Supplementary Materials

Development and Application of a Population Physiologically Based Pharmacokinetic Model for Penicillin G in Swine and Cattle for Food Safety Assessment

Miao Li, Ronette Gehring, Jim E. Riviere, Zhoumeng Lin*

Institute of Computational Comparative Medicine (ICCM), Department of Anatomy and Physiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506

Miao Li: miaoli@ksu.edu

Ronette Gehring: rgehring@ksu.edu

Jim E. Riviere: jriviere@ksu.edu

Zhoumeng Lin: zhoumeng@ksu.edu

* To whom correspondence should be addressed: zhoumeng@ksu.edu; Phone: +1-785-532-4087; Fax: +1-785-532-4953; Address: 1800 Denison Avenue, P200 Mosier Hall, Manhattan, KS 66506, USA. Website: http://www.k-state.edu/iccm/.

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Equations and Codes for the PBPK Model

1. **Two-Compartment Dissolution Model**

The undissolved procaine penicillin G acts as the depot of penicillin G, and maintains the therapeutic concentration of penicillin G for at least 24 hours (Papich and Riviere, 2009; Uboh et al., 2000). The IM and SC injections were simulated using a two-compartment injection site model with a dissolution process based on the approach used to simulate intramuscular absorption of long-acting oxytetracycline (Lin et al., 2015). This approach divides penicillin G into dissolved penicillin G moieties and undissolved procaine penicillin G acting as depot (**Figure 1**). **Equations S1-S7** that describe the process of IM injection are shown below:

$$\mathrm{Rpen} = \mathrm{Rinput} * (1\text{-}\mathrm{Frac}) \quad \text{(S1)}$$

$$\mathrm{Rppg} = \mathrm{Rinput} * \mathrm{Frac} \quad \text{(S2)}$$

$$\mathrm{Rdiss} = \mathrm{Appg}*\mathrm{Kdiss} \quad \text{(S3)}$$

$$\mathrm{Rim} = \mathrm{Kim}*\mathrm{Apen} \quad \text{(S4)}$$

$$d(Absorbim)/dt = \mathrm{Rim} \quad \text{(S5)}$$

$$d(Appg)/dt = \mathrm{Rppg} - \mathrm{Rdiss} \quad \text{(S6)}$$

$$d(Apen)/dt = \mathrm{Rpen} - \mathrm{Rim} + \mathrm{Rdiss} \quad \text{(S7)}$$

where Rinput is the administration rate of IM injection (mg/h); Rpen is the administration rate of penicillin G moieties (mg/h); Rppg is the administration rate of procaine penicillin G (mg/h); Frac is the fraction of procaine penicillin G stayed undissolved (unitless); Kdiss is the dissolution rate constant of procaine penicillin G into penicillin G moieties (h^{-1}); Rdiss is the dissolution rate of procaine penicillin G (mg/h); Rim is penicillin G absorption rate of IM route (mg/h); Appg is the amount of procaine penicillin G (mg); Apen is the amount of penicillin G moieties (mg); Kim

is the penicillin G absorption rate constant of IM administration (h^{-1}); Absorbim is the amount of penicillin G absorbed following IM injection (mg).

2. Hepatic Metabolism

A simplified first-order metabolic rate was used to simulate hepatic metabolism in this model (Fisher, 2000; Krishnan et al., 2009). The **Equation S8** describing the process of first-order liver metabolism is shown below:

$$Rmet = Kmet^* CL^*VL \quad (S8)$$

where Rmet is the metabolic rate of penicillin G in liver (mg/h); Kmet is the metabolic rate constant of penicillin G (/h); CL is the penicillin G concentration in liver tissue (mg/L); VL is the tissue volume for liver (L).

3. Urinary Elimination

A first-order urinary elimination equation in the kidney compartment was adapted from previous research (Craigmill, 2003). The **Equation S9** describing the process of first-order urinary elimination is shown below:

$$Rurine = Kurine * CVK \quad (S9)$$

where Rurine is the urine elimination rate of penicillin G (mg/h); Kurine is the urine elimination rate constant of penicillin G (L/h); CVK is the penicillin G concentration in kidney venous blood (mg/L).

4. Mass Balance Equations in Kidney

The rate of change for penicillin G in each tissue compartment was described using mass balance differential equations as we previously described (Leavens et al., 2012; Lin et al., 2015). Only penicillin G not bound to plasma proteins was considered as available for distribution. Kidney compartment was used as example for other tissue compartments. **Equations S10-S11** for mass

balance of penicillin G in flow-limited compartments using kidney as an example are described below.

$$RK = QK * (CAfree - CVK) – Rurine \quad (S10)$$

$$CVK = CK/PK \quad (S11)$$

where RK is the rate of distribution of penicillin G in the kidney (mg/h); QK is the volume of blood flow to the kidney per hour (L/h); CAfree is the arterial blood concentration of penicillin G not bound with plasma proteins (mg/L); CVK is the concentration of penicillin G in the kidney venous blood (mg/L); Rurine is the urinary elimination rate of penicillin G (mg/h); CK is the penicillin G concentration in the kidney (mg/L); PK is the kidney:plasma partition coefficient (unitless).

5. Sensitivity Analysis

A local sensitivity analysis was performed to determine which parameters were most influential on the 24-hour AUC of penicillin G concentrations in plasma, liver, kidney, and muscle. Each parameter was increased by 1%, 5%, or 10% and the corresponding 24-hour AUC of penicillin concentrations were computed. Normalized sensitivity coefficient (NSC) was calculated using **Equation S12** (Lin et al., 2011; Mirfazaelian et al., 2006) as shown below:

$$NSC = \Delta r/r * p/\Delta p \quad (S12)$$

where r is the response variable, and Δr is the change of the response variable resulting from 1%, 5%, or 10% increase in the parameter value, p is the original value of the parameter of interest, Δp is 1%, 5%, or 10% of the original value of the parameter of interest. The relative influence of each parameter on the response variables was categorized as: low: $|NSC|<0.2$; medium: $0.2\leq|NSC|<0.5$; high: $0.5\leq|NSC|$ (Lin et al., 2013; Yoon et al., 2009).

