Physiologically Based Pharmacokinetic Modeling 1: Predicting the Tissue Distribution of Moderate-to-Strong Bases

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ABSTRACT: Tissue-to-plasma water partition coefficients (Kpu's) form an integral part of whole body physiologically based pharmacokinetic (WBPBPK) models. This research aims to improve the predictability of Kpu values for moderate-to-strong bases ($pK_a \geq 7$), by developing a mechanistic equation that accommodates the unique electrostatic interactions of such drugs with tissue acidic phospholipids, where the affinity of this interaction is readily estimated from drug blood cell binding data. Additional model constituents are drug partitioning into neutral lipids and neutral phospholipids, and drug dissolution in tissue water. Major assumptions of this equation are that electrostatic interactions predominate, drugs distribute passively, and non-saturating conditions prevail. Resultant Kpu predictions for 28 moderate-to-strong bases were significantly more accurate than published equations with 89%, compared to 45%, of the predictions being within a factor of three of experimental values in rat adipose, bone, gut, heart, kidney, liver, muscle, pancreas, skin, spleen and thymus. Predictions in rat brain and lung were less accurate probably due to the involvement of additional processes not incorporated within the equation. This overall improvement in prediction should facilitate the further application of WBPBPK modeling, where time, cost and labor requirements associated with experimentally determining Kpu's have, to a large extent, deterred its application.
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INTRODUCTION

Obtaining rapid information regarding the pharmacokinetics of new drug candidates is a bottleneck in the discovery and development of new drugs, while unfavourable parameters are a common reason for the failure of many compounds. To expedite the acquisition of pharmacokinetic data, assist compound selection and ultimately reduce compound failures, a sound understanding as to the behaviour of a compound within the test system is required. Such knowledge improves the ability to predict *in vivo* pharmacokinetic behaviour from *in vitro* and *in silico* data. Prediction across compounds is facilitated by relating the pharmacokinetic behaviour of a compound to its structure and physicochemical properties.

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At present, pharmacokinetics is still predominantly descriptive in that the observed *in vivo* behaviour of a drug is characterised using predominantly empirical models, such as the sum of exponentials or compartmental models, based almost exclusively on the experimental drug data, mostly derived from measurements in plasma. A drawback of these empirically based models is that they have very restrictive predictive power; they are applied almost exclusively to the compound under study, with limited application to scaling across species and to other compounds. To improve on these predictive approaches it is necessary to move from empirically based methods towards more mechanistic models, which, unlike the former, can be applied to a wide array of structurally diverse compounds. Pharmacokinetic models that are mechanistically based are termed physiological based pharmacokinetic (PBPK) models, but a significant deterrent to their application within the pharmaceutical industry has been the vast quantities of data required for model construction. This problem is compounded when applying PBPK models to multiple tissues or compartments within the whole body (WBPBPK models) since experimental determination of tissue affinities (an essential input parameter), characterised by either the steady state tissue-to-plasma concentration ratio (Kp) or tissue-to-plasma water concentration ratio (Kpu), can be costly and time consuming, and require a substantial amount of compound, which is rarely available during discovery and early candidate selection. A solution to this problem would be to predict 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.

Such mechanistic equations have been developed by Poulin and coworkers, based on previous work in the environmental chemical area, and used to predict the Kp values of a diverse set of structurally unrelated acidic, basic and neutral compounds.[1–3] While a significant improvement over previous methods and generally successful, a drawback identified by these researchers was that Kp predictions for moderate-to-strong bases appeared to be less accurate than predictions for other compounds. To extend the application to moderate-to-strong bases we have developed a mechanistically-based equation that accommodates the unique binding interactions of this class of compound, and showed it to improve substantially the predictability over that of the published equations for 28 moderate-to-strong bases in 13 rat tissues. Issues surrounding the practical application and limitations of this new equation are also discussed.

METHODS

Underlying Considerations

Previous work with the individual enantiomers of a series of eight β-blocking drugs suggested that plasma protein binding and acidic phospholipid (phosphatidylserine (PS), mono- and diphosphatidylglycerol (PG), phosphatidylinositol (PI) and phosphatidic acid (PA)) concentrations in blood and tissue cells were the primary factors controlling the distribution of this drug class within the body.[4] The dominant interaction between β-blockers (pK_a of *ca*. 9.5) and acidic phospholipids is thought to be electrostatic since both components are predominantly ionised at physiological pH. As such, it can be assumed that in the absence of other specialised mechanisms, such as active transport and DNA inter-chelation, electrostatic interactions with acidic phospholipids will predominate for any basic compound that is sufficiently ionised within tissue cells. For the purpose of this research, such compounds have been defined as any base with at least one pK_a value ≥ 7, that is, at pH 7 (intracellular tissue pH) the drug will be at least 50% ionised. This assumption is supported by the findings of Yata et al.,[5] who demonstrated that propranolol, quinidine and imipramine (all bases with pK_a value > 8) have a very strong affinity for PS, PG and PI.

In addition to electrostatic interactions with acidic phospholipids, moderate-to-strong bases also dissolve in tissue water, and the unionised form can partition into neutral lipids and neutral phospholipids, processes that are generic to all drug types. The influence of these processes on the tissue distribution of bases such as the β-blocking drugs is minor when compared to electrostatic interactions, with the exception of adipose, a tissue with exceptionally high neutral lipid content and a much lower water content (Table 1). As such, these processes cannot be completely ignored and need to be considered along with electrostatic interactions. A generalised overview of the tissue distribution of moderate-to-strong bases is illustrated in Figure 1.

Table 1. Tissue Specific Input Parameters for the Mechanistic Equations Used to Predict Kpu Values in Rats*

Tissue	Fractional Tissue Volume[a]				Tissue Concentration of Acidic Phospholipids (mg/g)[d]
	Neutral Lipid	Neutral Phospholipid[b]	Extracellular Water	Intracellular Water[c]	
References	4,8–10		4,13,14		4–11
Blood cells	0.0017	0.0029	NA	0.603	0.50
Adipose	0.853	0.0016	0.135	0.017	0.40
Bone[e]	0.017	0.0017	0.100	0.346	0.67
Brain[f]	0.039	0.0015	0.162	0.620	0.40
Gut	0.038	0.0125	0.282	0.475	2.41
Heart	0.014	0.0111	0.320	0.456	2.25
Kidney	0.012	0.0242	0.273	0.483	5.03
Liver	0.014	0.0240	0.161	0.573	4.56
Lung	0.022	0.0128	0.336	0.446	3.91
Muscle	0.010	0.0072	0.118	0.630	1.53
Pancreas	0.041	0.0093	0.120	0.664	1.67
Skin	0.060	0.0044	0.382	0.291	1.32
Spleen	0.0077	0.0113	0.207	0.579	3.18
Thymus	0.017	0.0092	0.150	0.626	2.30

*Where more than one value was reported the mean has been taken.
[a]Based on the tissue wet weight.[3]
[b]Determined by converting the tissue concentration of acidic phospholipids in mg/g to a fractional tissue volume then subtracting this value from reported fractional tissue volumes of total phospholipid.[3,4,6–11] Where total phospholipid is quoted as lipid phosphorus a conversion factor of 25 was used.[5]
[c]Calculated by subtracting the fractional tissue volume of extracellular water from the fractional tissue volume of total tissue water.[4,8,10,12]
[d]The sum of phosphatidylserine, phosphatidylinositol, phosphatidylglycerol (mono- and di-) and phosphatidic acid tissue concentrations. Calculated from percentage values for each phospholipid and either total tissue phospholipid or phosphatidylserine levels.
[e]Value representative of bone marrow, cortical and trabular bone material.
[f]Uncertainty associated with phospholipid levels in brain (refer to text).

Derivation

Consider events within the body at steady state following a constant rate intravenous infusion of drug. For any tissue the Kpu is given by

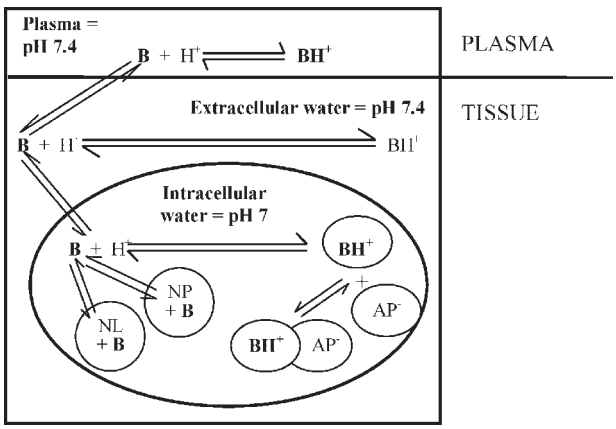

Figure 1. Illustration of the processes involved in the distribution of moderate-to-strong bases between tissue and plasma that have been accommodated in Equation 19 (see text for abbreviations and further information).

$$\mathrm{Kpu} = \frac{C_{\mathrm{T,ss}}}{Cu_{\mathrm{p,ss}}} \tag{1}$$

where $C_{\mathrm{T,ss}}$ is the steady state concentration of drug in a tissue outside of the blood perfusing it, and $Cu_{\mathrm{p,ss}}$ is the corresponding unbound concentration in plasma. Subsequently, the subscript ss is dropped, but inferred.

