

SUPPLEMENTARY MATERIAL

S. Grandoni et al. "Building in-house PBPK modelling tools for oral drug administration from literature information"

System-specific parameters values

Values of the physiological parameters used in the PBPK model described in the paper are here summarized. These values refer to a typical subject of 250 g for rats, 10 kg for dogs and 70 kg for man.

Rat parameters

| Fluxes [ml/min], [21,22] | | Volumes [ml], [21,22,23] | |
|--------------------------|-------|--------------------------|-------|
| Q_{brain} | 1.79 | V_{brain} | 1.43 |
| Q_{gut} | 11.92 | V_{gut} | 6.75 |
| Q_{spleen} | 0.8 | V_{spleen} | 0.5 |
| Q_{liver} | 14.6 | V_{liver} | 9.15 |
| Q_{muscle} | 24.91 | V_{muscle} | 101.8 |
| $Q_{adipose}$ | 6.27 | $V_{adipose}$ | 16.6 |
| Q_{heart} | 4.39 | V_{heart} | 0.83 |
| Q_{kidney} | 12.64 | V_{kidney} | 1.83 |
| $Q_{Restofthebody}$ | 23.1 | $V_{Restofthebody}$ | 72.36 |
| Cardiac Output | 89.6 | V_{lung} | 1.25 |
| | | V_{ven} | 10.12 |
| | | V_{art} | 3.38 |

| Gastrointestinal absorption model parameters | |
|--|------|
| Volumes of intestinal segments [ml], [25] | |
| $V_{stomach}$ | 3 |
| V_1 | 0.6 |
| V_2 | 0.66 |
| V_3 | 0.66 |
| V_4 | 0.41 |
| V_5 | 0.41 |
| V_6 | 0.41 |
| V_7 | 0.41 |
| V_{colon} | 3 |
| pH of intestinal segments, [25] | |
| $pH_{stomach}$ | 3 |
| pH_1 | 7.1 |
| pH_2 | 7.3 |
| pH_3 | 7.5 |
| pH_4 | 7.7 |
| pH_5 | 7.9 |

| | |
|------------------------|---------|
| pH_6 | 8 |
| pH_7 | 7.4 |
| pH_{colon} | 7.6 |
| MRT values | |
| <i>Stomach</i> | 10 min |
| <i>Small intestine</i> | 88 min |
| <i>Colon</i> | 228 min |

Rat tissue composition to apply the Poulin's methods, [35]

| Rat tissues | <i>Volume fraction of Phospholipids, Vph</i> | <i>Volume fraction of Neutral lipids, Vnl</i> | <i>Volume fraction of Water, Vw</i> | <i>Volume fraction of Interstitial space</i> |
|---------------------|--|---|-------------------------------------|--|
| <i>Adipose</i> | 0.002 | 0.853 | 0.12 | 0.715 |
| <i>Bone</i> | 0.0027 | 0.0273 | 0.446 | 0.42 |
| <i>Brain</i> | 0.0533 | 0.0392 | 0.788 | 0.162 |
| <i>Gut</i> | 0.0138 | 0.0292 | 0.749 | 0.39 |
| <i>Heart</i> | 0.0118 | 0.014 | 0.779 | 0.156 |
| <i>Kidney</i> | 0.0284 | 0.0123 | 0.771 | 0.346 |
| <i>Liver</i> | 0.0303 | 0.0138 | 0.705 | 0.159 |
| <i>Lung</i> | 0.014 | 0.0219 | 0.79 | 0.484 |
| <i>Muscle</i> | 0.009 | 0.01 | 0.756 | 0.115 |
| <i>Skin</i> | 0.018 | 0.0239 | 0.651 | 0.462 |
| <i>Spleen</i> | 0.0136 | 0.0077 | 0.771 | 0.264 |
| <i>Plasma</i> | 0.00083 | 0.00147 | 0.96 | 1 |
| <i>Erythrocytes</i> | - | - | - | - |

