Supporting Information

A computational framework for interspecies pharmacokinetics, exposure and toxicity assessment of gold nanoparticles

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1. Supplementary tables

Table 1. Pharmacokinetic studies used in the PBPK model calibration and evaluation.

Animal	Purpose	Sizes and coatings of AuNP	Administration method and dosage	Selected time points	Selected organs/tissues	Detection method	Reference
Adult mice	Calibration	13 nm; PEG	IV: 0.85 mg/kg	0.5, 4, 24 h, 7 days	Plasma, liver, spleen, kidneys, lungs	ICP-MS	Cho et al. (2010) [1]
Adult mice	Evaluation	13 nm; PEG	IV: 0.85 mg/kg	5, 30 min, 4, 24 h, 7 days	Plasma, liver, spleen	ICP-MS	Cho et al. (2009) [2]
Adult rats	Calibration (medium dose)	18.4 nm; citrate	IV: 0.6-1 mg/kg	0.5, 2, 6, 24 h	Blood, liver, spleen, kidneys, lungs	GFAAS	Morais et al. (2012) [3]
Adult rats	Evaluation (medium dose)	16.1 nm; citrate	IV: ~0.7 mg/kg	0.5 h, 28 days	Liver, spleen	GFAAS	Fraga et al. (2014) [4]
Adult rats	Calibration (low dose)	20 nm; NA	IV: 0.01 mg/kg	24 h, 7 days, 1, 2 months	Blood, liver, spleen, kidneys, lungs	ICP-MS	Balasubramanian et al. (2010) [5]
Juvenile swine	Calibration	15-20 nm; gum arabic	IV: 2 mg/kg	0.5, 1, 2, 4 h, 1, 3, 5, 7, 32 days	Plasma, liver, spleen, kidneys, lungs, urine	AAS	Fent et al. (2009) [6]
Juvenile swine	Evaluation	15-20 nm; gum arabic	IV: 0.8-1.88 mg/kg	0.5, 1, 2, 4, 24 h	Liver, spleen,	AAS and NAA	Kattumuri et al. (2007) [7]
Adult humans	Species extrapolation and evaluation	27 nm; PEG, rhTNF	IV: 0.001286-0.01726* mg/kg	5, 15, 30 min, 1, 2, 3, 4, 8, 24 h	Blood	ELISA	Libutti et al. (2010) [8]

Note: AAS: atomic absorption spectrometry; AuNP: gold nanoparticles; ELISA: enzyme-linked immunosorbent assay; GFAAS: graphite furnace atomic absorption spectrometry; ICP-MS: inductively coupled plasma mass spectrometry; IV: intravenous; NAA: neutron activation analysis. PBPK: physiologically based pharmacokinetic; PEG: polyethylene glycol; rhTNF: recombinant human tumor necrosis factor alpha; *: doses were calculated based on the injected amount (0.09-1.208 mg) and assuming the body weight of an adult human is 70 kg.

Table 2. Physiological parameters used in the PBPK model for gold nanoparticles in mice, rats, pigs, and humans.

Parameter	Symbol	Mouse	Rat	Pig	Human	Source
Body weight (kg)	BW	0.02	0.25	9.6-21	70	a, b, e, f
Cardiac output ($L/h/kg^{0.75}$)	QCC	16.5	15	5^	16.5	b, d, g
Blood flow to organ (fraction of cardiac output, unitless)						
Liver	QLC	0.161	0.183	0.2725	0.227	b, c, d, i
Spleen	QSC	0.011	0.0085	0.0151*	0.01375	a
Kidneys	QKC	0.091	0.141	0.12	0.175	b, c, d, i
Lungs	QLuC	1	1	1	1	b
Brain	QBRC	0.033	0.02	0.03	0.114	b, d
Rest of body	QrestC	0.704	0.6475	0.5624	0.47025	Calculated
Organ volumes (fraction of body weight, unitless)						
Liver	VLC	0.055	0.0366	0.0247	0.0257	b, c, d, i
Spleen	VSC	0.005	0.002	0.002	0.00257	a, b, d
Kidneys	VKC	0.017	0.0073	0.004	0.0044	b, c, d, i
Lungs	VLuC	0.007	0.005	0.01	0.008	b, d
Brain	VBRC	0.017	0.0057	0.004	0.02	b, d
Rest of body	VrestC	0.85	0.8694	0.8953	0.93933	Calculated
Blood	VBloodC	0.049	0.074	0.06	0.079	b, c, d, i
Plasma	VPlasmaC	0.029	0.047	0.04	0.044	a, c, h
Volume fraction of blood in organs (unitless)						
Liver	BVL	0.31	0.21	0.115	0.11	b, c
Spleen	BVS	0.17	0.22	0.3*	0.3*	b
Kidneys	BVK	0.24	0.16	0.105	0.36	b, c
Lungs	BVLu	0.5	0.36	0.3867*	0.3867*	b
Brain	BVBR	0.03	0.03	0.0275*	0.04	b
Rest of body	BVrest	0.04	0.04#	0.026#	0.01#	b, c

a: Davies and Morris (1993) [9]; b: Brown et al. (1997) [10]; c: Buur et al. (2005) [11]; d: Upton (2008) [12]; e: Fent et al. (2009) [6]; f: Balasubramanian et al. (2010) [5]; g: Crowell et al. (2011) [13]; h: Lin et al. (2013) [14]; i: Lin et al. (2015) [15]; ^: unit of this value is L/h/kg; *: average of other species, including mice, rats, dogs, and/or humans; #: assumed the same as the muscle.

Table 3. Nanoparticle-specific parameters used in the PBPK models of gold nanoparticles in mice, rats, pigs, and humans.

