow</td>
<td>60.82</td>
</tr>
</tbody>
</table>

$^a$ All parameter values were obtained from (Brown et al., 1997) except that $V_b$ was assigned to the average value of the reported blood volumes in the source literature where the modeling datasets were obtained. $V_t$ was calculated from $BW-V_b-V_h$ for the one-tissue mPBPK model, and from $V_t = V_{t1} + V_{t2}$ for the two-tissue mPBPK model.
Table S2. Parameter estimates for selected model compounds obtained by simultaneously fitting the blood and liver concentration-time data after single IV, oral and intraportal (DZP only) dosing by the mPBPK model extended with the hepatic SCM (n=1, 2, and 5)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Parameter Estimates (CV%)</th>
<th>SCM</th>
<th>$ER$</th>
<th>$f_{ub}CL_{int}$ (mL/min/kg)</th>
<th>$K_{ph}$ (mL/min/kg)</th>
<th>$K_{pt}$ (mL/min/kg)</th>
<th>$f_{d1}$</th>
<th>$f_{d2}$</th>
<th>$V_I$ (mL/kg)</th>
<th>$k_{a}$ (1/min)</th>
<th>$F_g$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotine</td>
<td></td>
<td>n=1</td>
<td>0.442 (4.42)</td>
<td>48.11 (7.92)</td>
<td>10.83 (11.93)</td>
<td>1.06 (10.04)</td>
<td>0.101 (20.17)</td>
<td>-</td>
<td>-</td>
<td>0.011 (17.14)</td>
<td>1 (fixed)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n=2</td>
<td>0.445 (4.29)</td>
<td>41.56 (6.74)</td>
<td>9.77 (10.59)</td>
<td>1.03 (10.08)</td>
<td>0.096 (20.52)</td>
<td>-</td>
<td>-</td>
<td>0.011 (16.87)</td>
<td>0.181 (17.32)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n=5</td>
<td>0.445 (3.92)</td>
<td>38.04 (5.66)</td>
<td>9.48 (7.96)</td>
<td>0.983 (9.95)</td>
<td>0.088 (20.22)</td>
<td>-</td>
<td>-</td>
<td>0.011 (16.19)</td>
<td>0.196 (17.61)</td>
</tr>
<tr>
<td>Diazepam</td>
<td></td>
<td>n=1</td>
<td>0.732 (3.43)</td>
<td>166.4 (12.79)</td>
<td>9.16 (14.25)</td>
<td>4.97 (16.44)</td>
<td>0.584 (58.01)</td>
<td>0.055 (15.33)</td>
<td>198 (17.28)</td>
<td>0.020 (19.96)</td>
<td>0.196 (17.61)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n=2</td>
<td>0.738 (3.35)</td>
<td>115.9 (9.66)</td>
<td>5.94 (13.08)</td>
<td>4.87 (15.17)</td>
<td>0.871 (68.53)</td>
<td>0.057 (15.10)</td>
<td>208 (16.40)</td>
<td>0.023 (21.51)</td>
<td>0.199</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n=5</td>
<td>0.735</td>
<td>92.65</td>
<td>4.61</td>
<td>4.74</td>
<td>0.945</td>
<td>0.055</td>
<td>211</td>
<td>0.023</td>
<td>0.239 (21.65)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n=1</td>
<td>0.753 (5.01)</td>
<td>185 (20.28)</td>
<td>34.93 (21.01)</td>
<td>2.92 (9.08)</td>
<td>0.326 (18.10)</td>
<td>0.066 (39.53)</td>
<td>392 (22.69)</td>
<td>0.020 (13.81)</td>
<td>0.239</td>
</tr>
<tr>
<td>Verapamil</td>
<td></td>
<td>n=2</td>
<td>0.742 (5.17)</td>
<td>118 (15.11)</td>
<td>22.27 (15.83)</td>
<td>3.12 (9.77)</td>
<td>0.322 (19.81)</td>
<td>0.070 (36.72)</td>
<td>361 (22.80)</td>
<td>0.031 (17.71)</td>
<td>0.234 (21.94)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n=5</td>
<td>0.737 (5.30)</td>
<td>93.02 (12.67)</td>
<td>17.76 (12.66)</td>
<td>3.26 (10.67)</td>
<td>0.332 (21.80)</td>
<td>0.072 (31.42)</td>
<td>336 (20.57)</td>
<td>0.099 (34.93)</td>
<td>0.256 (22.69)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n=1</td>
<td>0.824</td>
<td>285</td>
<td>17.87</td>
<td>4.18</td>
<td>0.833</td>
<td>0.167</td>
<td>237</td>
<td>0.125</td>
<td>1 (fixed)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n=2</td>
<td>0.826</td>
<td>170</td>
<td>10.71</td>
<td>4.17</td>
<td>0.829</td>
<td>0.172</td>
<td>232</td>
<td>0.132</td>
<td>1 (fixed)</td>
</tr>
<tr>
<td>Diltiazem</td>
<td></td>
<td>n=5</td>
<td>0.827</td>
<td>128</td>
<td>8.09</td>
<td>4.14</td>
<td>0.829</td>
<td>0.171</td>
<td>231</td>
<td>0.138</td>
<td>1 (fixed)</td>
</tr>
</tbody>
</table>
Table S3. Compound-specific physicochemical properties and blood partitioning parameters.

<table>
<thead>
<tr>
<th>Compound</th>
<th>BDDCS Class&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Solubility (mg/mL)&lt;sup&gt;a,g&lt;/sup&gt;</th>
<th>log &lt;i&gt;P&lt;/i&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
<th>log &lt;i&gt;D&lt;/i&gt;&lt;sup&gt;b&lt;/sup&gt; (pH 7.4)</th>
<th>MW&lt;sup&gt;b&lt;/sup&gt;</th>
<th>HA&lt;sup&gt;b&lt;/sup&gt;</th>
<th>HD&lt;sup&gt;b&lt;/sup&gt;</th>
<th>TPSA (Å&lt;sup&gt;2&lt;/sup&gt;)</th>
<th>&lt;i&gt;f&lt;/i&gt;&lt;sub&gt;up&lt;/sub&gt; (%)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>&lt;i&gt;R&lt;/i&gt;&lt;sub&gt;b&lt;/sub&gt;&lt;sup&gt;c&lt;/sup&gt;</th>
<th>&lt;i&gt;f&lt;/i&gt;&lt;sub&gt;ub&lt;/sub&gt; (%)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>&lt;i&gt;P&lt;/i&gt;&lt;sup&gt;d,e&lt;/sup&gt;</th>
<th>&lt;i&gt;f&lt;/i&gt;&lt;sub&gt;up&lt;/sub&gt;&lt;i&gt;P&lt;/i&gt;/&lt;i&gt;R&lt;/i&gt;&lt;sub&gt;b&lt;/sub&gt;&lt;sup&gt;d&lt;/sup&gt;</th>
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<td>13</td>
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<td>0</td>
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<td>18.4</td>
<td>0.93</td>
<td>19.8</td>
<td>14</td>
<td>2.77</td>
</tr>
</tbody>
</table>

<sup>a</sup> The Biopharmaceutics Drug Disposition Classification System (BDDCS) classification and solubility were obtained from (Benet et al., 2011).

