Supplementary Data

Probabilistic Physiologically Based Pharmacokinetic Model for Penicillin G in Milk from Dairy Cows following Intramammary or Intramuscular Administrations

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Contents

Equations and Codes for the PBPK Model

1. Equations for Milking Intervals

In pharmacokinetic studies and in actual practice, different milking intervals are used depending on the study designs and the individual farm's milking schedule, including 12+12 hour interval, 10+14 hour interval, and 15+9 hour interval. In order to better fit different experimental data sets, the two-dimensional array of size N×2 was created to customize each input of the milking intervals (N is the number of milkings). The first column of the array stands for the length of each milking interval (**Equation S1**), and the second column represents the total time elapsing at the ith milkings (**Equation S2**).

$$Tempus[i, 1] = Tmilking[i] \quad \text{(S1)}$$

$$Tempus[i, 2] = Tempus[i-1, 2] + Tmilking[i] \quad \text{(S2)}$$

where Tempus is the array created for milking intervals (h); Tempus[i, 1] represents the length of the ith milking interval (h); Tempus[i, 2] equals to the total time elapsing at the ith milking (h); Tmilking[i] is the length of the ith milking interval (h).

2. Equations for IMM Infusions

The input doses for IMM administration are fixed amounts for each quarter of udder, which are different from IM injections with doses scaling to the body weight (**Equation S3**). The SCHEDULE equations were applied to simulate the multiple-dose scenarios for IMM infusions. Different control factors were used to simulate the IMM infusions (**Equation S4-S7**). In pharmacokinetic experiments, the IMM infusion is typically done closely followed by the previous milking process, and they may both be marked as performed at the same time. To avoid the interference with milking process, the IMM infusion was achieved by using SCHEDULE equation (**Equation S4**) and the DELAY function (**Equation S5**) to make sure that the infusion happens slightly after the previous milking. The DELAY function in Berkeley Madonna helps to achieve the time delayed by 1.1×milking_time. The rate of change for the amount of penicillin G through IMM infusions was described in **Equation S7**.

$$Rimm = DOSEimm/Timm \quad (S3)$$

$$IMM1 = \text{if } MOD(time, Tinterval) < 1.1 \times milking\_time \text{ Then } 0 \text{ Else } 1 \quad (S4)$$

$$IMM2 = \text{if } MOD(delay(time, 1.1 \times milking\_time), Tinterval) >= Timm \text{ Then } 0 \text{ Else } 1 \quad (S5)$$

$$IMMstop = \text{if } time > Tdoses \times Tinterval \text{ Then } 0 \text{ Else } 1 \quad (S6)$$

$$Rinputimm = Rimm \times IMM1 \times IMM2 \times IMMstop \quad (S7)$$

where Rimm represents the rate of dosing through IMM infusion for each administration (mg/h); DOSEimm is the total dose of IMM infusion to the injected quarters (mg) (in practice only the affected quarters are injected with drug); Timm is the time duration for IMM infusion (h); IMM1 is the control factor to start IMM infusion after the preceding milking (unitless); Tinterval is the time interval for IMM infusion (h); milking_time is the time duration for milking process (h);

IMM2 is the control factor to stop each IMM infusion within the time limit of Timm (unitless); IMMstop represents the control factor to stop multiple dose scenarios of IMM infusions (unitless); Tdoses is the number of multiple infusions (unitless); Rinputimm is the actual input rate of penicillin G through IMM administration (mg/h).

3. Equations for lognormal transformation

The lognormal distribution is a continuous probability distribution of a random variable whose logarithm is normally distributed, so the inverse natural logarithmic transformation of the 'NORMAL' function can be used to produce lognormally distributed random numbers (**Equation S10**). To use **Equation S10**, the mean and the variance in lognormal distribution should be transformed into model parameters in normal distribution (**Equations S8-S9**).

$$\mu_{lnx} = ln\left(\frac{\mu_x^2}{\sqrt{\sigma_x^2+\mu_x^2}}\right) \quad \text{(S8)}$$

$$\sigma_{lnx} = \sqrt{ln\left(1+\frac{\sigma_x^2}{\mu_x^2}\right)} \quad \text{(S9)}$$

$$Lognormal\ (\mu_x, \sigma_x) = exp(Normal(\mu_{lnx}, \sigma_{lnx})) \quad \text{(S10)}$$

where μ_x is the mean for lognormal distribution; σ_x is the standard deviation for lognormal distribution; μ_{lnx} is the mean after the transformation to normal distribution; σ_{lnx} is the standard deviation after the transformation to normal distribution.