Parameter (unit)	Species	Liver	Spleen	Kidneys	Lungs	Brain	Rest of body
P_t (unitless)	All[a]	0.08	0.15	0.15	0.15	0.15	0.15
PAC_t (unitless)	All[a]	0.001	0.001	0.001	0.001	0.000001	0.000001
K_{max_t} (h^{-1})	Mouse	5	30	0.05	0.01	NA	0.4
	Rat (medium)	150	60	1	3	NA	15
	Rat (low)	20	10	0.5	1	NA	80
	Pig	1000	500	500	300	NA	0.05
	Human[b]	20	10	0.5	1	NA	80
K_{50_t} (h)	Mouse	48	48	24	24	NA	24
	Rat (medium)	24	24	24	24	NA	24
	Rat (low)	24	24	24	24	NA	24
	Pig	24	24	24	24	NA	24
	Human[b]	24	24	24	24	NA	24
n_t (unitless)	Mouse	5	5	5	5	NA	5
	Rat (medium)	0.1	0.1	0.1	0.1	NA	0.1
	Rat (low)	0.5	0.5	0.5	0.5	NA	0.5
	Pig	0.5	0.5	0.5	0.5	NA	0.5
	Human[b]	0.5	0.5	0.5	0.5	NA	0.5
$K_{release\_t}$ (h^{-1})	Mouse	0.02	0.005	0.01	0.002	NA	0.005
	Rat (medium)	0.25	0.25	0.25	0.07	NA	0.1
	Rat (low)	0.025	0.09	0.0075	0.07	NA	0.1
	Pig	0.005	0.02	0.04	0.02	NA	0.0001
	Human[b]	0.025	0.09	0.0075	0.07	NA	0.1
A_{cap_t} (μg/g tissue)	Mouse	100	200	15	15	NA	15
	Rat (medium)	195	150	330	150	NA	1.5
	Rat (low)	195	150	330	150	NA	1.5
	Pig	32.5	25	55	25	NA	0.25
	Human[b]	195	150	330	150	NA	1.5
K_{bileC} or K_{urineC} ($L/h/kg^{0.75}$)	Mouse	0.00003	NA	0.000003	NA	NA	NA
	Others[c]	0.00008	NA	0.0008	NA	NA	NA

P_t: tissue:plasma distribution; PAC_t: permeability coefficient; K_{max_t}: maximum uptake rate constant of phagocytic cells; K_{50_t}: time reaching half maximum uptake rate; n_t: Hill coefficient; $K_{release\_t}$: release rate constant of phagocytic cells; A_{cap_t}: uptake capacity per tissue weight; K_{bileC}: biliary excretion rate constant scalar; K_{urineC}: urinary excretion rate constant scalar.

[a] These values are from the mouse model [16] and the same across species.

[b] These parameters were set the same as those in the low-dose rat PBPK model.

[c] These values are the same across all species except the mouse.

NA: not applicable.

Table 4. Literature data on the toxicity of gold nanoparticles in primary human cells.

References*	Size[a] (nm)	Surface coating	Type of primary human cells	Dosage	Exposure duration	Method
Goodman et al. (2004) [17]	2	Quaternary ammonium, carboxylate	Red blood cells	0.27-833 µM	0.5 h	Hemolysis assay
Mironava et al. (2010) [18]	13, 45	Citrate	Dermal fibroblasts	13-190 µg/ml	3-6 d	Fluorescent microscopy
Sharma et al. (2011) [19]	3	Polyethylenimine	Corneal fibroblasts	3-10 µM	1 h	Trypan blue
Love et al. (2012) [20]	30	NA	Red blood cells	5-50 µg/ml	24-72 h	Hemolysis assay
Soenen et al. (2012) [21]	4	Poly(methacrylic acid)	Umbilical vein endothelial cells	1-500 nM	2-24 h	LDH
Moretti et al. (2013) [22]	50	PVP	Spermatozoa	30-500 µM	1, 2 h	eosin Y test
Ng et al. (2013) [23]	20	FBS	Small airway epithelial cells	0.25-2 nM	72 h	LDH, trypan blue
Pascarelli et al. (2013) [24]	50	PVP	Chondrocytes	20-250 µM	24 h	eosin Y test
DeRussy et al. (2014) [25]	30	Antibody	Foreskin-derived fibroblasts	50-100 ng/ml antibody	1 h	Trypan blue, MTT
Mironava et al. (2014) [26]	13, 45	Citrate	Adipose-derived stromal cells	13-190 µg/ml	7 days	Cell counting
Avalos et al. (2015) [27]	30-90	NA	Pulmonary fibroblasts	1-25 µg/ml	24-72 h	LDH, MTT
Bogdanov et al. (2015) [28]	10.4	MPEG-gPLL	Umbilical vein endothelial cells	0.5-500 µg/ml	24 h	WST
Ko et al. (2015) [29]	15-100	Citrate	Adipose-derived stem cells	1 µM	1-7 days	Cell counting kit-8
Lajunen et al. (2015) [30]	60X25[b], 50-60[c]	CTAB, PEG	Umbilical vein endothelial cells	50 µM	24 h	Alamar Blue
Schlinkert et al. (2015) [31]	7-10	Sodium citrate, chitosan	Bronchial epithelial cells	0.05-0.8 µg/cm^2	4-72 h	CellTiter-Blue, LDH

*: Among the published in vitro and in vivo toxicity studies of gold nanoparticles (AuNP), only in vitro studies conducted in primary human cells are listed in this table. Please refer to the review by Khlebstov and Dykman [32] for other toxicity studies of AuNP.
[a]: AuNP are spherical unless specifically mentioned; [b]: nanorods; [c]: nanostars.
CTAB: cetyltrimethylammonium bromide; FBS: fetal bovine serum; MPEG-gPLL: methoxypolyethylene glycol-graft poly-l-lysine copolymer; NA: not available; PEG: polyethylene glycol; PVP: polyvinylpyrrolidone.

