

**Minimal physiologically based pharmacokinetic-pharmacodynamic (mPBPK-PD)
model of GalNAc-conjugated siRNA disposition and gene silencing in preclinical
species and humans**

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Appendix S1. Supplemental Figures S1 – S3.

Figure S1. (a) Schematic for the receptor-mediated endocytosis of GalNAc-IL-2 in mice primary hepatocytes and (b and c) model-based characterization of *in vitro* data from pulse-chase experiments (Sato et al., 2002). In (a), free drug, free receptor, bound complex, and internalized complex are represented in the model, and binding and internalization processes are indicated by arrow lines. In (b) and (c), lines represent model fitted profiles whereas symbols are observed data [legend in (b)].

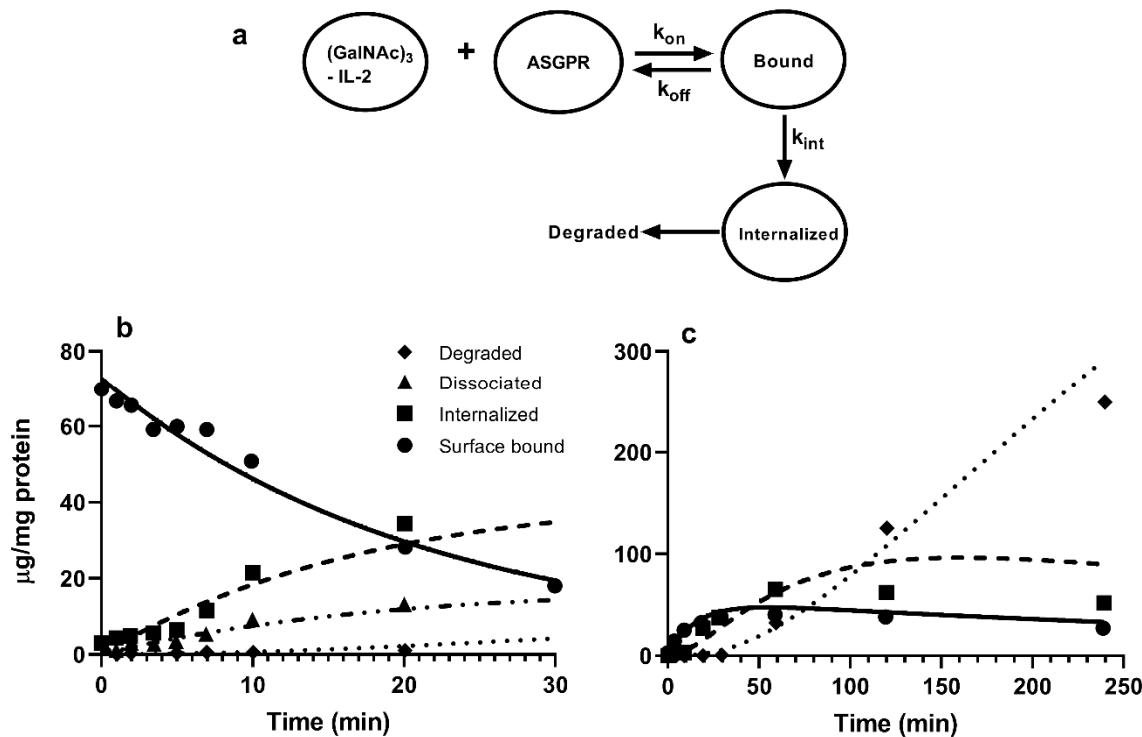


Figure S2. Model-based characterization of (a-c) plasma PK and (d) total liver PK following a single dose of ALN-AT3SC in mice. Solid lines and dots represent model fitted profiles as well as observed data. Dosing routes and levels are indicated by figure legends.

