

# An Evaluation of the Utility of Physiologically Based Models of Pharmacokinetics in Early Drug Discovery

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**ABSTRACT:** Generic physiologically-based models of pharmacokinetics were evaluated for early drug discovery. Plasma profiles after intravenous and oral dosing were simulated in rat for 68 compounds from six chemical classes. Input data consisted of structure based predictions of lipophilicity, ionization, and protein binding plus intrinsic clearance measured in rat hepatocytes, single measured values of aqueous solubility, and artificial membrane permeability. Log $P$  of compounds was high with a mean of 3.9 while free fraction in plasma (mean 9%) and solubility (mean 37  $\mu\text{g/mL}$ ) were low. Predicted and observed clearance and volume showed mean fold-error and  $R^2$  of 1.8, 0.56, and 1.9, 0.25 respectively. Predicted bioavailability showed strong bias to under prediction correlated to very low aqueous solubility and a theoretical correction for bile salt solubilization *in vivo* brought some improvement in average prediction error (to 31%). Overall, this evaluation shows that generic simulation may be applicable for typical drug-like compounds to predict differences in pharmacokinetic parameters of more than twofold based upon minimal measured input data. However verification of the simulations with *in vivo* data for a few compounds of each compound class is recommended since recent discovery compounds may have properties beyond the scope of the current generic models. © 2005 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 94:2327–2343, 2005

**Keywords:** pharmacokinetics; physiological model; simulations; computational ADME; absorption; GastroPlus; drug discovery

## INTRODUCTION

During drug discovery, considerable resources are required to assess the pharmacokinetic properties of potential drug candidates *in vivo* in animals and there is interest in optimizing the use of such testing by applying simulation.<sup>1–5</sup> Physiologically based pharmacokinetic (PBPK) models take *in vitro* and *in silico* data inputs and can predict concentration versus time profiles before any *in vivo* experiment is performed. If sufficiently reliable, such simulations could decrease the

turnaround time for delivery of information to medicinal chemists during the optimization phase and could also be used to prioritize compounds for the more costly *in vivo* testing. Equally importantly, the mechanistic framework provided by a PBPK model can integrate all available predictive data on a compound. Then, if comparison of simulation to *in vivo* data shows a large discrepancy, the need for further experiments to quantify additional processes may be indicated. Such integrative capabilities and mechanistic insights are not provided by the more commonly used noncompartmental or compartmental analysis and while pharmacokinetics in the rat is obviously not of ultimate interest for the pharmaceutical industry there is evidence that PBPK models are superior to other more empirical methods for interspecies scaling and prediction of human pharmacokinetics.<sup>6,7</sup>

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Thus a verified PBPK model in rat can be scaled to human to provide a basis for the rational selection of compounds for clinical development and if combined with a pharmacodynamic model allows prediction of the effective human dose.<sup>6,7</sup>

However, before such tools are routinely used they need to be extensively validated to define their accuracy and limitations. Here we assess the ability of generic PBPK models to predict plasma profiles in the rat for a set of 68 compounds taken from six chemical classes undergoing medicinal chemistry optimization in different drug discovery projects. The simulations are based upon minimal *in vitro* and *in silico* inputs such as are available at this early stage and the compounds were selected purely based on the availability of *in vivo* data. This study focuses on the practical utility of generic PBPK simulations and also discusses the required functionality of software for application in pharmaceutical drug discovery.

## METHODS

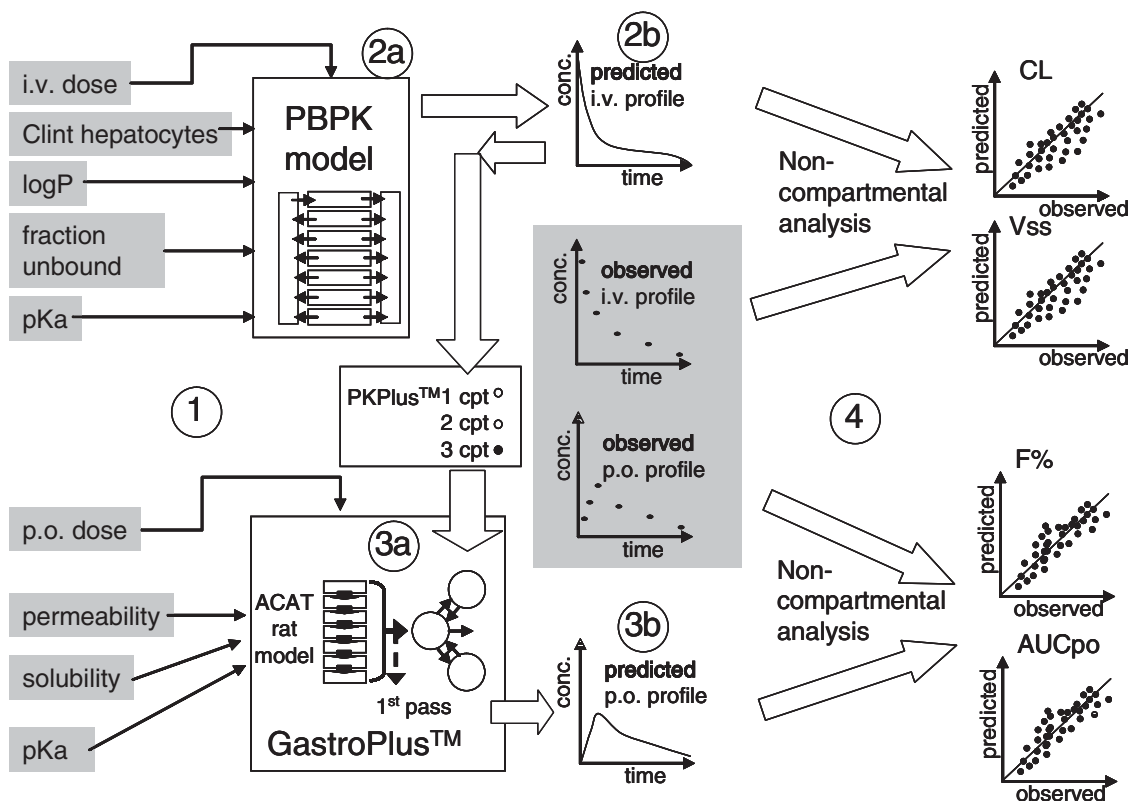
### Strategy

The steps taken are described below and are illustrated in Figure 1.

1. The data needed as input for the models are loaded into the simulation tools (experimental details are given below).
2. Use of the PBPK whole body disposition model involves:
  - a. Prediction of model parameters using established *in vitro* to *in vivo* scaling for clearance and mechanistic models of tissue distribution (described below).
  - b. Simulation of the concentration versus time profile of the compound after an intravenous bolus dose.
3. Simulation of plasma concentration versus time after an oral dose involves.
  - a. Simulation of the absorption versus time with a PBPK model of the GI tract.
  - b. Combining the predicted absorption with a compartmental disposition model fit to the simulated intravenous profile from 2b.
4. Comparison of simulated and observed plasma profiles and derived PK parameters.

