Kisitu et al.:

Chemical Concentrations in Cell Culture Compartments (C5) – Free Concentrations

Supplementary Data

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Box S1: Concentrations then and now

Dose: The concept of dose has been defined extensively before (Kisitu et al., 2019). It describes an absolute amount per experimental system (e.g., per mouse or per human patient). When the concept is applied to NAM, it describes the amount of chemical per cell culture well. Example, if a chemical concentration in the medium is 1 mM and the well contains 1 mL of medium, then the dose is 1 µmole; if the same well contains 2 mL medium, then the concentration is the same, but the dose doubles.

Weight-normalized doses: Already in Paracelsus' time it must have been clear that a dose tolerated by a tall and heavy adult may be lethal to a small child. This made clear that normalization to overall weight or volume is an important concept. Often normalized doses are expressed in dose per kg body weight (see Kisitu et al., 2019).

Nominal concentration: If a dose in an *in vitro* system is normalized to the volume of the system, then a nominal concentration is obtained. This measure indicates what the drug/toxicant concentration would be if all chemical was freely dissolved and no losses/distribution occurred. It can also be defined as the concentration that the experimental operator believes to have applied to the test system. It is liable to variations arising from volatility, plastic binding, and pipetting variability during the preparation of stock solutions and their addition to experimental compartments.

Free concentration: Relative to the nominal concentration, chemical molecules may be lost (e.g., by evaporation). Also, some chemical may be adsorbed to biomolecules. Only a fraction of the drug/toxicant will then be freely dissolved (free concentration). Free drug theory assumes that only the free fraction (f_u) of test compound is available for reversible interactions with "receptors" at the target site.

Receptors: We use here the term "receptor" in a wide sense, describing a biomolecule or biological structure (protein, DNA, carbohydrate) that shows affinity to a test chemical and usually binds it reversibly (irreversible interactions may also occur, see below). The concept does not differ between intentional targets and off-targets; it also does not consider what the result of the chemical-receptor interaction is (simple binding, triggering of a biological effect, transport of the chemical or metabolism of the chemical if the "receptor" is an enzyme). It is worth noting that receptors can be defined differently, e.g., in pharmacology, the definition would include the requirement that binding of a compound to a "receptor" evokes or prevents a biological response.

Macromolecular interactions: The concept that compounds only have a pharmacological or toxicological effect, when they interact with a biomolecule (= a "receptor" in the above broad sense) goes back to Paul Ehrlich's side chain theory. This provides the basis for the law of mass action that is used to define equilibrium constants. The concentrations of test compound and its receptor, together with the affinity constant, determine the effect – and this is why the considerations of concentrations are of such extreme importance.

Total concentrations and their effect: The total concentration (C_T) is the concentration of both bound and unbound compound in a given matrix. A very practical approach is to define it empirically as the concentration that can be measured in a body fluid, not taking into account which part was free or bound. We suggest here for NAM to define the total concentration as the amount (in moles) of chemical in a medium divided by the medium volume. Sometimes, the effect of a test chemical can be related to the total concentration (rather than to the free concentration). For biological effects to be dependent on the total concentration, the underlying processes should be slow relative to the binding/unbinding of chemical to protein/lipid. For instance, if test compound is metabolized or transported into cells, then more and more of the compound initially bound to biomolecules will be released (following physicochemical laws) and can therefore become available to transport or metabolism. Similarly, if a chemical triggers cytotoxicity by

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oxidizing cell constituents (and it is thereby "used-up"), then some of the bound chemical will shift to the free fraction and become available to maintain the irreversible reactions linked to cytotoxicity.

Concentrations *in vivo*: In a standard pharmacological or toxicological situation (oral, pulmonary or dermal exposure) and assuming that the bioavailability of the test compound is > 0 (and a first pass effect of < 100%), there is an initial uptake phase and a terminal elimination phase. This often leads to a concentration time course of the compound in blood that first increases, then reaches a peak (C_{max}), and later decreases (given a single exposure event). Such time courses can differ between body compartments, and there can even be pronounced differences between blood (which consists of about 42% cells) and plasma (cell-free part of blood). When scientists speak of "*in vivo* concentrations", they may mean C_{max} in plasma. However, this is not universally defined. Ideally, the time point and body compartment referred to should be indicated. Instead of C_{max}, other measures sometimes are used (e.g., the average concentration (C_{avg}) over a defined time period). For many bioactive compounds, it is not known whether the *in vivo* effect is most related to the C_{max}, the C_{avg} or some other, time-dependent concentration measure. This time- and compartment-dependency of *in vivo* concentrations adds to the complexity of free vs total concentrations and makes *in vivo* to *in vitro* comparisons challenging.

Irreversible effects of chemicals: The law of mass action assumes reversible interactions between drug/toxicant and its various receptors. However, in some cases, such interactions may be irreversible. For instance: the drug deprenyl interacts covalently with its target monoamine oxidase; the thrombin receptor on platelets is irreversibly modified by its ligand (proteolytic cleavage); biomolecules are oxidatively modified by hydrogen oxide (which is consumed by this process). In such reactions, not just the concentration, but also time plays a role (time is not a factor in the law of mass action after equilibrium has been reached). For the field of toxicology, it was recognized about 100 years ago (Haber's rule) that the damage is proportional to the concentration of the agent x time of exposure to the agent.

Qualitative vs quantitative hazard: For reversible interactions, hazard is a function of the compound concentration. This is universally accepted in toxicological research. However, the relationship needs not necessarily be monotonic. In the field of regulatory toxicology, hazard is often used with a different connotation. Here, it is meant to describe a theoretical propensity of a compound to trigger a certain type of damage. Often, such effects are seen at very high concentrations/doses only. The observation is accepted as true if it occurs in at least one of the test concentrations/doses/exposure situations. This approach has been criticized, as it neglects concentrations (doses). When animals were the only experimental systems used, there was at least some limitation of the concentrations that could be reached, as they are limited in animals by several factors (e.g., solubility in the dosing vehicle). The concept should not be transferred to NAM without giving consideration to the upper limits of the concentrations considered and without diligent controls for unspecific effects (e.g., cell death).

Derivation of the "extracellular biokinetics" formula

The concentration of a chemical in a culture dish is a theoretical construct, as explained earlier (Kisitu et al., 2019). It is obtained by dividing the amount of chemical added by the volume of medium in the dish (or by the volume of buffer in any type of vessel). More practically, it is derived from the nominal concentration of a chemical in a stock solution, divided by the dilution factor of stock in the medium. Both measures do not necessarily reflect the real free concentration of a chemical in the medium. One aspect may be that a chemical is taken up by cells, but this will not be considered here. In the present manuscript, only cell-free conditions are considered. Fisher et al. (2019) derived an equation for predicting the free fraction in an *in vitro* test system, and this will be illustrated here for non-specialists. The free concentration of a chemical can be calculated from Equation S12 (below) assuming no volatility, plastic binding or distribution into cells. Accordingly, the free fraction can be calculated from Equation S13.

Several different processes affect the free concentration, i.e., the concentration of molecules truly in solution and not bound (or lost) elsewhere (Fig. 1). These factors need to be taken into account to calculate the free concentration (C_f). For most biochemical or cell biological processes, C_f is the relevant physical quantity (= physical measure). For instance, it is relevant in the interaction with a "receptor" with which there is a non-covalent interaction. The fraction of chemical that is not free (e.g., bound to plastic) is normally considered to not interact with a toxicological or pharmacological target.

