Supplementary Materials

A physiologically based pharmacokinetic model of doxycycline for predicting tissue residues and withdrawal intervals in grass carp (*Ctenopharyngodon idella*)

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Additional method

1. Cardiac output determination and calculation

The concentration of Evans blue in each sample was detected by spectrophotometer, and then was used to calculate the area under concentration time curve (AUC) using the trapezoidal method. The semilog method was employed to better visualize the recirculation. Cardiac output was calculated for each fish using the equation described as follows:

$$Q = D / (AUC * BW) \quad (1)$$

where D is the amount of Evan blue (mg) injected into the fish, AUC (mg*min/mL) is the area-under concentration-time curve from the time of first dye appearance to the time of dye recirculation, and BW is the body weight (kg).

2. Equations for absorption from the gut

Grass carp do not have stomach. The fish gut is different from mammals, and it includes foregut, midgut, and hindgut. Food and drug absorption mainly occurs at foregut and midgut, and it occurs to some degrees in hindgut (Smith, 1980). In the present model, the gut structure was separated into two sections: one including foregut and midgut, and the other one representing hindgut. Additionally, enterohepatic circulation of DC was also considered in the model based on published studies (Lin et al., 2015; Zeng et al., 2017; Li et al., 2019). Oral absorption was described using the equations below.

Foregut and midgut:

$$RAI = RDOSEoral - Ka * AI – Kint * AI + Rbile - Kehc * AI \quad (2)$$

where RAI is the rate of change of DC in foregut and midgut (mg/h), RDOSEoral is the rate of DC following oral administration (mg/h), Ka is the absorption rate constant of DC in foregut and midgut (/h), AI is the amount of DC in foregut and midgut (mg), Kint is the transit rate constant from foregut/midgut to hindgut (/h), Rbile is the biliary excretion rate of DC (mg/h), and Kehc is the reabsorbed rate constant by the enterohepatic circulation (/h).

Hindgut:

RAIh = Kint *AI - Kah * AIh – Rfeces (3)

where RAIh is the rate of change of DC in hindgut (mg/h), Kah is the absorption rate constant of hindgut (/h), AIh is the amount of DC in hindgut (mg), and Rfeces is the rate of change of DC in feces.

3. Equations for calculating partition coefficients

Pt = AUCtissue/AUCplasma (4)

where Pt is partition coefficient in non-eliminating organs, including gill, muscle+skin and liver, AUCtissue is the AUC in the tissue, and AUCplasma is the AUC in plasma.

Pte = AUCtissue/(AUCplasma * (1-E)) (5)

where Pte is partition coefficient in the eliminating organ kidney, and E is the renal extraction ratio calculated as renal clearance divided by the blood flow to kidney.

Supplementary tables

Table S1. Summary of pharmacokinetic and tissue residue studies of doxycycline in grass carp (*Ctenopharyngodon idella*) used in the parameter calculation and model calibration

Species	Route	Temperature (°C)	Dose (mg/kg)	Repeat	Sex	n	Age (months)	BW (g)	Matrix	Assay	Ref.
Parameter calculation	IV	24	20	1	NA	6	12	400.5	P	UPLC	Present study
Parameter calculation	PO	24	20	1	NA	6	12	450.7	P, M, L, K, G	UPLC	Xu et al., 2019b
Model calibration	PO	24	20	3	NA	6	12	450.4	P, M, L, K, G	LC-MS/MS	Xu et al., 2019a

Note : The abbreviations for administration route: PO, per os; IV, intravenous administration. The abbreviations for matrix: P, plasma; M, muscle+skin; L, liver; K, kidney; G, gill. The abbreviations for determination: LC-MS/MS, liquid chromatography tandem mass spectrometry; UPLC, ultra-performance liquid chromatography. NA, not available.

Table S2. Pharmacokinetic parameters of doxycycline in grass carp (*Ctenopharyngodon idella*) following a single intravenous administration at 20 mg/kg

Parameters	Units	Values
A	mg/L	124.12
α	1/h	2.95
B	mg/L	23.32
β	1/h	0.02
$t_{1/2\alpha}$	h	0.24
$t_{1/2\beta}$	h	27.75
K10	1/h	0.15
$t_{1/2K10}$	h	4.59
K12	1/h	2.33
K21	1/h	0.49
$AUC_{0-\infty}$	h*mg/L	975.78
C_{max}	mg/L	147.44
V_{ss}	L/kg	0.79

Note: A, zero-time blood drug concentration intercept of distribution phase; α, distribution rate constant; B, zero-time blood drug concentration intercept of elimination phase; β, elimination rate constant; $t_{1/2\alpha}$, distribution half-life; $t_{1/2\beta}$, elimination half-life; K10, drug elimination rate constant from central compartment; $t_{1/2K10}$, half-life of drug leaving the body from the central compartment; K12, first-order transport rate constant from central compartment to peripheral compartment; K21, first-order transport rate constant from peripheral compartment to central compartment; $AUC_{0-\infty}$, area under concentration–time curve from 0 to ∞ ; C_{max}, peak concentration; Vss, volume of distribution at stead-state.

Supplementary figures

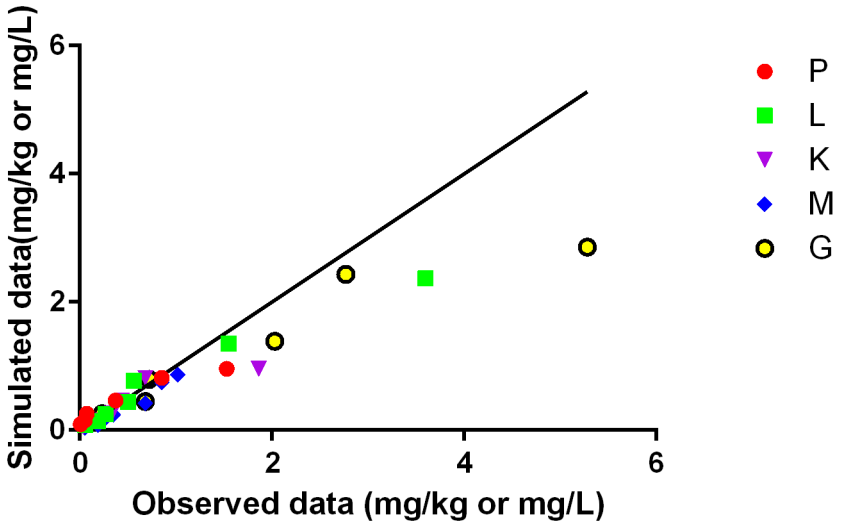

Figure S1. The regression analysis result between simulated data and measured data in plasma (P), liver (L), kidney (K), muscle+skin (M), and gill (G). The determination coefficient R^2 value is 0.93.

