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Use of partition coefficients in flow-limited physiologically-based pharmacokinetic modeling

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Abstract

Permeability-limited two-subcompartment and flow-limited, well-stirred tank tissue compartment models are routinely used in physiologically-based pharmacokinetic modeling. Here, the permeability-limited two-subcompartment model is used to derive a general flow-limited case of a two-subcompartment model with the well-stirred tank being a specific case where tissue fractional blood volume approaches zero. The general flow-limited two-subcompartment model provides a clear distinction between two partition coefficients typically used in PBPK: a biophysical partition coefficient and a well-stirred partition coefficient. Case studies using diazepam and cotinine demonstrate that, when the well-stirred tank is used with *a priori* predicted biophysical partition coefficients, simulations overestimate or underestimate total organ drug concentration relative to flow-limited two-subcompartment model behavior in tissues with higher fractional blood volumes. However, whole-body simulations show predicted drug concentrations in plasma and lower fractional blood volume tissues are relatively unaffected. These findings point to the importance of accurately determining tissue fractional blood volume for flow-limited PBPK modeling. Simulations using biophysical and well-stirred partition coefficients optimized with flow-limited two-subcompartment and well-stirred models, respectively, lead to nearly identical fits to tissue drug distribution data. Therefore, results of whole-body PBPK modeling with diazepam and cotinine indicate both flow-limited models are appropriate PBPK tissue models as long as the correct partition coefficient is used: the biophysical partition coefficient is for use with two-subcompartment models and the well-stirred partition coefficient is for use with the well-stirred tank model.

Keywords

Physiologically-based pharmacokinetics; Flow-limited; Permeability-limited; Well-stirred tank; Compartmental modeling; Partition coefficient; Biophysical; Diazepam; Cotinine

Introduction

Physiologically-based pharmacokinetic modeling has seen widespread use in toxicology and is emerging as an important tool in drug development [1]. The basic element of PBPK modeling is the tissue compartment model, and for over 30 years, the permeability-limited two-subcompartment model and its flow-limited counterpart, the well-stirred tank, have

defined the field of PBPK. The permeability-limited two-subcompartment model has a sound mechanistic basis, as it can be derived [2] from the one-dimensional advection equation [3] assuming a lumped or spatially homogenized treatment of the vascular region. Therefore, in making comparisons of the different tissue compartment models, the permeability-limited two-subcompartment model is assumed to be the most biophysically realistic model that is required to simulate ADME processes operating on slower timescales. In the recent work of Thompson and Beard [2], the development of permeability-limited two-region asymptotically reduced and flow-limited two-region asymptotically reduced tissue compartment models afforded the opportunity to reevaluate the permeability-limited two-subcompartment and well-stirred tank models. Herein, this work is further extended by considering the permeability-limited two-subcompartment model in the limit of higher permeation relative to flow. A flow-limited two-subcompartment model is proposed that distinguishes two types of partition coefficients used in PBPK which are evaluated using pharmacokinetic data from the rat.

Methods: theoretical

Case I (definition): biophysical partition coefficient

Physicochemical properties of drugs are used to predict and/or constrain PBPK model parameters such as the partition coefficient [4,5]. The biophysical partitioning of a drug between the blood and tissues is determined in part by the thermodynamically-driven transfer of drug molecules between lipid and water phases [6]. To simplify and implement this biophysical partitioning in a PBPK tissue model, partitioning between the blood and tissue can be minimally represented by a two-compartment, well-mixed system at equilibrium. Therefore, the biophysical partition coefficient is defined from this equilibrium partitioning as

$$P_{t,p} = \frac{c_{tissue}}{c_{plasma}} = \frac{c_2}{c_1} \quad (1)$$

where c_{tissue} is the concentration of drug in the tissue and c_{plasma} is the concentration of drug in the plasma. This definition is most appropriately applied to a two-subcompartment, vascular:extravascular model where c_{tissue} is the extravascular space, denoted by c_2 in the model below, and where c_{plasma} is the vascular space, denoted by c_1 in the model. Advantageously, the biophysical partition coefficient is amenable to experimental measurement with techniques such as vial-equilibration methods [7] using tissue homogenates to determine partition coefficients for PBPK modeling [8]. In addition, algorithms have been developed based on drug physicochemical properties and tissue composition to generate *a priori* predicted partition coefficients for PBPK modeling applications [4,5,9]. Since the term tissue:plasma partition coefficient ($P_{t,p}$) is used by Poulin, et al. [4] when discussing *a priori* predicted values, the biophysical partition coefficient used herein is denoted as $\lambda_{biophys}$ and is meant to imply a predicted value for $P_{t,p}$, an *in vitro* measurement, or a value arrived at through fitting data with a two-subcompartment model.

Case I (model): two-subcompartment PBPK tissue model for use with the biophysical partition coefficient ($\lambda_{biophys}$)

The biophysical partition coefficient used in PBPK modeling is most appropriately applied to the permeability-limited two-subcompartment model referred to as PLT by Thompson and Beard [2]. The permeability-limited two-subcompartment model is given by

$$\frac{dc_1}{dt} = \frac{Q}{V_1}(c_{in} - c_1) - \frac{PS}{V_1} \left(c_1 - \frac{c_2}{\lambda_{biophys}} \right) \quad (2)$$

and

$$\frac{dc_2}{dt} = + \frac{PS}{V_2} \left(c_1 - \frac{c_2}{\lambda_{biophys}} \right) \quad (3)$$

where Q is flow, c_{in} is the inflow concentration of drug, c_1 is the concentration of drug in the vascular subcompartment, V_1 is the vascular volume, c_2 is the concentration of drug in the extravascular subcompartment, V_2 is the extravascular volume, PS is the permeability-surface area product of the drug, and $\lambda_{biophys}$ is the partition coefficient of the drug relating c_2 to c_1 from Eq. 1.

Case II (definition): well-stirred partition coefficient

In 1984 [10], Rowland concisely discussed the issues of defining a steady-state partition coefficient for use in whole-body PBPK modeling and summarized the argument for the venous equilibrium partition coefficient as

$$K_p = \frac{c_T}{c_{out}} \quad (4)$$

where c_T is the average concentration of drug in a single compartment model and the outflowing venous blood, c_{out} , is at equilibrium with c_T . Herein, this steady-state, well-stirred partition coefficient will be denoted as $\lambda_{well-stirred}$.