Rat tissue composition, to apply the method of Rodgers, [37]

| Rat tissues | <i>Neutral phospholipids</i> | <i>Neutral lipids</i> | <i>Extracellular Water</i> | <i>Intracellular water</i> | <i>Tissue Concentration of Acidic Phospholipids (mg/g)</i> |
|--------------------|------------------------------|-----------------------|----------------------------|----------------------------|--|
| <i>Adipose</i> | 0.853 | 0.0016 | 0.135 | 0.017 | 0.40 |
| <i>Bone</i> | 0.017 | 0.0017 | 0.100 | 0.346 | 0.67 |
| <i>Brain</i> | 0.039 | 0.0015 | 0.162 | 0.620 | 0.40 |
| <i>Gut</i> | 0.038 | 0.0125 | 0.282 | 0.475 | 2.41 |
| <i>Heart</i> | 0.014 | 0.0111 | 0.320 | 0.475 | 2.25 |
| <i>Kidney</i> | 0.012 | 0.0242 | 0.273 | 0.483 | 5.03 |
| <i>Liver</i> | 0.014 | 0.0240 | 0.161 | 0.573 | 4.56 |
| <i>Lung</i> | 0.022 | 0.0128 | 0.336 | 0.446 | 3.91 |
| <i>Muscle</i> | 0.010 | 0.0072 | 0.118 | 0.630 | 1.53 |
| <i>Pancreas</i> | 0.041 | 0.0093 | 0.120 | 0.664 | 1.67 |
| <i>Skin</i> | 0.060 | 0.0044 | 0.382 | 0.291 | 1.32 |

| | | | | | |
|---------------|--------|--------|-------|-------|------|
| <i>Spleen</i> | 0.0077 | 0.0113 | 0.207 | 0.579 | 3.18 |
| <i>Thymus</i> | 0.017 | 0.0092 | 0.150 | 0.626 | 2.30 |

Tracheobronchial surface, S_{TB} : 81.75 cm² [14].

Hematocrit to compute the distribution: 0.46 [22].

Conversion factor to obtain the *in vivo* estimates of the hepatic clearance:

- MPPGL: 45 mg/g [25],
- HPGL: 125*10⁶ cells/g [25],
- Liver Weight: 9.15 g [21].

Filtration parameter to model the renal clearance:

- GFR: 1.31 ml/min [22].

Dog parameters

| Fluxes [ml/min], [21,22] | | Volumes [ml], [21,22,23] | |
|--------------------------|--------|--------------------------|-------|
| Q_{brain} | 21 | V_{brain} | 78 |
| Q_{gut} | 216 | V_{gut} | 368 |
| Q_{spleen} | 24 | V_{spleen} | 27 |
| Q_{liver} | 288 | V_{liver} | 329 |
| Q_{muscle} | 227.9 | V_{muscle} | 456.5 |
| $Q_{adipose}$ | 34 | $V_{adipose}$ | 1380 |
| Q_{heart} | 48.3 | V_{heart} | 78 |
| Q_{kidney} | 181.65 | V_{kidney} | 55 |
| $Q_{Restofthebody}$ | 246.8 | $V_{Restofthebody}$ | 1538 |
| Cardiac Output | 21 | V_{lung} | 82 |
| | | V_{ven} | 675 |
| | | V_{art} | 225 |

| Gastrointestinal absorption model parameters | |
|--|-------|
| Volumes of intestinal segments [ml], [25] | |
| $V_{stomach}$ | 14.54 |
| V_1 | 30.54 |
| V_2 | 32 |
| V_3 | 32 |
| V_4 | 20.1 |
| V_5 | 20.1 |
| V_6 | 20.1 |
| V_7 | 20.1 |
| V_{colon} | 290.9 |
| pH of intestinal segments, [25] | |
| $pH_{stomach}$ | 1.5 |
| pH_1 | 6 |
| pH_2 | 6 |
| pH_3 | 6 |
| pH_4 | 6.2 |
| pH_5 | 6.2 |
| pH_6 | 6.2 |
| pH_7 | 7.4 |
| pH_{colon} | 6.5 |

| MRT values | |
|-----------------|---------|
| Stomach | 30 min |
| Small intestine | 109 min |
| Colon | 9.4 h |

Tracheobronchial surface, $S_{TB} = 1176 \text{ cm}^2$, estimated with linear regression from the rat and man $BW-S_{TB}$ data [14].

Haematocrit: 0.42 [22].

Conversion factor to obtain the *in vivo* estimates of the hepatic clearance:

- MPPGL 43 mg/g [25],
- HPGL $120 \cdot 10^6$ cells/g [25],
- Liver Weight 329 g [21].