For each tissue, C_{T} is obtained by dividing the amount of drug in the tissue (A_{T}) by its volume (V_{T}). In turn A_{T} comprises the sum of amounts dissolved in the intracellular and extracellular tissue water ($A_{\mathrm{U,IW}}$ and $A_{\mathrm{U,EW}}$, respectively), that partitioned into neutral phospholipids and neutral lipids located within tissue cells ($A_{\mathrm{B,NP}}$ and $A_{\mathrm{B,NL}}$, respectively), and that associated with residual tissue components ($A_{\mathrm{B,REM}}$), as defined by Equation 2.

$$C_{\mathrm{T}} = \frac{A_{\mathrm{U,IW}} + A_{\mathrm{U,EW}} + A_{\mathrm{B,NP}} + A_{\mathrm{B,NL}} + A_{\mathrm{B,REM}}}{V_{\mathrm{T}}} \tag{2}$$

For moderate-to-strong bases given the dominant electrostatic binding to acidic phospholipids, $A_{B,REM}$ can be replaced by $A_{AP,REM}$, where the subscript AP refers to acidic phospholipids, a predominantly cellular constituent.

Expressing the amounts in terms of the respective concentrations (C) and volumes (V) of each phase yields Equation 3

$$C_T = \frac{\left[\begin{array}{l} C_{U,IW} \cdot V_{IW} + C_{U,EW} \cdot V_{EW} + C_{B,NL} \cdot V_{NL} \\ + C_{B,NP} \cdot V_{NP} + C_{B,AP} \cdot V_{B,REM} \end{array}\right]}{V_T} \tag{3}$$

or

$$\begin{aligned} C_T = {} & C_{U,IW} \cdot f_{IW} + C_{U,EW} \cdot f_{EW} + C_{B,NL} \cdot f_{NL} \\ & + C_{B,NP} \cdot f_{NP} + C_{B,AP} \cdot f_{REM} \end{aligned} \tag{4}$$

where f refers to fractional tissue volume (with $\sum f = 1$, so that $f_{REM} = 1 - f_{IW} - f_{EW} - f_{NL} - f_{NP}$), and subscripts U, B, IW, EW, NL, NP, AP and REM refer to unbound drug, bound drug, intracellular water, extracellular water, neutral lipid, neutral phospholipid, acidic phospholipid and residual tissue constituents, respectively.

The intracellular unbound concentration, $C_{U,IW}$, is the sum of the ionised (BH^+) and unionised (B) unbound species (Equation 5) which, for a monoprotic base, are related to the pK_a of the drug and the pH of the tissue *via* Equation 6.

$$C_{U,IW} = [B] + [BH^+] \tag{5}$$

$$pH = pK_a + \log \frac{[B]}{[BH^+]} \tag{6}$$

Combining Equations 5 and 6, yields expressions for $C_{U,IW}$ (Equation 7) and Cu_p (Equation 8).

$$\begin{aligned} C_{U,IW} &= [BH^+]_{U,IW} \cdot \left(1 + 10^{pH_{IW} - pK_a}\right) \\ &\equiv [B]_{U,IW} \cdot \left(1 + 10^{pK_a - pH_{IW}}\right) \end{aligned} \tag{7}$$

$$\begin{aligned} Cu_p &= [BH^+]_{U,P} \cdot \left(1 + 10^{pH_p - pK_a}\right) \\ &\equiv [B]_{U,P} \cdot \left(1 + 10^{pK_a - pH_p}\right) \end{aligned} \tag{8}$$

where subscript P refers to plasma, and pH_{IW} and pH_p refer to the pH of intracellular tissue water and plasma, respectively.

Equations 7 and 8 can then be combined by assuming that only unbound unionised drug permeates cell membranes, and that the concentrations of unbound unionised drug in the aqueous phase on each side of the cell membrane are equal at equilibrium, that is, $[B]_{U,IW} = [B]_{U,P}$. $C_{U,IW}$ for a monoprotic base is then given by:

$$C_{U,IW} = Cu_p \cdot \left(\frac{1 + 10^{pK_a - pH_{IW}}}{1 + 10^{pK_a - pH_p}}\right) \tag{9}$$

For polyprotic bases Equations 7–9 and all subsequent re-arrangements of these equations, must be modified in accordance with the Henderson–Hasselbalch equations.

Equation 9 can also be used to calculate $C_{U,EW}$ (Equation 4), which is equivalent to the unbound concentration in the tissue extracellular water, by replacing pH_{IW} with the pH of the extracellular tissue space (pH_{EW}). However, since pH_{EW} is generally the same as pH_p, that is, pH 7.4[15] then $C_{U,EW}$ equals Cu_p.

Previous research has shown that BH^+ (Equation 5) preferentially interacts with the acidic phospholipids PS, PG, PI, and PA,[4,5,16] which are located within cell membranes with the ionised head groups predominantly protruding into the intracellular tissue water.[17] Such an orientation suggests that the electrostatic interactions between unbound intracellular BH^+ ($[BH^+]_{U,IW}$) and the charged head groups of the acidic phospholipids occur at cell membrane–cytosol interfaces. The association constant (K_a) of basic compounds with these acidic phospholipids (AP^-) is given by Equation 10.

$$K_a = \frac{[AP^- \cdot BH^+]_{B,REM}}{[BH^+]_{U,IW} \cdot [AP^-]_{REM}} \tag{10}$$

where $[AP^- \cdot BH^+]_{B,REM}$ represents the ionised base–acidic phospholipid complex concentration, that is, $C_{B,AP}$, and $[AP^-]_{REM}$ is the concentration of available binding sites on the acidic phospholipids. Assuming all acidic phospholipids are totally ionised and non-saturating conditions prevail, such that few of the total AP sites are occupied, $[AP^-]_{REM}$ is taken to be the total AP concentration (which in turn is taken to be the sum of the PS, PG, PI and PA concentrations in the residual tissue matter, f_{REM}).

Rearranging Equation 10 find $C_{B,AP}$ and substituting $[BH^+]_{U,IW}$ with Equation 6 yields Equation 11 and since it has been assumed that $[B]_{U,IW} = [B]_{U,P}$ then Equation 11 can be combined with Equation 8 produce Equation 12 (for a monoprotic base).

$$C_{B,AP}=K_a \cdot [AP^-]_{REM} \cdot [B]_{U,IW} \cdot 10^{pK_a-pH_{IW}} \quad (11)$$

$$C_{B,AP}=\frac{K_a \cdot [AP^-]_{REM} \cdot Cu_p \cdot 10^{pK_a-pH_{IW}}}{1+10^{pK_a-pH_p}} \quad (12)$$

In addition to the aforementioned electrostatic interactions with acidic phospholipids, unionised moderate-to-strong bases also interact with intracellular neutral phospholipids and neutral lipids through hydrophobic interactions.

If we assume distribution into neutral lipids equates to the partitioning of unbound unionised drug between intracellular water and neutral lipids, then the affinity for neutral lipids equals the partition coefficient, where the *in vitro* surrogates for intracellular water and neutral lipid are assumed to be 'pure' water and *n*-octanol respectively, for all tissues except adipose. In adipose, vegetable oil was determined to be a better surrogate than *n*-octanol for neutral lipid by Poulin et al.,[2] so is used here. Partitioning into tissue neutral lipids can therefore be calculated using Equation 13, which after replacing $[B]_{U,P}$ with Equation 8 yields Equation 14 (for a monoprotic base).

$$[B]_{NL}=P \cdot [B]_{U,IW}=P \cdot [B]_{U,P} \quad (13)$$

$$[B]_{NL}=\frac{P \cdot Cu_p}{1+10^{pK_a-pH_p}} \quad (14)$$

where $[B]_{NL} \equiv Cu_P$ represents the concentration of unionised base in the neutral lipid of tissue cells and P is the *n*-octanol:water partition coefficient for all tissues except adipose, where it represents vegetable oil:water partition coefficient.

