Physiologically Based Pharmacokinetic Modelling 2: Predicting the Tissue Distribution of Acids, Very Weak Bases, Neutrals and Zwitterions

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Received 9 May 2005; revised 15 July 2005; accepted 7 August 2005

Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.20502

ABSTRACT: A key component of whole body physiologically based pharmacokinetic (WBPBPK) models is the tissue-to-plasma water partition coefficients (*K*pu's). The predictability of *K*pu values using mechanistically derived equations has been investigated for 7 very weak bases, 20 acids, 4 neutral drugs and 8 zwitterions in rat adipose, bone, brain, gut, heart, kidney, liver, lung, muscle, pancreas, skin, spleen and thymus. These equations incorporate expressions for dissolution in tissue water and, partitioning into neutral lipids and neutral phospholipids. Additionally, associations with acidic phospholipids were incorporated for zwitterions with a highly basic functionality, or extracellular proteins for the other compound classes. The affinity for these cellular constituents was determined from blood cell data or plasma protein binding, respectively. These equations assume drugs are passively distributed and that processes are nonsaturating. Resultant *K*pu predictions were more accurate when compared to published equations, with 84% as opposed to 61% of the predicted values agreeing with experimental values to within a factor of 3. This improvement was largely due to the incorporation of distribution processes related to drug ionisation, an issue that is not addressed in earlier equations. Such advancements in parameter prediction will assist WBPBPK modelling, where time, cost and labour requirements greatly deter its application. J Pharm Sci 95:1238–1257, 2006

Keywords: *in silico* modelling; pharmacokinetics; phospholipids; tissue partition; physiological model; physicochemical properties; partition coefficients; QSAR; PBPK modelling

INTRODUCTION

With the advent of high throughput procedures, medicinal chemists and biologists can rapidly screen novel chemical entities for favourable properties. One important set of properties concerns pharmacokinetic characteristics, which still require considerable effort and resource for assessment. While *in vitro* data can be used to gain a measure of intestinal permeability and hepatic metabolic stability, the issue of assessing likely tissue distribution remains problematic. Consequently the current common approach to gain the latter is through *in vivo* experiments, and describing the observation using empirical approaches, such as exponential equations or compartmental models. The resultant pharmacokinetic parameters for a series of compounds can then be regressed against physiological and physicochemical data, and used to make predictions of the

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Journal of Pharmaceutical Sciences, Vol. 95, 1238–1257 (2006)

likely behaviour of other drugs. A major drawback of these empirically based predictive approaches is that they are very restrictive, at best suitable only for structurally similar compounds.

Expediting pharmacokinetic evaluations requires a detailed understanding of the behaviour of structurally diverse compounds within the test system. Such knowledge would allow us to move from empirical towards more mechanistic approaches thereby permitting better *in vivo* pharmacokinetic predictions to be made from *in vitro* and *in silico* data. Mechanistically based pharmacokinetic models are often termed physiologically based pharmacokinetic (PBPK) models, which are constructed from physiological information (tissue blood flows, size and composition) and compound specific data (clearances, and tissue and blood affinities). When applied to multiple tissues or compartments within the whole body (WBPBPK models), the requirement for tissue affinity values from numerous tissues has acted as a deterrent in the use of these models in the pharmaceutical industry.

Tissue affinities are conventionally determined *in vivo* from steady-state tissue and plasma (*K*p) or plasma water (*K*pu) drug concentration data, requiring the development of appropriate specific assays. The cost and time involved in gathering this information are substantial and, together with the requirement for sizeable amounts of drug, present a serious problem in drug discovery and candidate selection. This hurdle could be overcome by predicting tissue affinities from *in vitro* and *in silico* data using equations derived from a mechanistic understanding of the underlying physiology of the animal species and the behaviour of drugs within this system.

Equations of this form were developed by Poulin and co-workers[1–3] and originally applied to small neutral molecules in the environmental industry.[4,5] These researchers later extended their equations to pharmaceutical acids, bases and neutrals, and on the whole obtained reasonable predictions of tissue affinities, with the exception of highly basic compounds. The reduced prediction accuracy for moderate-to-strong bases (taken to be those with at least one pKa value $\geq$7) was investigated by Rodgers basic and co-workers[6,7] and attributed to unique electrostatic interactions between these compounds and tissue acidic phospholipids. From this knowledge, these researchers[7] developed a new mechanistic equation that incorporated these interactions and the resultant tissue affinity predictions were considerably more accurate (prediction accuracy improved from 45% to 89%). We now report on an extension of this mechanistic equation to allow prediction of the *K*pu values of a range of additional compounds beyond moderate-to-strong bases, namely 7 very weak bases, 20 acids, 4 neutral drugs and 8 zwitterions in 13 rat tissues, and show that these predictions are a considerable improvement on those made by the equations of Poulin and co-workers.[1–3] Issues surrounding the practical application and limitations of these new equations are also discussed

METHODS

Underlying Considerations

In a previous study,[7] a mechanistic equation for predicting *K*pu values of moderate-to-strong bases was developed from the knowledge that these drugs preferentially interact with tissue acidic phospholipids (phosphatidylserine, phosphatidylglycerol (mono and di), phosphatidylinositol and phosphatidic acid) through electrostatic interactions. Such interactions are restricted to bases that are significantly ionised at physiological pH so will not apply to weaker bases and other drug classes. Additional factors incorporated into the equation for moderate-to-strong bases were drug dissolution in tissue water and the partitioning of unbound unionised drug into neutral lipids and neutral phospholipids. These considerations apply to all compound classes and the reader is referred to the previous publication[7] for details regarding the assumptions and mechanistic derivations of these distributional processes.

Another mechanism that should be considered for acids, neutrals and very weak bases is binding to extracellular proteins. This interaction was considered minimal for moderate-to-strong bases, since they tend to preferentially bind to α_1-acid glycoprotein, which is largely restricted to plasma.[8] In comparison, acids and very weak bases preferentially bind to albumin and lipophilic neutrals preferentially associate with lipoproteins. Both of these proteins are present in appreciable quantities in tissue extracellular water so interactions with albumin and lipoproteins will have a much greater impact upon the tissue distribution of these compounds.

Some drugs, however, are zwitterionic, and these can be divided into two categories. The first group (group 1) comprises compounds with at least one basic pKa $\geq$7; these compounds have

been assumed to undergo interactions with acidic phospholipids in a similar manner to moderate-to-strong bases.[7] All other zwitterionic compounds have been placed into a second group (group 2), and their distributional behaviour has been assumed comparable to acids and very weak bases.

A generalised overview of the interactions involved in the tissue distribution of acids, very weak bases, neutrals and zwitterions (group 2) are shown in Figure 1.

Derivation

*K*pu values are steady-state parameters that relate the concentration of unbound drug in tissues ($C_{T,SS}$) to unbound drug in plasma ($Cu_{P,SS}$) following a constant rate drug infusion, as described by Eq. 1. Fundamentally this equation is the same as that reported by Rodgers and co-workers[7] with the exception of an additional expression to account for interactions between drug molecules and extracellular tissue proteins ($C_{PR,EW} \times f_{EW}$).

$$K_{pu} = \frac{C_{T,SS}}{Cu_{P,SS}} = \frac{\begin{bmatrix} C_{U,IW} \cdot f_{IW} + (C_{U,EW} + C_{PR,EW}) \cdot f_{EW} \\ + C_{NL} \cdot f_{NL} + C_{NP} \cdot f_{NP} + C_{REM} \cdot f_{REM} \end{bmatrix}}{Cu_{P,SS}} \quad (1)$$

where f refers to fractional tissue volume, with $\sum f = 1$, so that $f_{REM} = 1 - f_{IW} - f_{EW} - f_{NL} - f_{NP}$; subscripts IW, EW, NL, NP, PR and REM refer to intracellular water, extracellular water, neutral lipid, neutral phospholipid, binding protein and residual tissue constituents respectively.

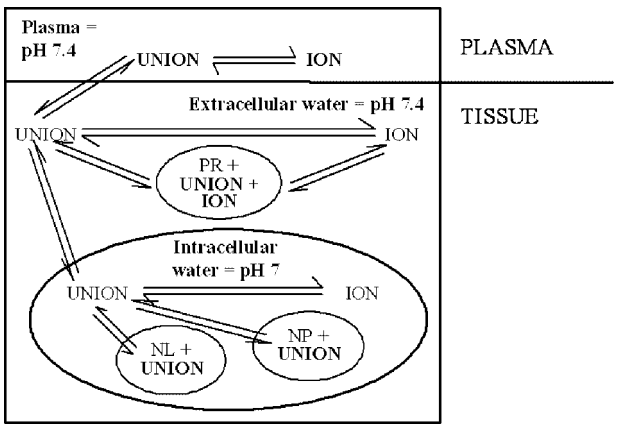

Figure 1. Illustration of the processes involved in the distribution of acids, very weak bases, neutrals and zwitterions (group 2), between tissues and plasma that have been accommodated in Eq. 14. UNION and ION refer to unionised and ionised drug respectively. For all other abbreviations, and further information, refer to the text.

For acids, neutrals and very weak bases, interactions with tissue constituents additional to those stated in Eq. 1 are deemed negligible, as such C_{REM} equates to zero. If additional compound-specific mechanisms occur, they could be accommodated by C_{REM}, but this issue has not been addressed in this study.

The concentrations of unbound drug (ionised and unionised) in the intracellular water ($C_{U,IW}$), and the concentrations of unbound unionised drug partitioned into neutral lipid (C_{NL}) and neutral phospholipids (C_{NP}) can be calculated using Eqs. 2–4, based on the assumption that only unionised unbound drug permeates membranes and that at equilibrium the concentrations of this permeating species are equal on both sides of the membrane.[7] In these Eqs. (2–4), X and Y differ as follows: for very weak monoprotic bases $X = 1 + 10^{pKa-pH_{IW}}$ and $Y = 1 + 10^{pKa-pH_p}$; for monoprotic acids $X = 1 + 10^{pH_{IW}-pKa}$ and $Y = 1 + 10^{pH_p-pKa}$; for neutral drugs X and Y equal 1 due to the lack of ionisation; and for zwitterions $X = 1 + 10^{pKa_{BASE}-pH_{IW}} + 10^{pH_{IW}-pKa_{ACID}}$ and $Y = 1 + 10^{pKa_{BASE}-pH_p} + 10^{pH_p-pKa_{ACID}}$.

$$C_{U,IW} = Cu_p \cdot \frac{X}{Y} \quad (2)$$

$$C_{NL} = \frac{P \cdot Cu_p}{Y} \quad (3)$$

$$C_{NP} = \frac{Cu_p}{Y} \cdot (0.3P + 0.7) \quad (4)$$

where pH_p and pH_{IW} refer to the pH of the intracellular water and plasma respectively, and P refers to the partition coefficient of the unionised drug. For all tissues except adipose P is the n-octanol:water partition coefficient; for adipose the vegetable oil:water partition coefficient was deemed more appropriate.[2,7] For polyprotic bases, acids and zwitterions X and Y should be refined in accordance with the corresponding Henderson–Hasselbalch equation.