Filtration parameter to model the renal clearance:

- GFR 61.3 ml/min [22].

Human parameters

| Fluxes [ml/min], [21,22] | | Volumes [ml], [21,22,23] | |
|---------------------------------|------|---------------------------------|-------|
| Q_{brain} | 745 | V_{brain} | 1400 |
| Q_{gut} | 1046 | V_{gut} | 1155 |
| Q_{spleen} | 160 | V_{spleen} | 182 |
| Q_{liver} | 1578 | V_{liver} | 1799 |
| Q_{muscle} | 1055 | V_{muscle} | 28000 |
| $Q_{adipose}$ | 310 | $V_{adipose}$ | 14994 |
| Q_{heart} | 248 | V_{heart} | 329 |
| Q_{kidney} | 1179 | V_{kidney} | 308 |
| $Q_{Restofthebody}$ | 1308 | $V_{Restofthebody}$ | 10801 |
| Cardiac Output | 6204 | V_{lung} | 532 |
| | | V_{ven} | 3900 |
| | | V_{art} | 1300 |

| Gastrointestinal absorption model parameters | |
|---|------|
| Volumes of intestinal segments [ml], [25] | |
| $V_{stomach}$ | 50 |
| V_1 | 105 |
| V_2 | 110 |
| V_3 | 110 |
| V_4 | 69 |
| V_5 | 69 |
| V_6 | 69 |
| V_7 | 69 |
| V_{colon} | 1000 |
| pH of intestinal segments | |
| $pH_{stomach}$ | 2 |
| pH_1 | 6 |
| pH_2 | 6.2 |
| pH_3 | 6.6 |
| pH_4 | 6.8 |
| pH_5 | 7 |
| pH_6 | 7.2 |
| pH_7 | 7.4 |
| pH_{colon} | 7 |

| MRT values | |
|------------------------|-----------|
| <i>Stomach</i> | 30 min |
| <i>Small intestine</i> | 199.2 min |
| <i>Colon</i> | 11 h |

Information available on human tissue composition, [35]

| Human tissues | <i>Volume fraction of Phospholipids, Vph</i> | <i>Volume fraction of Neutral lipids, Vnl</i> | <i>Volume fraction of Water, Vw</i> |
|----------------------|--|---|-------------------------------------|
| <i>Adipose</i> | 0.002 | 0.79 | 0.18 |
| <i>Bone</i> | 0.0011 | 0.074 | 0.439 |
| <i>Brain</i> | 0.0565 | 0.051 | 0.77 |
| <i>Gut</i> | 0.0163 | 0.0487 | 0.718 |
| <i>Heart</i> | 0.0166 | 0.0115 | 0.758 |
| <i>Kidney</i> | 0.0162 | 0.0207 | 0.783 |
| <i>Liver</i> | 0.0252 | 0.0348 | 0.751 |
| <i>Lung</i> | 0.009 | 0.003 | 0.811 |
| <i>Muscle</i> | 0.0072 | 0.0238 | 0.76 |
| <i>Skin</i> | 0.0111 | 0.0284 | 0.718 |
| <i>Spleen</i> | 0.0198 | 0.0201 | 0.788 |
| <i>Plasma</i> | 0.00225 | 0.0035 | 0.945 |
| <i>Erythrocytes</i> | - | - | - |

Tracheobronchial surface, S_{TB} : 8990 cm² [14].

Haematocrit: 0.44 [22].

Conversion factor to obtain the *in vivo* estimates of the hepatic clearance:

- MGPPGL: 32 mg/g [S1],
- HPGL: $99 \cdot 10^6$ cells/g [S1],
- Liver Weight: 1799 g [21].

Filtration parameter to model the renal clearance:

- GFR 125 ml/min [22].

Drug-related parameters relationships

In this section the equations to calculate the drug-specific parameters are reported.