Case II (model): single compartment well-stirred tank PBPK tissue model for use with the well-stirred partition coefficient ($\lambda_{well-stirred}$)

The single compartment well-stirred tank is the default flow-limited tissue model used in PBPK applications unless physicochemical properties or pharmacokinetic time courses for tissue distribution indicate a possible permeability limitation [2]. The well-stirred tank (WST) model is governed by

$$\frac{dc_T}{dt} = \frac{Q}{V_T} \left(c_{in} - \frac{c_T}{\lambda_{well-stirred}} \right) \quad (5)$$

where Q is flow, c_{in} is the inflow concentration of drug into the single compartment, c_T is the average or overall concentration of the well-stirred compartment, V_T is the total volume of the compartment, and $\lambda_{well-stirred}$ is the partition coefficient of drug at steady-state relating c_T to c_{out} from Eq. 4.

Use of well-stirred and biophysical partition coefficients

The biophysical and well-stirred partition coefficients are defined to varying degrees in the literature [11–13], and the relationship between them is often unclear [5]. Failing to clearly distinguish well-stirred and biophysical partition coefficients leads to instances where the two definitions are used interchangeably between permeability-limited and flow-limited models [14,15], even though they imply and express different mathematical relationships

between tissue and blood concentrations of drug based on the use of a two-subcompartment versus a single compartment tissue model.

Methods: flow-limited two-region model development

Recently, a PBPK tissue compartment modeling approach was developed by Thompson and Beard [2] that began to highlight this distinction. Using a singular perturbation analysis of the permeability-limited model (PLT), a new two-region asymptotically reduced model was developed that uses only one state variable. The permeability-limited two-region asymptotically reduced (P-TAR) model is given by

$$\frac{dc_2}{dt} = \frac{Q}{V_2} \left(\frac{PS/Q}{1+PS/Q} \right) \left(c_{in} - \frac{c_2}{\lambda_{biophys}} \right) \quad (6)$$

and c_1 is subsequently computed as

$$c_1 = \frac{c_{in} + (PS/Q)(c_2/\lambda_{biophys})}{1+PS/Q}. \quad (7)$$

In the flow-limiting case ($PS/Q \rightarrow \infty$), Eqs. 6 and 7 reduce to

$$\frac{dc_2}{dt} = \frac{Q}{V_2} \left(c_{in} - \frac{c_2}{\lambda_{biophys}} \right) \quad (8)$$

and

$$c_1 = \frac{c_2}{\lambda_{biophys}} \quad (9)$$

giving the flow-limited two-region asymptotically reduced (F-TAR) model. To conserve mass, the outflow concentration, c_{out} , is computed using the dynamic mass balance equation

$$Q(c_{in} - c_{out}) = V_1 \frac{dc_1}{dt} + V_2 \frac{dc_2}{dt} \quad (10)$$

to give c_{out} for the permeability-limited two-region asymptotically reduced case as

$$c_{out} = c_{in} - \frac{1}{Q} \left[\frac{V_1}{1+PS/Q} \frac{dc_{in}}{dt} + \left(\frac{V_1 PS/Q}{1+PS/Q \lambda_{biophys}} + V_2 \right) \frac{dc_2}{dt} \right] \quad (11)$$

and c_{out} for the flow-limited two-region asymptotically reduced case as

$$c_{out} = \left(1 + \frac{V_1}{\lambda_{biophys} V_2} \right) \frac{c_2}{\lambda_{biophys}} - c_{in} \frac{V_1}{\lambda_{biophys} V_2}. \quad (12)$$

Further conditional statements are discussed and applied in Thompson and Beard [2]. Equations 6 through 12 allow drug concentration to be simulated in both vascular and extravascular regions by solving only one differential equation for either case.

To evaluate the permeability-limited two-region asymptotically reduced model and the flow-limited two-region asymptotically reduced model, comparisons were made to the standard permeability-limited two-subcompartment (PLT) (Eqs. 2,3) and well-stirred tank (WST) (Eq. 5) models. As shown in Thompson and Beard [2], the permeability-limited two-region asymptotically reduced (P-TAR) and flow-limited two-region asymptotically reduced (F-TAR) tissue models closely approximate the behavior of the permeability-limited two-subcompartment model and the permeability-limited two-subcompartment model in the limit of $PS/Q \rightarrow \infty$, respectively. However, from this analysis, the well-stirred tank model appeared to not be an appropriate choice when drugs differentially partition between the blood and tissue spaces [2]. Further theoretical analysis of 75 drugs with a range of *a priori* predicted partition coefficients evaluated in a set of 8 rat tissues and organs showed that use of the well-stirred tank model to simulate flow-limited transport in whole-body PBPK modeling can potentially result in large differences in model simulations compared to the flow-limited two-region asymptotically reduced model [16].

As a result of these studies, herein we develop a model to simulate the flow-limited extreme of the permeability-limited two-subcompartment model that is as accurate as and somewhat more convenient than the asymptotic approximation of the flow-limited two-region asymptotically reduced model. The flow-limited two-subcompartment model formulation, which provides a clear theoretical framework to implement the biophysical partition coefficient and distinguish it from the well-stirred partition coefficient, is described and evaluated below.

Methods: flow-limited two-subcompartment model development

Consider the total concentration of solute in a two-subcompartment system:

$$c_T = \frac{V_1 c_1 + V_2 c_2}{V_T}, \quad (13)$$

where c_1 and c_2 are the concentrations of solute in the subcompartments, V_1 and V_2 are the volumes of the subcompartments, and $V_T = V_1 + V_2$. Taking the time derivative,

$$\frac{dc_T}{dt} = \frac{V_1}{V_T} \frac{dc_1}{dt} + \frac{V_2}{V_T} \frac{dc_2}{dt} \quad (14)$$

and substituting Eqs. 2 and 3, the governing equation for total concentration is

$$\frac{dc_T}{dt} = \frac{Q}{V_T} (c_{in} - c_1). \quad (15)$$

Assuming rapid equilibration between the vascular and extravascular spaces ($c_2 = \lambda_{biophys} c_1$), the outflow concentration (c_I) is given by

$$c_{out} = c_1 = \frac{c_T}{FBV + (1 - FBV)\lambda_{biophys}}, \quad (16)$$

where $FBV = V_1/V_T$ is the fractional blood volume of the compartment. Substituting into Eq. 15, we have

$$\frac{dc_T}{dt} = \frac{Q}{V_T} \left(c_{in} - \frac{c_T}{\text{FBV} + (1 - \text{FBV})\lambda_{biophys}} \right). \quad (17)$$

The flow-limited two-subcompartment model (Eq.17) can be re-expressed with

$$\lambda_{well-stirred} = \text{FBV} + (1 - \text{FBV})\lambda_{biophys} \quad (18)$$

to simulate the same time courses as the well-stirred tank (Eq. 5). Figure 1a diagrams the flow-limited two-subcompartment model with rapid mixing and equilibration between the vascular and extravascular spaces. The well-stirred tank is obtained from the flow-limited two-subcompartment model in the limit of $V_1/V_T \rightarrow 0$ (Fig. 1b) where the FBV approaches zero and the outflow concentration of the flow-limited two-subcompartment model approaches the outflow concentration of the well-stirred tank model. Therefore, the well-stirred tank model is a specific flow-limited case of the permeability-limited two-subcompartment model.