Within tissue cells, the neutral species of moderate-to-strong bases can also partition into phospholipids that possess no net charge, that is, neutral phospholipids. The strength of these hydrophobic unionised base–neutral phospholipid interactions cannot solely be described by P since phospholipids represent a mixture of 30% neutral lipid and 70% water.[18] The concentration of unionised base in neutral phospholipids ($[B]_{NP}$) can therefore be calculated using Equation 15, where $[B]_{NL}$ can be calculated using Equation 14 and $[B]_{U,P}$ using Equation 8, thereby producing Equation 16 (for a monoprotic base).

$$\begin{aligned}[B]_{NP} &= 0.3 \cdot [B]_{NL}+0.7 \cdot [B]_{U,IW} \\ &= 0.3 \cdot [B]_{NL}+0.7 \cdot [B]_{U,P}\end{aligned} \quad (15)$$

$$[B]_{NP}=\frac{Cu_p}{1+10^{pK_a-pH_p}} \cdot (0.3P+0.7) \quad (16)$$

Inserting the relevant terms into Equation 4, that is, remembering $C_{U,EW}$ equals Cu_p, and substituting $C_{U,IW}$ with Equation 9, $C_{B,AP}$ with Equation 12, C_{NL} ($[B]_{NL}$) with Equation 14 and C_{NP} ($[B]_{NP}$) with Equation 16, generates Equation 17.

$$C_T = Cu_p \cdot \left[\begin{array}{l} f_{EW}+\left(\dfrac{1+10^{pK_a-pH_{IW}}}{1+10^{pK_a-pH_p}} \cdot f_{IW}\right) \\ +\left(\dfrac{K_a \cdot [AP^-]_{REM} \cdot 10^{pK_a-pH_{IW}}}{1+10^{pK_a-pH_p}} \cdot f_{REM}\right) \\ +\left(\dfrac{(P \cdot f_{NL}+((0.3P+0.7) \cdot f_{NP}))}{1+10^{pK_a-pH_p}}\right) \end{array}\right] \quad (17)$$

Since $[AP^-]_{REM}$ is calculated by dividing the concentration of AP in the tissue ($[AP^-]_T$) by f_{REM} then f_{REM} cancels out to produce a mechanistic expression for C_T (Equation 18).

$$C_T = Cu_p \cdot \left[\begin{array}{l} f_{EW}+\left(\dfrac{1+10^{pK_a-pH_{IW}}}{1+10^{pK_a-pH_p}} \cdot f_{IW}\right) \\ +\left(\dfrac{K_a \cdot [AP^-]_T \cdot 10^{pK_a-pH_{IW}}}{1+10^{pK_a-pH_p}}\right) \\ +\left(\dfrac{(P \cdot f_{NL}+((0.3P+0.7) \cdot f_{NP}))}{1+10^{pK_a-pH_p}}\right) \end{array}\right] \quad (18)$$

So that

$$\begin{aligned}Kpu &= \frac{C_T}{Cu_p} \\ &= \left[\begin{array}{l} f_{EW}+\left(\dfrac{1+10^{pK_a-pH_{IW}}}{1+10^{pK_a-pHp}} \cdot f_{IW}\right) \\ +\left(\dfrac{K_a \cdot [AP^-]_T \cdot 10^{pK_a-pH_{IW}}}{1+10^{pK_a-pH_p}}\right) \\ +\left(\dfrac{(P \cdot f_{NL}+((0.3P+0.7) \cdot f_{NP}))}{1+10^{pK_a-pH_p}}\right) \end{array}\right]\end{aligned} \quad (19)$$

Tissue Input Parameters

Values for pH_p, pH_{IW} and pH_{BC} were taken to be 7.4, 7 and 7.22 (mean taken from references 15, 19 and 20), respectively. Regarding f_{IW}, f_{EW}, $f_{IW,BC}$, $f_{W,P}$ AP^-_T, AP^-_{BC}, AP^-_P, f_{NL}, f_{NP}, $f_{NL,BC}$, $f_{NP,BC}$, $f_{NL,P}$ and $f_{NP,P}$ values were obtained from the literature and are summarised in Table 1.

Compound Specific Input Parameters

The compound specific input parameters have been taken from the literature or predicted using

Table 2. Compound Specific Input Parameters for the Mechanistic Equations Used to Predict Kpu Values*

	pK_a		Log$P_{o:w}$[a]		Log$P_{vo:w}$[a,b]					
Compound	Exp[e]	Pred[f]	Exp[e]	Pred[g]	Exp	Pred	f_u	B:P[c]	Kpu_{BC}[d]	References
Acebutolol-R	9.7	9.0	1.87	1.27	0.74	0.07	0.79	1.09	1.53	4
Acebutolol-S	9.7	9.0	1.87	1.27	0.74	0.07	0.73	1.01	1.42	4
Betaxolol-R	9.4	9.2	2.59	3.00	1.54	2.00	0.53	2.06	6.29	4
Betaxolol-S	9.4	9.2	2.59	3.00	1.54	2.00	0.54	1.91	5.52	4
Biperiden	8.8	9.8	4.25	4.27	3.39	3.41	0.17	1.19	8.56	3,23
Bisoprolol-R	9.4	9.1	1.87	2.24	0.74	1.14	0.85	1.36	2.10	4
Bisoprolol-S	9.4	9.1	1.87	2.24	0.74	1.14	0.85	1.36	2.10	4
Carvedilol-R	8.1	7.5	4.19	3.12	3.32	2.13	0.019	0.81	31.4	3,24
Carvedilol-S	8.1	7.5	4.19	3.12	3.32	2.13	0.037	1.01	27.4	3,24
Fentanyl	9.0	8.6	4.05	3.97	3.17	3.08	0.15	0.89	5.05	3,25
Imipramine	9.5	9.4	4.80	4.75	4.00	3.95	0.24	1.67	10.2	3
Inaperisone	9.1[g]	9.1	3.72[g]	3.72	2.79	2.79	0.24	1.88	12.1	26
Lidocaine	8.0	7.8	2.44	2.12	1.37	1.01	0.38	1.27	4.18	3,27
Metoprolol-R	9.7	9.2	2.15	1.96	1.05	0.83	0.80	1.52	2.61	4
Metoprolol-S	9.7	9.2	2.15	1.96	1.05	0.83	0.81	1.51	2.53	4
Nicotine	7.8, 3.0	8.2: 3.6	1.17	1.28	−0.05	0.08	0.84	0.80	0.67	3
Oxprenolol-R	9.5	8.9	2.18	1.92	1.08	0.79	0.24	0.75	2.35	4
Oxprenolol-S	9.5	8.9	2.18	1.92	1.08	0.79	0.36	0.80	1.70	4
Pentazocine	8.5	9.3	3.31	4.65	2.34	3.84	0.46	1.55	4.77	3,28
Phencyclidine	9.4	9.9	4.96	4.76	4.10	3.96	0.47	1.12	2.68	3,29
Pindolol-R	8.8	9.2	1.75	1.69	0.60	0.53	0.51	1.23	2.94	4
Pindolol-S	8.8	9.2	1.75	1.69	0.60	0.53	0.76	1.48	2.67	4
Procainamide	9.2	8.8	0.88	1.26	−0.37	0.05	0.92	1.00	1.09	3
Propranolol-R	9.5	9.3	3.65	2.77	2.72	1.74	0.017	0.77	45.1	4
Propranolol-S	9.5	9.3	3.65	2.77	2.72	1.74	0.13	1.29	12.0	4
Quinidine	10.0, 5.4	9.0: 3.9	3.44	3.01	2.49	2.00	0.33	1.40	5.67	3,30,31
Timolol-S	9.2, 8.8	8.8	1.91	0.97	0.78	−0.27	0.63	1.10	1.93	4
Verapamil	8.5	8.5	3.79	4.94	2.88	4.16	0.05	0.85	13.5	3

*Input parameters for the published mechanistic equations[3] that are not stated above were determined using published procedures.[1–3] R and S denote the individual enantiomers.

[a]Antilog values used in mechanistic equations, o:w refers to octanol:water and vo:w, vegetable oil:water.

[b]Calculated from experimental (Exp) or predicted (Pred) octanol:water Log*P* values using published equations.[1]

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

[d]Affinity for blood cells calculated from f_u, B:P and the haematocrit[21] using standard equations.[22]

[e]Experimental values taken from the literature.

[f]Values predicted using the online package SPARC (ibmlc2.chem.uga.edu/sparc).

[g]Mean values predicted using the online packages KOWWIN (erc.syrres.com), Interactive Analysis (Log*P*.com) and SPARC (ibmlc2.chem.uga.edu/sparc).

online software (Log*P* and pK_a) and are summarised in Table 2, except for K_a for which values are not readily available. One approach to estimating K_a is to re-arrange Equation 19 and apply it to blood cells (BC; Equation 20), which also contain acidic phospholipids (Table 1). Recognising that blood cells do not posses an extracellular space ($f_{EW} = 0$) and that Kpu_{BC} (the blood cell to plasma water concentration ratio, that is, $C_{T(BC)}/Cu_p$) can be readily determined *in vitro* from the blood-to-plasma concentration ratio, fraction drug unbound in plasma, and the hematocrit.[22]

$$K_{aBC} = \left(\begin{array}{l} Kpu_{BC} - \left(\dfrac{1 + 10^{pK_a - pH_{BC}}}{1 + 10^{pK_a - pHp}} \cdot f_{IW,BC} \right) \\ - \left(\dfrac{\left(P \cdot f_{NL,BC} + (0.3P + 0.7) \cdot f_{NP,BC} \right)}{1 + 10^{pK_a - pH_p}} \right) \end{array} \right) \cdot \left(\frac{1 + 10^{pK_a - pH_p}}{[AP^-]_{BC} \cdot 10^{pK_a - pH_{BC}}} \right) \quad (20)$$

Then by assuming K_{aBC} is representative of the K_a in all tissues, the Kpu value for any tissue can be calculated by replacing K_a with the respective

K_{aBC} values, that is, inserting Equation 20 into Equation 19.

