The Constraints, Construction and Verification of a Strain-Specific Physiologically-Based Pharmacokinetic Rat Model

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Title Page:

The Constraints, Construction and Verification of a Strain-Specific Physiologically-Based Pharmacokinetic Rat Model

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Running Title Page

Running title: Simcyp Rat a PBPK IVIVE model

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Abstract

The use of in vitro-in vivo extrapolation (IVIVE) techniques, mechanistically incorporated within Physiologically-Based Pharmacokinetic (PBPK) models, can harness in vitro drug data and enhance understanding of in vivo pharmacokinetics. This study’s objective was to develop a user-friendly rat (250g, male Sprague-Dawley) IVIVE linked PBPK model. A 13-compartment PBPK model including mechanistic absorption models was developed, with required system data (anatomical, physiological and relevant IVIVE scaling factors) collated from literature and analysed. Overall, 178 system parameter values for the model are provided. This study also highlights gaps in available system data required for strain-specific rat PBPK model development. The model’s functionality and performance was assessed using prior literature sourced in vitro properties for diazepam, metoprolol and midazolam. The results of simulations were compared against observed pharmacokinetic rat data. Predicted and observed concentration profiles in 10 tissues for diazepam after a single i.v. dose making use of either observed i.v. clearance (CL_{iv}) or in vitro hepatocyte intrinsic clearance (CL_{int}) for simulations generally led to good predictions in various tissue compartments. Overall, all intravenous (i.v.) plasma concentration profiles were successfully predicted. However, there were challenges in predicting oral plasma concentration profiles for metoprolol and midazolam, the potential reasons and according solutions are discussed.

Keywords: Preclinical Pharmacokinetics, Physiological Model, Simulations, Clearance, Absorption, Distribution.
Introduction

In drug discovery numerous *in vitro* assays are routinely performed with new chemical entities (NCE’s) in order to characterize absorption, distribution, metabolism and excretion (ADME) properties. Such studies can include; dissolution tests, permeability assays, tissue-based experiments utilizing pre-clinical and human tissues and the use of recombinant systems, where specific enzymes and transporters are expressed. These data permit informed decisions as to the viability of an NCE to proceed to the next stage of the development pipeline. Data generated using these systems also has the potential to enable the quantitative prediction of the rate and extent of drug absorption and disposition in the intact organism. This is true whether it is human or a commonly used pre-clinical species such as the rat, dog, mouse and monkey.

Due to limited availability of human tissues, *in vitro* assays utilizing rat blood, plasma and tissues are routinely performed to characterize drug-tissue distribution, metabolic stability and toxicology of an NCE. At the drug discovery stage, in addition to *in vitro* assays, *in vivo* studies in pre-clinical species are also carried out to assess relationships between pharmacokinetics (PK), toxicokinetics (TK) and pharmacodynamics (PD). *In vivo* rat studies are often used to define dosages for first-in-human studies, and to characterize metabolite safety. However this may not always lead to a successful predictive outcome.

**Abbreviations:** ADAM (Advanced Dissolution Absorption & Metabolism (model)); AP (Acid phospholipids); B:P (Blood to Plasma ratio); CLint (intrinsic clearance); CLiv (Intravenous clearance); CLR (Renal Clearance); CLuHunt (unbound hepatic intrinsic clearance); EG (fraction of drug extracted/metabolised in the gut); EH (fraction of drug extracted/metabolised in the liver); EW (Extracellular Water); fu (fraction unbound in plasma); FG (Fraction of drug escaping gut metabolism); fHu (fraction of drug unbound in the enterocyte/gut); FH (Fraction of drug escaping hepatic metabolism); GI (Gastrointestinal); HPGL (Hepatocytes Per Gram of Liver); IW (Intracellular Water); IVIVE (*In Vitro – In Vivo Extrapolation*); KP,ALB (tissue-to-plasma Albumin ratio); KP,LPP (tissue-to-plasma Lipoprotein ratio); KP (tissue-to-plasma partition coefficient); MPPGI (Microsomal Protein Per Gram of Intestine); MPPGL (Microsomal Protein Per Gram of Liver); NL (Neutral Lipids); NP (Neutral Phospholipids); PBPK (Physiologically-Based Pharmacokinetic); Qill (Villus blood flow); CLperm (Clearance Permeability); Pat (effective jejunal permeability in rat); RIM (Rat Intestinal Microsomes); RIMe (Rat Intestinal Microsomes obtained by elution); RLM (Rat Liver Microsomes); RHep (Rat Hepatocytes); SEV (Surface Epithelial Volume); Vss (Volume of distribution at Steady State); WX (Weighted Mean)
when extrapolating from pre-clinical species to human via simple allometry, particularly if a metabolite unique to humans is identified.

A ‘bottom-up’, *in vitro - in vivo* Extrapolation (IVIVE) linked to whole body Physiologically-based Pharmacokinetic (PBPK) modelling approach acts as a cohesive mechanistic framework to harness and integrate *in vitro* data to predict drug disposition in a ‘virtual human population’. Using an IVIVE approach ‘drug’ data are used in conjunction with ‘system’ parameters *i.e.* the demographics, anatomy, physiology and genetics of the human individual or population along with ‘trial design’ data, *i.e.* dosage regimen, to predict the fate of a drug in a physiologically relevant ‘virtual human’. A challenge in drug discovery is that there is little or no *in vivo* human data to gauge the success of the IVIVE approach when employing human *in vitro* assays. Therefore, one approach is to gain confidence in an IVIVE/PBPK strategy through successful modelling of drug PK in a pre-clinical species such as the rat. The scope of employing data generated in discovery using an IVIVE rat PBPK model framework has been demonstrated, and the utility of rat PBPK in a drug discovery lead optimization has also been reported.

The heightened application of mechanistic IVIVE-PBPK in the pharmaceutical industry, together with the increasing acceptance or even requesting of submissions utilizing PBPK models, has led to a growing scrutiny on the ‘drug’- and ‘system’-specific parameters used to construct and verify the PBPK model. Recent regulatory guidance has been drafted that provides specific advice on the qualification and reporting of PBPK application in regulatory submissions. It is clear that sponsors, in conjunction with any providers of specialised PBPK tools should be in a position to provide suitable justification of the parameters employed to facilitate model qualification. The provision of robust, well qualified, system parameters is critical in this regard. Any alterations to ‘system’ parameters
made arbitrarily, could render it more challenging to qualify the models when incorporated into a regulatory submission.

Herein, we describe the Simcyp Rat Simulator (Version 12, Release 2); a generic whole body PBPK-IVIVE simulator principally designed for use in the drug discovery and toxicology setting. The rationale for undertaking the development and verification of the model will be described. The model structure and parameters are provided including physiologically-based system parameters, IVIVE scaling approaches, algorithm and differential equations that predict the relevant PK parameters, in order to facilitate qualification of PBPK models during regulatory submission, as well as determine temporal drug exposures within the tissues implemented within the rat model. The utility of the model will be explored and model performance verification will be detailed using in vitro and in vivo drug data collated from the literature.
Materials & Methods

The Model Framework

The aim of the model is to predict rat PK parameters and plasma and tissue concentration-time profiles by combining *in vitro* compound data with trial design regimes within a physiological framework. The model is based on an average 250g Sprague-Dawley rat. Laboratory rats are inbred and are used experimentally within narrow weight ranges (approximately 250g) to reduce experimental variability, thus one rat is assumed to differ insignificantly from another rat, unlike humans.

The model is based upon the free-drug hypothesis using well-stirred and perfusion limited assumptions, with the exception of the gastro-intestinal (GI) tract for which the permeability-limited, Advanced Dissolution Absorption and Metabolism (ADAM) model can be used. The PBPK model contains 13 compartments (adipose, bone, brain, gut, heart, kidney, liver, lung, muscle, skin, spleen, plasma and red blood cells), linked by blood flows (arterial and venous compartments), where ordinary differential equations govern the drug-tissue ‘mass balance’ allowing derivation of tissue specific drug concentration-time profiles. The model structure is similar to widely published PBPK models (with exceptions explained below) and the non-eliminating tissue concentration profiles are described as follows:

\[
\frac{dC_T}{dt} = \frac{Q_T}{V_T} \left( C_{ab} - \frac{C_T}{K_{p,T}} \cdot \frac{K_{p,T}}{B:P} \right)
\]

(1)

where \(V_T\) is the tissue volume, \(C_T\) is the drug concentration in the tissue, \(Q_T\) is the tissue blood flow and \(C_{ab}\) is the drug concentration in the arterial blood, \(K_{p,T}\) is the tissue to plasma partition coefficient and B:P is the blood to plasma concentration ratio of the drug.

The well-stirred liver differential equation is as follows:
\[
\frac{dC_{liver}}{dt} = \frac{1}{V_{liver}} \left[ \left( Q_{liver} - Q_{pv} \right) C_{ah} + Q_{pv} C_{pv} - \left( \frac{Q_{liver}}{K_{P_liver}} \frac{CL_{int\,Hep}}{B: P} + \frac{fu}{B: P} \right) C_{liver} \right]
\]

(2)

Where liver, pv, and ab subscripts refer to the liver, portal vein and arterial blood respectively. \(C, Q\) and \(V\) represent drug concentration, blood flow and tissue volume respectively, \(K_{P_liver}\) is the liver to plasma partition coefficient, \(fu\) is the fraction unbound in plasma, and \(CL_{int\,Hep}\) is the total hepatic intrinsic clearance (either as an intrinsic clearance (\(CL_{int}\)) or \(V_{max}/K_m\)).

The results of a simulation are dependent on the drug, system and trial parameters independently defined and entered into the model prior to running the simulation (see Table 1).

**System Parameters**

Analysis of a random selection of ten *in vivo* and ten *in vitro* rat pharmacokinetic studies published in the journal ‘Drug Metabolism and Disposition: the biological fate of chemicals’ from years 2005 – 2008 found that the Sprague-Dawley rat was the most commonly used strain with 75% (15/20) studies assessed utilizing this strain. It was therefore endeavored to build the model based on the Sprague-Dawley strain. Where physiological and anatomical system data were available for the Sprague-Dawley rat in the literature, these values were preferentially selected (c.f. data from other strains). In the absence of Sprague-Dawley-specific information, values from another rat strain were used. Original reports were used where possible rather than consensus/review articles (e.g., Davies & Morris\(^\text{18}\)). Using this approach, it was possible to scrutinize the materials, methods, results and study conclusions allowing informed decisions on elements such as rat size and strain. It also allowed for the correction of parameters to the standard rat weight (*i.e.* 250g) used in the
model, and the potential for removal of any error or ‘misquotation’ that might occur if citing from a secondary source. Where data from more than one study were available for a given parameter, a weighted mean value (WX) was calculated (3).

\[
WX = \frac{\sum_{j=1}^{J} (n_j \bar{x}_j)}{\sum_{j=1}^{J} n_j}
\]

(3)

where \(n_j\) is the number of reported studies and \(x_j\) is the mean value of the \(j^{th}\) study.

**Compound Parameters**

The model requires commonly used physicochemical and blood/plasma binding parameters such as molecular weight, octanol/buffer partition coefficient (log\(P_{o:w}\)), compound type (acid/base/ampholyte/neutral) and related pKa value(s), blood-to-plasma (B:P) partition ratio and fraction unbound in plasma (\(f_u\)).

The model can also handle a simple single compartment absorption model and the more advanced ADAM model. The former consists of a single well-stirred compartment characterized by an absorption rate constant (\(k_a\)) and the fraction of dose leaving the gut lumen (\(f_a\)). The latter is a physiologically-based, multi-compartmental model, where the drug is handled as unreleased (from formulation), undissolved or dissolved state; dissolution rate can be modeled along with supersaturation and precipitation \(^{17}\). There are nine gastrointestinal (GI) compartments viz. the stomach, seven small intestinal compartments and the colon. The rat small intestine comprises a single duodenal compartment, four jejunum compartments and two ileum compartments. The greater anatomical length of the jejunum as a proportion of the total small intestinal length in rats necessitates that the rat intestinal compartment structure is different to that of the human model \(^{17}\). For reference, the human jejunum is represented by two compartments, with the relatively longer ileum comprising
four compartments. In both the first order and ADAM models, $k_a$ can be predicted from effective jejunal permeability ($P_{\text{eff}}$) using (4)

$$k_a = \frac{2P_{\text{eff, rat}}}{R} \quad (4)$$

where $P_{\text{eff, rat}}$ is the effective jejunal permeability in rat ($10^{-4}$ cm/s) and $R$ (cm) is the radius of the small intestine. This $k_a$ can then be utilised in the prediction of $f_a$.

$P_{\text{eff, rat}}$ can be predicted from $P_{\text{eff, man}}$ (effective jejunal permeability in human) based upon a simple direct correlation between human and rat jejunal permeability described by Cao et al. $^{19}$ – verapamil, an outlier, was excluded by these authors from this relationship. There is a mistake in the reported equation, and Equation 5 in this article is the corrected version implemented in the model.

$$P_{\text{eff, rat}} = 0.6362 \times P_{\text{eff, man}}^{0.542} \quad (5)$$

Where both $P_{\text{eff, man}}$ and $P_{\text{eff, rat}}$ have the unit of $10^{-4}$ cm/sec. $P_{\text{eff, man}}$ can be predicted from $P_{\text{app}}$ (in vitro apparent permeability) measured using the in vitro cell monolayer systems MDCK-II and Caco-2 (pH 6.5–7.4), Parallel Artificial Membrane Permeability Assay (PAMPA) or the molecular descriptors polar surface area and hydrogen bond donor number.

The $Q_{\text{Gut}}$ model $^{20}$, is used with the first order absorption model to predict the extent of intestinal gut wall metabolism ($F_g$). This model is based upon the balance between whole gut $\text{CL}_{\text{int}}$, and a hybrid term ($Q_{\text{gut}}$) based upon villous blood flow ($Q_{\text{villi}}$) and a clearance permeability term ($\text{CL}_{\text{perm}}$) (6).

$$Q_{\text{gut}} = \frac{\text{CL}_{\text{perm}}Q_{\text{villi}}}{\text{CL}_{\text{perm}} + Q_{\text{villi}}} \quad (6)$$
where \( \text{CL}_{\text{perm}} \) is clearance permeability and its calculation is based on the effective permeability and the cylindrical surface area of the relevant GI tract \(^{20}\) and \( Q_{\text{villi}} \) is villus blood flow.

To describe the distribution of the compound, the steady state volume of distribution \((V_{ss})\) obtained from in vivo studies can be defined within the model. Alternatively, \( V_{ss} \) can be predicted by one of two mechanistic methods; The Poulin & Theil method \(^{21}\), as corrected by Berezhkovskiy \(^{22}\), or the Rodgers and Rowland method \(^{23-25}\). Both of these models use tissue composition and compound physicochemical data to calculate \( K_{p,T} \) values and thence, in conjunction with tissue volumes, \( V_{ss} \). The framework can handle two distribution models, namely a minimal “lumped” PBPK model and a full PBPK model. In the case of the minimal model, the gut, portal vein and liver are separate compartments with all other organs lumped together in a single compartment (systemic compartment), therefore it is not a solely empirical single compartment model, but is limited in complexity compared to the full PBPK model \(^{26}\). In order to use the full PBPK model the \( K_{p,T} \) values must be provided (either as measured or predicted values). This full PBPK model is comprised of thirteen compartments, with a number of differential equations describing the distribution of the drug into these tissues. These equations also describe the residence of the drug within each tissue, and its eventual return to the systemic circulation. These equations (1) and (2) have been described previously.

Depending on the availability of the data elimination within the model can be predicted via several options; input of an in vivo intravenous \((i.v.)\) or oral \((p.o.)\) clearance, plus the percentage of metabolite formed from in vivo studies, enabling the definition of concentration-time profiles of the metabolite; or from in vitro assays determining \( \text{CL}_{\text{int}} \). For an IVIVE strategy, unbound hepatic clearance \((\text{CL}_{\text{intH}})\) is predicted by rat liver microsome (RLM), or hepatocyte (R Hep) unbound intrinsic clearances \((\text{CL}_{\text{int}})\), which is scaled up via
rat microsomal protein per gram of liver (MPPGL, see Supporting Information, Table G), or via rat hepatocellularity (hepatocytes per gram of liver, (HPGL), Supporting Information, Table G), as shown in Equations 7 and 8, respectively.

$$\text{CL}_{\text{u int, H}} = \frac{\text{CL}_{\text{int}}}{\text{fu}_{\text{mic}}} \times \text{MPPGL} \times W_L$$

(7)

$$\text{CL}_{\text{u int, H}} = \frac{\text{CL}_{\text{int}}}{\text{fu}_{\text{inc}}} \times \text{HPGL} \times W_L$$

(8)

where $\text{fu}_{\text{mic}}$ is the fraction of drug unbound in microsomes, $\text{fu}_{\text{inc}}$ is the fraction of drug unbound within an incubation and $W_L$ is liver weight.