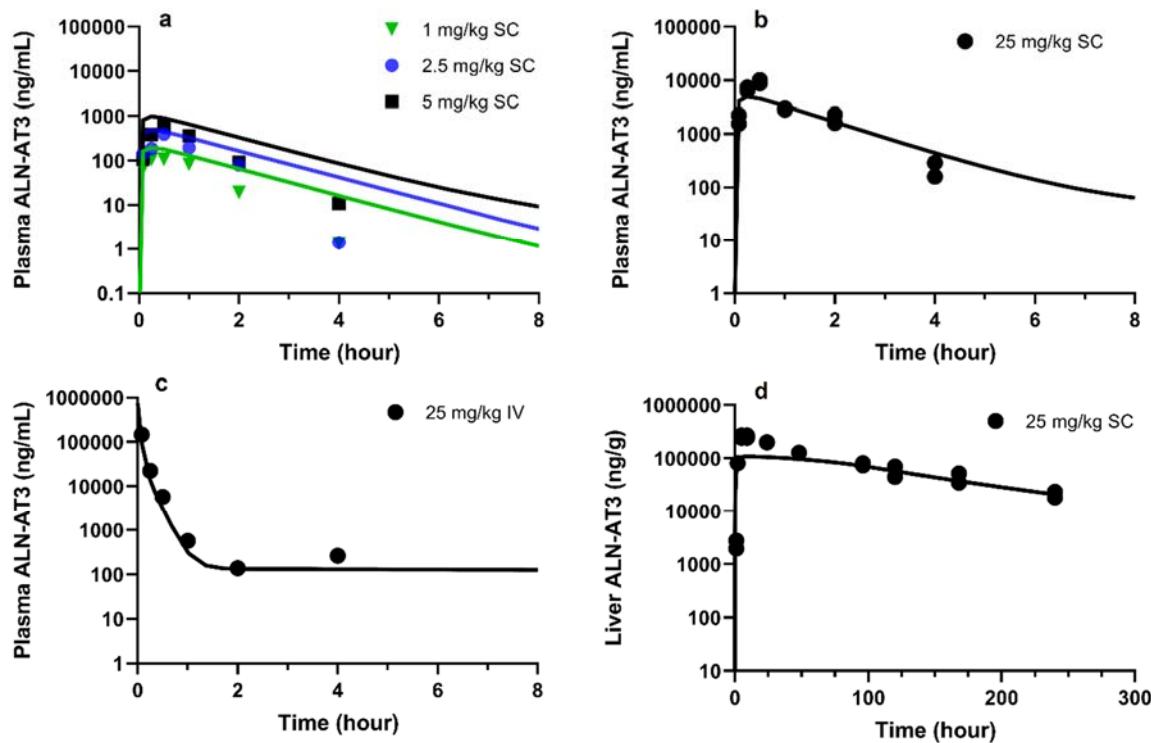
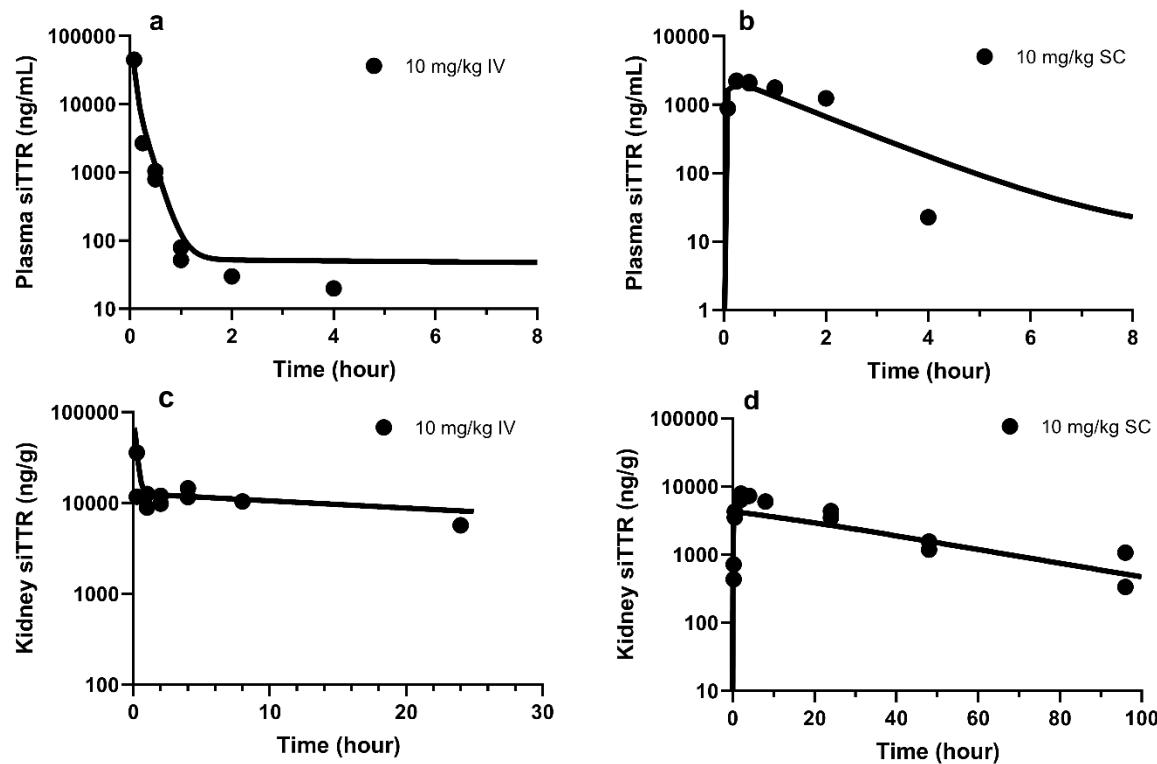