The concentration of drug in the extracellular water is the sum of the unbound concentration ($C_{U,EW}$) and concentration associated with extracellular proteins ($C_{PR,EW}$), where $C_{U,EW}$ equals Cu_p.[7] In general, the dominant binding protein in plasma for acids and very weak bases is albumin,[9] so albumin was assumed to be the dominant

 DOI 10.1002/jps

binding protein in tissues for these compound classes. Several binding sites have been reported for albumin, two of the major sites being the benzodiazepine and warfarin sites.[10–12] Benzodiazepines are very weak bases that are predominately unionised at physiological pH, whereas warfarin, being reasonably acidic (pKa 4.8), is essentially ionised. This indicates that both ionised and unionised acids and very weak bases are capable of interacting with albumin, and this assumption has been incorporated into the mechanistic equations. Regarding neutral drugs, lipoproteins are assumed to be the dominant binding protein.[9] The association constant of acids and very weak bases for albumin, and neutral drugs for lipoproteins ($K\mathrm{a}_{\mathrm{PR}}$) can be calculated using Eq. 5.

$$K\mathrm{a}_{\mathrm{PR}} = \frac{[\mathrm{PR}\cdot\mathrm{Drug}]_{\mathrm{EW}}}{[\mathrm{PR}]_{\mathrm{EW}}\cdot C_{\mathrm{U,EW}}} \tag{5}$$

where $[\mathrm{PR}\times\mathrm{Drug}]_{\mathrm{EW}}$ represents the protein-drug complex concentration in the extracellular space of the tissue, that is $C_{\mathrm{PR,EW}}$, and $[\mathrm{PR}]_{\mathrm{EW}}$ represents the concentration of sites still available for binding on the protein. Assuming nonsaturating conditions prevail, which occurs when few of the binding sites are occupied, then $[\mathrm{PR}]_{\mathrm{EW}}$ can be approximated to be the total concentration of binding protein in the tissue extracellular water.

Rearranging Eq. 5 finds $C_{\mathrm{PR,EW}}$, and inserting the assumption that $C_{\mathrm{U,EW}}$ equals $C\mathrm{u}_{\mathrm{P}}$ at equilibrium generates the following expression.

$$C_{\mathrm{PR,EW}} = K\mathrm{a}_{\mathrm{PR}}\cdot[\mathrm{PR}]_{\mathrm{EW}}\cdot C\mathrm{u}_{\mathrm{P}} \tag{6}$$

Inserting the relevant terms into Eq. 1, that is remembering $C_{\mathrm{U,EW}}$ equals $C\mathrm{u}_{\mathrm{p}}$ and C_{REM} equals zero, and substituting $C_{\mathrm{U,IW}}$ with Eq. 2, C_{NL} with Eq. 3, C_{NP} with Eq. 4 and $C_{\mathrm{PR,EW}}$ with Eq. 6, yields a generic expression (Eq. 7) for calculating $C_{\mathrm{T,SS}}$ for acids, very weak bases, neutrals and group 2 zwitterions.

$$C_{\mathrm{T,SS}} = C\mathrm{u}_{\mathrm{P,SS}}\cdot\left[\frac{X\cdot f_{\mathrm{IW}}}{Y} + (1+(K\mathrm{a}_{\mathrm{PR}}\cdot[\mathrm{PR}]_{\mathrm{EW}}))\cdot f_{\mathrm{EW}} + \left(\frac{P\cdot f_{\mathrm{NL}} + (0.3P+0.7)\cdot f_{\mathrm{NP}}}{Y}\right)\right] \tag{7}$$

Since $[\mathrm{PR}]_{\mathrm{EW}}$ is calculated by dividing the concentration of binding protein (albumin or lipoprotein) in the tissue ($[\mathrm{PR}]_{\mathrm{T}}$) by f_{EW}, then f_{EW} cancels out to produce Eq. 8.

$$C_{\mathrm{T,SS}} = C\mathrm{u}_{\mathrm{P,SS}}\cdot\left[\frac{X\cdot f_{\mathrm{IW}}}{Y} + K\mathrm{a}_{\mathrm{PR}}\cdot[\mathrm{PR}]_{\mathrm{T}} + f_{\mathrm{EW}} + \left(\frac{P\cdot f_{\mathrm{NL}} + (0.3P+0.7)\cdot f_{\mathrm{NP}}}{Y}\right)\right] \tag{8}$$

So that

$$K\mathrm{pu} = \left[\frac{X\cdot f_{\mathrm{IW}}}{Y} + K\mathrm{a}_{\mathrm{PR}}\cdot[\mathrm{PR}]_{\mathrm{T}} + f_{\mathrm{EW}} + \left(\frac{P\cdot f_{\mathrm{NL}} + (0.3P+0.7)\cdot f_{\mathrm{NP}}}{Y}\right)\right] \tag{9}$$

For group 1 zwitterionic drugs, we assume that electrostatic interactions with acidic phospholipids dominate, so the mechanistic equation developed for moderate-to-strong bases[7] applies. This equation has been modified in accordance with the Henderson–Hasselbalch equations for application to group 1 zwitterions (Eq. 10).

$$K\mathrm{pu} = \left[f_{\mathrm{EW}} + \frac{X\cdot f_{\mathrm{IW}}}{Y} + \left(\frac{P\cdot f_{\mathrm{NL}} + (0.3P+0.7)\cdot f_{\mathrm{NP}}}{Y}\right) + \left(\frac{K\mathrm{a}_{\mathrm{AP}}\cdot[\mathrm{AP}^-]_{\mathrm{T}}\cdot 10^{\mathrm{pKa_{BASE}}-\mathrm{pH_{IW}}} + 10^{\mathrm{pH_{IW}}-\mathrm{pKa_{ACID}}}}{Y}\right)\right] \tag{10}$$

where $[\mathrm{AP}^-]_{\mathrm{EW}}$ is the concentration of acidic phospholipids in the tissue, and $K\mathrm{a}_{\mathrm{AP}}$ is the association constant of a drug for acidic phospholipids, which can be calculated from the blood cell-to-plasma water partition coefficient ($K\mathrm{pu}_{\mathrm{BC}}$), knowing the concentration of acidic phospholipids in blood cells.[7]

Tissue Specific Input Parameters

The tissue specific input parameters for rats are detailed in Table 1 and Reference 7. Values of 0.0023 and 0.0013 were used for $f_{\mathrm{NL,P}}$ and $f_{\mathrm{NP,P}}$ respectively.[3,13,14]

Compound Specific Input Parameters

The compound specific input parameters have been taken from the literature or predicted using online software (Log*P* and pKa) and are summarised in Tables 2 and 3, with the exception of $K\mathrm{a}_{\mathrm{PR}}$ for which values are not readily available.

Table 1. Rat Tissue Composition Data Used in Equations 10 and 14 for Predicting *K*pu Values

	Residual Blood Adjusted Fractional Tissue Volumes[a]			Tissue-to-Plasma[b]	
	Neutral Lipid	Neutral Phospholipid	Tissue Water	Albumin Ratio	Lipoprotein Ratio
Adipose	0.0016	0.853	0.144	0.049	0.068
Bone	0.0174	0.0016	0.417	0.100	0.050
Brain	0.0391	0.0015	0.753	0.048	0.041
Gut	0.0375	0.0124	0.738	0.158	0.141
Heart	0.0135	0.0106	0.568	0.157	0.160
Kidney	0.0121	0.0240	0.672	0.130	0.137
Liver	0.0135	0.0238	0.642	0.086	0.161
Lung	0.0215	0.0123	0.574	0.212	0.168
Muscle	0.0100	0.0072	0.726	0.064	0.059
Pancreas	0.0403	0.0090	0.641	0.060	0.060
Skin	0.0603	0.0044	0.658	0.277	0.096
Spleen	0.0071	0.0107	0.562	0.097	0.207
Thymus	0.0168	0.0092	0.752	0.075	0.075

[a]Values adjusted from Reference 7 in accordance with Appendix A to account for contributions from residual blood.

[b]Data are the means of reported values.[15,16] Values were converted from extracellular fluid-to-plasma ratios into tissue-to-plasma ratios by multiplying the former by the fractional extracellular volumes reported by Rodgers and co-workers.[7]

One approach to estimating $K\mathrm{a}_{\mathrm{PR}}$ is to incorporate the mechanistic expressions for binding to the various tissue constituents into the following standard equation.

$$\mathrm{fu} = \frac{C\mathrm{u}_{\mathrm{P}}}{C_{\mathrm{P}}} = \frac{C\mathrm{u}_{\mathrm{P}}}{C\mathrm{u}_{\mathrm{P}} + C_{\mathrm{Bd,P}}} \tag{11}$$

where subscript P refers to plasma, $C_{\mathrm{Bd,P}}$ refers to the concentration of drug bound in plasma and fu is the fraction drug unbound in plasma.

Inserting expressions for partitioning into plasma neutral lipids (Eq. 3) and plasma neutral phospholipids (Eq. 4), together with association of compound with plasma albumin or lipoprotein (Eq. 6) yields Eq. 12, which can be rearranged and simplified to find $K\mathrm{a}_{\mathrm{PR}}$ (Eq. 13).

$$\mathrm{fu} = \frac{1}{1 + \left[\left(\frac{P \cdot f_{\mathrm{NL,P}} + (0.3P + 0.7) \cdot f_{\mathrm{NP,P}}}{Y}\right) + K\mathrm{a}_{\mathrm{PR}} \cdot [\mathrm{PR}]_{\mathrm{P}}\right]} \tag{12}$$

$$K\mathrm{a}_{\mathrm{PR}} = \left[\frac{1}{\mathrm{fu}} - 1 - \left(\frac{P \cdot f_{\mathrm{NL,P}} + (0.3P + 0.7) \cdot f_{\mathrm{NP,P}}}{Y}\right)\right] \cdot \frac{1}{[\mathrm{PR}]_{\mathrm{P}}} \tag{13}$$

where $[\mathrm{PR}]_{\mathrm{P}}$ refers to the concentration of albumin or lipoprotein in plasma (the assumptions for $[\mathrm{PR}]_{\mathrm{EW}}$ and $[\mathrm{PR}]_{\mathrm{T}}$ apply).