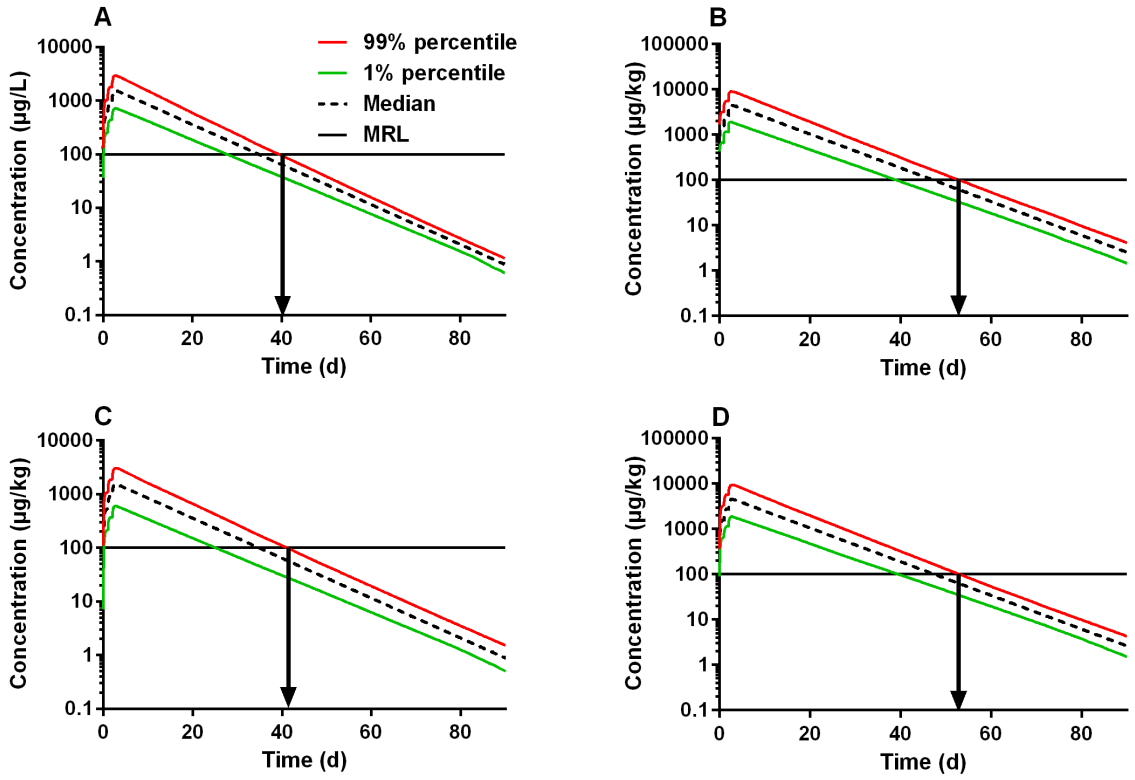

Figure S2. Monte Carlo simulation result for doxycycline concentrations in grass carp (*Ctenopharyngodon idella*) based on sensitive parameters by examining influence on 24-h AUC of plasma and tissues using the label dose of 20 mg/kg. The median value (black dash lines), 99th percentile (red solid lines) and 1th percentile (green solid lines) of model predictions for doxycycline concentrations in plasma (A), liver (B), kidney (C), and gill (D) of grass carp (*Ctenopharyngodon idella*) following daily oral administration at 20 mg/kg for 3 days are shown in the figure. The horizontal black line represents the maximum residue limit of 100 µg/kg for doxycycline in fish in Europe and China.