Equations 19 and 20 were then used to predict the Kpu values for 28 moderate-to-strong bases in rat adipose, bone, brain, gut, heart, kidney, liver, lung, muscle, pancreas, thymus, skin and spleen, and the predictions were compared to *in vivo* experimentally determined Kpu values, were available. In addition, Kpu values predicted using experimentally determined Log*P* (*n*-octanol:water) and pK_a values were compared with those made using mean Log*P* (*n*-octanol:water) values predicted using KOWWIN (esc.syrres.com), Interactive Analysis (Log*P*.com) and SPARC (ibmlc2.chem.uga.edu/sparc), and using pK_a values predicted using SPARC (ibmlc2.chem.uga.edu/sparc).

Comparison With Published Equations

Poulin and Theil[1–3] developed mechanistic equations for acidic, basic and neutral drugs by taking into account the solubility of a drug in the lipid and water phases of plasma and tissues, and its ability to bind to the macromolecules present within these matrices. These equations were used to predict the Kpu values (Kp value predicted *via* the published equations was divided by the f_u values in Table 2) for the same set of 28 moderate-to-strong bases in rat adipose, bone, brain, gut, heart, kidney, liver, lung, muscle, pancreas, thymus, skin and spleen. The accuracy of these predictions was then compared to that of the newly developed equation.

RESULTS

New Equation (Equation 19)

Overall, the accuracy of the Kpu predictions for 28 moderate-to-strong bases in 13 rat tissues ($n = 261$) was good, and little difference was observed between Kpu values predicted using experimental compared to predicted Log*P* and pK_a values (Figures 2 and 3, Tables 3 and 4), with 85.1% compared to 86.3% of the predicted values being within a factor of three of experimentally determined Kpu's. Predictions of brain and lung Kpu values were less accurate with *ca*67% of the predictions agreeing with experimental Kpu values to within a factor of three. The prediction accuracy in the remaining 11 tissues (rat adipose, bone, gut, heart, kidney, liver, muscle, pancreas, thymus, skin and spleen) was considerably higher with *ca*88% of the predicted values deviating from

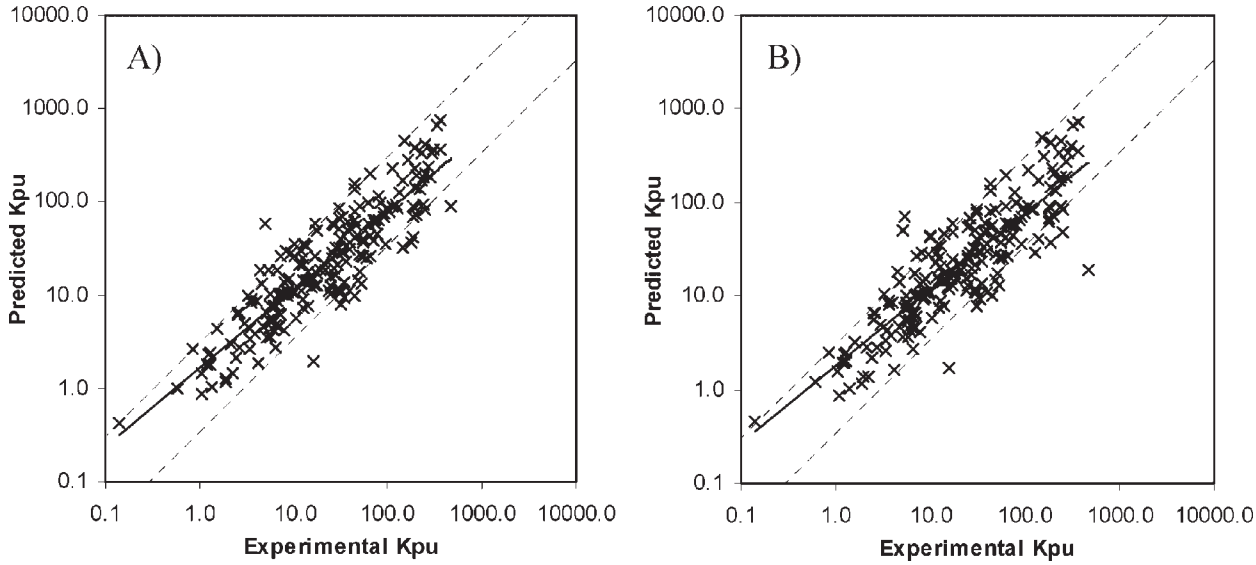

Figure 2. Relationship between predicted (Equation 19—K_a from blood cell Kpu) and experimentally determined Kpu values for 28 moderate-to-strong bases in rat adipose, bone, gut, heart, kidney, liver, muscle, pancreas, thymus, skin and spleen. The dashed lines represent a factor of 3 either side of the line of identity and the solid line is the line of best fit through the data. The Log*P* and pK_a values used in Equations 19 and 20 were experimentally determined for data in Figure 2A ($y = 1.66^{0.84}$, $R^2 = 0.79$) and predicted for data in Figure 2B ($y = 1.79^{0.82}$, $R^2 = 0.76$).

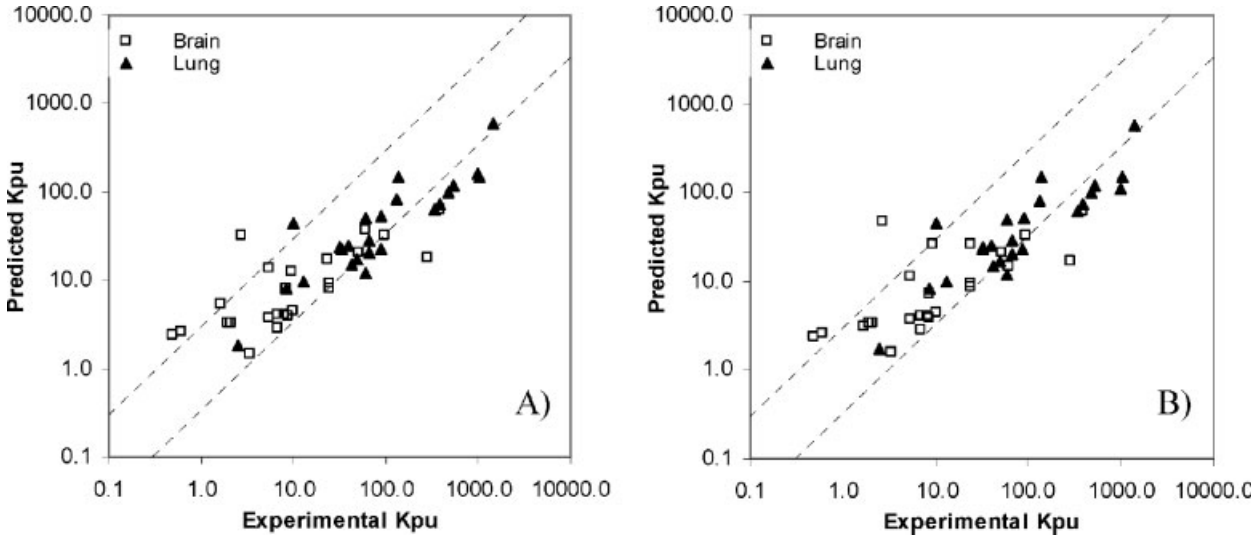

Figure 3. Relationship between predicted (Equation 19—K_a from blood cell Kpu) and experimentally determined Kpu values for 28 moderate-to-strong bases in rat brain and lung, where the dashed lines represent a factor of 3 either side of the line of identity. The Log$P$ and pK_a values used in Equations 19 and 20 were experimentally determined for data in Figure 3A and predicted for data in Figure 3B.

Table 3. Comparison of the Accuracy of the Kpu Predictions, Using Predicted or Experimental Log$P$ and pK_a Values in the New and Published Mechanistic Equations

	Percentage of Predicted Kpu Values Agreeing With Experimental Values to Within Factors of:				Mean (±SD) Predicted to Experimental Kpu Ratio
	<2	2–3	3–4	>4	
Overall ($n = 261$)					
New-predicted[a]	67.0	19.3	8.5	5.2	1.37 ± 1.35
New-experimental[b]	62.5	22.6	6.9	8.1	1.27 ± 1.28
Published-predicted[c]	24.5	15.1	14.6	45.8	0.62 ± 1.09
Published-experimental[d]	24.5	21.1	9.2	45.2	0.73 ± 1.24
11 tissues[e] ($n = 212$)					
New-predicted[a]	61.3	21.5	8.0	9.2	1.34 ± 1.64
New-experimental[b]	67.9	21.2	7.1	3.8	1.31 ± 1.09
Published-predicted[c]	24.1	16.9	12.6	46.4	0.67 ± 1.17
Published-experimental[d]	25.0	20.3	11.3	43.4	0.65 ± 1.00
Brain and lung ($n = 49$)					
New-predicted[a]	36.7	30.6	6.1	26.5	1.22 ± 2.54
New-experimental[b]	38.8	28.6	6.1	26.5	1.12 ± 1.92
Published-predicted[c]	22.4	24.5	4.1	49.0	0.88 ± 1.45
Published-experimental[d]	22.4	24.5	0.0	53.1	1.09 ± 1.93

[a]Using Equations 19 and 20, inserting predicted values for Log$P$ (n-octanol:water) and pK_a.
[b]Using Equations 19 and 20, inserting experimental values for Log$P$ (n-octanol:water) and pK_a.
[c]Using published equations,[3] inserting predicted values for Log$P$ (n-octanol:water) and pK_a.
[d]Using published equations,[3] inserting experimental values for Log$P$ (n-octanol:water) and pK_a.
[e]Refers to adipose, bone, gut, heart, kidney, liver, muscle, pancreas, skin, spleen, and thymus.