Then, by assuming that $K\mathrm{a}_{\mathrm{PR}}$ determined from plasma data is representative of the $K\mathrm{a}_{\mathrm{PR}}$ in all tissues, the *K*pu value for any tissue can be calculated by inserting Eq. 13 into Eq. 9 yielding Eq. 14. Namely,

$$K\mathrm{pu} = \frac{X \cdot f_{\mathrm{IW}}}{Y} + f_{\mathrm{EW}} + \left(\frac{P \cdot f_{\mathrm{NL}} + (0.3P + 0.7) \cdot f_{\mathrm{NP}}}{Y}\right) + \left[\left(\frac{1}{\mathrm{fu}} - 1 - \left(\frac{P \cdot f_{\mathrm{NL,P}} + (0.3P + 0.7) \cdot f_{\mathrm{NP,P}}}{Y}\right)\right) \cdot \frac{[\mathrm{PR}]_{\mathrm{T}}}{[\mathrm{PR}]_{\mathrm{P}}}\right] \tag{14}$$

*K*pu values were then predicted using Eq. 14 for 7 very weak bases, 20 acids, 4 neutral drugs and 2 group 2 zwitterions, and using Eq. 10 for 6 group 1 zwitterions, in rat adipose, bone, brain, gut, heart, kidney, liver, lung, muscle, pancreas, skin, spleen and thymus. The predictions were then compared to the corresponding *in vivo* experimentally determined *K*pu values, where available. In addition, *K*pu values predicted using experimentally determined Log*P* (n-octanol:-water) and pKa values were compared with those made using mean Log*P* (n-octanol: water) values

Table 2. Compound Specific Input Parameters for Very Weak Bases, Acids and Neutrals for the Mechanistic Equations Used to Predict *K*pu Values[a]

	pKa		Log$P_{o:w}$[b]			
Compound	Exp	Pred[c]	Exp	Pred[d]	fu	References[e]
Very weak bases						
Alfentanil	6.5	5.1	2.2	1.3	0.11	3,18
Alprazolam	2.4	2.8	2.3	2.8	0.35	19
Chlordiazepoxide	4.7	NC	2.4	2.6	0.15	19
Diazepam	3.4	2.5	2.8	2.8	0.14	3,19,20
Flunitrazepam	1.8	2.0	2.1	2.1	0.25	19
Midazolam	6.0	2.3	3.1	4.0	0.059	3,19,21
Triazolam	2.0	2.5	2.4	3.2	0.28	19
5-n-alkyl-5-ethyl barbituric acids						
Methyl	8.1	NC	0.05	0.2	1.0	3,16
Ethyl	7.9	NC	0.7	0.6	0.95	3,16
Propyl	7.8	NC	0.8	1.1	0.87	3,16
Butyl	7.8	NC	1.7	1.6	0.61	3,16
Pentyl	8.0	NC	2.2	2.1	0.50	3,16
Hexyl	7.7	NC	2.8	2.6	0.19	3,16
Heptyl	7.8	NC	3.3	3.2	0.066	3,16
Octyl	7.8	NC	3.8	3.7	0.026	3,16
Nonyl	7.8	NC	4.1	4.3	0.0093	3,16
Other acids						
Cefazolin	2.3	2.5	0.3	−1.9	0.16	3,22,23
Dideoxyinosine	9.1	8.0	−1.2	−1.0	0.97	3
Etodolac	4.7	4.3	3.6	3.5	0.0046(R), 0.017(S)	23–25
Penicillin	2.7	3.5	1.6	1.0	0.15	1,23
Phenobarbital	7.4	NC	1.6	1.5	0.64	3,23,26
Phenytoin	8.3	NC	2.5	2.1	0.19	2,23,26
Salicyclic acid	3.0	3.1	2.2	2.1	0.14	23,27–29
Tenoxicam	5.3	6.7 (acid), 2.9 (base)	1.9	2.4	0.017	23,30,31
Thiopental	7.5	1.0	2.9	2.0	0.13	3,23
Tolbutamide	5.3	3.5	2.4	2.2	0.27	23,26,32
Valproate	4.6	4.8	2.8	2.7	0.37	23,26,33
Neutrals						
Cyclosporine	NA	NA	2.9	−1.3[f]	0.083[g]	3,34
Digoxin	NA	NA	1.2	−0.6	0.61	2,23
Ethoxybenzamide	NA	NA	0.8	1.1	0.59	3,35
Ftorafur	NA	NA	−0.3	−0.2	0.78	36[f]

Exp refers to experimental values obtained from the literature. Pred refers to predicted values. NA stands for not applicable and NC refers to situations where the software could not compute a value.

[a]Input parameters for the published mechanistic equations[3,17] that are not stated above were determined using published procedures.[1–3]

[b]Antilog values used in mechanistic equations, o:w refers to octanol:water.

[c]Predicted pKa values calculated using the online software package SPARC (http://ibmlc2.chem.uga.edu).

[d]Mean values predicted using the online packages KOWWIN (http://erc.syrres.com), Interactive Analysis (http://www.logp.com) and SPARC (http://ibmlc2.chem.uga.edu/sparc).

[e]In addition to the references cited in the table values were also obtained online from www.medicinescomplete.com/mc and www.chemfinder.com.

[f]Mean from two online packages since SPARC could not compute the Log*P* for cyclosporine.

[g]Calculated from the high dose *K*p and *K*pu data in Reference 34.

predicted using a combination of KOWWIN (http://esc. syrres.com), Interactive Analysis (http://www.logP.com) and SPARC (http://ibmlc2.chem.uga.edu/sparc), together with pKa values predicted using SPARC (http://ibmlc2.chem. uga.edu/sparc).

Comparison with Published Equations

Mechanistic equations developed by Poulin and co-workers[1–3] for predicting *K*p values were recently cited as being 'erroneous' by Berezhkovskiy[17]

Table 3. Compound Specific Input Parameters for Zwitterions for the Mechanistic Equations Used to Predict *K*pu Values[a]

Compound	pKa[c]	Log$P^{b}_{o:w}$ Exp	Log$P^{b}_{o:w}$ Pred[d]	fu	*B*:*P*[f]	*K*pu$_{BC}$[g]	References[e]
Group 1 zwitterions							
Enoxacin							
Exp	8.7 (basic), 6.1 (acidic)	0.1	0.2	0.66	0.94[h]	1.33	23,37,38
Pred	7.2 (basic), 4.8 (acidic)						
Lomefloxacin							
Exp	9.3 (basic), 5.8 (acidic)	−0.3	1.2	0.72	0.96	1.26	37,38[j]
Pred	8.1 (basic), 4.9 (acidic)						
Ofloxacin							
Exp	8.2 (basic), 6.1 (acidic)	−0.4	0.9	0.77	0.92	1.08	37,38[j]
Pred	7.3 (basic), 4.9 (acidic)						
Pefloxacin							
Exp	7.6 (basic), 6.3 (acidic)	1.2	1.2	0.80	0.94[h]	1.09	23,37,38[j]
Pred	7.0 (basic), 5.0 (acidic)						
Pipemidic acid							
Pred	7.0, 3.5 (basic), 4.9 (acidic)[k]	−2.2	−0.6	0.79	0.94[h]	1.11	37[j]
Tetracycline							
Exp	9.7 (basic), 7.7, 3.3 (acidic)	0.03	−1.8	0.50	1.0[i]	2.00	1,39[j]
Pred	5.8 (basic), 9.7, 7.2, 3.4 (acidic)						
Group 2 zwitterions							
Ceftazidime							
Exp	3.8 (basic), 2.5, 1.9 (acidic)	−0.5	−0.9	0.10	—	—	3,23
Pred	5.3, 3.8 (basic), 3.8, 2.8 (acidic)						
Nalidixic acid							
Pred	3.3 (basic), 5.1 (acidic)[k]	1.1	1.6	0.29	—	—	23

Exp refers to experimental values obtained from the literature. Pred refers to predicted values.

[a,b,c,d,e] see Table 2 footnotes.

[f] Blood to plasma concentration ratio(B:P).

[g] Affinity for blood cells calculated from fu, B:P and the haematocrit[40] using standard equations.[41]

[h] Experimental values could not be found in the literature so a mean value from lomefloxacin and ofloxacin was used due to the physicochemical similarities.[37]

[i] Experimental values could not be found in the literature so a value of one was assumed that is the value in humans (www.medicinescomplete.com/mc).

[j] KOWWIN experimental value database (http://esc.syrres.com).

[k] Experimental values could not be found in the literature.

and thereby subjected to minor modifications. These published equations account for the solubility of a drug in the lipid and water phases of plasma and tissues, and the ability of a drug to bind to the macromolecules present within these matrices. The Berezhkovskiy[17] modified equations were used to predict the *K*pu values (*K*p value predicted via the published equations was divided by the fu values in Tabs. 2 and 3) for the same set of acids, very weak bases, neutrals and zwitterions (groups 1 and 2) in the same rat tissues. The accuracy of these predictions was then compared to that of the newly developed equations.