Figure S3. Model-based characterization of (a-b) plasma PK and (d) total kidney PK following a 10 mg/kg SC/IV single dose of siTTR-2 in mice. Solid lines and dots represent model fitted profiles as well as observed data. Dosing routes and levels are indicated by figure legends.



Appendix S2. Supplemental Tables S1 – S3.**Table S1.** Glossary of the state variables used in the model.

Model variable	Description	Unit
C_p	Drug concentration in plasma	ng/mL
C_{rest}	Drug concentration in the remaining organs	ng/mL
$C_{k,vas}$	Drug concentration in the kidney vasculature	ng/mL
C_k	Drug concentration in the kidney tissue	ng/mL
$C_{l,vas}$	Drug concentration in the liver vasculature	ng/mL
$A_{deep,k}$	Drug amount in kidney bound pool	ng
C_{lisf}	Free GalNAc-siRNA concentration in liver interstitial fluid (ISF)	nM
$A_{deep,l}$	Drug amount in liver bound pool	nmol
R_f and BR	Free (unbound) and bound ASGPR concentrations in liver	nM
F_{vol}	Volume correction factor for interaction at endosome and ISF interface	-
$R_{f,endo}$ and BR_{endo}	Free (unbound) ASGPR and bound ASGPR in liver endosome	nM
$C_{f,endo}$ and $C_{f,cytoplasm}$	Free (unbound) siRNA in liver endosome and in cytoplasm	nM
B_RISC	RISC-loaded siRNA in the liver	nM
$RISC_{tot}$	Total RISC concentration in hepatocytes	nM
$mRNA$ and $mRNA_0$	Relative target mRNA over time and when $t = 0$	%
$Protein$ and $Protein_0$	Relative target protein over time and when $t = 0$	%

Table S2. Physiological parameter values used in mice, rats, monkeys, and humans.

Parameter	Description	Unit	Mice (28 g)	Rat (280 g)	NHP (6.2 kg)	Human (71 kg)
Q_{rest}	Plasma flow to remainder tissue	mL/h	204	1761	10358	97677
V_{rest}	Extracellular volume of remainder tissue	mL	5.1	52.6	1087	12394
V_p	Plasma volume in circulation	mL	0.94	9.1	187	3126
Q_{kidey}	Plasma flow to kidney	mL/h	68.5	365	3237	36402
V_k	Interstitial and cellular volume of kidney	mL	0.47	2.2	24.4	296
V_{k,vas}	Plasma volume in kidney vasculature	mL	0.029	0.132	1.5	18.2
Q_{liver}	Plasma flow to liver	mL/h	100	820	8838	47844
V_{l,c}	Cellular volume of liver	mL	1.23	10.7	120	1371
V_{l,isf}	Interstitial volume of liver	mL	0.39	2.6	37.4	429
V_{l,vas}	Plasma volume in liver vasculature	mL	0.16	1.3	15.9	183
GFR	Glomerular filtration rate	mL/h	13.8	168	1138	7200
f_u	Plasma free fraction of GalNAc-siRNA	-	0.15	0.15	0.15	0.15

Table S3. Model parameter values and approach for mPBPK-PD based scale-up from mice to rats, monkeys, and humans.