<sup>b</sup> The molecular descriptors used for the prediction of PAMPA <i>P</i>, e.g., molecular weight (MW), log <i>P</i>, log <i>D</i>, the number of hydrogen bond donors (HD) and acceptors (HA), and the topological polar surface area (TPSA) were obtained from http://www.chemspider.com/ base on the chemical structure of each compound.

<sup>c</sup> The <i>f</i><sub>up</sub> and <i>R</i><sub>b</sub> values were available from (Li and Jusko, 2022), and the <i>f</i><sub>ub</sub> were calculated from <i>f</i><sub>up</sub>/<i>R</i><sub>b</sub>.

<sup>d</sup> PAMPA <i>P</i> and apparent PAMPA <i>P</i> (i.e., <i>f</i><sub>up</sub><i>P</i>/<i>R</i><sub>b</sub>) are listed as ×10<sup>-6</sup> cm/s.

<sup>e</sup> The PAMPA <i>P</i> for CQ, QD, VEM, DLZ, and PPN were calculated from the observed log<i>P</i><sub>app,PAMPA</sub> values listed in the Supplementary Table SII from (Jeong et al., 2022) and those for the remaining compounds were predicted applying Eq. 4 in (Jeong et al., 2022).

<sup>f</sup> CQ is assigned as a class 1 drug in Biopharmaceutics Classification System (BCS).
Supplemental Methods

ADAPT Code

1) One-tissue mPBPK with SCM (n=5) and nonlinear tissue binding (IV PK of QD)

******************************************************************************
C                           ADAPT                                     *
C                         Version 5                                   *
C****************************************
C                                                                     *
C                           MODEL                                     *
C                                                                     *
C    This file contains Fortran subroutines into which the user       *
C    must enter the relevant model equations and constants.           *
C    Consult the User's Guide for details concerning the format for  *
C    entered equations and definition of symbols.                    *
C                                                                     *
C       1. Symbol- Parameter symbols and model constants             *
C       2. DiffEq- System differential equations                     *
C       3. Output- System output equations                          *
C       4. Varmod- Error variance model equations                    *
C       5. Covmod- Covariate model equations (ITS,MLEM)              *
C       6. Popinit- Population parameter initial values (ITS,MLEM)   *
C       7. Prior - Parameter mean and covariance values (ID,NPD,STS) *
C       8. Sparam- Secondary parameters                              *
C       9. Amat - System state matrix                               *
C                                                                     *
C**********************************************************************
C######################################################################C

Subroutine SYMBOL
Implicit None

Include 'globals.inc'
Include 'model.inc'

******************************************************************************
NDEqs = 13 ! Enter # of Diff. Eqs.
NSParam = 7 ! Enter # of System Parameters.
NVparam = 2 ! Enter # of Variance Parameters.
NSecPar = 1 ! Enter # of Secondary Parameters.
NSecOut = 0 ! Enter # of Secondary Outputs (not used).
Ieqsol = 1 ! Model type: 1 - DIFFEQ, 2 - AMAT, 3 - OUTPUT only.
Descr = 'mPBPK with one tissue blood liver nonlinear Kp SCM5'

C Enter Symbol for Each System Parameter (eg. Psym(1)='Keli')
Psym(1)='fd'
Psym(2)='Kp1'
Psym(3)='CLint'
Psym(4)='Rb'
Psym(5)='Bmax'
Psym(6)='Kd'
Psym(7)='fup'

C Enter Symbol for Each Variance Parameter (eg. PVsym(1)='Sigma')
PVsym(1)='intercept'
PVsym(2)='sigma'

C Enter Symbol for Each Secondary Parameter (eg. PSsym(1)='CLt')
PSsym(1)='ER'

C Return
End

Subroutine DIFFEQ(T,X,XP)
Implicit None
Include 'globals.inc'
Include 'model.inc'

Real*8 T,X(MaxNDE),XP(MaxNDE)
Real*8 fd,Kp1,Kp2,CLb,Vt,Qco,Vb,Rb,CLint,Qliver,Vliver
Real*8 Bmax,Kd,fup,Kp3,Kp4,Kp5,Kp6,Kp7

C Enter Differential Equations Below {e.g. XP(1) = -P(1)*X(1) } C
C---------------------------------------------------------------------C

fd = P(1)
Kp1 = P(2)
CLint = P(3)
Rb = P(4)
Bmax = P(5)
Kd = P(6)
fup = P(7)

!Tissue volume mL/kg
Vb=78 !from Kp DMD manuscript
Vt=885.7  ! BW-Vb-Vliver=1000-78-36.6
Vliver=36.6

!Cardiac output (L/min) = 0.235(BW)^0.75 from Brown et al
Qco=332.34 !mL/min/kg
Qliver=60.82 !mL/min/kg