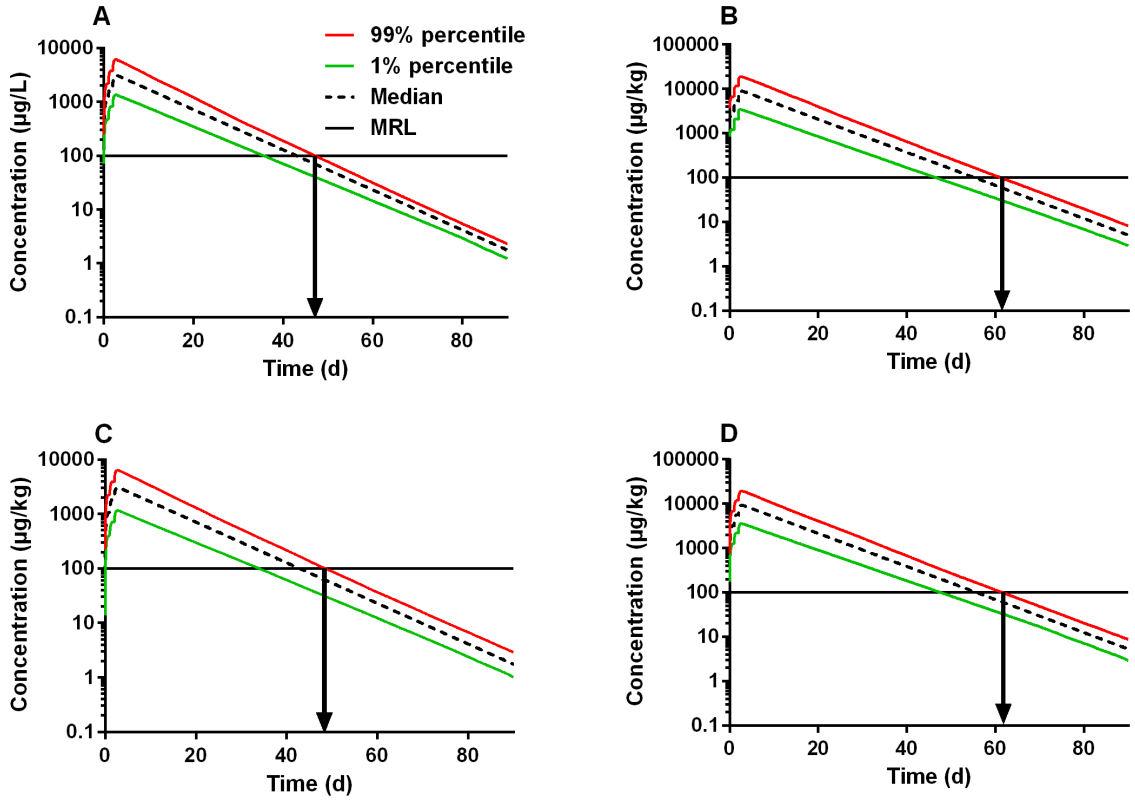

Figure S3. Monte Carlo simulation result for doxycycline concentrations in grass carp (*Ctenopharyngodon idella*) based on sensitive parameters by examining influence on 24-h AUC of plasma and tissues using the extra-label dose of 40 mg/kg. The median value (black dash lines), 99th percentile (red solid lines) and 1th percentile (green solid lines) of model predictions for doxycycline concentrations in plasma (A), liver (B), kidney (C), and gill (D) of grass carp (*Ctenopharyngodon idella*) following daily oral administration at 40 mg/kg for 3 days are shown in the figure. The horizontal black line represents the maximum residue limit of 100 µg/kg for doxycycline in fish in Europe and China.

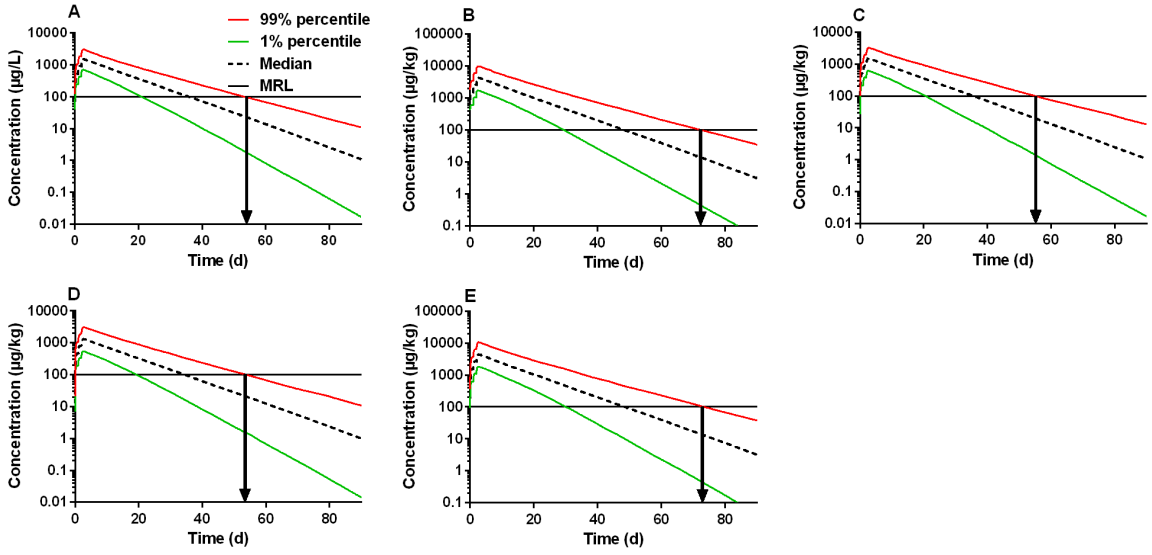

Figure S4. Monte Carlo simulation result for doxycycline concentrations in grass carp (*Ctenopharyngodon idella*) based on sensitive parameters by examining influence on 1008-h AUC of plasma and tissues using the label dose of 20 mg/kg. The median value (black dash lines), 99th percentile (red solid lines) and 1th percentile (green solid lines) of model predictions for doxycycline concentrations in plasma (A), liver (B), kidney (C), muscle + skin (D) and gill (E) of grass carp (*Ctenopharyngodon idella*) following daily oral administration at 20 mg/kg for 3 days are shown in the figure. The horizontal black line represents the maximum residue limit of 100 µg/kg for doxycycline in fish in Europe and China.

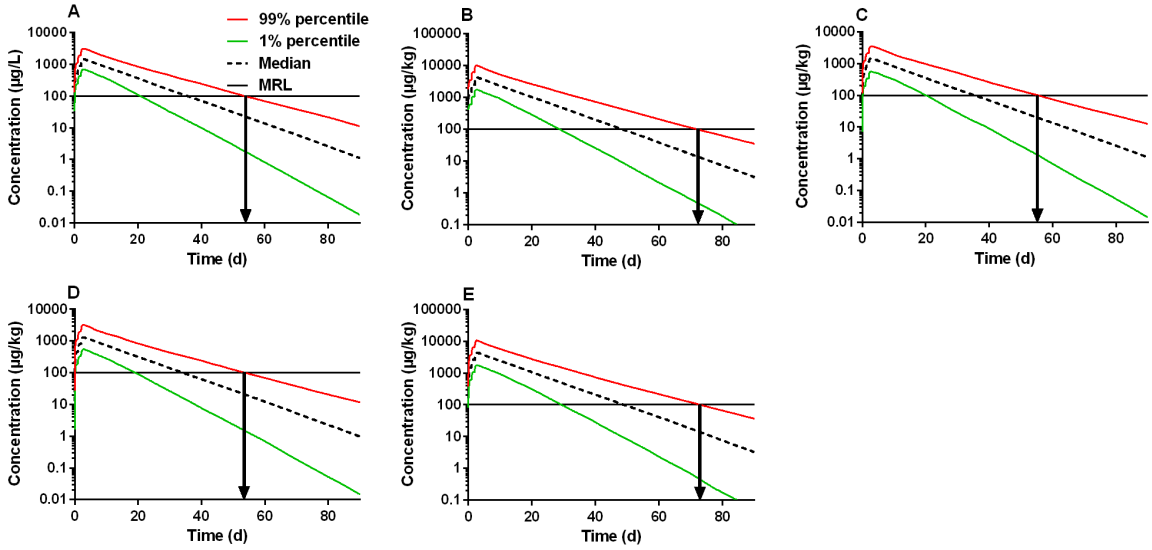

Figure S5. Monte Carlo simulation result for doxycycline concentrations in grass carp (*Ctenopharyngodon idella*) based on combined sensitive parameters by examining influence on 24-h AUC and 1008-h AUC of plasma and tissues using the label dose of 20 mg/kg. The median value (black dash lines), 99th percentile (red solid lines) and 1th percentile (green solid lines) of model predictions for doxycycline concentrations in plasma (A), liver (B), kidney (C), muscle + skin (D) and gill (E) of grass carp (*Ctenopharyngodon idella*) following daily oral administration at 20 mg/kg for 3 days are shown in the figure. The horizontal black line represents the maximum residue limit of 100 µg/kg for doxycycline in fish in Europe and China.