The predicted $\text{CL}_{\text{u int, H}}$ value is incorporated into well-stirred model in order to predict $F_H$ and hepatic clearance ($\text{CL}_{H}$)\textsuperscript{27}.

For prediction of unbound gut intrinsic clearance ($\text{CL}_{\text{u int,G}}$), rat intestinal microsomes are scaled via microsomal protein per gram of rat intestine (Equation 9; for details of MPPGI, see Supporting Information, Table G).

$$\text{CL}_{\text{u int,Gut}} = \frac{\text{CL}_{\text{int}}}{\text{fu}_{\text{mic}}} \times \text{MPPGI} \times \sum_{i=1}^{7} (\text{wet wt.} \times L_i \times \text{c.f})$$

(9)

Where wet wt. is the wet weight of the intestinal ($i$) segment (g/cm), where there are 7 small intestinal segments, $L_i$ is the length of the intestinal segment, $\text{c.f}$ is the correction factor accounting for differences in metabolic capacity of intestinal segments. For the purposes of this study both the in vitro data and required scaling factors were obtained via elution methods, so no additional correction factors are required.

The $\text{c.f}$ term in equation 9, depicting the region-specific gut metabolic capacity (see Supporting Information, Table G) is not compound specific but reflects the differential distribution of CYP450 enzymes along the small intestine of the rat. To estimate the fraction of drug escaping gut wall metabolism ($F_g$) the ‘$Q_{Gut}$’ model, $\text{fu}_{\text{gut}}$ and $\text{CL}_{\text{u int,Gut}}$ are incorporated into a well-stirred equation of intestinal metabolism\textsuperscript{20} or the ADAM model is
used. There are also options for assigning additional unbound hepatic $\text{CL}_{\text{int}}$, a multiplicative scaling factor accounting for active uptake into hepatocytes, a non-metabolic renal clearance ($\text{CL}_{\text{R}}$) and an additional systemic clearance. The model contains similar features for metabolites to those for substrates.

**Drug Parameter Inputs**

For rat IVIVE, drug-specific data routinely generated in the development process are required. Ten substrate compounds were developed. For one compound, caffeine, the active metabolite paraxanthine is available. The minimum data required for IVIVE compound development are: compound type (acid, base etc.) and associated pKa values, log$\text{P}_{\text{o:w}}$, fraction of drug unbound in rat plasma ($f_{\text{u}}$), blood-to-plasma ratio (B:P), an *in vitro*-derived hepatic $\text{CL}_{\text{int}}$ (rat liver microsomes or hepatocytes) and an estimate of $V_{\text{ss}}$. In oral dosing simulations the unbound fraction in enterocyte ($f_{\text{gut}}$) of 1 was used. To permit the verification of model performance the *i.v.* (or *p.o.*) clearance and $V_{\text{ss}}$ are required from *in vivo* rat studies. The relative paucity of rat compound data compared to that published for humans limits the number of compound files that can be developed, verified and provided as examples.

**Trial Design**

Information on the design of the rat study can be incorporated into the model including the route and frequency of dosing, sampling duration and whether the drug is administered with food.

**Performance Verification**

Model performance verification was undertaken with Simcyp Rat (Version 12 Release 2) where simulated time courses were compared to studies available in the literature.
Diazepam concentration-time profiles were available in multiple tissues following *i.v.* administration \(^{28}\). Plasma concentration-time profiles were available for metoprolol \(^{29}\) and midazolam \(^{30}\) following both *i.v.* and *p.o.* administration. Observed concentration-time profile data from *in vivo* PK studies was extracted by graphical digitization techniques (Get Data Graph Digitizer, http://www.getdata-graph-digitizer.com). IVIVE simulations for all compounds used combinations of physicochemical descriptors, permeability assays, *in vitro* hepatocyte and microsomal assay data. The associated references are provided in the Supporting Information, Tables H-J. Clearances were calculated using Equation 10.

\[
\text{Clearance} = \frac{\text{Dose}}{\text{AUC}}
\]

where AUC is the area under the concentration-time curve.
Results

System Parameters

In order to construct the model, 178 anatomical and physiological ‘system parameters’ were collected from the literature; the final values originated from 42 references (including a personal communication). The system parameter values, the strain of rat from which the values were obtained, and source references are provided in the Supporting Information, Tables A-G. Of the system parameter values (excluding tissue composition parameters due to data complexity, see ‘Results - Tissue Composition Parameters’ below for further detail) 36/93 (39%) were taken from Sprague-Dawley rat measurements, 40/93 (43%) were measured from Wistar rats, with a large proportion, 31/51 (61%) of parameters specific to the gastrointestinal tract and ADAM model, being derived from experimental measurements in this strain. The remaining values (17/93, 18%) were obtained from one of the following categories: a strain of rat other than Sprague-Dawley or Wistar; the strain was not known; the value was scaled from human, or a value was calculated based upon other parameters, due to the lack of experimental values. These data highlight the challenges associated in building a strain-specific rat model, particularly in relation to the paucity of applicable GI parameters available in the literature for the Sprague-Dawley strain. If an i.v. dosing strategy is used for a simulation, i.e. not requiring employment of GI/ADAM parameters, a model with greater inclusivity of Sprague-Dawley ‘systemic’ parameters, 29/42 (69%) is utilised, with a lower proportion of Wistar parameters 9/42 (21%).

Tissue volumes

The volumes of 13 tissues were collected from 8 articles, where 7 values were obtained from studies in Sprague-Dawley rats (see Supporting Information, Table A). All tissue volumes were corrected to a 250g standard rat weight, assuming linear scaling from the
weight of the rat used in the study. Corrections for weight were required for 6 studies. Where possible, data for tissue volumes were obtained from original studies rather than consensus articles, however the volume of bone (16.47 mL) was obtained from a study where the parameter was not directly measured and cannot be traced to an original article. Therefore, the veracity of this value can be questioned, leading to uncertainty surrounding this model parameter. The 16.47 mL bone volume was used in the absence of verifiable data from an original study.

**Tissue blood flows & cardiac output**

The blood flows connecting the tissue compartments are based on the percentage of blood flow routed to each tissue from cardiac output and only studies where the animals were conscious or had recovered from an anaesthetic regimen (if anaesthesia was performed) were included. This is due to the potential haemodynamic effects of anaesthetic agents. It was possible to obtain Sprague-Dawley specific cardiac outputs from 8 studies \( (n = 99) \). A weighted mean analysis followed and lead to a weighted mean cardiac output of 80 mL/min/250g with each organ receiving a percentage of cardiac output (Supporting Information, Table B). All values except villus blood flow were measured in Sprague-Dawley rats. The villus blood flow is not a rat specific value and is a calculated parameter based on the villus receiving 60% of the small intestinal blood flow. The lung receives 100% of cardiac output therefore this value does not require further verification from the literature. Five of the parameters, heart, kidney, liver (arterial & portal vein) and spleen were obtained from multiple reference sources, therefore the parameter values are provided as weighted means. It is critical to obtain accurate estimates of liver blood flow, as drugs for which extraction is limited by perfusion are particularly sensitive to this parameter. In the model, a total liver blood flow of 19.4 mL/min/250g, equating to 24.2% of cardiac output, is utilized.
The haematocrit is important in defining the drug B:P threshold, as B:P cannot be less than 1 minus the haematocrit. The haematocrit value of 43.9% is taken from Wistar rats, although a Sprague-Dawley rat value was available \(^{31}\). As this value appears to be particularly high at 56%, the decision was taken to utilize the Wistar value. The splanchnic fed/fasted ratio is the final blood flow parameter required and is relevant to the modelling of the effects of food intake on drug absorption and disposition.

**Gastrointestinal parameters**

The anatomical and physiological GI system parameters are described in the Supporting Information, Table C. In the absence of rat-specific values, the total intestinal enterocyte volume was scaled from human values for the surface epithelial volume (SEV) per m\(^2\) of intestinal cylindrical surface area. The SEV term includes the additional surface area provided by the villi but excludes additional surface area related to the presence of plicae circulares, which are present in humans but not rats. This scaling approach assumes that the villus surface expansion is the same in rats as in humans.

The anatomical lengths and diameters of each small intestinal segment are required for allocating the appropriate dimensions to each of 7 small intestinal compartments in the ADAM model. Surprisingly, there are relatively few studies that specifically focus on determining the lengths of the duodenum, jejunum and ileum, as many studies measured the total small intestinal length. Kararli \(^{32}\) stated that the ileum length is 2.5-3.5 cm, however, an ileal length of 20cm (adult Wistar rat, personal communication, Dr. Emma McConnell, The School of Pharmacy, University of London) was implemented. Dr. McConnell also provided data relating to unpublished initial stomach fluid volumes in the fed and fasted state and fasted state gastric emptying time *via* personal communication. Basal small and large intestinal fluid volumes required for intestinal compartment fluid dynamics \(^{17}\), and stomach
and small and large intestinal pH were provided in published work from McConnell et al., 2008 (see Supporting Information for reference). Interestingly, rat stomach pH in the fasted state appears to be more alkaline than in the fed state, whereas observed values in humans show the opposite. Segmental blood flows ($Q_{villi}$ %) were measured in Sprague-Dawley male rats ($n=14$). However, the blood flows given in the study results required converting from a blood flow per gram of tissue to a percentage of total blood flow for each of the 7 small intestinal segments in the model (Supporting Information, Table C footnote). It is assumed that there are no differences in small intestinal transit rate through each intestinal segment therefore transit rates are calculated based on segmental lengths as a proportion of total small intestinal length (Supporting Information, Table C footnote). The transit values do not directly relate to absolute transit rates from the literature, i.e. rate of movement of luminal contents through each segment, as there are no appropriate data available for 250g Sprague-Dawley rats. The compartmental wet weights required for metabolic scaling in the small intestine (equations 7 and 8) are from the Wistar rat control group in Sha et al. 34.

Tissue composition parameters

All the tissue composition parameters in Supporting Information Tables D & E were sourced from publications by Rodgers and Rowland 23-25. However the parameter values provided were not directly measured within these studies, having been assembled from data available in the literature. These data are complex; with no one study summarizing the full composition of one tissue, and very few reporting one parameter for all required tissues. The data are therefore sourced from a number of different studies, each of which may contain details of total water, extracellular water, total lipids and/or individual lipids for specific tissues. These are then combined to calculate individual values for extracellular water (EW), intracellular water (IW), neutral lipids (NL), neutral phospholipids (NP) and acid
phospholipids (AP). If more than one reference was available for a value then a mean was taken; interested readers can refer to the original Rodgers et al. references. Where possible, original references and calculations were independently cross-checked, allowing a level of confidence in the cited data, and an indication of the strains used in determination of these values, as mentioned previously. Due to the nature of the data, no one tissue was determined fully from one strain of rat, although Sprague-Dawley and Wistar were the most commonly used.

At present, the tissue composition data required to predict $V_{ss}$ and $Kp_{T}$ values are almost exclusively determined from rat, with only minor exceptions, including the use of human plasma proteins in the determination of the tissue-to-plasma Albumin ratio ($Kp_{ALB}$) and the tissue-to-plasma Lipoprotein ratio ($Kp_{LPP}$). This is dissimilar to other species such as human, where data are sparse and a full complement of system parameter data are not available.

Similar to human, rat plasma and extracellular is pH 7.4, whereas the intracellular compartment of tissues (measured as whole body intracellular pH) and red blood cells are slightly more acidic at pH 6.9 and 7.27, respectively.

**Metabolic scaling factors**

Sprague-Dawley specific rat liver metabolic scalars MPPGL and HPGL were incorporated into meta-analyses that included 4 studies ($n = 29$) for HPGL, and 3 studies for MPPGL including 26 samples (Supporting Information, Table G), a considerably lower number of studies than those measured for human. When compared to an average 25 year old human male, Sprague-Dawley rat scalars are similar; with HPGL for rat of $107 \times 10^6$ compared to a human value of $117.5 \times 10^6$ cells/g liver, and a rat MPPGL of 46 mg/g, close to the 40 mg/g liver measured in humans. Also included in liver metabolic scaling is liver
density which was derived from a single study using 3 Sprague-Dawley rats. The GI-specific metabolic scalar MPPGI of 15.5 mg/g was derived from a single study of 6 rats (for references and derivation of the implemented values see Supporting Information, Table G). Included within the system parameters is the region-specific gut metabolic capacity, reflecting the differential distribution of cyp450 enzymes along the small intestine. Cyp450 absolute abundance data from 4 male Wistar rats was used, where the abundance of cyp2b1, cyp3a, cyp2c6 and cyp2d1 was assessed by immuno-blotting. The relative activity for the 4 jejunal segments is set to the maximum level of 1 with duodenum at 0.8 and the 2 ileal compartments having the lowest activity of 0.5. At present the model does not account for colonic metabolism therefore a scalar is not required for the large intestine.

Performance Verification

Concentration-time profiles following a single dose of diazepam (i.v.)

Predicted and observed concentration profiles in 10 tissues for diazepam after a single i.v. dose of 1.2 mg/kg are shown in Figure 1. Model inputs and the references associated to these simulations are provided in the Supporting Information, Table H. Using the observed i.v. clearance (CL_{iv}) for simulations lead to good predictions in various tissue compartments. Predictions based on in vitro hepatocyte CL_{int} data and Rodgers & Rowland V_{ss} predictions also provided a good agreement between predicted and observed profiles in most tissues, although there was a 2.1-fold under-prediction in plasma clearance (Dose/AUC). In the brain, GI and skin, the model over-predicted the tissue concentrations. The Rodgers & Rowland method predicts V_{ss} within 1.5-fold (5.2 L/kg observed vs. predicted 3.63 L/kg). It should be noted that the observed data were obtained from a pharmacokinetic study in Wistar rats.
Concentration-time profiles following a single dose of metoprolol (i.v. & p.o.)

Three doses of i.v. administered metoprolol (0.5, 1 & 2 mg/kg) were simulated and compared to observed data (Figure 2). Model inputs and the associated references are provided in the Supporting Information, Table I. For this compound, the original study reported AUC extrapolated to infinity, all simulations were extended to 72h, thus the clearance of the compound was complete (negligible concentration reported) and comparisons were made to the published values. For clarity, the figures show the comparisons of the simulated plasma concentration-time profile to the observed data, complete to the last observed data point. At all doses, a minimal PBPK model using an observed $V_{ss}$ did not adequately describe the observed disposition of metoprolol, as shown in Figure 2. Predictions using a full PBPK model generated profiles with good prediction at all doses. Full PBPK simulations using observed $CL_{iv}$ and IVIVE using RLM were undertaken demonstrating a reasonably good recovery of the observed plasma profiles. For the simulations with p.o. metoprolol given as solution (Figure 3), the aim was to identify if the incorporation of data generated early in the drug development pipeline (i.e. Caco-2 permeability, intestinal and liver microsomal data) could be used to predict metoprolol concentration-time profiles at 3 different doses (1, 2 and 5 mg/kg), using a simple first order absorption or the ADAM model. Intestinal permeability was predicted using Caco-2 $P_{app}$ data generating a $P_{eff, rat}$ of $1.54 \times 10^{-4}$ cm/sec and a predicted $f_a$ of 1. For 1 and 2 mg/kg doses using either the first order or ADAM model the clearance and AUC values are predicted within 2-fold. However, the $C_{max}$ for the first order method is under-predicted (0.4 - 0.41 – fold), while it is much better predicted for the ADAM model (0.69 - 0.72 fold). This under-prediction of $C_{max}$ could be linked to the 2.7-fold over-prediction of $V_{ss}$ (predicted value of 10.97 versus the observed value of 4.08). Data for intestinal and hepatic extraction ratios ($E_G$ and $E_H$, respectively) were provided for the observed study. The predicted $E_H$ of 0.41 is
reasonably close to the $E_H$ of 0.59 for the observed data. Compared to the observed $E_G$ (0.49) this parameter is predicted well for the ADAM model (0.46), however, there is a considerable over-prediction in $E_G$ for the first order simulation ($E_G$ 0.72). Adjusting the gastric emptying for simulations to 0.11 h in the ADAM gives more accurate predictions of $T_{\text{max}}$. This is to be expected, as the original study gave the dose via intra-duodenal administration and decreasing the gastric emptying time will correct for this to some extent. The observed data at 5 mg/kg metoprolol shows dose non-linearity (a greater than proportional AUC change with dose) which the model fails to capture in all simulation conditions (Figure 3C). The model is capable of accounting for non-linear or saturable kinetics for whole organ scaling methods. Simulations using the ADAM model allow the estimation of the enterocyte concentration of metoprolol in all gut segments. This information combined with knowledge of intestinal microsomal $K_m$ allows investigation as to whether if intestinal metabolism saturation is the likely cause of dose non-linearity. The $K_m$ estimated in intestinal microsomes from Yoon’s study 29 (24 μM) was utilized in this model to perform this investigation. However, in this case, the non-linearity could not be recovered, with only a slight increase in $C_{\text{max}}$ and AUC observed. Unfortunately, the observed study data for $E_G$ and $E_H$ were not provided for the 5 mg/kg dose therefore a comparison for these parameters was not possible.