Table 4. Tissue-to-Plasma Water Partition Coefficients (Kpu) in Various Rat Tissues for 28 Moderate-to-Strong Bases—*In Vivo* Experimental Versus Predicted Values

	Experimentally Determined Kpu[a] Followed by Kpu Predicted *via* Equations 19 and 20[b]; Published[c] Mechanistic Equations												
Compound	Adipose	Bone	Brain	Gut[d]	Heart	Kidney	Liver	Lung	Muscle	Pancreas	Skin	Spleen	Thymus
Acebutolol-R[4]	1.38	0.07	0.61	112	7.23	29.7	39.7	13.1	6.28	—	3.83	—	6.85
	0.82; 0.18	2.34; 1.69	2.57; 3.52	6.45; 3.65	6.10; 2.00	11.8; 2.20	11.0; 2.27	9.67; 2.53	4.84; 1.59	—	3.85;4.97	—	6.45;2.16
Acebutolol-S[4]	1.07	0.06	0.49	125	5.88	44.6	34.2	8.42	6.09	—	3.37	—	6.00
	0.87; 0.18	2.10; 1.77	2.42; 3.68	5.57; 3.82	5.28; 2.09	10.0; 2.30	9.32; 2.37	8.21; 2.76	4.28; 1.67	—	3.37;5.20	—	5.61;2.26
Betaxolol-R[4]	5.57	24.9	24.5	75.9	44.5	110	247	384	26.0	—	12.3	—	64.3
	7.53; 0.44	12.9; 5.26	9.18; 11.4	44.7; 12.0	41.7; 5.47	91.3; 6.29	83.1; 6.61	72.7; 7.92	28.9; 3.97	—	24.9;17.4	—	42.8;6.10
Betaxolol-S[4]	5.29	23.6	24.2	70.0	39.7	101	200	338	25.2	—	11.4	—	55.2
	6.52; 0.44	11.2; 5.19	8.13; 11.3	38.5; 11.8	35.9; 5.41	78.4; 6.21	71.4; 6.53	62.5; 7.83	25.0; 3.93	—	21.5;17.2	—	36.9;6.02
Biperiden[23]	464	16.5	60.3	89.3	62.4	78.7	—	474	31.2	—	32.5	—	—
	88.0; 63.8	26.3; 23.1	36.8; 50.6	77.1; 53.4	57.6; 23.0	114; 26.8	—	97.6; 34.4	40.0; 16.1	—	69.1;78.9	—	—
Bisoprolol-R[4]	1.21	5.74	1.93	31.2	7.63	29.3	26.8	49.2	6.35	—	2.56	—	10.1
	1.79; 0.20	3.61; 1.62	3.36; 3.38	11.0; 3.51	10.4; 1.92	21.4; 2.12	19.6; 2.18	17.2; 2.53	7.73; 1.53	—	6.37;4.77	—	10.8;2.07
Bisoprolol-S[4]	1.20	5.21	2.10	30.2	7.87	29.2	27.0	49.4	6.15	—	2.60	—	8.67
	1.79; 0.20	3.61; 1.62	3.36; 3.37	11.0; 3.50	10.4; 1.92	21.4; 2.11	19.6; 2.17	17.2; 2.52	7.73; 1.52	—	6.37;4.76	—	10.8;2.07
Carvediolol-R[24]	—	—	—	—	196	149	244	—	43.7	—	—	161	—
	—	—	—	—	226; 177	453; 207	419; 219	—	155; 124	—	—	284;118	—
Carvediolol-S[24]	—	—	—	—	201	189	312	—	43.4	—	—	271	—
	—	—	—	—	196; 90.3	386; 105	358; 111	—	134; 63.3	—	—	241;60.2	—
Fentanyl[25]	178	—	23.5	55.7	30.0	80.6	25.3	90.0	20.6	142	13.9	184	—
	36.0; 28.6	—	17.5; 53.8	40.5; 56.8	32.0; 24.5	64.2; 28.6	59.4; 30.2	54.1; 36.6	22.5; 17.2	33.0;59.4	33.5;83.9	40.9;16.4	—
Imipramine[2,5]	43.8	—	95.8	97.9	91.3	190	216	531	36.7	182	—	239	—
	78.3; 59.7	—	32.4; 37.6	86.6; 39.6	70.4; 17.1	146; 19.9	134; 21.1	121; 25.5	48.6; 11.9	68.1;41.5	—	92.3;11.4	—
Inaperisone[26]	64.6	—	51.3	—	30.8	242	141	139	17.1	—	26.3	—	—
	26.2; 11.4	—	21.1; 35.4	—	85.3; 16.2	186; 18.8	170; 19.9	149; 24.1	58.5; 11.3	—	56.1;55.1	—	—
Lidocaine[27]	—	—	8.53	8.20	7.18	45.3	30.3	10.0	4.42	—	6.79	12.6	—
	—	—	8.03; 12.2	29.1; 12.8	26.1; 5.97	55.6; 6.83	50.9; 7.15	44.9; 8.54	18.3; 4.39	—	18.3;18.4	35.7;4.26	—
Metoprolol-R[4]	1.31	6.46	8.09	16.4	8.62	33.4	50.1	31.9	7.05	—	3.97	—	14.5
	2.46; 0.20	4.74; 2.44	4.06; 5.20	15.1; 5.44	14.2; 2.71	29.9; 3.05	27.3; 3.17	24.4; 3.74	10.3; 2.06	—	8.60;7.66	—	14.7;2.97
Metoprolol-S[4]	1.23	6.55	8.60	13.7	7.74	33.4	55.1	32.6	6.86	—	3.61	—	13.3
	2.35; 0.20	4.56; 2.43	3.95; 5.16	14.5; 5.40	13.6; 2.69	28.5; 3.03	26.1; 3.15	22.9; 3.72	9.90; 2.05	—	8.25;7.61	—	14.1;2.95
Nicotine[32]	0.60	—	3.33	—	2.26	15.8	4.17	2.50	1.90	—	1.31	—	—
	0.99; 0.92	—	1.44; 1.44	—	1.43; 1.11	1.92; 1.14	1.84; 1.14	1.86; 1.26	1.19; 0.99	—	1.81;1.68	—	—
Oxprenolol-R[4]	2.42	7.77	5.38	52.9	15.2	59.1	35.6	66.2	12.7	—	5.72	—	17.2
	2.15; 0.23	4.17; 5.84	3.72; 12.4	13.1; 13.0	12.2; 6.43	25.5; 7.25	23.4; 7.55	20.5; 8.93	8.99; 4.88	—	7.49;18.4	—	12.7;7.07
Oxprenolol-S[4]	1.93	6.46	6.87	31.1	12.3	48.9	34.5	59.0	10.6	—	4.74	—	14.2
	1.29; 0.23	2.73; 4.27	2.83; 9.10	7.84; 9.52	7.37; 4.70	14.7; 5.30	13.6; 5.52	11.9; 6.53	5.70; 3.57	—	4.64;13.5	—	7.75;5.17
Pentazocine[28]	5.39	11.8	9.43	10.1	11.8	43.8	5.05	59.0	12.8	—	10.1	—	-
	18.5; 13.3	11.4; 9.07	12.4; 19.8	35.7; 20.9	30.2; 9.11	62.9; 10.6	57.7; 11.2	51.7; 13.5	21.2; 6.43	—	25.7;30.8	—	—
Phencyclidine[29]	131	—	5.46	—	4.66	25.1	17.1	87.2	3.22	—	—	—	—
	128;100	—	13.8; 22.8	—	13.2; 10.3	10.4; 12.1	12.5; 12.8	22.8; 15.5	10.0; 7.24	—	—	—	—
Pindolol-R[4]	2.13	5.33	10.0	51.0	27.4	92.9	28.3	65.7	15.7	—	5.62	—	17.8
	2.98; 0.28	5.49; 1.88	4.54; 3.88	17.9; 4.01	16.7; 2.31	35.5; 2.52	34.4; 2.58	28.4; 2.97	12.0; 1.88	—	10.2;5.34	—	17.3;2.47
Pindolol-S[4]	0.84	3.02	6.80	24.2	12.4	39.4	9.54	39.8	9.57	—	3.60	—	8.93
	2.63; 0.28	4.89; 1.47	4.17; 3.03	15.7; 3.14	14.7; 1.81	31.0; 1.97	28.4; 2.02	24.9; 2.32	10.6; 1.47	—	8.97;4.18	—	15.2;1.94