In summary, we propose the following: 1) the flow-limited two-subcompartment model of Eq. 17 is the appropriate flow-limited counterpart to the permeability-limited two-subcompartment model for use in PBPK modeling when using the biophysical definition of the partition coefficient ($\lambda_{biophys}$); 2) the well-stirred tank is an appropriate PBPK tissue model when using the well-stirred definition for the partition coefficient ($\lambda_{well-stirred}$); and 3) the two partition coefficients, $\lambda_{biophys}$ and $\lambda_{well-stirred}$, are related by a tissue's fractional blood volume, assuming drug transport is flow-limited in a tissue with rapid equilibration between subcompartments.

Methods: PBPK tissue compartment model analysis

To demonstrate the effect of using a biophysical partition coefficient with the well-stirred tank, an open loop circulatory model [16] is used to simulate inflow concentration with

$$\frac{dc_{dose}}{dt} = -k_a c_{dose}, \quad \frac{dc_{in}}{dt} = k_a c_{dose} - k_{el} c_{in}, \quad (19)$$

where k_a ($=0.001 \text{ sec}^{-1}$) is the absorption rate constant, k_{el} ($=0.0005 \text{ sec}^{-1}$) is the elimination rate constant, and c_{dose} is set to 2 arbitrary units (a.u.) of concentration. A worst-case scenario is assessed using a model representing the human kidney. Physiological parameter values are from Brown, et al. [17]. The human kidney receives a mean flow distribution of cardiac output equal to 17.5%, or 1138 ml/min for a cardiac output of 6.5 l/min. For a 70 kg person, the kidney weight is 308 g (0.44% of the body weight) or 293 ml (kidney density of 1.05 g/ml). The fractional blood volume for the human kidney has been reported to be up to 50% [17] with a mean value of 36%. Simulations (Fig. 2) use a value of 50% for kidney fractional blood volume. Drug-specific parameters are $\lambda_{biophys} = 0.25$ and 25, with the low and high values selected based on the range of $\lambda_{biophys}$ in 8 rat tissues and organs from a previous analysis of *a priori* predicted $P_{t,p}$ for 75 structurally-unrelated drugs [16]. The $\lambda_{well-stirred}$ value is calculated based on Eq.18. Both C_{max} and area under the curve (AUC) are assessed with the percent difference of the well-stirred model (using $\lambda_{biophys}$) expressed relative to the flow-limited two-subcompartment model (using the same $\lambda_{biophys}$).

Though this set of simulations shows how model agreement depends on the partition coefficient definition used, evaluation of rat pharmacokinetic data for two drugs, diazepam

and cotinine, provides an opportunity to study PBPK tissue model behavior in a whole-body context.

Methods: case study of diazepam ($\lambda > 1$) in the rat

Diazepam is a well-studied benzodiazepine with several tissue distribution studies in the literature [18–21] forming the basis of diazepam PBPK modeling papers on optimization [18,22], fuzzy simulation [19,23], and Bayesian approaches [20]. The PBPK model structure (Fig. 3) proposed for diazepam analysis [20] is used herein to evaluate the new flow-limited two-subcompartment model (using $\lambda_{biophys}$) compared to the well-stirred tank (using $\lambda_{biophys}$ and $\lambda_{well-stirred}$). The physicochemical properties of diazepam (logP value of 2.99 [5] and an unbound fraction (f_u) in the plasma of 0.15 [19]), result in predicted partition coefficients greater than 1 for all tissues (Table 2) based on the method of Poulin, et al. [4,5] with the correction by Berezhkovskiy [24]. The ratio of blood to plasma diazepam is set equal to 1 [18]. Tissue time course data for diazepam in the rat (1 mg/kg, intravenous infusion) are from the work of Gueorguieva et al. [20]. Rat tissue blood flows and tissue masses are obtained from Gueorguieva, et al. [20] and can be found in Table 1. Fractional blood volume (FBV) is obtained from the work of Everett, et al. [25] or Brown, et al. [17], with values given for both the mean and range of fractional blood volume when available, as indicated in Table 1. Values for the human [17,20] are also provided in Table 1 for reference.

The well-stirred tank model equations governing liver metabolism of diazepam are based on Gueorguieva et al. [20]. Herein, the equations for the well-stirred model are presented as

$$\text{Inflow}_{\text{liver}} = Q_{\text{hepatic}} C_{\text{in,arterial}} + Q_{\text{stomach}} C_{\text{out,stomach}} + Q_{\text{splanchnic}} C_{\text{out,splanchnic}} \quad (20)$$

$$Q_{\text{liver}} = Q_{\text{hepatic}} + Q_{\text{stomach}} + Q_{\text{splanchnic}} \quad (21)$$

$$V_{\text{liver}} \frac{dc_{T,\text{liver}}}{dt} = \text{Inflow}_{\text{liver}} - Q_{\text{liver}} \frac{c_{T,\text{liver}}}{\lambda_{\text{well-stirred}}} - CL_{\text{INT}} \frac{c_{T,\text{liver}}}{\lambda_{\text{well-stirred}} / fu_b} \quad (22)$$

where R is the blood to plasma ratio, f_u is the unbound fraction in plasma, $fu_b = fu/R$, and CL_{INT} is the intrinsic clearance. Liver blood flow (Q_{liver}) is the sum of hepatic artery blood flow (Q_{hepatic}), stomach blood flow (Q_{stomach}), and splanchnic blood flow ($Q_{\text{splanchnic}}$). The governing equation for liver metabolism of diazepam for the flow-limited two-subcompartment liver tissue model is given by

$$V_{\text{liver}} \frac{dc_{T,\text{liver}}}{dt} = \text{Inflow}_{\text{liver}} - Q_{\text{liver}} \frac{c_{T,\text{liver}}}{FBV + (1-FBV)\lambda_{biophys}} - CL_{\text{INT}} \frac{c_{T,\text{liver}}}{[FBV + (1-FBV)\lambda_{biophys}] / fu_b} \quad (23)$$

with Eq. 23 reducing to Eq. 22 when FBV approaches zero or $\lambda_{biophys} = 1$.