2. Supplementary figures

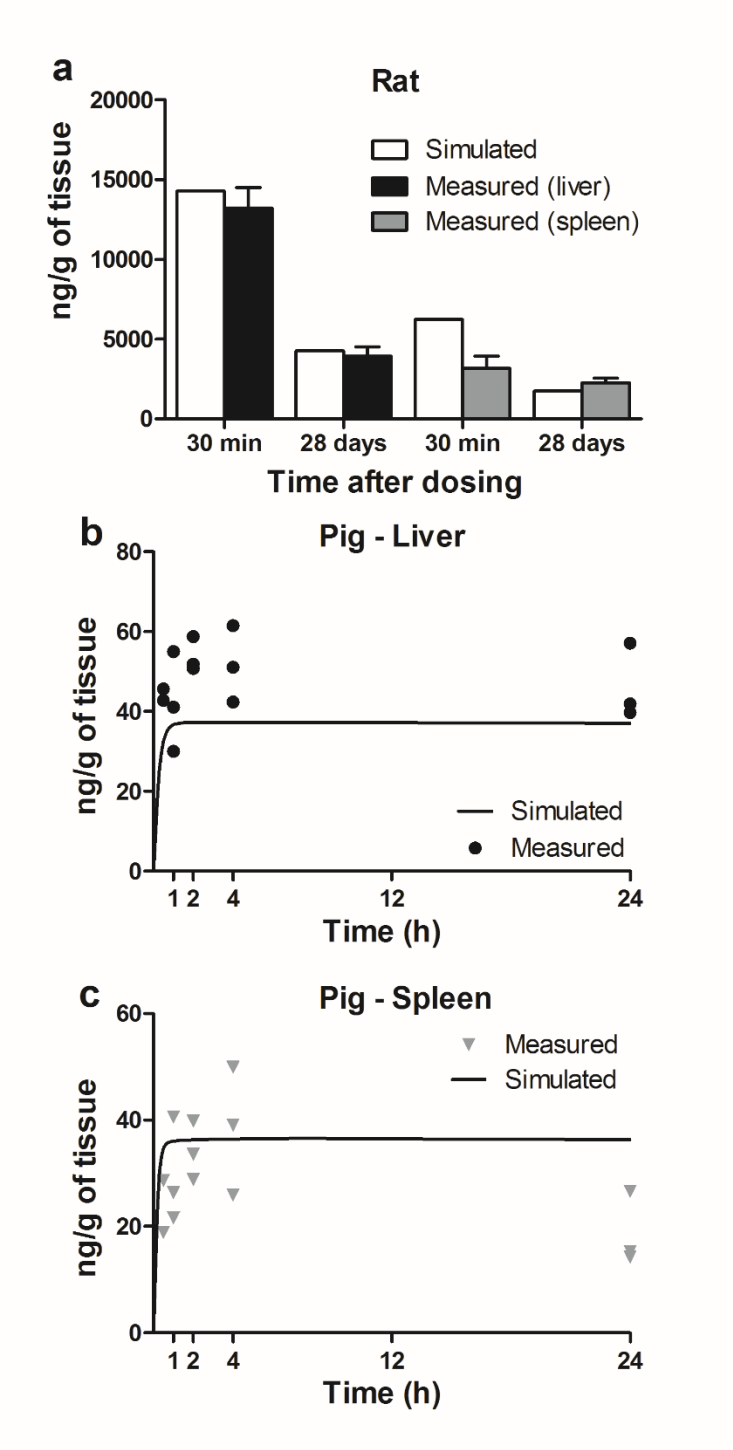

Figure 1. Rat (medium-dose) and pig PBPK model evaluation. Plots of experimentally determined [4, 7] versus rat (panel a) and pig (panels b-c) model-predicted concentrations of AuNP in the liver and spleen.