Assumptions 1

- Chemicals bind reversibly to other medium components (B). The law of mass action applies.
- The concentration of B ([B]) is very high (compared to chemical C): no binding processes are saturated.
- The equilibrium constant K of the reversible binding (i.e., the interaction constant between the chemical and, e.g., protein (albumin), lipids, culture well surface, medium-air) is the ratio of adducts (B x C_f) and product (the bound concentration C_b).
- The tested concentrations are within the aqueous solubility range of the test compounds in the culture medium.

These assumptions allow defining by the following equation:

$$C_b = K \times C_f \times [B] \qquad (S1)$$

Considering the binding to several components within the *in vitro* system, the nominal concentration is defined as the sum of the individual binding processes and the free concentration.

$$C_t = C_f + \{[bound\ to\ protein] + [bound\ to\ lipids] + [bound\ to\ culture\ well\ plastic] + [evaporated]\} \quad (S2a)$$

For a total concentration C_t, a chemical's free concentration (C_f) is:

$$C_f = C_t - C_b \quad (S2b)$$

simply expanded as

$$C_f = C_t - \{[bound\ to\ protein] + [bound\ to\ lipids] + [bound\ to\ culture\ well\ plastic] + [evaporated]\} \quad (S2c)$$

or put in terms of C_b in Equation S1 and defining the interaction terms for proteins, lipids, plastic and the headspace:

$$C_f = C_t - \left\{\left[K_{prot} \times C_f \times [P]\right] + \left[P_{ow} \times C_f \times [L]\right] + [Bound\ to\ plastic] + [Evaporated]\right\} \quad (S2d)$$

Assumptions 2

- Binding to albumin and lipid in complete culture media are the only significant processes limiting the availability of test compound for distribution into the treated cells.
- Loss of compound due to volatility or binding to the plastics used in cell culture is not accounted for here. It is assumed that logPow >>>>>K_{aw} & $K_{plastic}$ (the air-water and water-plastic distribution equilibria constants).
- Lipid is a little-defined term. It may be the triglyceride fraction (TG) or the cholesterol fraction (Chol) or a combined fraction, or whatever measure is available.

Considering Assumptions 2 and Equation S1, Equation S2d then becomes:

$$C_f = C_t - \{K_{prot} \times C_f \times [P] + P_{ow} \times C_f \times [L]\}$$

With the latter two terms standing for the concentration bound to protein and the concentration bound to lipid.
This can further be transformed to

$$C_f = \frac{C_t}{1 + K_{prot} \times [P] + P_{ow} \times [L]} \quad (S3)$$

Where:
K_{prot} = protein binding constant
[P] = concentration of protein present in the medium
P_{ow}: octanol-water partition coefficient
[L] = concentration of lipids present in the medium

Medium is often supplemented with serum or a serum substitute (FCS) that acts as a source of protein and lipids. Notably, the extracellular matrix may also act as a binding target. In human plasma, the predominant drug-binding protein is albumin. Amongst plasma lipids, TG form the major lipid component of lipoproteins (Nichols, 1969).

Assumptions 3

- Binding in the protein phase is predominantly to albumin while that in the lipid phase is to neutral lipids (TG).
- The binding to the neutral lipids is considered only for the non-ionized form of the chemical.

The total concentration of protein in the medium [P] is given by:

$[P] = \frac{mass\ of\ protein}{total\ vol.\ of\ medium}$, this translates to: $= \frac{V_{protein} \times D_{protein}}{total\ vol.\ of\ medium}$, or simply:

$$[P] = f_{protein} \times D_{protein} \quad (S4a)$$

Where:
$f_{protein}$ is the volume fraction of proteins in the medium. $V_{protein}$ is the protein volume and $D_{protein}$ is the protein density

Strictly speaking, albumin is not the only relevant protein. Within the bulk of the overall protein phase, the relative concentration of albumin is related to that of other proteins by its partial specific volume (PSV_{alb}).
The concentration of albumin [Alb] as a component of total protein is given by:
$[Alb] = f_{protein} \times D_{protein} \times PSV_{alb}$
If albumin is the dominant protein in the medium, then, $D_{protein} \sim D_{alb}$ and $f_{protein} \sim f_{alb}$.
$[Alb] = f_{alb} \times D_{alb} \times PSV_{alb}$ (*S4b*)

Note:

1) The specific volume of a solution/mixture is the ratio of its volume to the mass of the mixture/solution, i.e., specific volume is inversely proportional to density.
2) The specific volume is the sum of the partial specific volumes of the components of the mixture or solution.

By substituting $D_{alb} = \frac{1}{PSV_{alb}}$ in (S4b) above, the concentration of albumin in the medium can thus be taken to be directly proportional or represented by its volume fraction;

$[Alb] \propto f_{alb}$ (*S5*)

Where:

f_{alb} is the volume fraction of albumin in the culture medium

Assumptions 4

- Chemicals bind only to neutral lipids ([L]NL).
- Neutral lipids are assumed to constitute the majority of the lipid phase in the medium (others to be neglected).

The neutral lipid concentration is similarly derived from the total lipid concentration as follows:
$[L] = \frac{mass\ of\ lipids}{total\ vol.of\ medium}$

$[L] = \frac{V_L.D_L}{total\ vol.of\ medium}$, or simply: $\approx f_L \times D_L$

Where, [L], f_L and D_L are the total lipid concentration, the volume fraction and density of lipids in the culture medium respectively.
The concentration of neutral lipids in the bulk lipid phase is related to the total lipid concentration by the PSV_{NL}.

$[L]_{NL} = f_L \times D_L \times PSV_{NL}$

If neutral lipids are assumed to dominate over other lipids, such that;

$D_{NL} \approx D_L\ and\ f_{NL} \approx f_L$ then
$[L]_{NL} \approx f_{NL} \times PSV_{NL} \times D_{NL}\ OR\ f_{NL} \times PSV_{NL} \times \frac{1}{PSV_{NL}}$, from which

$[L]_{NL} \propto f_{NL}$ (*S6*)

Where:
V_L: is the volume of lipids in medium
D_L and D_{NL} are the densities of lipids and neutral lipids in the medium
PSV_{NL} are the partial specific volume fractions of trioleate, a TG representative of neutral lipids, in mL/g
F_L and F_{NL} is the volume fraction of total lipids and neutral lipids in the culture medium
Fitting the new parameters derived in Equations S4-S6 into Equation S3, the free concentration is predicted by the following equation:

$C_f = \frac{C_t}{1+K_{alb} \times f_{alb} + P_{ow} \times f_{NL}}$ (*S7*)

Correcting for Kow

The terms octanol-water distribution constant (K_{ow} or P_{ow}) and the volume fraction of neutral lipids (f_{NL}) need further consideration and modification (Fisher et al., 2019). First, f_{NL} will be addressed by considering that:

a) the binding to neutral lipids is limited to the non-ionized species. Thus, the distribution coefficient (P_{ow}) needs to be corrected for to account only for the non-ionized form of the chemical that is present at a certain pH.

b) the ratio (*Y*) of the ionized species and non-ionized species can be calculated by the Henderson-Hasselbalch equation.