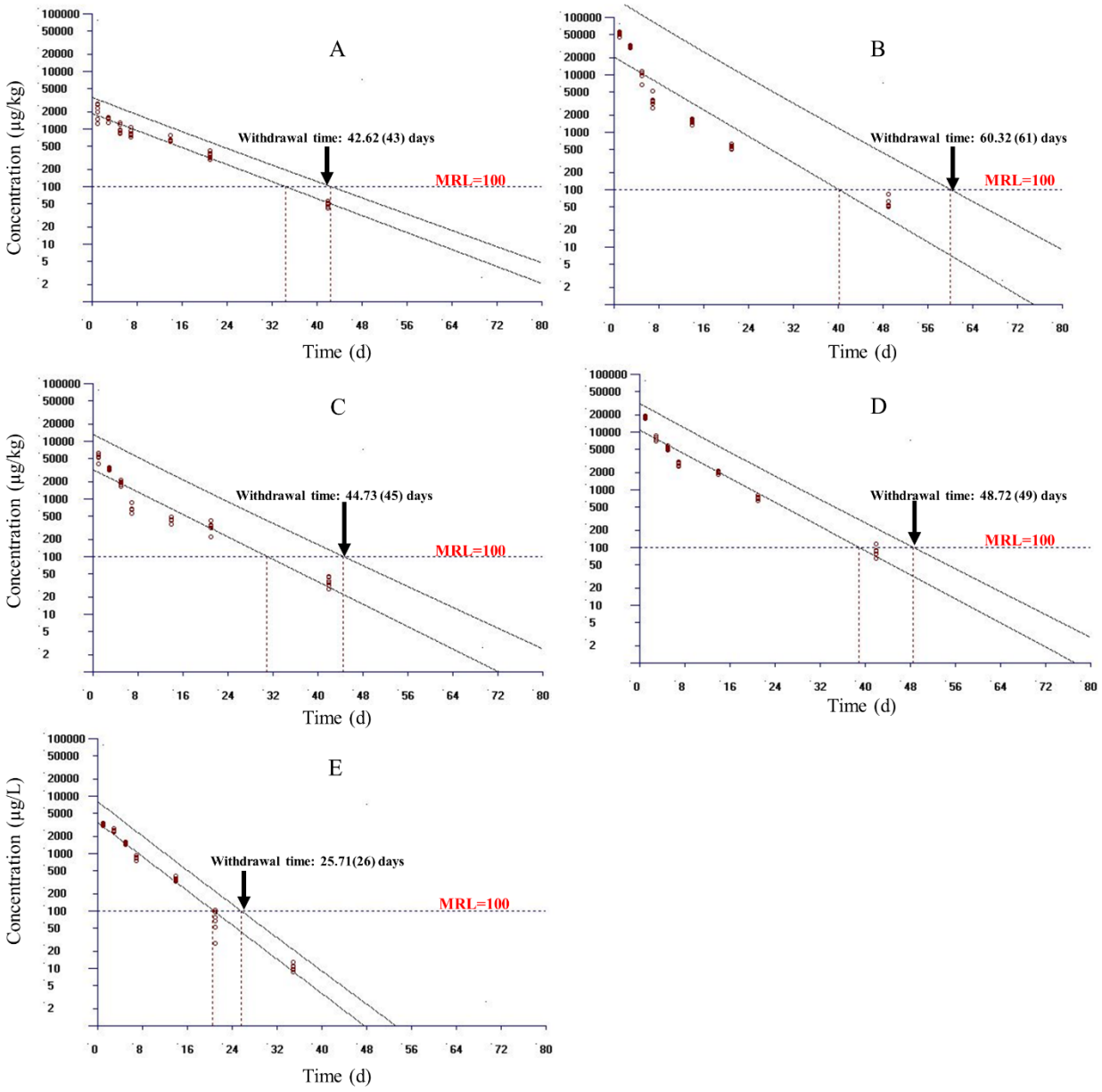

Figure S6. Estimated withdrawal times for doxycycline in grass carp (*Ctenopharyngodon idella*) using experimental data from oral administrations at 20 mg/kg for 3 days (Xu et al., 2019a) based on the EMA method using the WT 1.4 software (A for muscle+skin, B for liver, C for kidney, D for gill, and E for plasma) with a tolerance limit of 99th percentile with a 95% confidence level. MRL: maximum residue limits for doxycycline from the European Medicines Agency (EMA) (EMA, 2018). If the calculated withdrawal time was a fraction of a day, the estimated withdrawal time was rounded up to the next whole day shown in the parenthesis.