Supplementary Figures

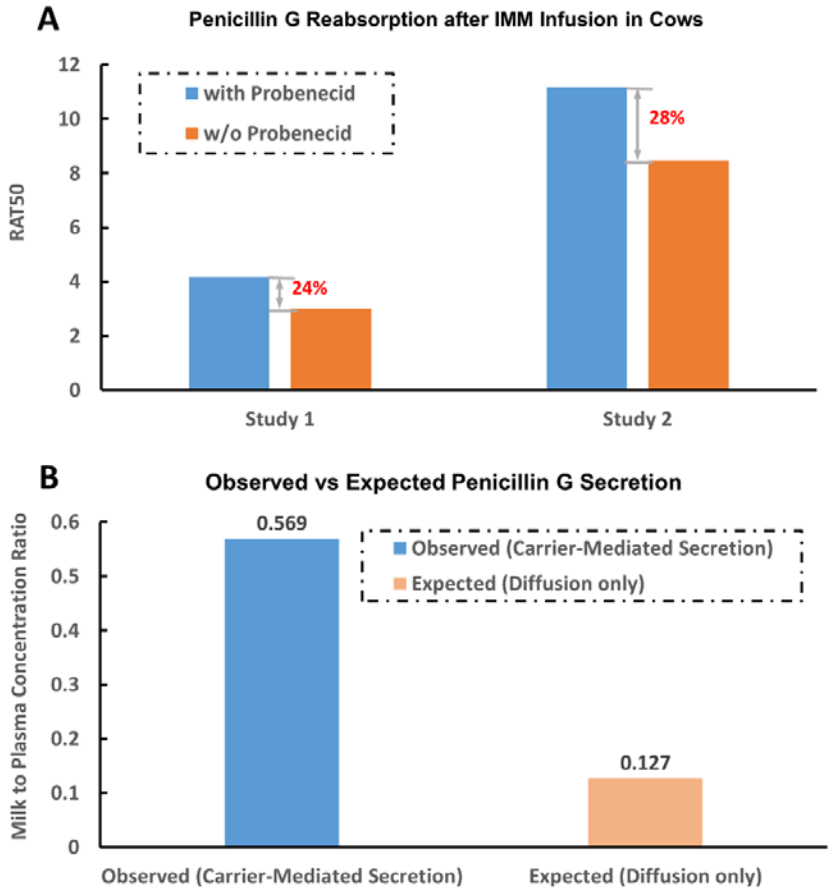

Figure S1. The passive diffusion and active carrier-mediated transport in the reabsorption and secretion of penicillin G in the mammary gland. The experimental data were extracted and adapted from the study reported by Schadewinkel-Scherkl et al. (1993). The carrier-mediated secretion could be inhibited by probenecid. In the panel A, 'RAT50' is the abbreviation of the ratio of the absorption half-lives, which represent the half-life for penicillin G reabsorption. The increase of RAT50 indicates that the rate of the reabsorption of penicillin G is decreased. In the panel B, the expected value was calculated based on passive diffusion only. Compared to the expected value, the observed value was much higher due to the active secretion. Based on these results, the passive diffusion was used as the mechanism for penicillin G absorption from milk to udder tissues, and carrier-mediated transport was considered as the mechanism for penicillin G secretion in the mammary gland.

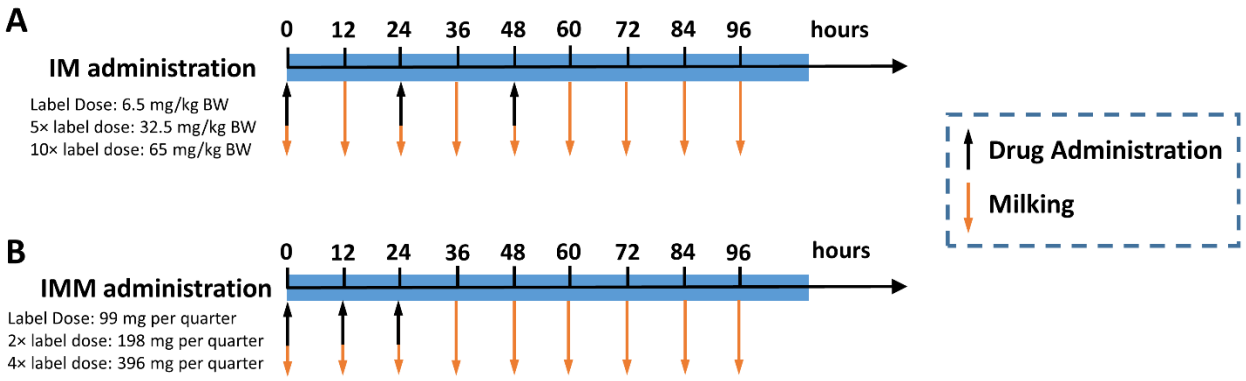

Figure S2. Dosing regimens for applications of the model with IM and IMM administrations. Both administration routes are simulated with milking process with 12-hour intervals. To avoid drug administrations and milking process happen simultaneously, DELAY functions are applied. A. Dosing regimens for 3 repeated doses of IM administrations with 24-hour intervals at the label dose 6,000 IU/kg (6.5 mg/kg) and two commonly used extralabel doses (5× label dose, 32.5 mg/kg; 10× label dose, 65 mg/kg). B. Dosing regimens for 3 repeated doses of IMM infusions with 12-hour intervals at the label dose 100,000 IU (99 mg per quarter of udders) and two commonly used extralabel doses (2× label dose, 198 mg per quarter; 4× label dose, 396 mg per quarter) to two of the four quarters.