Parameter	Description	Mouse	Rat	Monkey	Human
R_{tot} (nM)	Baseline ASGPR density in mouse liver	633	513 ^{a,b}	339 ^{b,c}	340 ^b
k_{deg} (h ⁻¹)	Degradation rate constant for ASGPR	0.04			
k_{syn} (nM/h)	Synthesis rate for ASGPR	27.9	Calculated ($k_{syn} = R_{tot} \cdot k_{deg}$)		
k_{off} (h ⁻¹)	Dissociation rate constant for GalNAc to ASGPR	1.32			
k_{on} (nM ⁻¹ ·h ⁻¹)	Association rate constant for GalNAc to ASGPR	0.53			
k_{int} (h ⁻¹)	Internalization rate constant for bound ASGPR	2.4	- 0.25 ^d		
k_{rec} (h ⁻¹)	ASGPR recycling constant	13.8			
$k_{deg,R}$ (h ⁻¹)	Degradation rate constant for endosomal ASGPR	1.53			
k_{cle} (h ⁻¹)	Rate constant for GalNAc cleavage from siRNA	1.32			
k_a (h ⁻¹)	SC absorption rate constant	0.7	0.54 ^e	0.35 ^e	0.06 (5.5)
F (%)	SC bioavailability	33	50 ^e	33 ^f	28 ^g
$f_{u,p}$	Plasma fraction unbound	0.15			
PS (mL/h)	Kidney permeability flow rate	49.6	0.75 ^d		
$f_{u,k}$	Kidney fraction unbound	0.07			
$k_{ass,kid}$ (h ⁻¹)	Kidney association rate constant for binding	9.9			
$k_{dis,kid}$ (h ⁻¹)	Kidney dissociation rate constant for binding	0.026			
CL_{up,liv_in} (mL/h)	Liver uptake clearance	207	0.75 ^d		
CL_{up,liv_eff} (mL/h)	Liver release clearance	0.0025	0.75 ^d		
$k_{ass,liv}$ (h ⁻¹)	Liver association rate constant for binding	0.0032			
$k_{dis,liv}$ (h ⁻¹)	Liver dissociation rate constant for binding	0.0034			
$K_{P,rest}$	Partition coefficient for remaining organs	0.1			
f_{esc}	Fraction siRNA in endosome escaping into cytosol	0.01			
$k_{deg,d}$ (h ⁻¹)	Rate constant for endosomal siRNA degradation	0.012	0.012 ^f	- 0.25 ^d	
$k_{on,RISC}$ (nM ⁻¹ ·h ⁻¹)	Association rate constant of siRNA to RISC	0.00023	0.00016 (15) ^h	0.00023 ^f	
$k_{off,RISC}$ (h ⁻¹)	Dissociation rate constant of siRNA from RISC	10 ⁻⁷			
$RISC_{tot}$ (nM)	Total RISC concentration in hepatocytes	30			
k_{DR} (h ⁻¹)	Degradation rate constant for loaded RISC	0.005	0.008 ^h	0.005 ^f	0.0007 ^g
$k_{deg,c}$ (h ⁻¹)	Degradation rate constant for free cytosolic siRNA	0.1			
$k_{deg,m}$ (h ⁻¹)	Degradation rate constant for AT mRNA	0.06	NA	0.06 ^f	0.04 ⁱ
$k_{deg,p}$ (h ⁻¹)	Degradation rate constant for AT protein	0.05	NA	0.01 (9.0)	0.0096 ^j
S_{max}	Maximal stimulation of target mRNA degradation	14.2	NA	14.2	14.2
SC_{50} (nM)	Bound RISC at half maximal effect	2.4	NA	5.3 (9.6)	3.4 (7.4)

γ	Gamma coefficient for mRNA-protein translation	1.5	NA	1.5	1.5
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^a Total ASGPR density calculated based on 1.8 million receptors/cell (assumed same as mice; see Appendix S4).

^b Corrected for HPPGL and liver weight.

^c Total ASGPR density calculated based on 1.1 million receptors/cell (assumed same as human; see Appendix S4).

^d Estimate in mice scaled using species body weight and listed allometric exponent.

^e Calibrated using plasma PK for undisclosed siRNA (not shown).

^f Assumed same as mice.

^g Local sensitivity analysis for lowest OFV.

^h Parameter value specific to givosiran.

ⁱ Calculated (Yang et al., 2003).

^j Calculated (Menache et al., 1990).

Note: Values listed within parentheses reflect % CV of estimate.