For a monoprotic acid:

$$Y_{monoprotic\ acid} = \frac{[ionized\ species]}{[non-ionized\ species]} = 10^{(pH-pKa)}$$

For other compound types,

$$Y_{neutral} = 0 \qquad Y_{monoprotic\ base} = 10^{(pKa-pH)}$$

For a diprotic acid/base and ampholyte, Y is the summation of the sub-fractions (Y_1 and Y_2) from the ionizing species (Berezhkovskiy, 2011)

$$Y_{diprotic\ acid}: \mathrm{Y1} = 10^{(pH-pKa1)} + 10^{(pH-pKa2)} \text{ and } \mathrm{Y2} = 10^{2pH-(pKa1+pKa2)}$$
$$Y_{diprotic\ base}: \mathrm{Y1} = 10^{(pKa1-pH)} + 10^{(pKa2-pH)} \text{ and } \mathrm{Y2} = 10^{(pKa1+pKa2)-2pH}$$

For ampholytes or zwitterions (i.e., considering one site to be acidic and the other basic), Y is defined as:

$$Y_{ampholyte}: \mathrm{Y1a} = 10^{(pH-pKa1)}, \mathrm{Y1b} = 10^{(pKa2-pH)} \text{ and } \mathrm{Y2} = 10^{(pKa2-pKa1)}$$

In this case, pKa1 and pKa2 correspond to the acidic and basic groups respectively.
Here, we exemplify further steps with a monoprotic acid (e.g. valproic acid)

$$\frac{[ionized\ species]}{[non-ionized\ species]} = 10^{(pH-pKa)}$$

N.B.: The ratio of the concentration of ionized species to the non-ionized species is the same as the ratio of the ionized fraction (I) to the non-ionized fraction (Ni*)*
Then, $l = N_i \times 10^{pH-pKa}$
But also, $l = 1 - Ni$
Therefore,

$$1 - Ni = N_i \times 10^{pH-pKa}$$
$$N_i = \frac{1}{1+10^{pH-pKa}}$$

From which

$$N_i = \frac{1}{1+Y} \qquad (S8)$$

The distribution coefficient, expressed as D^{pH}, is a pH-dependent simple descriptor for ionizable solutes and results from the weighted contributions of all electrical forms/spp present at this pH, as illustrated by the following equation (Caron et al*.,* 2007):

$$D^{pH} = P^N \times F^N + \sum P^i \times F^i$$

Where:
P^N and P^i are the respective partition coefficients for the neutral and ionized spp. P^N would be the same as the P_{ow}
F^N and F^i are the respective molar fractions of the neutral and ionized forms.

Here, we come back to the assumption that the binding to neutral lipids is only for the non-ionized component of the compound.
Based on this assumption, the equation by (Caron et al*.,* 2007) can be reduced to

$$D^{pH} = P^N \times F^N$$

From Equation S8, F^N can be defined as: $F^N = \frac{1}{1+Y}$

From which, $D^{pH} = P^N \times \frac{1}{1+Y}$ OR simply $D^{pH} = P_{ow} \times \frac{1}{1+Y}$ $(S9)$

Substituting the correction for P_{ow} in Equation S9 into Equation S7, the free concentration in the medium is given by:

$$C_f = \frac{C_t}{1+K_{alb}\times f_{alb}+\frac{P_{ow}\times f_{NL}}{1+Y}} \quad (S10)$$

A further correction for K_{ow} is necessary as octanol is not an ideal representative of cellular membranes and lipids. Olive oil was proposed to be a better surrogate for neutral lipids than n-octanol, and a way of obtaining an olive oil corrected value of P_{ow} was reported by Poulin and Theil (2002) to be of the following relationship:

$$logD_{vow} = 1.115 \times logP_{ow} - 1.35 \quad (S11)$$

Where:
P_{ow}: n-octanol: water partition coefficient of non-ionized species.
D_{vow}: the olive oil-water partition coefficient of the non-ionized species. It is used in the derived equation as an anti-log of log D_{vow}. Since we consider here the binding of the non-ionized form of the compound to be predominantly to the neutral lipids, log D_{vow} can be referred to as the neutral lipid partition coefficient.
Therefore, the free concentration in the complete culture medium can be calculated as:

$$C_f = \frac{C_t}{1+K_{alb}\times f_{alb}+\frac{D_{vow}\times f_{NL}}{1+Y}} \quad (S12)$$

The D_{vow} required for Equation S12 can be derived from logPow values (easier to find in databases) from Equation S11.
Equation S12 can be further transformed to give the *in vitro* free fraction (f_u) of the compound:

$$f_u = \frac{C_f}{C_t} \quad \text{or} = \frac{1}{1+K_{alb}\times f_{alb}+\frac{D_{vow}\times f_{NL}}{1+Y}} \quad (S13)$$

The culture medium can have negligible amounts of lipids or protein. In this case, the equation can be simplified as follows:
Only proteins in the medium:

$$f_u = \frac{1}{1+K_{alb}\times f_{alb}} \quad (S14)$$

Only lipids in the medium:

$$f_u = \frac{1}{1+\frac{D_{vow}\times f_{NL}}{1+Y}} \quad (S15)$$

Deriving albumin (f_{alb}) and lipid (f_{NL}) fractions
f_{alb} and f_{NL} are calculated using the experimentally determined partial specific volume values of these biomolecules, i.e., F_{NL}: the volumetric fraction of medium comprised of neutral lipids as TG.
The total volume (V) of cell culture medium at constant temperature (T) and pressure (P) can be expressed using the partial specific volumes (PSV in units of mL/g) of the number of component biomolecules (n) and their masses in grams (g) (Durchschlag, 1986).

$$V = \sum_{i\longrightarrow 1}^{n} PSV_i \times g_i \quad (S16)$$

Where PSV_i x g_i represents the volume contribution of a specific component *i* in the system.
The change in volume attribute to the addition of component *i* can thus be expressed as

$$PSV_i = \left(\frac{\Delta V}{\Delta g_i}\right) \quad \text{Or simply } \Delta V = PSV_i \times \Delta g_i \quad (S17)$$

Assumptions 5

- To take into account the total volume of the system, we hereby express the mass of the component *i* as a concentration (C in mg/mL)
- To further simplify the equation, we define the volume fraction (V_f) of a specific component biomolecule (the term 1000 being a conversion factor between mg and g):

$$V_f = \frac{PSV_i \times C_i}{1000} \quad (S18)$$

Taking neutral lipids to be represented mainly by TG in the culture medium, the volume fraction of neutral lipids is defined here as:

$$f_{NL} \approx f_{TAG} = \frac{[TAG] \times PSV_{TAG}}{1000} \quad (S19)$$

f_{alb}: the volumetric fraction of medium comprised of protein, mainly being present as albumin, is defined as:

$$f_{alb} = \frac{[albumin] \times PSV_{albumin}}{1000} \quad (S20)$$

Where:
PSV_{TG} is 1.093 mL/g;
[TG] is expressed in mg/mL;
$PSV_{albumin}$ is 0.73 mL/g;
[albumin] is expressed in mg/mL.