Methods: case study of cotinine ($\lambda < 1$) in the rat

Cotinine is a polar metabolite of nicotine that has been used as a biomarker for smoking exposure [26] with previous PBPK modeling indicating cotinine has flow-limited distribution to tissues in the rat [27]. Cotinine is predicted to have partition coefficients less than 1 for all tissues (Table 4) based on the method of Poulin, et al. [4] with the correction by Berezhkovskiy [24]. The ratio of blood to plasma cotinine is equal to 0.88 [28].

Physicochemical properties of cotinine include a logP value of -0.3 [29] and an unbound fraction (f_u) in the plasma of 0.97 [4].

Tissue time course data for cotinine (0.5 mg, intravenous bolus) in the rat are obtained from the published work of Gabrielsson, et al. [27], digitized (ScanIt V1.04, AmsterCHEM, Almeria, Spain), and analyzed with the same model structure used for diazepam (Fig. 3). Based on the tissues reported [27], the level of detail in the PBPK model structure is preserved without lumping; however, the values for partition coefficients used for the skin, stomach, and testes are assumed to be the same as the remainder compartment, both during optimization as well as simulation with *a priori* predicted values (Table 4).

All simulations and optimization using Monte Carlo and fmincon approaches are carried out in Matlab v.R2010a, (Mathworks, Natick, MA).

Results and Discussion

Comparing models using biophysical and well-stirred partition coefficients

As shown previously [2], the permeability-limited two-region asymptotically reduced model closely follows permeability-limited two-subcompartment model behavior and the flow-limited two-region asymptotically reduced model closely follows a numerically-approximated ($PS/Q \rightarrow \infty$), flow-limited version of the permeability-limited two-subcompartment model. Neither the numerically-approximated flow-limited version of the permeability-limited two-subcompartment model nor the flow-limited two-region asymptotically reduced model agreed with the well-stirred tank model except at values of $\lambda = 1$ [2,16]. To further investigate this issue, a general flow-limited case of the permeability-limited two-subcompartment model, the flow-limited two-subcompartment tissue compartment model, is developed herein, with the well-stirred tank model being a specific case of the flow-limited two-subcompartment model where $V_1/V_T \rightarrow 0$. The hierarchy of tissue models (the permeability-limited two-subcompartment leading to the flow-limited two-subcompartment leading to the well-stirred tank) demonstrates that all of the tissue models are biophysically appropriate; however, the models differ regarding the interpretation of the partition coefficient.

Simulations show that the flow-limited two-subcompartment model agrees well with the numerically-approximated, flow-limited version of the permeability-limited two-subcompartment model, as well as the flow-limited two-region asymptotically reduced model regardless of the value of the $\lambda_{biophys}$ partition coefficient. However, when the same partition coefficient, $\lambda_{biophys}$, is used in the well-stirred tank model, the well-stirred tank overestimates c_T (Fig. 2B, solid black line indicated by black arrowhead) when $\lambda_{biophys}$ is greater than 1 and underestimates c_T (Fig. 2A, solid black line indicated by black arrowhead) when $\lambda_{biophys}$ is less than 1. The worst-case result of not correcting *a priori* predicted partition coefficients prior to use in the well-stirred tank model is explored with an open circulatory loop approach, here using physiological parameters for the human kidney. Both the peak concentration (C_{max}) and area under the curve (AUC) substantially differ between well-stirred tank and flow-limited two-subcompartment model simulations. Well-stirred tank C_{max} percent difference ranges from -60% to $+86\%$ and well-stirred tank AUC percent difference ranges from -60% to $+103\%$ (when $\lambda_{biophys}$ is set to 0.25 and 25 , respectively). If the $\lambda_{biophys}$ value used with the well-stirred tank is adjusted according to Eq. 18, the well-stirred simulations (Fig. 2A,B; solid light gray lines) match flow-limited two-subcompartment simulations (Fig. 2A,B; dashed black lines).

Tissues and organs, such as the skin, brain, adipose, and skeletal muscle have relatively small fractional blood volumes and the differences will be substantially less [16]. However,

spleen, heart, lung, and tissues and organs that play key roles in ADME processes, such as liver and kidney, have higher fractional blood volumes [17] that may impact estimation of metabolic clearance or elimination rates (rat FBV range: liver [0.12–0.27], lung [0.26–0.52], kidney [0.11–0.27]; human FBV range: kidney [0.22–0.50]). Thus theoretically, proper matching of a flow-limited model with a partition coefficient is important. However, whole-body analysis of pharmacokinetic data is needed to evaluate these theoretical considerations.

Diazepam case study: pharmacokinetics in the rat

Simulations using a priori predicted partition coefficients for diazepam

Since the well-stirred tank is a reasonable approximation of the flow-limited two-subcompartment model when tissue fractional blood volume is low (adipose, brain, muscle, skin, splanchnic, stomach, and testes), well-stirred simulations (Fig. 4; solid black lines) are very close to flow-limited two-subcompartment simulations (Fig. 4; dashed gray lines). Area under the curve values are listed by tissue in Table 3. The percent difference for these tissues averages -2.7% (range of -1.9% to -3.6%). Simulations of plasma concentration of diazepam also show well-stirred tank simulations (Fig. 4; solid black lines) being nearly identical to flow-limited two-subcompartment simulations (Fig. 4; dashed gray lines). This observation implies that selection of an inappropriate partition coefficient for use with the well-stirred tank PBPK tissue model has minimal impact on the systemic kinetics of plasma diazepam (Table 3, area under the curve). This finding may be partly explained by the PBPK model structure used herein, where approximately 87% of tissue mass has a fractional blood volume of less than or equal to 0.05. Counter to findings in low fractional blood volume tissues and the plasma, tissues and organs with higher fractional blood volumes, plotted in Fig. 4 (heart, kidney, liver, lung), do differ more when *a priori* values are used with both models. Area under the curve values (Table 3) for these organs differ by an average of -21.3% (range of -12.5% to -35.7%). Also, in the tissues with higher fractional blood volume, the flow-limited two-subcompartment model simulations using *a priori* predicted partition coefficients are closer to the experimental data, indicating that consideration of fractional blood volume by the flow-limited two-compartment model may improve model prediction for diazepam.