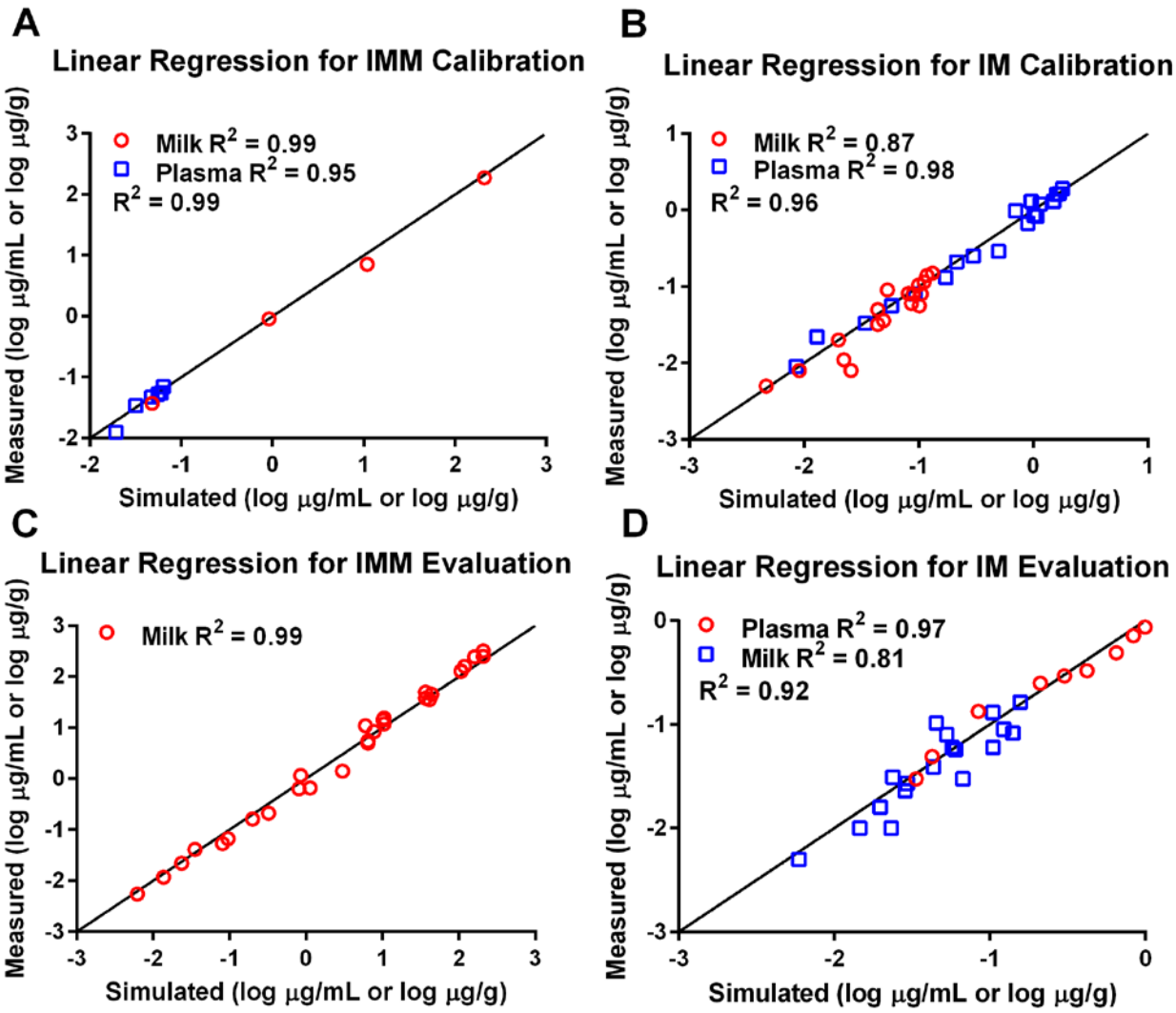

Figure S3. Linear regression analyses of the model calibration and evaluation results. Linear regression analyses were carried out for all the results of the calibration and evaluation via IM and IMM administrations. The results of a regression analysis between log-transformed values of model-simulated and measured penicillin G concentrations in the plasma and milk from dairy cows are shown on panels A, B, C and D. R^2 values for the milk, plasma and all together, and the lines of equality are shown in the panel.

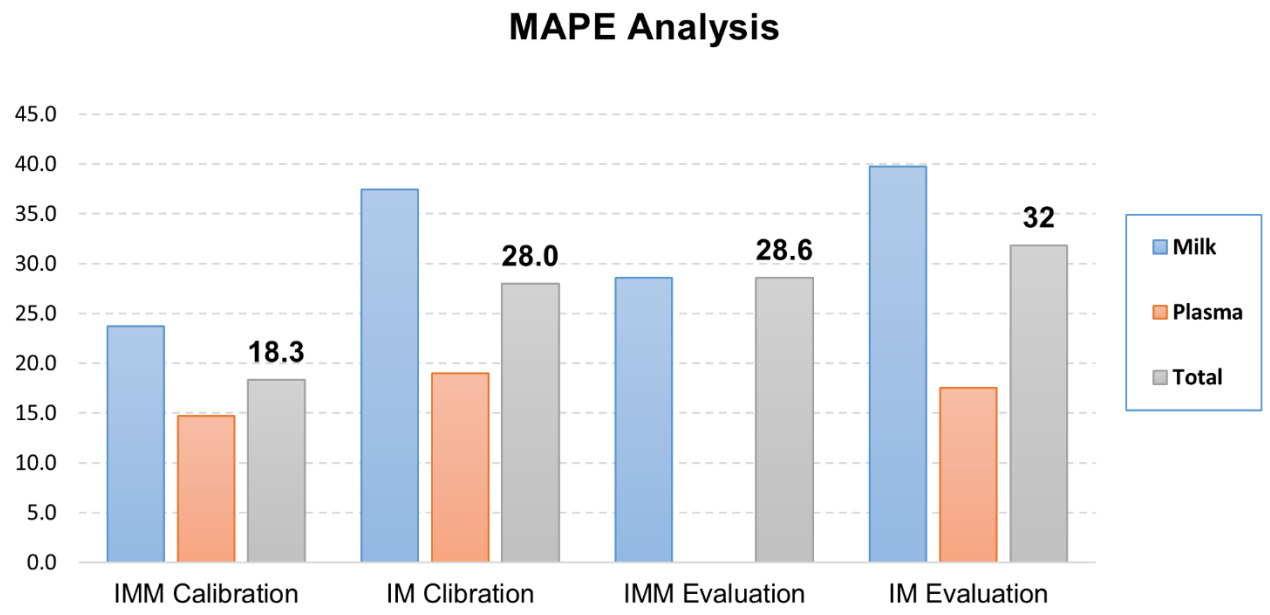

Figure S4. The mean absolute percentage error (MAPE) analysis for results of the model calibration and evaluation. MAPE values were calculated for all the results of the calibration and evaluation via IM and IMM administrations. The MAPE values for the milk, plasma and all together were shown respectively for each of the groups. All the values were lower than 40%.