### Simulation of Disposition Profiles

A previously described whole body PBPK simulation tool has been developed for generic



**Figure 1.** Steps taken in this evaluation.

application<sup>8</sup> and was applied in this study. The model includes 11 tissue compartments (adipose, bone, brain, gut, heart, kidney, liver, lung, muscle, skin, and spleen) linked together by the blood circulation. Tissue distribution is estimated using mechanistic tissue composition equations for prediction of *in vivo* partition coefficients.<sup>9,10</sup> These equations assume that drug distributes homogeneously into tissues and partitioning may be described by two processes: (1) nonspecific binding to lipids estimated from lipophilicity, and (2) specific reversible binding to common proteins present in plasma and tissue and estimated from the unbound fraction in plasma.

The input data for each compound are molecular weight,  $\log P$ ,  $pK_a$ , fraction unbound in plasma, blood/plasma ratio, clearance, and dose. Flow limited, well-stirred tissue models were used for all predictive simulations here but some exploration of the effect of a permeability limitation was made. (An overview of PBPK whole body models including equations for flow limited and diffusion limited tissue models is given in<sup>11</sup> and<sup>12</sup>).

For all simulations, the liver was assumed to be the only site of elimination and hepatic metabolic clearance was estimated by physiologically based scaling of the hepatocyte intrinsic clearance in  $\mu\text{L}/\text{min}/10^6$  cells accounting for hepatocellularity and liver weight.<sup>13</sup> For scaling *in vitro* intrinsic clearance to *in vivo*, the well-stirred liver model was applied<sup>14</sup> with the assumption that binding to plasma proteins *in vivo* is the same as binding in the hepatocyte incubation medium containing diluted serum. Thus the fraction unbound in plasma is cancelled out by the fraction unbound in the incubation and does not affect the final calculated clearance. The choice of this method is based on work (not shown here) where predictions of *in vivo* intrinsic clearance with the well-stirred model with and without consideration of plasma protein binding were compared for a range of compound classes. The liver blood flow in rat was taken as 60 mL/min/kg.

### Simulation of Absorption Profiles

The software GastroPlus<sup>TM</sup> 3.2.03 (Simulations Plus Inc., [www.simulationsplus.com](http://www.simulationsplus.com)) was used to simulate plasma profiles after oral dosing. The model underlying GastroPlus<sup>TM</sup> is known as the Advanced Compartmental Absorption & Transit model.<sup>15</sup> It is a physiologically based model consisting of nine compartments corresponding to different segments of the gastrointestinal tract.

GastroPlus<sup>TM</sup> takes drug specific input data for solubility, permeability,  $\log P$ ,  $pK_a$ , particle size, and dose and uses these in models describing the processes of dissolution, absorption, and intestinal transit to yield the amount absorbed into the portal vein as a function of time. Simulation of plasma concentration versus time profiles combines the predicted absorption with the simulated disposition. For use in GastroPlus<sup>TM</sup> the simulated disposition profile was fit with a 1, 2, or 3 compartment model with elimination taking place from the central compartment. This fitting was facilitated by a GastroPlus<sup>TM</sup> add-on module called PKPlus<sup>TM</sup> using an objective function based on the weighted sum of squared deviations of predicted from observed. The weighting was the reciprocal of the square of the predicted concentration and the choice of the best model was based on the Akaike information index.

For prediction of absorption a single permeability value was input for each compound and GastroPlus<sup>TM</sup> calculated the absorption rate for each compartment based on the physiological variation in regional surface area in the rat intestine. The variation of solubility with different intestinal regions was assumed to depend only on the pH of that region and was calculated from the ionization predicted from the  $pK_a$  values of the drug according to the Henderson-Hasselbalch equation<sup>16</sup> (a feature built in to GastroPlus<sup>TM</sup>). Dissolution was calculated assuming a suspension of spherical particles all with initial radius of 1 micron (dissolution according to a Noyes-Whitney<sup>17</sup> model is built in to GastroPlus<sup>TM</sup>). The first pass effect (FPE) was assumed to occur only in the liver and was calculated from the hepatic blood clearance predicted from hepatocytes. If  $CL_h$  is the scaled *in vivo* hepatic blood clearance and  $Q_h$  is the liver blood flow then the FPE was estimated as  $\text{FPE}[\%] = 100 * CL_h / Q_h$ .

### In Vivo Data

Plasma concentration versus time profiles for each drug were obtained from at least two male Wistar rats for each route of administration. Test compound was given intravenously by bolus injection through a catheter implanted into the jugular vein at doses ranging from 0.8 to 5 mg/kg and orally by gavage at doses ranging from 1 to 10 mg/kg. Blood samples were collected via catheter up to 48 h after administration. Analysis of parent compound was carried out in plasma using LC-MS/MS.

### *In Silico* and *In Vitro* Input Data

Structure based predictions of unbound fraction to plasma proteins were obtained with an artificial neural network model in QMPRPlus™ version 3.0.0 (Simulations Plus). Estimates of  $pK_a$  values were made with pKalc (CompuDrug International) and estimates of octanol/water partition coefficient were made with in-house software based on the atom/fragment method.<sup>18</sup>

The intrinsic clearance ( $\mu\text{L}/\text{min}/10^6$  cells) in rat hepatocytes ( $N=1$  or  $N=2$ ) was measured in a screening mode at an initial concentration of 10  $\mu\text{M}$ . Intrinsic clearance was obtained from the initial concentration of parent divided by the area under the concentration versus time curve extrapolated to infinity. Permeability measurements were obtained in a Parallel Artificial Membrane Permeation Assay (PAMPA).<sup>19,20</sup>

As GastroPlus™ expects values for human jejunal permeability as input it was necessary to transform the PAMPA values via a correlation built for 18 reference drugs with known human permeability.  $\text{Log}(\text{Human Peff}) = 3.47 + 1.31 * \text{Log}(\text{PAMPA})$  ( $N=18$ ,  $r^2=0.6$ ,  $\text{SE}=0.3$ ) (and when simulating absorption in the rat a correlation of rat to human permeability is built in to GastroPlus™.  $\text{Peff}_{\text{rat}} = 1.14 * \text{Peff}_{\text{man}} - 0.0643$ ).

Solubility was measured in a high throughput lyophilisation assay where stock DMSO solution is evaporated and the remaining amorphous powder is used to determine the thermodynamic solubility at pH 6.5 after overnight incubation. Fourteen compounds had solubility below the assay limit of detection of 1  $\mu\text{g}/\text{mL}$  and these were set to 1  $\mu\text{g}/\text{mL}$  for simulations.

For *in vivo* solubility estimation, a correction factor accounting for the enhancement due to bile salts in intestinal fluids was applied. This relationship between the increase in solubility and the lipophilicity of the drug was developed for a series of six steroids and a further five compounds of diverse chemical structures.<sup>21</sup> If the solubilization ratio is defined as  $\text{SR} = \text{SC}_{\text{bs}}/\text{SC}_{\text{aq}}$  (where  $\text{SC}_{\text{bs}}$  is the solubilization capacity of the bile salt in moles of drug per mole of bile salt and  $\text{SC}_{\text{aq}}$  is the solubilization capacity of pure water) then Mithani found that the hydrophobicity of the drug was the driving force for solubilization and obtained the following relationship:  $\text{Log SR} = 2.09 + 0.64 \text{ log } P$  ( $N=11$ ,  $R^2=0.951$ ). A concentration of bile salts of 15 mmol/L corresponding to the fed state in human was used in the development of this relationship

but for use here a concentration relevant for the fasted state in rat is needed. In line with reported bile salt concentration in the fasted proximal human small intestine of 3–5 mmol/L<sup>22</sup> and the fact that a more diluted bile is secreted in rat due to the lack of a gall bladder<sup>23</sup> a concentration of 2 mmol/L was chosen.