Appendix S3. Model Differential Equations.

Plasma (ng/mL)

$$\frac{dC_p}{dt} = \frac{\text{Input}}{V_p} - (Q_{rest} + Q_{liver} + Q_{kidney}) \cdot \frac{C_p}{V_p} + Q_{rest} \cdot \frac{C_{rest}}{K_{p,rest} \cdot V_p} + Q_{liver} \cdot \frac{C_{l,vas}}{V_p} + Q_{kidney} \cdot \frac{C_{k,vas}}{V_p} \quad (1)$$

Input = Dose (IV) or $ka \cdot Dose \cdot F (SC)$

Remainder (ng/mL)

$$\frac{dC_{rest}}{dt} = \frac{Q_{rest} \cdot C_p - Q_{rest} \cdot \frac{C_{rest}}{K_{p,rest}}}{V_{rest}} \quad (2)$$

Kidney - vascular (ng/mL)

$$\frac{dC_{k,vas}}{dt} = \frac{(Q_{kidney} \cdot (C_p - C_{k,vas}) - PS \cdot (f_u \cdot C_{k,vas} - f_{u,k} \cdot C_k) - GFR \cdot f_u \cdot C_{k,vas})}{V_{k,vas}} \quad (3)$$

Kidney - tissue (ng/g)

$$\frac{dC_k}{dt} = PS \cdot \frac{(f_u \cdot C_{k,vas} - f_{u,k} \cdot C_k)}{V_k} - k_{ass,k} \cdot f_{u,k} \cdot C_k + k_{dis,k} \cdot \frac{A_{deep,k}}{V_k} \quad (4)$$

Kidney - bound pool (ng)

$$\frac{dA_{deep,k}}{dt} = k_{ass,k} \cdot f_{u,k} \cdot C_k \cdot V_k - k_{dis,k} \cdot A_{deep,k} \quad (5)$$

Liver - vascular (ng/mL)

$$\frac{dC_{l,vas}}{dt} = \frac{Q_{liver} \cdot (C_p - C_{l,vas}) - (CL_{up,liv_{in}} \cdot f_u \cdot C_{l,vas} - CL_{up,liv_{eff}} \cdot C_{l,ISF} \cdot \frac{MW}{1000})}{V_{l,vas}} \quad (6)$$

Where $MW = 15000 \frac{g}{mol}$

Liver - interstitial fluid (nM)

$$\begin{aligned} \frac{dC_{l,ISF}}{dt} = & \frac{CL_{up,liv_{in}} \cdot f_u \cdot \frac{C_{l,vas}}{MW \cdot 1000} - CL_{up,liv_{eff}} \cdot \frac{C_{l,ISF}}{1000}}{V_{l,ISF}} - k_{on} \cdot C_{l,ISF} \cdot R_f + k_{off} \cdot BR \quad (\text{contd.}) \\ & - k_{ass,l} \cdot C_{l,ISF} + k_{dis,l} \cdot \frac{A_{deep,k}}{V_{l,ISF}} \end{aligned} \quad (7)$$

Note: $V_{l,ISF}$ in L

Liver – bound pool (nmol)

$$\frac{dA_{deep,l}}{dt} = k_{ass,l} \cdot C_{l,isf} \cdot V_{l,isf} - k_{dis,l} \cdot A_{deep,l} \quad (8)$$

Note: $V_{l,isf}$ in L

Liver - free ASGPR (nM)

$$\frac{dR_f}{dt} = k_{syn} - k_{deg} \cdot R_f - k_{on} \cdot C_{l,ISF} \times R_f + k_{off} \cdot BR + k_{recycle} \cdot R_{f,endo} \cdot F_{vol} \quad (9)$$

where $F_{vol} = V_{l,c} \cdot \frac{0.8}{V_{l,isf}}$

Liver - bound ASGPR (nM)

$$\frac{dB_R}{dt} = k_{on} \cdot C_{l,ISF} \cdot R_f - k_{off} \cdot BR - k_{int} \cdot BR \quad (10)$$

Liver - bound endosomal ASGPR (nM)

$$\frac{dBR_{endo}}{dt} = k_{int} \cdot BR/F_{vol} - k_{cle} \cdot BR_{endo} \quad (11)$$