Concentration-time profiles following a single dose of midazolam (i.v. & p.o.)

Observed plasma concentration profiles for a single i.v. dose 5 mg/kg of midazolam 30 were compared to simulated predictions (Figure 4). Model inputs and the references associated for these simulations are provided in the Supporting Information, Table J. As for the metoprolol, reported values were obtained from AUC extrapolated to infinity, so simulations were adjusted to account for this. For both observed $\text{CL}_{\text{iv}}$ and IVIVE simulations using RHep and RIme $\text{CL}_{\text{int}}$ elimination data, the profile shows reasonable agreement to the
observed values with the clearance predicted within 1.03 – 1.66 fold and AUC predicted within 0.98-1.67 fold.

For p.o. simulations (single dose, 15 mg/kg) using in vitro data, clearance is approximately 2- to 4-fold under-predicted leading to a 5- to 6-fold over-prediction in $C_{\text{max}}$ and a 2- to 4-fold over-prediction in AUC, showing that for midazolam, the available in vitro elimination parameter inputs combined with the additional systemic clearance input, cannot predict the observed in vivo clearance. Using the observed $CL_{po}$ with the ADAM model improves predictions for both $C_{\text{max}}$ and AUC (Figure 5).
Discussion

The primary objective of this study was to build a strain-specific virtual rat model as a tool that can be utilised to assess the IVIVE approach in drug discovery, using prior drug physicochemical properties and \textit{in vitro} ADME data. It is highly unlikely that \textit{in vivo} human pharmacokinetic data are available to verify a human IVIVE approach at the discovery stage, therefore the availability of both rat \textit{in vitro} data and \textit{in vivo} pharmacokinetic studies can be used to assess possible outcomes of IVIVE approaches. The history of generic PBPK models goes back to 1937 \cite{40}. In the late 1990s and early 2000s methods to predict tissue to plasma partition coefficients were developed \cite{41,42}.

When developing a model it is important that uncertainties surrounding system and drug parameters are minimised. Therefore, obtaining robust physiological system parameters that underpin PBPK model structure, together with comprehensive \textit{in vitro} drug data, is essential to gaining confidence when employing an IVIVE approach. This is reflected in the collation and review of the individual system parameters required for the model. Original references were sought and scrutinized in preference to consensus or review articles in order to allow informed decisions to be made about the quality and suitability of the data to be selected and utilized. As the knowledge of the physiological processes governing the pharmacokinetics of a drug has advanced, a reciprocal advancement in a PBPK models infrastructure is required to meet these demands. Given the current model structure, one hundred and seventy-eight system parameters are required to generate the rat PBPK model described herein. With the current availability of data in the literature, it is a considerable challenge to build a model specific to the Sprague-Dawley rat strain, and for five parameters, no strain was specified. It is noteworthy that GI physiological parameter values were predominantly obtained from studies using Wistar rats, as there was no applicable data available for the Sprague-Dawley strain. Ideally, for each parameter, a meta-analysis containing numerous studies is performed.
in order to limit the potential bias when implementing a value from a single study. Due to the paucity of suitable data, 6 system parameters rely on a value from a single study. The certainty of a parameter is especially critical if simulation outcome is sensitive to a change in the value. For example, in the case of a high hepatic extraction compound, with a hepatic clearance greater than the implemented organ blood flow, the simulated clearance will be limited leading to an over-prediction in AUC. In the current model a liver blood flow rate of 19.4 mL/min/250g is used, which is greater than the values used in other rat PBPK models, \textit{i.e.} 11.8 mL/min/250g \textsuperscript{12,43}. A sensitivity analysis could be undertaken to identify whether liver blood flow is limiting hepatic clearance at the values used for simulation.

To verify the model performance, 3 compound models using key data to run IVIVE simulations were constructed from literature data. For diazepam, RHep CL\textsubscript{int} data \textsuperscript{44,45} was preferentially used for simulation due to the better predictive performance when compared to rat liver microsomal CL\textsubscript{int}, however, clearance is still under-predicted when using the weighted mean hepatocyte CL\textsubscript{int}. There were good predictions of tissue concentration-time profiles. It is known that clearance predictions using \textit{in vitro} CL\textsubscript{int} data are generally under-predicted when using an IVIVE scaling approach, which is more prevalent when incorporating a protein binding term (fu) \textsuperscript{46}. If clearance is not predictive, it is recommended that a CL\textsubscript{int} is calculated in a retrograde manner from the \textit{in vivo} clearance \textsuperscript{27}. There has been reasonable success in predicting V\textsubscript{ss} using a variety of approaches \textsuperscript{47}, including PBPK, which has been applied in an independent study when using Simcyp Rat \textsuperscript{48}. The predicted V\textsubscript{ss} for diazepam using the Rodgers & Rowland method was within two-fold of the observed (Supporting Information, Table H). The observed data \textsuperscript{28} also describe a considerable partitioning of diazepam into adipose tissue with a Kp\textsubscript{T} of 20.5 compared to a predicted Kp\textsubscript{T} of 11.05. It is this disparity, combined with adipose tissue having a relatively large volume (17.5 mL, Table A, Supporting Information) that is likely to lead in-part to the 1.5 fold under-
prediction. Adjusting the adipose $K_{p,T}$ by using the observed value of 20.5 improves $V_{ss}$ prediction to 4.29 L/kg.

The intravenous plasma concentration-time profiles were reasonably successfully predicted at 3 different doses for metoprolol (Figure 2) and for the 1 and 2 mg/kg $p.o.$ studies (Figure 3A & B) using the first order absorption model. Simulations using the ADAM model predict a delayed $T_{\text{max}}$ compared to the observed data. Predictions can be improved by reducing the gastric emptying time from 0.25h to 0.11h. This is to be expected since the $in$ $vivo$ data come from a study where the solution is administered directly into the duodenum, meaning gastric emptying will not delay absorption. The intestinal extraction of metoprolol using rat intestinal microsomes was predicted within 2-fold for both the first order and ADAM absorption models. However, there seems to be an under-prediction of the absorption phase, probably due to the 0.11h gastric emptying time not being sufficient to describe direct intra-duodenal administration. Enterocyte drug concentrations can be simulated in the ADAM model. For metoprolol, the $C_{\text{max}}$ in the proximal jejunum enterocyte compartment at 1 and 2 mg/kg is predicted to be 16.2 and 32.4 $\mu$M, respectively. The reported intestinal microsomal $K_{m}$ is 24 $\mu$M, therefore at 1 mg/kg metoprolol levels are below the concentration expected for metabolic saturation within these systems. Yet, at 2 mg/kg, the predicted enterocyte concentration is approaching the concentration required for metabolic saturation, however, the observed data does not exhibit dose non-linearity $^{29}$. At the highest dose (5 mg/kg), the observed data does show dose non-linearity, which could be due to saturation of metabolism. The predicted enterocyte concentration at 5 mg/kg in the proximal jejunum segment is 81.0 $\mu$M, a 3.4-fold higher concentration than intestinal microsomal $K_{m}$, indicative of metabolic saturation in this segment. However, the model fails to capture the observed dose non-linearity using the whole organ metabolic clearance option for the $V_{\text{max}}$ and $K_{m}$ generated from Yoon et al. $^{29}$, although there is an observed increase in the $C_{\text{max}}$ and
AUC (Figure 5). This could be due to the under-prediction of the absorption phase, or other ongoing processes not currently accounted for in the model.

When intravenously dosing midazolam, the IVIVE approach predicts the concentration-time profile reasonably successfully; this is not the case when midazolam is dosed orally. Despite in vitro and in vivo additional organ elimination data being available (i.e. pulmonary clearance), overall clearance is under-predicted by up to 5-fold. This could be due to a number of reasons. Considering the previous i.v. simulations, although predictions were reasonable, there was a slight under-prediction of clearance; in turn, this might contribute to the under-prediction observed in the p.o. simulations. Another possibility is that the midazolam permeability data from MDCK cell monolayers is not sufficient to describe the effective permeability in the intestine. The absorption phase is poorly described (Figure 5), with a potential over-prediction of $k_a$, even where the in vivo $CL_{po}$ is utilised, which could be another contributing factor to the over-prediction of AUC and $C_{max}$. Also, in the original study $^{30}$, the rats used were larger (300-350g) than the standard rat of 250g described in this model, and increased in weight throughout the study, which may indicate that the simulations are no longer representative of the study. Finally, any uncertainty surrounding the in vitro drug data can lead to uncertainty in the results. In this case, the additional clearance value for pulmonary elimination came from a single study using data from Wistar rats $^{49}$. Uncertainty in this parameter may impact on the simulation results; therefore further studies investigating the pulmonary elimination of midazolam in rats are warranted. In the case of the simulations run in this study, elimination data from hepatocytes were utilised, with a single study found to determine midazolam $CL_{int}$ in rat hepatocytes. However, a search of the literature reveals inconsistencies in midazolam elimination data from rat liver microsomes, with values varying from 99 $^{44}$ to 1290 $^{49}$ µL/min/mg. This highlights the requirement for robust in vitro data for use in predictive simulations. While it is possible that predictions for midazolam
could be improved if the additional clearance was normalised to the observed \textit{in vivo} data, the goal of this study was to attempt to predict the \textit{in vivo} pharmacokinetics from \textit{in vitro} data. Therefore, this approach was not utilised in this instance, which clearly highlights the challenges of predictive IVIVE in certain cases.

Using an IVIVE approach in toxicity testing is advocated\textsuperscript{50}. By characterizing and combining a molecule’s metabolism, transport or binding \textit{in vitro}, with the knowledge of the concentrations of that molecule that produces deleterious effects, the whole body exposure of the molecule for an organism can be predicted using an IVIVE approach linked to a PBPK model (IVIVE-PBPK) strategy. In the same manner as for pre-clinical therapeutics, this concept could be utilized to further characterize exposure levels in early toxicity screens in both environmental and pharmaceutical settings. In these cases, the IVIFE approach should be verified and refined in conjunction with \textit{in vivo} exposure data. In addition, idiosyncratic adverse drug reactions in humans are particularly challenging to predict from pre-clinical toxicity testing, unless the appropriate pre-clinical model is employed in discovery-based toxicological studies\textsuperscript{51}. Using allometric scaling of rat PK parameters such as clearance to predict clearance in human is commonplace, a comprehensive assessment of allometric performance have been undertaken recently\textsuperscript{46}, however using allometric scaling methods even if successfully translated into humans, does not enlighten discovery scientists as to the mechanisms acting to contribute to a PK outcome.
Conclusion

The framework and mechanistic utility of a rat PBPK model has been demonstrated. The IVIVE-PBPK approach in pre-clinical species should be advantageous in a drug discovery setting; 1) to gain confidence in the IVIVE scaling strategy, in order to apply the approach to human IVIVE when using human in vitro assays \(^{52}\), 2) to refine PK study design in rats leading to a reduction in the number of animals, 3) to obtain a greater mechanistic insight into the processes that govern the ADME properties of the investigation molecule and 4) by using the model to aid in elucidating the mechanism underlying any disparity in extrapolation predictions, i.e. sensitivity analysis \(^{12}\) where altering drug, system or trial parameters could provide an insight into the potential mechanisms underlying disparity in predictions. Any mechanistic knowledge gaps can be identified and model performance can be reassessed, a ‘predict, learn, confirm and apply’ paradigm.

In summary, the data presented here highlight an approach for obtaining the system parameters required to construct a strain-specific rat PBPK model and the challenges associated with this process. The utility of such a model is described for use within drug discovery; for identifying the mechanisms responsible for PK outcomes, and how the IVIVE approach can be employed to identify where further in vitro data are required to refine model predictions. This work focused on the models and algorithms dealing with the metabolism and passive transport processes. Incorporating active transporter mechanisms may improve predictions and provide better insight in drug disposition and effects.
Acknowledgements

This work was funded by Simcyp Limited (a Certara Company). The Simcyp Simulator is freely available, following completion of the training workshop, to approved members of academic institutions and other non-for-profit organizations for research and teaching purposes. The authors also thank Dr. Oliver Hatley for useful discussions around the scaling factors and elimination data. The help of Eleanor Savill and Jessica Waite in preparing the manuscript is appreciated.

Conflict of Interest/Disclosure

Musther, Harwood, Turner and Jamei are employees of Simcyp Limited (a Certara company). Yang is the CEO of Mosim Co Ltd, Shanghai, China. A. Rostami-H. is an employee of the University of Manchester and part-time secondee to Simcyp Limited (a Certara Company).

Authorship Contributions

Participated in study design: Musther, Harwood, Yang, Turner, Jamei, Rostami-Hodjegan

Conducted experiments: Musther, Harwood, Yang.

Performed data analysis: Musther, Harwood, Yang.

Contributed to writing the manuscript: Musther, Harwood, Jamei, Turner.

Musther and Harwood contributed equally to this work.
References


Figure legends

Figure 1. Comparison of simulated and observed tissue concentration profiles following a single intravenous dose of diazepam (1.2 mg/kg). Observed data given as solid triangles, simulations using observed clearance and predicted $V_{ss}$ given as dashed lines, and IVIVE simulations using RHep $CL_{int}$ given as solid lines.

Figure 2. Comparison of simulated and observed plasma concentration-time profiles following intravenous dosing at (A) 0.5 mg/kg, (B) 1 mg/kg and (C) 2 mg/kg metoprolol. Observed data given as solid triangles, simulations using observed $V_{ss}$ in a minimal PBPK model and observed clearance given as dotted lines, simulations using a Rodgers & Rowland predicted $V_{ss}$ in a full PBPK model and observed clearance given as dashed lines, and IVIVE simulations using a Rodgers & Rowland predicted $V_{ss}$ in a full PBPK model and rat liver microsomal $CL_{int}$ given as solid lines.

Figure 3. Comparison of simulated and observed plasma concentration-time profiles following oral dosing at (A) 1 mg/kg, (B) 2 mg/kg and (C) 5 mg/kg metoprolol. Observed data given as solid triangles, simulations using a first order absorption model and RLM and RIMe $CL_{int}$ are given as dashed lines, the ADAM model with RLM and RIMe $CL_{int}$ with gastric emptying rate at 0.25h given as solid lines, and ADAM model with adjusted gastric emptying (0.11h) given as a dot-dashed line. ADAM model with adjusted gastric emptying, and $K_m-V_{max}$ data are shown as dotted line.

Figure 4. Comparison of simulated and observed plasma concentration-time profiles following a single intravenous dose of midazolam (5 mg/kg). Observed data given as solid triangles, simulations using observed clearance and predicted $V_{ss}$ given as dotted lines, IVIVE
simulations using rat liver microsomal CL_{int} given as solid lines and simulations using RHep
CL_{int} given as dashed lines.