Optimization of diazepam partition coefficients and clearance parameters with well-stirred tank and flow-limited two-subcompartment models

Partition coefficients optimized using the well-stirred tank model differ from coefficients optimized using the flow-limited two-subcompartment model in a predictable manner (Table 2). Higher fractional blood volume organs (heart, 20.4%; lung, 27.4%) differ the most in optimized partition coefficient values. Simulations using the optimized values (Fig. 5; well-stirred simulation, solid black line; flow-limited two-subcompartment, dashed gray line) and area under the curve data (Table 3) show the models produce similar results, including simulation of diazepam plasma concentration. Therefore, for drugs with partition coefficients greater than 1, optimization with either the flow-limited two-subcompartment model (using $\lambda_{biophys}$) or with the well-stirred tank (using $\lambda_{well-stirred}$) produce similar fits to experimental data.

Cotinine case study: pharmacokinetics in the rat

Simulations using a priori predicted partition coefficients for cotinine

Similar to findings for diazepam, well-stirred tank simulations (Fig. 6; solid medium gray lines) are very close to flow-limited two-subcompartment simulations (Fig. 6; dashed dark gray lines) for low fractional blood volume tissues. However, simulations for tissues with higher fractional blood volumes are also similar because the *a priori* predicted coefficients

are relatively close to 1 (Table 4, cotinine partition coefficients). The AUC percent difference for tissues averages 3.4% (range of 0.9% to 7.3%). Simulations of plasma concentration of cotinine are even closer (0.5%, Table 5), again showing that use of a biophysical partition coefficient in the well-stirred tank model has minimal impact on the systemic kinetics of plasma cotinine. However, fits of *a priori* based simulations to the experimental data are poor, as *a priori* prediction overestimated the values for the tissue partition coefficients. This leads to larger uptake by tissues and lower plasma concentrations than observed in the rat pharmacokinetic study. As seen in Table 4, the difference between *a priori* values and either biophysical or well-stirred coefficients is greater than the difference between the biophysical and well-stirred partition coefficients. As a result, *a priori* predicted values for cotinine do not provide a reference point to which flow-limited two-subcompartment optimized partition coefficients can readily be compared. This result is not entirely unexpected as Rodgers, et al. noted that methods to predict partition coefficients are considered reasonably accurate if within a factor of 3 of the experimentally determined values [30].

Optimization of cotinine well-stirred partition coefficients using the well-stirred tank model and translation of well-stirred partition coefficients to biophysical partition coefficients

In contrast to the case study of diazepam, only the well-stirred partition coefficients are optimized for cotinine. The optimized well-stirred partition coefficients are then adjusted based on Eq.18 to give the biophysical partition coefficients for the flow-limited two subcompartment model simulations, with the clearance parameter kept the same between the models (Table 4). In this situation, well-stirred simulations (Fig. 7; solid light gray lines) are nearly identical to flow-limited two-subcompartment simulations (Fig. 7; dashed dark gray lines) in all tissues (Table 5, AUC percent difference) with one exception. The optimized well-stirred partition coefficient for cotinine in the lung is lower than the fractional blood volume for the lung. The relationship between $\lambda_{well-stirred}$ and $\lambda_{biophys}$, as described by Eq. 18, yields a value for $\lambda_{biophys}$ that would be negative. Since a negative concentration ratio is not reasonable in PBPK, an arbitrarily low value for $\lambda_{biophys}$ is used in the simulation. As seen in Fig. 7, the flow-limited two-subcompartment model (dashed dark gray line) cannot fit the lung data, unlike the well-stirred tank (solid light gray line). This finding implies either: 1) the fractional blood volume of the lung in the Gabrielsson, et al. [27] study was less than the reported value of Brown, et al. [17] and less than the well-stirred partition coefficient for cotinine in the lung; or 2) cotinine distribution to the lung is actually not flow-limited but rather permeability-limited. In this latter case, the optimized parameter for the lung is clearly only meaningful if the appropriate tissue model structure is used. If a permeability-limitation exists, but a flow-limited model is used to fit pharmacokinetic data, the meaningfulness of the model parameters is questionable. The lung cotinine example demonstrates the potential benefit to using the flow-limited two-subcompartment model along with the well-stirred tank to better understand drug pharmacokinetics through incorporation of more biophysical and physiological detail into PBPK tissue models.

Implication for physiological measurements

The relationship between the biophysical and well-stirred partition coefficients is summarized in Fig. 8. The ratio of $\lambda_{well-stirred}$ to $\lambda_{biophys}$ varies between 1 and 1-FBV when $\lambda_{biophys} > 1$, and the percent difference of $\lambda_{biophys}$ versus $\lambda_{well-stirred}$ varies between 0 and 100*FBV when $\lambda_{biophys} > 1$. For a drug with a biophysical partition coefficient of >1 in the rat lung, where mean fractional blood volume is 0.36 [16], the lowest possible ratio of $\lambda_{well-stirred}$ to $\lambda_{biophys}$ is 0.64 and the largest percent difference is 36%. As an example, for diazepam in the rat lung (Table 2, FLT-optimized and WST-optimized partition coefficients), the ratio is 0.73 and the percent difference is 27.4%. However, if the drug

partition coefficient is <1 , the ratio of $\lambda_{\text{well-stirred}}$ to λ_{biophys} and the absolute percent difference can become very large.