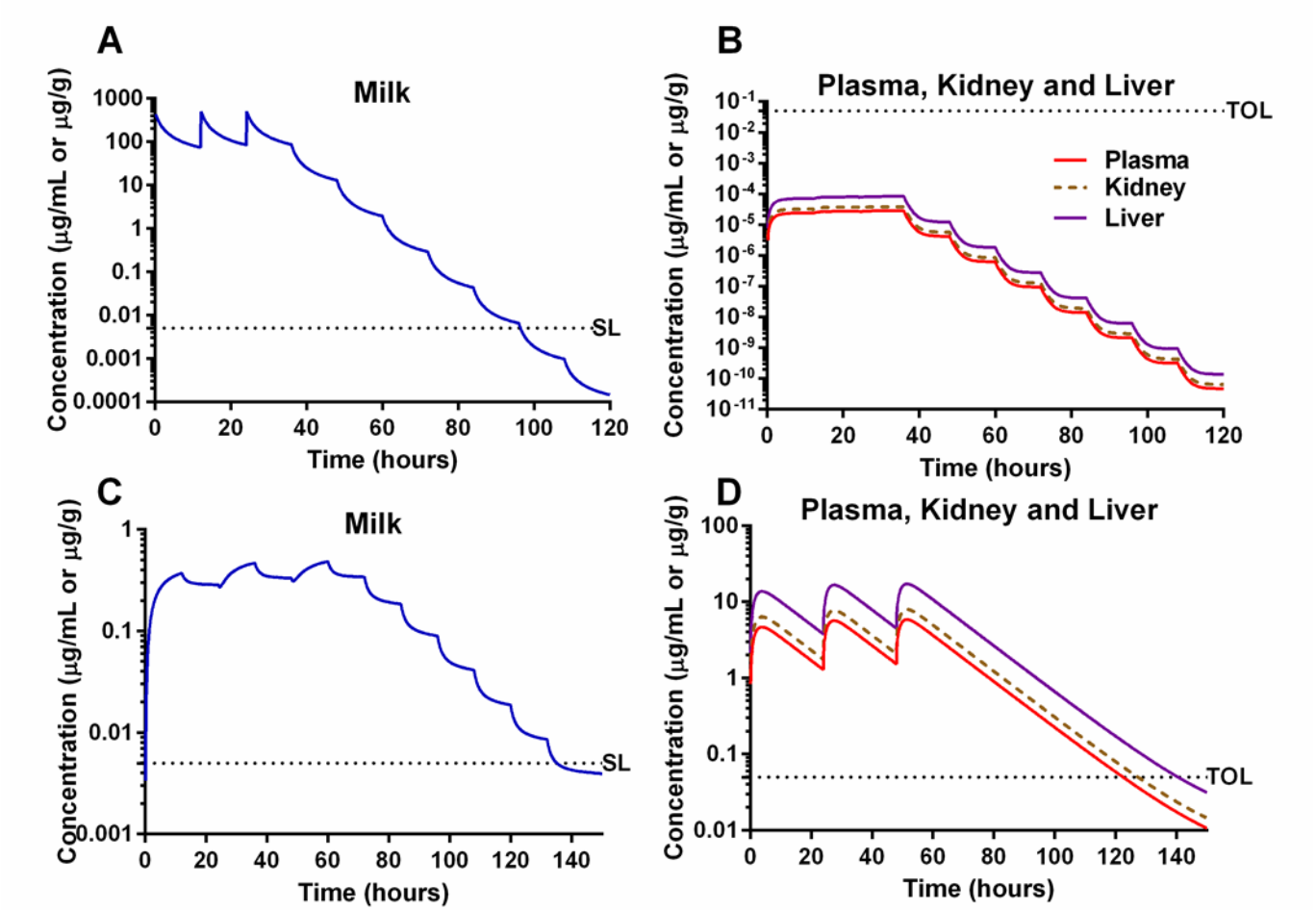

Figure S5. Application of the PBPK model for penicillin G in milk to predict milk and tissue residues. The simulations for concentrations of penicillin G in the milk and tissues of dairy cows exposed to procaine penicillin G via IMM infusions for repeated 3 doses with 12-hour intervals (4× label dose, 396 mg per quarter for two quarters) are shown on panels A and B. The simulations for concentrations of penicillin G in the milk and tissues of dairy cows by IM injections for repeated 3 doses with 24-hour intervals (10× label dose, 65 mg/kg BW) are shown on panels C and D. Though zero tolerance was established for penicillin G in milk, the safe level (SL) of 5 µg/kg was used by FDA in actual practice. Tolerances (TOL) for penicillin G in edible tissues are 50 µg/kg.