Figure 5. Comparison of simulated and observed plasma concentration-time profiles
following a single oral dose of midazolam (15 mg/kg). Observed data given as solid triangles,
simulations using the first order absorption and Q_{gut} model with IVIVE RHep/RIMe CL_{int}
elimination given as a dashed line, simulations using observed CL_{po} with the ADAM model
given as the dotted line and IVIVE RHep/RIMe CL_{int} ADAM simulations given as a solid line.
Table 1. The functional capabilities of the Simcyp Rat Simulator

<table>
<thead>
<tr>
<th>Function</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Absorption</strong></td>
<td>A first order absorption model driven by mass transfer rate constants ((k_a)). Capability to utilise <em>in vitro</em> permeability assays to predict intestinal absorption. Advanced dissolution absorption and metabolism (ADAM) model</td>
</tr>
<tr>
<td><strong>Distribution</strong></td>
<td>Minimal or full (13 compartment) PBPK models (<em>In vivo</em> steady state volume of distribution (Vss) input, or mechanistic prediction of Vss by Poulin &amp; Theil (corrected by Berezhkovskiy) ‘Method 1’ or Rodgers &amp; Rowland ‘Method 2’. Experimentally derived tissue partition ((K_p, T)) values can be input for each organ if available</td>
</tr>
<tr>
<td><strong>Elimination</strong></td>
<td>IVIVE scaling of drug metabolism in liver and gut utilising data generated in <em>in vitro</em> systems; - Rat liver microsomes (-) Rat hepatocytes (-) Rat intestinal microsomes (-) Rat intestinal slices Additional hepatic elimination and non-metabolic renal clearance.</td>
</tr>
<tr>
<td><strong>PK profiles</strong></td>
<td>Simulation of full concentration-time profiles for substrate and metabolite ((for the metabolite only the minimal PBPK model is available) Tissue-specific concentration-time profiles available in full PBPK model</td>
</tr>
<tr>
<td><strong>Species Library</strong></td>
<td>Based on a 250g rat, Sprague-Dawley physiological ‘system’ data preferred where available from the literature Flexibility to change system parameters to create your own rat.</td>
</tr>
<tr>
<td><strong>Trial Design</strong></td>
<td>Single &amp; multiple oral, intravenous and infusion dosing options Fed &amp; fasted states to incorporate food effects</td>
</tr>
<tr>
<td><strong>Simulation Tools</strong></td>
<td><strong>Automated sensitivity analysis</strong> permitting 2 parameters to be simultaneously analysed to establish the sensitivity of the simulation to changing the parameter(s) by assessing multiple PK outcomes.</td>
</tr>
<tr>
<td></td>
<td>An <strong>automated compound import</strong> function in which multiple Simcyp compound files can be created using an excel template, which is particularly useful for handling large numbers of compounds. Compound parameters specific to the simulator can be imported from excel into Simcyp rat.</td>
</tr>
<tr>
<td></td>
<td>A <strong>batch processor application</strong> allows a large number of simulations to be performed in an automated fashion to generate dedicated customised output sheets</td>
</tr>
</tbody>
</table>
Figure 1. Comparison of simulated and observed tissue concentration profiles following a single intravenous dose of diazepam (1.2 mg/kg). Observed data given as solid triangles, simulations using observed clearance and predicted Vss given as dashed lines, and IVIVE simulations using RHep CLint given as solid lines.
Figure 2. Comparison of simulated and observed plasma concentration-time profiles following intravenous dosing at (A) 0.5 mg/kg, (B) 1 mg/kg and (C) 2 mg/kg metoprolol. Observed data given as solid triangles, simulations using observed Vss in a minimal PBPK model and observed clearance given as dotted lines, simulations using a Rodgers & Rowland predicted Vss in a full PBPK model and observed clearance given as dashed lines, and IVIVE simulations using a Rodgers & Rowland predicted Vss in a full PBPK model and rat liver microsomal CLint given as solid lines.
Figure 3. Comparison of simulated and observed plasma concentration-time profiles following oral dosing at (A) 1 mg/kg, (B) 2 mg/kg and (C) 5 mg/kg metoprolol. Observed data given as solid triangles, simulations using a first order absorption model and RLM and RIMe CLint are given as dashed lines, the ADAM model with RLM and RIMe CLint with gastric emptying rate at 0.25h given as solid lines, and ADAM model with adjusted gastric emptying (0.11h) given as a dot-dashed line. ADAM model with adjusted gastric emptying, and Km-Vmax data are shown as dotted line.
Figure 4. Comparison of simulated and observed plasma concentration-time profiles following a single intravenous dose of midazolam (5 mg/kg). Observed data given as solid triangles, simulations using observed clearance and predicted Vss given as dotted lines, IVIVE simulations using rat liver microsomal CLint given as solid lines and simulations using RHep CLint given as dashed lines.
Figure 5. Comparison of simulated and observed plasma concentration-time profiles following a single oral dose of midazolam (15 mg/kg). Observed data given as solid triangles, simulations using the first order absorption and $Q_{\text{gut}}$ model with IVIVE $R_{\text{Hep}}/R_{\text{Ime}} CL_{\text{int}}$ elimination given as a dashed line, simulations using observed $CL_{\text{po}}$ with the ADAM model given as the dotted line and IVIVE $R_{\text{Hep}}/R_{\text{Ime}} CL_{\text{int}}$ ADAM simulations given as a solid line.
Supporting Information

Table A. Tissue Volumes with rat strains and references.

<table>
<thead>
<tr>
<th>Tissue Compartment</th>
<th>Tissue Volumes (mL)</th>
<th>Rat Strain</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adipose</td>
<td>17.5</td>
<td>Sprague-Dawley</td>
<td>1</td>
</tr>
<tr>
<td>Bone</td>
<td>16.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Wistar</td>
<td>2 (via 3)</td>
</tr>
<tr>
<td>Brain</td>
<td>1.3</td>
<td>Wistar</td>
<td>4</td>
</tr>
<tr>
<td>Gut</td>
<td>6.5</td>
<td>Sprague-Dawley</td>
<td>5</td>
</tr>
<tr>
<td>Heart</td>
<td>1.1</td>
<td>Wistar</td>
<td>4</td>
</tr>
<tr>
<td>Kidneys</td>
<td>2.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Sprague-Dawley</td>
<td>6 (via 7 and 8)</td>
</tr>
<tr>
<td>Liver</td>
<td>9</td>
<td>Sprague-Dawley</td>
<td>9</td>
</tr>
<tr>
<td>Lung</td>
<td>1.3</td>
<td>Wistar</td>
<td>4</td>
</tr>
<tr>
<td>Muscle</td>
<td>122&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Sprague-Dawley</td>
<td>6 (via 7 and 8)</td>
</tr>
<tr>
<td>Skin</td>
<td>41.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>No strain specified</td>
<td>10</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Sprague-Dawley</td>
<td>6 (via 7 and 8)</td>
</tr>
<tr>
<td>Plasma</td>
<td>10.55</td>
<td>Sprague-Dawley</td>
<td>11</td>
</tr>
<tr>
<td>Red Blood Cells</td>
<td>5.55</td>
<td>Sprague-Dawley</td>
<td>11</td>
</tr>
</tbody>
</table>

<sup>a</sup> – The value for bone is possibly derived from Kawai et al<sup>3</sup>, however in this study using Wistar rats bone volume is 15.8 mL.  
<sup>b</sup> – no strain data given.  
<sup>c</sup> – data was reported from a 250g rat in Meno-Tetang et al<sup>6</sup> from Bernareggi & Rowland<sup>7</sup> (using Sprague-Dawley rats in their pharmacokinetic study) and the review paper by Davies & Morris<sup>8</sup>, who cite King et al<sup>12</sup> (where the volume data is based on those volumes cited in Bischoff et al<sup>1971</sup>  13, where no rat strain is specified) and Bernareggi & Rowland<sup>7</sup>. 

(continues...
Table B. Cardiac output, tissue blood flow with rat strains and references.

<table>
<thead>
<tr>
<th>Cardio-vascular Parameter (Units)</th>
<th>Parameter Value</th>
<th>Rat Strain</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac output (mL/min)</td>
<td>80(^a)</td>
<td>Sprague-Dawley</td>
<td>5,14-20</td>
</tr>
</tbody>
</table>

**Tissue Blood Flow (% cardiac output)**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Value</th>
<th>Rat Strain</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adipose</td>
<td>5.9</td>
<td>Sprague-Dawley</td>
<td>5</td>
</tr>
<tr>
<td>Bone</td>
<td>10.1</td>
<td>Sprague-Dawley</td>
<td>5</td>
</tr>
<tr>
<td>Brain</td>
<td>1.4</td>
<td>Sprague-Dawley</td>
<td>5</td>
</tr>
<tr>
<td>Stomach &amp; Oesophagus</td>
<td>1.7</td>
<td>Sprague-Dawley</td>
<td>21</td>
</tr>
<tr>
<td>Small Intestine</td>
<td>6.2</td>
<td>Sprague-Dawley</td>
<td>21</td>
</tr>
<tr>
<td>Villi</td>
<td>3.7</td>
<td>See footnote b</td>
<td>22,23</td>
</tr>
<tr>
<td>Large Intestine</td>
<td>2.2</td>
<td>Sprague-Dawley</td>
<td>21</td>
</tr>
<tr>
<td>Heart</td>
<td>4.0(^a)</td>
<td>Sprague-Dawley</td>
<td>14,21</td>
</tr>
<tr>
<td>Kidney</td>
<td>14.5(^a)</td>
<td>Sprague-Dawley</td>
<td>14,17,20,21</td>
</tr>
<tr>
<td>Liver (Arterial)</td>
<td>1.6(^a)</td>
<td>Sprague-Dawley</td>
<td>5,14,15,21</td>
</tr>
<tr>
<td>Liver (Portal)</td>
<td>22.6(^a)</td>
<td>Sprague-Dawley</td>
<td>14,15,20</td>
</tr>
<tr>
<td>Lung</td>
<td>100</td>
<td>No reference required(^c)</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>23.7</td>
<td>Sprague-Dawley</td>
<td>5</td>
</tr>
<tr>
<td>Skin</td>
<td>5.1</td>
<td>Sprague-Dawley</td>
<td>5</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.1(^a)</td>
<td>Sprague-Dawley</td>
<td>14,17,21</td>
</tr>
</tbody>
</table>

**Splanchnic Fed :Fasted (Ratio)**

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Value</th>
<th>Rat Strain</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Splanchnic Fed :Fasted (Ratio)</td>
<td>1.6</td>
<td>No strain specified</td>
<td>24</td>
</tr>
</tbody>
</table>

Haematocrit (%)

<table>
<thead>
<tr>
<th>Haematocrit (%)</th>
<th>Value</th>
<th>Rat Strain</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematocrit (%)</td>
<td>43.9</td>
<td>Wistar</td>
<td>11</td>
</tr>
</tbody>
</table>

\(^a\) – Where multiple references are cited, a weighted mean using each value from each reference, correcting for sample number per study was calculated. \(^b\) – villus blood flow was calculated as a percentage of small intestinal blood flow routed to the villus, which was suggested to be 60% of small intestinal blood flow, though the reference texts are not specifically related to rat villus blood flow. \(^c\) – the lung receives 100% of cardiac output from the heart via the pulmonary arteries prior to blood oxygenation.
<table>
<thead>
<tr>
<th>Gastrointestinal Parameter (Unit)</th>
<th>Parameter Value</th>
<th>Rat Strain</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small Intestine Enterocyte Volume (mL)</td>
<td>3.4(^a)</td>
<td>Scaled via human</td>
<td>25,26</td>
</tr>
<tr>
<td>Colon Enterocyte Volume (mL)</td>
<td>0.238</td>
<td>Scaled from Small Intestine</td>
<td></td>
</tr>
<tr>
<td>Duodenal Length (cm)</td>
<td>7</td>
<td>Lister</td>
<td>27</td>
</tr>
<tr>
<td>Jejunal Length (cm)</td>
<td>90</td>
<td>No strain specified</td>
<td>28</td>
</tr>
<tr>
<td>Ileum length (cm)</td>
<td>20(^b)</td>
<td>Wistar</td>
<td>E.McConnell (Personal Communication)</td>
</tr>
<tr>
<td>Duodenal Diameter (cm)</td>
<td>0.275</td>
<td>Lister</td>
<td>27</td>
</tr>
<tr>
<td>Jejunal Diameter (cm)</td>
<td>0.4</td>
<td>No strain specified</td>
<td>28</td>
</tr>
<tr>
<td>Ileal Diameter (cm)</td>
<td>0.4</td>
<td>No strain specified</td>
<td>28</td>
</tr>
<tr>
<td>Basal Volume of Stomach Fluid (mL) - Fasted</td>
<td>0.26(^b)</td>
<td>Wistar</td>
<td>E.McConnell (Personal Communication)</td>
</tr>
<tr>
<td>Initial Volume of Stomach Fluid (mL) – Fed State</td>
<td>0.86(^b)</td>
<td>Wistar</td>
<td>E.McConnell (Personal Communication)</td>
</tr>
<tr>
<td>Small Intestine Basal Fluid Volumes (mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td>0.184</td>
<td></td>
<td>29</td>
</tr>
<tr>
<td>Jejunum</td>
<td>0.335</td>
<td></td>
<td>29</td>
</tr>
<tr>
<td>Ileum</td>
<td>0.485</td>
<td>Wistar</td>
<td>29</td>
</tr>
<tr>
<td>Caecum</td>
<td>1.246</td>
<td></td>
<td>29</td>
</tr>
<tr>
<td>Proximal Colon</td>
<td>0.326</td>
<td></td>
<td>29</td>
</tr>
<tr>
<td>Distal Colon</td>
<td>0.393</td>
<td></td>
<td>29</td>
</tr>
<tr>
<td>Mean Gastric Emptying – Fasted (h)</td>
<td>0.25(^b)</td>
<td>Wistar</td>
<td>E.McConnell (Personal Communication)</td>
</tr>
<tr>
<td>Mean Gastric Emptying – Fed (h)</td>
<td>0.55(^b)</td>
<td>Wistar</td>
<td>E.McConnell (Personal Communication)</td>
</tr>
<tr>
<td>Small Intestinal Transit Time (h)</td>
<td>1.57(^b)</td>
<td>Wistar</td>
<td>30,31</td>
</tr>
<tr>
<td>Colon Transit Time (h)</td>
<td>19.7</td>
<td>Wistar</td>
<td>30,32</td>
</tr>
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</table>
### pH Fasted

<table>
<thead>
<tr>
<th>Region</th>
<th>pH</th>
</tr>
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<tbody>
<tr>
<td>Stomach</td>
<td>3.9</td>
</tr>
<tr>
<td>Duodenum</td>
<td>5.9</td>
</tr>
<tr>
<td>Jejunum I</td>
<td>6.1</td>
</tr>
<tr>
<td>Jejunum II</td>
<td>6.1</td>
</tr>
<tr>
<td>Jejunum III</td>
<td>6.1</td>
</tr>
<tr>
<td>Jejunum IV</td>
<td>6.1</td>
</tr>
<tr>
<td>Ileum I</td>
<td>5.9</td>
</tr>
<tr>
<td>Ileum II</td>
<td>5.9</td>
</tr>
<tr>
<td>Colon</td>
<td>6.2</td>
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</table>

<table>
<thead>
<tr>
<th>Data Source</th>
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<tbody>
<tr>
<td>Wistar</td>
<td>29</td>
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</table>

### pH Fed

<table>
<thead>
<tr>
<th>Region</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>3.2</td>
</tr>
<tr>
<td>Duodenum</td>
<td>5</td>
</tr>
<tr>
<td>Jejunum I</td>
<td>5.1</td>
</tr>
<tr>
<td>Jejunum II</td>
<td>5.1</td>
</tr>
<tr>
<td>Jejunum III</td>
<td>5.1</td>
</tr>
<tr>
<td>Jejunum IV</td>
<td>5.1</td>
</tr>
<tr>
<td>Ileum I</td>
<td>5.9</td>
</tr>
<tr>
<td>Ileum II</td>
<td>5.9</td>
</tr>
<tr>
<td>Colon</td>
<td>6.2</td>
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</table>

<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar</td>
<td>29</td>
</tr>
</tbody>
</table>

### Regional Villous Blood Flow (Q\textsubscript{villi} %)

<table>
<thead>
<tr>
<th>Region</th>
<th>Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td>8</td>
</tr>
<tr>
<td>Jejunum I</td>
<td>17</td>
</tr>
<tr>
<td>Jejunum II</td>
<td>17</td>
</tr>
<tr>
<td>Jejunum III</td>
<td>17</td>
</tr>
<tr>
<td>Jejunum IV</td>
<td>17</td>
</tr>
<tr>
<td>Ileum I</td>
<td>12</td>
</tr>
<tr>
<td>Ileum II</td>
<td>12</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Data Source</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sprague-Dawley</td>
<td>33</td>
</tr>
</tbody>
</table>