RESULTS

New Equations (Equations 10 and 14)

Overall, the accuracy of the *K*pu predictions for 7 very weak bases, 20 acids, 4 neutral drugs, 2 group 2 zwitterions and 6 group 1 zwitterions in 13 rat tissues ($n = 370$ observations in total) was good, with mean predicted-to-experimental *K*pu ratios of 0.91–1.62 for the different drug classes thereby indicating no significant bias in the mechanistic predictions. Additionally, little difference was generally observed between *K*pu values predicted using experimental compared to

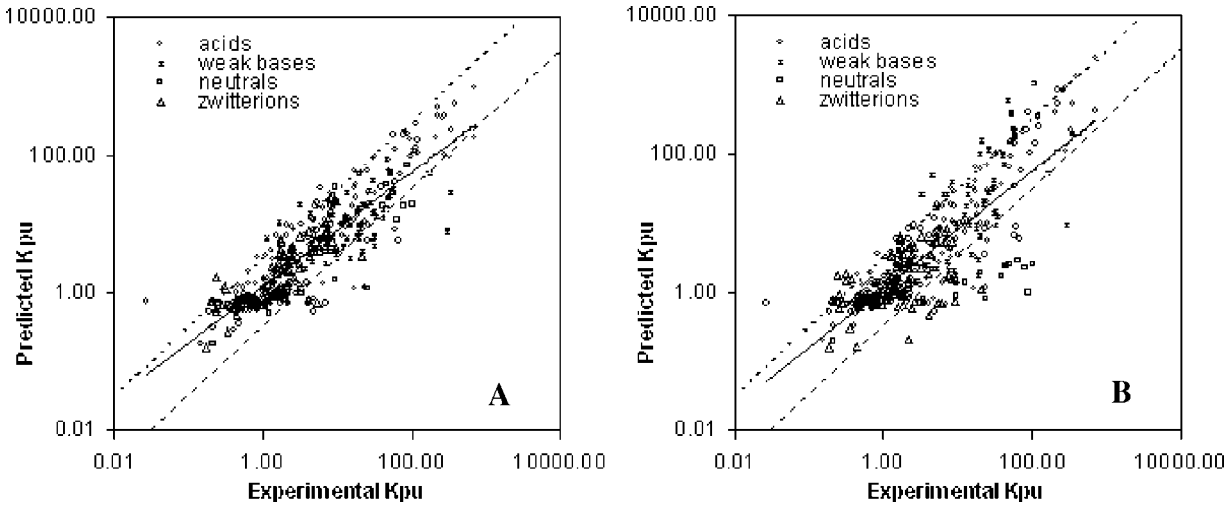

Figure 2. Relationship between predicted (Eqs. 10 and 14) and experimentally determined *K*pu values for 7 very weak bases, 20 acids, 4 neutral drugs, 2 group 2 zwitterions and 6 group 1 zwitterions in 13 rat tissues ($n = 370$). The dashed lines represent a factor of 3 either side of the line of unity, and the solid line represents the line of best fit through the data. In (A) the Log*P* and pKa values were experimentally determined and the line of best fit equates to $y = 1.29x^{0.82}$, $R^2 = 0.78$. In (B) the Log*P* and pKa values were predicted and the line of best fit equates to $y = 1.13x^{0.85}$, $R^2 = 0.67$.

predicted Log*P* (n-octanol:water) and pKa values, with 84% compared to 77% of the predicted values being within a factor of 3 of experimentally determined *K*pu's (Fig. 2, Tabs. 4 and 5). However, for some compounds, the prediction accuracy was lower using predicted compared to experimentally determined Log*P* (n-octanol:-water) and pKa values in response to significant differences between these parameters (Tabs. 2 and 3).

Published Equations

For the same data set ($n = 370$, Tab. 4), the *K*pu values predicted using published equations[3,17] were found to be less accurate (Fig. 3, Tabs. 4 and 5), compared to Eqs. 10 and 14. Of these 370 predicted *K*pu values, only 61% were within a factor of three of the experimental values, using experimental Log*P* (n-octanol:water) and pKa values. Inaccuracies were most pronounced for the acidic drugs as reflected by the mean predicted-to-experimental *K*pu ratio of 8.17, the high standard deviation (24.5) and a maximal over-prediction of a factor of 220, indicating a high positive bias in the predictions and a wide spread of accuracies. Additionally, significant under-predictions were observed for the two group 1 zwitterions.

DISCUSSION

Many of the issues discussed in a preceding publication[7] are relevant to this research and have been briefly summarised here, along with areas of interest relating to the compounds detailed in this study. However, the reader is still encouraged to peruse this earlier publication.

One issue generic to both this and the preceding publication[7] is variability and uncertainty inherent to the biological system and parameter determinations. These variables are not currently accommodated in mechanistic equations, which are deterministic in nature. Boundaries either side of the mean were therefore devised to account for this variability and uncertainty, and a global value of $\pm$ a factor of 3 was considered appropriate and subsequently utilised. Predicted *K*pu values were therefore deemed accurate if they fell within these boundaries.[1,7]

The overall accuracy of *K*pu predictions in rats using the currently developed equations was considerably higher than that achieved with the published equations,[3,17] with 84% compared to 61% respectively ($n = 370$, Tab. 4) of the predicted *K*pu values being within a factor of three of the *in vivo* *K*pu values (similar differences in prediction accuracy were also observed when predicted

Table 4. Comparison of the *K*pu Prediction Accuracy in 13 Rat Tissues Using Predicted or Experimental Log*P* (n-octanol:water) and pKa Values in the New and Published Mechanistic Equations

	Percentage of Predicted *K*pu Values Agreeing with Experimental Values to Within Factors of				Mean (±SD) Predicted to Experimental *K*pu Ratio
	<2	2–3	3–4	>4	
20 Acidic drugs ($n = 205$)					
New-experimental[a]	68.8	17.1	7.3	6.8	1.62 ± 2.17
New-predicted[b]	65.9	20.0	6.3	7.8	1.49 ± 1.94
Published-experimental[c]	45.4	10.2	9.3	35.1	8.17 ± 24.5
7 Very weakly basic drugs ($n = 58$)					
New-experimental[a]	55.2	22.4	5.2	17.2	1.09 ± 1.32
New-predicted[b]	32.8	25.9	12.1	29.3	2.35 ± 2.94
Published-experimental[c]	55.2	20.7	12.1	12.1	2.26 ± 4.06
4 Neutral drugs ($n = 37$)					
New-experimental[a]	51.4	27.0	8.1	13.5	0.91 ± 0.79
New-predicted[b]	48.6	8.1	0.0	43.2	0.68 ± 0.68
Published-experimental[c]	64.9	18.9	5.4	10.8	1.26 ± 1.37
8 Zwitterionic drugs (groups 1 and 2 combined) ($n = 70$)					
New-experimental[a]	65.7	20.0	5.7	8.6	1.47 ± 1.12
New-predicted[b]	58.6	18.6	5.7	17.1	0.96 ± 0.75
Published-experimental[c]	21.4	30.0	20.0	28.6	0.92 ± 1.23
Overall for 49 structurally unrelated drugs ($n = 370$)					
New-experimental[a]	63.2	20.8	6.5	9.5	1.34 ± 1.58
New-predicted[b]	57.6	19.5	6.5	16.5	1.44 ± 1.95
Published-experimental[c]	44.3	16.5	11.4	27.8	5.18 ± 18.6

[a]Using Eqs. 10 and 14, inserting experimental values for Log*P* (n-octanol:water) and pKa.
[b]Using Eqs. 10 and 14, inserting predicted values for Log*P* (n-octanol:water) and pKa.
[c]Using published equations,[3,17] inserting experimental values for Log*P* (n-octanol:water) and pKa.

and experimental *K*p values were compared). This divergence in prediction accuracy was anticipated since published equations[3,17] make no allowances for drug ionisation, as reflected by the poor prediction accuracy of highly ionised acids, for example salicyclic acid, etodolac and tolbutamide, and the group 1 zwitterions (i.e. those that behave as moderate-to-strong bases), for example, tetracycline. However, the accuracy of the *K*pu predictions for neutral compounds was similar by both approaches, which was anticipated since the equations of Poulin and Theil[3] were originally developed for small neutral lipophilic molecules.[4,5]

Drug Binding, Transport and Elimination Issues

The published equations[3,17] utilised in this study assume that tissue distribution is governed by dissolution and lipid partitioning, and that the ionised drug species behaves in an identical manner to the unionised form. This latter assumption is untrue since ionised bases electrostatically interact with acidic phospholipids[7] and, in contrast to unionised molecules, ionised drug species do not partition into tissue lipids. In accordance with Eq. 14, the major distribution mechanisms for ionised acids therefore become interactions with extracellular albumin and dissolution in tissue water, which is inline with Rowland and Tozers[53] description of small volume of distribution acids. The ionisation omissions in the published equations[3,17] were therefore the likely cause of the under-predictions observed for group 1 zwitterions and the over-predictions for ionised acids. Regarding the group 1 zwitterions, we have assumed the basic functionality of the molecule, with a pKa value ≥ 7, drives tissue distribution in an identical manner to moderate-to-strong bases.[6,7] Incorporating this assumption into the mechanistic calculations resulted in a pronounced increase in the *K*pu prediction accuracy when using Eq. 10 compared to those predicted using published equations.[3,17] For the ionised acids, the inaccurate assumptions of the published equations[3,17] led to over-predictions for many of the *K*pu values. This issue was partly

Table 5. Tissue-to-Plasma Water Partition Coefficients (*K*pu) in Various Rat Tissues for Acidic, Very Weakly Basic, Neutral and zwitterionic drugs–*In Vivo* Experimental Versus Predicted Values