The binding strength of a compound to albumin is described as its albumin binding affinity. In this equation, since we are working with volumetric fractions of proteins (albumin), we here express the binding of a compound to albumin as an albumin-water partition coefficient in the aqueous environment of an *in vitro* culture system. The binding affinity and albumin-water partition coefficient can be interconverted based on the assumptions considered here that the concentration of compound-bound albumin is far less than the total albumin concentration. Thus, taking K_{alb} as an albumin-water partition coefficient, it is derived from the octanol-water partition coefficient (logPow) as reported by Endo and Goss (2011) such that:

If $logP_{ow} < 4.5$, then; $logk_{albumin} = 1.08 \times logP - 0.7$ (S21)
If $logP_{ow} \geq 4.5$, then; $logk_{albumin} = 0.37 \times logP + 2.56$ (S22)

Box S2: Example use of Equation S13 with an acidic and basic drug

Test method terms	UKN5 test method	Compound related terms	Valproic acid	Amphetamine
Protein	3.3 mg/mL	logPow	2.75	1.76
Lipid	0.025 mg/mL	pKa	4.8	10
f_{alb}	2.43 x10^{-3}	K_{alb}	186	16
f_{NL}	2.73 x 10^{-5}	D_{vow}	52	4.1
		y	398	398
		Predicted f_u	0.69	0.96

The terms f_{alb} and f_{NL} can be derived from the concentrations of protein and lipid (see Equations S5 and S6 in the supplementary material). To convert the concentrations (standard test system information) to the volume fractions, one needs to have information on the density of the biomolecules. More specifically, one uses the partial specific volume (PSV) values as shown in Equations S19 and S20. For all test systems and situations, the same fixed, experimentally determined values (Durchschlag, 1986; Redgrave and Calson, 1979; Fisher et al., 2019) can be used, i.e., PSV_{TG} = 1.093 mL/g (lipid density) and $PSV_{albumin}$ = 0.73 mL/g (protein density). More data and background information is given in Table S2 (below) and Fisher et al. (2019). Using this calculation approach, f_{alb} of the UKN5 test is 2.43 x 10^{-3} (with a protein content of 3.3 mg per mL of the medium). This means that within 1 mL of medium, albumin (protein) occupies a volume of 2.43 µL. Similarly, F_{NL} can be calculated to be 2.73 x 10^{-5}. Thus, the volume attribute to neutral lipids in 1 mL medium would be only 0.027 µL (with a lipid content in UKN5 medium of 0.025 mg/mL).

Taking an example of 1 mM VPA, with Y (Y = $10^{(pH-pKa)}$) at pH = 7.4, this would mean that 0.9974 mM is ionized and 0.0026 mM is in the non-ionized form. Or, for 50 µM VPA, this would mean that there is 49.87 µM negatively charged drug and 0.13 µM in the neutral form. Only the unionized fraction binds to lipids. If one returns to Equation S13, you appreciate that the correction term Y is only linked to the lipid-binding part of the equations, but not to the protein binding part. The explanation is that the equation assumes that proteins (albumin) bind both the ionized and non-ionized forms of a drug.

If we choose amphetamine as a drug, the f_u prediction would be 0.96, i.e., only 4% of the drug would be bound. In this we note that:

a) amphetamine (logPow 1.76) is less hydrophobic than VPA, with a smaller D_{vow} (olive oil-water distribution)
b) the drug contains a basic amino group. At pH 7.4, most of the amino group would be protonated (positively charged ammonium group). Here Y is calculated as Y= ($10^{(pKa-pH)}$). From the table above, this means that only a 400th part of the dissolved amphetamine is available for lipid binding.

It needs to be noted that this is a simplified model. It may apply to cell culture media that contain mainly neutral lipids. In the presence of cells, bases may actually bind extensively to negatively-charged phospholipids.

Tab. S1: Cell composition data essential for biokinetics calculations

Cell type	Cell weight [ng/cell]	Cell volume [pl]	Cell protein [pg/cell]	Cell lipid [pg/cell]	Cell_lipid [s] [pg/cell]	Cell water (pL) [% of cell weight]
Hek 293Tcells	7.40[a]	7.30[q]	450[a]	130[a]	104	6.60[a] [89]
Hek 293Hcells	3.00[a]	3.00[q]	170[a]	150[a]	39	2.50[a] [83]
HepG2	3.00[a]	3.00[q]	210[a]	180[a]	48	2.70[a] [90]
HCT116	2.60[a]	2.60[q]	220[a]	184[a]	51	2.30[a] [88]
Me-180	4.00[a]	4.00[q]	290[a]	135[a]	67	3.30[a] [83]
MDA-MB231	3.24[o]	2.70[b]	415[b]		95	2.70[n]
A549	2.88[o]	2.38[b]	659[b]		152	2.36[n]
MIA Paca-2	3.12[o]	2.6[b]	757[b]		174	2.56[n]
HepG2	3.36[o]	2.83[b]	1192[b]		274	2.76[n]
HepaRGTM	1.80[c]	1.67[c]	480[c]	22[c]	110	1.30[c] [72]
Cardiomyocytes (ICellTM)	15.6[c]	7.85[c]	4180[c]	187[c]	961	11.0[c] [71]
Sperm cells			9		2	
Balb/c 3T3 cells	2.53[p] 3.14[p]		370[e] 460[f]	85[e,r] 106[f,r]	85 106	2.10[n] 2.60[n]
RTgill-Wi	1.43[p]		210[f]	48[f, r]	48	1.43[n]
LUHMES	2.00[g]	1.4-1.6[g]	70[g]		16	1.64[n]
IMA	3.96[o]	3.3[h]				3.3[n]
Oct3-IMA	2.76[o]	2.3[h]				2.3[n]
HL-60	0.77[o]	0.32[m]	170[i]		39	0.63[n]
U-937	1.1[o]	0.47[m]	110[i]		25	0.90[n]
MCF-7	2.04[o]	0.85[m]	404[i]		93	1.67[n]
MCF-7-p51	2.2[o]	0.92[m]	625[i]		144	1.80[n]
MIA-Paca-2	2.4[o]	1.01[m]	730[i]		168	1.97[n]
PC-3	3.48[o]	1.45[m]	724[i]		167	2.85[n]
Neuroepithelial cells	3.35	1.75[h]				2.75[n]
Astrocytes (cat)	0.7[o]	0.57[i]/0.32[k]				0.57[n]
Normal mononuclear cells	0.23[o]	0.19[l]				0.19[n]
Normal T cells	0.22[o]	0.18[l]				0.18[n]
Normal B cells	0.23[o]	0.19[l]				0.19[n]
Normal monocytes	0.49[o]	0.41[l]				0.41[n]
Reactive lymph nodes	0.23[o]	0.19[l]				0.19[n]
Chronic lymphocytic leukemia	0.20[o]	0.17[l]				0.17[n]
Acute lymphocytic leukemia	0.30[o]	0.24[l]				0.24[n]
Burkitt's lymphoma	0.48[o]	0.40[l]				0.40[n]
Hairy cell leukemia	0.48[o]	0.40[l]				0.40[n]

MDA-MB231, human mammary adenocarcinoma; A549, adenocarcinoma alveolar epithelial; MIA PaCa-2, human pancreatic carcinoma; RTgill-Wi, gill epithelial cells; HL-60, human promyelocytic leukemia; U-937, human histiocytic leukemia; MCF-7, mammary adenocarcinoma; MCF-7-p51, mammary adenocarcinoma GPx4 overexpressor; PC-3, prostate adenocarcinoma; HCT116, human colon carcinoma cell line; Me-180, human cervical cancer cells; IMA, immortalised mouse astrocytes; oct3-IMA, immortalized mouse astrocytes with an introduced organic cation transporter 3.