Supplementary Figures

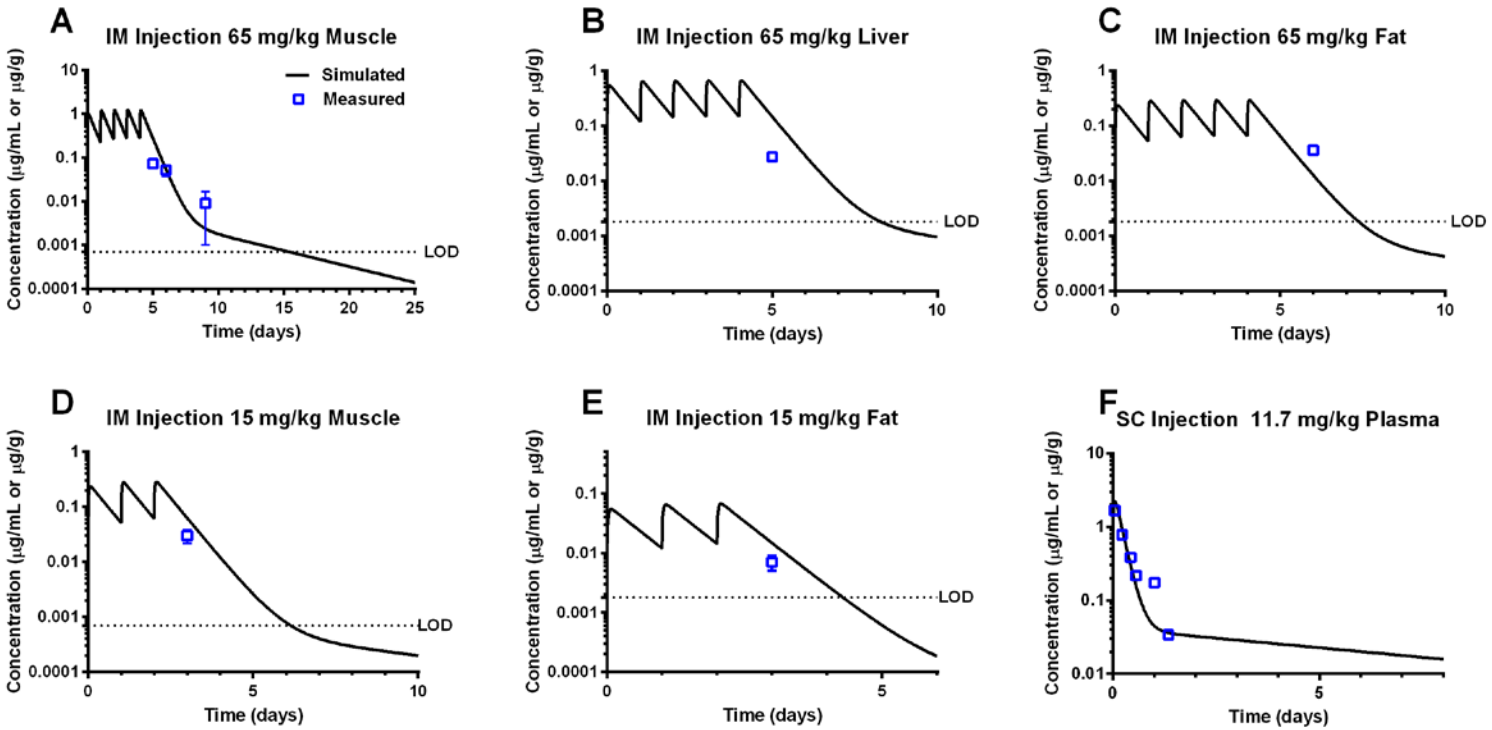

Figure S1. Calibration of the swine model. Comparison of model predictions (solid line) and observed data (blue squares) for penicillin G concentrations in the plasma, liver, fat and muscle of pigs exposed to procaine penicillin G via IM repeated 5 doses (65 mg/kg, A, B, C), IM repeated 3 doses (15 mg/kg, D, E), and single dose SC injection (11.7 mg/kg, F) are shown. Experimental data (mean ± SEM) are from previous studies (A-E) (Korsrud et al., 1998) and (F) (Ranheim et al., 2002). Limit of detection (LOD) is shown on each of the six panels using dotted line. LOD for the plasma is 1.5 ng/g, for the fat is 1.8 ng/g, for the liver is 1.8 ng/g, and for the muscle is 0.7 ng/g (Lupton et al., 2014).

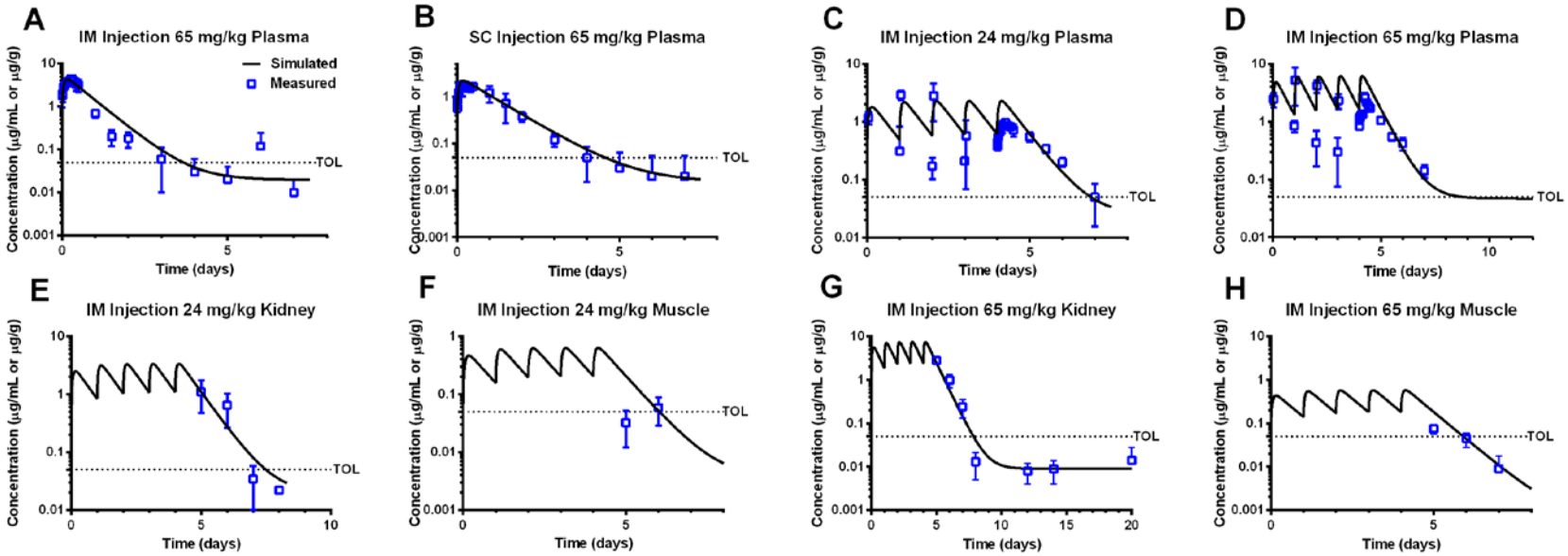

Figure S2. Calibration of the cattle model. Comparison of model predictions (solid line) and observed data (blue squares) for penicillin G concentrations in the plasma, muscle, and kidney of cattle exposed to procaine penicillin G via IM single dose (65 mg/kg, A), SC single dose (65 mg/kg, B), IM with 5 repeated doses (24 mg/kg, C, E, F; 65 mg/kg, D, G, H) is shown. Experimental data for A, B, C, D (mean ± SEM) are from the previous study (Papich et al., 1993), and E, F, G, H (mean ± SEM) are from the study by Korsrud et al. (Korsrud et al., 1993). Tolerance (TOL) of penicillin G (0.05 µg/g) is shown on each of the panels using dotted line.