Absorption

The Henderson-Hasselbalch equations to calculate the solubility at a certain pH are here reported

Monoprotic acids

$$C_{spH} = S_{int}(1 + 10^{(pH - pKa1)}) \quad (s1)$$

Monoprotic bases

$$C_{spH} = S_{int}(1 + 10^{(-pH + pKa1)}) \quad (s2)$$

Diprotic acids

$$C_{spH} = S_{int} (1 + 10^{(-pH + pKa1)} + 10^{(2pH - pKa1 - pKa2)}) \quad (s3)$$

Diprotic bases

$$C_{spH} = S_{int} (1 + 10^{(-pH + pKa1)} + 10^{(-2pH + pKa1 + pKa2)}) \quad (s4)$$

Neutrals

$$C_{spH} = S_{int} \quad (s5)$$

Zwitterions

$$C_{spH} = S_{int}(1 + 10^{(-pH + pKaA)} + 10^{(pH - pKaB)}) \quad (s6)$$

where pKaA is the acidic pKa and pKaB is the basic pKa.

Partition coefficients

This subsection contains the equations needed to calculate $P_{T:B}$ values with the method of Poulin [35,36] and of Rodgers [37,38]. For the latter the equations for each chemical species are reported.

Poulin's Method

The fractional volumes of phospholipids (V_{ph}), neutral lipids (V_{nl}) and water (V_w), required to apply the method, are reported in the *species-specific parameters* section. In the following P indicates plasma and T tissue.

$$Pow = 10^{\log P}$$

$$Dow = 10^{\log D}$$

$$f_{uT} = 1 / (1 + (1 - f_{uP}) / f_{uP} 0.5)$$

For non-adipose tissues

$$P_{T:p} = [(Pow(V_{nlT} + 0.3V_{phT}) + (V_{wT} + 0.7V_{phT}))] / [Pow(V_{nlp} + 0.3V_{php}) + (V_{wp} + 0.7V_{php})] (f_{uP} / f_{uT}) \quad (s7)$$

For adipose tissues

$$P_{T:p} = [(Dow(V_{nlT} + 0.3V_{phT}) + (V_{wT} + 0.7V_{phT}))] / [Dow(V_{nlp} + 0.3V_{php}) + (V_{wp} + 0.7V_{php})] f_{uP} \quad (s8)$$

To obtain the values of $P_{T:B}$ from the $P_{T:P}$, the tissue to plasma partition coefficient, can be applied the following equation:

$$P_{T:B} = P_{T:p} / BP \quad (s9)$$

Distribution

Rodger's Method

The volumes related to tissues composition in terms of neutral lipids (nl), neutral phospholipids (nph), extracellular water (ew), intracellular water (iw), the ratios such as the lipoprotein ratio (lr), the albumin ratio (ar) and the tissue concentration of acidic phospholipids (ap) are reported in the *species-specific parameters* section. In the notation, T indicates the tissue and B the blood. The values of pH_p, pH_w and pH_{bc} are fixed, as reported by the authors, to 7.4, 7 and 7.22 respectively. The values for fNL_p and fNP_p are fixed as 0.0023 and 0.0013 respectively, as reported in the paper. For all tissues, except adipose ones, the value P in the subsequent equations is the n-octanol:water partition coefficient (here reported as P1); for the adipose tissues the vegetable oil:water partition coefficient was deemed more appropriate (here indicated as P2). To obtain the value of P_{T:B} from the K_{pu} (tissue to plasma unbound partition coefficient) the following equation can be applied:

$$P_{T:B} = K_{pu} f_{up} / BP \quad (s10)$$

$$P1 = 10^{\log P} \quad (s11)$$

$$\log P_{veg} = 1.115 \log P - 1.35 \quad (s12)$$

$$P2 = 10^{\log P_{veg}} \quad (s13)$$

Acids

$$X = 1 + 10^{(pH_{iw} - pK_a)}$$

$$Y = 1 + 10^{(pH_p - pK_a)}$$

$$K_{puT} = ew_T + X iw_T / Y + ((P nl_T + (0.3 P + 0.7) nph_T) / Y) + (1 / f_{up} - 1 - (P fNL_p + (0.3 P + 0.7) fNP_p) / Y) ar_T \quad (s14)$$

Diprotic acids

In this equations $pK_{a1} < pK_{a2}$

$$X = 1 + 10^{(pH_{iw} - pK_{a1})} + 10^{(-pK_{a2} - pK_{a1} + 2pH_{iw})}$$

$$Y = 1 + 10^{(pH_p - pK_{a1})} + 10^{(-pK_{a2} - pK_{a1} + 2pH_p)}$$

$$K_{puT} = ew_T + X iw_T / Y + ((P nl_T + (0.3 P + 0.7) nph_T) / Y) + (1 / f_{up} - 1 - (P fNL_p + (0.3 P + 0.7) fNP_p) / Y) ar_T \quad (s15)$$