### Calculation of Pharmacokinetic Parameters

Noncompartmental analysis was performed for both observed and simulated profiles using the software WinNonLin® Professional version 3.1 (Pharsight). Clearance was calculated from the relationship:  $\text{CL} = \text{Dose}/\text{AUC}$  and volume of distribution at steady state was calculated as:  $V_{\text{ss}} = \text{CL} \times \text{AUMC}/\text{AUC}$ . The area under the curve, AUC, and area under the first moment curve, AUMC, were calculated using the logarithmic trapezoidal rule, and then extrapolated to time infinity by adding  $c/\beta$  to AUC and  $t \times c/\beta + c/\beta^2$  to AUMC, where  $c$  is the predicted concentration at the last sampling time  $t$  and  $\beta$  is the slope of the terminal phase, determined by log-linear regression of the last three or four data points.

The bioavailability was estimated as the ratio of dose normalized AUC after oral and intravenous administration using the mean of individual AUCs.

### Assessment of the Predictions

The following statistics were used to assess prediction accuracy.

The fold-error:

$$\text{fold\_error} = \begin{cases} \frac{\text{observed}}{\text{predicted}} & \text{if observed} > \text{predicted} \\ \frac{\text{predicted}}{\text{observed}} & \text{if predicted} > \text{observed} \end{cases}$$

The arithmetic average of the fold errors was used as a measure of bias for a compound class.

The geometric mean fold-error giving equal weight to under and over estimates is often used to assess predicted pharmacokinetic parameters. This was calculated as:

$$\begin{aligned} &\text{Geometric mean fold - error} \\ &= 10^{\sum_{i=1}^n |\log(\text{predicted}/\text{observed})|/n} \end{aligned}$$

The commonly used  $R^2$  statistic (square of the Pearson product moment correlation coefficient) was used to quantify the extent of linear relationship between predicted and observed parameters.

The Mean Error was used for assessment of the bias in bioavailability predictions.

$$\text{Mean error} = \frac{\sum_{i=1}^N (\text{observed} - \text{predicted})}{N}$$

The Root Mean Squared Error (RMSE) was used for assessment of the accuracy of bioavailability predictions:

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^N (\text{observed} - \text{predicted})^2}{N}}$$

## RESULTS

### Physicochemical Properties of the Compounds (Table 1)

Molecular weight ranged from 300 to 670, with a mean of 438. The lipophilicity was high with mean calculated  $\log P$  of 3.9 and values ranging between 0.6 and 7.5. Seven of the compounds were acids with a  $\text{p}K_a < 8$ , 14 were bases with a  $\text{p}K_a > 6$ , and the remainder were neutral at physiological pH. The free fraction in plasma was low with a mean of 9% and values ranging from less than 1% to 37%. The estimated human jejunal permeability ranged between 0.3 and  $8.9 \times 10^{-4}$  cm/s indicating medium or high permeability for all compounds. Aqueous solubility was low for all compounds with the average being 37  $\mu\text{g/mL}$  and values ranging from  $<1$   $\mu\text{g/mL}$  to 190  $\mu\text{g/mL}$ .

### Pharmacokinetic Parameters of the Compounds

Pharmacokinetic parameters of the 68 compounds analyzed are shown in Table 2. The clearance ranged from very low to greater than liver blood flow (1–123 mL/min/kg) with an average value of 27 mL/min/kg. Volume exceeded extracellular water (0.2 L/kg) for all compounds and mostly exceeded total body water (0.7 L/kg) ranging from 0.5 to 17 L/kg with an average of 3.7 L/kg. The bioavailability ranged from 2% to over 100% with a mean of 66%. Six compounds showed a bioavailability considerably in excess of 100% which may indicate nonlinearity in pharmacokinetics or could be related to experimental or analytical problems. These values were set to 100% in calculation of statistics.

### Variability and Uncertainty

The error in *in silico* and *in vitro* input data is clearly a source of error in the final prediction of

pharmacokinetics. We estimate that calculated  $\log P$  and  $\text{p}K_a$  have a standard error of 0.5 log units when applied to novel structural classes and predicted protein binding is very approximate showing a RMSE of 0.27 for  $\log(\%$  unbound) ( $N = 33$ ) (QMPRPlus, Simulations Plus). Our correlation of PAMPA permeability to human jejunal permeability shows a standard error of 0.3 log units.

Pharmacokinetic parameters calculated from the *in vivo* data are also associated with error. The geometric mean of the maximum divided by minimum of the observed values of clearance and volume for the 68 compounds was 1.4 in both cases. Bioavailability showed a mean range of 23%.

### Comparison of Simulated and Observed Disposition Pharmacokinetics

The results for clearance and volume are shown in Table 3 and Figures 2 and 3.

For clearance (Fig. 2) the mean fold-error was 1.8 and 59% of predictions were within twofold of the observed value. The  $R^2$  was 0.56. For comparison if the mean observed clearance of 27 mL/min/kg is taken as predicted value for all compounds the mean fold-error is 3.2 and 31% are within twofold. Many of the compounds in the E project showed a total *in vivo* clearance exceeding liver blood flow and so were under predicted while D project compounds tended to be over predicted. No clear trends were noticed based on physicochemical properties of the compounds. Considering the variability in the *in vivo* profiles showed that although 28 of the 68 predictions fall outside of a twofold range of the mean observed clearance 13 of these compounds would be within twofold of one observed value.

For volume of distribution (see Fig. 3) the mean fold-error was 1.9 and 62% of predictions were within twofold of the observed volume. The  $r^2$  value was 0.25. For comparison, if the mean observed volume of 3.7 L/kg is taken as predicted value for all compounds the mean fold-error is 2.1 and 50% are within twofold. E project compounds tended to be under predicted while volume was often over predicted by more than twofold for D and F project compounds. Compounds in both of these projects show very high lipophilicity and very low free fraction in plasma. A general trend for increasing volume with  $\log D$  was seen up to  $\log D$  of 4 but for values above 4 the trend did not hold for these 68 compounds (see Fig. 4). In terms of charge, the F compounds were acids and were all