Compound [Reference]	Experimentally Determined *K*pu[a] Followed by *K*pu Predicted via Equations 10 or 14[b]; Published[c] Mechanistic Equations												
	Adipose	Bone	Brain	Gut[d]	Heart	Kidney	Liver	Lung	Muscle	Pancreas	Skin	Spleen	Thymus
Very weak bases													
Alfentanil	19.1	—	1.14	19.2	5.00	7.47	9.05	7.03	2.78	8.65	1.65	6.65	—
[18,42]	9.20;8.92	—	6.30;24.1	7.33;25.3	3.97;11.4	4.24;13.7	4.05;14.3	5.48;16.3	2.88;9.46	6.71;25.7	10.7;35.7	2.70;8.17	—
Alprazolam	4.51	—	7.03	6.14	—	11.8	31.1	—	9.66	—	8.46	—	—
[19]	14.2;14.1	—	8.71;11.1	9.17;11.7	—	4.71;6.21	4.89;6.48	—	3.23;4.16	—	13.3;16.7	—	—
Chlordiazepoxide	28.7	—	5.00	13.1	17.4	18.0	32.3	—	5.13	—	3.20	—	—
[19]	20.4;19.7	—	11.9;26.2	12.8;27.5	5.94;12.0	6.63;14.4	6.75;15.1	—	4.38;9.40	—	18.9;39.7	—	—
Diazepam	168	—	13.2	26.2	34.3	29.9	32.1	22.5	14.4	—	30.3	—	—
[19,20]	56.3;49.2	—	28.3;39.8	29.9;42.0	12.8;17.8	14.6;21.3	15.3;22.4	18.9;26.5	9.38;13.3	—	44.4;61.4	—	—
Flunitrazepam	294	—	6.80	19.8	4.72	1.68	15.0	—	3.80	—	—	—	—
[19]	7.83;7.82	—	5.42;10.4	5.90;10.9	2.92;5.04	3.25;6.05	3.26;6.26	—	2.29;4.27	—	—	—	—
Midazolam	106	—	52.2	52.7	53.0	54.8	342	57.0	25.6	—	46.6	41.0	—
[19,21]	120;89.0	—	55.1;107	58.6;113	25.2;47.1	28.5;56.4	29.8;59.6	37.4;70.9	18.0;34.7	—	87.7;166	15.8;31.0	—
Triazolam	21.5	—	—	42.5	—	30.1	13.4	—	21.5	—	19.5	—	—
[19]	19.3;18.8	—	—	11.9;16.0	—	6.01;8.40	6.25;8.79	—	4.04;5.51	—	17.4;23.1	—	—
5-n-alkyl-5-ethyl barbituric acids													
Methyl	0.15	—	0.67	0.56	0.83	1.30	0.80	0.87	1.10	0.67	0.53	0.70	0.75
[16]	0.18;0.19	—	0.73;0.83	0.74;0.83	0.56;0.62	0.66;0.74	0.63;0.71	0.58;0.64	0.68;0.77	0.63;0.72	0.69;0.76	0.54;0.61	0.72;0.81
Ethyl	0.21	—	0.67	0.54	0.82	1.70	0.77	0.75	0.90	0.63	0.51	0.55	0.74
[16]	0.31;0.32	—	0.81;0.99	0.83;1.00	0.60;0.69	0.70;0.83	0.66;0.81	0.64;0.75	0.69;0.84	0.75;0.90	0.85;1.01	0.56;0.66	0.74;0.90
Propyl	0.49	—	0.78	0.76	1.10	2.30	1.10	0.94	0.98	0.91	0.70	0.61	0.90
[16]	0.34;0.35	—	0.82;1.09	0.85;1.09	0.62;0.75	0.71;0.90	0.66;0.87	0.67;0.81	0.68;0.89	0.74;0.99	0.90;1.13	0.56;0.70	0.74;0.97
Butyl	1.60	—	1.50	1.55	1.80	3.90	2.40	1.20	1.50	1.80	1.40	0.59	1.30
[16]	2.50;2.56	—	2.21;3.46	2.35;3.59	1.26;1.81	1.43;2.17	1.42;2.21	1.63;2.39	1.13;1.69	2.25;3.57	3.15;4.78	0.96;1.41	1.45;2.22
Pentyl	3.30	—	1.80	1.55	1.80	4.50	3.40	1.30	1.70	2.20	2.20	0.66	1.10
[16]	9.76;9.81	—	6.21;8.02	6.51;8.42	2.96;3.77	3.40;4.52	4.18;5.41	2.38;3.06	6.59;8.60	9.35;12.0	2.02;2.65	3.45;4.49	32.4;30.3
Hexyl	17.0	—	6.30	5.30	5.60	13.0	21.0	5.00	5.70	8.40	9.20	4.40	2.60
[16]	32.4;30.3	—	16.8;30.1	17.8;31.8	7.79;13.5	8.84;16.2	9.19;17.0	11.4;20.1	5.72;10.2	18.1;32.8	26.4;46.4	5.00;9.00	8.81;15.9
Heptyl	60.0	—	21.0	17.5	18.0	33.0	58.0	18.0	16.0	26.0	32.0	19.0	8.10
[16]	123;90.6	—	54.4;101	57.7;107	24.7;44.5	27.9;53.3	29.2;56.3	36.6;67.1	17.6;32.7	59.0;110	86.4;157	15.4;29.2	27.8;52.1
Octyl	225	—	79.0	57.5	52.0	98.0	290	116	51.0	88.0	117	72.0	34.0
[16]	490;194	—	186;274	197;289	82.3;120	93.8;144	98.9;152	123;182	58.7;87.3	202;300	295;427	51.1;78.1	93.9;140
Nonyl	737	—	272	233	122	210	723	355	124	283	390	332	93.0
[16]	958;238	—	341;769	364;812	156;337	176;404	183;427	232;510	109;245	371;842	548;1200	96.6;219	174;394
Other acids													
Cefazolin	—	0.57	—	1.11	0.43	17.1	4.84	1.03	0.40	—	1.89	—	—
[22,43]	—	0.74;1.66	—	1.28;3.05	1.23;2.25	1.10;2.71	0.79;2.60	1.52;2.35	0.69;2.80	—	1.92;2.88	—	—
Dideoxyinosine	—	—	0.027	0.53	—	7.07	0.79	—	0.72	0.74	—	0.41	—
[44]	—	—	0.75;0.80	0.75;0.79	—	0.69;0.74	0.66;0.70	—	0.73;0.78	0.64;0.69	—	0.57;0.61	—
Etodolac-R	14.9	—	6.81	—	39.6	26.4	26.4	—	—	—	—	—	—
[45]	11.6;0.91	—	11.1;1491	—	35.0;654	29.1;784	19.3;828	—	—	—	—	—	—
Etodolac-S	10.1	—	2.72	—	26.6	23.1	25.4	—	—	—	—	—	—
[45]	3.74;0.91	—	3.45;406	—	9.69;178	8.15;214	5.49;226	—	—	—	—	—	—
Penicillin	—	—	—	6.44	0.63	24.7	1.67	1.05	0.41	—	—	0.64	—
[22]	—	—	—	1.36;9.14	1.31;4.74	1.17;5.68	0.84;5.76	1.63;6.15	0.72;4.55	—	—	0.90;3.77	—

Table 5. (*Continued*)

Compound [Reference]	Experimentally Determined *K*pu[a] Followed by *K*pu Predicted via Equations 10 or 14[b]; Published[c] Mechanistic Equations												
	Adipose	Bone	Brain	Gut[d]	Heart	Kidney	Liver	Lung	Muscle	Pancreas	Skin	Spleen	Thymus
Phenobarbital	0.47	—	—	2.82	1.42	1.14	2.82	1.21	1.55	—	1.88	—	—
[42]	1.23;1.26	—	—	1.43;2.85	0.88;1.50	0.97;1.81	0.92;1.82	1.07;1.93	0.79;1.47	—	1.84;3.69	—	—
Phenytoin	9.30	—	4.70	12.9	6.20	8.27	11.9	4.91	5.68	—	8.79	—	—
[42]	21.2;20.4	—	12.0;22.5	12.9;23.7	5.81;10.3	6.55;12.3	6.74;12.9	8.39;15.1	4.33;7.97	—	19.0;34.3	—	—
Salicyclic acid	—	1.04	0.44	1.50	1.36	3.20	1.66	1.40	0.85	—	1.78	—	—
[46]	—	0.85;9.25	0.70;19.9	1.46;20.8	1.41;9.45	1.25;11.3	0.89;11.8	1.77;13.4	0.76;7.81	—	2.24;29.5	—	—
Tenoxicam	1.82	6.06	0.85	11.8	10.9	61.2	67.8	18.8	4.85	7.27	9.09	4.24	—
[31]	3.11;313	6.20;48.3	3.26;102	9.93;107	9.81;51.2	8.21;61.3	5.48;63.1	13.1;70.0	4.17;45.3	3.93;107	17.0;146	6.12;38.4	—
Thiopental	59.5	—	5.34	9.93	8.40	23.7	17.6	8.40	3.82	7.63	6.11	4.58	—
[47]	40.9;37.3	—	20.2;45.5	21.6;47.9	9.51;20.2	10.7;24.2	11.1;25.5	13.9;30.3	6.83;15.1	21.8;49.6	32.2;70.3	6.04;13.4	—
Tolbutamide	—	—	0.36	0.45	1.01	—	—	0.93	0.49	—	0.82	0.71	—
[42]	—	—	0.60;15.2	0.97;16.0	0.88;7.02	—	—	1.06;10.2	0.56;5.54	—	1.36;23.1	0.63;4.85	—
Valproate	0.41	—	0.19	1.23	1.17	4.10	4.92	1.15	0.44	—	1.28	—	—
[42]	0.30;0.23	—	0.52;17.4	0.77;18.3	0.71;7.80	0.68;9.35	0.52;9.84	0.82;11.6	0.48;5.88	—	1.03;26.8	—	—
Neutrals													
Cyclosporine	89.8	—	9.55	46.5	45.9	80.5	103	50.7	13.0	—	20.4	65.7	40.7
[34]	69.4;58.4	—	34.0;68.3	36.2;72.0	15.9;30.4	17.9;36.4	19.2;38.4	23.0;45.5	11.3;22.6	—	52.7;106	11.0;20.1	17.7;35.7
Digoxin	—	—	—	9.69	3.31	3.39	24.9	3.43	2.28	—	—	—	—
[48,49]	—	—	—	1.53;1.91	0.96;1.14	1.10;1.37	1.11;1.35	1.11;1.35	0.97;1.24	—	—	—	—
Ethoxybenzamide	1.26	—	1.58	0.97	1.74	2.19	—	1.57	1.32	—	1.73	1.47	—
[35,50]	0.48;0.45	—	1.03;1.38	1.10;1.39	0.79;0.95	0.91;1.14	—	0.86;1.04	0.85;1.12	—	1.11;1.45	0.78;0.89	—
Ftorafur	0.22	—	0.52	0.46	0.49	0.87	0.50	0.33	0.64	0.26	0.51	0.54	—
[36]	0.19;0.17	—	0.79;0.92	0.81;0.91	0.63;0.69	0.74;0.83	0.72;0.80	0.65;0.71	0.76;0.87	0.69;0.79	0.72;0.82	0.64;0.68	—
Zwitterions													
Ceftazidime	0.18	—	—	0.46	0.24	5.38	0.28	0.49	0.21	—	0.43	—	—
[51]	0.14;0.15	—	—	0.48;0.84	0.44;0.64	0.45;0.77	0.36;0.73	0.45;0.65	0.37;0.81	—	0.52;0.75	—	—
Nalidixic acid	0.34	0.99	0.75	1.67	1.67	1.84	1.98	1.13	1.23	—	1.19	1.16	—
[37]	0.27;0.16	0.47;1.45	0.52;2.83	0.86;2.88	0.80;1.78	0.75;2.14	0.57;2.11	0.95;2.06	0.52;1.99	—	1.17;3.27	0.59;1.59	—
Enoxacin	—	2.18	—	—	1.62	6.99	4.87	1.73	2.20	—	2.06	2.47	—
[37]	—	1.74;0.58	—	—	4.62;0.78	9.88;0.94	9.04;0.90	7.61;0.81	3.74;0.98	—	3.16;0.97	6.33;0.77	—
Lomefloxacin	0.37	2.19	0.30	2.26	1.90	6.72	3.19	1.72	2.24	—	1.30	2.40	—
[37]	0.76;0.15	1.68;0.53	1.83;0.96	4.81;0.95	4.12;0.73	8.68;0.87	8.00;0.83	6.67;0.74	3.55;0.92	—	2.90;0.86	5.62;0.71	—
Ofloxacin	0.25	1.84	0.31	—	2.31	8.30	2.65	1.76	2.23	—	1.55	2.50	—
[37]	0.60;0.15	1.15;0.51	1.16;0.92	—	3.09;0.70	6.47;0.84	5.89;0.80	5.03;0.71	2.44;0.88	—	2.17;0.82	4.15;0.69	—
Pefloxacin	—	—	0.24	—	3.11	5.16	6.68	2.56	3.22	—	—	4.34	—
[37]	—	—	1.00;1.58	—	2.79;0.97	5.79;1.16	5.24;1.15	4.54;1.14	2.12;1.05	—	—	3.70;0.85	—
Pipemidic acid	0.43	4.12	0.20	—	1.59	9.38	5.83	1.88	2.68	—	—	2.49	—
[37]	0.48;0.15	0.79;0.49	0.74;0.89	—	2.29;0.68	4.72;0.82	4.24;0.78	3.72;0.69	1.66;0.87	—	—	3.01;0.68	—
Tetracycline	2.20	21.5	—	7.50	—	8.10	9.40	—	4.10	—	—	—	—
[52]	0.94;0.15	1.58;0.68	—	5.40;1.23	—	1.06;1.11	9.60;1.07	—	3.48;1.16	—	—	—	—