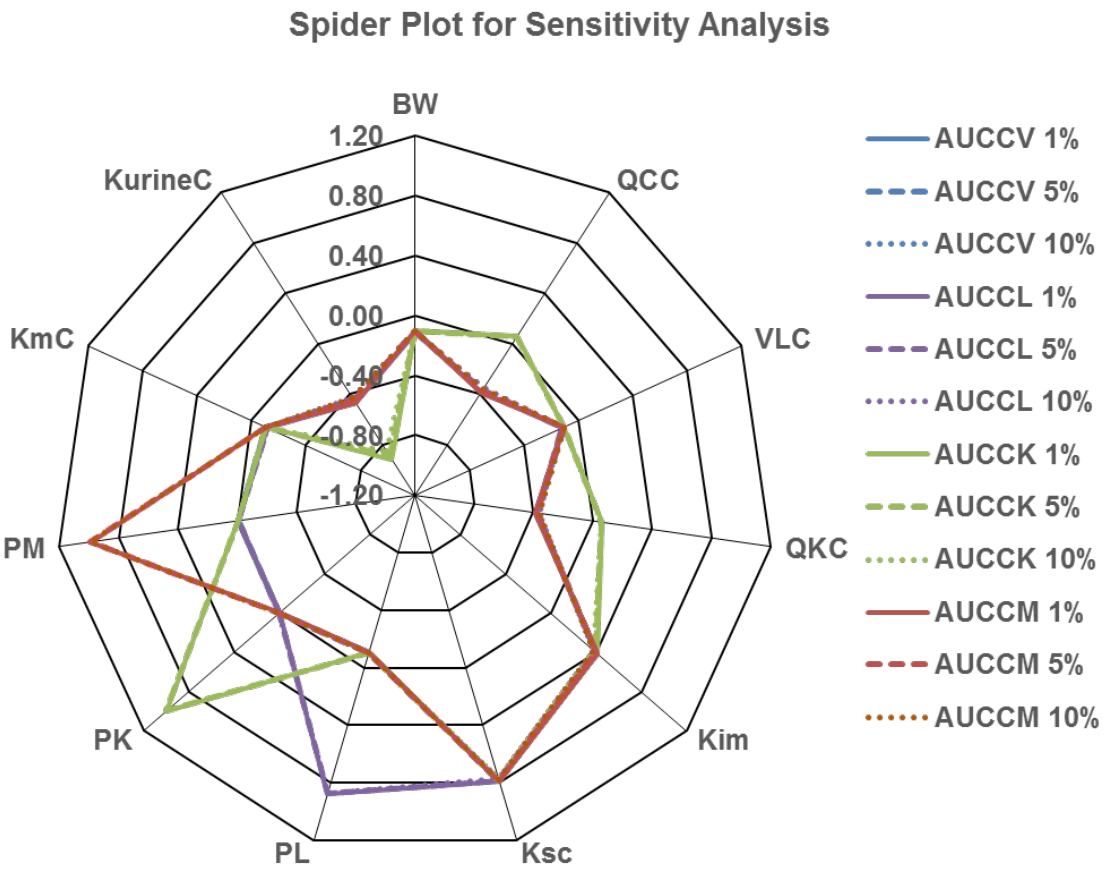

Figure S3. Comparison of the changes of NSCs (normalized sensitivity coefficients) by 1%, 5% and 10% variations in parameter values. Only the sensitive parameters with at least one absolute value of NSC greater than 0.1 are plotted in the graph. Due to superimposing with other lines, the lines for the AUCCV are hard to be identified.

Supplementary Tables

Table S1. Normalized sensitivity coefficients of representative parameters using AUCs for concentrations of penicillin G in plasma, liver, kidney and muscle as the dose metrics.

Parameters	AUCs of Penicillin Concentrations			
	AUCCV	AUCCL	AUCCK	AUCCM
BW	-0.10	-0.12	-0.10	-0.10
QCC	-0.39	-0.38	0.06	-0.39
QKC	-0.39	-0.39	0.06	-0.39
VLC	-0.11	-0.12	-0.11	-0.11
Ksc	0.79	0.79	0.79	0.79
Kim	0.41	0.41	0.41	0.41
PL	-0.11	0.88	-0.11	-0.11
PK	0.00	0.00	1.00	0.00
PM	-0.01	-0.01	-0.01	0.99
KmC	-0.10	-0.12	-0.10	-0.10
KurineC	-0.46	-0.46	-0.92	-0.46

Notes: Only parameters with at least one absolute value of NSC greater than 0.1 are shown in the table. AUCCV, AUCCL, AUCCK, and AUCCM represent 24-hour area under concentration curves of penicillin in plasma, liver, kidney and muscle. Please refer to **Table 2** for abbreviations of parameters.