### Transit Time (% of Total for SI)

<table>
<thead>
<tr>
<th>Region</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td>6\textsuperscript{c}</td>
</tr>
<tr>
<td>Jejunum I</td>
<td>19.2\textsuperscript{c}</td>
</tr>
<tr>
<td>Jejunum II</td>
<td>19.2\textsuperscript{c}</td>
</tr>
<tr>
<td>Jejunum III</td>
<td>19.2\textsuperscript{c}</td>
</tr>
<tr>
<td>Jejunum IV</td>
<td>19.2\textsuperscript{c}</td>
</tr>
</tbody>
</table>

No literature values
For Peer Review

ID 17-225 - MUSTHER

a – Where rat enterocyte volume is scaled down using morphometric principles outlined by Crowe & Marsh \(^{25}\) and Sugano \(^{26}\).

b – personal communications with Emma McConnell a PhD student with Professor Abdul Basit, School of Pharmacy, University of London from the period June 2008 – August 2008.

c – Intestinal segment transit time is calculated by the total transit time divided by the intestinal segment length as a proportion of total small intestinal length (segmental transit time is required for the ADAM model), calculated as follows:

**Duodenum** – The total small intestinal length is **117cm**
The duodenum is **7cm** in length which is **6%** (6cm/117cm x100) of total small intestinal length, thus the duodenum accounts for **6%** **percentage of total small intestinal transit.**

**Jejunum** – The jejunum is **90cm** which is **76.9%** (90cm/117cm x100) of total small intestinal length. There are 4 jejunal compartments thus each compartment accounts for **19.2%** (76.9%/4) of **total small intestinal transit.**

**Ileum** – The ileum is **20cm** in length which is **17.1%** (20cm/117cm x100) of total small intestinal length. There are 2 ileal compartments thus each compartment accounts for **8.6%** (17.1%/2) of **total small intestinal transit.**

d – Baseline intestinal compartmental blood flows were calculated by taking the blood flow to the duodenum, jejunum and ileum provided in Klemm & Moody \(^{33}\), combined with the wet weight of tissue data from Sha et al \(^{34}\), calculated as follows:

**Duodenum** - 2.54 (mL/min/g) x (0.17 (g/cm) x 7 (cm))
Blood flow x (wet weight x segment length)
2.54 x 1.19 g (duodenal wet weight) = **3.02 mL/min** to whole duodenum

**Jejunum** - 2.4 (mL/min/g) x (0.12 (g/cm) x 90 (cm))
2.4 x 10.8 g (jejunal wet weight) = **25.92 mL/min** to whole jejunum
There are 4 model compartments in the jejunum, thus 25.92/4 = **6.48 mL/min** per jejunal compartment.

**Ileum** - 4.17 (mL/min/g) x (0.11 (g/cm) x 20 (cm))
4.17 x 2.2 g (ileal wet weight) = **9.174 mL/min** to whole ileum
There are 2 model compartments for the ileum, thus 9.174/2 = **4.59 mL/min** per ileal compartment.

Total blood flow to the small intestine is the sum of the blood flows given for the duodenum, jejunum and ileum and is **38.11 mL/min per whole small intestine.**

The above values for each compartment of the small intestine were converted into a percentage of total blood flow perfusing the whole small intestine and were implemented into the model thus;

**Duodenum** – **3.02 (mL/min/duodenum)/38.11 (mL/min/whole small intestine) x 100 = 8%**
**Jejunum** – **6.48 (mL/min/jejenum)/38.11 (mL/min/whole small intestine) x 100 = 17%**
**Ileum** – **4.59 (mL/min/ileum)/38.11 (mL/min/whole small intestine) x 100 = 12%**
Table D – Tissue Composition: Relative volume of extracellular and intracellular water, neutral lipid and phospholipid wet tissue weight expressed as relative volume of wet tissue (%).

<table>
<thead>
<tr>
<th>Organs</th>
<th>Extracellular Water</th>
<th>Intracellular Water</th>
<th>Neutral Lipids</th>
<th>Neutral Phospholipids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adipose</td>
<td>13.5</td>
<td>1.7</td>
<td>84.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.16</td>
</tr>
<tr>
<td>Bone</td>
<td>10</td>
<td>34.6</td>
<td>1.7</td>
<td>0.17</td>
</tr>
<tr>
<td>Brain</td>
<td>16.2</td>
<td>62</td>
<td>3.9</td>
<td>0.15</td>
</tr>
<tr>
<td>Gut</td>
<td>28.2</td>
<td>47.5</td>
<td>3.8</td>
<td>1.25</td>
</tr>
<tr>
<td>Heart</td>
<td>32</td>
<td>45.6</td>
<td>1.4</td>
<td>1.11</td>
</tr>
<tr>
<td>Kidney</td>
<td>27.3</td>
<td>48.3</td>
<td>1.2</td>
<td>2.42</td>
</tr>
<tr>
<td>Liver</td>
<td>16.1</td>
<td>57.3</td>
<td>1.4</td>
<td>2.4</td>
</tr>
<tr>
<td>Lung</td>
<td>33.6</td>
<td>44.6</td>
<td>2.2</td>
<td>1.28</td>
</tr>
<tr>
<td>Muscle</td>
<td>11.8</td>
<td>63</td>
<td>1</td>
<td>0.72</td>
</tr>
<tr>
<td>Skin</td>
<td>38.2</td>
<td>29.1</td>
<td>6</td>
<td>0.44</td>
</tr>
<tr>
<td>Spleen</td>
<td>20.7</td>
<td>57.9</td>
<td>0.77</td>
<td>1.13</td>
</tr>
<tr>
<td>Plasma</td>
<td>94.5</td>
<td>-</td>
<td>0.23&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.13&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Red Blood Cells</td>
<td>-</td>
<td>60.3</td>
<td>0.17</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Unless otherwise indicated values taken from Rodgers et al., 2005<sup>35</sup>. *from Rodgers & Rowland 2007<sup>2</sup>.

<sup>a</sup> - Neutral lipids given as 85.3 % in reference<sup>36</sup>, however for the model it was scaled back to 84.6% as the tissue component fractions cannot add up to > 100%. The wet tissue data from Rodgers and Rowland 2005 is from a mixture of Sprague-Dawley, Wistar and Albino rat strains see references 4, 8-10, 13 & 14 in Rodgers & Rowland 2005 for further details.
Table E. Tissue Composition: Acid phospholipid concentration, tissue extracellular water- to-plasma albumin and lipoprotein ratios and references.

<table>
<thead>
<tr>
<th>Organs</th>
<th>Acidic Phospholipids (mg/g tissue)</th>
<th>Tissue-to-plasma Albumin Ratio (Kp,ALP)</th>
<th>Tissue-to-plasma Lipoprotein Ratio (Kp,LPP)</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adipose</td>
<td>0.4*</td>
<td>0.049†</td>
<td>0.068†</td>
<td>35,36</td>
</tr>
<tr>
<td>Bone</td>
<td>0.67*</td>
<td>0.1†</td>
<td>0.05†</td>
<td>35,36</td>
</tr>
<tr>
<td>Brain</td>
<td>0.4*</td>
<td>0.048†</td>
<td>0.041†</td>
<td>35,36</td>
</tr>
<tr>
<td>Gut</td>
<td>2.41*</td>
<td>0.158†</td>
<td>0.141†</td>
<td>35,36</td>
</tr>
<tr>
<td>Heart</td>
<td>2.25*</td>
<td>0.157†</td>
<td>0.16†</td>
<td>35,36</td>
</tr>
<tr>
<td>Kidney</td>
<td>5.03*</td>
<td>0.13†</td>
<td>0.137†</td>
<td>35,36</td>
</tr>
<tr>
<td>Liver</td>
<td>4.56*</td>
<td>0.086†</td>
<td>0.161†</td>
<td>35,36</td>
</tr>
<tr>
<td>Lung</td>
<td>3.91*</td>
<td>0.212†</td>
<td>0.168†</td>
<td>35,36</td>
</tr>
<tr>
<td>Muscle</td>
<td>1.53*</td>
<td>0.064†</td>
<td>0.059†</td>
<td>35,36</td>
</tr>
<tr>
<td>Skin</td>
<td>1.32*</td>
<td>0.277†</td>
<td>0.096†</td>
<td>35,36</td>
</tr>
<tr>
<td>Spleen</td>
<td>3.18*</td>
<td>0.097†</td>
<td>0.207†</td>
<td>35,36</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.057</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Red Blood Cells</td>
<td>0.5*</td>
<td>-</td>
<td>-</td>
<td>35</td>
</tr>
</tbody>
</table>

*denotes value derived from Rodgers et al 2005, † denotes value derived from Rodgers & Rowland 2006.

The rat strain data where acidic phospholipid content provided is derived from multiple sources, see references 20-31 in Rodgers et al 2005, the strains which the data were derived are Sprague-Dawley, Wistar, Albino, Buffalo & Blue Spruce. The rat strains where tissue:plasma albumin (ratio) and tissue:plasma lipoprotein (ratio) are provided were derived from Sprague-Dawley and Wistar rats.
Table F. Tissue Compartment pH with rat strains and references.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Parameter Value</th>
<th>Rat Strain</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>7.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Sprague-Dawley</td>
<td>35,37-41</td>
</tr>
<tr>
<td>Extracellular</td>
<td>7.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Sprague-Dawley</td>
<td>35,37-41</td>
</tr>
<tr>
<td>Intracellular water</td>
<td>6.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Sprague-Dawley</td>
<td>37-39</td>
</tr>
<tr>
<td>Red blood cell intracellular water</td>
<td>7.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Sprague-Dawley</td>
<td>41,42</td>
</tr>
</tbody>
</table>
Table G. Metabolic scaling factors with the rat strains and references from where they were obtained.

<table>
<thead>
<tr>
<th>Scaling Parameter (Unit)</th>
<th>Parameter Value</th>
<th>Rat Strain</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPGL (10^6 cells/g liver)</td>
<td>10^8^a</td>
<td>Sprague-Dawley</td>
<td>43-46</td>
</tr>
<tr>
<td>MPPGL (mg/g liver)</td>
<td>46^a</td>
<td>Sprague-Dawley</td>
<td>45,47,48</td>
</tr>
<tr>
<td>Liver Density (g/mL)</td>
<td>1.05</td>
<td>Sprague-Dawley</td>
<td>46</td>
</tr>
<tr>
<td>MPPGI (mg/g gut)</td>
<td>15.5</td>
<td>Wistar</td>
<td>49</td>
</tr>
<tr>
<td>Intestinal region-specific relative CYPP450 activity</td>
<td></td>
<td>Wistar</td>
<td>50</td>
</tr>
<tr>
<td>Duodenum</td>
<td>0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jejunum</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ileum</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HPGL – Hepatocytes Per Gram of Liver, MPPGL – Microsomal Protein Per Gram of Liver, MPPGI – Microsomal Protein Per Gram of Intestine

^a - Where multiple references are cited, a weighted mean using each value from each reference, correcting for sample number per study was calculated. In some cases a positive or negative weighted was applied to the study upon discretion by the scientist analysing the data, (see System Parameters section in Materials and Methods).

^b - The microsomal protein per intestine (MPPGI) was extracted from Dawson & Bridges^49 study which used a total of 60cm of small intestinal length, half the length of 120cm used within the model. The total weight of the intestine is 3.5g therefore half the intestine weighs 1.75g. Correcting the value MPPGI is 27.2 mg/g gut in the study for the weight of half the intestine gives 15.5 mg/g gut implemented in the model.
Table H. Parameter values for diazepam simulations.

<table>
<thead>
<tr>
<th>Compound Parameter</th>
<th>Parameter Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/kg)</td>
<td>1.2</td>
<td>51</td>
</tr>
<tr>
<td>B:P</td>
<td>0.79</td>
<td>52,53</td>
</tr>
<tr>
<td>fu</td>
<td>0.17</td>
<td>52-54</td>
</tr>
<tr>
<td>$V_{ss}$ (L/kg)</td>
<td>3.63*</td>
<td>Predicted by Rodgers &amp; Rowland method</td>
</tr>
<tr>
<td>$CL_{iv}$ (mL/min/250g)</td>
<td>18.875</td>
<td>51</td>
</tr>
<tr>
<td>RHep (uL/min/10^6 cells)</td>
<td>115.6</td>
<td>52,55</td>
</tr>
<tr>
<td>Renal Clearance (mL/min)</td>
<td>0.17</td>
<td>56</td>
</tr>
</tbody>
</table>

* Observed $V_{ss}$ from Igari et al. is 5.3 L/kg
Table I. Parameter values for metoprolol simulations.

<table>
<thead>
<tr>
<th>Compound Parameter</th>
<th>Parameter Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doses (i.v.) (mg/kg)</td>
<td>0.5, 1, &amp; 2 mg/kg</td>
<td>57</td>
</tr>
<tr>
<td>Doses (oral) (mg/kg)</td>
<td>1, 2 &amp; 5 mg/kg</td>
<td>57</td>
</tr>
<tr>
<td>B:P</td>
<td>1.52</td>
<td>2</td>
</tr>
<tr>
<td>fu</td>
<td>0.81</td>
<td>2</td>
</tr>
<tr>
<td>P_{eff} \text{, rat (x 10}^{-4} \text{ cm/sec})</td>
<td>1.54</td>
<td>Predicted from Caco-2</td>
</tr>
<tr>
<td>Caco-2 P_{app} (x 10^{-6} \text{ cm/sec})</td>
<td>34.58</td>
<td>58</td>
</tr>
<tr>
<td>Ka (1/h)</td>
<td>5.56</td>
<td>Predicted from Caco-2 P_{app}</td>
</tr>
<tr>
<td>V_{ss} (L/kg) Predicted</td>
<td>10.97</td>
<td>Predicted by Rodgers &amp; Rowland method</td>
</tr>
<tr>
<td>V_{ss} (L/kg) Observed</td>
<td>4.08*</td>
<td>57</td>
</tr>
<tr>
<td>CL_{iv} (mL/min/250g)</td>
<td>18.33</td>
<td>57</td>
</tr>
<tr>
<td>CL_{po} (mL/min/250g)</td>
<td>79.04^a</td>
<td>57</td>
</tr>
<tr>
<td>RLM (uL/min/mg)</td>
<td>58.17</td>
<td>57</td>
</tr>
<tr>
<td>RIM(enterocytes) (uL/min/mg)</td>
<td>11.81</td>
<td>57</td>
</tr>
<tr>
<td>RIM Km (µM)</td>
<td>24^b</td>
<td>57</td>
</tr>
<tr>
<td>Renal Clearance (mL/min)</td>
<td>2.56</td>
<td>57</td>
</tr>
</tbody>
</table>

*Yoon et al\textsuperscript{57} observed V_{ss} (L/kg) 4.08, a – data calculated from 1 and 2 mg/kg dose using clearance = dose/AUC (equation 9), b – Vmax calculated using CLint and Km.
Table J. Parameter values for midazolam simulations.