(*Continued*)

Table 4. (*Continued*)

Compound	Experimentally Determined Kpu[a] Followed by Kpu Predicted *via* Equations 19 and 20[b]; Published[c] Mechanistic Equations												
	Adipose	Bone	Brain	Gut[d]	Heart	Kidney	Liver	Lung	Muscle	Pancreas	Skin	Spleen	Thymus
Procainamide[33]	0.14	—	—	—	2.68	6.91	3.45	—	3.36	—	—	—	—
	0.42; 0.16	—	—	—	2.80; 0.94	4.49; 0.96	4.32; 0.94	—	2.60; 0.87	—	—	—	—
Propranolol-R[4]	38.0	82.2	383	369	227	364	327	1426	111	—	63.8	—	290
	62.1; 4.05	99.3; 185	63.3; 406	357;428	332; 185	739; 216	671; 228	587; 276	226; 130	—	197;631	—	339;208
Propranolol-S[4]	18.9	53.3	281	182	124	278	231	1037	74.3	—	40.5	—	191
	18.6; 4.05	26.0; 27.5	18.3; 60.2	91.3; 63.5	84.4; 27.5	186; 32.0	169; 33.9	148; 41.0	57.9; 19.3	—	51.8;93.6	—	86.4;30.9
Quinidine[5]	—	—	2.73	30.6	17.6	62.7	50.0	130	13.0	26.4	—	72.7	—
	—	—	32.2; 26.2	65.9; 27.6	50.0; 12.0	97.7; 14.0	91.0; 14.8	83.6; 17.8	35.7; 8.44	55.6;28.9	—	62.5;8.05	—
Timolol-S[4]	1.03	1.57	1.66	32.0	8.50	21.3	14.2	42.6	6.59	-	2.52	—	8.71
	1.44; 0.16	4.31; 2.03	5.32; 4.25	10.6; 4.42	10.0; 2.39	18.6; 2.64	17.6; 2.72	15.3; 3.16	8.72; 1.89	—	6.23;6.05	—	11.1;2.58
Verapamil[34]	—	—	—	—	120	250	—	1000	70.0	—	—	—	—
	—	—	—	—	94.8; 66.2	201; 77.2	—	165; 98.8	65.1; 46.5	—	—	—	—

[a]Where more than one value was reported the mean has been taken and where tissue-to-plasma values are reported Kpu values have been calculated using the f_u values in Table 2.
[b]New equation, using experimentally determined Log*P* (*n*-octanol:water) and pK_a values.
[c]Taken from Poulin & Theil,[3] using experimentally determined Log*P* (*n*-octanol:water) and pK_a values.
[d]Where stomach and/or individual segments of the intestine are reported the mean has been taken.

experimental Kpu's by less than a factor of three. However, for acebutolol significant under-predictions were seen for gut and over-predictions for bone by factors of *ca*20 and 33, respectively (values excluded from accuracy assessments and Figures 2 and 3).

Published Equations

For the same data set ($n = 261$,Table 4), the Kpu values predicted using published equations[3] were found to be far less accurate (Figures 4 and 5, Tables 3 and 4), compared to the current equation (Equation 19). Of these 261 predicted Kpu values only *ca*43% were within a factor of three of the experimental values, using experimental Log*P* and pK_a, with the maximum deviant being a factor of 69.

DISCUSSION

At present the mechanistic equations used for Kpu predictions are deterministic, in that they compute a fixed mean with no measure of the inherent variability and uncertainty associated with the biological system and parameter deter-

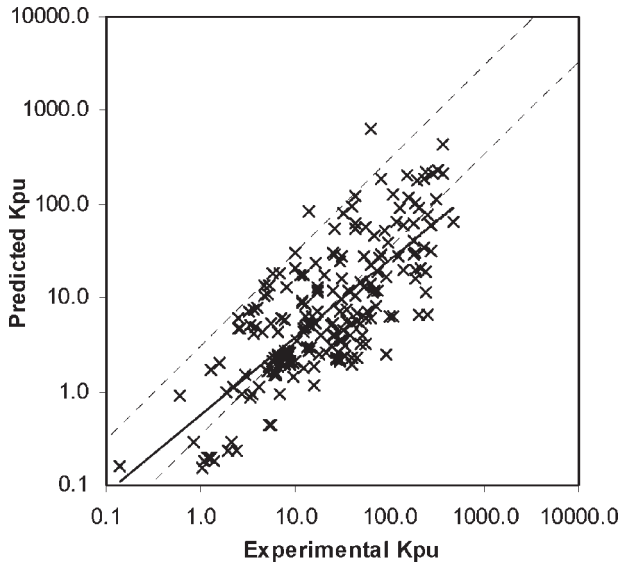

Figure 4. Relationship between published predicted[3] and experimentally determined Kpu values for 28 moderate-to-strong bases in rat adipose, bone, gut, heart, kidney, liver, muscle, pancreas, thymus, skin and spleen (experimentally determined Log*P* and pK_a values were used). The dashed lines represent a factor of 3 either side of the line of identity and the solid line is the line of best fit through the data ($y = 0.56^{0.83}$, $R^2 = 0.58$).

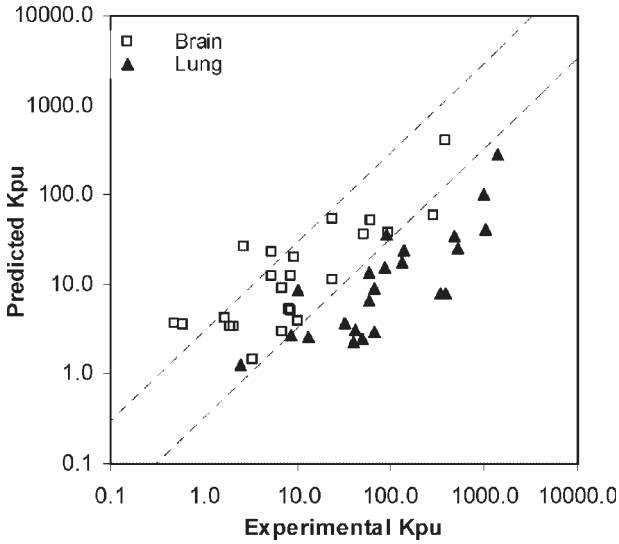

Figure 5. Relationship between published predicted[3] and experimentally determined Kpu values for 28 moderate-to-strong bases in rat brain and lung, where the dashed lines represent a factor of 3 either side of the line of identity.

minations. So to assess the accuracy of the Kpu predictions point estimates have been compared with mean *in vivo* Kpu values, but due to the aforementioned variability and uncertainty the likelihood of these two values being identical is minimal. As such, an arbitrary global level of variability and uncertainty needs to be considered, and has been suggested by Poulin et al.[1] to be a factor of three either side of the mean after identifying that *in vivo* tissue distribution determinations can differ within these limits between animals and between laboratories. For the purpose of the current research, predicted Kpu values were therefore deemed accurate if they agree with estimated experimental values to within a factor of three.

The majority of the tissue Kpu values for the β-blockers included in the current analysis were obtained directly, in that tissue concentrations were related to measured unbound drug concentration[4]. In most cases, however, investigators determine instead Kp values, by relating tissue concentration to the corresponding plasma concentration, C_P. The two parameters are related through the fraction of drug unbound in plasma, f_u. Namely

$$\mathrm{Kp} = \frac{C_T}{C_P} = f_u \cdot \mathrm{Kpu} \qquad (21)$$

To bring all values into a common frame, in cases where Kp values were reported, Kpu values were calculated using independently determined f_u values. The reason for wishing to predict Kpu rather than Kp are several fold. First, fundamentally, only unbound drug is able to distribute across cell membranes, so using a parameter that relates to unbound drug in plasma is preferred. Second, when extrapolating across animal species, plasma protein binding of drugs often differs considerably, and more so than tissue binding, perhaps because plasma binding frequently involves the interaction of drug with a specific binding site on a specific protein, which differs structurally from one species to another, whereas tissue binding involves the interaction with many constituents, thereby reflecting an average value. This is supported by the observation that using the same Kpu value for a tissue across species, and correcting for differences in plasma protein binding, tends to produce better prediction of tissue distribution than when no correction for differences in plasma protein binding is made.[35] Third, large differences in Kp values between enantiomers, for example propranolol and pindolol in rat, essentially disappears when considering Kpu values, signifying that the major difference in Kp lies in a difference in plasma protein binding rather than tissue binding.[4]

Equation 20 brings into focus the importance of the fraction unbound in plasma, f_u. Various methods exist to determine f_u; essentially all depend on the separation of the small ligand from the much larger protein bound complex. Notwithstanding the methodology employed, the lower the f_u the more uncertain is its value. This is particularly true for compounds with $f_u \ll 0.01$, both with respect to potential bias, produced for example by extensive adsorption of very hydrophobic compounds onto the apparatus used in their determination, and precision. This uncertainty in the estimate of f_u may be a substantial cause of failure in accurately predicting Kp values when inserting Equation 19 for Kpu in Equation 20 and also needs to be kept in mind when regressing the resultant equation against experimental Kp values to estimate parameters of Equation 19.