PBPK Model Code

Note: The Berkeley Madonna model code below is a general physiologically based pharmacokinetic (PBPK) model for procaine penicillin G in cattle and swine. Parameter values used in the model code are for procaine penicillin G in cattle. All parameter values in swine and cattle are summarized in **Tables 2-3.** Physiological parameter values of cattle and swine reported in our earlier paper by Lin et al. (2016) were for an average animal collected from previously published PBPK models. In the present penicillin G modeling work, in order to conduct population analysis, we need to have distributions of all parameters, so we conducted more extensive literature search on the physiological parameters of cattle and swine. As a result, some of the physiological parameters have been updated in this study. The updated physiological parameter values will be used in our subsequent models. Overall, the mean value of each physiological parameter is still quite close to the value reported in Lin et al. (2016) for both cattle and swine.

```
{
Penicillin PBPK model for Cattle (flow-limited model, linear metabolism equation, plasma protein binding)
The PBPK model code is based on the Oxytetracycline.mmd from Lin et al. (2015)
}

METHOD RK4

STARTTIME = 0
STOPTIME= 100                   ; h
DT = 0.000391
DTOUT = 0.1

{Physiological Parameters}
; Blood Flow Rates
QCC = 5.97                      ; L/h/kg, Cardiac Output (Doyle et al., 1960)

; Fracion of blood flow to organs (unitless)
QLC =  0.405                    ; Fraction of blood flow to the liver (Doyle et al., 1960; Lescoat et al., 1996)
QKC = 0.09                      ; Fraction of blood flow to the kidneys (Lin et al., 2016)
QFC = 0.08                      ; Fraction of blood flow to the fat (Lin et al., 2016)
QMC = 0.18                      ; Fraction of blood flow to the muscle (Lin et al., 2016)
QLuC = 1                        ; Fraction of blood flow to the lung (Achenbach, 1995)
QrestC = 1-QLC-QKC-QFC-QMC      ; Fraction of blood flow to the rest of body (total sum equals to 1)

; Tissue Volumes
BW = 300                        ; Body Weight (kg) (Mirzaei et al., 2011)
; Fractional organ tissue volumes (unitless)
VLC = 0.014                     ; Fractional liver tissue (Swett et al., 1933)
VKC = 0.002                     ; Fractional kidney tissue (Swett et al., 1933)
VFC = 0.15                      ; Fractional fat tissue (Lin et al., 2016; Leavens et al., 2014)
VMC = 0.27                      ; Fractional muscle tissue (Lin et al., 2016; Leavens et al., 2014)
VLuC = 0.008                    ; Fractional lung tissue (Lin et al., 2016; Leavens et al., 2014; Swett et al., 1933)
VvenC = 0.0296                  ; Venous blood volume, fraction of blood volume (Lin et al., 2016)
VartC = 0.0104                  ; Arterial blood volume, fraction of blood volume (Lin et al., 2016)
VrestC = 1-VLC-VKC-VFC-VMC-VLuC-VvenC-VartC; Fractional rest of body (total sum equals to 1)

{Mass Transfer Parameters (Chemical-Specific Parameters)}
; Partition Coefficients (PC, tissue:plasma)
PL = 3                          ; Liver:plasma PC (0.157, Tsuji et al., 1983, Table 4, in rats)
PM = 0.3                        ; Muscle:plasma PC (0.062, Tsuji et al., 1983, Table 4, in rats)
PF = 0.04                       ; Fat:plasma PC (0.062, adapted from muscle partition coefficient)
PK = 2.5                        ; Kidney:plasma PC (3.70, Tsuji et al., 1983, Table 4, in rats)
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PLu = 0.18                  ; Lung:plasma PC (0.157, Tsuji et al., 1983, Table 4, in rats)
Prest = 0.479               ; Rest of body:plasma PC (Cao et al. 2012, Table 1, estimated value in human)

{Kinetic Constants}
; IM Absorption Rate Constants
Kim = 0.07                  ; /h, IM absorption rate constant

; SC Absorption Rate Constants
Ksc = 0.02                  ; /h, SC absorption rate constant

; Percentage Plasma Protein Binding unitless
PB = 0.483                  ; Percentage of drug bound to plasma proteins (Keen, 1965)
Free = 1-PB

; Urinary Elimination Rate Constants
KurineC = 0.45              ; L/h/kg

; Metabolic Rate Constant
KmC = 0.0025                ; /h/kg

{Parameters for Various Exposure Scenarios}
PDOSEsc = 0                 ; (mg/kg)
PDOSEim = 65                ; (mg/kg)

{Cardiac output and blood flow to tissues (L/h)}
QC = QCC*BW                 ; Cardiac output
QL = QLC*QC                 ; Liver
QK = QKC*QC                 ; Kidney
QF = QFC*QC                 ; Fat
QM = QMC*QC                 ; Muscle
QLu = QLuC*QC               ; Lung
QR = QrestC*QC              ; Rest of body

{Tissue volumes (L)}
VL = VLC*BW                 ; Liver
VK = VKC*BW                 ; Kidney
VF = VFC*BW                 ; Fat
VM = VMC*BW                 ; Muscle
VLu = VLuC*BW               ; Lung
VR = VrestC*BW              ; Rest of body
Vven = VvenC*BW             ; Venous Blood
Vart = VartC*BW             ; Arterial Blood