**Table 1.** *In Vitro* and *In Silico* Data for the 68 Compounds

Compound	Project	Molecular Weight	Intrinsic Clearance (mL/min/10 <sup>6</sup> Cells)	Calculated LogP	Calculated Fraction Unbound	Acid/Base/Neutral	pK <sub>a</sub>	Aqueous Solubility (μg/mL)	Estimated Human Jejunal Permeability (10 <sup>-4</sup> cm/s)	Blood/Plasma Ratio
1	A	454	2.2	2.6	0.18	B	9	78	0.4	
2	A	391	6.3	2.0	0.21	N		74	3.1	
3	A	407	2.3	2.5	0.18	N		59	2.6	
4	A	420	6.4	4.2	0.11	N		188	1.4	
5	A	421	5.6	2.9	0.16	N		40	2.3	
6	A	409	4.3	3.3	0.17	N		190	0.3	
7	A	419	3.8	2.6	0.16	N		19	2.2	
8	A	419	7.7	2.6	0.16	N		40	2.3	
9	A	419	5.5	2.6	0.16	N		43	2.0	
10	B	302	1.3	2.1	0.15	N		<1	6.0	0.73
11	B	324	2.7	1.5	0.12	N		21	8.1	
12	B	334	2.3	1.8	0.10	N		11	6.1	0.81
13	B	342	3.5	1.6	0.11	N		16	4.6	
14	B	314	0.6	0.7	0.15	N		16	6.2	
15	B	342	6.0	1.7	0.10	N		15	5.7	
16	B	320	2.8	2.3	0.12	N		<1	2.0	
17	B	342	6.1	1.6	0.11	N		102	3.2	
18	B	342	3.1	1.6	0.11	N		129	3.1	
19	B	342	4.8	1.7	0.10	N		117	1.6	
20	B	342	1.0	1.7	0.10	N		3	3.2	0.93
21	B	342	1.2	1.7	0.10	N		2	3.3	1.17
22	B	342	0.9	1.7	0.10	N		29	3.9	1.42
23	B	353	1.5	1.7	0.10	N		20	3.6	1.04
24	B	342	3.9	1.7	0.10	N		105	1.6	
25	B	342	6.9	1.7	0.10	N		23	1.6	
26	C	374	1.2	2.8	0.16	B	8	<1	0.8	1.00
27	C	381	1.8	4.4	0.05	N		<1	3.6	0.91
28	C	479	2.3	5.4	0.03	N		<1	2.7	1.05
29	C	428	0.7	3.2	0.09	B	8	<1	2.7	
30	C	396	1.2	5.3	0.04	N		<1	3.1	
31	C	409	0.7	5.5	0.03	N		<1	3.3	1.15
32	C	439	0.4	5.5	0.03	N		<1	2.6	
33	C	395	2.1	5.0	0.04	N		<1	4.0	0.86
34	C	423	2.1	6.0	0.03	B	6	<1	4.8	1.00



**Table 2.** Pharmacokinetic Data for the 68 Compounds

Compound	Project	IV Dose (mg/kg)	Observed Blood Clearance (mL/min/kg)	Predicted Blood Clearance (mL/min/kg)	Observed Volume (L/kg)	Predicted Volume (L/kg)	PO Dose (mg/kg)	Observed Bioavailability %	Predicted Bioavailability %	Observed Oral AUC/Dose (hr·µg/mL/mg)	Predicted Oral AUC/Dose (hr·µg/mL/mg)
1	A	1.0	8.0	8.6	1.2	2.3	10.0	91	42	7.6	2.5
2	A	1.0	12.0	20.2	1.3	1.3	1.0	30	66	1.7	4.6
3	A	5.0	12.0	8.9	1.6	2.3	5.0	101	85	5.6	4.8
4	A	2.0	21.0	20.3	3.5	7.8	2.0	71	66	2.3	3.0
5	A	2.0	20.0	18.3	3.1	3.8	2.0	76	69	2.5	2.7
6	A	5.0	4.0	15.0	1.2	5.7	5.0	51	63	8.5	12.0
7	A	3.0	16.0	13.7	2.6	2.6	3.0	58	77	2.4	2.3
8	A	3.0	37.0	23.2	3	2.6	1.8	71	61	1.3	1.3
9	A	3.0	9.0	18.2	0.9	2.6	2.3	61	69	4.6	5.7
10	B	1.0	5.0	4.0	1.3	1.3	5.0	76	21	10.2	0.7
11	B	1.0	19.0	10.2	1.8	0.6	3.0	37	83	1.3	2.1
12	B	1.0	8.0	7.2	2	0.9	5.0	78	87	6.5	3.1
13	B	1.0	19.0	12.5	1.5	0.7	3.0	82	79	2.9	1.5
14	B	1.1	2.0	2.7	0.7	0.4	1.6	55	94	18.2	21.0
15	B	1.0	19.0	19.2	1.4	0.7	3.0	48	68	1.7	1.3
16	B	1.0	3.0	10.5	0.5	1.6	3.0	54	12	11.9	1.0
17	B	1.0	21.0	19.7	1.7	0.7	3.0	55	67	1.7	4.1
18	B	1.0	10.0	11.6	0.9	0.7	3.0	47	81	3.1	5.1
19	B	1.0	15.0	11.1	2.2	0.7	2.8	76	81	3.4	12.7
20	B	1.0	2.0	3.7	1	0.8	3.0	69	47	23.2	3.5
21	B	1.0	2.0	5.9	0.9	0.8	2.4	40	39	13.4	2.6
22	B	1.0	4.0	5.3	1	0.7	3.0	66	91	11.0	12.8
23	B	1.0	3.0	6.2	0.8	0.7	2.5	95	89	21.0	10.6
24	B	1.1	4.0	3.6	1.1	0.7	2.5	157	93	26.2	16.3
25	B	1.0	10.0	21.3	1.3	0.7	2.9	76	64	5.1	2.6
26	C	1.3	8.0	6.4	5.7	2.9	10.0	46	2	3.9	0.1
27	C	2.5	23.0	10.9	3.4	5.9	10.0	3	8	0.1	0.1
28	C	2.0	14.0	12.2	5.8	5.4	9.0	16	6	0.8	0.2
29	C	2.5	8.0	3.7	6.5	3.8	10.0	95	5	7.9	2.4
30	C	2.5	9.0	5.9	4.5	5.8	9.0	45	8	3.3	0.3
31	C	2.0	6.0	3.5	4.9	5.5	7.2	101	9	11.2	0.3
32	C	5.0	5.0	2.4	6.6	5.5	8.5	177	8	23.6	0.8
33	C	1.0	18.0	12.6	1.3	5.8	7.2	15	11	0.5	0.2
34	C	2.5	6.0	8.4	6	5.3	9.0	55	11	6.1	0.7
35	C	1.0	17.0	7.9	6.7	6.2	6.4	293	7	11.5	0.7
36	D	2.0	16.0	21.8	2.4	4.7	2.0	36	24	1.5	0.1
37	D	5.0	6.0	4.4	4.4	6.2	5.0	34	72	3.7	2.0
38	D	2.5	6.0	18.7	1.8	4.7	2.5	78	26	8.7	0.4
39	D	2.5	3.0	7.3	1.2	4.7	2.5	53	31	11.9	0.9
40	D	2.8	15.0	20.9	4.2	4.7	4.8	70	17	3.1	0.1
41	D	2.5	5.0	16.7	1.9	4.8	5.0	43	17	5.7	0.5
42	D	5.4	13.0	23.0	2.8	4.7	5.9	73	13	3.8	0.2
43	D	5.0	5.0	15.1	2.1	4.7	5.0	72	17	9.7	0.4
44	D	1.5	8.0	14.3	2	4.9	5.0	62	24	5.2	0.1