Since the biophysical and well-stirred partition coefficients are related to each other by tissue fractional blood volume, fractional blood volume takes on newfound importance as a fixed parameter in flow-limited PBPK tissue models, whereby previous use of fractional blood volume was largely restricted to permeability-limited two-subcompartment settings. The potential importance of fractional blood volume was noted previously by Khor and Mayersohn [31,32] in correcting partition coefficients for residual blood in the tissues. However, few papers exist which cite Khor and Mayersohn and apply the correction to pharmacokinetic data. In order to meaningfully convert between the biophysical and well-stirred partition coefficients using fractional blood volume, values for fractional blood volume the literature must be carefully selected. The work of Brown, et al. [17] summarizes tissue fractional blood volumes across species and serves as a major reference for permeability-limited modeling. If the flow-limited two-subcompartment model is adopted for use in PBPK, reassessment of available data on fractional blood volume, as well as the methods to accurately determine fractional blood volume in a range of tissues, may be needed. Experimental techniques used to measure the vascular space require use of a substance impermeant to the endothelium with examples including the work of Everett, et al. [25] using erythrocytes tagged with iron-59 and the recent work of Boswell et al. labeling erythrocytes with technetium-99 in mice [33]. Reconsidering the use and measurement of fractional blood volume will also benefit other two-subcompartment models, such as the permeability-limited two-subcompartment intracellular:extracellular model where interstitial fluid volume is required in addition to vascular volume [34,35].

Summary and implications for PBPK modeling research

The development of the flow-limited two-subcompartment model has highlighted the need to clearly distinguish between two partition coefficients for use in PBPK tissue models: 1) a partition coefficient (λ_{biophys}) for tissue:plasma drug partitioning arrived at using *in vitro* approaches and prediction algorithms or analyzing experimental data with a two-subcompartment model; and 2) a well-stirred partition coefficient ($\lambda_{\text{well-stirred}}$) for use in analyzing experimental data with the well-stirred tank model. The flow-limited two-subcompartment model shows how the partition coefficients can be interconverted to allow agreement of two-subcompartment and well-stirred tank model simulations. When using *in vitro* or *a priori* predicted tissue:plasma partition coefficients with the well-stirred tank model, the partition coefficient (λ_{biophys}) should be converted to $\lambda_{\text{well-stirred}}$. Though plasma kinetics do not appear to be substantially impacted by choice of partition coefficient for use in the well-stirred tank, simulation of target tissue drug concentration needed for carrying out mechanistic pharmacodynamic and toxicodynamic modeling may be significantly impacted. A potential advantage to implementing a flow-limited two-subcompartment model is that c_2 can be estimated, with the potential to influence pharmacokinetic-pharmacodynamic modeling.

The work presented herein clarifies previous findings from Thompson and Beard regarding the well-stirred tank model [2,16]: the well-stirred tank and the flow-limited two-subcompartment model are both appropriate flow-limited PBPK tissue models as long as the correct partition coefficient is used. The development of the flow-limited two-subcompartment model also highlights the need to reconsider the importance of physiological parameters, such as tissue fractional blood volume, that may improve PBPK model predictability and interpretation of drug pharmacokinetics and pharmacodynamics.

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Figure 1. Flow-limited two-subcompartment and well-stirred PBPK tissue compartment model diagrams

A) Flow-limited two-subcompartment (FLT) model, Eq.17; and B) well-stirred tank (WST) model, Eq.5. The flow-limited two-subcompartment model has both vascular (V_1) and extravascular (V_2) spaces and simulates total concentration in the compartment, c_T , as a function of the volume and concentrations in each subcompartment. The outflow concentration differs between the flow-limited two-subcompartment and well-stirred tank models depending on the type of partition coefficient used ($\lambda_{biophys}$ or $\lambda_{well-stirred}$) and the fractional blood volume (FBV) of the compartment. The flow-limited two-subcompartment model is a general flow-limited case of the PLT model. The well-stirred tank model is a specific flow-limited case of the PLT model where $FBV \rightarrow 0$.

Figure 2. Flow-limited model simulations using a biophysical partition coefficient in a model representing the human kidney: a worst-case scenario

A,B) Well-stirred tank with $\lambda_{biophys}$ (solid black lines, indicated by black arrowheads), well-stirred tank with $\lambda_{well-stirred}$ (solid light gray lines); flow-limited two-subcompartment with $\lambda_{biophys}$ (dashed black lines). Simulations are based on Eq.19 with $k_a = 0.001 \text{ sec}^{-1}$ and $k_{el} = 0.0005 \text{ sec}^{-1}$; physiological parameters: $V_T = 293 \text{ ml}$, $FBV = 0.5$, $Q = 1138 \text{ ml} \cdot \text{min}^{-1}$; drug-specific parameter: A) $\lambda_{biophys} = 0.25$ with $\lambda_{well-stirred}$ calculated based on Eq.18; B) $\lambda_{biophys} = 25$ with $\lambda_{well-stirred}$ calculated based on Eq.18. Arbitrary units (a.u.) of concentration are plotted versus time in hours. Insets show overlapping peak concentration-time curves for the well-stirred tank model with $\lambda_{well-stirred}$ and the flow-limited two-subcompartment model with $\lambda_{biophys}$.

Figure 3. PBPK model structure

Diazepam PBPK model structure has 12 tissue compartments and 2 blood compartments (arterial side and venous side of circulation) as implemented by Gueorguieva et al. [19]. Cotinine simulations use the same model structure.

Figure 4. Simulated concentration-time curves using *a priori* predicted partition coefficients for diazepam

Plasma, adipose, brain, heart, kidney, liver, lung, muscle, skin, splanchnic, stomach, and testes concentration-time curves are simulated using *a priori* predicted partition coefficients based on the method of Poulin et al. [4] with the correction by Berezhkovskiy [24]. Well-stirred tank (solid black lines) and flow-limited two-subcompartment (dashed gray lines) model simulations are plotted against pharmacokinetic data (open circles) in the rat from Gueorguieva et al. [20]. Diazepam partition coefficient values are reported in Table 2, and AUC values are reported in Table 3.

Figure 5. Simulated concentration-time curves using well-stirred and flow-limited two-subcompartment model-optimized partition coefficients for diazepam

Lower fractional blood volume tissues (adipose, brain, muscle, skin, splanchnic, stomach, and testes), higher fractional blood volume tissues (heart, kidney, liver, lung), and plasma concentration-time curves are simulated using optimized partition coefficients. The well-stirred model is used to optimize diazepam well-stirred partition coefficients, and the flow-limited two-subcompartment model is used to optimize diazepam biophysical partition coefficients (Table 2). Well-stirred tank (solid black lines) and flow-limited two-subcompartment (dashed gray lines) model simulations are plotted against pharmacokinetic data (open circles) in the rat from Gueorguieva et al. [20]. AUC values are reported in Table 3.