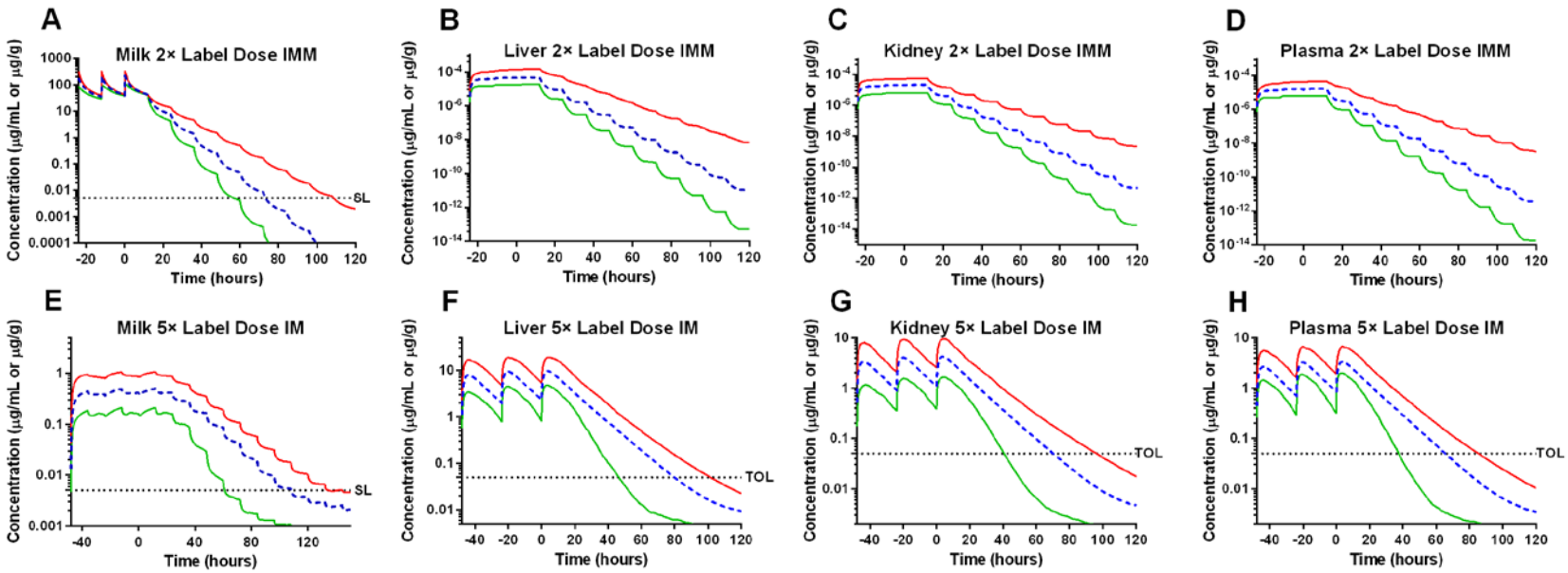

Figure S6. Probabilistic analysis for penicillin G concentrations in the milk, liver, kidney and plasma through IMM infusions with 2× label dose and IM administrations with 5× label dose. IMM infusions with 2× label dose (198 mg per quarter for two quarters) for 3 repeated doses were simulated as the therapeutic scenario for dairy cows (A-D). IM injections with commonly used extralabel dose (5× label dose, 32.5 mg/kg) for 3 repeated doses were simulated as the therapeutic scenario for dairy cows (E-H). The milking intervals were 12 hours. Tolerances (TOL) of penicillin G in edible tissues are 50 µg/kg, and the safe level (SL) of penicillin G in milk is 5 µg/kg.

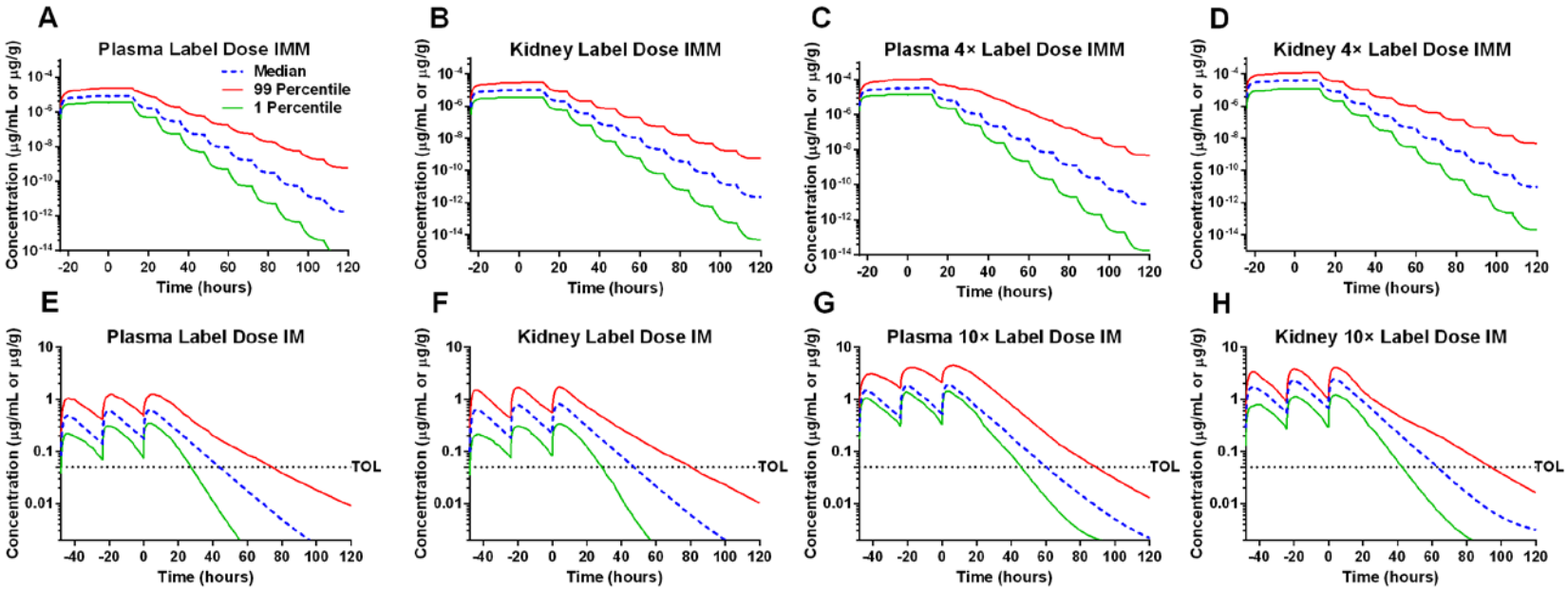

Figure S7. Probabilistic analysis for penicillin G concentrations in the plasma and kidney through IMM and IM administrations. IMM infusions with label dose (99 mg per quarter for two quarters) and 4× label dose (396 mg per quarter for two quarters) for 3 repeated doses were simulated as the therapeutic scenario for dairy cows (A-D). IM injections with label dose (6.5 mg/kg) and 10× label dose (65 mg/kg) for 3 repeated doses were simulated as the therapeutic scenario for dairy cows (E-H). Tolerances (TOL) of Penicillin G in edible tissues are 50 µg/kg.