45	E	1.0	121.0	44.9	7.9	5.9	10.0	6	22	0.0	0.1
46	E	1.0	123.0	44.4	8.1	5.6	10.0	7	32	0.1	0.3
47	E	1.0	88.0	41.0	13	5.6	10.0	6	21	0.0	0.1
48	E	1.0	120.0	33.0	5.6	3.8	10.0	30	4	0.3	0.2
49	E	1.0	107.0	41.2	6.3	5.0	10.0	2	9	0.1	0.4
50	E	1.0	114.0	45.2	8.5	7.1	10.0	13	25	0.3	0.3
51	E	1.0	58.0	36.4	7.5	5.3	10.0	33	61	0.3	0.8
52	E	0.9	22.0	41.1	10.3	4.6	8.3	15	67	0.1	0.4
53	E	1.0	66.0	42.9	4.9	6.6	10.0	68	40	4.1	2.9
54	E	1.0	29.0	39.7	15	6.0	10.0	42	3	0.6	0.2
55	E	1.0	77.0	23.6	2.6	0.6	8.5	35	62	0.2	0.3
56	E	0.8	99.0	19.0	4.4	0.6	6.1	10	9	0.4	0.1
57	E	1.0	11.0	36.0	1.5	0.8	6.1	49	10	1.2	0.1
58	E	1.0	45.0	32.8	17.1	4.5	6.1	144	0	4.6	0.0
59	E	1.0	105.0	22.8	2.2	0.7	8.5	91	43	0.9	0.5
60	E	0.8	16.0	18.6	4.3	4.3	7.5	99	88	66.2	48.4
61	E	1.0	27.0	9.3	4.5	1.7	5.5	206	74	68.5	35.6
62	F	1.1	21.0	18.6	2.3	4.3	3.0	81	95	10.8	12.0
63	F	1.1	66.0	24.7	2.6	4.3	9.0	106	24	17.6	1.6
64	F	1.0	1.0	3.6	1.3	4.3	6.4	110	31	12.3	1.6
65	F	1.1	2.0	1.1	0.5	4.3	8.0				
66	F	1.0	5.0	2.7	2.5	4.3	6.8				
67	F	1.0	4.0	2.8	2.4	4.3	10.0				
68	F	0.9	6.0	12.1	0.8	4.3	8.1				

over predicted, bases in the E and C projects tended to be under predicted while neutral compounds showed both under and over predictions.

Considering the variability in the *in vivo* profiles showed that, although 26 of the 68 predictions fell outside of a twofold error range of the mean observed volume, 10 of these compounds would be within a twofold range of one observed value.

The impact of error in input data on the simulated pharmacokinetics was investigated by noting the sensitivity of predicted volume to changes in the two key input parameters of  $\log P$  and fraction unbound. A change in  $\log P$  of 0.5 log unit resulted in an average change in volume of 30% while a change of 0.27 in  $\log(\% \text{unbound})$  resulted in an average change of 12%. However this sensitivity is very dependent upon the compound class since above values of 4 the tissue distribution equations are insensitive to changes in  $\log P$  and similarly for fraction unbound below 1%. For compound classes such as A with more moderate lipophilicity the same changes in  $\log P$  and  $\log(\% \text{unbound})$  produced changes of 60% and 20% in predicted volume.

The correlation of predicted versus observed plasma concentrations is shown in Figure 5. 58% of predicted concentrations were within twofold of the observed concentration at that time point. A trend for under prediction of higher concentrations (earlier time points) and over prediction of lower concentrations was apparent. This pattern could be consistent with permeability limited distribution into tissues as was verified with a simplified model including just two tissues (muscle and fat) with a diffusion barrier between extracellular and cellular spaces. Reducing the permeability-surface area parameter by a 100-fold changes the distribution behavior from perfusion limited to diffusion limited (Fig. 6). Noncompartmental analysis of these simulated profiles showed the estimates of volume of distribution at steady state changes from 3.3 L/kg for the perfusion limited case to 0.6 L/kg with a large permeability limitation. The estimate of clearance is also affected but the change is less pronounced (10.9 to 10.2 mL/min/kg).

#### Comparison of PK Parameters from PO Profiles

Simulations could be made for only 64 of the 68 compounds due to missing oral profiles for four compounds.

**Table 3.** Statistics for Predicted Clearance and Volume of Distribution

	Clearance				Volume of Distribution			
	Arithmetic Average Fold Error	Geometric Mean Fold Error	% Within Twofold Error	$R^2$	Arithmetic Average Fold Error	Geometric Mean Fold Error	% Within Twofold Error	$R^2$
A	0.3	1.5	78	0.39	1.2	1.7	67	0.22
B	0.5	1.5	81	0.55	-1.5	1.8	63	0.04
C	-1.4	1.7	60	0.60	0.0	1.5	90	0.10
D	1.9	2.0	56	0.52	2.2	2.1	44	0.35
E	-1.7	2.4	35	0.10	-2.2	2.0	53	0.36
F	-0.5	1.8	43	0.76	3.5	2.8	57	0.04
All projects	-0.3	1.8	59	0.56	-0.1	1.9	62	0.25

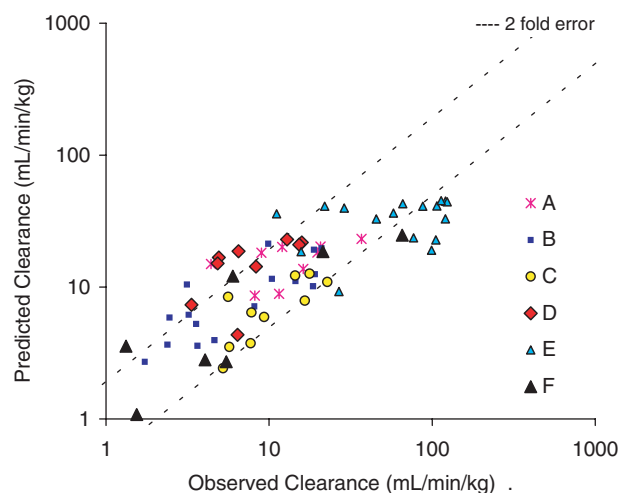
### Bioavailability

The graph of predicted versus observed bioavailability is shown in Figure 7 and the project by project statistics are given in Table 4. The RMSE in prediction is 40% and there is a tendency for under prediction with 64% of compounds under predicted and a mean error of 16%. Compounds in projects C, F, and D all showed under predictions. The under prediction of bioavailability is strongly correlated to low solubility as shown in Figure 7. 27 of 29 compounds with solubility less than 6  $\mu\text{g/mL}$  have their bioavailability under predicted. No obvious trend with ionization of compounds (acid, base, neutral) or permeability was seen but there was a clear trend for under prediction with  $\log P$  values above 4. On repeating the simulations with solubility adjusted for bile salt solubilization, the overall trend for under predic-

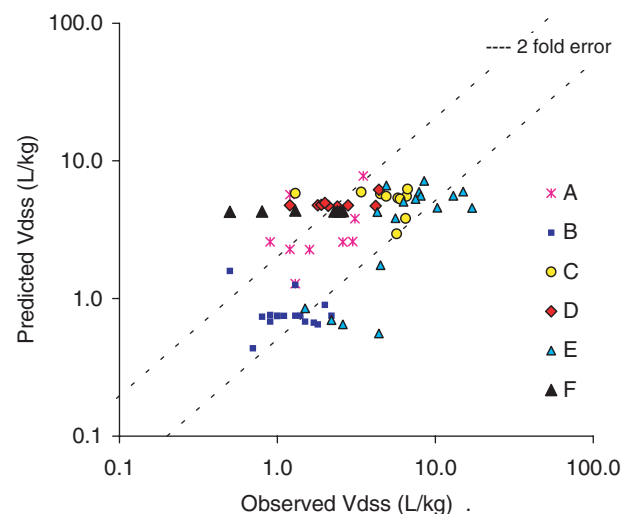
tion of bioavailability was removed (see Tab. 4). However a RMSE of 31% remains and the correlation of predicted and observed is still weak ( $R^2 = 0.16$ ).