Bases

$$X = 1 + 10^{(pK_a - pH_{iw})}$$

$$Y = 1 + 10^{(pK_a - pH_p)}$$

$$X1 = 1 + 10^{(pK_a - pH_{bc})}$$

$$Y1 = 1 + 10^{(pK_a - pH_p)}$$

$$X2 = 10^{(pK_a - pH_{bc})}$$

$$K_{puBC} = (BP - 1 + haematocrit) / haematocrit / f_{up}$$

$$KaAP = (K_{puBC} - (X1 / Y1 iw_b) - (P nl_b + (0.3 P + 0.7) nph_b) / Y1) (Y1 / ap_b / X2)$$

$$K_{puT} = ew_T + X iw_T / Y + (P nl_T + (0.3 P + 0.7) nph_T) / Y + (KaAP ap_T (X - 1)) / Y \quad (s16)$$

Very weak bases

$$X = 1 + 10^{(pK_a - pH_{iw})}$$

$$Y = 1 + 10^{(pK_a - pH_p)}$$

$$K_{puT} = ew_T + X iw_T / Y + ((P nl_T + (0.3 P + 0.7) nph_T) / Y) + (1 / f_{up} - 1 - (P fNL_p + (0.3 P + 0.7) fNP_p) / Y) ar_T \quad (s17)$$

Diprotic bases

In these equations $pK_{a1} < pK_{a2}$

$$X = 1 + 10^{(pK_{a2} - pH_{iw})} + 10^{(pK_{a2} + pK_{a1} - 2 pH_{iw})}$$

$$Y = 1 + 10^{(pK_{a2} - pH_p)} + 10^{(pK_{a2} + pK_{a1} - 2 pH_p)}$$

$$X_1 = 1 + 10^{(pK_{a2} - pH_{bc})} + 10^{(pK_{a2} + pK_{a1} - 2 pH_{bc})}$$

$$X_2 = 10^{(pK_{a2} - pH_{bc})} + 10^{(pK_{a2} + pK_{a1} - 2 pH_{bc})}$$

$$Y_1 = 1 + 10^{(pK_{a2} - pH_p)} + 10^{(pK_{a2} + pK_{a1} - 2 pH_p)}$$

$$K_{puBC} = (BP - 1 + \text{haematocrit}) / \text{haematocrit} / f_{up}$$

$$K_{aAP} = (K_{puBC} - (X_1/Y_1) i_{w_b}) - ((P n_{l_b} + (0.3 P + 0.7) n_{ph_b}) / Y_1) (Y_1 / a_{p_b} / X_2)$$

$$K_{puT} = e_{w_T} + X i_{w_T} / Y + (P n_{l_T} + (0.3 P + 0.7) n_{ph_T}) / Y + (K_{aAP} a_{p_T} (X - 1)) / Y \quad (s18)$$

Very weak diprotic bases

In these equations $pK_{a1} < pK_{a2}$

$$X = 1 + 10^{(pK_{a2} - pH_{iw})} + 10^{(pK_{a2} + pK_{a1} - 2 pH_{iw})}$$

$$Y = 1 + 10^{(pK_{a2} - pH_p)} + 10^{(pK_{a2} + pK_{a1} - 2 pH_p)}$$

$$K_{puT} = e_{w_T} + X i_{w_T} / Y + ((P n_{l_T} + (0.3 P + 0.7) n_{ph_T}) / Y) + (1 / f_{up} - 1 - (P f_{NLp} + (0.3 P + 0.7) f_{NPP}) / Y) a_{r_T} \quad (s19)$$

Neutrals

$$X = 1$$

$$Y = 1$$

$$K_{puT} = X i_{w_T} / Y + e_{w_T} + ((P n_{l_T} + (0.3 P + 0.7) n_{ph_T}) / Y) + (1 / f_{up} - 1 - (P f_{NLp} + (0.3 P + 0.7) f_{NPP}) / Y) l_{r_T} \quad (s20)$$