[a]Where more than one value was reported the mean has been taken and where tissue-to-plasma values are reported *K*pu values have been calculated using the fu values in Table 2.
[b]New equations, using experimentally determined Log*P* (n-octanol:water) and pKa values.
[c]Equations from References 3 and 17, using experimentally determined Log*P* (n-octanol:water) and pKa values.
[d]Where stomach and/or individual segments of the intestine are reported the mean has been taken.

DOI 10.1002/jps

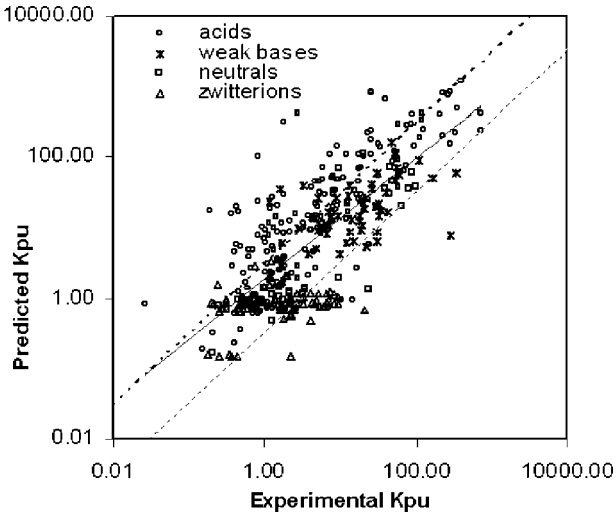

Figure 3. Relationship between published predicted[3,17] and experimentally determined *K*pu values for 7 very weak bases, 20 acids, 4 neutral drugs, 2 group 2 zwitterions and 6 group 1 zwitterions in 13 rat tissues ($n = 370$). The dashed lines represent a factor of 3 either side of the line of unity, and the solid line represents the line of best fit through the data ($y = 1.84x^{0.86}$, $R^2 = 0.57$).

addressed by Poulin and Theil,[1,3] who devised an alternative equation for such compounds that assumes no intracellular distribution. Using this approach, the prediction accuracy was improved but distinct outliers were still evident (see Fig. 4), partly because intracellular distribution is likely to impact upon the *K*pu values for the more lipophilic acids. The advantage of Eq. 14 is that ionisation and lipophilicity are incorporated, as opposed to just the latter, which subsequently determines the degree of intracellular penetration instead of assuming an all-or-nothing scenario. Applying this new equation to ionised acids improved the prediction accuracy even further (Fig. 4), with the added advantage of removing *a priori* segregation. However, outliers were still evident particularly for brain, liver and kidney, although their magnitude was significantly reduced (Fig. 4), indicating that additional processes may be contributing towards tissue distribution in certain instances.

Within brain there is an abundance of active transport mechanisms that can influence brain penetration. These transport processes should ideally be incorporated into the mechanistic equations, which currently assume passive diffusion, but at present parameters capable of accurately describing these events are extremely limited. It therefore comes as no surprise to see inaccurate *K*pu predictions (Eqs. 10 and 14) in brain for some compounds, namely, alfentanil, lomefloxacin, pefloxacin and dideoxyinosine. For these compounds over-predictions (by factors of 5.5, 4.2, 7.3 and 28 respectively) were evident, and this has been attributed to active efflux for the quinolones[54] and dideoxyinosine.[55]

In eliminating organs, arterial blood concentrations need to be adjusted for extraction across the organ of elimination (E, extraction ratio) since *K*pu (and *K*p) values reference drug concentrations in tissues to those in the emerging venous blood, and in most instances arterial, as opposed to venous, blood matrices are quantified. In the absence of this correction, the *in vivo* *K*pu will be underestimated, which will be reflected by an apparent over-prediction of the *K*pu values for eliminating organs. These inaccuracies will be more pronounced for highly cleared drugs since variability and uncertainty in clearance values and blood flows through the eliminating organs will have a significant impact on the prediction of E. To overcome this issue, allowances were made for E, with the exception salicyclic acid,[29] thiopental,[47] tetracycline[52] and etodolac.[25] As anticipated, the lack of E adjustments for these drugs did not result in over-predictions of the *K*pu values in kidney and liver since they are all low clearance compounds. However, under-predictions were evident for several compounds in these eliminating organs indicating additional mechanisms may be contributing towards their distribution.

First, pronounced under-predictions were evident for many of the highly ionised acids in kidney and liver, namely, cefazolin, penicillin, tenoxicam and valproate (by factors of 6–21). It has been suggested that such compounds can bind to ligandin, a cellular anion binding protein found in appreciable concentrations in kidney and liver,[22,56,57] and since ligandin binding was not accounted for in Eq. 14 this could explain the resultant under-predictions. Ligandin binding may also have contributed towards the kidney *K*pu under-prediction for ceftazidime (factor of 7), a group 2 zwitterion containing two highly acidic moieties. An additional contributory factor in these liver and kidney *K*pu under-predictions may be active influx mechanisms, for example organic anion transporting polypeptides and organic anion transporters could potentially be involved.[58,59]

Under-predictions were also evident for the very weak bases alprazolam, chlordiazepoxide, flunitrazepam and midazolam, (by factors of 6.4,

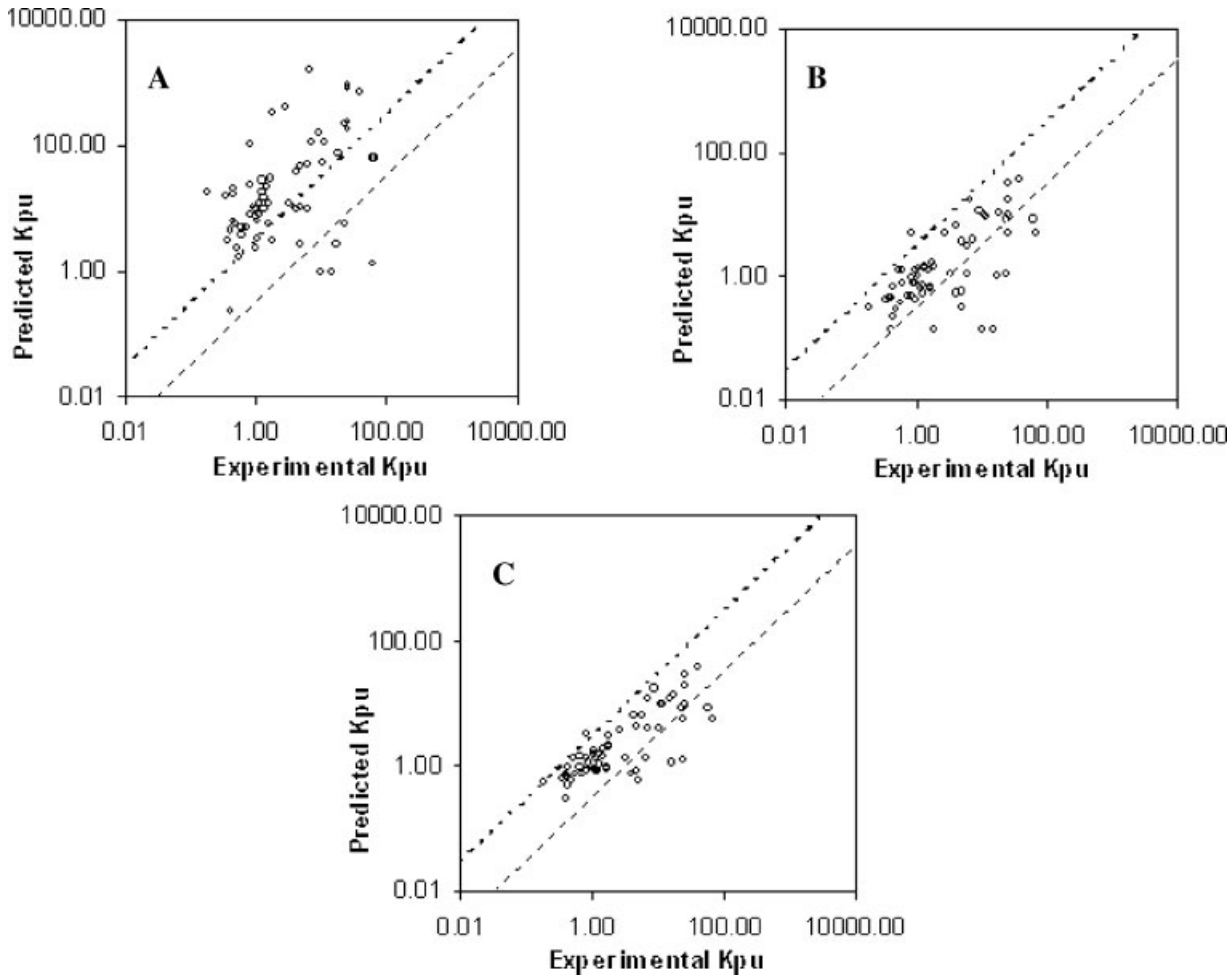

Figure 4. Relationship between predicted and experimentally determined *K*pu values for 8 acids with pKa values <7 in 13 rat tissues ($n = 62$), using (A) the generic published equation,[3,17] (B) the alternative published equation[3] and (C) the new equation (Eq. 14). The dashed lines represent a factor of 3 either side of the line of unity.