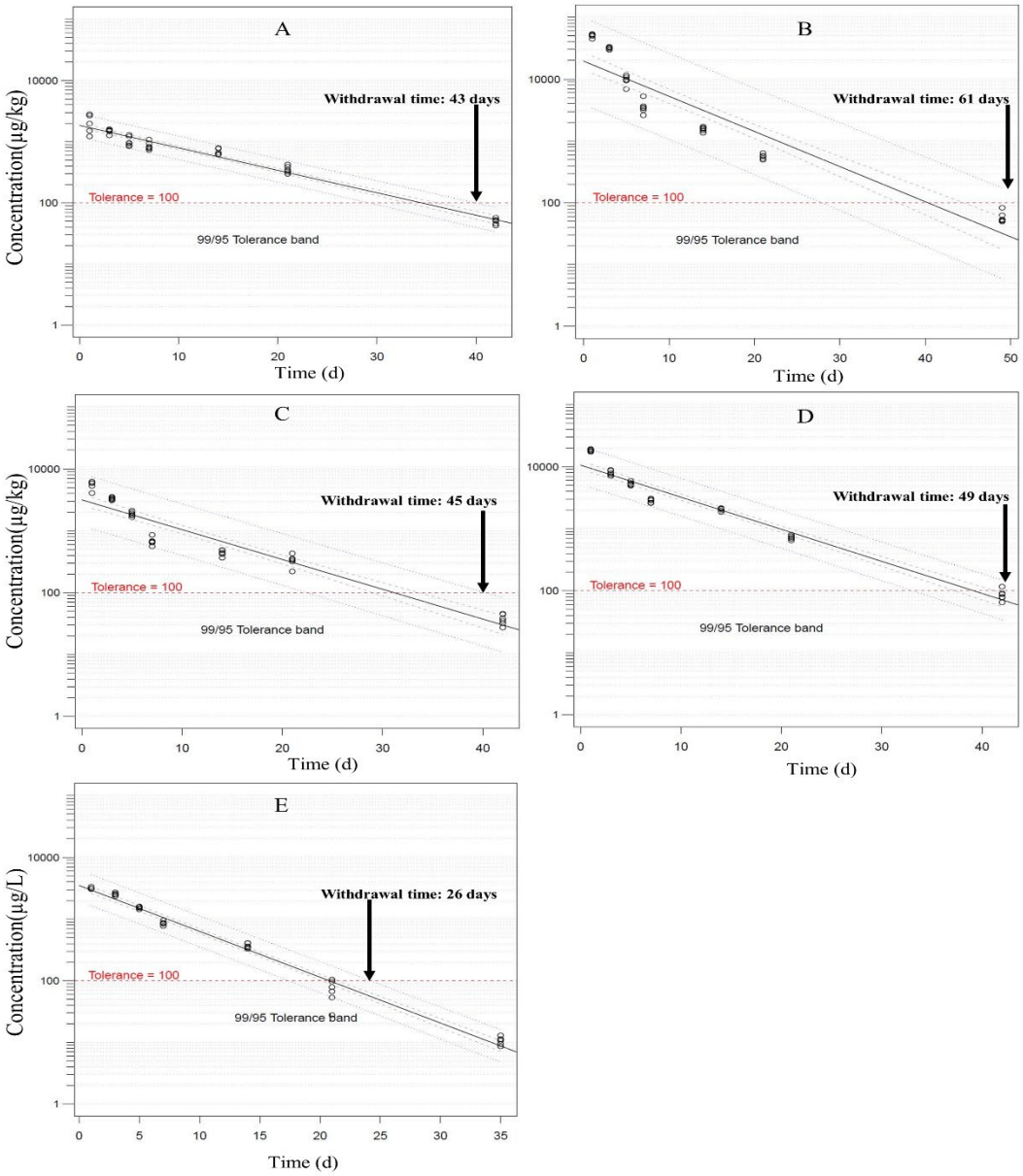

Figure S7. Estimated withdrawal times for doxycycline in grass carp (*Ctenopharyngodon idella*) using experimental data from oral administrations at 20 mg/kg for 3 days (Xu et al., 2019a) using FDA's tolerance limit method coded in the "rescheme" package (A for muscle+skin, B for liver, C for kidney, D for gill, and E for plasma). The withdrawal time was calculated based on the maximum residue limit (MRL) of 100 µg/kg for DC in fish plasma and tissues with a tolerance limit of 99th percentile with a 95% confidence level.