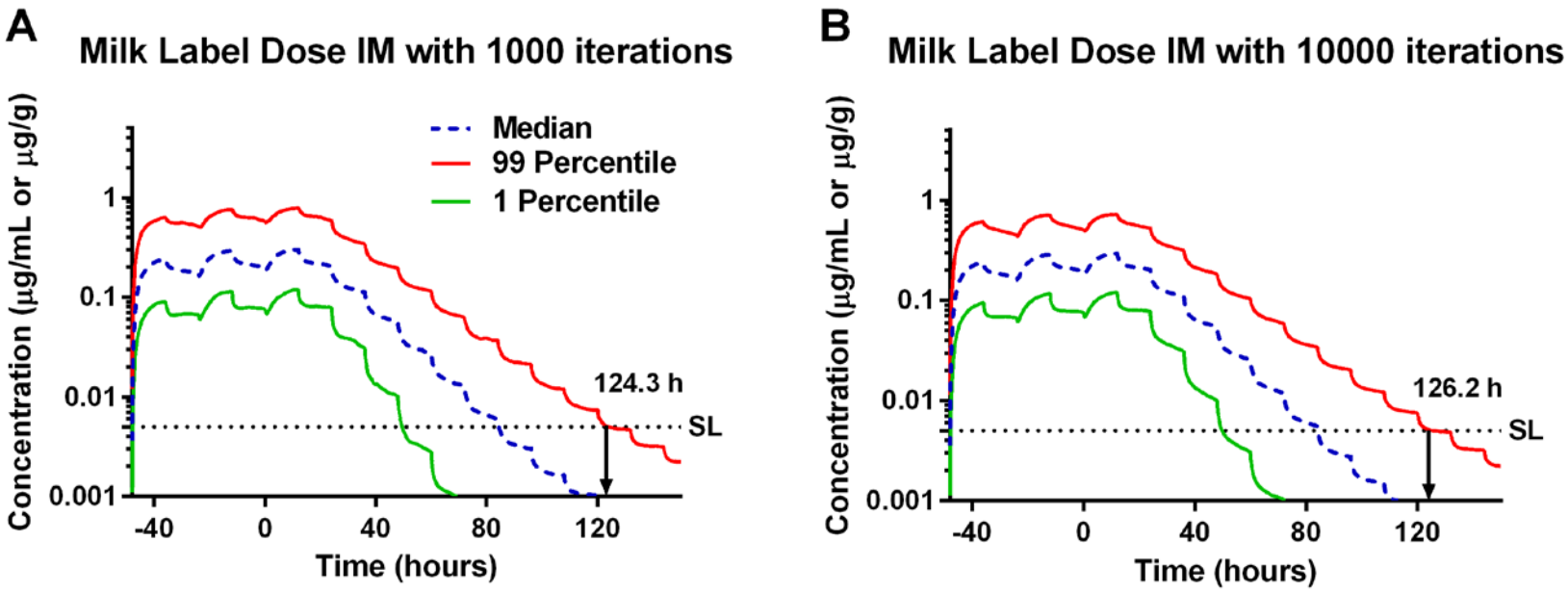

Figure S8. The comparison of probabilistic analysis for 1,000 iterations with 10,000 iterations. The three-repeated doses of IM administrations with label dose were used as an example to compare the differences of simulations between 1,000 and 10,000 iterations. A is the result of 1,000 iterations, and B is the result of 10,000 iterations. The safe level (SL) of 5 µg/kg was used by FDA in actual practice.

Supplementary Tables

Table S1. Normalized sensitivity coefficients of relatively sensitive parameters on the area under the concentrations (AUCs) of penicillin G in plasma, liver, kidney and milk.

Sensitive Parameter	Normalized sensitivity coefficients (NSCs)			
	AUCCV	AUCCL	AUCCK	AUCCmilk
BW	0.00	0.00	0.00	0.99
QCC	-0.37	-0.35	0.08	-0.25
QKC	-0.36	-0.37	0.09	-0.24
VMmilksp	0.00	0.00	0.00	-0.99
s	0.05	0.05	0.05	0.61
F	0.07	0.07	0.07	-0.24
KabC	0.98	0.98	0.98	-0.02
PL	0.00	1.00	0.00	0.00
PK	0.00	0.00	1.00	0.00
KmetC	-0.16	-0.18	-0.16	-0.10
KurineC	-0.44	-0.44	-0.90	0.00
Kim	0.41	0.41	0.41	0.34
Frac	-1.50	-1.50	-1.50	-1.06
VmaxC	0.00	0.00	0.00	1.00
Km	0.00	0.00	0.00	-0.70

Notes: Only parameters with at least one absolute value of NSC greater than 0.15 are shown in the table. We defined these parameters as relatively sensitive parameters on the selected key dose metrics. AUCCV, AUCCL, AUCCK, and AUCCmilk represent 24-hour area under concentration curves of penicillin G in plasma, liver, kidney and milk, respectively. Please refer to **Table 2** for definition of parameter abbreviations.