4.8, 4.6 and 11 respectively) in the liver and triazolam in the kidney (factor of 5), and for the weak acid dideoxyinosine in the kidney (factor of 10). These compounds have low to high clearance values, so while discrepancies in the estimation of extraction ratios may be a contributory factor in the under-predictions for some of these drugs, other unidentified mechanisms may be more influential, for example active influx or binding to additional tissue constituents. The liver *K*pu value for the neutral drug digoxin was also significantly under-predicted by a factor of 22, and in light of the findings of Funakoshi and co-workers[49] the most probable cause is active influx by organic anion transporting polypeptide 2 (oatp2).

Finally, the kidney, liver and spleen *K*pu values for cyclosporine were under-predicted by factors of 4.5, 5.4 and 6.0 respectively, which can be attributed to interactions between cyclosporine and the cellular protein cyclophilin,[34] a process not accounted for in the mechanistic calculations. Interestingly, the *K*pu prediction accuracy in all tissues increased with increasing cyclosporine dose (1.2–30 mg/kg, higher dose utilised in this publication) probably due to saturation of cyclophilin binding. Additionally, cyclosporine is a known p-glycoprotein substrate[34] so pronounced over-predictions of the brain *K*pu would be anticipated, but this was not observed (over-predicted by only a factor of 3.6). One explanation for this observation is that the experimental *K*pu values utilised in this study refer to a high cyclosporine dose (30 mg/kg) and at this high dose, p-glycoprotein efflux may become saturated. Additionally, there may be a balance between active p-glycoprotein efflux (resulting in over-predictions)

and cyclophilin binding (resulting in under-predictions), both of which are not incorporated into Eq. 14.

Another tissue where under-predictions were observed as a result of additional binding mechanisms is the bone. In bone, tetracycline has been reported to interchelate with bone apaptite crystals, and bind to the bone matrix and calcium.[60] These additional binding constituents were not accounted for in Eq. 10 and are the probable cause of the 6.2-fold under-prediction of the bone *K*pu for tetracycline. A similar under-prediction (factor of 5.2) of the bone *K*pu was also observed for pipemidic acid, but evidence of similar binding mechanisms could not be found.

When determining *in vivo* gut *K*pu values, gut tissue is frequently analysed along with its contents, for experimental ease. Under these conditions, the gut *K*pu is no longer purely distributional; instead, it may be a composite of distribution and elimination processes if for example, parent drug is significantly excreted in bile, glucuronide metabolites are metabolically converted back into parent compound by gut glucuronidases, enterohepatic recirculation transpires or gastrointestinal secretion occurs. Such processes increase the concentration of parent drug in the gut and will result in under-predictions of the gut *K*pu when using Eqs. 10 and 14. These elimination processes cannot at present be incorporated into Eqs. 10 and 14, and are the likely cause of the under-predictions of gut *K*pu values for penicillin (factor of 4.7), for which enterohepatic recirculation was indicated,[22] and for digoxin (factor of 6.3), for which gastrointestinal secretion via p-glycoprotein was proposed.[48,61] In addition, both compounds are extensively secreted into bile but this was not a contributory process in these under-predictions since experiments were performed on bile duct ligated animals.[22,48,61] The under-predictions for penicillin and digoxin in the gut can be attributed to like not being compared with like, that is gut tissue (predicted *K*pu) being compared to gut tissue plus luminal contents (*in vivo* *K*pu). As such, the predicted gut *K*pu values for these drugs are likely to be accurate estimates of gut distribution, since for the other compounds investigated predictions were generally accurate.

Global Influences

In addition to the aforementioned processes, the *K*pu prediction accuracy can also be globally influenced by the accuracy of the input parameters of the mechanistic equation, the accuracy of *in vivo* *K*pu determinations, and situations in which the assumptions of the equations are not upheld. For example, physiological uncertainty due to the use of varying strains, weights, sex and ages of rats, interlaboratory differences, *in vivo* experiments conducted under nonsteady-state conditions, nonlinear pharmacokinetic behaviour, plasma protein binding uncertainty (utilised for Ka_{PR} determinations) and the Log*P* solvent system, all of which have been discussed in more detail in an earlier publication.[7]

An additional factor that can influence the experimentally determined *K*pu values is the residual blood within tissues. This can have a significant impact on *K*pu values for highly vascular tissues, for example heart, lung and spleen, for drugs with low volumes of distribution, for example highly ionised acids with extensive protein binding. Under these conditions, blood can significantly contribute towards the concentration of drug in the tissue. Standard equations exist for adjusting tissue concentrations for residual blood drug levels[16] but these were not applied to all published data sets for poorly distributed drugs. This omission can result in an over-estimate of the *in vivo* *K*pu value, which will be reflected by an apparent under-prediction of the mechanistically predicted *K*pu's. For example, the published tissue concentrations for S-etodolac[45] were not adjusted for drug levels in residual blood, and the *K*pu values were under-predicted by a factor of 4.6 in liver (Tab. 5). After incorporating residual blood S-etodolac concentrations into the mechanistic *K*pu predictions using Eq. B5 (Appendix B), experimental and predicted *K*pu values now only differed by a factor of 2.5. This Eq. B5 was not used to correct for residual blood content in situations where *in vivo* *K*pu values reflected a composite of blood and tissue because, while Eq. B5 improves the prediction accuracy, the resultant *K*pu estimate is not a true measure of the affinity of a drug for a particular tissue.

Inaccurate *K*pu predictions can also arise when using raw published tissue composition data since the neutral lipid, neutral phospholipid and total tissue water contents refer to tissue plus residual blood. The impact of using uncorrected tissue composition data was investigated and found to be relatively minor, even for low distribution compounds, since the fractional contribution of blood constituents on the tissue composition data was largely negligible, for example fractional

neutral lipid levels in blood adjusted and unadjusted heart were 0.0135 and 0.0140 respectively. However, since adjustments are possible (Appendix A) they were performed and the blood adjusted values (Tab. 1) were subsequently utilised in the *K*pu predictions (Eqs. 10 and 14 and published equations[3,17]).

The use of predicted, as opposed to experimentally measured, Log*P* (n-octanol:water) and pKa values would remove uncertainty associated with interlaboratory differences in the determination of these parameters and reduce the experimentation required for mechanistic *K*pu calculations. This approach was utilised for moderate-to-strong bases and a good agreement between the two sets of *K*pu predictions was reported.[7] Regarding the compounds studied in the current research, the overall *K*pu prediction accuracy for the two data sets was generally good but distinct outliers were observed (Tabs. 2 and 3). Namely, the predicted and experimental Log*P* (n-octanol:water) values differed significantly for the very weak bases alfentanil, midazolam and triazolam, and the neutral drug cyclosporine, and the predicted basic pKa of tetracycline (a group 1 zwitterion) was much lower than the experimental value, that is 5.8 versus 9.7 respectively. The consequence of this tetracycline pKa disparity was that the assumptions regarding tissue binding differed in response to the different pKa values, thereby invoking the utilisation of Eq. 10 for the experimental pKa's and Eq. 14 for the predicted pKa values. This resulted in inaccurate *K*pu predictions when using the predicted pKa values for tetracycline. Alternative software packages may remove these disparities but they were not available for this research.

Another input parameter that warrants further discussion is the affinity constant of a drug for binding proteins in the extracellular space of tissues. It was assumed that the major binding protein for neutral drugs is lipoprotein, and that for acids and very weak bases is albumin. However, many drugs can also interact with additional plasma and tissue proteins to varying extents. If these interactions are minor in comparison to the assumed major protein–drug complexes, or if other binding mechanisms dominate, for example lipid partitioning, then these processes will have a minimal impact on the *K*pu predictions. In contrast, if the additional protein interactions have a significant impact on tissue distribution then *K*pu values will be under-predicted using Eqs. 10 and 14, as mentioned earlier for ligandin binding of acidic drugs and cyclophilin binding of cyclosporine. However, if the major binding protein in plasma and extracellular fluid is albumin as opposed to lipoprotein (or visa versa), for example the neutral drugs digitoxin and dexamethasone (not studied in this research) predominantly bind to albumin not lipoprotein,[9] then this will have a limited impact on the *K*pu prediction accuracy in most tissues, exceptions being bone, liver, skin and spleen where the tissue-to-plasma albumin and lipoprotein ratios differ by factors of 2 or greater (Tab. 1). Under these circumstances, the appropriate ratios can be utilised if the major binding protein has already been identified.