Zwitterions, with at least one basic $pK_{a} > 7$

$$X = 1 + 10^{(pK_{aB} - pH_{iw})} + 10^{(pH_{iw} - pK_{aA})}$$

$$Y = 1 + 10^{(pK_{aB} - pH_p)} + 10^{(pH_p - pK_{aA})}$$

$$X_1 = 1 + 10^{(pK_{aB} - pH_{bc})} + 10^{(pH_{bc} - pK_{aA})}$$

$$Y_1 = 1 + 10^{(pK_{aB} - pH_p)} + 10^{(pH_p - pK_{aA})}$$

$$X_2 = 10^{(pK_{aB} - pH_{bc})} + 10^{(pH_{bc} - pK_{aA})}$$

$$K_{puBC} = (BP - 1 + \text{haematocrit}) / \text{haematocrit} / f_{up};$$

$$K_{aAP} = (K_{puBC} - (X_1/Y_1) i_{w_b}) - (P n_{l_b} + (0.3 P + 0.7) n_{ph_b}) / Y_1 (Y_1 / a_{p_b} / X_2)$$

$$K_{puT} = e_{w_T} + X i_{w_T} / Y + (P n_{l_T} + (0.3 P + 0.7) n_{ph_T}) / Y + ((K_{aAP} a_{p_T} 10^{(pK_{aB} - pH_{iw})} + 10^{(pH_{iw} - pK_{aA})}) / Y) \quad (s21)$$

All other zwitterions

$$X = 1 + 10^{(pK_{aB} - pH_{iw})} + 10^{(pH_{iw} - pK_{aA})}$$

$$Y = 1 + 10^{(pK_{aB} - pH_p)} + 10^{(pH_p - pK_{aA})}$$

$$K_{puT} = e_{w_T} + X i_{w_T} / Y + ((P n_{l_T} + (0.3 P + 0.7) n_{ph_T}) / Y) + (1 / f_{up} - 1 - (P f_{NLp} + (0.3 P + 0.7) f_{NPP}) / Y) a_{r_T} \quad (s22)$$

Metabolism and Elimination

The equations to apply the "Q_{gut}" model [33], with the related scaling factors, to obtain F_{GUT} in humans from measurement of *in vitro* intrinsic clearance from HLM, for CYP3A metabolizers are here reported. The fraction of drug escaping the first pass metabolism can be calculated as follows:

$$F_{Gut} = Q_{villi} / (Q_{villi} + f_{uGUT} CL_{uint,GUT} (1 + Q_{villi} / CL_{perm})) \quad (s23)$$

where Q_{villi} is the intestinal villi blood flow that for humans is 300 ml/min; f_{UGUT} is the unbound drug fraction in gut, if not available can be supposed equal to 1; $CL_{\text{uint,GUT}}$ is the net metabolic intrinsic clearance based on the unbound drug concentration, this last term can be obtained from the HLM as follows:

$$CL_{\text{uint,GUT}} = (CL_{\text{uint}} / \text{PEMP}) \text{NEWI} \quad (\text{s24})$$

where CL_{uint} is the unbound hepatic intrinsic clearance obtained from HLM and expressed in microliter/minute/milligram of protein, PEMP is the Picomol of CYP3A Enzymes for Milligram of Protein that is 155 picomol/milligram of protein, NEWI is the value of Nanomol of Enzyme for the Whole Intestine that is 70.5 nanomol [33].

The value of CL_{perm} , can be obtained as:

$$CL_{\text{perm}} = \text{Peff}_{\text{human}} A \quad (\text{s25})$$

where A is the area of the intestine, for humans 6600 cm² obtained supposing a radius of 1.75 cm and a length of 6 m [33].

Additional References

[s1] Zoe E. Barter, Martin K. Bayliss, Philip H. Beaune, Alan R. Boobis, David J. Carlile, Robert J. Edwards, J. Brian Houston, Brian G. Lake, John C. Lipscomb, Olavi R. Pelkonen, Geoffrey T. Tucker¹ and Amin Rostami-Hodjegan. Scaling Factors for the Extrapolation of In Vivo Metabolic Drug Clearance From In Vitro Data: Reaching a Consensus on Values of Human Microsomal Protein and Hepatocellularity Per Gram of Liver. *Current Drug Metabolism* **8** (2007) 33-45.