PBPK Model Code

Note: The Berkeley Madonna model code below is a general physiologically based pharmacokinetic (PBPK) model for procaine penicillin G in dairy cows. Parameter values used in the model code are for procaine penicillin G via the IM administration. All parameter values in dairy cows are summarized in **Table 2.**

```
{
Penicillin PBPK milk model in dairy cows (flow-limited model, linear metabolism equation, plasma protein binding,
milking model with Hill-Langmuir Equation, and systematic absorption from mammary gland)
The PBPK model code is based on the penicillin G model in cattle and swine by Li et al. (2017)
}

METHOD RK4

STARTTIME = 0
STOPTIME= 100                    ; h
DT = 0.00025
DTOUT = 0.1

{Physiological parameters of cattle and swine reported in Lin et al. (2016) only had an average value in each
species. Later on, in our subsequent paper (Li et al., 2017), 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 the paper by Li et al.
(2017) that describes a population PBPK model for penicillin G in cattle and swine. Overall, the value of each
physiological parameter in Li et al. (2017) is still quite close to the value reported in Lin et al. (2016). Physiological
parameters used in the present milk model is based on the updated values from Li et al. (2017).}

; The physiological parameters involve some parameters impacted by lactating status (VvenC, VartC)
; Blood Flow Rates
QCC = 5.97                       ; L/h/kg, Cardiac Output (Li et al. 2017; Doyle et al. 1960)

; Fraction of blood flow to organs (unitless)
QLC = 0.405                      ; Fraction of blood flow to the liver (Li et al. 2017; Lescoat et al. 1996;
Doyle et al. 1960)
QKC = 0.090                      ; Fraction of blood flow to the kidneys (Li et al. 2017; Lin et al. 2016)
QMC = 0.180                      ; Fraction of blood flow to the muscle (Li et al. 2017; Lin et al. 2016)
QFC = 0.080                      ; Fraction of blood flow to the fat (Li et al. 2017; Lin et al. 2016)
QLuC = 1                         ; Fraction of blood flow to the lung (Li et al. 2017; Achenbach 2000)
QUC = 0.081                      ; Fraction of blood flow to the udder (Campbell et al. 2016 Dairy
Production and processing page 145)
QrestC = 1-QLC-QKC-QFC-QMC-QUC   ; Fraction of blood flow to the rest of body (total sum equals to 1)

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

; Fractional organ tissue volumes (unitless)
VLC = 0.014                      ; Fractional liver tissue (Li et al. 2017; Swett et al. 1933)
VKC = 0.002                      ; Fractional kidney tissue (Li et al. 2017; Swett et al. 1933)
VFC = 0.150                      ; Fractional fat tissue (Li et al. 2017; Lin et al. 2016)
VMC = 0.270                      ; Fractional muscle tissue (Li et al. 2017; Lin et al. 2016)
VLuC = 0.008                     ; Fractional lung tissue (Li et al. 2017; Lin et al. 2016)
VvenC =  0.037                   ; 0.030 non-lactating; Venous blood volume, fraction of blood
volume (Li et al. 2017; Lin et al. 2016) total blood increase from 3.8% to 4.9% of body weight (Campbell et al. 2016
Dairy Production and processing page 145)
```

```
VartC =  0.012                                  ; 0.010 non-lactating ; Arterial blood volume, fraction of blood volume (Li et al. 2017; Lin et al. 2016)
VUC = 0.008                                     ; Fractional udder tissue (Gionbelli et al. 2015 PMC4368534)
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 (Li et al. 2017)
PM = 0.3                        ; Muscle:plasma PC  (Li et al. 2017)
PF = 0.04                       ; Fat:plasma PC  (Li et al. 2017)
PK = 2.5                        ; Kidney:plasma PC  (Li et al. 2017))
PLu = 0.18                      ; Lung:plasma PC  (Li et al. 2017)
PU = 0.2                        ; Udder tissue:plasma PC (estimated)
Prest = 0.479                   ; Rest of body:plasma PC  (Li et al. 2017)

{Kinetic Constants}
; IM Absorption Rate Constants
Kim = 0.070                     ; /h, IM absorption rate constant
Frac = 0.600                    ; the fraction of procaine penicillin G stayed undissolved (unitless)
Kdiss = 1e-5                    ; /h

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

{Metabolic Rate Constant}
KmetC = 0.0025                  ; /h/kg (Li et al. 2017)

; Urinary Elimination Rate Constants
KurineC = 0.45                  ; L/h/kg (Li et al. 2017)

{Parameters for Various Exposure Scenarios}
PDOSEim = 10                    ; 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
QU = QUC*QC                     ; Udder
Qrest = 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
VU = VUC*BW                     ; Udder
Vrest = VrestC*BW               ; Rest of body
Vven = VvenC*BW                 ; Venous Blood
Vart = VartC*BW                 ; Arterial Blood

; Urinary Glomerular Filtration Rate Constants
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```
Kurine = KurineC*BW              ; L/h

; Metabolism rate constants
Kmet = KmetC*BW                  ; /h

{Dosing}
; Dosing calculation based on BW
DOSEim = PDOSEim*BW              ; (mg)
DOSEimm = 0                      ; (mg) Label dose for two quarters

; Dosing, repeated doses
Tinterval = 24                   ; Varied dependent on the exposure paradigm (h)
Tdoses = 1                       ; Number of multiple injections

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

{Penicillin distribution in each compartment}
; Penicillin in venous blood compartment
RV = (QL*CVL+QK*CVK+QF*CVF+QM*CVM+Qrest*CVrest+Rim+QU*CVU)-QC*CV; RV the rate of change
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 rate of change 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 = CA*Free                 ; CAfree concentration of unbound drug in the arterial blood (mg/L)
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 rate of change 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)
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 rate of change 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 rate of change 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 rate of change 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
Rrest = Qrest*(CAfree-CVrest)  ; Rrest the rate of change of the amount of drug in the rest of the body (mg/h)
d/dt(Arest) = Rrest            ; Arest amount of the drug in the rest of the body (mg)
init Arest = 0
Crest = Arest/Vrest            ; Crest drug concentration in the rest of the body (mg/L)
CVrest = Arest/(Vrest*Prest)
d/dt(AUCCrest) = Crest         ; AUCCrest AUC of drug concentration in the rest of the body (mg*h/L)
init AUCCrest = 0

; Penicillin in lung compartment, flow-limited model
RLu = QLu*(CV-CVLu)            ; RLu the rate of change 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

;; Milk volume
; Milking constants (Whittem et al. 2012)
```

```
milking_time = 0.1                  ; h, Time for milking process (To milk a cow by hand takes 10 to 15 min; A milking machine can do in less than 5 min. {http://farmerdave.calgarystampede.com/farmer-dave-answers/dairy-cattle.html})
Timm = 0.01                         ; h, Time for IMM injection (estimated)
VMmilksp = 26.8                     ; L, Total volume of milk space
s = 23.2                            ; h, Time to get half of the total volume of milk space
F = 0.04                            ; unitless, Milk residue ratio after milking
t0 = 8                              ; h, starting time for milking
n = 6                               ; number of milkings

; milking time intervals and total time (h)
; n+1 by 2 (13 by 2 array in this code) array is created for milking time. Tempus[i, 1] stands for milking intervals, Tempus[i, 2] stands for total time at the ith milking
; Tempus[i,2] is created to help reset time (tm) for milk secretion process
Tempus[1,1] = t0                                    ; first milking after IMM injection
Tempus[1,2]= t0                                     ; total time before first milking
Tempus[2..n,2] = Tempus[i-1,2]+Tempus[i,1]          ; total time before ith milking
Tempus[n+1, 2]=STOPTIME                             ; the total time before simulation stops
Tempus[2,1]=16                                      ; milking interval between the first and second milking
Tempus[3,1]=8                                       ; milking interval between the 2nd and 3rd milking
Tempus[4,1]=16                                      ; milking interval between the 3rd and 4th milking
Tempus[5,1]=8                                       ; milking interval between the 4th and 5th milking
Tempus[6,1]=16                                      ; milking interval between the 5th and 6th milking
;Tempus[7,1]=8                                      ; milking interval between the 6th and 7th milking
;Tempus[8,1]=8                                      ; milking interval between the 7th and 8th milking
;Tempus[9,1]=8                                      ; milking interval between the 8th and 9th milking
;Tempus[10,1]=8                                     ; milking interval between the 9th and 10th milking
;Tempus[11,1]=8                                     ; milking interval between the 10th and 11th milking
;Tempus[12,1]=8                                     ; milking interval between the 11th and 12th milking
;Tempus[13,1]=8                                     ; additional milking intervals for backup
;Tempus[14,1]=8
;Tempus[15,1]=8
Tempus[n+1, 1]=STOPTIME-Tempus[n,2]                 ; time after the last milking

; milk secretion after each preceding milking
Control[2..n] = if time > Tempus[i,2] then 0 else 1                          ; milk secretion stop factor for each milking interval after the first milking
tm[2..(n+1)] = if time< Tempus[i-1,2] then 0 else time-Tempus[i-1,2]         ; milk secretion start factor for each milking interval after the first milking
Vmilkinga[1] = if time <t0 then time*VMmilksp/(time+s) else 0                ; L, volume of milk secreted before the first milking
Vmilkinga[2..n] = if tm[i]>0 then Control[i]*(tm[i]*VMmilksp)/(tm[i]+s) else 0 ; L, volume of milk secreted before the ith milking
Vmilkinga[n+1] = if time< Tempus[n,2] then 0 else (tm[i]*VMmilksp)/(tm[i]+s); L, volume of milk secreted after the last milking
Vmilking = arraysum(Vmilkinga[*])                                            ; L, volume of milk secreted after the preceding milking, the sum of each milk secretion interval creates the volume of milking secreted
Vresidue = VMmilksp*F                                                        ; L, volume of residue milk after preceding milking
Vmilksp = arraysum(Vmilkinga[*])+Vresidue                                    ; L, volume of total milk in milk space

;; Penicillin G in milk
; milking using schedule (this is the important part where problems be), this part of code simulates the penicillin amount change in milk by episodic milking
; The amount of penicillin G eliminated by milking depends on the ratio of the volume of secreted milk and total milk in udders at each milking time point. The ratio is a confirmed number for the milking_time. But the dynamic of milk volume change is simulated separated from the dynamic of penicillin G amount change. During the
```