Modified Approaches

Despite the limitations of the current mechanistic approach, and the uncertainty and variability of the parameter values, the equations accurately predicted the majority of the *K*pu values for 7 very weak bases, 20 acids, 4 neutral drugs and 8 zwitterions in 13 rat tissues. In these predictions, the affinity of a drug for acidic phospholipids (group 1 zwitterions) or extracellular binding proteins (all other compound types) were determined from $K\text{pu}_{\text{BC}}$ or fu, respectively. The advantage of using these parameters for affinity calculations is that they can be determined *in vitro*[41] and the only biological matrix required is whole blood, which is relatively easy to obtain for any species. An alternative approach for determining the $K\text{a}_{\text{AP}}$ of drug-acidic phospholipid complex was proposed by Rodgers and co-workers,[7] which permitted the distribution into one tissue to be predicted from that in another thereby increasing the flexibility of the mechanistic predictions. The tissue investigated for this purpose by these researchers was muscle since a good correlation between the distribution into muscle and the distribution into other tissues has been reported[1,62] and good *in vitro*-to-*in vivo* correlations have been cited for muscle *K*p and *K*pu values.[16,62,63] This last approach was also investigated for the compounds utilised in this research (Fig. 5, values not tabulated) and the accuracy of the *K*pu predictions using muscle *K*pu data for affinity calculations was also similar to that obtained using plasma or blood cell data.

Within this research, the mechanistic equations have been restricted to rats due to the scarcity of tissue composition data in other species and experimental *K*pu values with which to challenge the *K*pu predictions. If such information were to

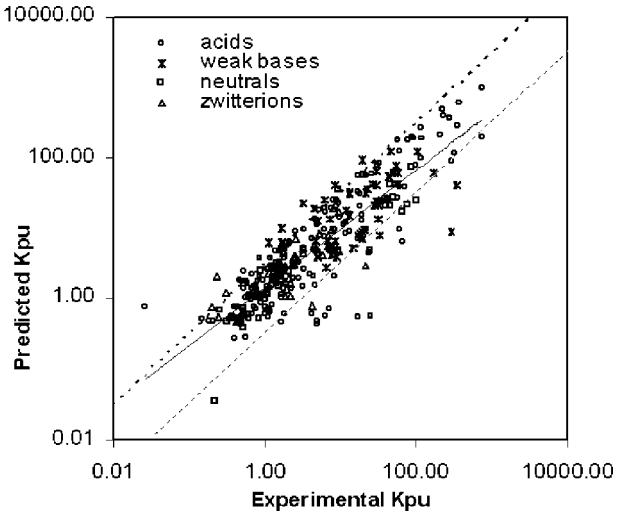

Figure 5. Relationship between predicted (Eqs. 10 and 14—$K\mathrm{a}_{\mathrm{AP}}$ or $K\mathrm{a}_{\mathrm{PR}}$ from muscle *K*pu values, using experimental Log*P* and pKa values) and experimentally determined *K*pu values for 7 very weak bases, 20 acids, 4 neutral drugs, 2 group 2 zwitterions and 6 group 1 zwitterions in 13 rat tissues. The dashed lines represent a factor of 3 either side of the line of identity (data within these lines represent 82% of 314 predictions) and the solid line is the line of best fit through the data ($y = 1.48x^{0.83}$, $R^2 = 0.76$).

become available then these equations could be further tested with the future possibility of *a priori* *K*pu predictions in various animal species and humans. Additionally, the data set employed here is somewhat restrictive for certain drug classes, for example neutral drugs ($n = 4$ compounds), and should ideally be expanded but this was not possible in this research due to the limited availability of experimental data. Such expansions would have been of particular interest for compounds that are restricted to extracellular water and do not bind to any tissue constituents. Compounds for which this is likely to apply are hydrophilic neutral drugs, since the hydrophilic and neutral compound sucrose is used as a marker for measuring extracellular tissue volumes.[64] For such compounds, it is anticipated that modifications to Eq. 14 would be required since in its current form, the smallest *K*pu value it can predict is equivalent to total tissue water.

In summary, the time, cost and labour intensity of experimental *K*pu determinations have restricted the use of multicompartmental WBPBPK models in the pharmaceutical industry, and promoted investigations into model reduction and parameter predictions. With model reduction, the dimensionality and complexity of the model are reduced but essential information can be irretrievably lost. Regarding parameter predictions, the most noticeable benefits come from mechanistic predictions of the *K*pu values for multiple tissues, which permits model conservation and avoids losing tissue-specific information. Using published equations, pronounced under and over-predictions of *K*pu values were evident for zwitterions containing a basic functionality with a pKa value ≥ 7 and ionised acids respectively, since ionisation and its impact on distribution were not accounted for. This spurred the development of new equations to accommodate drug ionisation and their application significantly improved the accuracy of mechanistic *K*pu predictions.

ACKNOWLEDGMENTS

Financial support for this project was provided by the following Centre for Applied Pharmacokinetic Research (The University of Manchester) Consortium members, GlaxoSmithKline, Novartis, Pfizer, Servier and Eli Lilly.

APPENDIX A

Adjusting the Mechanistic Equation Input Parameters for Contributions from the Residual Blood in Tissues

Published values for the levels of neutral lipids and phospholipids in tissues are based on tissue plus residual blood, whereas tissue *K*pu values are a measure of the affinity of a drug for the tissue only, that is not including contributions from residual blood. To account for this disparity, the tissue lipid levels have been adjusted for contributions from residual blood using the following equation and these values have been reported in Table 1.

$$f_{\mathrm{NL,T}} = f_{\mathrm{NL,T}}^{\mathrm{published}} - (f_{\mathrm{residual}} \cdot (f_{\mathrm{NL}}^{\mathrm{plasma}} \cdot (1 - H) + f_{\mathrm{NL}}^{\mathrm{blood}} \cdot H)) \quad \text{(A1)}$$

where $f_{\mathrm{NL,T}}$ and $f_{\mathrm{NL,T}}^{\mathrm{published}}$ are the fractional volumes of neutral lipid in the residual blood adjusted and unadjusted tissues respectively, $f_{\mathrm{NL}}^{\mathrm{blood}}$ and $f_{\mathrm{NL}}^{\mathrm{plasma}}$ are the fractional volumes of neutral lipid in blood and plasma respectively, f_{residual} is the fractional volume of residual blood in the tissue and H is the haematocrit.

For neutral phospholipids, fractional volumes for neutral phospholipids replaced all values relating to neutral lipid, that is subscript NL. Values for the fractional volumes of neutral lipid and neutral phospholipid in tissues were not adjusted for residual blood, and f_{NL}^{blood} were taken from Reference 7, $f_{residual}$ was taken from Reference 16 and f_{NL}^{plasma} can be found in the text.

Regarding the fractional water content of the tissues these were adjusted for the contribution from residual blood using Eq. A2.

$$f_{W,T} = f_{W,T}^{published} - (f_{residual} \cdot (f_{W}^{plasma} \cdot (1 - H) + f_{IW}^{blood} \cdot H)) \quad \text{(A2)}$$

where $f_{W,T}$ and $f_{W,T}^{published}$ are the fractional volumes of water in the residual blood adjusted and unadjusted tissues respectively, f_{IW}^{blood} is the fractional volume of intracellular water in blood cells and f_{W}^{plasma} is the fractional volumes of water in plasma.

APPENDIX B

Adjusting the Predicted *K*pu Values for Residual Blood in Tissues Using a Mechanistic Expression (for Accuracy Comparisons Only)

The residual blood in tissues can have a significant impact upon the *K*pu values for poorly distributed drugs, particularly in highly vascular tissues. Standard equations[16] exist for correcting tissue concentrations for contributions from blood but in the absence of raw data, that is for many of the compounds studied in this research, this approach was not possible. An alternative approach to making such adjustments is to modify the mechanistic equations presented in this work.

In blood, drugs can associate with both blood cells and plasma constituents, as shown by Eq. B1.

$$C_{blood} = \frac{A_{blood}}{V_{blood}} + \frac{A_{BC} + A_{P}}{V_{blood}} = \frac{C_{BC} \cdot V_{BC} + C_{P} \cdot V_{P}}{V_{blood}} = C_{BC} \cdot H + C_{P} \cdot (1 - H) \quad \text{(B1)}$$

where V, A and C refer to volume, amount and concentration respectively, and subscripts BC and P refer to blood cells and plasma respectively.

The concentration of drug in blood cells can then be determined by applying Eq. 7 blood cells, by recognising that blood cells have no extracellular space and assuming blood cells contain no albumin or lipoproteins (Eq. B2).

$$C_{BC} = Cu \cdot \left[\frac{X \cdot f_{IW,BC}}{Y} + \left(\frac{P \cdot f_{NL,BC} + (0.3P + 0.7) \cdot f_{NP,BC}}{Y}\right)\right] \quad \text{(B2)}$$

where subscript BC refers to blood cells and X and Y have been previously defined but in the case of X, pH_{IW} refers to the intracellular pH of blood cells, that is 7.22.[7]

In plasma, drug concentrations can be predicted using the standard equation $\left(C_P = {}^{Cu}/_{fu}\right)$, inserting this information along with Eq. B2 into Eq. B1 yields the following.

$$C_{blood} = \frac{Cu \cdot (1 - H)}{fu} + Cu \cdot H \cdot \left[\frac{X \cdot f_{IW,BC}}{Y} + \left(\frac{P \cdot f_{NL,BC} + (0.3P + 0.7) \cdot f_{NP,BC}}{Y}\right)\right] \quad \text{(B3)}$$

The predicted *K*pu values can then be adjusted to remove contributions from residual blood K_{puadj}) using Eq. B4.

$$Kpu_{adj} = Kpu + \frac{A_{blood}}{V_T \cdot Cu} = Kpu + \frac{C_{blood} \cdot V_{residual}}{V_T \cdot Cu} = Kpu + \frac{C_{blood} \cdot f_{residual}}{Cu} \quad \text{(B4)}$$

where *K*pu refers to *K*pu values calculated using Eq. 14, $V_{residual}$ is the volume of residual blood in the tissue and $f_{residual}$ is the fractional volume of residual blood in the tissue.

Inserting Eq. B3 into Eq. B4 and simplifying it by cancelling out the term *C*u generates a mechanistic expression for accounting for residual blood contributions to the *K*pu (Eq. B5).

$$Kpu_{adj} = Kpu + f_{residual} \cdot \left[\frac{(1 - H)}{fu} - H \cdot \left[\frac{X \cdot f_{IW,BC}}{Y} + \left(\frac{P \cdot f_{NL,BC} + (0.3P + 0.7) \cdot f_{NP,BC}}{Y}\right)\right]\right] \quad \text{(B5)}$$

This expression is used purely for assessing prediction accuracy when the *in vivo* *K*pu value has not been adjusted for tissue blood, and should not be used for predicting tissue *K*pu's.

 DOI 10.1002/jps

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