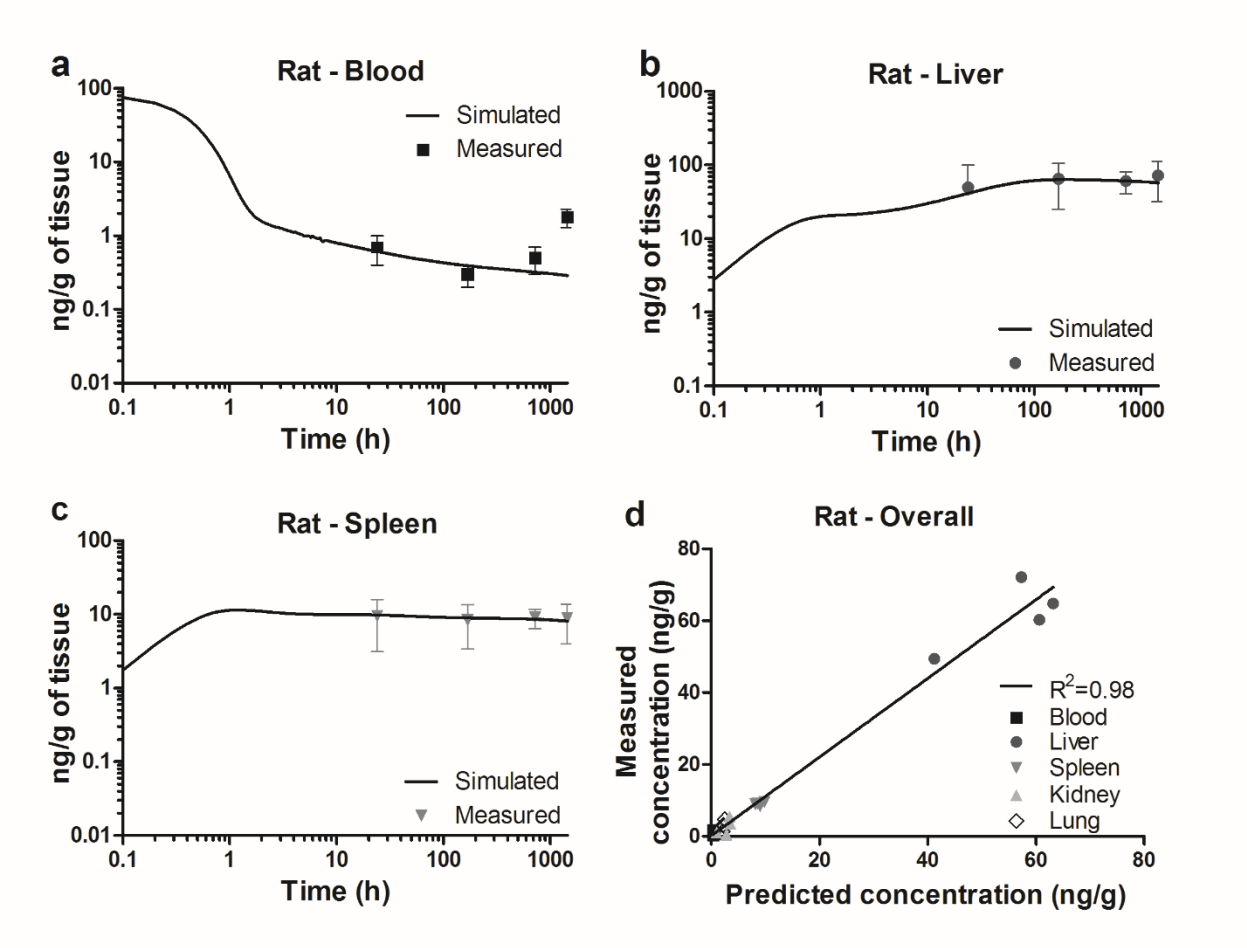

Figure 2. Rat (low-dose) PBPK model calibration. Plots of experimentally determined [5] (symbols) versus rat (panels a-c) model-predicted (lines) concentrations of AuNP in blood, liver and spleen. Panel d represents overall regression analysis results between measured and simulated data. The straight line in panel d represents regression line. R^2 means determination coefficient.