### Area-Under-the-Curve (AUC)

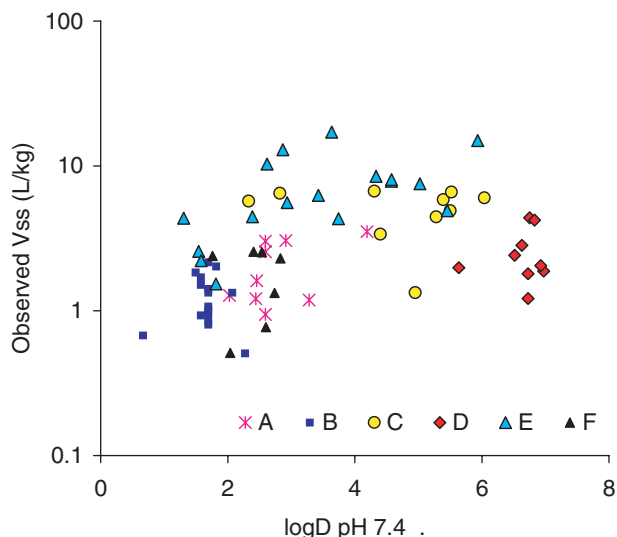
Simulated and observed dose normalized AUC is shown in Table 5. The mean fold-error was 3.5, 36% of predictions were within twofold of the observed AUC and the  $R^2$  value is 0.56. With use of the predicted *in vivo* solubility the mean fold-error is reduced to 2.6, 48% of compounds are within twofold of observed and the  $R^2$  increases slightly to 0.6 (Fig. 8). This improvement is mainly due to the compounds in the C, D, and F projects where the mean fold-error is improved considerably by the solubility correction.



**Figure 2.** Predicted versus observed clearance for 68 compounds. Symbols indicate different chemical classes.



**Figure 3.** Predicted versus observed volume for 68 compounds. Symbols indicate different chemical classes.



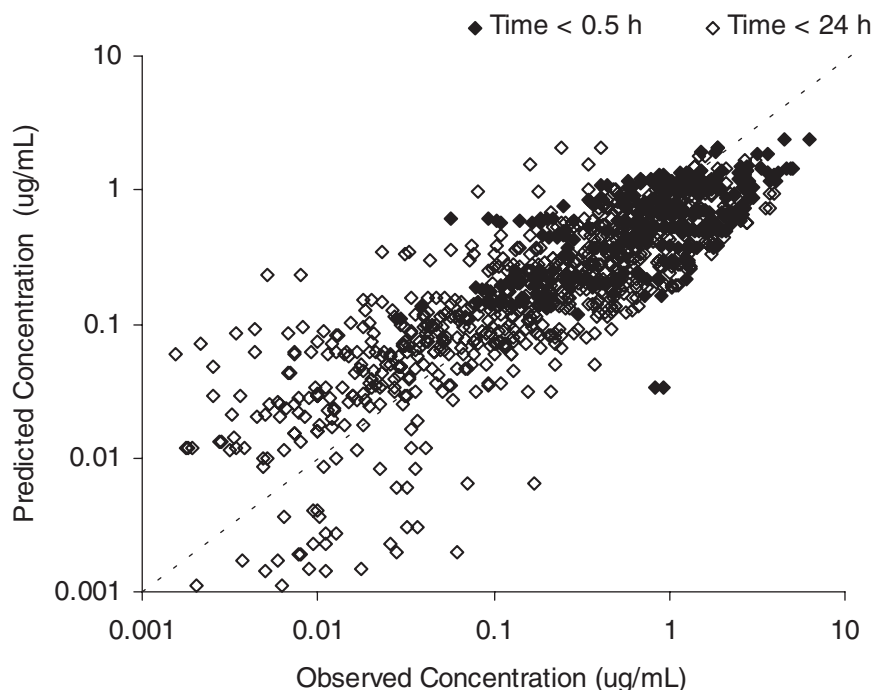
**Figure 4.** Observed volume versus  $\log D$  for 68 compounds. Symbols indicate different chemical classes.

## DISCUSSION

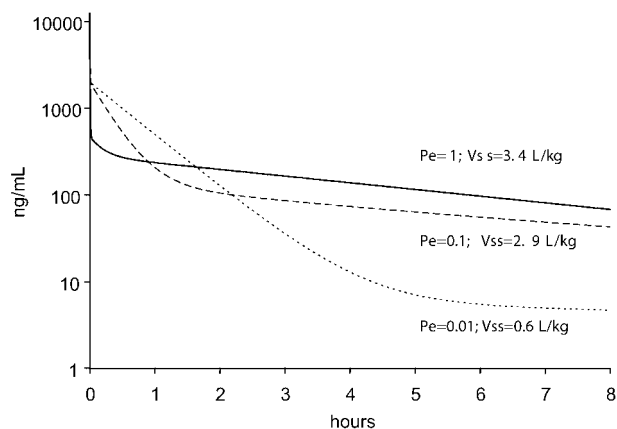
Many previous evaluations of generic physiologically based models of pharmacokinetics have been carried out with data sets consisting largely of marketed drugs.<sup>24–26</sup> Such drugs tend to have different physicochemical properties to

compounds considered as candidates for clinical development.<sup>27</sup> Therefore, to provide the most useful practical information on the potential and limitations of models for use in drug discovery an evaluation with early discovery compounds is needed. The 68 compounds in this study were selected on the basis of availability of *in vivo* data and are representative of compounds now being considered as potential clinical development candidates. The compounds show very high lipophilicity with  $\log P$  ranging from 0.6 to 7.5 and free fraction in plasma and aqueous solubility are very low.

This study was based upon very limited measured data. This is representative of the situation during early drug discovery when the physicochemical nature of compounds and the need for high throughput often limits the possibility of *in vitro* measurement. The use of calculated properties instead of measured ones could clearly contribute to error in the predictions. Concerning the predictions of volume of distribution, for compounds of moderate lipophilicity such as the A class a sensitivity analysis with changes in  $\log P$  and fraction unbound of the magnitude of the known prediction errors in these inputs showed a change of more than 60% in predicted volume. This level of sensitivity implies that, even if the model



**Figure 5.** Predicted and observed concentrations for 68 compounds. Filled symbols are times before 30 min.



**Figure 6.** Simulations of the theoretical effect of a switch from perfusion limited to permeability limited tissue distribution on the profile after an intravenous bolus dose. A simplified PBPK model of rat including muscle ( $K_p = 1.2$ ) and fat ( $K_p = 2$ ) compartments was used. Steady state volume was calculated by noncompartmental analysis of each simulated profile.

assumptions are largely valid, a fine discrimination of similar compounds to better than twofold cannot be expected. In other compound classes such as D and F the sensitivity of predicted volume to inputs is slight and we can assume that prediction error is due to invalid model assumptions. In the original work of Poulin<sup>25</sup> it was found that, for a set of 123 marketed drugs, 80% of the predicted volumes were within twofold of the observed value. In the current study, this worsened to 62% and this may well be related to the different properties of the compound sets. The mean  $\log D$  (pH 7.4) and unbound fraction for the dataset in this study are 3.2 and 9% compared to 1.4 and 38% for the set used by Poulin.