<table>
<thead>
<tr>
<th>Compound Parameter</th>
<th>Parameter Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (i.v.) (mg/kg)</td>
<td>5</td>
<td>59</td>
</tr>
<tr>
<td>Dose (oral) (mg/kg)</td>
<td>15</td>
<td>59</td>
</tr>
<tr>
<td>B:P</td>
<td>1.03</td>
<td>60</td>
</tr>
<tr>
<td>fu</td>
<td>0.07</td>
<td>52</td>
</tr>
<tr>
<td>$P_{eff}$, rat ($x 10^{-4}$ cm/sec)</td>
<td>2.10</td>
<td>Predicted from MDCK-II</td>
</tr>
<tr>
<td>MDCK II $P_{app}$ ($x 10^{-6}$ cm/sec)</td>
<td>70.1</td>
<td>61</td>
</tr>
<tr>
<td>$ka$ (1/h)</td>
<td>7.55</td>
<td>Predicted from MDCK-II $P_{app}$</td>
</tr>
<tr>
<td>$V_{ss}$ (L/kg)</td>
<td>6.04</td>
<td>Predicted by Rodgers &amp; Rowland Method</td>
</tr>
<tr>
<td>$CL_{iv}$ (mL/min/250g)</td>
<td>19.75</td>
<td>59</td>
</tr>
<tr>
<td>$CL_{po}$ (mL/min/250g)</td>
<td>263.5</td>
<td>59</td>
</tr>
<tr>
<td>RLM (uL/min/mg)</td>
<td>131$^\dagger$</td>
<td>59</td>
</tr>
<tr>
<td>RHep (uL/min/10$^6$ cells)</td>
<td>74</td>
<td>52</td>
</tr>
<tr>
<td>RIM (enterocytes) (uL/min/mg)</td>
<td>34.92$^\dagger$</td>
<td>59</td>
</tr>
<tr>
<td>Additional CL (mL/min)</td>
<td>8.14</td>
<td>62</td>
</tr>
</tbody>
</table>

*Kotegawa et al* 59 observed $V_{ss}$ (L/kg) 3.1. $^\dagger$ an fumic of 0.88 was used based on data from human microsomal incubations 63
Supplemental References


Title Page:

**Simcyp Rat**: The Constraints, Construction and Verification of a Strain-Specific Physiologically-Based Pharmacokinetic Rat Model

Authors:

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*Musther and Harwood contributed equally to this project.
Running Title Page

Running title: Simcyp Rat a PBPK IVIVE model

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Abstract

The use of in vitro-in vivo extrapolation (IVIVE) techniques, mechanistically incorporated within Physiologically-Based Pharmacokinetic (PBPK) models, can harness in vitro drug data and enhance understanding of in vivo pharmacokinetics. This study’s objective was to develop a user-friendly rat (250g, male Sprague-Dawley) IVIVE linked PBPK model. A 13-compartment PBPK model including mechanistic absorption models was developed, with required system data (anatomical, physiological and relevant IVIVE scaling factors) collated from literature and analysed. Overall, 178 system parameter values for the model are provided. This study also highlights gaps in available system data required for strain-specific rat PBPK model development. The model’s functionality and performance was assessed using prior literature sourced in vitro properties for diazepam, metoprolol and midazolam. The results of simulations were compared against observed pharmacokinetic rat data. Predicted and observed concentration profiles in 10 tissues for diazepam after a single i.v. dose making use of either observed i.v. clearance (CLiv) or in vitro hepatocyte intrinsic clearance (CLint) for simulations generally led to good predictions in various tissue compartments. Overall, all intravenous (i.v.) plasma concentration profiles were successfully predicted. However, there were challenges in predicting oral plasma concentration profiles for metoprolol and midazolam, the potential reasons and according solutions are discussed.

Keywords: Preclinical Pharmacokinetics, Physiological Model, Simulations, Clearance, Absorption, Distribution.
Introduction

In drug discovery numerous in vitro assays are routinely performed with new chemical entities (NCE’s) in order to characterize absorption, distribution, metabolism and excretion (ADME) properties. Such studies can include; dissolution tests, permeability assays, tissue-based experiments utilizing pre-clinical and human tissues and the use of recombinant systems, where specific enzymes and transporters are expressed. These data permit informed decisions as to the viability of an NCE to proceed to the next stage of the development pipeline. Data generated using these systems also has the potential to enable the quantitative prediction of the rate and extent of drug absorption and disposition in the intact organism. This is true whether it is human or a commonly used pre-clinical species such as the rat, dog, mouse and monkey.

Due to limited availability of human tissues, in vitro assays utilizing rat blood, plasma and tissues are routinely performed to characterize drug-tissue distribution, metabolic stability and toxicology of an NCE. At the drug discovery stage, in addition to in vitro assays, in vivo studies in pre-clinical species are also carried out to assess relationships between pharmacokinetics (PK), toxicokinetics (TK) and pharmacodynamics (PD). In vivo rat studies are often used to define dosages for first-in-human studies, and to characterize metabolite safety. However this may not always lead to a successful predictive outcome.

Abbreviations: ADAM (Advanced Dissolution Absorption & Metabolism (model)); AP (Acid phospholipids); B:P (Blood to Plasma ratio); CLint (intrinsic clearance); CLiv (Intravenous clearance); CLr (Renal Clearance); CLu_all (unbound hepatic intrinsic clearance); Ef (fraction of drug extracted/metabolised in the gut); Ef (fraction of drug extracted/metabolised in the liver); EW (Extracellular Water); fu (fraction unbound in plasma); F (Fraction of drug escaping gut metabolism); fum (fraction of drug unbound in the enterocyte/gut); Fh (Fraction of drug escaping hepatic metabolism); GI (Gastrointestinal); HPGL (Hepatocytes Per Gram of Liver); IW (Intracellular Water); IVIVE (In Vitro – In Vivo Extrapolation); Kp,ALB (tissue-to-plasma Albumin ratio); Kp,LPP (tissue-to-plasma Lipoprotein ratio); KP (tissue-to-plasma partition coefficient); MPPGI (Microsomal Protein Per Gram of Intestine); MPPGL (Microsomal Protein Per Gram of Liver); NL (Neutral Lipids); NP (Neutral Phospholipids); PBPK (Physiologically-Based Pharmacokinetic); Q_sili (Villus blood flow); CLPerm (Clearance Permeability); Peff (effective jejunal permeability in rat); RIM (Rat Intestinal Microsomes); RIMe (Rat Intestinal Microsomes obtained by elution); RLM (Rat Liver Microsomes); RHep (Rat Hepatocytes); SEV (Surface Epithelial Volume); Vd (Volume of distribution at Steady State); WX (Weighted Mean)
when extrapolating from pre-clinical species to human via simple allometry, particularly if a metabolite unique to humans is identified.

A ‘bottom-up’, in vitro - in vivo Extrapolation (IVIVE) linked to whole body Physiologically-based Pharmacokinetic (PBPK) modelling approach acts as a cohesive mechanistic framework to harness and integrate in vitro data to predict drug disposition in a ‘virtual human population’. Using an IVIVE approach ‘drug’ data are used in conjunction with ‘system’ parameters i.e. the demographics, anatomy, physiology and genetics of the human individual or population along with ‘trial design’ data, i.e. dosage regimen, to predict the fate of a drug in a physiologically relevant ‘virtual human’ . A challenge in drug discovery is that there is little or no in vivo human data to gauge the success of the IVIVE approach when employing human in vitro assays. Therefore, one approach is to gain confidence in an IVIVE/PBPK strategy through successful modelling of drug PK in a pre-clinical species such as the rat. The scope of employing data generated in discovery using an IVIVE rat PBPK model framework has been demonstrated, and the utility of rat PBPK in a drug discovery lead optimization has also been reported.

The heightened application of mechanistic IVIVE-PBPK in the pharmaceutical industry, together with the increasing acceptance or even requesting of submissions utilizing PBPK models, has led to a growing scrutiny on the ‘drug’- and ‘system’-specific parameters used to construct and verify the PBPK model. Recent regulatory guidance has been drafted that provides specific advice on the qualification and reporting of PBPK application in regulatory submissions. It is clear that sponsors, in conjunction with any providers of specialised PBPK tools should be in a position to provide suitable justification of the parameters employed to facilitate model qualification. The provision of robust, well qualified, system parameters is critical in this regard. Any alterations to ‘system’ parameters
made arbitrarily, could render it more challenging to qualify the models when incorporated into a regulatory submission.

Herein, we describe the Simcyp Rat Simulator (Version 12, Release 2); a generic whole body PBPK-IVIVE simulator principally designed for use in the drug discovery and toxicology setting. The rationale for undertaking the development and verification of the model will be described. The model structure and parameters are provided including physiologically-based system parameters, IVIVE scaling approaches, algorithm and differential equations that predict the relevant PK parameters, in order to facilitate qualification of PBPK models during regulatory submission, as well as determine temporal drug exposures within the tissues implemented within the rat model. The utility of the model will be explored and model performance verification will be detailed using in vitro and in vivo drug data collated from the literature.
Materials & Methods

The Model Framework

The aim of the model is to predict rat PK parameters and plasma and tissue concentration-time profiles by combining in vitro compound data with trial design regimes within a physiological framework. The model is based on an average 250g Sprague-Dawley rat. Laboratory rats are inbred and are used experimentally within narrow weight ranges (approximately 250g) to reduce experimental variability, thus one rat is assumed to differ insignificantly from another rat, unlike humans.

The model is based upon the free-drug hypothesis using well-stirred and perfusion limited assumptions, with the exception of the gastro-intestinal (GI) tract for which the permeability-limited, Advanced Dissolution Absorption and Metabolism (ADAM) model can be used.

The PBPK model contains 13 compartments (adipose, bone, brain, gut, heart, kidney, liver, lung, muscle, skin, spleen, plasma and red blood cells), linked by blood flows (arterial and venous compartments), where ordinary differential equations govern the drug-tissue ‘mass balance’ allowing derivation of tissue specific drug concentration-time profiles. The model structure is similar to widely published PBPK models (with exceptions explained below) and the non-eliminating tissue concentration profiles are described as follows:

\[
\frac{dC_T}{dt} = \frac{Q_T}{V_T} \left( C_{ab} - \frac{C_T}{K_{p,T}} \right)
\]

where \( V_T \) is the tissue volume, \( C_T \) is the drug concentration in the tissue, \( Q_T \) is the tissue blood flow and \( C_{ab} \) is the drug concentration in the arterial blood, \( K_{p,T} \) is the tissue to plasma partition coefficient and B:P is the blood to plasma concentration ratio of the drug.

The well-stirred liver differential equation is as follows:
\[
\frac{dC_{\text{liver}}}{dt} = \frac{1}{V_{\text{liver}}} \left[ (Q_{\text{liver}} - Q_{\text{pv}}) C_{\text{ab}} + Q_{\text{pv}} C_{\text{pv}} - \left( \frac{Q_{\text{liver}}}{K_{P_{\text{liver}}} B : P} - \frac{Q_{\text{pv}}}{K_{P_{\text{liver}}} B : P} \right) \frac{\text{fu}}{B : P} C_{\text{liver}} \right]
\]  

(2)

Where liver, pv, and ab subscripts refer to the liver, portal vein and arterial blood respectively. \( C \), \( Q \) and \( V \) represent drug concentration, blood flow and tissue volume respectively, \( K_{P_{\text{liver}}} \) is the liver to plasma partition coefficient, \( \text{fu} \) is the fraction unbound in plasma, and \( CL_{\text{intHep}} \) is the total hepatic intrinsic clearance (either as an intrinsic clearance (\( CL_{\text{int}} \)) or \( V_{\text{max}}/K_m \)).

The results of a simulation are dependent on the drug, system and trial parameters independently defined and entered into the model prior to running the simulation (see Table 1).

**System Parameters**

Analysis of a random selection of ten *in vivo* and ten *in vitro* rat pharmacokinetic studies published in the journal ‘Drug Metabolism and Disposition: the biological fate of chemicals’ from years 2005 – 2008 found that the Sprague-Dawley rat was the most commonly used strain with 75% (15/20) studies assessed utilizing this strain. It was therefore endeavored to build the model based on the Sprague-Dawley strain. Where physiological and anatomical system data were available for the Sprague-Dawley rat in the literature, these values were preferentially selected (c.f. data from other strains). In the absence of Sprague-Dawley-specific information, values from another rat strain were used. Original reports were used where possible rather than consensus/review articles (e.g., Davies & Morris\(^\text{18}\)). Using this approach, it was possible to scrutinize the materials, methods, results and study conclusions allowing informed decisions on elements such as rat size and strain. It also allowed for the correction of parameters to the standard rat weight (i.e. 250g) used in the
model, and the potential for removal of any error or ‘misquotation’ that might occur if citing from a secondary source. Where data from more than one study were available for a given parameter, a weighted mean value (WX) was calculated (3).

\[
WX = \frac{\sum_{j=1}^{J} (n_j \cdot \bar{x}_j)}{\sum_{j=1}^{J} n_j}
\]

(3)

where \(n_j\) is the number of reported studies and \(x_j\) is the mean value of the \(j^{th}\) study.

**Compound Parameters**

The model requires commonly used physicochemical and blood/plasma binding parameters such as molecular weight, octanol-buffer partition coefficient (logP_{o:w}), compound type (acid/base/ampholyte/neutral) and related pKa value(s), blood-to-plasma (B:P) partition ratio and fraction unbound in plasma (fu).

The model can also handle a simple single compartment absorption model and the more advanced ADAM model. The former consists of a single well-stirred compartment characterized by an absorption rate constant (\(k_a\)) and the fraction of dose leaving the gut lumen (\(f_a\)). The latter is a physiologically-based, multi-compartmental model, where the drug is handled as unreleased (from formulation), undissolved or dissolved state; dissolution rate can be modeled along with supersaturation and precipitation\(^{17}\). There are nine gastrointestinal (GI) compartments viz. the stomach, seven small intestinal compartments and the colon. The rat small intestine comprises a single duodenal compartment, four jejunum compartments and two ileum compartments. The greater anatomical length of the jejunum as a proportion of the total small intestinal length in rats necessitates that the rat intestinal compartment structure is different to that of the human model\(^{17}\). For reference, the human jejunum is represented by two compartments, with the relatively longer ileum comprising
four compartments. In both the first order and ADAM models, \( k_a \) can be predicted from effective jejunal permeability \( (P_{\text{eff}}) \) using (4)

\[
k_a = \frac{2P_{\text{eff}, \text{rat}}}{R}
\]

(4)

where \( P_{\text{eff}, \text{rat}} \) is the effective jejunal permeability in rat \( (10^{-4} \text{ cm/s}) \) and \( R \) (cm) is the radius of the small intestine. This \( k_a \) can then be utilised in the prediction of \( f_a \).

\( P_{\text{eff}, \text{rat}} \) can be predicted from \( P_{\text{eff}, \text{man}} \) (effective jejunal permeability in human) based upon a simple direct correlation between human and rat jejunal permeability described by Cao et al. \( ^{19} \) – verapamil, an outlier, was excluded by these authors from this relationship. There is a mistake in the reported equation, and Equation 5 in this article is the corrected version implemented in the model.

\[
P_{\text{eff}, \text{rat}} = 0.6362 \times P_{\text{eff}, \text{man}}^{0.542}
\]

(5)

Where both \( P_{\text{eff}, \text{man}} \) and \( P_{\text{eff}, \text{rat}} \) have the unit of \( 10^{-4} \text{ cm/sec} \). \( P_{\text{eff}, \text{man}} \) can be predicted from \( P_{\text{app}} \) (in vitro apparent permeability) measured using the in vitro cell monolayer systems MDCK-II and Caco-2 (pH6.5-7.4), Parallel Artificial Membrane Permeability Assay (PAMPA) or the molecular descriptors polar surface area and hydrogen bond donor number. The Q\text{Gut} model \( ^{20} \), is used with the first order absorption model to predict the extent of intestinal gut wall metabolism (\( F_g \)). This model is based upon the balance between whole gut CL\text{ins}, and a hybrid term (Q\text{gut}) based upon villous blood flow (Q\text{villi}) and a clearance permeability term (CL\text{perm}) (6).

\[
Q_{\text{gut}} = \frac{\text{CL}_{\text{perm}} Q_{\text{villi}}}{\text{CL}_{\text{per}} + Q_{\text{villi}}}
\]

(6)
where \( CL_{perm} \) is clearance permeability and its calculation is based on the effective permeability and the cylindrical surface area of the relevant GI tract \(^{20}\) and \( Q_{villi} \) is villus blood flow.

To describe the distribution of the compound, the steady state volume of distribution \((V_{ss})\) obtained from \textit{in vivo} studies can be defined within the model. Alternatively, \( V_{ss} \) can be predicted by one of two mechanistic methods; The Poulin & Theil method \(^{21}\), as corrected by Berezhkovskiy \(^{22}\), or the Rodgers and Rowland method \(^{23-25}\). Both of these models use tissue composition and compound physicochemical data to calculate \( Kp,T \) values and thence, in conjunction with tissue volumes, \( V_{ss} \). The framework can handle two distribution models, namely a minimal “lumped” PBPK model and a full PBPK model. In the case of the minimal model, the gut, portal vein and liver are separate compartments with all other organs lumped together in a single compartment (systemic compartment), therefore it is not a solely empirical single compartment model, but is limited in complexity compared to the full PBPK model \(^{26}\). In order to use the full PBPK model the \( Kp,T \) values must be provided (either as measured or predicted values). This full PBPK model is comprised of thirteen compartments, with a number of differential equations describing the drug distribution of the drug into these tissues. These equations also describe the residence of the drug within each tissue, and its eventual return to the systemic circulation. These equations (1) and (2) have been described previously.