PBPK model code for an average individual

The model code as described below is for the physiologically based pharmacokinetic (PBPK) of doxycycline (DC) in grass carp (*Ctenopharyngodon idella*). All physiological and chemical-specific parameter values are shown in Table 1. The cardiac output and organ weight fractions were experimentally measured in the present study. The fractional blood flows in various tissues were cited form the corresponding values in rainbow trout (Law et al., 1991). The blood hematocrit in grass carp was used in the model (Yavuzcan-Yıldız and Kırkavgaç-Uzbilek, 2001). Fractional arterial plasma and venous plasma were respectively set as 0.2 and 0.8 (Lin et al., 2016). The tissue/plasma partition coefficients were calculated using pharmacokinetic data of DC in grass carp (Xu et al., 2019b). Other unknown chemical-specific parameters were obtained by model fitting to residue data of DC in grass carp (Xu et al., 2019a).

```
METHOD RK4

STARTTIME = 0
STOPTIME= 1400
DT = 0.0025
DTOUT = 0.01

; Physiological parameters
; Blood flow rates
QCC = 3.738                    ; Cardiac output (L/h/kg)
QLC = 0.181                    ; Fractional blood flow to the liver
QKC = 0.102                    ; Fractional blood flow to the kidney
QMC = 0.398                    ; Fractional blood flow to the muscle+skin
QGC = 1                        ; Fractional blood flow to the gill
QRC = 0.010                    ; Fractional blood flow to the richly perfused tissues
QSC = 0.309                    ; Fractional blood flow to the slowly perfused tissues

; Body weight (kg)
k= 3.896                                    ; The slope of the equation
Factor = 1000                               ; Conversion from g to kg
Hoursinaday = 24                            ; Conversion from a day to hours
BW_0 = 0.45                                 ; kg, The intial body weight
BW = BW_0 + (k*(TIME/Hoursinaday)) / Factor  ; The equation of body weight growth

; Tissue/Organ volumes
BW = 0.450                     ; Body weight (kg)
VLC = 0.004                    ; Fractional liver
VKC = 0.004                    ; Fractional kidney
VMC = 0.386                    ; Fractional muscle+skin
VGC = 0.037                    ; Fractional gill
VRC = 0.030                    ; Fractional richly perfused tissues
VSC = 0.465                    ; Fractional slowly perfused tissues
VartC = 0.015                  ; Fractional arterial blood
VvenC = 0.059                  ; Fractional venous blood
Hematocrit = 0.254             ; The blood hematocrit in grass carp

; Mass Transfer Parameters (Chemical-specific parameters)
; Partition coefficients (PC, tissue:plasma)
PL = 2.821                     ; Liver:plasma PC
```

```
PK = 1.064                          ; Kidney:plasma PC
PM = 0.901                          ; Muscle+skin:plasma PC
PG = 2.981                          ; Gill:plasma PC
PR = 2.821                          ; Richly perfused tissues:plasma
PS = 0.901                          ; Slowly perfused tissues:plasma


;Cardiac output and blood flows to tissues (L/h)
QC = QCC * BW * (1 - Hematocrit)                        ; Cardiac output
QL = QLC * QC                                           ; Liver
QK = QKC * QC                                           ; Kidney
QG = QGC * QC                                           ; Gill
QM = QMC * QC                                           ; Muscle+skin
QR = QRC * QC                                           ; Richly perfused tissues
QS = (1- QLC - QKC - QMC - QRC) * QC                    ; Slowly perfused tissues

; Tissue/Organ volumes
VL = VLC * BW                                           ; Liver
VK = VKC * BW                                           ; Kidney
VG = VGC * BW                                           ; Muscle+skin
VM = VMC * BW                                           ; Blood
Vart = VartC * BW * (1 - Hematocrit)                    ; Arterial plasma
Vven = VvenC * BW * (1 - Hematocrit)                    ; Venous plasma
VR = VRC * BW                                           ; Richly perfused tissues
VS = (1 - VLC - VKC - VGC - VMC - VartC - VartC - VRC) * BW  ; Slowly perfused tissues

; Kinetic constants
; Repeated oral absorption rate constants
tlen = 0.01                                             ; Length of oral gavage exposure (h/day)
tinterval = 24                                          ; Varied dependent on the exposure paradigm (h)
Tdose = 3                                               ; Number of dosings for multiple oral gavage
REPEAT [1..Tdose] = SQUAREPULSE (0 + (i - 1) * tinterval, tlen)
Exposure = ARRAYSUM (REPEAT[*])

; Oral absorption and fecal elimination rate constants
Ka = 0.007                          ; /h, Absorption rate constant from foregut and midgut
Kah = 0.001                         ; /h, Absorption rate constant from hindgut
Kint = 3.100e-3                     ; /h, Gut transit rate constant
Kfeces = 0.025                      ; /h , Fecal elimination rate constant

; Rate constant for the enterohepatic circulation
KehcC = 0.016                       ; /h/kg

; Biliary elimination rate Constant
KbileC = 0.480                      ; L/h/kg

; Metabolic rate
Kehc = KehcC * BW                   ; /h

; Biliary elimination rate
Kbile = KbileC * BW                 ; L/h

; Percentage of plasma protein binding, measured in the present study
PB = 0.900                          ; Percentage of DC bound to plasma proteins
```

```
; IV infusion rate constant
Timeiv = 0.01                                   ; IV injection/infusion time (h)

; Urinary elimination rate constant
KurineC = 0.019                                 ; L/h/kg

; Urinary elimination rate
Kurine = KurineC * BW                           ; L/h

; Parameters for exposure scenarios
PDOSEiv = 0                                     ; mg/kg
PDOSEoral = 20                                  ; mg/kg

; Dosing
DOSEiv = PDOSEiv * BW                           ; mg
DOSEoral = PDOSEoral * BW                       ; mg

; Oral dosing model
RDOSEoral = (DOSEoral / tlen) * Exposure
RAI = RDOSEoral - Ka * AI – Kint * AI + Rbile - Kehc * AI
d/dt (AI) = RAI
init AI = 0
RAIh = Kint * AI - Kah * AIh - Rfeces
d/dt (AIh) = RAIh
init AIh = 0
Rfeces = Kfeces * AIh
d/dt (Afeces) = Rfeces
init Afeces = 0
RAO = Ka * AI + Kah * AIh
d/dt (AAO) = RAO
init AAO = 0

; DC iv injection to the venous
IVR = DOSEiv / Timeiv
Riv = IVR * (1.-step(1,Timeiv))
d/dt (Aiv) = Riv
init Aiv = 0

; DC in plasma compartment
RV = QL * CVL + QK * CVK + QM * CVM + QR * CVR + QS * CVS + Riv  - QC * CV
d/dt (AV) = RV
init AV = 0
CV = AV / Vven
d/dt (AUCCV) = CV
init AUCCV = 0

RA = QC * (CVG - CAfree)
d/dt (AA) = RA
init AA = 0
CA = AA / Vart
CAfree = CA * (1 - PB)

Aplasma = AV + AA

; DC in gill compartment
RG = QC * (CV - CVG)
```

```
d/dt (AG) = RG
init AG = 0
CG = AG / VG ;
CVG = AG / (VG * PG)
d/dt (AUCCG) = CG
init AUCCG = 0

; DC in liver compartment
RL = QL * (CAfree - CVL) + RAO - Rbile + Rehc
d/dt (AL) = RL
init AL = 0
CL = AL / VL ;
CVL = AL / (VL * PL)
d/dt (AUCCL) = CL
init AUCCL = 0

Rehc = Kehc * AI
d/dt (Aehc) = Rehc
init Aehc = 0

Rbile = Kbile * CVL
d/dt (Abile) = Rbile
init Abile = 0

; DC in kidney compartment
RK = QK * (CAfree - CVK) - Rurine
d/dt (AK) = RK
init AK = 0
CK = AK / VK
CVK = AK / (VK * PK)
d/dt (AUCCK) = CK
init AUCCK = 0

; Urinary excretion of DC
Rurine = Kurine * CVK
d/dt (Aurine) = Rurine
init Aurine = 0

; DC in muscle+skin compartment
RM = QM * (CAfree - CVM)
d/dt (AM) = RM
init AM = 0
CM = AM / VM
CVM = AM / (VM * PM)
d/dt (AUCCM) = CM
init AUCCM = 0

; DC in richly perfused tissue compartment
RR = QR * (CAfree - CVR)
d/dt (AR) = RR
init AR = 0
CR = AR / VR
CVR = AR/(VR * PR)

; DC in slowly perfused tissue compartment
RS = QS * (CAfree - CVS)
```

```
d/dt (AS) = RS
init AS = 0
CS = AS / VS
CVS = AS / (VS * PS)

; Mass balance
Qbal = QC – QL – QK – QM – QR – QS
Tmass = Aplasma + AL + AK + AG + AM + AR + AS + Aurine + Abile
Bal = Aiv + AAO + Aehc – Tmass
```