C ------ nonlinear liver Kp -------------------------
Kp3=fup*(-(X(3)-Kd-Bmax)+((X(3)-Kd-Bmax)**2+4*Kd*X(3))**0.5)
  x   /(2*Kd)
Kp4=fup*(-(X(4)-Kd-Bmax)+((X(4)-Kd-Bmax)**2+4*Kd*X(4))**0.5)
  x   /(2*Kd)
Kp5=fup*(-(X(5)-Kd-Bmax)+((X(5)-Kd-Bmax)**2+4*Kd*X(5))**0.5)
  x   /(2*Kd)
Kp6=fup*(-(X(6)-Kd-Bmax)+((X(6)-Kd-Bmax)**2+4*Kd*X(6))**0.5)
  x   /(2*Kd)
\[\text{Kp7} = \text{fup} \times \left( -(X(7) - K_d - B_{max}) + ((X(7) - K_d - B_{max})^2 + 4 \times K_d \times X(7))^2 \right)^{0.5} \times \frac{1}{(2 \times K_d)} \]

```
c -------- Blood -----------------------------------------
  IF(fd .gt. 1) then
    XP(1) = 0
  else
    XP(1) = \((\text{Qco} - \text{Qliver}) \times \text{fd} \times (X(2) \times R_b / K_p1 - X(1))\)
    + \text{Qliver} \times (X(7) \times R_b / K_p7 - X(1)) / V_b
  endif

c -------- Compartment 1 -----------------------------
  XP(2) = (\text{Qco} - \text{Qliver}) \times \text{fd} \times (X(1) - X(2) \times R_b / K_p1) / V_t

c -------- Liver SCM-5 -------------------------------
  XP(3) = (\text{Qliver} \times (X(1) - X(3) \times R_b / K_p3) \times -(C_{L_{int}} / 5) \times X(3) \times R_b / K_p3 / (V_{liver} / 5))
  XP(4) = (\text{Qliver} \times (X(3) \times R_b / K_p3 - X(4) \times R_b / K_p4) \times -(C_{L_{int}} / 5) \times X(4) \times R_b / K_p4 / (V_{liver} / 5))
  XP(5) = (\text{Qliver} \times (X(4) \times R_b / K_p4 - X(5) \times R_b / K_p5) \times -(C_{L_{int}} / 5) \times X(5) \times R_b / K_p5 / (V_{liver} / 5))
  XP(6) = (\text{Qliver} \times (X(5) \times R_b / K_p5 - X(6) \times R_b / K_p6) \times -(C_{L_{int}} / 5) \times X(6) \times R_b / K_p6 / (V_{liver} / 5))
  XP(7) = (\text{Qliver} \times (X(6) \times R_b / K_p6 - X(7) \times R_b / K_p7) \times -(C_{L_{int}} / 5) \times X(7) \times R_b / K_p7 / (V_{liver} / 5))
```