The overall accuracy of Kpu predictions in rats using the currently developed equation was considerably higher than the accuracy achieved with the published equations,[3] with *ca*89% compared to *ca*45%, respectively ($n = 261$), of the predicted Kpu values being within a factor of three of *in vivo* Kpu values (similar differences in prediction accuracy were observed when predicted and experimental Kp values were compared). This

divergence in prediction accuracy was anticipated since published equations make no allowances for electrostatic interactions of drugs with acidic phospholipids, which have been identified as the primary driving force in the tissue distribution of moderate-to-strong bases.[4,5] However, in rat brain and lung other additional, and possibly tissue-specific processes, appear to be contributing towards the distribution of moderate-to-strong bases since, although application of Equation 19 improved the accuracy of the predicted Kpu values, under-predictions still prevailed in lung and both under- and over-predictions were apparent in brain (Figures 3 and 5).

In lungs, extensive distribution is a common feature of drugs with moderate to high basicity and lipophilicity,[5,34,36,37] but the additional mechanisms involved in this distribution remain unclear at present. One possibility is a contribution from lysosomal trapping, since these acidic organelles are abundant in certain tissues, including lungs, and extensive accumulation within lysosomes has been reported for many basic drugs.[38,39] However, the impact of lysosomal trapping on the distribution of compounds into lung is unclear since lung concentrations of these organelles could not be found in the literature or quantitatively compared with other tissues, and Kpu under-predictions were not prevalent in other lysosome-rich tissues such as liver and kidney. An alternative contributory factor may be pulmonary phospholipidosis, a process that results in the elevation of lung phospholipid levels and the formation of additional inclusion bodies, such as lysosomes. Pulmonary phospholipidosis can occur when phospholipid breakdown is suppressed by chronic dose administration of lysosomotrophic amines,[40,41] a class of compounds that includes many moderate-to-strong bases of sufficient lipophilicity to permeate the lysosomal membrane.[38,39] As such, lung phospholipid concentrations in animals chronically dosed with lysosomotrophic amines are likely to be higher than in control animals and, since control values are used in the mechanistic equation, under-predictions of lung Kpu values would be anticipated. This makes pulmonary phospholipidosis a plausible hypothesis but one that, in the absence of specific tissue lysosomal data in animals receiving the study drugs, cannot be accommodated within the mechanistic equation.

In brain, reported tissue levels of the acidic phospholipids vary by a factor of *ca*25 between researchers,[5,7] thereby introducing a great deal of uncertainty into the Kpu predictions. Despite this discrepancy, brain Kpu values were still reasonably accurately predicted using the lower of the two acidic phospholipid concentrations (Tables 3 and 4, Figure 3) suggesting that the lower reported value maybe the more accurate of the two. However, some under and over-predictions were still evident, which came as no surprise, since in comparison to other tissues brain penetration is more restrictive and selective. As such, active transport mechanisms are likely to play a key role in brain distribution and should ideally be incorporated into the mechanistic equation, which assumes passive diffusion. This would require accurate parameters characterising such transport events, and preferably structure–function relationships, which are to a large extent are currently lacking. Active transport processes are not restricted to the brain and are the probable cause of inaccuracies associated with Kpu predictions (Equation 19) in other tissues. For acebutolol, Kpu values were over-predicted in brain and bone (factors of *ca*4.5 and 33, respectively) possibly due to active efflux mechanisms. The causal transporter in brain is likely to be *p*-glycoprotein, since acebutolol has been postulated as a substrate for this efflux transporter,[42] which is present in relatively high levels within the brain.[43] However, efflux of acebutolol from the bone appears to be more pronounced but evidence of pronounced *p*-glycoprotein levels in bone could not be found, suggesting the involvement of other unknown efflux mechanisms.

Gut Kpu values were also significantly under-predicted for acebutolol, and to a lesser extent for oxprenolol (factors of *ca*20 and 4, respectively). These divergences may be a consequence of the experimental procedures employed in determining these Kpu values, since the gut tissue was analysed along with its contents for many of the drugs studied.[4] Under these conditions, the gut Kpu value is no longer purely distributional; instead, it is a composite of distribution and elimination processes. For example, biliary secretion, intestinal re-absorption, enterohepatic recirculation, and the metabolic conversion of glucuronide metabolites back to parent compound by gut glucuronidases, all processes which may serve to increase the concentration of parent compound in the gut. These elimination processes cannot at present be incorporated into Equation 19 and are the probable cause of the differences between experimental and predicted gut Kpu values for acebutolol, for which appreciable biliary secre-

tion[44] and intestinal re-absorption[45,46] have been reported, and for oxprenolol, for which significant glucuronidation of unchanged oxprenolol occurs.[47–49] Under such circumstances the difference between predicted and experimental Kpu values does not imply inaccurate predictions, since like is not being compared with like, that is, gut tissue (predicted Kpu) is being compared with gut tissue plus luminal contents (experimental Kpu). Alternatively, the predicted Kpu values for acebutolol and oxprenolol may be accurate estimates of distribution into gut tissue, since for the other compounds investigated predictions were generally accurate.

In WBPBPK modeling, Kpu or Kp values are used to relate amount (and concentration) in a tissue to the emerging venous drug concentration, and are estimated on that basis. Most tissues and organs of the body do not eliminate drug and for these tissues at steady state the input and venous concentrations, as well as that in the blood within the tissue, are equal so that estimating Kpu (and Kp) values, which are generally referenced to concentration in systemic arterial blood (C_A), presents no problem. The problem arises for eliminating organs, such as the liver and kidney, and for them, because it is rarely measured directly, it is necessary to calculate the venous concentration (C_V) from the arterial concentration, before estimating Kpu, by taking into account the extraction ratio of the drug by the organ (E). Namely, $C_V = C_A(1-E)$.

In the absence of such corrections for E the experimental Kpu will be under-estimated, which will be reflected by an apparent over-prediction of the Kpu values for eliminating organs that will be more pronounced for highly cleared drugs. To overcome this issue, allowances have been made for E for all compounds investigated, with the exception of phencyclidine. Regarding phencyclidine, kidney and liver Kpu predictions were within a factor of three of experimental Kpu's despite this lack of E adjustment.[29] For nicotine, adjustments were made for E but the kidney Kpu was under-predicted (low renal E, under-predicted in kidney by a factor of 8.2), indicating other mechanisms may be contributing towards the distribution of nicotine into the kidney, such as active uptake into kidney cells. In contrast, a significant over-prediction (factor of 11) of the experimental value was apparent for the liver Kpu for pentazocine. Possible causes for this over-prediction could be that there was an appreciable delay between liver excision and termination of subsequent bio-transformations, which the researchers achieved by homogenisation in Krebs-Ringer solution.[28] Alternatively the animals were used under urethane anaesthesia, which may have altered the pharmacokinetic behaviour of pentazocine, a phenomenon that has been reported for thiamine by Pipkin and Stella.[50]

In addition to the aforementioned tissue and/or compound specific issues, the accuracy of the Kpu predictions, and the assessment of this accuracy, are globally influenced by the accuracy of the input parameters of the mechanistic equation, the accuracy of *in vivo* Kpu determinations, and situations in which the assumptions of the equations are not upheld. Accurate input parameters are obviously imperative for accurate Kpu predictions, and although on an individual basis these parameters appear accurate, collectively there are issues of accuracy and precision, since the values utilised (Tables 1 and 2) have been determined in different research facilities, in different strains of rat, and in animals of varying age and weight ranges. These aforementioned factors may also influence the reference *in vivo* values and if like is not compared with like, for example, tissue-specific parameters from a 250 g Sprague-Dawley rat are used to predict the Kpu values for a 400 g Wistar rat, Kpu predictions may appear inaccurate when in fact the disparity reflects a true physiological or inter-laboratory difference. This highlights a need for future experiments to quantitatively assess the variability and uncertainty associated with the input parameters of the mechanistic equation.

To reduce the number of experimentally determined input parameters, values for Log$P$ (n-octanol:water) and pK_a were predicted using readily available online software packages, and the accuracy of the resultant Kpu predictions was compared with that using experimentally determined Log$P$ and pK_a values. Little difference was seen in the accuracy of the two data sets as shown in Table 3, suggesting predicted Log$P$ (n-octanol:water) and pK_a values may be used in the mechanistic predictions of Kpu, which has the added advantage of removing inter-laboratory differences in the experimental determination of these input parameters. Regarding Log$P$, we have assumed n-octanol:water is a good surrogate for lipid:tissue water partitioning in all tissues except adipose where vegetable oil:water was used. On a global basis this appears to give accurate predictions of Kpu values, but the possibility of using alternative solvent systems should not be ignored

and further investigations may reveal suitable alternatives.