Population PBPK model code

The code used to run Monte Carlo analysis is based on combined sensitive parameters by examining influence on 24-h AUC and 1008-h AUC of plasma, liver, kidney, muscle+skin, and gill.

```
METHOD RK4

STARTTIME = 0
STOPTIME= 1400
DT = 0.0025
DTOUT = 0.01

;Physiological parameters
;Blood flow rates
QCC = 3.738                          ; Cardiac output (L/h/kg)
QLC = 0.181                          ; Fractional blood flow to the liver
QKC = 0.102                          ; Fractional blood flow to the kidney
QMC = 0.398                          ; Fractional blood flow to the muscle+skin
QGC = 1                              ; Fractional of blood flow to the gill
QRC = 0.010                          ; Fractional blood flow to richly perfused tissues
QSC = 0.309                          ; Fractional blood flow to slowly perfused tissues

; Body weight (kg)
k= 3.896                                    ; The slope of the equation
Factor = 1000                               ; Conversion from g to kg
Hoursinaday = 24                            ; Conversion from a day to hours
BW_0 = 0.45                                 ; kg, The intial body weight
BW = BW_0 + (k*(TIME/Hoursinaday)) / Factor  ; The equation of body weight growth


;Tissue volumes
BW = 0.450                           ; Body weight (kg)
VLC = 0.004                          ; Fractional liver
VKC = 0.004                          ; Fractional kidney
VMC = 0.386                          ; Fractional muscle+skin
VGC = 0.037                          ; Fractional gill
VRC = 0.030                          ; Fractional richly perfused tissues
VSC = 0.465                          ; Fractional slowly perfused tissues
VartC = 0.015                        ; Fractional arterial blood
VvenC = 0.059                        ; Fractional venous blood
Hematocrit = 0.254                   ; The blood hematocrit in grass carp
```

```
; Mass transfer parameters (Chemical-specific parameters)
; Partition coefficients (PC, tissue:plasma)
PL = 2.821                          ; Liver:plasma PC
PK = 1.064                          ; Kidney:plasma PC
PM = 0.901                          ; Muscle+skin:plasma PC
PG = 2.981                          ; Gill :plasma PC
PR = 2.821                          ; Richly perfused tissues:plasma
PS = 0.901                          ; Slowly perfused tissues:plasma

; Kinetic constants
; Repeated oral absorption rate constants
tlen = 0.01                                          ; Length of oral gavage exposure (h/day)
tinterval = 24                                       ; Varied dependent on the exposure paradigm (h)
Tdose = 3                                            ; Number of doses for multiple oral gavage
REPEAT[1..Tdose] = SQUAREPULSE (0+(i - 1) * tinterval, tlen)
Exposure = ARRAYSUM (REPEAT[*])

; Oral absorption and fecal elimination rate constants
Ka = 0.007                          ; /h, Absorption rate constant from foregut and midgut
Kah = 0.001                         ; /h, Absorption rate constant from hindgut
Kint = 3.100e-3                     ; /h, Gut transit rate constant
Kfeces = 0.025                      ; /h , Fecal elimination rate constant

; Rate constant for the enterohepatic circulation
KehcC = 0.016                       ; /h/kg,

; Biliary elimination rate constant
KbileC = 0.480                      ; L/h/kg,

; Metabolic rate constant
Kehc = KehcCm * BW                  ; /h

; Biliary elimination rate constant
Kbile = KbileCm * BW                ; L/h

; Percentage of plasma protein binding, measured in the present study
PB = 0.900                          ; Percentage of DC bound to plasma proteins

; IV infusion rate contant
Timeiv = 0.01                       ; IV injection/infusion time (h)

; Urinary elimination rate constant
KurineC = 0.019                ; L/h/kg

; Urinary elimination rate
Kurine = KurineCm * BW                      ; L/h


; Parameters for exposure scenarios
PDOSEiv = 0                         ; mg/kg
PDOSEoral = 20                      ; mg/kg

; Dosing
DOSEiv = PDOSEiv * BW               ; mg
DOSEoral = PDOSEoral * BW           ; mg
```

```
; Variances of Parameters
BW_sd = 5.320e-2                    ; Standard deviation of Body Weight
PL_sd = 5.642e-1                    ; Standard deviation of PL
PK_sd = 2.128e-1                    ; Standard deviation of PK
PM_sd = 1.802e-1                    ; Standard deviation of PM
PG_sd = 5.926e-1                    ; Standard deviation of PG
Ka_sd = 2.100e-3                    ; Standard deviation of Ka
PB_sd = 2.700e-1                    ; Standard deviation of PB
KehcC_sd = 4.800e-3                 ; Standard deviation of KehcC
KbileC_sd = 1.440e-1                ; Standard deviation of KbileC
Kint_sd = 9.300e-4                  ; Standard deviation of Kint
KurineC_sd = 5.580e-3               ; Standard deviation of KurineC

; Generation of Parameters based on Normal Distribution
init BWm = Normal(BW, BW_sd)        ; Generation of the BWm based on normal distribution

; Assignment of the Values to Parameters
next BWm = BWm                      ; Assignment of the first created value to BWm, without this step BWm
will change at each integration time step

; Lognormal Transformation of Parameters
PL_ln = logn(PL^2/(PL_sd^2+PL^2)^0.5)                    ; Lognormal transformation of PL values
PL_lnsd = (logn(1+PL_sd^2/PL^2))^0.5
PK_ln = logn(PK^2/(PK_sd^2+PK^2)^0.5)                    ; Lognormal transformation of PK values
PK_lnsd = (logn(1+PK_sd^2/PK^2))^0.5
PM_ln = logn(PM^2/(PM_sd^2+PM^2)^0.5)                    ; Lognormal transformation of PM values
PM_lnsd = (logn(1+PM_sd^2/PM^2))^0.5