[h] Own data – the cell volume was estimated by taking a million cells in an Eppendorf vial and filling up a parallel vial with an equal volume of medium. The volume of medium was then equated to be the volume of a million cells (Schildknecht et al., 2015);

[j] Total cell volume of soma plus processes

[k] Cell volume of soma; cell density was assumed to be the same as that of water (1 g/mL or 1 ng/pL).

[m] Cell volume calculated assuming the cell in 2D cultures take up a dome shape (half-sphere) = (4/3ππr3) x 0.5; cell diameter was obtained from the corresponding publication.

[n] Water content by weight was found to average ≈ 82% of the cell weight. Cell water volume was calculated from the given cell weight as a 0.82 fraction by weight and a water density of 1 g/mL or 1 ng/pL.

[o] Cell weight (in cases not reported; it was taken to be 82% of total cell weight) was derived from cell volume and water density (V x ρ); total cell weight was then scaled to 100% by multiplying by 1.2 = (100/82). In calculating the cell weight from cell volume, the cell volume is taken for a full sphere.

[p] Cell weight derived from the fraction of cell lipid + protein ≈ 18%.

[q] Cell volume was calculated as the sum of cell lipid, cell protein and cell water volumes.

[r] Lipid content in the Balb/c 3T3 cells and RTgill-W1 was calculated as assumed by both Gulden et al. (2002) and Kramer et al. (2012) that there is 0.23 mg lipid/mg protein in the cells.

[s] Cell_Lipid-lipid content extrapolated from cellular protein.

[a] Fischer et al., 2017; [b] Doskey et al., 2015; [c] Worth et al., 2017; [d] Gülden et al., 2001; [e] Gülden et al., 2002; [f] Kramer et al., 2012; [g] Delp et al., 2018; [h] own data; [i] Wagner et al., 2011; [j,k] Williams et al., 1980; [l] Chapman et al., 1981

Tab. S2: Measured and predicted human plasma f_u values

Drug	CAS	logPow	Predicted f_u	Measured f_u	Reference
Acebutolol	37517-30-9	1.53	0.79	0.74	Varma et al., 2010
Acyclovir	59277-89-3	-1	1.00	0.91	Varma et al., 2010
Adefovir	106941-25-7	-4.5	0.81	0.96	Varma et al., 2010
Adinazolam	37115-32-5	2.24	0.38	0.31	Varma et al., 2010
Alfentanil	71195-58-9	2.81	0.13	0.09	Varma et al., 2010
Allopurinol	000315-30-0	0.031	0.99	0.97	Varma et al., 2010
Alprazolam	28981-97-7	2.37	0.31	0.29	Varma et al., 2010
Alprenolol	13655-52-2	2.69	0.18	0.18	Varma et al., 2010
Amantadine[a]	768-94-5	*2.44*	0.28	0.33	Varma et al., 2010
Amiodarone	1951-25-3	7.64	7E-05	0.0002	Varma et al., 2010
Amisulpride	71675-85-9	0.25	0.99	0.84	Varma et al., 2010
Amitriptyline	50-48-6	4.81	0.002	0.07	Varma et al., 2010
Amlodipine[a]	88150-42-9	*3*	0.090	0.005	Varma et al., 2010
Amoxicillin	26787-78-0	-2.3	1.00	0.85	Varma et al., 2010
Amphotericin B	1397-89-3	-2.3	1.00	0.04	Varma et al., 2010
Ampicillin	69-53-4	-2	1.00	0.85	Varma et al., 2010
Antipyrine	000060-80-0	1.22	0.89	0.93	Varma et al., 2010
Atenolol	29122-68-7	0.43	0.98	0.94	Varma et al., 2010
Atomoxetine	83015-26-3	3.81	0.013	0.02	Varma et al., 2010
Atovaquone	95233-18-4	5	0.001	0.001	Varma et al., 2010
Atropine	51-55-8	1.57	0.78	0.61	Varma et al., 2010
Azithromycin	83905-01-5	2.44	0.28	0.71	Wishart et al., 2006
Aztreonam	78110-38-0	*-0.68*	1.00	0.44	Wishart et al., 2006
Betaxolol	63659-18-7	2.54	0.24	0.4	Varma et al., 2010
Biperiden	514-65-8	3.54	0.025	0.097	Varma et al., 2010
Bisoprolol	66722-44-9	2.2	0.42	0.66	Varma et al., 2010
Bromazepam	1812-30-2	2.54	0.23	0.3	Wishart et al., 2006
Bromfenac	91714-94-2	3.66	0.019	0.11	Varma et al., 2010
Budesonide	51333-22-3	2.73	0.15	0.13	Varma et al., 2010
Buflomedil	55837-25-7	1.88	0.62	0.4	Varma et al., 2010
Bufuralo	54340-62-4	2.99	0.092	0.19	Varma et al., 2010
Bumetanide	28395-03-1	*2.57*	0.22	0.031	Varma et al., 2010
Bupivacaine	2180-92-9	4.52	0.002	0.056	Varma et al., 2010
Busulphan	55-98-1	-0.76	1.00	1	Varma et al., 2010
Caffeine	58-08-2	-0.55	1.00	0.64	Varma et al., 2010
Captopril	62571-86-2	0.73	0.97	0.73	Varma et al., 2010
Carbaryl	63-25-2	2.5	0.24	0.69	CompTox[b]
Carboplatin	41575-94-4	0	0.99	1	Varma et al., 2010
Carvedilol	72956-09-3	3.42	0.034	0.05	Wishart et al., 2006
Cefadroxil	50370-12-2	*-0.6*	1.00	0.79	Wishart et al., 2006
Cefatrizine	51627-14-6	*-0.07*	1.00	0.4	Varma et al., 2010
Cefazolin[a]	25953-19-9	*-0.58*	1.00	0.18	Varma et al., 2010
Cefepime	88040-23-7	-4.3	1.00	0.78	Varma et al., 2010
Cefetamet	65052-63-3	-0.65	1.00	0.78	Varma et al., 2010
Cefixime[a]	79350-37-1	-0.4	1.00	0.35	Wishart et al., 2006
Ceftriaxone[a]	073384-59-5	-1.7	1.00	0.054	Varma et al., 2010
Cefuroxime[a]	55268-75-2	*-0.16*	1.00	0.5	Wishart et al., 2006
Cephalexin	15686-71-2	-2.1	1.00	0.9	Wishart et al., 2006
Cephradine	38821-53-3	-2.4	1.00	0.95	Varma et al., 2010
Cerivastatin[a]	145599-86-6	3.4	0.035	0.01	Varma et al., 2010
Chlorambucil	305-03-3	3.94	0.009	0.01	Varma et al., 2010
Chloramphenicol[a]	56-75-7	*1.14*	0.91	0.5	Wishart et al., 2006
Chlordiazepoxide	58-25-3	*5.5*	0.0006	0.056	Varma et al., 2010
Chloroquine[a]	54-05-7	*4.63*	0.002	0.26	Wishart et al., 2006
Chlorpheniramine[a]	132-22-9	3.38	0.04	0.28	Wishart et al., 2006
Chlorpromazine[a]	50-53-3	5.41	0.001	0.056	Varma et al., 2010
Chlorpropamide[a]	94-20-2	*2.27*	0.38	0.03	Varma et al., 2010
Chlorthalidone	77-36-1	*1.77*	0.67	0.25	Wishart et al., 2006
Cibenzoline	53267-01-9	3	0.09	0.5	Varma et al., 2010
Cidofovir[a]	113852-37-2	-3.9	1.00	1	Varma et al., 2010
Cilomilast	153259-65-5	3.9	0.010	0.006	Varma et al., 2010
Cimetidine[a]	051481-61-9	0.4	0.98	0.85	Wishart et al., 2006
Ciprofloxacin	85721-33-1	1.55	0.78	0.8	Wishart et al., 2006
Citalopram[a]	59729-33-8	3.76	0.015	0.2	Varma et al., 2010
Clarithromycin[a]	81103-11-9	3.16	0.062	0.23	Varma et al., 2010