One area where improvement in the tissue composition based approach of Poulin may be required

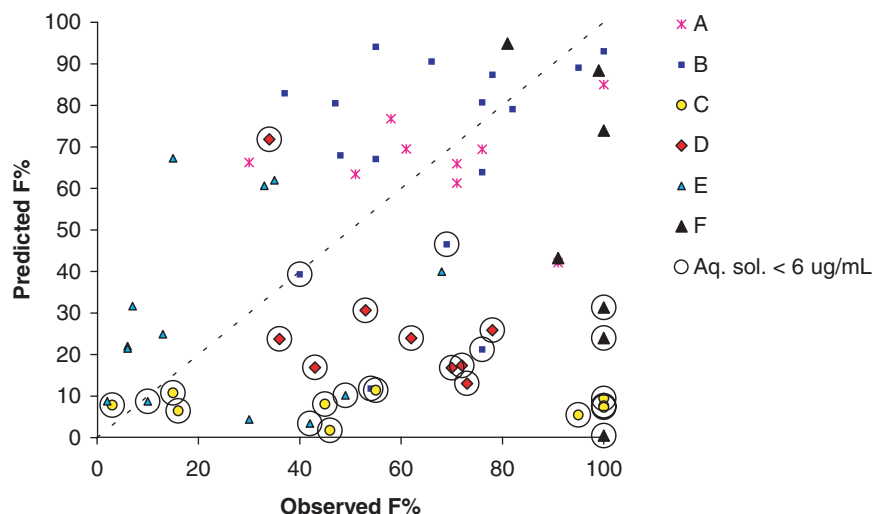
is to account for different charge states. The acids in this study showed volumes of distribution less than predicted for a homogeneous distribution but larger than extracellular space. Bases tended to be under predicted, which may be related to their binding to negatively charged phospholipids in tissue membranes.<sup>28,29</sup> Further work to include consideration of charge in the mechanistic equations could lead to improvement since a pure *in silico* method based upon calculated  $\log P$  and charge yielded a reasonable mean fold-error of 2.96 for a set of 328 drugs.<sup>30</sup> High lipophilicity also causes problems for the prediction of volume (Fig. 4). Compounds in the D project has very high  $\log P$  values ( $>5$ ) and showed predicted volumes more than twofold higher than observed values. This class is neutral at physiological pH, has relatively high molecular weights (560–661) and low free fraction in plasma. The PAMPA permeability of these compounds was good but *in vivo* the free concentration is low and as this determines the effective tissue permeation a permeability limitation may be present. A decrease in brain tissue penetration with very high lipophilicity has been reported<sup>31,32</sup> and was suggested to be related to unfavorable partitioning from the endothelium into underlying tissue. A separate investigation showed that passive diffusion through Caco-2 cells could be predicted from  $\log D$  and molecular weight<sup>33</sup> and that restricted membrane diffusion occurred for molecular weights above 500.

One possible contributor to the discrepancy between predicted and observed volume could be the estimation of steady state volume from plasma concentrations measured after a single bolus dose. A permeability limited distribution into tissues can reduce significantly the volume estimated in this way, even though the true steady state parti-

**Table 4.** Statistics for Predicted Bioavailability

	Bioavailability				Bioavailability After Solubility Increase			
	% Under Predictions	Mean Prediction Error %	RMSE %	$R^2$	% Under Predictions	Mean Prediction Error %	RMSE %	$R^2$
A	56	1	23	0.00	56	0	21	0.00
B	50	−3	27	0.07	50	−3	27	0.07
C	90	50	62	0.00	50	11	45	0.01
D	90	31	43	0.32	40	−16	27	0.19
E	38	−4	28	0.02	31	−8	27	0.01
F	86	45	58	0.26	86	22	34	0.04
All Projects	64	16	40	0.06	0	0	31	0.16

*In vivo* solubility enhancement due to the presence of bile salts was calculated according to Ref. <sup>21</sup>



**Figure 7.** Predicted and observed bioavailability for 64 compounds.

tioning does not change (Fig. 6). Another possible source of discrepancy is the over-estimation of  $V_{ss}$  by noncompartmental analysis of total plasma concentrations in the case of high protein binding with slow dissociation.<sup>34</sup> Measured tissue concentrations would be needed to make a distinction between an error in estimation of tissue partition coefficients and an error due to neglecting permeability limitations.

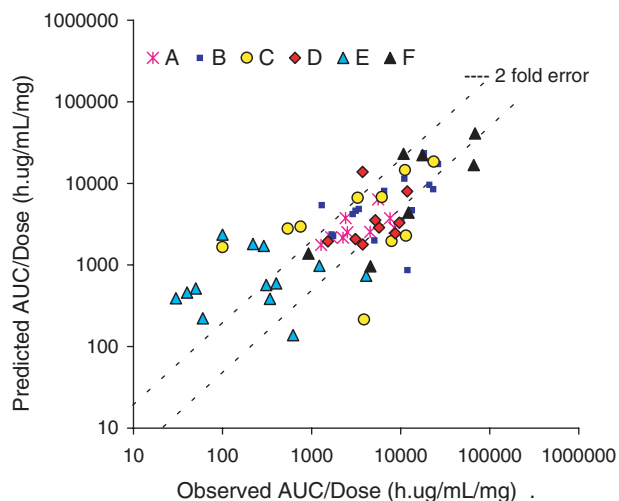
However, the trend for under prediction of plasma concentrations at earlier time points (Fig. 5) may also be related to a false assumption of flow limited distribution into tissues. A detailed physiological model for a cyclosporin derivative (molecular weight >1200,  $\log P = 2.9$ ) found that membrane transport limitation was needed to describe the measured tissue concentrations<sup>35</sup> and a significant capillary membrane permeability limitation has been invoked for highly protein

bound antibiotics dicloxacillin and ceftriaxone.<sup>36</sup> Even for the more typical drugs thiopental<sup>37</sup> and alfentanil<sup>38</sup> (molecular weights 242 and 417,  $\log P$  values 3 and 2 respectively) it was shown that the well stirred model could not adequately describe measured concentrations in several tissues at early time points. Therefore it seems possible that membrane transport needs to be accounted for in modeling of these early discovery compounds with their tendency to higher molecular weight and low free fraction in plasma. Extension of a generic PBPK tool to include permeability limited tissues has been described superficially<sup>39</sup> but the details of this commercial tool, (PKSIM, [www.pk-sim.com](http://www.pk-sim.com)), and validation data, are kept proprietary. The PKQuest model<sup>40</sup> ([www.pkquest.com](http://www.pkquest.com)), also includes a permeability limitation. For generic use within drug discovery further work is needed to enable prediction of compound specific

**Table 5.** Statistics for Predicted AUC/Dose

	Oral AUC/Dose			Oral AUC/Dose After Solubility Increase		
	Mean Fold Error	% Within Twofold Error	$R^2$	Mean Fold Error	% Within Twofold Error	$R^2$
A	1.5	78	0.16	1.5	78	0.20
B	2.1	56	0.43	2.1	56	0.43
C	5.7	20	0.70	3.7	30	0.61
D	7.8	0	0.00	2.2	50	0.02
E	5.0	23	0.00	4.8	31	0.00
F	5.0	29	0.56	2.3	43	0.49
All Projects	3.5	36	0.56	2.6	48	0.60

*In vivo* solubility enhancement due to the presence of bile salts was calculated according to Ref.<sup>21</sup>



**Figure 8.** Predicted and observed dose normalized AUC for 64 compounds after adjustment of solubility values.

membrane permeability *in vivo* together with characterization of the physiological terms for membrane surface areas of tissues. Such estimation of the permeability surface area products for all model tissues could then facilitate early predictions and also be useful for mechanistic explorations at the later stages of drug discovery and development when more data become available.