; Urinary Elimination Rate Constants
Kurine = KurineC*BW

; Metabolic Rate Constant
Kmet = KmC*BW

{Dosing}
; Dosing caculation based on BW
DOSEsc = PDOSEsc*BW         ; (mg)
DOSEim = PDOSEim*BW         ; (mg)

Kdiss = 1e-5                ; /h
Kdisssc = 1e-4              ; /h
```

```
Frac = 0.6

; Dosing, repeated doses
tinterval = 24                  ; Varied dependent on the exposure paradigm (h)
Tdoses = 5                      ; times for multiple oral gavage

dosingperiod = if time < Tdoses*tinterval-DT then 1 else 0

; Dosing, IM, intramuscular
Rinputim = pulse(DOSEim,0,tinterval)*dosingperiod
Rpenim = Rinputim*(1-Frac);
Rppgim =  Rinputim*Frac;
Rim = Kim*Amtsiteim
d/dt(Absorbim) = Rim
init Absorbim = 0
d/dt(Amtsiteim) = Rpenim- Rim + Kdiss* DOSEppgim
init Amtsiteim = 0
d/dt(DOSEppgim) = Rppgim-Kdiss* DOSEppgim
init DOSEppgim = 0

; Dosing, SC, subcutaneous
Rinputsc = pulse(DOSEsc,0,tinterval)*dosingperiod
Rpensc = Rinputsc*(1-Frac);
Rppgsc =  Rinputsc*Frac;
Rsc = Ksc*Amtsitesc
d/dt(Absorbsc) = Rsc
init Absorbsc = 0
d/dt(Amtsitesc) = Rpensc- Rsc + Kdisssc* DOSEppgsc
init Amtsitesc = 0
d/dt(DOSEppgsc) = Rppgsc-Kdisssc* DOSEppgsc
init DOSEppgsc = 0

{Penicillin distribution in each compartment}
; Penicillin in venous blood compartment
RV = (QL*CVL+QK*CVK+QF*CVF+QM*CVM+QR*CVR+Rsc+Rim)-QC*CV; RV the changing rate in the
venous blood (mg/h)
d/dt(AV) = RV                   ; AV the amount of the drug in the venous blood (mg)
init AV = 0
CV = AV/Vven                    ; CV drug concentration in the venous blood (mg/L)
RA = QC*(CVLu-CAfree)           ; RA the changing rate in the arterial blood (mg/h)
d/dt(AA) = RA
init AA = 0                     ; AA the amount of the drug in the arterial blood (mg)
CA = AA/Vart                    ; CAfree concentration of unbound drug in the arterial blood (mg/L)
CAfree = CA*Free
d/dt(AUCCV) = CV                ; AUCCV AUC of drug concentration in the venous blood (mg*h/L)
init AUCCV = 0

ABlood = AA+AV

; Penicillin in liver compartment, flow-limited model
RL = QL*(CAfree-CVL)-Rmet       ; RL the changing rate of the amount of drug in liver (mg/h)
d/dt(AL) = RL                   ; AL amount of drug in liver (mg)
init AL = 0
CL = AL/VL                      ; CL drug concentration in liver (mg/L)
CVL= AL/(VL*PL)                 ; CVL drug concentration in venous blood from liver (mg/L)
d/dt(AUCCL) = CL                ; AUCCL area under the curve of drug concentration in liver (mg*h/L)
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```
init AUCCL = 0

; Metabolism of Penicillin in liver compartment
Rmet = Kmet*CL*VL              ; Rmet the metabolic rate in liver (mg/h)
d/dt(Amet) = Rmet              ; Amet the amount of drug metabolized in liver (mg)
init Amet = 0

; Penicillin in kidney compartment, flow-limited model
RK = QK*(CAfree-CVK)-Rurine    ; RK the changing rate of the amount of drug in kidney (mg/h)
d/dt(AK) = RK                  ; AK amount of drug in kidney (mg)
init AK = 0
CK = AK/VK                     ; CK drug concentration in kidney (mg/L)
CVK = AK/(VK*PK)
d/dt(AUCCK) = CK               ; AUCCK AUC of drug concentration in kidney (mg*h/L)
init AUCCK = 0

; Penicillin urinary excretion
Rurine = Kurine*CVK
d/dt(Aurine) = Rurine
init Aurine = 0

; Penicillin in muscle compartment, flow-limited model
RM = QM*(CAfree-CVM)           ; RM the changing rate of the amount of drug in muscle (mg/h)
d/dt(AM) = RM                  ; AM amount of the drug in muscle (mg)
init AM = 0
CM = AM/VM                     ; CM drug concentration in muscle (mg/L)
CVM = AM/(VM*PM)
d/dt(AUCCM) = CM
init AUCCM = 0

; Penicillin in fat compartment, flow-limited model
RF = QF*(CAfree-CVF)           ; RF the changing rate of the amount of drug in fat (mg/h)
d/dt(AF) = RF                  ; AF amount of the drug in fat (mg)
init AF = 0
CF = AF/VF                     ; CF drug concentration in fat (mg/L)
CVF = AF/(VF*PF)
d/dt(AUCCF) = CF               ; AUCCF AUC of drug concentration in fat (mg*h/L)
init AUCCF = 0

; Penicillin in the compartment of rest of body, flow-limited model
RR = QR*(CAfree-CVR)  ; Rrest the changing rate of the amount of drug in the rest of the body (mg/h)
d/dt(AR) = RR                  ; Arest amount of the drug in the rest of the body (mg)
init AR = 0
CR = AR/VR                     ; Crest drug concentration in the rest of the body (mg/L)
CVR = AR/(VR*Prest)
d/dt(AUCCR) = CR               ; AUCCrest AUC of drug concentration in the rest of the body (mg*h/L)
init AUCCR = 0

; Penicillin in lung compartment, flow-limited model
RLu = QLu*(CV-CVLu)            ; RLu the changing rate of the amount of drug in the lung (mg/h)
d/dt(ALu) = RLu                ; ALu amount of the drug in the lung (mg)
init ALu = 0
CLu = ALu/VLu                  ; CLu drug concentration in the rest of the lung (mg/L)
CVLu = ALu/(VLu*PLu)
d/dt(AUCCLu) = CLu             ; AUCCLu AUC of drug concentration in the lung (mg*h/L)
init AUCCLu = 0
```