Clavulanic Acid[a]	58001-44-8	-2.3	1.00	0.91	Varma et al., 2010
Clinafloxacin	105956-97-6	-0.42	1.00	0.96	Varma et al., 2010
Clindamycin[a]	18323-44-9	2.16	0.44	0.4	Wishart et al., 2006
Clofibrate	637-07-0	3	0.085	0.05	CompTox[b]
Clonazepam[a]	1622-61-3	2.41	0.29	0.18	Wishart et al., 2006
Clozapine[a]	5786-21-0	3.23	0.051	0.055	Varma et al., 2010
Colchicine	64-86-8	*1.46*	0.81	0.66	Wishart et al., 2006
Conivaptan	210101-16-9	6.3	0.0002	0.01	Varma et al., 2010
Cyclophosphamide[a]	000050-18-0	0.8	0.96	0.87	Varma et al., 2010
Cyclosporine A	59865-13-3	2.92	0.10	0.068	Wishart et al., 2006
Dapsone[a]	80-08-0	1.31	0.86	0.3	Wishart et al., 2006
Desipramine	50-47-5	3.64	0.004	0.08	Wishart et al., 2006
Dexamethasone[a]	50-02-2	1.83	0.63	0.32	Varma et al., 2010
Dexloxiglumide	119817-90-2	3.37	0.038	0.024	Varma et al., 2010
Diazepam	439-14-5	3.08	0.071	0.023	Varma et al., 2010
Diazoxide[a]	364-98-7	1.81	0.65	0.1	Wishart et al., 2006
Diclofenac[a]	15307-86-5	4.51	0.002	0.005	Varma et al., 2010
Dicloxacillin[a]	3116-76-5	2.91	0.11	0.033	Varma et al., 2010
Didanosine[a]	69655-05-6	-1.24	1.00	0.95	Varma et al., 2010
Digoxin	20830-75-5	1.26	0.88	0.75	Wishart et al., 2006
Diltiazem[a]	33286-22-5	2.8	0.14	0.18	Varma et al., 2010
Disopyramide[a]	05/09/3737	2.58	0.22	0.16	Varma et al., 2010
Domperidone[a]	57808-66-9	3.9	0.010	0.082	Varma et al., 2010
Doxifluridine	05/09/3094	0.07	0.99	0.61	Varma et al., 2010
Doxorubicin[a]	23214-92-8	1.27	0.88	0.28	Varma et al., 2010
Doxycycline[a]	564-25-0	*0.63*	0.97	0.12	Varma et al., 2010
Drotaverine	14009-24-6	3.54	0.024	0.12	Varma et al., 2010
Enalaprilat	76420-72-9	1.73	0.70	0.62	Varma et al., 2010
Encainide	66778-36-7	*4*	0.0082	0.26	Varma et al., 2010
Entacapone[a]	130929-57-6	2.8	0.14	0.02	Varma et al., 2010
Epristeride	119169-78-7	3.93	0.0097	0.03	Varma et al., 2010
Eprosartan	133040-01-4	3.9	0.01	0.017	Varma et al., 2010
Erythromycin[a]	114-07-8	2.6	0.21	0.2	Wishart et al., 2006
Etilefrine	709-55-7	0.23	0.99	0.77	Varma et al., 2010
Etoposide	33419-42-0	*0.67*	0.97	0.12	Varma et al., 2010
Felodipine[a]	72509-76-3	3.86	0.01	0.0036	Varma et al., 2010
Finasteride[a]	98319-26-7	3.03	0.08	0.095	Varma et al., 2010
Flecainide	54143-55-4	*2.8*	0.14	0.52	Varma et al., 2010
Fleroxacin[a]	79660-72-3	0.24	0.99	0.73	Varma et al., 2010
Fluconazole[a]	86386-73-4	0.5	0.98	0.89	Varma et al., 2010
Flucytosine[a]	2022-85-7	-1.1	1.00	1	Varma et al., 2010
Flumazenil[a]	78755-81-4	1	0.93	0.58	Varma et al., 2010
Flupirtine	56995-20-1	2.67	0.18	0.15	Varma et al., 2010
Fluvastatin[a]	93957-54-1	4.5	0.002	0.0079	Varma et al., 2010
Folinic acid	1492-18-8	-1.31	1.00	0.87	Varma et al., 2010
Foscarnet	63585-09-1	-2.1	1.00	0.85	Varma et al., 2010
Fosfomycin[a]	23155-02-4	-1.6	1.00	1	Varma et al., 2010
Frovatriptan[a]	158747-02-5	0.9	0.95	0.85	Varma et al., 2010
Furosemide	54-31-9	2.03	0.52	0.04	Wishart et al., 2006
Gabapentin[a]	60142-96-3	1.25	0.88	0.97	Varma et al., 2010
Ganciclovir[a]	82410-32-0	-1.66	1.00	0.99	Varma et al., 2010
Gatifloxacin	160738-57-8	1.73	0.70	0.8	Varma et al., 2010
Gentamicin[a]	1405-41-0	-3.1	1.00	1	Varma et al., 2010
Glimepiride[a]	93479-97-1	3.12	0.068	0.005	Varma et al., 2010
Glipizide	29094-61-9	*3.35*	0.040	0.02	Varma et al., 2010
Glyburide[a]	10238-21-8	3.754	0.015	0.021	Varma et al., 2010
Granisetron[a]	109889-09-0	2.6	0.21	0.35	Varma et al., 2010
Guanfacine	29110-47-2	1.89	0.60	0.28	Varma et al., 2010
Haloperidol[a]	52-86-8	4.3	0.0038	0.08	Varma et al., 2010
Hexachlorophene[a]	70-30-4	7.54	0.0001	0.08	Wishart et al., 2006
Hydroxyurea[a]	127-07-1	-1.8	1.00	1	Varma et al., 2010
Ibuprofen	15687-27-1	3.8	0.013	0.01	Wishart et al., 2006
Ifosfamide[a]	3778-73-2	0.86	0.95	1	Varma et al., 2010
Imipenem	64221-86-9	-3.9	1.00	0.86	Varma et al., 2010
Indomethacin[a]	53-86-1	4.27	0.0042	0.01	Varma et al., 2010
Isosorbide-5-Mononitrate[a]	16051-77-7	-0.15	1.00	1	Varma et al., 2010
Isoxicam[a]	34552-84-6	*2.83*	0.13	0.035	Varma et al., 2010
Itraconazole[a]	84625-61-6	5.66	0.0005	0.002	Varma et al., 2010