The predictions of clearance described here with a mean fold-error of 1.8 and 59% of compounds within a twofold error are comparable to results previously published for direct physiologically based scaling from rat hepatocytes.<sup>24</sup> Several factors may contribute to the error in clearance prediction. The hepatocyte screening data is based on a single batch of hepatocytes measured at a single concentration and partitioning of compound into blood cells was often neglected. Also, there was no information available on extra-hepatic clearance and so the predicted hepatic clearance has been compared to the total plasma clearance. Extrahepatic metabolism is a probable reason for the under prediction of the E project compounds many of which show total clearances greater than liver blood flow. However, the level of information available at the optimization stage does not usually allow the prediction of alternate clearance pathways such as biliary excretion, extra-hepatic metabolism, or renal excretion of unchanged drug.

The GastroPlus<sup>TM</sup> program was applied based on typical higher throughput screening data related to the key properties determining oral absorption. Many assumptions and approximations were made in the simulation of plasma exposures after

an oral dose and the error in predicted bioavailability was large. However it does seem that a major factor leading to error in prediction of oral absorption is the use of aqueous solubility for these very low solubility compounds. Almost half of the compounds studied showed solubility lower than 6  $\mu\text{g/mL}$  and there was a very clear trend for the bioavailability of these compounds to be under predicted. Our experience with GastroPlus<sup>TM</sup> applied at later stages of development shows that simulated oral absorption based on solubility measured in physiologically relevant media such as fasted state simulating intestinal fluid (FaSSIF)<sup>41</sup> becomes much more realistic. Published work also confirms the importance of such media for accurate simulations.<sup>42</sup> One factor determining the solubility *in vivo* is micellar solubilization due to bile salts and the use of a lipophilicity based calculated solubility lead to an overall improvement for the compounds in this study. The remaining 31% RMSE in predicted bioavailability could be due to a number of factors. PAMPA provides an estimate of the passive transcellular component of permeability but the pH dependence, which could be important for ionizable drugs<sup>43</sup> has been neglected. Calculation of solubility versus pH profiles with the Henderson–Hasselbalch equation gives only a rough estimate<sup>44</sup> and the calculated  $\text{pK}_a$  values used are also subject to error. In addition the prediction of bioavailability requires an estimate of FPE. This becomes very sensitive to small changes when the hepatic clearance is high (as with E project compounds) and can be under estimated if intestinal metabolism is important. A previous evaluation of GastroPlus<sup>TM</sup> for prediction of the fraction absorbed for a set of 28 marketed drugs<sup>26</sup> showed an RMSE of 24%.

In the prediction of oral AUC both the predicted clearance and absorption are important. For both the A and B projects the predicted AUC are within twofold of observed for most compounds. Other projects show more divergence from the observed data although after adjustment of the solubility input the D and F predictions are improved.

The potential of generic PBPK to be applied prospectively during drug discovery for compound prioritization has recently received attention.<sup>2,4,39,45</sup> The suggestion is that a generic PBPK approach based upon limited screening data may be used to select compounds for more detailed characterization and *in vivo* experiments. Within a discovery project the main effort in optimization takes place within a close series of structures and considering the statistics (Tabs. 3–5) it is clear

that within class correlations are often too poor to distinguish compounds by the predicted PK parameter. In some cases, (e.g. in volume predictions for class C) this may be because the compounds are all very similar in this respect and the observed difference may not be significant in the context of the project. However, for the same class C, the bioavailability prediction was too poor to be useful and additional work would be needed to determine the probable cause of the mismatch and possibly to improve the simulation. This highlights an important factor to consider in the use of the generic simulation which is the more extreme properties of many of these early compounds which can put them beyond the range of validity of the models. Therefore, it is suggested that in practice the utility of the approach should be assessed on a class by class basis. In the two projects with more moderate physicochemical properties, A and B, the simulated pharmacokinetics showed reasonable agreement with observation and it could be questioned whether the measured *in vivo* data is really necessary for all compounds. Also in the D and F projects corrections to the solubility input lead to improved agreement with *in vivo* data and simulation then became more useful. Thus, at present it would seem that for each new interesting class of compounds, *in vivo* data for a small set of compounds is necessary to build confidence in simulations. Such data may also allow the construction of compound class specific models and structure property relations to improve predictions within a series.

In the light of this study, it seems that generic simulation will be inadequate for prospective prediction in a significant number of early projects since data on processes important to describe the pharmacokinetics is missing. Modeling of such compounds, based upon later more mechanistic studies will, for the foreseeable future, remain the domain of the specialist. However other projects may be more tractable at the early stage and then, if simulation is to be used more widely, an important prerequisite is availability of software providing a facile user interface with seamless links to the databases holding the needed *in silico*, *in vitro* and *in vivo* data. Typically, project team members can only devote a fraction of their time to this task and if usability is a significant barrier then simulation remains restricted to specialists. However this aspect has seen considerable developments in recent years with several user-friendly applications appearing and some competition between the various suppliers as software devel-

ops rapidly. The GastroPlus 4.0 model used in this evaluation will be extended in the next version to include a full physiological model allowing both iv and po simulations and such capabilities are already present in two other commercial software tools (PK-Sim, [www.pksim.com](http://www.pksim.com)), (Cloe-PK, [www.cypotex.com](http://www.cypotex.com)). These capabilities are also already present in two other commercial software tools (Bayer Technology Services, 2003), (Jones et al., 2004). The developments in this area were recently reviewed and the technical capabilities of the different software packages compared.<sup>39</sup>

As projects move on through preclinical development more detailed characterization occurs for certain compounds from each class. Thus, we now have more information which sheds light on the mismatches seen between prediction and observation. For example, for the A compound class we have evidence that biliary excretion of parent compound and subsequent enterohepatic recirculation occurs while for the B class specific binding to receptor has an influence on volume of distribution. Such processes are not included in the generic model and are not normally possible to quantify during early drug discovery.

Overall, this study indicates that generic simulation of pharmacokinetics at the lead optimization stage could be useful to predict differences in pharmacokinetic parameters of twofold or more based upon minimal measured input data. However, it is recommended that verification of the simulations with *in vivo* data for a few compounds of each new compound class will allow an assessment of the error in prediction, will identify invalid model assumptions, and may allow for construction of class specific corrections.

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