C -------- Partial AUC -------------------------------
  XP(8) = X(1) / R_b !AUCplasma
  XP(9) = X(3) !AUCliver1
  XP(10) = X(4) !AUCliver2
  XP(11) = X(5) !AUCliver3
  XP(12) = X(6) !AUCliver4
  XP(13) = X(7) !AUCliver5
```
Subroutine OUTPUT(Y,T,X)
Implicit None
Include 'globals.inc'
Include 'model.inc'
Real*8 Y(MaxNOE),T,X(MaxNDE)
Real*8 Rb,fup,Bmax,Kd

Rb = P(4)
Bmax = P(5)
Kd = P(6)
fup = P(7)

Y(1) = X(1) !blood
Y(2) = X(3)
Y(3) = X(4)
Y(4) = X(5)
Y(5) = X(6)
Y(6) = X(7)
Y(7) = (X(3)+X(4)+X(5)+X(6)+X(7))/5 !liver
Y(8) = (((X(9)+X(10)+X(11)+X(12)+X(13))/5)/X(8) !Kp,AR
Y(9) = fup*(-(X(3)+X(4)+X(5)+X(6)+X(7))/5-Kd-Bmax) x +((X(3)+X(4)+X(5)+X(6)+X(7))/5-Kd-Bmax)**2 x +4*Kd*(X(3)+X(4)+X(5)+X(6)+X(7))/5)**0.5)/(2*Kd) !nonlinear Kp for the whole liver
Subroutine VARMOD(V,T,X,Y)

Implicit None

Include 'globals.inc'
Include 'model.inc'

Real*8 V(MaxNOE),T,X(MaxNDE),Y(MaxNOE)
Real*8 intercept, sigma

C

Enter Variance Model Equations Below
{e.g. V(1) = (PV(1) + PV(2)*Y(1))**2 }

C
C
C

intercept=PV(1)

sigma=PV(2)

V(1:9) = (intercept + sigma*Y(1:9))**2

C

C

----------------------------------

C

Return
End

C

Subroutine COVMOD(Pmean, ICmean, PC)

C Defines any covariate model equations (MLEM, ITS)

Implicit None

Include 'globals.inc'
Include 'model.inc'

Real*8 PC(MaxNCP)
Real*8 Pmean(MaxNSP+MaxNDE), ICmean(MaxNDE)
Subroutine POPINIT(PmeanI, ICmeanI, PcovI, ICcovI, PCI)

Initial parameter values for population program parameters (ITS, MLEM)

Implicit None

Include 'globals.inc'
Include 'model.inc'

Integer I, J
Real*8 PmeanI(MaxNSP+MaxNDE), ICmeanI(MaxNDE)
Real*8 PcovI(MaxNSP+MaxNDE, MaxNSP+MaxNDE), ICcovI(MaxNDE, MaxNDE)
Real*8 PCI(MaxNCP)
Subroutine PRIOR(Pmean,Pcov,ICmean,ICcov)
C Parameter mean and covariance values for MAP estimation (ID,NPD,STS)
Implicit None

Include 'globals.inc'
Include 'model.inc'

Integer I,J
Real*8 Pmean(MaxNSP+MaxNDE), ICmean(MaxNDE)
Real*8 Pcov(MaxNSP+MaxNDE,MaxNSP+MaxNDE), ICcov(MaxNDE,MaxNDE)
Manuscript number: DMD-AR-2023-001403

CC
C---------------------------------------------C
C Enter Nonzero Elements of Prior Mean Vector
C      { e.g. Pmean(1) = 10.0 }
C---------------------------------------------C

CC
C---------------------------------------------C
C Enter Nonzero Elements of Covariance Matrix (Lower Triang.)
C      { e.g. Pcov(2,1) = 0.25 }
C---------------------------------------------C

C---------------------------------------------C
C---------------------------------------------C
C
      Return
      End

C#******************************************************************************C
Subroutine SPARAM(PS,P,IC)
Implicit None

   Include 'globals.inc'

   Real*8 PS(MaxNSECP), P(MaxNSP+MaxNDE), IC(MaxNDE)
   Real*8 CLint,Rb,Vb,Vt,Kp,Dose,Qliver

CC
C---------------------------------------------C
C Enter Equations Defining Secondary Parameters
C      { e.g. PS(1) = P(1)*P(2) }
C---------------------------------------------C

      CLint = P(3)
      Qliver=60.82 !mL/min/kg
      PS(1) = 1-(Qliver/(Qliver+P(3)/5))**5 !5SCM ER

C---------------------------------------------C
Subroutine AMAT(A)
Implicit None

Include 'globals.inc'
Include 'model.inc'

Integer I,J
Real*8 A(MaxNDE,MaxNDE)

DO I=1,Ndeqs
   Do J=1,Ndeqs
      A(I,J)=0.0D0
   End Do
End Do
2) Two-tissue mPBPK extended with the SCM (n=5) (IV, oral and intraportal PK of DZP)

**********************************************************************
C                           ADAPT                                     *
C                         Version 5                                   *
C***************          C                                                                     *
C                           MODEL                                     *  
C                                                                     *
C       1. Symbol- Parameter symbols and model constants             *
C       2. DiffEq- System differential equations                     *
C       3. Output- System output equations                           *
C       4. Variomod- Error variance model equations                  *
C       5. Covmod- Covariate model equations (ITS,MLEM)             *
C       6. Popinit- Population parameter initial values (ITS,MLEM)   *
C       7. Prior- Parameter mean and covariance values (ID,NPD,STS)   *
C       8. Sparam- Secondary parameters                              *
C       9. Amat- System state matrix                                 *
C                                                                     *
C**********************************************************************
C######################################################################C

Subroutine SYMBOL
Implicit None

Include 'globals.inc'
Include 'model.inc'

CC
C----Enter as Indicated
C----
NDEqs = 15 ! Enter # of Diff. Eqs.
NSParam = 8 ! Enter # of System Parameters.
Manuscript number: DMD-AR-2023-001403

NVparam = 2 ! Enter # of Variance Parameters.
NSecPar = 1 ! Enter # of Secondary Parameters.