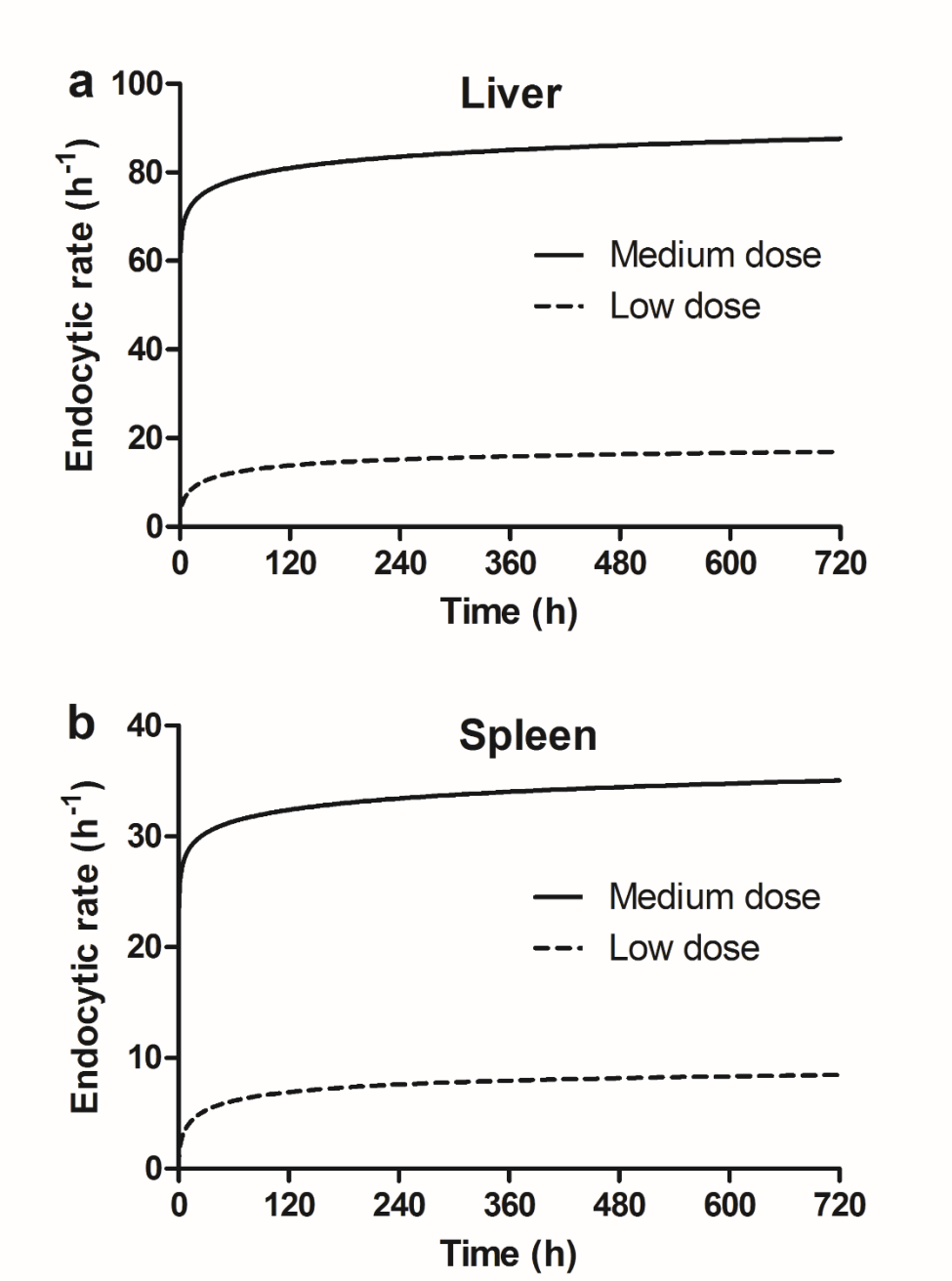

Figure 3. Rat model prediction of dose-dependent endocytosis of AuNP in liver and spleen. Solid and dashed lines represent simulation results from the medium-dose model and the low-dose model, respectively.

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4. PBPK model code in CSL format

```
PROGRAM
! Gold nanoparticle PBPK model in humans, derived from the low-dose rat model
! Model structure and code for other species are exactly the same as this human model, except
! species- and dose-specific parameters, which are given in Supplementary Tables 2 and 3
! Human model double-checked on April 24, 2015

INITIAL
! code that is executed once at the beginning of a simulation run goes here
! Blood flow rate (Fraction of cardiac output, unitless)
CONSTANT QCC = 16.5 ! Cardiac output (L/h/kg^0.75) (Brown et al.,1997; Upton, 2008;
Crowell et al., 2011)
CONSTANT QLC = 0.227 ! Fraction of blood flow to liver (Brown et al.,1997; Buur et al., 2005;
Upton, 2008; Lin et al., 2015)
CONSTANT QBRC = 0.114 ! Fraction of blood flow to brain (Brown et al., 1997; Upton, 2008)
CONSTANT QKC = 0.175 ! Fraction of blood flow to kidneys (Brown et al.,1997; Buur et al.,
2005; Upton, 2008; Lin et al., 2015)
CONSTANT QSC = 0.01375 ! Fraction of blood flow to spleen (Davies and Morris, 1993)

! Tissue volumes (Fraction of body weight, unitless)
CONSTANT BW = 70 ! Body weight (kg) (Davies and Morris, 1993; Brown et al., 1997; Fent et
al., 2009; Balasubramanian et al., 2010)
CONSTANT VLC = 0.0257 ! Liver (Brown et al.,1997; Buur et al., 2005; Upton, 2008; Lin et al.,
2015)
CONSTANT VBRC = 0.02 ! Brain (Brown et al., 1997 Table 21; Upton, 2008)
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CONSTANT VKC = 0.0044 ! Kidneys (Brown et al.,1997; Buur et al., 2005; Upton, 2008; Lin et al., 2015)

CONSTANT VSC = 0.00257 ! Spleen (Davies and Morris,1993; Brown et al., 1997; Upton, 2008)

CONSTANT VLuC = 0.008 ! Lungs (Brown et al., 1997, Table 21; Upton, 2008)

CONSTANT VBloodC = 0.079 ! Blood (Brown et al., 1997; Buur et al., 2005; Upton, 2008; Lin et al., 2015)

CONSTANT VPlasmaC = 0.079 ! If plasma, use 0.44; if blood, use 0.079, Hematocrit is 0.44; (Davies and Morris, 1993, Buur et al., 2005; Lin et al., 2013)

! Blood volume fraction in organs and tissues (percentage of organs/tissues, unitless)

CONSTANT BVL = 0.11 ! Liver (Brown et al. 1997, Table 30; Buur et al., 2005)

CONSTANT BVBR = 0.04 ! Brain (Brown et al., 1997, Table 30)

CONSTANT BVK = 0.36 ! Kidneys (Brown et al., 1997, Table 30; Buur et al., 2005)

CONSTANT BVS = 0.3 ! Spleen (Brown et al., 1997, Table 30, average of 3 species)

CONSTANT BVLu = 0.3867 ! Lungs (Brown et al., 1997, Table 30, average of 3 species)

CONSTANT BVrest = 0.01 ! Rest of body (Brown et al., 1997, Table 30, assume equal to the muscle)

! Tissue:plasma distribution coefficients (PCs), unitless; these values were from our published mouse PBPK model for gold nanoparticles (Lin et al., in press)

CONSTANT PL = 0.08 ! Liver

CONSTANT PBR = 0.15 ! Brain

CONSTANT PK = 0.15 ! Kidneys

CONSTANT PS = 0.15 ! Spleen

CONSTANT PLu = 0.15 ! Lungs

CONSTANT Prest = 0.15 ! Rest of body

! Membrane-limited permeability coefficient constants, unitless; these values were from our published mouse PBPK model for gold nanoparticles (Lin et al., in press)

CONSTANT PALC = 0.001 ! Liver

CONSTANT PABRC = 0.000001 ! Brain

CONSTANT PAKC = 0.001 ! Kidneys

CONSTANT PASC =0.001 ! Spleen

CONSTANT PALuC = 0.001 ! Lungs

CONSTANT PArestC = 0.000001 ! Rest of body

! Endocytic parameters; RES represent phagocytic cells; L, S, K, Lu, rest represent liver, spleen, kidneys, lungs, and rest of body, respectively.

! Compared to the original mouse model, uptake capacity parameters were added to simulate the dose-dependence;

! Endocytosis in the rest of body compartment was added because this compartment contains certain tissues (e.g., bone marrow) that can also uptake NPs.

CONSTANT KLRESrelease = 0.025 ! Release rate constant of phagocytic cells, (h-1)

CONSTANT KLRESmax = 20 ! Maximum uptake rate constant of phagocytic cells, (h-1)

CONSTANT KLRES50 = 24 ! Time reaching half maximum uptake rate, (h)

CONSTANT KLRESn = 0.5 ! Hill coefficient, (unitless)

CONSTANT ALREScap = 195 ! Uptake capacity per tissue weight (ug/g tissue)

CONSTANT KSRESrelease = 0.09 ! Release rate constant of phagocytic cells, (h-1)

CONSTANT KSRESmax = 10 ! Maximum uptake rate constant of phagocytic cells, (h-1)

CONSTANT KSRES50 = 24 ! Time reaching half maximum uptake rate, (h)

CONSTANT KSRESn = 0.5 ! Hill coefficient, (unitless)

CONSTANT ASREScap = 150 ! Uptake capacity per tissue weight (ug/g tissue)

CONSTANT KKRESrelease = 0.0075 ! Release rate constant of phagocytic cells, (h-1)

CONSTANT KKRESmax = 0.5 ! Maximum uptake rate constant of phagocytic cells, (h-1)

CONSTANT KKRES50 = 24 ! Time reaching half maximum uptake rate, (h)

CONSTANT KKRESn = 0.5 ! Hill coefficient, (unitless)

CONSTANT AKREScap = 330 ! Uptake capacity per tissue weight (ug/g tissue)

CONSTANT KLuRESrelease = 0.07 ! Release rate constant of phagocytic cells, (h-1)

CONSTANT KLuRESmax = 1 ! Maximum uptake rate constant of phagocytic cells, (h-1)

CONSTANT KLuRES50 = 24 ! Time reaching half maximum uptake rate, (h)

CONSTANT KLuRESn = 0.5 ! Hill coefficient, (unitless)

CONSTANT ALuREScap = 150 ! Uptake capacity per tissue weight (ug/g tissue)

CONSTANT KrestRESrelease = 0.1 ! Release rate constant of phagocytic cells, (h-1)

CONSTANT KrestRESmax = 80 ! Maximum uptake rate constant of phagocytic cells, (h-1)

CONSTANT KrestRES50 = 24 ! Time reaching half maximum uptake rate, (h)

CONSTANT KrestRESn = 0.5 ! Hill coefficient, (unitless)

CONSTANT ArestREScap = 1.5 ! Uptake capacity per tissue weight (ug/g tissue)

! Biliary excretion

CONSTANT KbileC = 0.0008 ! Biliary clearance (L/hr/kg^0.75)

!L/hr/kg changed to L/h/kg^0.75 for interspecies extrapolation

! Urine excretion

CONSTANT KurineC = 0.0008 ! Urine clearance (L/hr/kg^0.75)

!L/hr changed to L/h/kg^0.75 for interspecies extrapolation