Depending on the availability of the data elimination within the model can be predicted via several options; input of an \textit{in vivo} intravenous (\textit{i.v.}) or oral (\textit{p.o.}) clearance, plus the percentage of metabolite formed from \textit{in vivo} studies, enabling the definition of concentration-time profiles of the metabolite; or from \textit{in vitro} assays determining \( CL_{int} \). For an IVIVE strategy, unbound hepatic clearance (\( CL_{intH} \)) is predicted by rat liver microsome (RLM), or hepatocyte (R Hep) unbound intrinsic clearances (\( CL_{int} \)), which is scaled up via
rat microsomal protein per gram of liver (MPPGL, see Supporting Information, Table G), or via rat hepatocellularity (hepatocytes per gram of liver, (HPGL), Supporting Information, Table G), as shown in Equations 7 and 8, respectively.

\[
CL_{\text{u int, H}} = \frac{CL_{\text{int}}}{fu_{\text{mic}}} \times \text{MPPGL} \times W_L \tag{7}
\]

\[
CL_{\text{u int, H}} = \frac{CL_{\text{int}}}{fu_{\text{inc}}} \times \text{HPGL} \times W_L \tag{8}
\]

where \(fu_{\text{mic}}\) is the fraction of drug unbound in microsomes, \(fu_{\text{inc}}\) is the fraction of drug unbound within an incubation and \(W_L\) is liver weight.

The predicted \(CL_{\text{u int, H}}\) value is incorporated into well-stirred model in order to predict \(F_H\) and hepatic clearance (\(CL_H\)) \(^{27}\).

For prediction of unbound gut intrinsic clearance (\(CL_{\text{u int,G}}\)), rat intestinal microsomes are scaled via microsomal protein per gram of rat intestine (Equation 9; for details of MPPGI, see Supporting Information, Table G).

\[
CL_{\text{u int, Gut}} = \frac{CL_{\text{int}}}{fu_{\text{mic}}} \times \text{MPPGI} \times \sum_{i=1}^{7} (\text{wet wt.} \times L_i \times c.f) \tag{9}
\]

Where wet wt. is the wet weight of the intestinal \((i)\) segment (g/cm), where there are 7 small intestinal segments, \(L_i\) is the length of the intestinal segment, \(c.f\) is the correction factor accounting for differences in metabolic capacity of intestinal segments. For the purposes of this study both the in vitro data and required scaling factors were obtained via elution methods, so no additional correction factors are required.

The \(c.f\) term in equation 9, depicting the region-specific gut metabolic capacity (see Supporting Information, Table G) is not compound specific but reflects the differential distribution of CYP450 enzymes along the small intestine of the rat. To estimate the fraction of drug escaping gut wall metabolism (\(F_g\)) the ‘\(Q_{\text{Gut}}\)’ model, \(fu_{\text{gut}}\) and \(CL_{\text{u int,Gut}}\) are incorporated into a well-stirred equation of intestinal metabolism \(^{20}\) or the ADAM model is
used. There are also options for assigning additional unbound hepatic $\text{CL}_{\text{int}}$, a multiplicative scaling factor accounting for active uptake into hepatocytes, a non-metabolic renal clearance ($\text{CL}_{\text{R}}$) and an additional systemic clearance $^{27}$. The model contains similar features for metabolites to those for substrates.

**Drug Parameter Inputs**

For rat IVIVE, drug-specific data routinely generated in the development process are required. Ten substrate compounds were developed. For one compound, caffeine, the active metabolite paraxanthine is available. The minimum data required for IVIVE compound development are: compound type (acid, base etc.) and associated pKa values, log$P_{\text{o/w}}$, fraction of drug unbound in rat plasma (fu), blood-to-plasma ratio (B:P), an in vitro-derived hepatic $\text{CL}_{\text{int}}$ (rat liver microsomes or hepatocytes) and an estimate of $V_{ss}$. In oral dosing simulations the unbound fraction in enterocyte (fu$_{\text{gut}}$) of 1 was used. To permit the verification of model performance the *i.v.* (or *p.o.*) clearance and $V_{ss}$ are required from *in vivo* rat studies. The relative paucity of rat compound data compared to that published for humans limits the number of compound files that can be developed, verified and provided as examples.

**Trial Design**

Information on the design of the rat study can be incorporated into the model including the route and frequency of dosing, sampling duration and whether the drug is administered with food.

**Performance Verification**

Model performance verification was undertaken with Simcyp Rat (Version 12 Release 2) where simulated time courses were compared to studies available in the literature.
Diazepam concentration-time profiles were available in multiple tissues following *i.v.* administration \(^{28}\). Plasma concentration-time profiles were available for metoprolol \(^{29}\) and midazolam \(^{30}\) following both *i.v.* and *p.o.* administration. Observed concentration-time profile data from *in vivo* PK studies was extracted by graphical digitization techniques (Get Data Graph Digitizer, http://www.getdataygraph-digitizer.com). IVIVE simulations for all compounds used combinations of physicochemical descriptors, permeability assays, *in vitro* hepatocyte and microsomal assay data. The associated references are provided in the Supporting Information, Tables H-J. Clearances were calculated using Equation 10.

\[
Clearance = \frac{Dose}{AUC}
\]  

(10)

where AUC is the area under the concentration-time curve.
Results

System Parameters

In order to construct the model, 178 anatomical and physiological ‘system parameters’ were collected from the literature; the final values originated from 42 references (including a personal communication). The system parameter values, the strain of rat from which the values were obtained, and source references are provided in the Supporting Information, Tables A-G. Of the system parameter values (excluding tissue composition parameters due to data complexity, see ‘Results - Tissue Composition Parameters’ below for further detail) 36/93 (39%) were taken from Sprague-Dawley rat measurements, 40/93 (43%) were measured from Wistar rats, with a large proportion, 31/51 (61%) of parameters specific to the gastrointestinal tract and ADAM model, being derived from experimental measurements in this strain. The remaining values (17/93, 18%) were obtained from one of the following categories: a strain of rat other than Sprague-Dawley or Wistar; the strain was not known; the value was scaled from human, or a value was calculated based upon other parameters, due to the lack of experimental values. These data highlight the challenges associated in building a strain-specific rat model, particularly in relation to the paucity of applicable GI parameters available in the literature for the Sprague-Dawley strain. If an i.v. dosing strategy is used for a simulation, i.e. not requiring employment of GI/ADAM parameters, a model with greater inclusivity of Sprague-Dawley ‘systemic’ parameters, 29/42 (69%) is utilised, with a lower proportion of Wistar parameters 9/42 (21%).

Tissue volumes

The volumes of 13 tissues were collected from 8 articles, where 7 values were obtained from studies in Sprague-Dawley rats (see Supporting Information, Table A). All tissue volumes were corrected to a 250g standard rat weight, assuming linear scaling from the
weight of the rat used in the study. Corrections for weight were required for 6 studies. Where possible, data for tissue volumes were obtained from original studies rather than consensus articles, however the volume of bone (16.47 mL) was obtained from a study where the parameter was not directly measured and cannot be traced to an original article. Therefore, the veracity of this value can be questioned, leading to uncertainty surrounding this model parameter. The 16.47 mL bone volume was used in the absence of verifiable data from an original study.

**Tissue blood flows & cardiac output**

The blood flows connecting the tissue compartments are based on the percentage of blood flow routed to each tissue from cardiac output and only studies where the animals were conscious or had recovered from an anaesthetic regimen (if anaesthesia was performed) were included. This is due to the potential haemodynamic effects of anaesthetic agents. It was possible to obtain Sprague-Dawley specific cardiac outputs from 8 studies ($n = 99$). A weighted mean analysis followed and lead to a weighted mean cardiac output of 80 mL/min/250g with each organ receiving a percentage of cardiac output (Supporting Information, Table B). All values except villus blood flow were measured in Sprague-Dawley rats. The villus blood flow is not a rat specific value and is a calculated parameter based on the villus receiving 60% of the small intestinal blood flow. The lung receives 100% of cardiac output therefore this value does not require further verification from the literature. Five of the parameters, heart, kidney, liver (arterial & portal vein) and spleen were obtained from multiple reference sources, therefore the parameter values are provided as weighted means. It is critical to obtain accurate estimates of liver blood flow, as drugs for which extraction is limited by perfusion are particularly sensitive to this parameter. In the model, a total liver blood flow of 19.4 mL/min/250g, equating to 24.2% of cardiac output, is utilized.
The haematocrit is important in defining the drug B:P threshold, as B:P cannot be less than 1 minus the haematocrit. The haematocrit value of 43.9% is taken from Wistar rats, although a Sprague-Dawley rat value was available. As this value appears to be particularly high at 56%, the decision was taken to utilize the Wistar value. The splanchnic fed/fasted ratio is the final blood flow parameter required and is relevant to the modelling of the effects of food intake on drug absorption and disposition.

**Gastrointestinal parameters**

The anatomical and physiological GI system parameters are described in the Supporting Information, Table C. In the absence of rat-specific values, the total intestinal enterocyte volume was scaled from human values for the surface epithelial volume (SEV) per m² of intestinal cylindrical surface area. The SEV term includes the additional surface area provided by the villi but excludes additional surface area related to the presence of plicae circulares, which are present in humans but not rats. This scaling approach assumes that the villus surface expansion is the same in rats as in humans.

The anatomical lengths and diameters of each small intestinal segment are required for allocating the appropriate dimensions to each of 7 small intestinal compartments in the ADAM model. Surprisingly, there are relatively few studies that specifically focus on determining the lengths of the duodenum, jejunum and ileum, as many studies measured the total small intestinal length. Kararli stated that the ileum length is 2.5-3.5 cm, however, an ileal length of 20cm (adult Wistar rat, personal communication, Dr. Emma McConnell, The School of Pharmacy, University of London) was implemented. Dr. McConnell also provided data relating to unpublished initial stomach fluid volumes in the fed and fasted state and fasted state gastric emptying time via personal communication. Basal small and large intestinal fluid volumes required for intestinal compartment fluid dynamics, and stomach
and small and large intestinal pH were provided in published work from McConnell et al., 2008 (see Supporting Information for reference). Interestingly, rat stomach pH in the fasted state appears to be more alkaline than in the fed state, whereas observed values in humans show the opposite. Segmental blood flows (Q_villi %) were measured in Sprague-Dawley male rats (n=14). However, the blood flows given in the study results required converting from a blood flow per gram of tissue to a percentage of total blood flow for each of the 7 small intestinal segments in the model (Supporting Information, Table C footnote). It is assumed that there are no differences in small intestinal transit rate through each intestinal segment therefore transit rates are calculated based on segmental lengths as a proportion of total small intestinal length (Supporting Information, Table C footnote). The transit values do not directly relate to absolute transit rates from the literature, i.e. rate of movement of luminal contents through each segment, as there are no appropriate data available for 250g Sprague-Dawley rats. The compartmental wet weights required for metabolic scaling in the small intestine (equations 7 and 8) are from the Wistar rat control group in Sha et al.

**Tissue composition parameters**

All the tissue composition parameters in Supporting Information Tables D & E were sourced from publications by Rodgers and Rowland 23-25. However the parameter values provided were not directly measured within these studies, having been assembled from data available in the literature. These data are complex; with no one study summarizing the full composition of one tissue, and very few reporting one parameter for all required tissues. The data are therefore sourced from a number of different studies, each of which may contain details of total water, extracellular water, total lipids and/or individual lipids for specific tissues. These are then combined to calculate individual values for extracellular water (EW), intracellular water (IW), neutral lipids (NL), neutral phospholipids (NP) and acid
phospholipids (AP). If more than one reference was available for a value then a mean was taken; interested readers can refer to the original Rodgers et al. references. Where possible, original references and calculations were independently cross-checked, allowing a level of confidence in the cited data, and an indication of the strains used in determination of these values, as mentioned previously. Due to the nature of the data, no one tissue was determined fully from one strain of rat, although Sprague-Dawley and Wistar were the most commonly used.

At present, the tissue composition data required to predict $V_{ss}$ and $K_{p,T}$ values are almost exclusively determined from rat, with only minor exceptions, including the use of human plasma proteins in the determination of the tissue-to-plasma Albumin ratio ($K_{p,ALB}$) and the tissue-to-plasma Lipoprotein ratio ($K_{p,LPP}$)\textsuperscript{35,36}. This is dissimilar to other species such as human, where data are sparse and a full complement of system parameter data are not available.

Similar to human, rat plasma and extracellular is pH 7.4, whereas the intracellular compartment of tissues (measured as whole body intracellular pH) and red blood cells are slightly more acidic at pH 6.9 and 7.27, respectively.

**Metabolic scaling factors**

Sprague-Dawley specific rat liver metabolic scalars MPPGL and HPGL were incorporated into meta-analyses that included 4 studies ($n = 29$) for HPGL, and 3 studies for MPPGL including 26 samples (Supporting Information, Table G), a considerably lower number of studies than those measured for human. When compared to an average 25 year old human male, Sprague-Dawley rat scalars are similar; with HPGL for rat of $107 \times 10^6$ compared to a human value of $117.5 \times 10^6$ cells/g liver, and a rat MPPGL of $46 \text{ mg/g}$, close to the $40 \text{ mg/g}$ liver measured in humans \textsuperscript{37}. Also included in liver metabolic scaling is liver
density which was derived from a single study using 3 Sprague-Dawley rats. The GI-specific metabolic scalar MPPGI of 15.5 mg/g was derived from a single study of 6 rats (for references and derivation of the implemented values see Supporting Information, Table G). Included within the system parameters is the region-specific gut metabolic capacity, reflecting the differential distribution of cyp450 enzymes along the small intestine. Cyp450 absolute abundance data from 4 male Wistar rats was used, where the abundance of cyp2b1, cyp3a, cyp2c6 and cyp2d1 was assessed by immuno-blotting. The relative activity for the 4 jejunal segments is set to the maximum level of 1 with duodenum at 0.8 and the 2 ileal compartments having the lowest activity of 0.5. At present the model does not account for colonic metabolism therefore a scalar is not required for the large intestine.

Performance Verification

Concentration-time profiles following a single dose of diazepam (i.v.)

Predicted and observed concentration profiles in 10 tissues for diazepam after a single i.v. dose of 1.2 mg/kg are shown in Figure 1. Model inputs and the references associated to these simulations are provided in the Supporting Information, Table H. Using the observed i.v. clearance (CL_{iv}) for simulations lead to good predictions in various tissue compartments. Predictions based on in vitro hepatocyte CL_{int} data and Rodgers & Rowland V_{ss} predictions also provided a good agreement between predicted and observed profiles in most tissues, although there was a 2.1-fold under-prediction in plasma clearance (Dose/AUC). In the brain, GI and skin, the model over-predicted the tissue concentrations. The Rodgers & Rowland method predicts V_{ss} within 1.5-fold (5.2 L/kg observed vs. predicted 3.63 L/kg). It should be noted that the observed data were obtained from a pharmacokinetic study in Wistar rats.
Concentration-time profiles following a single dose of metoprolol (i.v. & p.o.)