NSecOut = 0 ! Enter # of Secondary Outputs (not used).
Ieqsol = 1 ! Model type: 1 - DIFFEQ, 2 - AMAT, 3 - OUTPUT only.
Descr = ' DZP iv oral intraportal 2tissues SCMSNP'D

CC
C Enter Symbol for Each System Parameter (eg. Psym(1)='Kel')
C------------------------------------------------------------------
Psym(1)= 'fd1'
Psym(2)='Kp1'
Psym(3)= 'CLInt'
Psym(4)= 'Kp2'
Psym(5)= 'fd2'
Psym(6)= 'Vt1'
Psym(7)= 'ka'
Psym(8)= 'Fpo'

CC
C Enter Symbol for Each Variance Parameter {eg: PVsym(1)='Sigma'}
C------------------------------------------------------------------
PVsym(1)= 'intercept'
PVsym(2)= 'sigma'

CC
C Enter Symbol for Each Secondary Parameter {eg: PSsym(1)='CLt'}
C------------------------------------------------------------------
PSsym(1)= 'ER'

C
Return
End

Subroutine DIFFEQ(T,X,XP)
Implicit None
Include 'globals.inc'
Include 'model.inc'

Real*8 T,X(MaxNDE),XP(MaxNDE)
Real*8 fd,Kp1,Kp2,CLb,Vt,Qco,Vb,Rb,CLint,Qliver,Vliver
Real*8 ka,Fpo,kinf,fd2,Vt1,Vt2,fd1

C Enter Differential Equations Below {e.g. XP(1) = -P(1)*X(1) } C

fd1 = P(1)
Kp1 = P(2)
CLint = P(3)
Kp2 = P(4)
fd2 = P(5)
Vt1 = P(6)
ka= P(7)
Fpo=P(8)
Rb =1.04

!Tissue volume mL/kg
Vb=78 !from Kp DMD manuscript
Vliver=36.6
Vt2=1000-Vb-Vt1-Vliver
!Cardiac output (L/min) = 0.235(BW)^0.75 from Brown et al
Qco=332.34 mL/min/kg
Qliver=60.82 mL/min/kg
fd=fd1+fd2

C-define different dosing regimens
if (subjIND.eq.2) then !intalportal 1.2 mg/kg Igari et al
   ka = 0
elseif (subjIND.eq.3) then !iv 1.2 mg/kg Igari
   ka = 0
endif

CC---Drug Absorption compartment
XP(1) = -ka*X(1)
c --------- Liver SCM-5 -------------------------------------

XP(2) = (ka*X(1)*Fpo +Qliver*(X(7)-X(2)*Rb/Kp2)}
\[ x = -(\text{CLint}/5)\cdot(\text{X}(2)\cdot\text{Rb}/\text{Kp2})/(\text{Vliver}/5) \]

\[ \text{XP}(3) = (\text{Qliver}\cdot(\text{X}(2)\cdot\text{Rb}/\text{Kp2} - \text{X}(3)\cdot\text{Rb}/\text{Kp2}) \]
\[ x = -(\text{CLint}/5)\cdot(\text{X}(3)\cdot\text{Rb}/\text{Kp2})/(\text{Vliver}/5) \]

\[ \text{XP}(4) = (\text{Qliver}\cdot(\text{X}(3)\cdot\text{Rb}/\text{Kp2} - \text{X}(4)\cdot\text{Rb}/\text{Kp2}) \]
\[ x = -(\text{CLint}/5)\cdot(\text{X}(4)\cdot\text{Rb}/\text{Kp2})/(\text{Vliver}/5) \]

\[ \text{XP}(5) = (\text{Qliver}\cdot(\text{X}(4)\cdot\text{Rb}/\text{Kp2} - \text{X}(5)\cdot\text{Rb}/\text{Kp2}) \]
\[ x = -(\text{CLint}/5)\cdot(\text{X}(5)\cdot\text{Rb}/\text{Kp2})/(\text{Vliver}/5) \]

\[ \text{XP}(6) = (\text{Qliver}\cdot(\text{X}(5)\cdot\text{Rb}/\text{Kp2} - \text{X}(6)\cdot\text{Rb}/\text{Kp2}) \]
\[ x = -(\text{CLint}/5)\cdot(\text{X}(6)\cdot\text{Rb}/\text{Kp2})/(\text{Vliver}/5) \]

\[ \text{c----blood compart-----} \]
\[ \text{IF}(\text{fd} > 1) \text{ then} \]
\[ \text{XP}(7) = 0 \]
\[ \text{else} \]
\[ \text{XP}(7) = ((\text{Qco} - \text{Qliver})\cdot\text{fd1}\cdot(\text{X}(8)\cdot\text{Rb}/\text{Kp1} - \text{X}(7)) \]
\[ + (\text{Qco} - \text{Qliver})\cdot\text{fd2}\cdot(\text{X}(9)\cdot\text{Rb}/\text{Kp1} - \text{X}(7)) \]
\[ + \text{Qliver}\cdot(\text{X}(6)\cdot\text{Rb}/\text{Kp2} - \text{X}(7))/\text{Vb} \]
\[ \text{endif} \]

\[ \text{c----Compart1------} \]
\[ \text{XP}(8) = (\text{Qco} - \text{Qliver})\cdot\text{fd1}\cdot(\text{X}(7) - \text{X}(8)\cdot\text{Rb}/\text{Kp1})/\text{Vt1} \]

\[ \text{c----Compart2------} \]
\[ \text{XP}(9) = (\text{Qco} - \text{Qliver})\cdot\text{fd2}\cdot(\text{X}(7) - \text{X}(9)\cdot\text{Rb}/\text{Kp1})/\text{Vt2} \]

\[ \text{C -------- Partial AUC --------------------} \]

\[ \text{XP}(10) = \text{X}(7)/\text{Rb} \text{ !AUCplasma} \]
\[ \text{XP}(11) = \text{X}(2) \text{ !AUCliver1} \]
\[ \text{XP}(12) = \text{X}(3) \text{ !AUCliver2} \]
\[ \text{XP}(13) = \text{X}(4) \text{ !AUCliver3} \]
\[ \text{XP}(14) = \text{X}(5) \text{ !AUCliver4} \]
\[ \text{XP}(15) = \text{X}(6) \text{ !AUCliver5} \]

\[ \text{C------------------------------------------------------------------------C} \]
\[ \text{C------------------------------------------------------------------------C} \]
\[ \text{C} \]
\[ \text{Return} \]
\[ \text{End} \]
Subroutine OUTPUT(Y,T,X)
Implicit None

Include 'globals.inc'
Include 'model.inc'

Real*8 Y(MaxNOE),T,X(MaxNDE)

Y(1) = X(7)
Y(2) = X(2)
Y(3) = X(3)
Y(4) = X(4)
Y(5) = X(5)
Y(6) = X(6)
Y(7) = (X(2)+X(3)+X(4)+X(5)+X(6))/5 !liver
Y(8) = X(10) !AUCplasma
Y(9) = ((X(11)+X(12)+X(13)+X(14)+X(15))/5)/X(10) !Kp,AR

Return
End

Subroutine VARMOD(V,T,X,Y)
Implicit None

Include 'globals.inc'
Include 'model.inc'

Real*8 V(MaxNOE),T,X(MaxNDE),Y(MaxNOE)
Real*8 intercept, sigma
Subroutine COVMOD(Pmean, ICmean, PC)
C Defines any covariate model equations (MLEM, ITS)
Implicit None

Include 'globals.inc'
Include 'model.inc'

Real*8 PC(MaxNCP)
Real*8 Pmean(MaxNSP+MaxNDE), ICmean(MaxNDE)

CC
C-----------------------------C
C Enter # of Covariate Parameters C
C----c-------------------------------C

NCparam = 0     ! Enter # of Covariate Parameters.

CC
C-----------------------------C
C Enter Symbol for Covariate Params {eg: PCsym(1)='CLRenal'} C
C----c-------------------------------C