```
! IV dosing
CONSTANT Timeiv = 0.005 ! IV infusion time (h), set, approximately 15-20 seconds, on
average 18 sec
CONSTANT PDOSEiv = 0.01726 ! mg/kg

END ! INITIAL

DYNAMIC

ALGORITHM IALG = 2
NSTEPS    NSTP = 10
MAXTERVAL MAXT = 1.0e9
MINTERVAL MINT = 1.0e-9
CINTERVAL CINT = 0.1

DERIVATIVE
! code for calculating the derivative goes here
! Scaled parameters
! Cardiac output and regional blood blow (L/h)
!BW = 9.6+0.015*t ! This equation is for medium-dose rat model 28-day study because juvenile
rats were used; it is valid only up to 28 days after injection
!More complex equations describing body weight and organ weight growth could be found in Lin
et al. (2013)
QC = QCC*BW**0.75 ! Cardiac output
QL = QC*QLC ! Blood flow to liver
```

```
QBR = QC*QBRC ! Blood flow to brain

QK = QC*QKC ! Blood flow to kidneys

QS = QC*QSC ! Blood flow to spleen

Qrest = QC-QL-QBR-QK-QS ! Blood flow to rest of body

Qbal = QC-QL-QBR-QK-QS-Qrest ! Blood flow balance equation


! Tissue volumes (L)

VL = BW*VLC ! Liver

VBR = BW*VBRC ! Brain

VK = BW*VKC ! Kidneys

VS = BW*VSC ! Spleen

VLu = BW*VLuC ! Lungs

VBlood = BW*VBloodC

VPlasma = BW*VPlasmaC

Vrest = BW-VL-VBR-VK-VS-VLu-VPlasma

Vbal = BW-VL-VBR-VK-VS-VLu-VPlasma-Vrest

VLb = VL*BVL ! Weight/volume of capillary blood in liver compartment

VLt = VL-VLb ! Weight/volume of tissue in liver compartment

VBRb = VBR*BVBR ! Weight/volume of capillary blood in brain compartment

VBRt = VBR-VBRb ! Weight/volume of tissue in brain compartment

VKb = VK*BVK ! Weight/volume of capillary blood in kidney compartment

VKt = VK-VKb ! Weight/volume of tissue in kidney compartment

VSb = VS*BVS ! Weight/volume of capillary blood in spleen compartment

VSt = VS-VSb ! Weight/volume of tissue in spleen compartment

VLub = VLu*BVLu ! Weight/volume of capillary blood in lung compartment

VLut = VLu-VLub ! Weight/volume of tissue in lung compartment
```

```
Vrestb = Vrest*BVrest ! Weight/volume of capillary blood in rest of body compartment
Vrestt = Vrest-Vrestb ! Weight/volume of tissue in rest of body compartment