Liver - free endosomal ASGPR (nM)

$$\frac{dR_{f,endo}}{dt} = k_{cle} \cdot BR_{endo} - k_{rec} \cdot R_{f,endo} - k_{deg,R} \cdot R_{f,endo} \quad (12)$$

Liver - free endosomal siRNA (nM)

$$\frac{dC_{f,endo}}{dt} = k_{cle} \cdot BR_{endo} - k_{esc} \cdot C_{f,endo} - k_{deg,D} \cdot C_{f,endo} \quad (13)$$

Liver - free cytoplasmic siRNA (nM)

$$\begin{aligned} \frac{dC_{f,cytoplasm}}{dt} = & f_{esc} \cdot k_{deg,D} \cdot C_{f,endo} - k_{deg,C} \cdot C_{f,cytoplasm} - k_{on,RISC} \cdot C_{f,cytoplasm} \cdot (RISC_{tot} - B_RISC) \\ & + k_{off,RISC} \cdot B_{RISC} \end{aligned} \quad (14)$$

Liver – siRNA bound to RISC (nM)

$$\frac{dB_RISC}{dt} = k_{on,RISC} \cdot C_{f,cytoplasm} \cdot (RISC_{tot} - B_RISC) - k_{off,RISC} \cdot B_RISC - k_{DR} \cdot B_RISC \quad (15)$$

Liver - target mRNA knockdown (%)

$$k_{syn,mRNA} = k_{deg,mRNA} \cdot mRNA_0 \quad (16)$$

$$\frac{dmRNA}{dt} = k_{syn,mRNA} - k_{deg,mRNA} \cdot \left(1 + \frac{S_{max} \cdot B_RISC}{SC_{50} + B_RISC}\right) \cdot mRNA \quad (17)$$

Liver - target protein knockdown (%)

$$k_{syn,protein} = k_{deg,protein} \cdot Protein_0 \quad (18)$$

$$\frac{dProtein}{dt} = k_{syn,protein} \cdot \left(\frac{mRNA}{mRNA_0}\right)^{\gamma} - k_{deg,protein} \cdot Protein \quad (19)$$

Output – Plasma concentration (ng/mL)

$$Output_{plasma} = C_p \quad (20)$$

Output – Kidney concentration (ng/g, perfused tissue)

$$Output_{kidney} = (C_k \cdot V_k + A_{deep,k}) / (V_{k,vas} + V_k + V_{k,BC,vas}) \quad (21)$$

$V_{k,BC,vas}$ is the blood cell volume in kidney vasculature

Output – Liver concentration (ng/g, perfused tissue)

$$\begin{aligned} Output_{Liver} \\ &= (((C_{l,isf} + BR) \cdot V_{l,isf} / 1000 + (BR_{endo} + C_{f,endo} + C_{f,cytoplasm}) \cdot V_{l,c} \cdot \frac{0.8}{1000} + A_{deep,l}) \cdot MW) / (V_{l,isf} + V_{l,c} \\ &\quad + V_{l,vas} + V_{l,BC,vas}) \end{aligned} \quad (22)$$

$V_{l,BC,vas}$ is the blood cell volume in liver vasculature

Assuming hepatocytes account for 80% cells in liver

Output – RISC-bound siRNA (ng/g)

$$Output_{RISC_bound_siRNA} = B_{RISC} \cdot 6.65 \quad (21)$$

Assumes a MW of 13.3 kDa for double-stranded siRNA (cleaved from GalNAc) and 6.65 kDa for RISC-bound antisense strand.

Appendix S4. Calculation of liver ASGPR density and interspecies scaling.

$$\text{Liver ASGPR density} = \frac{\frac{\text{receptors}}{\text{cell}} \times \text{HPPGL} \left(\frac{\text{cells}}{\text{g}} \right) \times \text{liver wt. (g)} \times 10^9}{\text{avogadro's constant} \times \text{cell vol. of liver (L)}}$$

Receptors per cell taken from (Bon et al., 2017) for mice (assumed same in rats) and from (Miki et al., 2001) for humans (assumed same in cyno monkeys). HPPGL represents hepatocytes per gram of liver, which is 135 million in mice, 117 million in rats, and 120 million in monkey and humans (Sohlenius-Sternbeck, 2006). Liver weights (wt.) and cellular volume (vol.) of liver for each species is listed in Table S2 of Appendix S2.

References

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