Subroutine POPINIT(PmeanI, ICmeanI, PcovI, ICcovI, PCI)
C Initial parameter values for population program parameters (ITS, MLEM)

Implicit None

Include 'globals.inc'
Include 'model.inc'

Integer I, J
Real*8 PmeanI(MaxNSP+MaxNDE), ICmeanI(MaxNDE)
Real*8 PcovI(MaxNSP+MaxNDE,MaxNSP+MaxNDE), ICcovI(MaxNDE,MaxNDE)
Real*8 PCI(MaxNCP)

C Enter Initial Values for Population Means
{ e.g. PmeanI(1) = 10.0 }

C Enter Initial Values for Pop. Covariance Matrix (Lower Triang.)
{ e.g. PcovI(2,1) = 0.25 }
Subroutine PRIOR(Pmean, Pcov, ICmean, ICcov)
C Parameter mean and covariance values for MAP estimation (ID, NPD, STS)
Implicit None

Include 'globals.inc'
Include 'model.inc'

Integer I, J
Real*8 Pmean(MaxNSP+MaxNDE), ICmean(MaxNDE)
Real*8 Pcov(MaxNSP+MaxNDE, MaxNDE), ICcov(MaxNDE, MaxNDE)

C Enter Values for Covariate Model Parameters
{  e.g. PCI(1) = 2.0  }

C Enter Nonzero Elements of Prior Mean Vector
{  e.g. Pmean(1) = 10.0  }

C Enter Nonzero Elements of Covariance Matrix (Lower Triang.)
{  e.g. Pcov(2,1) = 0.25  }

Return
End
Subroutine SPARAM(PS,P,IC)
Implicit None
Include 'globals.inc'
Real*8 PS(MaxNSECP), P(MaxNSP+MaxNDE), IC(MaxNDE)
Real*8 CLint,Rb,Vb,Vt,Kp,Dose,Qliver

CLint = P(3)
Qliver=60.82 !mL/min/kg
PS(1) = 1-(Qliver/(Qliver+P(3)/5))**5 !5-SCM ER

Subroutine AMAT(A)
Implicit None
Include 'globals.inc'
Include 'model.inc'
Integer I,J
Real*8 A(MaxNDE,MaxNDE)

DO I=1,Ndeqs
   Do J=1,Ndeqs
      A(I,J)=0.0D0
   End Do
End Do

C----c--------------------------------------------------C
C   Enter non zero elements of state matrix (e.g. A(1,1) = -P(1) ) C
C----c--------------------------------------------------C

C---c--------------------------------------------------C

Return
End

C###############################################################################C
3) One-tissue mPBPK with absorption delays (IV and oral PK of CyA)

This file contains Fortran subroutines into which the user must enter the relevant model equations and constants. Consult the User's Guide for details concerning the format for entered equations and definition of symbols.

1. Symbol - Parameter symbols and model constants
2. DiffEq - System differential equations
3. Output - System output equations
4. Varmod - Error variance model equations
5. Covmod - Covariate model equations (ITS,MLEM)
6. Popinit - Population parameter initial values (ITS,MLEM)
7. Prior - Parameter mean and covariance values (ID,NPD,STS)
8. Sparam - Secondary parameters
9. Amat - System state matrix

Subroutine SYMBOL
Implicit None
Include 'globals.inc'
Include 'model.inc'

NDEqs = 9 ! Enter # of Diff. Eqs.
NSParam = 6 ! Enter # of System Parameters.
NVParam = 2 ! Enter # of Variance Parameters.
NSecPar = 1 ! Enter # of Secondary Parameters.
NSecOut = 0 ! Enter # of Secondary Outputs (not used).
Ieqsol = 1 ! Model type: 1 - DIFFEQ, 2 - AMAT, 3 - OUTPUT only.
Descr = 'CyA iv 2oral SCM1 3 transit final'

Psym(1)='fd'
Psym(2)='Kp1'
Psym(3)='CLint'
Psym(4)='ktr'
Psym(5)='Kp2'
Psym(6)='Fg'

PVsym(1)='intercept'
PVsym(2)='sigma'

PSym(1)='ER'

Subroutine DIFFEQ(T,X,XP)
Implicit None
Include 'globals.inc'
Include 'model.inc'

Real*8 T,X(MaxNDE),XP(MaxNDE)
Real*8 fd,Kp1,Kp2,CLb,Vt,Qco,Vb,CLint,Qliver,Vliver
Real*8 ka,Fg,Tlag,LNFAC,Dose1,Dose2,Ktr,n,MTT

C Enter Differential Equations Below {e.g. XP(1) = -P(1)*X(1) }
C----c-----------------------------------------------C
fd = P(1)
Kp1 = P(2)
CLint = P(3)
ktr = P(4)
Kp2 = P(5)
Fg = P(6) !prehepatic availability
ka=ktr

!Tissue volume mL/kg
Vb=78 !from Kp DMD manuscript
Vt=885.7 ! BW-Vb-Vliver=1000-78-36.6
Vliver=36.6
!Cardiac output (L/min) = 0.235(BW)^0.75 from Brown et al
Qco=332.34 !mL/min/kg
Qliver=60.82 !mL/min/kg
Rb = 1.23

C-define different dosing regimens
if (subjIND.eq.3) then !IV infusion 5.9mg/kg 2min
  ktr = 0
endif

C---Transit compartments for absorption delay
XP(1) = -ktr*X(1)
XP(2) = ktr*(X(1)-X(2))
XP(3) = ktr*(X(2)-X(3))

C---Drug Absorption compartment
XP(4) = ktr*X(3)-ka*X(4)
cc---Liver Compartment SCM1

\[
XP(5) = (ka\times X(4)\times Fg + Qliver\times (X(6) - X(5)\times Rb/Kp2)\times -(CLint)\times X(5)\times Rb/Kp2)/Vliver
\]

cc----blood compart------

IF(fd .gt. 1) then
XP(6) = 0
else
XP(6) = (R(1)+(Qco-Qliver)\times fd\times (X(7)\times Rb/Kp1-X(6))\times +Qliver\times (X(5)\times Rb/Kp2-X(6)))/Vb
endif

cc----Compart1------

XP(7) = (Qco-Qliver)\times fd\times (X(6)-X(7)\times Rb/Kp1)/Vt

cc----AUC----------

XP(8) = X(5) !AUCliver
XP(9) = X(6)/Rb !AUCplasma

C---------------------------------------------------------------C
C---------------------------------------------------------------C
C
Return
End
C
C#%%%%%%%%%%%%%%%%%%%%%Subroutine OUTPUT(Y,T,X)
C#Subroutine OUTPUT(Y,T,X)
C Implicit None
C Include 'globals.inc'
C Include 'model.inc'
C Real*8 Y(MaxNOE),T,X(MaxNDE)

CC
C Enter Output Equations Below {e.g. Y(1) = X(1)/P(2) } C
C----c---------------------------------------------------------------C

Y(1) = X(6)
\[ Y(2) = X(5) \]
\[ Y(3) = X(9) \text{ (AUCplasma)} \]
\[ Y(4) = X(8)/X(9) \text{ (Kp,AR)} \]