! Permeability coefficient-surface area cross-product (L/h)
PAL = PALC*QL ! Liver
PABR = PABRC*QBR ! Brain
PAK = PAKC*QK ! Kidneys
PAS = PASC*QS ! Spleen
PALu = PALuC*QC ! Lungs
PArest = PArestC*Qrest ! Rest of body

! Endocytosis rate (h-1)
KLRESUP = (KLRESmax*T^KLRESn)/(KLRES50^KLRESn+T^KLRESn)*(1-
(ALRES/(ALREScap*VL))) ! Liver
KSRESUP = (KSRESmax*T^KSRESn)/(KSRES50^KSRESn+T^KSRESn)*(1-
(ASRES/(ASREScap*VS))) ! Spleen
KKRESUP = (KKRESmax*T^KKRESn)/(KKRES50^KKRESn+T^KKRESn)*(1-
(AKRES/(AKREScap*VK))) ! Kidneys
KLuRESUP = (KLuRESmax*T^KLuRESn)/(KLuRES50^KLuRESn+T^KLuRESn)*(1-
(ALuRES/(ALuREScap*VLu))) ! Lungs
KrestRESUP = (KrestRESmax*T^KrestRESn)/(KrestRES50^KrestRESn+T^KrestRESn)*(1-
(ArestRES/(ArestREScap*Vrest))) ! Rest of body

! Dosing
DOSEiv = PDOSEiv*BW !mg
IVR = DOSEiv/Timeiv !mg/h
```

```
RIV = IVR*(1.-step(Timeiv))

AIV = Integ(RIV, 0.0)

! Elimination

Kbile = KbileC*BW**0.75 !L/h

Kurine = KurineC*BW**0.75 !L/h

! Blood compartment

! CA = Arterial blood concentration (mg/L or ug/ml)

RA = QC*CVLu - QC*CA

AA = Integ(RA, 0.0)

!CA = AA/(VBlood*0.2)

CA = AA/(VPlasma*0.2)

AUCCA = Integ(CA,0.0)

CA1000 = CA*1000 ! ng/g, ng/ml, ug/L

AUCCA1000 = Integ(CA1000,0.0)

! CV = Venous blood concentration (mg/L or ug/ml)

RV = QL*CVL + QBR*CVBR + QK*CVK + Qrest*CVrest + RIV - QC*CV

AV = Integ(RV, 0.0)

!CV = AV/(VBlood*0.8)

CV = AV/(VPlasma*0.8)

CV1000 = CV*1000

APlasma = AA+AV

APlasmaperc = 100*(APlasma/Doseiv)/(VPlasma*1000)

Abloodperc = 100*(APlasma/Doseiv)/(VBlood*1000)
```

```
!! Lung compartment

! Membrane-limited model

RLub = QC*(CV-CVLu) - PALu*CVLu + (PALu*CLut)/PLu + RLuRESrelease -KLuRESup*ALub

ALub = Integ(RLub,0.0)

CVLu = ALub/VLub


RLut = PALu*CVLu - (PALu*CLut)/PLu

ALut = Integ(RLut,0.0)

CLut = ALut/VLut

ALutotal = ALub+ALut

CLu = ALutotal/VLu

CLu1000 = CLu*1000 ! ng/g, ng/ml, ug/L


RLuRES = KLuRESUP*ALub-KLuRESrelease*ALuRES

RLuRESUP = KLuRESUP*ALub !*1000

RLuRESrelease = KLuRESrelease*ALuRES

ALuRES = INTEG(RLuRES,0.0)

CLung = (ALutotal+ALuRES)/VLu

CLungtissue = (ALut+ALuRES)/VLut

Clungtissue1000 = 1000*(ALut+ALuRES)/VLut

Alungtissue1000 = 1000*(ALut+ALuRES)

CLung1000 = CLung*1000


ALungtissue = ALut+ALuRES

ALungtissueperc = 100*(ALungtissue/Doseiv)/(VLut*1000)
```

!! Brain compartment

! Membrane-limited model

RBRb = QBR*(CA-CVBR) - PABR*CVBR + (PABR*CBRt)/PBR

ABRb = Integ(RBRb,0.0)

CVBR = ABRb/VBRb

RBRt = PABR*CVBR - (PABR*CBRt)/PBR

ABRt = Integ(RBRt,0.0)

CBRt = ABRt/VBRt

ABRtotal = ABRb+ABRt

CBR = ABRtotal/VBR

!! Rest of body compartment

! Membrane-limited model, endocytosis is included in this compartment because it contains certain tissues (e.g., bone marrow) that can uptake non-PEG NPs.

Rrestb = Qrest*(CA-CVrest) - PArest*CVrest + (PArest*Crestt)/Prest + RrestRESrelease - KrestRESUP*Arestb

Arestb = Integ(Rrestb,0.0)

CVrest = Arestb/Vrestb

Rrestt = PArest*CVrest - (PArest*Crestt)/Prest

Arestt = Integ(Rrestt,0.0)

Crestt = Arestt/Vrestt

Aresttotal = Arestb+Arestt

Crest = Aresttotal/Vrest