{Mass balance equations}
Qbal = QC-QM-QR-QF-QK-QL
Tmass = ABlood+AM+ALu+AR+AF+AK+AL+Aurine+Amet
Input = Absorbim+Absorbsc
Bal = Input-Tmass

Population PBPK Model Code

Note: The Berkeley Madonna model code below is a population physiologically based pharmacokinetic (PBPK) model for procaine penicillin G in cattle and swine. Parameter values used in the model code are for procaine penicillin G in cattle. All parameter values used for population model in swine and cattle are summarized in **Tables 2-3.** Physiological parameter values of cattle and swine reported in our earlier paper by Lin et al. (2016) were for an average animal collected from previously published PBPK models. In the present penicillin G modeling work, in order to conduct population analysis, we need to have distributions of all parameters, so we conducted more extensive literature search on the physiological parameters of cattle and swine. As a result, some of the physiological parameters have been updated in this study. The updated physiological parameter values will be used in our subsequent models. Overall, the mean value of each physiological parameter is still quite close to the value reported in Lin et al. (2016) for both cattle and swine.

```
{
Monte Carlo Analysis based on Penicillin PBPK model for Cattle (flow-limited model, linear metabolism equation, plasma protein binding)
The PBPK model code is based on the Oxytetracycline.mmd from Zhoumeng Lin
}

METHOD RK4

STARTTIME = 0
STOPTIME= 250                ; h, 24
DT = 0.000125
DTOUT = 0.1

{Physiological Parameters}
; Blood Flow Rates
QCC = 5.970                  ; L/h/kg, Cardiac Output (1960 Doyle)

; Fracion of blood flow to organs (unitless)
QLC =  0.405                 ; Fraction of blood flow to the liver (1996 Lescoat, 1960 Dlyle)
QKC = 0.090                  ; Fraction of blood flow to the kidneys (2016 Lin)
QMC = 0.180                  ; Fraction of blood flow to the muscle (2016 Lin)
QFC = 0.080                  ; Fraction of blood flow to the fat (2016 Lin)
QLuC = 1                     ; Fraction of blood flow to the lung considered to be 1
QrestC = 0.245               ; Fraction of blood flow to the rest of body (total sum equals to 1)

; Tissue Volumes
BW = 299.96                  ; Body Weight (kg) (2011 Mirzaei)

; Fractional organ tissue volumes (unitless)
VLC = 0.014                  ; Fractional liver tissue (1933 Swett)
VKC = 0.002                  ; Fractional kidney tissue (1933 Swett)
VFC = 0.150                  ; Fractional fat tissue (2016 Lin, 2014 Leavens)
VMC = 0.270                  ; Fractional muscle tissue (2016 Lin, 2014 Leavens)
VLuC = 0.008                 ; Fractional lung tissue (2016 Lin, 2014 Leavens)
VvenC = 0.030                ; Venous blood volume, fraction of blood volume (2016 Lin; 2008 Leavens)
VartC = 0.010                ; Arterial blood volume, fraction of blood volume (2016 Lin; 2008 Leavens)
VrestC = 0.516               ; Fractional rest of body (total sum equals to 1)

{Mass Transfer Parameters (Chemical-Specific Parameters)}
; Partition Coefficients (PC, tissue:plasma)
PL = 3.000                   ; Liver:plasma PC (0.157, Tsuji et al., 1983, Table 4, in rats)
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PK = 2.500                  ; Kidney:plasma PC (3.70, Tsuji et al., 1983, Table 4, in rats)
PM = 0.300                  ; Muscle:plasma PC (0.062, Tsuji et al., 1983, Table 4, in rats)
PF = 0.040                  ; Fat:plasma PC (0.062, adapted from muscle partition coefficient)
PLu = 0.180                 ; Lung:plasma PC (0.157, Tsuji et al., 1983, Table 4, in rats)
Prest = 0.479               ; Rest of body:plasma PC (Cao et al. 2012, Table 1, estimated value in human)

{Kinetic Constants}
; IM Absorption Rate Constants
Kim = 0.070                 ; /h, IM absorption rate constant
Frac = 0.600
Kdiss = 1e-5                ; /h

; SC Absorption Rate Constants
Ksc = 0.020                 ; /h, SC absorption rate constant
Fracsc = 0.700
Kdisssc = 1e-4

; Percentage Plasma Protein Binding unitless
PB = 0.483                  ; Percentage of drug bound to plasma proteins (1965 Keen)
Free = 1-PBm                ; Percentage of drug not bound to plasma protein

{Metabolic Rate Constant}
KmC = 0.0025                ; /h/kg, metabolic rate constant

; Urinary Elimination Rate Constants
KurineC = 0.450             ; L/h/kg

{Parameters for Various Exposure Scenarios}
PDOSEsc = 0                 ; (mg/kg)
PDOSEim = 6.5               ; (mg/kg)

{Variances of Parameters}
QCC_sd = 1.99               ; Standard deviation of QCC
QLC_sd = 0.1942             ; Standard deviation of QLC
QKC_sd = 0.027              ; Standard deviation of QKC
QMC_sd = 0.054              ; Standard deviation of QMC
QFC_sd = 0.024              ; Standard deviation of QFC
QrestC_sd = 0.0736          ; Standard deviation of QrestC
BW_sd = 46.18               ; Standard deviation of Body Weight
VLC_sd = 1.63e-3            ; Standard deviation of VLC
VKC_sd = 4.321e-4           ; Standard deviation of VKC
VMC_sd = 8.1e-2             ; Standard deviation of VMC
VFC_sd = 4.5e-2             ; Standard deviation of VFC
VLuC_sd = 1.696e-3          ; Standard deviation of VLuC
VrestC_sd = 0.1548          ; Standard deviation of VrestC
VvenC_sd = 8.88e-3          ; Standard deviation of VvenC
VartC_sd = 3.12e-3          ; Standard deviation of VartC
PL_sd = 0.6                 ; Standard deviation of PL
PK_sd = 0.50                ; Standard deviation of PK
PM_sd =0.060                ; Standard deviation of PM
PF_sd = 8e-3                ; Standard deviation of PF
PLu_sd = 3.6e-2             ; Standard deviation of PLu
Prest_sd = 9.58e-2          ; Standard deviation of Prest
Kim_sd = 2.10e-2            ; Standard deviation of Kim
Frac_sd = 0.06              ; Standard deviation of Frac
Kdiss_sd = 3e-6             ; Standard deviation of Kdiss
```

```
Ksc_sd = 6e-3                    ; Standard deviation of Ksc
Fracsc_sd = 0.07                 ; Standard deviation of Fracsc
Kdisssc_sd = 3e-5                ; Standard deviation of Kdisssc
PB_sd = 0.1449                   ; Standard deviation of PB
KmC_sd = 7.5e-4                  ; Standard deviation of KmC
KurineC_sd = 0.135               ; Standard deviation of KurineC

{Generation of Parameters based on Normal Distribution}