PG_ln = logn(PG^2/(PG_sd^2+PG^2)^0.5)                    ; Lognormal transformation of PG values
PG_lnsd = (logn(1+PG_sd^2/PG^2))^0.5
PB_ln = logn(PB^2/(PB_sd^2+PB^2)^0.5)                    ; Lognormal transformation of PB values
PB_lnsd = (logn(1+PB_sd^2/PB^2))^0.5
Ka_ln = logn(Ka^2/(Ka_sd^2+Ka^2)^0.5)                    ; Lognormal transformation of Ka values
Ka_lnsd = (logn(1+Ka_sd^2/Ka^2))^0.5
KehcC_ln = logn(KehcC^2/(KehcC_sd^2+KehcC^2)^0.5)        ; Lognormal transformation of KehcC values
KehcC_lnsd = (logn(1+KehcC_sd^2/KehcC^2))^0.5
KbileC_ln = logn(KbileC^2/(KbileC_sd^2+KbileC^2)^0.5)    ; Lognormal transformation of KbileC values
KbileC_lnsd = (logn(1+KbileC_sd^2/KbileC^2))^0.5
Kint_ln = logn(Kint^2/(Kint_sd^2+Kint^2)^0.5)            ; Lognormal transformation of Kint values
Kint_lnsd = (logn(1+Kint_sd^2/Kint^2))^0.5
KurineC_ln = logn(KurineC^2/(KurineC_sd^2+KurineC^2)^0.5) ; Lognormal transformation of KurineC values
KurineC_lnsd = (logn(1+KurineC_sd^2/KurineC^2))^0.5

; Creation of Parameters based on Lognormal Distribution
init PLm = exp(Normal(PL_ln, PL_lnsd)) next PLm = PLm       ; Generation of PLm based on lognormal distribution
init PMm = exp(Normal(PM_ln, PM_lnsd)) next PMm = PMm       ; Generation of PMm based on lognormal distribution
init PKm = exp(Normal(PK_ln, PK_lnsd)) next PKm = PKm       ; Generation of PKm based on lognormal distribution
init PGm = exp(Normal(PG_ln, PG_lnsd)) next PGm = PGm       ; Generation of PGm based on lognormal distribution
init Kam = exp(Normal(Ka_ln, Ka_lnsd)) next Kam = Kam       ; Generation of Kam based on lognormal distribution
init PBm = exp(Normal(PB_ln, PB_lnsd)) next PBm = PBm       ; Generation of PBm based on lognormal distribution
init KehcCm = exp(Normal(KehcC_ln, KehcC_lnsd)) next KehcCm = KehcCm     ; Generation of KehcCm based on
lognormal distribution
init KbileCm = exp(Normal(KbileC_ln, KbileC_lnsd)) next KbileCm = KbileCm    ; Generation of KbileCm based on
lognormal distribution
init Kintm = exp(Normal(Kint_ln, Kint_lnsd)) next Kintm = Kintm              ; Generation of Kintm based on
lognormal distribution
```

```
init KurineCm = exp(Normal(KurineC_ln, KurineC_lnsd)) next KurineCm = KurineCm  ; Generation of KurineCm based on lognormal distribution

; Limit the parameter values within the lower and upper bounds
limit BWm >= 0.346
limit BWm <= 0.554
limit Kam >=0.004
limit Kam <= 0.012
limit KehcCm >= 0.009
limit KehcCm <= 0.027
limit KbileCm >= 0.259
limit KbileCm <= 0.817
limit PLm >= 1.876
limit PLm <= 4.078
limit PKm >= 0.708
limit PKm <= 1.538
limit PMm >= 0.599
limit PMm <= 1.303
limit PGm >= 1.983
limit PGm <= 4.309
limit PBm >=0.458
limit PBm <=0.990
limit Kintm >= 0.002
limit Kintm <= 0.005
limit KurineCm >= 0.010
limit KurineCm <= 0.032

;Cardiac output and blood flows to tissues (L/h)
QC = QCC * BWm * (1 - Hematocrit)          ; Cardiac output
QL = QLC * QC                              ; Liver
QK = QKC * QC                              ; Kidney
QG = QGC * QC                              ; Gill
QM = QMC * QC                              ; Muscle+skin
QR = QRC *QC                               ; Richly perfused tissues
QS = QSC * QC                              ; Slowly perfused tissues

; Tissue volume
VL = VLC * BWm                             ; Liver
VK = VKC * BWm                             ; Kidney
VG = VGC * BWm                             ; Gill
VM = VMC * BWm                             ; Muscle+skin
Vart = VartC * BWm * (1 - Hematocrit)      ; Arterial plasma
Vven = VvenC * BWm * (1 - Hematocrit)      ; Venous plasma
VR = VRC * BWm                             ; Richly perfused tissues
VS = VSC * BWm                             ; Slowly perfused tissues

; Oral Dosing model
RDOSEoral = (DOSEoral / tlen) * Exposure
RAI = RDOSEoral - Kam * AI – Kintm *AI + Rbile - Kehc * AI
d/dt (AI) = RAI
init AI = 0
RAIh = Kintm * AI - Kah * AIh - Rfeces
d/dt (AIh) = RAIh
init AIh = 0
Rfeces = Kfeces * AIh
d/dt (Afeces) = Rfeces
```

```
init Afeces = 0
RAO = Kam * AI + Kah * AIh
d/dt (AAO) = RAO
init AAO = 0

; DC iv injection to the venous
IVR = DOSEiv / Timeiv
Riv = IVR * (1.-step(1,Timeiv))
d/dt (Aiv) = Riv
init Aiv = 0

; DC in plasma compartment
RV = QL * CVL + QK * CVK + QM * CVM + QR * CVR + QS * CVS + Riv – QC * CV
d/dt (AV) = RV
init AV = 0
CV = AV / Vven
d/dt (AUCCV) = CV
init AUCCV = 0

RA = QC * (CVG - CAfree)
d/dt (AA) = RA
init AA = 0
CA = AA / Vart ;
CAfree = CA * (1-PBm)

Aplasma = AV + AA

; DC in gill compartment
RG = QC * (CV - CVG)
d/dt (AG) = RG ;
init AG = 0
CG = AG / VG ;
CVG = AG / (VG * PGm)
d/dt (AUCCG) = CG
init AUCCG = 0

; DC in liver compartment
RL = QL* (CAfree - CVL) + RAO - Rbile + Rehc
d/dt (AL) = RL
init AL = 0
CL = AL / VL
CVL = AL/(VL* PLm)
d/dt (AUCCL) = CL
init AUCCL = 0

Rehc = Kehc * AI
d/dt (Aehc) = Rehc
init Aehc = 0

Rbile = Kbile * CVL
d/dt (Abile) = Rbile
init Abile = 0

; DC in kidney compartment
RK = QK * (CAfree - CVK) - Rurine
d/dt (AK) = RK
```

```
init AK = 0
CK = AK / VK
CVK = AK / (VK * PKm)
d/dt (AUCCK) = CK
init AUCCK = 0

; Urinary excretion of DC
Rurine = Kurine * CVK
d/dt (Aurine) = Rurine
init Aurine = 0

; DC in muscle+skin compartment
RM = QM * (CAfree - CVM)
d/dt (AM) = RM
init AM = 0
CM = AM / VM
CVM = AM / (VM * PMm)
d/dt (AUCCM) = CM
init AUCCM = 0

; DC in richly perfused tissue compartment
RR = QR * (CAfree - CVR)
d/dt (AR) = RR
init AR = 0
CR = AR / VR
CVR = AR / (VR * PR)

; DC in slowly perfused tissue compartment
RS = QS * (CAfree - CVS)
d/dt (AS) = RS
init AS = 0
CS = AS / VS
CVS = AS / (VS * PS)

; Mass balance
Qbal = QC – QL – QK – QM – QR - QS
Tmass = Aplasma + AL + AK + AG + AM + AR + AS + Aurine + Abile
Bal = Aiv + AAO + Aehc - Tmass
```

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