Three doses of i.v. administered metoprolol (0.5, 1 & 2 mg/kg) were simulated and compared to observed data \(^{29}\) (Figure 2). Model inputs and the associated references are provided in the Supporting Information, Table I. For this compound, the original study reported AUC extrapolated to infinity, all simulations were extended to 72h, thus the clearance of the compound was complete (negligible concentration reported) and comparisons were made to the published values. For clarity, the figures show the comparisons of the simulated plasma concentration-time profile to the observed data, complete to the last observed data point. At all doses, a minimal PBPK model using an observed \(V_{ss}\) did not adequately describe the observed disposition of metoprolol, as shown in Figure 2. Predictions using a full PBPK model generated profiles with good prediction at all doses. Full PBPK simulations using observed \(CL_{iv}\) and IVIVE using RLM were undertaken demonstrating a reasonably good recovery of the observed plasma profiles. For the simulations with p.o. metoprolol given as solution (Figure 3), the aim was to identify if the incorporation of data generated early in the drug development pipeline (i.e. Caco-2 permeability, intestinal and liver microsomal data) could be used to predict metoprolol concentration-time profiles at 3 different doses (1, 2 and 5 mg/kg), using a simple first order absorption or the ADAM model. Intestinal permeability was predicted using Caco-2 \(P_{app}\) data generating a \(P_{eff,rat}\) of \(1.54 \times 10^{-4}\) cm/sec and a predicted \(f_a\) of 1. For 1 and 2 mg/kg doses using either the first order or ADAM model the clearance and AUC values are predicted within 2-fold. However, the \(C_{max}\) for the first order method is under-predicted (0.4 - 0.41 - fold), while it is much better predicted for the ADAM model (0.69 - 0.72 fold). This under-prediction of \(C_{max}\) could be linked to the 2.7-fold over-prediction of \(V_{ss}\) (predicted value of 10.97 versus the observed value of 4.08 \(^{29}\)). Data for intestinal and hepatic extraction ratios (\(E_G\) and \(E_H\), respectively) were provided for the observed study. The predicted \(E_H\) of 0.41 is
reasonably close to the \( E_H \) of 0.59 for the observed data. Compared to the observed \( E_G \) (0.49) this parameter is predicted well for the ADAM model (0.46), however, there is a considerable over-prediction in \( E_G \) for the first order simulation (\( E_G \) 0.72). Adjusting the gastric emptying for simulations to 0.11h in the ADAM gives more accurate predictions of \( T_{\text{max}} \). This is to be expected, as the original study gave the dose via intra-duodenal administration and decreasing the gastric emptying time will correct for this to some extent. The observed data at 5 mg/kg metoprolol shows dose non-linearity (a greater than proportional AUC change with dose) which the model fails to capture in all simulation conditions (Figure 3C). The model is capable of accounting for non-linear or saturable kinetics for whole organ scaling methods. Simulations using the ADAM model allow the estimation of the enterocyte concentration of metoprolol in all gut segments. This information combined with knowledge of intestinal microsomal \( K_m \) allows investigation as to whether if intestinal metabolism saturation is the likely cause of dose non-linearity. The \( K_m \) estimated in intestinal microsomes from Yoon’s study \(^{29} \) (24 \( \mu \)M) was utilized in this model to perform this investigation. However, in this case, the non-linearity could not be recovered, with only a slight increase in \( C_{\text{max}} \) and AUC observed. Unfortunately, the observed study data for \( E_G \) and \( E_H \) were not provided for the 5 mg/kg dose therefore a comparison for these parameters was not possible.

**Concentration-time profiles following a single dose of midazolam (i.v. & p.o.)**

Observed plasma concentration profiles for a single i.v. dose 5 mg/kg of midazolam\(^{30} \) were compared to simulated predictions (Figure 4). Model inputs and the references associated for these simulations are provided in the Supporting Information, Table J. As for the metoprolol, reported values were obtained from AUC extrapolated to infinity, so simulations were adjusted to account for this. For both observed CL\(_{iv}\) and IVIVE simulations using RHep and RIMe CL\(_{int}\) elimination data, the profile shows reasonable agreement to the
observed values with the clearance predicted within 1.03 – 1.66 fold and AUC predicted within 0.98-1.67 fold.

For p.o. simulations (single dose, 15 mg/kg \(^{30}\)) using \textit{in vitro} data, clearance is approximately 2- to 4-fold under-predicted leading to a 5- to 6-fold over-prediction in C\(_{\text{max}}\) and a 2- to 4-fold over-prediction in AUC, showing that for midazolam, the available \textit{in vitro} elimination parameter inputs combined with the additional systemic clearance input, cannot predict the observed \textit{in vivo} clearance. Using the observed CL\(_{\text{po}}\) with the ADAM model improves predictions for both C\(_{\text{max}}\) and AUC (Figure 5).
Discussion

The primary objective of this study was to build a strain-specific virtual rat model as a tool that can be utilised to assess the IVIVE approach in drug discovery, using prior drug physicochemical properties and *in vitro* ADME data. It is highly unlikely that *in vivo* human pharmacokinetic data are available to verify a human IVIVE approach at the discovery stage, therefore the availability of both rat *in vitro* data and *in vivo* pharmacokinetic studies can be used to assess possible outcomes of IVIVE approaches. The history of generic PBPK models goes back to 1937\(^40\). In the late 1990s and early 2000s methods to predict tissue to plasma partition coefficients were developed\(^41,42\).

When developing a model it is important that uncertainties surrounding system and drug parameters are minimised. Therefore, obtaining robust physiological system parameters that underpin PBPK model structure, together with comprehensive *in vitro* drug data, is essential to gaining confidence when employing an IVIVE approach. This is reflected in the collation and review of the individual system parameters required for the model. Original references were sought and scrutinized in preference to consensus or review articles in order to allow informed decisions to be made about the quality and suitability of the data to be selected and utilized. As the knowledge of the physiological processes governing the pharmacokinetics of a drug has advanced, a reciprocal advancement in a PBPK models infrastructure is required to meet these demands. Given the current model structure, one hundred and seventy-eight system parameters are required to generate the rat PBPK model described herein. With the current availability of data in the literature, it is a considerable challenge to build a model specific to the Sprague-Dawley rat strain, and for five parameters, no strain was specified. It is noteworthy that GI physiological parameter values were predominantly obtained from studies using Wistar rats, as there was no applicable data available for the Sprague-Dawley strain. Ideally, for each parameter, a meta-analysis containing numerous studies is performed.
in order to limit the potential bias when implementing a value from a single study. Due to the paucity of suitable data, 6 system parameters rely on a value from a single study. The certainty of a parameter is especially critical if simulation outcome is sensitive to a change in the value. For example, in the case of a high hepatic extraction compound, with a hepatic clearance greater than the implemented organ blood flow, the simulated clearance will be limited leading to an over-prediction in AUC. In the current model a liver blood flow rate of 19.4 mL/min/250g is used, which is greater than the values used in other rat PBPK models, i.e. 11.8 mL/min/250g \(^{12,43}\). A sensitivity analysis could be undertaken to identify whether liver blood flow is limiting hepatic clearance at the values used for simulation.

To verify the model performance, 3 compound models using key data to run IVIVE simulations were constructed from literature data. For diazepam, RHep CL\(_{int}\) data \(^{44,45}\) was preferentially used for simulation due to the better predictive performance when compared to rat liver microsomal CL\(_{int}\), however, clearance is still under-predicted when using the weighted mean hepatocyte CL\(_{int}\). There were good predictions of tissue concentration-time profiles. It is known that clearance predictions using in vitro CL\(_{int}\) data are generally under-predicted when using an IVIVE scaling approach, which is more prevalent when incorporating a protein binding term (fu) \(^{46}\). If clearance is not predictive, it is recommended that a CL\(_{int}\) is calculated in a retrograde manner from the in vivo clearance \(^{27}\). There has been reasonable success in predicting V\(_{ss}\) using a variety of approaches \(^{47}\), including PBPK, which has been applied in an independent study when using Simcyp Rat \(^{48}\). The predicted V\(_{ss}\) for diazepam using the Rodgers & Rowland method was within two-fold of the observed (Supporting Information, Table H). The observed data \(^{28}\) also describe a considerable partitioning of diazepam into adipose tissue with a Kp\(_{T}\) of 20.5 compared to a predicted Kp\(_{T}\) of 11.05. It is this disparity, combined with adipose tissue having a relatively large volume (17.5 mL, Table A, Supporting Information) that is likely to lead in-part to the 1.5 fold under-
prediction. Adjusting the adipose Kp,T by using the observed value of 20.5 improves Vss prediction to 4.29 L/kg.

The intravenous plasma concentration-time profiles were reasonably successfully predicted at 3 different doses for metoprolol (Figure 2) and for the 1 and 2 mg/kg p.o. studies (Figure 3A & B) using the first order absorption model. Simulations using the ADAM model predict a delayed T\text{max} compared to the observed data. Predictions can be improved by reducing the gastric emptying time from 0.25h to 0.11h. This is to be expected since the \textit{in vivo} data come from a study where the solution is administered directly into the duodenum, meaning gastric emptying will not delay absorption. The intestinal extraction of metoprolol using rat intestinal microsomes was predicted within 2-fold for both the first order and ADAM absorption models. However, there seems to be an under-prediction of the absorption phase, probably due to the 0.11h gastric emptying time not being sufficient to describe direct intra-duodenal administration. Enterocyte drug concentrations can be simulated in the ADAM model. For metoprolol, the C\text{max} in the proximal jejunum enterocyte compartment at 1 and 2 mg/kg is predicted to be 16.2 and 32.4 \mu M, respectively. The reported intestinal microsomal K\text{m} is 24 \mu M, therefore at 1 mg/kg metoprolol levels are below the concentration expected for metabolic saturation within these systems. Yet, at 2 mg/kg, the predicted enterocyte concentration is approaching the concentration required for metabolic saturation, however, the observed data does not exhibit dose non-linearity \textsuperscript{29}. At the highest dose (5 mg/kg), the observed data does show dose non-linearity, which could be due to saturation of metabolism. The predicted enterocyte concentration at 5 mg/kg in the proximal jejunum segment is 81.0 \mu M, a 3.4-fold higher concentration than intestinal microsomal K\text{m}, indicative of metabolic saturation in this segment. However, the model fails to capture the observed dose non-linearity using the whole organ metabolic clearance option for the V\text{max} and K\text{m} generated from Yoon \textit{et al.} \textsuperscript{29}, although there is an observed increase in the C\text{max} and
AUC (Figure 5). This could be due to the under-prediction of the absorption phase, or other ongoing processes not currently accounted for in the model.

When intravenously dosing midazolam, the IVIVE approach predicts the concentration-time profile reasonably successfully; this is not the case when midazolam is dosed orally. Despite *in vitro* and *in vivo* additional organ elimination data being available (*i.e.* pulmonary clearance), overall clearance is under-predicted by up to 5-fold. This could be due to a number of reasons. Considering the previous *i.v.* simulations, although predictions were reasonable, there was a slight under-prediction of clearance; in turn, this might contribute to the under-prediction observed in the *p.o.* simulations. Another possibility is that the midazolam permeability data from MDCK cell monolayers is not sufficient to describe the effective permeability in the intestine. The absorption phase is poorly described (Figure 5), with a potential over-prediction of $k_a$, even where the *in vivo* $CL_{po}$ is utilised, which could be another contributing factor to the over-prediction of AUC and $C_{max}$. Also, in the original study\(^{30}\), the rats used were larger (300-350g) than the standard rat of 250g described in this model, and increased in weight throughout the study, which may indicate that the simulations are no longer representative of the study. Finally, any uncertainty surrounding the *in vitro* drug data can lead to uncertainty in the results. In this case, the additional clearance value for pulmonary elimination came from a single study using data from Wistar rats\(^ {49}\). Uncertainty in this parameter may impact on the simulation results; therefore further studies investigating the pulmonary elimination of midazolam in rats are warranted. In the case of the simulations run in this study, elimination data from hepatocytes were utilised, with a single study found to determine midazolam $CL_{int}$ in rat hepatocytes. However, a search of the literature reveals inconsistencies in midazolam elimination data from rat liver microsomes, with values varying from 99\(^{44}\) to 1290\(^ {49}\) µL/min/mg. This highlights the requirement for robust *in vitro* data for use in predictive simulations. While it is possible that predictions for midazolam
could be improved if the additional clearance was normalised to the observed in vivo data, the objective of this study was to attempt to predict the in vivo pharmacokinetics from in vitro data. Therefore, this approach was not utilised in this instance, which clearly highlights the challenges of predictive IVIVE in certain cases.

Using an IVIVE approach in toxicity testing is advocated 50. By characterizing and combining a molecule’s metabolism, transport or binding in vitro, with the knowledge of the concentrations of that molecule that produces deleterious effects, the whole body exposure of the molecule for an organism can be predicted using an IVIVE approach linked to a PBPK model (IVIVE-PBPK) strategy. In the same manner as for pre-clinical therapeutics, this concept could be utilized to further characterize exposure levels in early toxicity screens in both environmental and pharmaceutical settings. In these cases, the IVIVE approach should be verified and refined in conjunction with in vivo exposure data. In addition, idiosyncratic adverse drug reactions in humans are particularly challenging to predict from pre-clinical toxicity testing, unless the appropriate pre-clinical model is employed in discovery-based toxicological studies 51. Using allometric scaling of rat PK parameters such as clearance to predict clearance in human is commonplace, a comprehensive assessment of allometric performance have been undertaken recently 46, however using allometric scaling methods even if successfully translated into humans, does not enlighten discovery scientists as to the mechanisms acting to contribute to a PK outcome.
Conclusion

The framework and mechanistic utility of a rat PBPK model has been demonstrated. The IVIVE-PBPK approach in pre-clinical species should be advantageous in a drug discovery setting; 1) to gain confidence in the IVIVE scaling strategy, in order to apply the approach to human IVIVE when using human in vitro assays, 2) to refine PK study design in rats leading to a reduction in the number of animals, 3) to obtain a greater mechanistic insight into the processes that govern the ADME properties of the investigation molecule and 4) by using the model to aid in elucidating the mechanism underlying any disparity in extrapolation predictions, i.e. sensitivity analysis where altering drug, system or trial parameters could provide an insight into the potential mechanisms underlying disparity in predictions. Any mechanistic knowledge gaps can be identified and model performance can be reassessed, a ‘predict, learn, confirm and apply’ paradigm.

In summary, the data presented here highlight an approach for obtaining the system parameters required to construct a strain-specific rat PBPK model and the challenges associated with this process. The utility of such a model is described for use within drug discovery; for identifying the mechanisms responsible for PK outcomes, and how the IVIVE approach can be employed to identify where further in vitro data are required to refine model predictions. This work focused on the models and algorithms dealing with the metabolism and passive transport processes. Incorporating active transporter mechanisms may improve predictions and provide better insight in drug disposition and effects.
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Conflict of Interest/Disclosure

Musther, Harwood, Turner and Jamei are employees of Simcyp Limited (a Certara company). Yang is the CEO of Mosim Co Ltd, Shanghai, China. A. Rostami-H. is an employee of the University of Manchester and part-time secondee to Simcyp Limited (a Certara Company).

Authorship Contributions

Participated in study design: Musther, Harwood, Yang, Turner, Jamei, Rostami-Hodjegan

Conducted experiments: Musther, Harwood, Yang.

Performed data analysis: Musther, Harwood, Yang.

Contributed to writing the manuscript: Musther, Harwood, Jamei, Turner.

Musther and Harwood contributed equally to this work.
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Figure legends

Figure 1. Comparison of simulated and observed tissue concentration profiles following a single intravenous dose of diazepam (1.2 mg/kg). Observed data given as solid triangles, simulations using observed clearance and predicted $V_{ss}$ given as dashed lines, and IVIVE simulations using RHep CL$_{int}$ given as solid lines.

Figure 2. Comparison of simulated and observed plasma concentration-time profiles following intravenous dosing at (A) 0.5 mg/kg, (B) 1 mg/kg and (C) 2 mg/kg metoprolol. Observed data given as solid triangles, simulations using observed $V_{ss}$ in a minimal PBPK model and observed clearance given as dotted lines, simulations using a Rodgers & Rowland predicted $V_{ss}$ in a full PBPK model and observed clearance given as dashed lines, and IVIVE simulations using a Rodgers & Rowland predicted $V_{ss}$ in a full PBPK model and rat liver microsomal CL$_{int}$ given as solid lines.

Figure 3. Comparison of simulated and observed plasma concentration-time profiles following oral dosing at (A) 1 mg/kg, (B) 2 mg/kg and (C) 5 mg/kg metoprolol. Observed data given as solid triangles, simulations using a first order absorption model and RLM and RIMe CL$_{int}$ are given as dashed lines, the ADAM model with RLM and RIMe CL$_{int}$ with gastric emptying rate at 0.25h given as solid lines, and ADAM model with adjusted gastric emptying (0.11h) given as a dot-dashed line. ADAM model with adjusted gastric emptying, and $K_m$-$V_{max}$ data are shown as dotted line.

Figure 4. Comparison of simulated and observed plasma concentration-time profiles following a single intravenous dose of midazolam (5 mg/kg). Observed data given as solid triangles, simulations using observed clearance and predicted $V_{ss}$ given as dotted lines, IVIVE
simulations using rat liver microsomal CL\textsubscript{int} given as solid lines and simulations using RHep CL\textsubscript{int} given as dashed lines.

Figure 5. Comparison of simulated and observed plasma concentration-time profiles following a single oral dose of midazolam (15 mg/kg). Observed data given as solid triangles, simulations using the first order absorption and Q\textsubscript{gut} model with IVIVE RHep/RIMe CL\textsubscript{int} elimination given as a dashed line, simulations using observed CL\textsubscript{po} with the ADAM model given as the dotted line and IVIVE RHep/RIMe CL\textsubscript{int} ADAM simulations given as a solid line.