; Generation of Parameters based on Normal Distribution
init QCCm = Normal(QCC, QCC_sd)              ; Generation of the QCCm based on normal distribution
init QLCm = Normal(QLC, QLC_sd)              ; Generation of the QLCm based on normal distribution
init QKCm = Normal(QKC, QKC_sd)              ; Generation of the QKCm based on normal distribution
init QFCm = Normal(QFC, QFC_sd)              ; Generation of the QFCm based on normal distribution
init QMCm = Normal(QMC, QMC_sd)              ; Generation of the QMCm based on normal distribution
init QrestCm = Normal(QrestC, QrestC_sd)     ; Generation of the QrestCm based on normal distribution
init BWm = Normal(BW, BW_sd)                 ; Generation of the BWm based on normal distribution
init VLCm = Normal(VLC, VLC_sd)              ; Generation of the VLCm based on normal distribution
init VKCm = Normal(VKC, VKC_sd)              ; Generation of the VKCm based on normal distribution
init VMCm = Normal(VMC, VMC_sd)              ; Generation of the VMCm based on normal distribution
init VFCm = Normal(VFC, VFC_sd)              ; Generation of the VFCm based on normal distribution
init VLuCm = Normal(VLuC, VLuC_sd)           ; Generation of the VLuCm based on normal distribution
init VrestCm = Normal(VrestC, VrestC_sd)     ; Generation of the VrestCm based on normal distribution
init VvenCm = Normal(VvenC, VvenC_sd)        ; Generation of the VvenCm based on normal distribution
init VartCm = Normal(VartC, VartC_sd)        ; Generation of the VvartCm based on normal distribution

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

; Creation of Adjust Factor
AdjustF = QLCm+QKCm+QFCm+QMCm+QrestCm                                   ; Adjust factor to keep the
sum of blood flow fractions to 1
AdjustF1=VLCm+VKCm+VMCm+VFCm+VLuCm+VrestCm+VvenCm+VartCm                ; Adjustment factor to
make sure the sum of fractions of organ tissue volumes to be 1

; Creation of Adjusted Parameters
next QLCm = QLCm/AdjustF                     ; Adjustment of QLCm based on the adjust factor
next QKCm = QKCm/AdjustF                     ; Adjustment of QKCm
next QFCm = QFCm/AdjustF                     ; Adjustment of QFCm
next QMCm = QMCm/AdjustF                     ; Adjustment of QMCm
next QrestCm = QrestCm/AdjustF               ; Adjustment of QrestCm
next VLCm=VLCm/AdjustF1                      ; Adjustment of VLCm based on the adjust factor
next VKCm=VKCm/AdjustF1                      ; Adjustment of VKCm based on the adjust factor
next VMCm=VMCm/AdjustF1                      ; Adjustment of VMCm based on the adjust factor
next VFCm=VFCm/AdjustF1                      ; Adjustment of VFCm based on the adjust factor
next VLuCm=VLuCm/AdjustF1                    ; Adjustment of VLuCm based on the adjust factor
next VrestCm=VrestCm/AdjustF1                ; Adjustment of VrestCm based on the adjust factor
next VvenCm=VvenCm/AdjustF1                  ; Adjustment of VvenCm based on the adjust factor
next VartCm=VartCm/AdjustF1                  ; Adjustment of VartCm based on the adjust factor

{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
PF_ln = logn(PF^2/(PF_sd^2+PF^2)^0.5)                    ; Lognormal transformation of PF values
PF_lnsd = (logn(1+PF_sd^2/PF^2))^0.5
PLu_ln = logn(PLu^2/(PLu_sd^2+PLu^2)^0.5)                ; Lognormal transformation of PLu values
PLu_lnsd = (logn(1+PLu_sd^2/PLu^2))^0.5
Prest_ln = logn(Prest^2/(Prest_sd^2+Prest^2)^0.5)        ; Lognormal transformation of Prest values
Prest_lnsd = (logn(1+Prest_sd^2/Prest^2))^0.5
Kim_ln = logn(Kim^2/(Kim_sd^2+Kim^2)^0.5)                ; Lognormal transformation of Kim value
Kim_lnsd = (logn(1+Kim_sd^2/Kim^2))^0.5
Frac_ln = logn(Frac^2/(Frac_sd^2+Frac^2)^0.5)            ; Lognormal transformation of Frac value
Frac_lnsd = (logn(1+Frac_sd^2/Frac^2))^0.5
Kdiss_ln = logn(Kdiss^2/(Kdiss_sd^2+Kdiss^2)^0.5)        ; Lognormal transformation of Kdiss value
Kdiss_lnsd = (logn(1+Kdiss_sd^2/Kdiss^2))^0.5
Ksc_ln = logn(Ksc^2/(Ksc_sd^2+Ksc^2)^0.5)                ; Lognormal transformation of Ksc value
Ksc_lnsd = (logn(1+Ksc_sd^2/Ksc^2))^0.5
Fracsc_ln = logn(Fracsc^2/(Fracsc_sd^2+Fracsc^2)^0.5)    ; Lognormal transformation of Fracsc value
Fracsc_lnsd = (logn(1+Fracsc_sd^2/Fracsc^2))^0.5
Kdisssc_ln = logn(Kdisssc^2/(Kdisssc_sd^2+Kdisssc^2)^0.5) ; Lognormal transformation of Kdisssc value
Kdisssc_lnsd = (logn(1+Kdisssc_sd^2/Kdisssc^2))^0.5
PB_ln = logn(PB^2/(PB_sd^2+PB^2)^0.5)                    ; Lognormal transformation of PB
PB_lnsd = (logn(1+PB_sd^2/PB^2))^0.5
KmC_ln = logn(KmC^2/(KmC_sd^2+KmC^2)^0.5)                ; Lognormal transformation of KmC
KmC_lnsd = (logn(1+KmC_sd^2/KmC^2))^0.5
KurineC_ln = logn(KurineC^2/(KurineC_sd^2+KurineC^2)^0.5) ; Lognormal transformation of KurineC
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
init PFm = exp(Normal(PF_ln, PF_lnsd)) next PFm = PFm                                  ; Generation of PFm
init PKm = exp(Normal(PK_ln, PK_lnsd)) next PKm = PKm                                  ; Generation of PKm
init PLum = exp(Normal(PLu_ln, PLu_lnsd)) next PLum = PLum                             ; Generation of
PLum
init Prestm = exp(Normal(Prest_ln, Prest_lnsd)) next Prestm = Prestm                   ; Generation of Prestm
init Kimm = exp(Normal(Kim_ln, Kim_lnsd)) next Kimm = Kimm                             ; Generation of
Kimm
init Fracm = exp(Normal(Frac_ln, Frac_lnsd)) next Fracm = Fracm                        ; Generation of Fracm
init Kdissm = exp(Normal(Kdiss_ln, Kdiss_lnsd)) next Kdissm = Kdissm                   ; Generation of
Kdissm
init Kscm = exp(Normal(Ksc_ln, Ksc_lnsd)) next Kscm = Kscm                             ; Generation of
Kscm
init Fracscm = exp(Normal(Fracsc_ln, Fracsc_lnsd)) next Fracscm = Fracscm              ; Generation of Fracscm
init Kdissscm = exp(Normal(Kdisssc_ln, Kdisssc_lnsd)) next Kdissscm = Kdissscm        ; Generation of Kdissscm
init PBm = exp(Normal(PB_ln, PB_lnsd)) next PBm = PBm                                  ; Generation of PBm
init KmCm = exp(Normal(KmC_ln, KmC_lnsd)) next KmCm = KmCm                             ; Generation of
KmCm
init KurineCm = exp(Normal(KurineC_ln, KurineC_lnsd)) next KurineCm = KurineCm         ; Generation of KurineCm

{limit the parameter values within the lower and upper bounds}
limit BWm >= 209.45
limit BWm <= 390.464
limit QCCm >= 2.07
limit QCCm <= 9.87
```