Figure 6. Simulated concentration-time curves using *a priori* predicted partition coefficients for cotinine

Adipose, brain, heart, kidney, liver, lung, muscle, and splanchnic concentration-time curves are simulated using *a priori* predicted partition coefficients based on the method of Poulin et al. [4] with the correction by Berezhkovskiy [24]. Well-stirred tank (solid medium gray lines) and flow-limited two-subcompartment (dashed dark gray lines) model simulations are plotted against rat pharmacokinetic data from Gabrielsson, et al. [27] (filled dark gray circles, mean; vertical bars, range). Plasma concentration-time curves are also plotted in each tissue panel (well-stirred, solid light gray lines; flow-limited two-subcompartment, dashed medium gray lines) against rat plasma time course data (filled light gray squares, mean; vertical bars, range). Partition coefficient values for cotinine are reported in Table 4, and AUC values are reported in Table 5.

Figure 7. Simulated concentration-time curves for the well-stirred tank and flow-limited two-subcompartment model using well-stirred model-optimized and flow-limited two-subcompartment model-calculated partition coefficients for cotinine

Lower fractional blood volume tissues (adipose, brain, muscle, splanchnic) and higher fractional blood volume tissues (heart, kidney, liver, lung) are simulated (well-stirred tank, solid light gray lines; flow-limited two-subcompartment, dashed dark gray lines) and plotted against rat pharmacokinetic data from Gabrielsson, et al. [27] (filled dark gray circles, mean; vertical bars, range). The well-stirred model is used to optimize cotinine well-stirred partition coefficients, and cotinine biophysical partition coefficients are calculated from optimized well-stirred partition coefficients based on Eq.18 (Table 4). Plasma concentration-time curves are also plotted in each panel (well-stirred, solid light gray lines; flow-limited two-subcompartment, dashed medium gray lines) against rat plasma time course data (filled light gray squares, mean; vertical bars, range). AUC values are reported in Table 5.

Figure 8. Relationship between the biophysical and well-stirred partition coefficients at a set fractional blood volume

A,B) The fractional blood volume is arbitrarily set to 0.36 (rat lung). The shaded gray areas indicate the biophysical partition coefficient is <1 . A) The ratio of $\lambda_{well-stirred}$ to $\lambda_{biophys}$ versus $\lambda_{biophys}$. The ratio is always >1 when $\lambda_{biophys}$ is <1 . When $\lambda_{biophys}$ is >1 , the upper and lower dashed lines are the bounds on the ratio of $\lambda_{well-stirred}$ to $\lambda_{biophys}$ for a given fraction blood volume (FBV). B) The percent difference between $\lambda_{biophys}$ and $\lambda_{well-stirred}$ versus $\lambda_{biophys}$. The percent difference is always negative when $\lambda_{biophys}$ is <1 . When $\lambda_{biophys}$ is >1 , the upper and lower dashed lines are the bounds on the percent difference for a given fraction blood volume (FBV).

Table 1

Physiological parameter values for a 250 g rat

Tissue	Rat ^a		Fractional Blood Volume ^b (FBV)	
	Flow	Weight	mean	range
	ml/min	g		
Adipose	2.55	10	0.02(h)	0.02–0.03(h)
Brain	0.78	1.2	0.03(r), 0.04(h)	0.02–0.04(r), 0.03–0.1(h)
Heart	4.2	1	0.26(r)	-
Kidney	16.61	2	0.16(r), 0.36(h)	0.11–0.27(r), 0.22–0.5(h)
Liver	3.55	11	0.21(r), 0.11(h)	0.12–0.27(r)
Lung	80	1.2	0.36(r)	0.26–0.52(r)
Muscle	16.25	125	0.04(r), 0.01(h)	0.01–0.09(r)
Skin	7.1	43.8	0.02(r), 0.08(h)	-
Splanchnic	20.25	15	0.035(r) ^c	-
Stomach	1.9	1.1	0.035(r) ^c	-
Testes	1.9	2.5	0.017(r) ^c	-
Remainder	4.91	15.8	0.05 ^d	-
Venous	80	13.6	-	-
Arterial	80	6.8	-	-
Whole Body	80	250	-	-

^aGueorguiev a, et al. [20]^bBrown, et al. [17], r-rat, h-human^cestimated from Everett, et al. [25], r-rat^dassumed

Table 2

Partition coefficients and clearance for diazepam case study

Tissue	FBV ^a	Partition Coefficients for Diazepam ^b		
		λ_{apriori}	λ_{biophys}	$\lambda_{\text{well-stirred}}$
Adipose	0.02	15.2	23.1	23.1
Brain	0.03	7.1	2.2	2.2
Heart	0.26	2.5	5.4	4.3
Kidney	0.16	2.9	5.7	5.2
Liver	0.21	3.1	9.7	9.1
Lung	0.36	3.5	7.3	5.3
Muscle	0.04	1.9	2.5	2.4
Remainder ^c	0.05	4.9	16.0	16.5
Skin	0.02	3.9	3.1	3.2
Splanchnic	0.035	4.4	4.1	4.3
Stomach	0.035	4.9	4.5	4.5
Testes	0.017	4.9	4.6	4.7
CL _{INT} (ml/min)				
Clearance		83 ^d	374 ^e	422 ^e

^aMean rat fractional blood volumes (FBV) from Table I^b λ_{apriori} is the *a priori* predicted tissue:plasma partition coefficients for diazepam estimated using the method of Poulin et al. [4] with the correction by Berezhkovskiy [24]; λ_{biophys} is the biophysical partition coefficient between the two subcompartments; $\lambda_{\text{well-stirred}}$ is the well-stirred partition coefficient of the single compartment well-stirred tank; the partition coefficients, λ_{biophys} and $\lambda_{\text{well-stirred}}$, are determined via optimization^cStomach and testes compartments are given the same λ_{apriori} partition coefficient values as the remainder ($\lambda_{\text{apriori}} = 4.9$)^dPredicted intrinsic clearance from Poulin, et al. [5]^eOptimized intrinsic clearance value corresponding to each given set of optimized partition coefficients

Table 3
Area under the curve (AUC) for diazepam simulations with the flow-limited two-subcompartment and well-stirred tank models