```
Crest1000 = Crest*1000 ! ng/g, ng/ml, ug/L

RrestRES = KrestRESUP*Arestb-KrestRESrelease*ArestRES
RrestRESUP = KrestRESUP*Arestb !*1000
RrestRESrelease = KrestRESrelease*ArestRES
ArestRES = INTEG(RrestRES,0.0)
Crestall = (Aresttotal+ArestRES)/Vrest
Cresttissue1000 = 1000*(Arestt+ArestRES)/Vrestt
Cresttissue = (Arestt+ArestRES)/Vrestt
Aresttissue1000 = 1000*(Arestt+ArestRES)
Crestall1000 = Crestall*1000

Aresttissue = Arestt+ArestRES
Aresttissueperc = 100*(Aresttissue/Doseiv)/(Vrestt*1000)

!! Kidney compartment
! Membrane-limited model
RKb = QK*(CA-CVK) - PAK*CVK + (PAK*CKt)/PK - Rurine + RKRESrelease - KKRESUP*AKb
AKb = Integ(RKb,0.0)
CVK = AKb/VKb

RKt = PAK*CVK - (PAK*CKt)/PK
AKt = Integ(RKt,0.0)
CKt = AKt/VKt
AKtotal = AKb+AKt
CK = AKtotal/VK
```

CK1000 = CK*1000 ! ng/g, ng/ml, ug/L

! Urinary excretion

Rurine = Kurine*CVK !mg/h

Aurine = Integ(Rurine,0.0)

Aurine2 = Aurine*1000

!RKRES = KKRESUP*AA-KKRESrelease*AKRES

RKRES = KKRESUP*AKb-KKRESrelease*AKRES

RKRESUP = KKRESUP*AKb!*1000

RKRESrelease = KKRESrelease*AKRES

AKRES = INTEG(RKRES,0.0)

CKidney = (AKtotal+AKRES)/VK

CKidneytissue = (AKt+AKRES)/VKt

CKidneytissue1000 = 1000*(AKt+AKRES)/VKt

AKidneytissue1000 = 1000*(AKt+AKRES)

CKidney1000 = CKidney*1000

AKidneytissue = AKt+AKRES

AKidneytissueperc = 100*(AKidneytissue/Doseiv)/(VKt*1000)

!! Spleen compartment

! Membrane-limited model

RSb = QS*(CA-CVS) - PAS*CVS + (PAS*CSt)/PS + RSRESrelease - KSRESUP*ASb

ASb = Integ(RSb,0.0)

CVS = ASb/VSb

```
RSt = PAS*CVS - (PAS*CSt)/PS

ASt = Integ(RSt,0.0)

CSt = ASt/VSt

AStotal = ASb+ASt

CS = AStotal/VS

CS1000 = CS*1000 ! ng/g, ng/ml, ug/L


!RSRES = KSRESUP*AA-KSRESrelease*ASRES

RSRES = KSRESUP*ASb-KSRESrelease*ASRES

RSRESUP = KSRESUP*ASb!*1000

RSRESrelease = KSRESrelease*ASRES

ASRES = INTEG(RSRES,0.0)

CSpleen = (AStotal+ASRES)/VS

Cspleentissue = (ASt+ASRES)/VSt

CSpleentissue1000 = 1000*(ASt+ASRES)/VSt

AUCCSpleentissue1000 = Integ(CSpleentissue1000,0.0)

ASpleentissue1000 = 1000*(ASt+ASRES)

CSpleen1000 = CSpleen*1000

ASpleentissue = ASt+ASRES

ASpleentissueperc = 100*(ASpleentissue/Doseiv)/(VSt*1000)


!! Liver compartment

! Membrane-limited model

RLb = QL*(CA-CVL) + QS*CVS - PAL*CVL + (PAL*CLt)/PL - Rbile + RLRESrelease -

KLRESUP*ALb
```

```
ALb = Integ(RLb,0.0)

CVL = ALb/VLb


RLt = PAL*CVL - (PAL*CLt)/PL

ALt = Integ(RLt,0.0)

CLt = ALt/VLt

ALtotal = ALb+ALt

CL = ALtotal/VL

CL1000 = CL*1000 ! ng/g, ng/ml, ug/L


!RLRES = KLRESUP*AA-KLRESrelease*ALRES

RLRES = KLRESUP*ALb-KLRESrelease*ALRES

RLRESUP = KLRESUP*ALb!*1000

RLRESrelease = KLRESrelease*ALRES

ALRES = INTEG(RLRES,0.0)

CLiver = (ALtotal+ALRES)/VL

CLivertissue1000 = 1000*(ALt+ALRES)/VLt

AUCCLivertissue1000 = Integ(CLivertissue1000,0.0)

CLivertissue = (ALt+ALRES)/VLt

ALivertissue1000 = 1000*(ALt+ALRES)

CLiver1000 = CLiver*1000


ALivertissue = ALt+ALRES

ALivertissueperc = 100*(ALivertissue/Doseiv)/(VLt*1000)


! Biliary excretion
```

```
Rbile = Kbile*CVL ! mg/h
Abile = Integ(Rbile,0.0)

! Mass balance
Tmass =
AA+AV+ALtotal+ABRtotal+AKtotal+ALutotal+Aresttotal+AStotal+Abile+Aurine+ALRES+ASRES
+ALuRES+AKRES+ArestRES
Bal = AIV-Tmass

END ! DERIVATIVE

! Add discrete events here as needed
!  DISCRETE
!  END

! code that is executed once at each communication interval goes here

CONSTANT TSTOP = 770!4325!240.0
TERMT (T .GE. TSTOP, 'checked on communication interval: REACHED TSTOP')
END ! DYNAMIC
TERMINAL
! code that is executed once at the end of a simulation run goes here
END ! TERMINAL
END ! PROGRAM
```