limit VartCm >= 0.004
limit VartCm <= 0.017
limit VvenCm >= 0.012
limit VvenCm <= 0.047
limit VLCm >= 0.010
limit VLCm <= 0.017
limit VKCm >= 0.002
limit VKCm <= 0.003
limit VMCm >= 0.111
limit VMCm <= 0.429
limit VFCm >= 0.062
limit VFCm <= 0.238
limit VLuCm >= 0.005
limit VLuCm <= 0.011
limit VrestCm >=0.213
limit VrestCm <=0.819
limit QLCm >=0.024
limit QLCm <=0.785
limit QKCm >=0.037
limit QKCm <=0.143
limit QMCm >=0.074
limit QMCm <=0.286
limit QFCm >=0.033
limit QFCm <=0.127
limit QrestCm >=0.101
limit QrestCm <=0.390
limit Kimm >=0.038
limit Kimm <= 0.119
limit Fracm >= 0.491
limit Fracm <= 0.726
limit Kdissm >=5.388e-6
limit Kdissm <= 1.703e-5
limit Kscm >= 0.011
limit Kscm <= 0.034
limit Fracscm >= 0.573
limit Fracscm <= 0.847
limit Kdissscm >= 5.388e-5
limit Kdissscm <= 1.703e-4
limit PLm >= 1.995
limit PLm <= 4.337
limit PKm >= 1.663
limit PKm <= 3.614
limit PMm >= 0.2
limit PMm <= 0.434
limit PFm >= 0.027
limit PFm <= 0.058
limit PLum >= 0.12
limit PLum <= 0.26
limit Prestm >= 0.319
limit Prestm <= 0.692
limit KmCm >=0.001
limit KmCm <=0.004
limit PBm >=0.260
limit PBm <=0.822
limit KurineCm >= 0.242
limit KurineCm <= 0.766

```
{Cardiac output and blood flow to tissues (L/h)}
QC = QCCm*BWm                    ; Cardiac output
QL = QLCm*QC                     ; Liver
QK = QKCm*QC                     ; Kidney
QF = QFCm*QC                     ; Fat
QM = QMCm*QC                     ; Muscle
QLu = QLuC*QC                    ; Lung
QR = QrestCm*QC                  ; Rest of body

{Tissue volumes (L)}
VL = VLCm*BWm                    ; Liver
VK = VKCm*BWm                    ; Kidney
VF = VFCm*BWm                    ; Fat
VM = VMCm*BWm                    ; Muscle
VLu = VLuCm*BWm                  ; Lung
VR = VrestCm*BWm                 ; Rest of body
Vven = VvenCm*BWm                ; Venous Blood
Vart = VartCm*BWm                ; Arterial Blood

; Metabolism Rate Constants
Kmet = KmCm*BWm

; Urinary Elimination Rate Constants
Kurine = KurineCm*BWm

{Dosing}
; Dosing caculation based on BW
DOSEsc = PDOSEsc*BWm        ; (mg)
DOSEim = PDOSEim*BWm        ; (mg)

; Dosing, repeated doses
tinterval = 24              ; Varied dependent on the exposure paradigm (h)
Tdoses = 5                  ; times for multiple oral gavage

dosingperiod = if time < Tdoses*tinterval-DT then 1 else 0

; Dosing, IM, intramuscular
Rinputim = pulse(DOSEim,0,tinterval)*dosingperiod
Rpenim = Rinputim*(1-Fracm);
Rppgim =  Rinputim*Fracm;
Rim = Kimm*Amtsiteim
d/dt(Absorbim) = Rim
init Absorbim = 0
d/dt(Amtsiteim) = Rpenim- Rim + Kdissm* DOSEppgim
init Amtsiteim = 0
d/dt(DOSEppgim) = Rppgim-Kdissm* DOSEppgim
init DOSEppgim = 0

; Dosing, SC, subcutaneous
Rinputsc = pulse(DOSEsc,0,tinterval)*dosingperiod
Rpensc = Rinputsc*(1-Fracscm);
Rppgsc =  Rinputsc*Fracscm;
Rsc = Kscm*Amtsitesc
d/dt(Absorbsc) = Rsc
init Absorbsc = 0
```

```
d/dt(Amtsitesc) = Rpensc- Rsc + Kdissscm* DOSEppgsc
init Amtsitesc = 0
d/dt(DOSEppgsc) = Rppgsc-Kdissscm* DOSEppgsc
init DOSEppgsc = 0

{Penicillin distribution in each compartment}
; Penicillin in venous blood compartment
RV = (QL*CVL+QK*CVK+QF*CVF+QM*CVM+QR*CVR+Rsc+Rim)-QC*CV; RV the changing rate in the
venous blood (mg/h)
d/dt(AV) = RV                ; AV the amount of the drug in the venous blood (mg)
init AV = 0
CV = AV/Vven                 ; CV drug concentration in the venous blood (mg/L)
RA = QC*(CVLu-CAfree)        ; RA the changing rate in the arterial blood (mg/h)
d/dt(AA) = RA
init AA = 0                  ; AA the amount of the drug in the arterial blood (mg)
CA = AA/Vart                 ; CAfree concentration of unbound drug in the arterial blood (mg/L)
CAfree = CA*Free
d/dt(AUCCV) = CV             ; AUCCV AUC of drug concentration in the venous blood (mg*h/L)
init AUCCV = 0

ABlood = AA+AV

; Penicillin in liver compartment, flow-limited model
RL = QL*(CAfree-CVL)-Rmet    ; RL the changing rate of the amount of drug in liver (mg/h)
d/dt(AL) = RL                ; AL amount of drug in liver (mg)
init AL = 0
CL = AL/VL                   ; CL drug concentration in liver (mg/L)
CVL= AL/(VL*PLm)             ; CVL drug concentration in venous blood from liver (mg/L)
d/dt(AUCCL) = CL             ; AUCCL area under the curve of drug concentration in liver (mg*h/L)
init AUCCL = 0

; Metabolism of Penicillin in liver compartment
Rmet = Kmet*CL*VL            ; Rmet the metabolic rate in liver (mg/h)
d/dt(Amet) = Rmet            ; Amet the amount of drug metabolized in liver (mg)
init Amet = 0

; Penicillin in kidney compartment, flow-limited model
RK = QK*(CAfree-CVK)-Rurine  ; RK the changing rate of the amount of drug in kidney (mg/h)
d/dt(AK) = RK                ; AK amount of drug in kidney (mg)
init AK = 0
CK = AK/VK                   ; CK drug concentration in kidney (mg/L)
CVK = AK/(VK*PKm)
d/dt(AUCCK) = CK             ; AUCCK AUC of drug concentration in kidney (mg*h/L)
init AUCCK = 0

; Penicillin urinary excretion
Rurine = Kurine*CVK
d/dt(Aurine) = Rurine
init Aurine = 0

; Penicillin in muscle compartment, flow-limited model
RM = QM*(CAfree-CVM)         ; RM the changing rate of the amount of drug in muscle (mg/h)
d/dt(AM) = RM                ; AM amount of the drug in muscle (mg)
init AM = 0
CM = AM/VM                   ; CM drug concentration in muscle (mg/L)
CVM = AM/(VM*PMm)
```

```
d/dt(AUCCM) = CM
init AUCCM = 0

; Penicillin in fat compartment, flow-limited model
RF = QF*(CAfree-CVF)          ; RF the changing rate of the amount of drug in fat (mg/h)
d/dt(AF) = RF                 ; AF amount of the drug in fat (mg)
init AF = 0
CF = AF/VF                    ; CF drug concentration in fat (mg/L)
CVF = AF/(VF*PFm)
d/dt(AUCCF) = CF              ; AUCCF AUC of drug concentration in fat (mg*h/L)
init AUCCF = 0

; Penicillin in the compartment of rest of body, flow-limited model
RR = QR*(CAfree-CVR)          ; Rrest the changing rate of the amount of drug in the rest of the body (mg/h)
d/dt(AR) = RR                 ; Arest amount of the drug in the rest of the body (mg)
init AR = 0
CR = AR/VR                    ; Crest drug concentration in the rest of the body (mg/L)
CVR = AR/(VR*Prestm)
d/dt(AUCCR) = CR              ; AUCCrest AUC of drug concentration in the rest of the body (mg*h/L)
init AUCCR = 0

; Penicillin in lung compartment, flow-limited model
RLu = QLu*(CV-CVLu)           ; RLu the changing rate of the amount of drug in the lung (mg/h)
d/dt(ALu) = RLu               ; ALu amount of the drug in the lung (mg)
init ALu = 0
CLu = ALu/VLu                 ; CLu drug concentration in the rest of the lung (mg/L)
CVLu = ALu/(VLu*PLum)
d/dt(AUCCLu) = CLu            ; AUCCLu AUC of drug concentration in the lung (mg*h/L)
init AUCCLu = 0

{Mass balance equations}
Qbal = QC-QM-QR-QF-QK-QL
Tmass = ABlood+AM+ALu+AR+AF+AK+AL+Aurine+Amet
Input = Absorbim+Absorbsc
Bal = Input-Tmass
```

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