Ketanserin	74050-98-9	3.61	0.021	0.055	Varma et al., 2010
Ketoprofen[a]	22071-15-4	3.12	0.068	0.008	Varma et al., 2010
Ketorolac[a]	74103-06-3	2.1	0.48	0.0068	Varma et al., 2010
Lamivudine[a]	134678-17-4	*-1.4*	1.00	0.94	Varma et al., 2010
Lansoprazole	103577-45-3	3.03	0.079	0.021	Varma et al., 2010
Letrozole[a]	112809-51-5	*2.5*	0.24	0.41	Varma et al., 2010
Levofloxacin	100986-85-4	2.1	0.48	0.62	Wishart et al., 2006
Linezolid	165800-03-3	0.9	0.95	0.69	Varma et al., 2010
Lisinopril	76547-98-3	-1.01	1.00	1	Varma et al., 2010
Lorazepam[a]	846-49-1	2.39	0.30	0.25	Wishart et al., 2006
Lorcainide[a]	59729-31-6	4.85	0.002	0.15	Varma et al., 2010
Lormetazepam	848-75-9	3.26	0.05	0.12	Varma et al., 2010
Losartan	114798-26-4	5.32	0.001	0.01	Varma et al., 2010
Lovastatin[a]	075330-75-5	4.08	0.006	0.043	Varma et al., 2010
Mebendazole[a]	31431-39-7	2.83	0.12	0.086	Varma et al., 2010
Melagatran	159776-70-2	-1.3	1.00	0.93	Varma et al., 2010
Meloxicam[a]	71125-38-7	*3.43*	0.033	0.01	Wishart et al., 2006
Metformin[a]	657-24-9	-2.6	1.00	1	Varma et al., 2010
Methadone[a]	76-99-3	3.93	0.010	0.1	Wishart et al., 2006
Methotrexate	59-05-2	0.17	0.99	0.53	Wishart et al., 2006
Methyldopa[a]	555-30-6	-1.7	1.00	0.85	Varma et al., 2010
Metoclopramide	364-62-5	1.09	0.92	0.7	Wishart et al., 2006
Metolazone	17560-51-9	2.92	0.10	0.05	Varma et al., 2010
Metoprolol	37350-58-6	1.49	0.81	0.88	Varma et al., 2010
Metronidazole[a]	000443-48-1	-0.02	0.99	0.96	Varma et al., 2010
Midazolam	59467-70-8	3.33	0.04	0.03	Wishart et al., 2006
Miglitol[a]	72432-03-2	-2.7	1.00	1	Varma et al., 2010
Milrinone	78415-72-2	1.17	0.90	0.35	Varma et al., 2010
Mirtazapine[a]	61337-67-5	2.9	0.11	0.15	Varma et al., 2010
Moclobemide	71320-77-9	1.1	0.91	0.77	Varma et al., 2010
Montelukast[a]	158966-92-8	7.9	0.0001	0.002	Varma et al., 2010
Moxifloxacin	354812-41-2	1.85	0.63	0.6	Varma et al., 2010
Moxonidine	75438-57-2	1.54	0.78	0.9	Wishart et al., 2006
MPP^{+}[a]		-2.28	1.00		
Nadolol[a]	42200-33-9	0.81	0.96	0.7	Wishart et al., 2006
Naratriptan[a]	121679-13-8	1.6	0.76	0.72	Wishart et al., 2006
Nateglinide	105816-04-4	3.91	0.01	0.02	Wishart et al., 2006
Nefazodone	83366-66-9	4.7	0.0016	0.01	Varma et al., 2010
Nevirapine	129618-40-2	2.5	0.24	0.32	Varma et al., 2010
Nicardipine	55985-32-5	3.82	0.013	0.01	Varma et al., 2010
Nicotine[a]	54-11-5	1.17	0.90	0.95	Varma et al., 2010
Nifedipine[a]	21829-25-4	2.2	0.41	0.08	Wishart et al., 2006
Nimodipine[a]	66085-59-4	3.05	0.08	0.05	Wishart et al., 2006
Nisoldipine[a]	63675-72-9	3.26	0.05	0.01	Wishart et al., 2006
Nitrazepam	146-22-5	2.25	0.38	0.13	Varma et al., 2010
Nizatidine	76963-41-2	1.1	0.91	0.65	Varma et al., 2010
Nomifensine	24526-64-5	2.62	0.20	0.4	Varma et al., 2010
Nortriptyline[a]	894-71-3	3.9	0.01	0.07	Wishart et al., 2006
Ofloxacin	82419-36-1	1.51	0.80	0.75	Varma et al., 2010
Omeprazole[a]	73590-58-6	2.23	0.39	0.05	Varma et al., 2010
Ondansetron[a]	99614-02-5	2.4	0.30	0.27	Varma et al., 2010
Oseltamivir acid	187227-45-8	-1.8	1.00	0.97	Varma et al., 2010
Oxazepam	604-75-1	2.98	0.09	0.11	Wishart et al., 2006
Pantoprazole[a]	102625-70-7	2.05	0.50	0.38	Varma et al., 2010
Papaverine	58-74-2	3	0.09	0.073	Varma et al., 2010
Paracetamol	103-90-2	1.1	0.91	0.9	Wishart et al., 2006
Paraquat	1910-42-5	-4.22	1.00	1	Houzé et al.,1990
Paricalcitol[a]	131918-61-1	4.5	0.002	0.0016	Varma et al., 2010
Pefloxacin[a]	70458-92-3	0.27	0.99	0.8	Wishart et al., 2006
Penciclovir[a]	39809-25-1	-1.1	1.00	0.84	Varma et al., 2010
Phencyclidine[a]	77-10-1	4.69	0.002	0.35	Varma et al., 2010
Phenobarbital	50-06-6	1.56	0.78	0.8	Wishart et al., 2006
Phenoxymethylpenicillin	000087-08-1	2.09	0.49	0.45	Varma et al., 2010
Pindolol[a]	13523-86-9	1.75	0.69	0.6	Wishart et al., 2006
Pirmenol	61447-94-9	3.93	0.010	0.13	Varma et al., 2010
Practolol[a]	6673-35-4	0.79	0.96	0.01	Varma et al., 2010
Pravastatin	81093-37-0	2.18	0.43	0.52	Wishart et al., 2006
Prazosin	19216-56-9	1.45	0.82	0.06	Varma et al., 2010