Tissue	AUC for Diazepam ^d					
	FLT ($\lambda_{apriori}$) ^b ($\times 10^{10}$)	WST ($\lambda_{apriori}$) ^c ($\times 10^{10}$)	%diff ^d	FLT-opt ($\lambda_{biophys}$) ^e ($\times 10^{10}$)	WST-opt ($\lambda_{well-stirred}$) ^f ($\times 10^{10}$)	%diff ^g
Adipose	14.0	14.3	-1.9	9.90	9.70	2.1
Brain	4.93	5.09	-3.4	0.50	0.48	3.2
Heart	1.35	1.61	-19.3	0.94	0.92	2.5
Kidney	1.66	1.86	-12.5	1.10	1.08	1.5
Liver	1.19	1.40	-17.6	0.58	0.59	-1.9
Lung	1.67	2.26	-35.7	1.09	1.09	0.2
Muscle	1.39	1.42	-2.6	0.71	0.68	3.7
Remainder	3.55	3.73	-5.1	6.02	6.37	-5.9
Skin	3.05	3.11	-2.0	0.92	0.91	0.6
Splanchnic	2.83	2.93	-3.5	0.91	0.95	-4.0
Stomach	3.16	3.27	-3.6	1.00	0.98	2.6
Testes	3.34	3.41	-2.0	1.10	1.08	1.5
Plasma (art) ^h	0.64	0.64	-0.7	0.22	0.21	5.0
Plasma (ven) ^h	0.64	0.64	-0.6	0.22	0.21	5.0

^a Area under the curve (AUC) for time courses plotted in Figs. 4 and 5.

^b AUC values based on using diazepam $\lambda_{apriori}$ values in the flow-limited two-subcompartment (FLT) model

^c AUC values based on using diazepam $\lambda_{apriori}$ values in the well-stirred tank model (WST)

^d Percent difference in AUC between FLT and WST models using $\lambda_{apriori}$ calculated as [(AUC_{FLT} - AUC_{WST})/AUC_{FLT}] $\times 100$

^e AUC values based on using diazepam $\lambda_{biophys}$ optimized with the flow-limited two-subcompartment model

^f AUC values based on using diazepam $\lambda_{well-stirred}$ optimized with the well-stirred tank model

^g Percent difference in AUC between FLT and WST models using optimized partition coefficients calculated as [(AUC_{FLT} - AUC_{WST})/AUC_{FLT}] $\times 100$

^h Arterial compartment (art) and venous compartment (ven)

Table 4

Partition coefficients and clearance for cotinine case study

Tissue	FBV ^a	Partition Coefficients for Cotinine ^b		
		λ_{apriori}	$\lambda_{\text{biophys}}^c$	$\lambda_{\text{well-stirred}}^d$
Adipose	0.02	0.61	0.02	0.04
Brain	0.03	0.87	0.25	0.27
Heart	0.26	0.82	0.03	0.28
Kidney	0.16	0.82	0.67	0.72
Liver	0.21	0.76	0.23	0.39
Lung	0.36	0.83	0.001*	0.30
Muscle	0.04	0.79	0.26	0.29
Remainder ^e	0.05	0.78	0.14	0.18
Splanchnic	0.035	0.79	0.28	0.31
CL _{INT} (ml/min)				
Clearance		0.21	0.13 ^f	0.13 ^g

^aMean rat fractional blood volumes (FBV) from Table I^b λ_{apriori} is the *a priori* predicted tissue:plasma partition coefficients for cotinine estimated using the method of Poulin et al. [4] with the correction by Berezhkovskiy [24]; λ_{biophys} is the biophysical partition coefficient between the two subcompartments; $\lambda_{\text{well-stirred}}$ is the well-stirred partition coefficient of the single compartment well-stirred tank.^cThe λ_{biophys} partition coefficients are calculated from optimized $\lambda_{\text{well-stirred}}$ values using Eq.18 with the partition coefficient for lung being set arbitrarily low (indicated by *)^dThe $\lambda_{\text{well-stirred}}$ partition coefficients are determined via optimization^eSkin, stomach, and testes were kept as compartments in the model but were given the same respective partition coefficient value as the remainder ($\lambda_{\text{apriori}} = 0.78$, $\lambda_{\text{biophys}} = 0.14$, or $\lambda_{\text{well-stirred}} = 0.18$)^fWell-stirred tank-optimized intrinsic clearance value is used in flow-limited two-subcompartment simulations^gWell-stirred tank-optimized intrinsic clearance value

Table 5

Area under the curve (AUC) for cotinine simulations with the flow-limited two-subcompartment and well-stirred tank models

Tissue	AUC for Cotinine ^a					
	FLT ($\lambda_{apriori}^b$) ($\times 10^{11}$)	WST ($\lambda_{apriori}^c$) ($\times 10^{11}$)	%diff ^d	FLT-calc ($\lambda_{biophys}^e$) ($\times 10^{11}$)	WST-opt ($\lambda_{well-stirred}^f$) ($\times 10^{11}$)	%diff ^g
Adipose	3.31	3.25	1.7	0.23	0.23	-0.1
Brain	4.68	4.63	0.9	1.77	1.77	-0.1
Heart	4.63	4.36	5.8	1.85	1.86	-0.1
Kidney	4.53	4.36	3.8	4.76	4.76	-0.1
Liver	4.29	4.01	6.7	2.56	2.57	-0.1
Lung	4.76	4.41	7.3	2.37	2.00	15.7
Muscle	4.29	4.23	1.5	1.89	1.89	-0.1
Remainder	4.24	4.16	1.8	1.21	1.21	-0.1
Splanchnic	4.26	4.20	1.4	2.03	2.04	-0.1
Plasma (art) ^h	5.34	5.32	0.5	6.58	6.59	-0.1
Plasma (ven) ^h	5.34	5.32	0.5	6.58	6.59	-0.1

^a Area under the curve (AUC) for time courses plotted in Figs. 6 and 7.

^b AUC values based on using cotinine $\lambda_{apriori}$ values in the flow-limited two-subcompartment model (FLT)

^c AUC values based on using cotinine $\lambda_{apriori}$ values in the well-stirred tank model (WST)

^d Percent difference in AUC between FLT and WST models using $\lambda_{apriori}$ calculated as $[(AUC_{FLT} - AUC_{WST})/AUC_{FLT}] \times 100$

^e AUC values based on using calculated cotinine $\lambda_{biophys}$ (calculated from optimized $\lambda_{well-stirred}$ values using Eq.18)

^f AUC values based on using optimized cotinine $\lambda_{well-stirred}$ (optimized with the well-stirred tank model)

^g Percent difference in AUC between FLT and WST models calculated as $[(AUC_{FLT} - AUC_{WST})/AUC_{FLT}] \times 100$

^h Arterial compartment (art) and venous compartment (ven)