```
C-------------------------C
C-------------------------C
C
   Return
   End

C*******************************************************************************C
Subroutine VARMOD(V,T,X,Y)
    Implicit None
    Include 'globals.inc'
    Include 'model.inc'

    Real*8 V(MaxNOE),T,X(MaxNDE),Y(MaxNOE)
    Real*8 intercept, sigma

    C Enter Variance Model Equations Below
    C (e.g. V(1) = (PV(1) + PV(2)*Y(1))**2 )

    intercept=PV(1)
    sigma=PV(2)
    V(1:4) = (intercept + sigma*Y(1:4))**2

C*******************************************************************************C
Subroutine COVMOD(Pmean, ICmean, PC)
    C Defines any covariate model equations (MLEM, ITS)
    Implicit None
```
Include 'globals.inc'
Include 'model.inc'

Real*8 PC(MaxNCP)
Real*8 Pmean(MaxNSP+MaxNDE), ICmean(MaxNDE)

CC
C-------------------------------
C Enter # of Covariate Parameters
C-------------------------------
NCparam = 0    ! Enter # of Covariate Parameters.

CC
C-------------------------------
C Enter Symbol for Covariate Params {eg: PCsym(1)='CLRenal'}
C-------------------------------

CC
C-------------------------------
C For the Model Params. that Depend on Covariates Enter the Equation
C {e.g. Pmean(1) = PC(1)*R(2) }
C-------------------------------

C-------------------------------
C Return
C End

C# Subroutine POPINIT(PmeanI, ICmeanI, PcovI, ICcovI, PCI)  
C Initial parameter values for population program parameters (ITS, MLEM)
Integer I,J
Real*8  PmeanI(MaxNSP+MaxNDE), ICmeanI(MaxNDE)
Real*8  PcovI(MaxNSP+MaxNDE,MaxNSP+MaxNDE), ICcovI(MaxNDE,MaxNDE)
Real*8  PCI(MaxNCP)

C Enter Initial Values for Population Means
{ e.g. PmeanI(1) = 10.0 }

C Enter Initial Values for Pop. Covariance Matrix (Lower Triang.)
{ e.g. PcovI(2,1) = 0.25 }

C Enter Values for Covariate Model Parameters
{ e.g. PCI(1) = 2.0 }

C Return
End

Subroutine PRIOR(Pmean,Pcov,ICmean,ICcov)
C Parameter mean and covariance values for MAP estimation (ID,NPD,STS)
Implicit None

Include 'globals.inc'
Include 'model.inc'


Integer I, J
Real*8 Pmean(MaxNSP+MaxNDE), ICmean(MaxNDE)
Real*8 Pcov(MaxNSP+MaxNDE,MaxNSP+MaxNDE), ICcov(MaxNDE,MaxNDE)

CC
C-------------------------------------------------------------------C
C Enter Nonzero Elements of Prior Mean Vector                          C
C { e.g. Pmean(1) = 10.0 }                                              C
C-------------------------------------------------------------------C

CC
C-------------------------------------------------------------------C
C Enter Nonzero Elements of Covariance Matrix (Lower Triang.)          C
C { e.g. Pcov(2,1) = 0.25 }                                             C
C-------------------------------------------------------------------C

C-------------------------------------------------------------------C
C-------------------------------------------------------------------C
C
Return
End

CC###################################################################C
Subroutine SPARAM(PS,P,IC)
Implicit None

Include 'globals.inc'

Real*8 PS(MaxNSECP), P(MaxNSP+MaxNDE), IC(MaxNDE)
Real*8 CLint,Rb,Vb,Vt,Kp,Dose,Qliver,Ktr,n,MTT

CC
C-------------------------------------------------------------------C
C Enter Equations Defining Secondary Parameters                      C
C { e.g. PS(1) = P(1)*P(2) }                                          C
C-------------------------------------------------------------------C
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\[ \text{CLint} = P(3) \]
\[ \text{Qliver} = 60.82 \text{ mL/min/kg} \]
\[ \text{FS}(1) = 1 - \left( \frac{\text{Qliver}}{\text{Qliver} + P(3)/1} \right)^{**1} \text{ SCM ER} \]

Subroutine AMAT(A)

\begin{verbatim}
Implicit None
Include 'globals.inc'
Include 'model.inc'

Integer I,J
Real*8 A(MaxNDE,MaxNDE)

DO I=1,Ndeqs
   Do J=1,Ndeqs
      A(I,J)=0.0D0
   End Do
End Do

Subroutine AMAT(A)

Enter non zero elements of state matrix \{e.g. \( A(1,1) = -P(1) \) \}

Return
End
\end{verbatim}
4) One-tissue mPBPK model with nonlinear hepatic metabolism (IV PK of DPH)

**********************************************************************
C                           ADAPT                                     *
C                         Version 5                                   *
C****************************************
C                                                                     *
C                           MODEL                                     *
C                                                                     *
C    This file contains Fortran subroutines into which the user       *
C    must enter the relevant model equations and constants.           *
C    Consult the User's Guide for details concerning the format for   *
C    entered equations and definition of symbols.                    *
C                                                                     *
C       1. Symbol- Parameter symbols and model constants             *
C       2. DiffEq- System differential equations                     *
C       3. Output- System output equations                           *
C       4. Varmod- Error variance model equations                    *
C       5. Covmod- Covariate model equations (ITS,MLEM)              *
C       6. Popinit- Population parameter initial values (ITS,MLEM)   *
C       7. Prior - Parameter mean and covariance values (ID,NPD,STS) *
C       8. Sparam- Secondary parameters                              *
C       9. Amat - System state matrix                                *
C                                                                     *
C**********************************************************************
C######################################################################C

Subroutine SYMBOL
Implicit None

Include 'globals.inc'
Include 'model.inc'

CC
C                                                                     *
C Enter as Indicated                                                  *
C                                                                     *
NDEqs = 5 ! Enter # of Diff. Eqs.
NSParam = 5 ! Enter # of System Parameters.
NVparam = 2 ! Enter # of Variance Parameters.

41
NSecPar = 0 ! Enter # of Secondary Parameters.
NSecOut = 0 ! Enter # of Secondary Outputs (not used).
Ieqsol = 1 ! Model type: 1 - DIFFEQ, 2 - AMAT, 3 - OUTPUT only.
Descr = 'DPH 1 tissue 6 doses SCM1 NPD nonlinear metabolism iv'

Psym(1)= 'fd'
Psym(2)= 'Kp1'
Psym(3)= 'Vmax'
Psym(4)= 'Kp2'
Psym(5)= 'Km'

PVsym(1)= 'intercept'
PVsym(2)= 'sigma'

Return
End

Subroutine DIFFEQ(T,X,XP)
Implicit None
Include 'globals.inc'
Include 'model.inc'
Real*8 T,X(MaxNDE),XP(MaxNDE)
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Real*8 fd,Kp1,Kp2,CLb,Vt,Qco,Vb,Rb,Qliver,Vliver
Real*8 ka,Ppo,kinf,fd2,Vt1,Vt2,fd1,Vmax,Km,CLint,fup

CC
C----------------------------------------------C
C Enter Differential Equations Below {e.g. XP(1) = -P(1)*X(1) } C
C-----------------------------------------------C

fd = P(1)
Kp1 = P(2)
Vmax = P(3)
Kp2 = P(4)
Km=P(5)
Rb = 0.99
fup=0.227
!Tissue volume mL/kg
Vb=78 !from Kp DMD manuscript
Vt=885.7  ! BW-Vb-Vliver=1000-78-36.6
Vliver=36.6
!Cardiac output (L/min) = 0.235(BW)^0.75 from Brown et al
Qco=332.34 !mL/min/kg
Qliver=60.82 !mL/min/kg

C-define different dosing regimens
 c subj1: oral 30mg/kg Neetati et al;
 c subj2: oral 30mg/kg Burstein et al;
 c subj3-5: iv 40,25,10 mg/kg Gerber et al;
 c subj6: iv 10mg/kg blood&liver Itoh et al;

cc---Liver Compartment SCM1

XP(1) = (Qliver*(X(2)-X(1)*Rb/Kp2)
  x - (Vmax/(Km+fup*X(1)/Kp2))*fup*X(1)/Kp2)/Vliver