Prednisolone	50-24-8	1.62	0.74	0.35	Wishart et al., 2006
Prednisone	53-03-2	2.21	0.40	0.5	Wishart et al., 2006
Probenecid[a]	57-66-9	3.21	0.06	0.05	Wishart et al., 2006
Procainamide[a]	51-06-9	0.88	0.95	0.84	Varma et al., 2010
propranolol	525-66-6	3.48	0.029	0.04	Wishart et al., 2006
Propylthiouracil	51-52-5	1.14	0.91	0.18	Varma et al., 2010
Pyridostigmine	155-97-5	-3.16	1.00	1	Varma et al., 2010
Quinaprilat	82768-85-2	3.16	0.06	0.32	Varma et al., 2010
Quinidine	56-54-2	2.88	0.12	0.12	Wishart et al., 2006
Quinine	130-95-0	2.32	0.35	0.3	Varma et al., 2010
Rabeprazole	117976-89-3	*2.99*	0.09	0.037	Varma et al., 2010
Ranitidine	66357-35-5	0.88	0.95	0.95	Varma et al., 2010
Reboxetine[a]	98769-81-4	3.1	0.07	0.019	Varma et al., 2010
Remoxipride	80125-14-0	2.1	0.48	0.16	Varma et al., 2010
Repaglinide	135062-02-1	5.04	0.001	0.015	Varma et al., 2010
Rifabutin	72559-06-9	3.58	0.022	0.15	Wishart et al., 2006
Rifampin	13292-46-1	2.7	0.17	0.2	Varma et al., 2010
Risedronate[a]	105462-24-6	-3.3	1.00	0.76	Varma et al., 2010
Risperidone[a]	106266-06-2	2.63	0.20	0.1	Varma et al., 2010
Rosiglitazone	122320-73-4	3.13	0.066	0.002	Varma et al., 2010
Rosuvastatin	287714-41-4	2.05	0.51	0.12	Varma et al., 2010
Rotenone[a]	83-79-4	4.01	0.0075	0.02	CompTox[b]
salbutamol	18559-94-9	0.61	0.97	0.92	Varma et al., 2010
Saquinavir[a]	127779-20-8	3.8	0.013	0.028	Varma et al., 2010
Selegiline[a]	14611-52-0	2.7	0.17	0.13	Varma et al., 2010
Sematilide	101526-62-9	0.11	0.99	0.96	Varma et al., 2010
Sildenafil[a]	139755-83-2	2.75	0.15	0.04	Varma et al., 2010
Sitafloxacin	127254-12-0	2.18	0.43	0.51	Varma et al., 2010
Sitagliptin[a]	790712-60-6	1.5	0.80	0.62	Varma et al., 2010
Solifenacin	242478-38-2	3.98	0.009	0.02	Varma et al., 2010
Sotalol	3930-20-9	0.16	0.99	1	Wishart et al., 2006
Sparfloxacin	110871-86-8	2.4	0.31	0.55	Wishart et al., 2006
Sufentanil	56030-54-7	3.17	0.06	0.075	Varma et al., 2010
Sulfadiazine	68-35-9	0.37	0.99	0.44	Varma et al., 2010
Sulfamethoxazole	723-46-6	1.04	0.93	0.3	Wishart et al., 2006
Sulfinpyrazone	57-96-5	3.3	0.04	0.02	Wishart et al., 2006
Sulfisoxazole[a]	127-69-5	*1.01*	0.93	0.079	Varma et al., 2010
Sulpiride[a]	15676-16-1	0.57	0.98	0.72	Varma et al., 2010
Sumatriptan[a]	103628-46-2	0.93	0.94	0.83	Varma et al., 2010
Suprofen	40828-46-4	3.28	0.05	0.006	Varma et al., 2010
Suramin	145-63-1	3.48	0.03	0.003	Varma et al., 2010
Tacrolimus[a]	104987-11-3	3.3	0.04	0.01	Varma et al., 2010
Talinolol	57460-41-0	2.8	0.14	0.39	Varma et al., 2010
Tamsulosin	106133-20-4	2.47	0.27	0.01	Varma et al., 2010
Taxol	33069-62-4	3.3	0.042	0.02	Wishart et al., 2006
Tebuconazole	80443-41-0	3.3	0.042	0.07	CompTox[b]
Tegaserod	145158-71-0	3.81	0.01	0.02	Varma et al., 2010
Telmisartan[a]	144701-48-4	7.7	0.0001	0.004	Varma et al., 2010
Tenoxicam	59804-37-4	2.4	0.29	0.01	Wishart et al., 2006
Terazosin	60-87-7	1.47	0.81	0.1	Wishart et al., 2006
Terbutaline	23031-25-6	1.16	0.91	0.75	Varma et al., 2010
Terodiline	15793-40-5	5.01	0.0013	0.08	Varma et al., 2010
Tesaglitazar	251565-85-2	3.05	0.08	0.0011	Varma et al., 2010
Tetracycline	60-54-8	-1.3	1.00	0.78	Varma et al., 2010
Theophylline[a]	58-55-9	-0.02	0.99	0.61	Varma et al., 2010
Tiagabine	115103-54-3	5.28	0.001	0.04	Varma et al., 2010
Tilidine	20380-58-9	3.35	0.04	0.21	Varma et al., 2010
Timolol	26839-75-8	1.17	0.90	0.9	Varma et al., 2010
Tinidazole	19387-91-8	-0.64	1.00	0.88	Wishart et al., 2006
Tizanidine[a]	51322-75-9	1.72	0.70	0.7	Wishart et al., 2006
Tocainide[a]	41708-72-9	0.76	0.96	0.9	Wishart et al., 2006
Tolbutamide	64-77-7	2.3	0.36	0.05	Wishart et al., 2006
Tolterodine[a]	124937-51-5	5.6	0.0008	0.03	Wishart et al., 2006
Torsemide[a]	56211-40-6	3.356	0.04	0.01	Wishart et al., 2006
Tramadol[a]	27203-92-5	1.34	0.86	0.8	Wishart et al., 2006
Triazolam	28911-01-5	3.31	0.04	0.1	Varma et al., 2010
Trimethoprim	738-70-5	1.05	0.92	0.56	Wishart et al., 2006
Triphenylphosphate	115-86-6	5.6	0.0006	0.01	CompTox[b]

Trovafloxacin	146836-84-2	2.69	0.18	0.24	Wishart et al., 2006
Valproic acid	99-66-1	2.75	0.16	0.2	Wishart et al., 2006
Valsartan	137862-53-4	1.499	0.026	0.05	Wishart et al., 2006
Vancomycin	123409-00-7	-2.27	1.00	0.7	Varma et al., 2010
Vardenafil	224785-90-4	*2.96*	0.09	0.05	Varma et al., 2010
Venlafaxine	93413-69-5	2.25	0.39	0.73	Varma et al., 2010
Verapamil	52-53-9	3.79	0.014	0.06	Wishart et al., 2006
Vinblastine[a]	865-21-4	3.7	0.02	0.014	Varma et al., 2010
Warfarin	81-81-2	2.7	0.17	0.015	Varma et al., 2010
Xamoterol	81801-12-9	-0.82	1.00	0.97	Varma et al., 2010
Zaleplon	151319-34-5	1.59	0.76	0.4	Varma et al., 2010
Zanamivir[a]	139110-80-8	-3	1.00	0.86	Varma et al., 2010
Ziprasidone[a]	146939-27-7	3.8	0.01	0.0012	Varma et al., 2010
Zolmitriptan[a]	139264-17-8	1.792	0.67	0.75	Varma et al., 2010
Zolpidem[a]	82626-48-0	3.02	0.08	0.08	Varma et al., 2010
Zopiclone	43200-80-2	1.54	0.78	0.55	Wishart et al., 2006

[a]Compound logPow taken from EpiSuite database V.4.1; [b]CompTox, https://comptox.epa.gov/dashboard/

Predictions are as detailed in Equation S13 above at a system pH of 7.4. A graphical display is shown in Figure 2.

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