Another input parameter that warrants further discussion is the affinity constant of a drug for acidic phospholipids in tissues (K_a). This parameter represents a global affinity for phosphatidylserine, phosphatidylglycerol (mono and di), phosphatidylinositol and phosphatidic acid, and assumes the affinity for this acidic phospholipid mixture is the same across tissues and in blood cells. Testing this assumption by means of regression analysis was not performed since it would be hindered by the aforementioned uncertainty in acidic phospholipid tissue concentrations and the variability in the relative proportions of the individual acidic phospholipids across tissues[4] and the affinity of a drug for each acidic phospholipid.[5,16] Despite these variations and parameter uncertainty the overall accuracy of the Kpu predictions for the compounds investigated was good when this global affinity constant was calculated from blood cell data (Equation 20) and applied to other tissues. However, it is likely that the accuracy could be further improved by experimentally determining the affinity of the drugs for the individual acidic phospholipids, but the compound requirements and time investments would largely defeat the aims of these mechanistic predictions, especially during drug discovery when time and resources for a compound are limited.

Inaccurate predictions will also prevail if the assumptions of the equation are not met, as in the case of active, as opposed to passive, transport processes. An additional source of non-conformity may arise if a drug extensively binds to tissue constituents that are not accommodated in the mechanistic equation. A prime example is seen with doxorubicin, a moderate-to-strong base (pK_a 8.15, Log$P$ 1.27) reported to extensively interact with tissue DNA,[51,52] and since this was not accounted for in Equation 19, Kpu values for doxorubicin were significantly under-predicted (by up to a factor of 15 in all 8 tissues investigated, data not shown).

An additional tissue constituent with which moderate-to-strong bases can interact is α_1-acid glycoprotein, but an expression for this interaction was not incorporated into Equation 19. The reason for this omission was in part because tissue levels of this protein are unavailable. Moreover, α_1-acid glycoprotein is primarily located in plasma, as judged by a volume of distribution not much larger than plasma volume[53] (implying that extracellular fluid concentrations in tissues are relatively minor), and moderate-to-strong bases have an exceptionally high affinity for acidic phospholipids. As such, the impact of α_1-acid glycoprotein binding within tissues on the regional distribution of these drugs can be considered minimal.

Further limitations of Equation 19 are that inaccuracies are likely to arise for compounds displaying non-linear pharmacokinetic behaviour, although if due to saturation of the acidic phospholipids, it is possible to modify Equation 19 appropriately and use the associated nonlinear parameters estimated from blood cell binding experiments explored over a wide concentration range. Another potential problem arises when *in vivo* Kpu values, challenged with Equation 19, are estimated under non-steady state conditions, such as following a bolus dose, which was the case for some of the test compounds, that is, carvediolol,[24] inaperisone[26] and phencyclidine.[29] Although when non-saturating conditions prevail, Kpu values based on area-under-the-concentration-time curve values after a bolus dose should theoretically equate to those at steady state, there are many potential sources of error. For example, poor sampling times and errors arising from tissue sampling being inherently destructive, with only one concentration-time value per tissue from each animal, add uncertainty to the estimate of area.

Despite these limitations as well as uncertainty and variability in parameter values, the current mechanistic equation accurately predicted the majority of the Kpu values for 28 moderate-to-strong bases in 13 rat tissues, by using the Kpu value for blood cells (Kpu_{BC}) to determine the affinity of a drug for acidic phospholipids (K_a). The advantage in using Kpu_{BC} is that it can be easily determined *in vitro*[22] and the only biological matrix required is whole blood, which is relatively easy to obtain for any species. However, literature shows distribution into muscle generally correlates well with the distribution into other tissues,[1,54] suggesting muscle Kpu values could be used as a surrogate for predicting the Kpu values of other tissues (Equations 19 and 20). This was performed for all 28 moderate-to-strong bases using *in vivo* muscle Kpu values (Table 4), and the accuracy of these predictions (Figure 6, values not tabulated) was very similar to that obtained using blood cell data (Figure 2). A drawback of this alternate approach is the requirement for in-life experiments to calculate the *in vivo* muscle Kpu values, which largely defeats the objectives of Kpu prediction, although it would

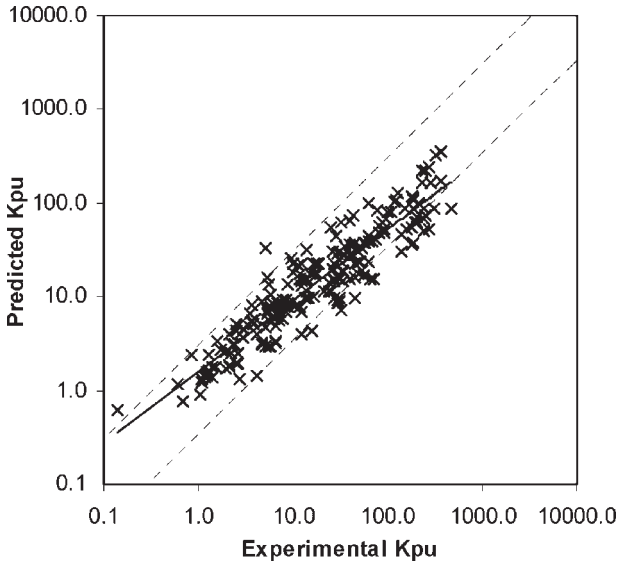

Figure 6. Relationship between predicted (Equation 19—K_a from muscle Kpu, using experimental Log*P* and pK_a values) and experimentally determined Kpu values for 28 moderate-to-strong bases in rat adipose, blood cells, bone, gut, heart, kidney, liver, pancreas, thymus, skin and spleen. The dashed lines represent a factor of 3 either side of the line of identity (data within these lines represents 88% of 212 predictions) and the solid line is the line of best fit through the data ($y = 1.58^{0.76}$, $R^2 = 0.86$).

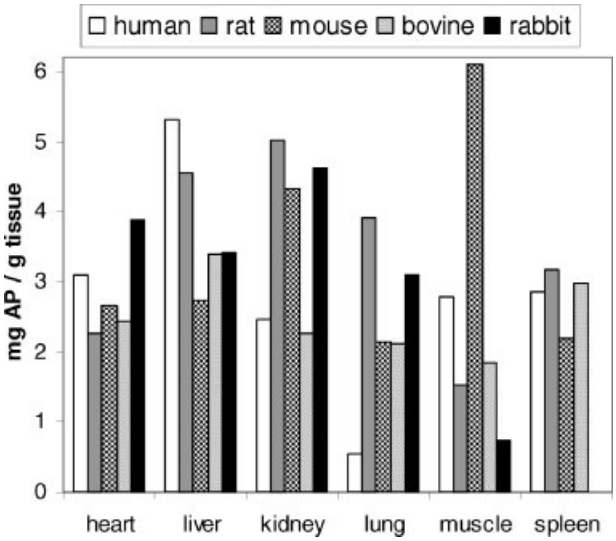

Figure 7. Possible inter-species variability in the concentration of acidic phospholipids from various tissues (values are not from a single source leading to uncertainty in the degree of inter-species differences)[7,57]. Tissue concentrations are expressed as milligram acidic phospholipid (AP)/g of tissue.

greatly reduce the number of tissues that need to be assayed. However, good *in vitro*-to-*in vivo* correlations have been reported for muscle Kp and Kpu values for some drugs[54–56] raising the possibility of using *in vitro* muscle Kpu's for K_a determinations. This refinement would require fresh muscle tissue, which although easy to obtain for rat, may be restrictive for other species, for example, human. However, the use of muscle Kpu, as opposed to Kpu_{BC}, does offer a useful alternative that increases the flexibility of Equation 19 and offers a means of predicting distribution into one tissue from that of another using a mechanistic, instead of empirical[1], approach.

The mechanistic equations developed and employed here can potentially be applied to any species, but have been restricted here to rat due to the limited availability of tissue-specific input parameters and experimental values in other species with which to compare the predictions. The ability to predict regional tissue distribution in any species would immensely benefit WBPBPK modeling, especially in large animals and humans where ethics and expense greatly restrict Kpu determinations. As such, in scaling tissue distribution from rat to larger animals and humans, Kpu values are generally assumed identical to those of rat. However, since inter-species variability in the acidic phospholipid content of tissues is indicated (Figure 7)[7,57] the accuracy of this assumption is uncertain, although appropriate corrections can now be made using Equation 19 by inserting the appropriate species-specific input parameters.

In summary, the multi-compartmental structure of WBPBPK models has been a deterrent in its application within the pharmaceutical industry, and swayed researchers towards model reduction, but in doing so essential information may be irretrievable lost. An alternative way of reducing the time, cost and labour requirements is to predict the compound-specific input parameters of these models, with the most noticeable benefit coming from mechanistic predictions of Kpu values in multiple tissues. Using published equations, under-predictions prevailed for moderate-to-strong bases since a major binding mechanism was omitted. This spurred the development of a new equation that accommodates the unique electrostatic interactions of moderate-to-strong bases with the acidic phospholipids present within tissue cells, and a marked improvement in the resultant Kpu predictions was observed.

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