 cc---blood compart-----
  IF(fd .gt. 1) then
  XP(2) = 0
  else
  XP(2) = ((Qco-Qliver)*fd*(X(3)*Rb/Kp1-X(2))
  x +Qliver*(X(1)*Rb/Kp2-X(2)))/Vb
  endif

cc---Compartment1-----

XP(3) = (Qco-Qliver)*fd*(X(2)-X(3)*Rb/Kp1)/Vt
Subroutine OUTPUT(Y,T,X)
Implicit None
Include 'globals.inc'
Include 'model.inc'
Real*8 Y(MaxNOE),T,X(MaxNDE)
Real*8 CLint,Rb,Vb,Vt,Kp,Dose,Qliver,Vmax,Km,fup,Kp2

Y(1) = X(2)
Y(2) = X(1)
Y(3) = X(4)

Vmax = P(3)
Kp2 = P(4)
Km = P(5)
fup=0.227
Rb=0.99
Qliver=60.82 mL/min/kg

CLint = Vmax/(Km+fup*X(1)/Kp2) !nonlinear metabolism

Y(4) = 1-(Qliver/(Qliver+(CLint*fup/Rb)/1))**1 !1-SCM ER
Subroutine VARMOD(V,T,X,Y)
Implicit None
Include 'globals.inc'
Include 'model.inc'

Real*8 V(MaxNOE),T,X(MaxNDE),Y(MaxNOE)
Real*8 intercept, sigma

C--------------------------------------------------------------------
C   Enter Variance Model Equations Below                               C
C         {e.g. V(1) = (PV(1) + PV(2)*Y(1))**2 }                       C
C------------------------------------------------------------------

intercept=PV(1)
sigma=PV(2)
V(1:4) = (intercept + sigma*Y(1:4))**2

C--------------------------------------------------------------------
C------------------------------------------------------------------

Return
End

Subroutine COVMOD(Pmean, ICmean, PC)
C Defines any covariate model equations (MLEM, ITS)
Implicit None
Include 'globals.inc'
Include 'model.inc'

Real*8 PC(MaxNCP)
Real*8 Pmean(MaxNSP+MaxNDE), ICmean(MaxNDE)

CC
C---------------------------------------------------------------C
C      Enter # of Covariate Parameters                         C
C---------------------------------------------------------------C

NCparam = 0    ! Enter # of Covariate Parameters.

CC
C---------------------------------------------------------------C
C     Enter Symbol for Covariate Params {eg: PCsym(1)='CLRenal'}  C
C---------------------------------------------------------------C

CC
C---------------------------------------------------------------C
C     For the Model Params. that Depend on Covariates Enter the Equation C
C         {e.g. Pmean(1) = PC(1)*R(2) }                         C
C---------------------------------------------------------------C

C---------------------------------------------------------------C
C---------------------------------------------------------------C
C
Return
End

C########################################################################
Subroutine POPINIT(PmeanI,ICmeanI,PcovI,ICcovI, PCI)
C Initial parameter values for population program parameters (ITS, MLEM)

Implicit None

Include 'globals.inc'
Include 'model.inc'

Integer I,J
Real*8 PmeanI(MaxNSP+MaxNDE), ICmeanI(MaxNDE)
Real*8 PcovI(MaxNSP+MaxNDE,MaxNSP+MaxNDE), ICcovI(MaxNDE,MaxNDE)
Subroutine PRIOR(Pmean,Pcov,ICmean,ICcov)
C Parameter mean and covariance values for MAP estimation (ID,NPD,STS)
   Implicit None
   Include 'globals.inc'
   Include 'model.inc'

   Integer I,J
   Real*8 Pmean(MaxNSP+MaxNDE), ICmean(MaxNDE)
   Real*8 Pcov(MaxNSP+MaxNDE,MaxNSP+MaxNDE), ICcov(MaxNDE,MaxNDE)
Manuscript number: DMD-AR-2023-001403

CC
C*******************************************************************************C
C Enter Nonzero Elements of Prior Mean Vector                                 C
C { e.g. Pmean(1) = 10.0 }                                                   C
C*******************************************************************************C

CC
C*******************************************************************************C
C Enter Nonzero Elements of Covariance Matrix (Lower Triang.)                C
C { e.g. Pcov(2,1) = 0.25 }                                                 C
C*******************************************************************************C

C*******************************************************************************C
C*******************************************************************************C
C*******************************************************************************C
C*******************************************************************************C
C*******************************************************************************C
C*******************************************************************************C
C*******************************************************************************C
C*******************************************************************************C
C*******************************************************************************C
C*******************************************************************************C
C*******************************************************************************C
C Return
End
C*******************************************************************************C
C*******************************************************************************C
CC
C*******************************************************************************C
C Enter Equations Defining Secondary Parameters                              C
C { e.g. PS(1) = P(1)*P(2) }                                                C
C*******************************************************************************C

Subroutine SPARAM(PS,P,IC)
Implicit None

Include 'globals.inc'

Real*8 PS(MaxNSECP), P(MaxNSP+MaxNDE), IC(MaxNDE)
Real*8 CLint,Rb,Vb,Vt,Kp,Dose,Qliver,Vmax,Km,fup,Kp2

CC
C*******************************************************************************C
C*******************************************************************************C
C*******************************************************************************C
C*******************************************************************************C
C*******************************************************************************C
C*******************************************************************************C
C*******************************************************************************C
C*******************************************************************************C
C*******************************************************************************C
C*******************************************************************************C
C*******************************************************************************C

C

        Return
        End

C###########################################################################C

        Subroutine AMAT(A)
        Implicit None

        Include 'globals.inc'
        Include 'model.inc'

        Integer I, J
        Real*8 A(MaxNDE, MaxNDE)

        DO I=1, Ndeqs
           Do J=1, Ndeqs
              A(I, J)= 0.0D0
           End Do
        End Do

CC

C###########################################################################C

C      Enter non zero elements of state matrix { e.g. A(1, 1) = - P(1) } C
C###########################################################################C

C###########################################################################C

C        Return
        End

C###########################################################################C
References


Jeong YS, Kim MS, and Chung SJ (2022) Determination of the number of tissue groups of kinetically distinct transit time in whole-body physiologically based pharmacokinetic (pbpk) models II: practical application of tissue lumping theories for pharmacokinetics of various compounds. *AAPS J* **24**:91.

Li X and Jusko WJ (2022) Assessing liver-to-plasma partition coefficients and in silico calculation methods: when does the hepatic model matter in PBPK? *Drug Metab Dispos.*