milking_time, the dynamic changes of secreted milk volume and total milk volume have impact on penicillin G eliminated by milking. So the MAX combined with DELAY function was used to make the milking ratio a confirmed number

```
Rmilkingex = (max(Amilk, delay(Amilk, milking_time))*Vmaxmilking)/(milking_time*Vmaxmilk)
        ; Rmilkingex stands for the rate of penicillin G eliminated by milking
StartFM[2..n+1] = if MOD(tm[i], Tempus[i,1]) >= milking_time then 0 else 1                    ; Start
factor for each milking process
StopFM[2..n+1] = if time-Tempus[i-1,2] > Tempus[i,1] then 0 else 1                            ; Stop
factor for each milking process after each milking interval
Stop[2..n+1] = if time<Tempus[i-1,2] then 0 else 1                                            ; Stop
factor for each milking process before each milking interval
Rmilkinga[2..n+1] = Rmilkingex*StopFM[i]*StartFM[i]*Stop[i]
        ; Rmilkinga is the array of Rmilking
Rmilking = arraysum(Rmilkinga[*])
        ; Rmilking, the rate of excretion of the amount of drug via milk (mg/h)
; Vmaxmilking = max(Vmilking,delay(Vmilking, milking_time))
        ; It is OK to use only two numbers for getting the Vmaxmilking, using more in delay function is to reduce
the step length
Vmaxmilking = max(Vmilking,delay(Vmilking, 0.1*milking_time),delay(Vmilking,
0.2*milking_time),delay(Vmilking, 0.3*milking_time),delay(Vmilking, 0.4*milking_time),delay(Vmilking,
0.5*milking_time),delay(Vmilking, 0.6*milking_time),delay(Vmilking, 0.7*milking_time),delay(Vmilking,
0.8*milking_time),delay(Vmilking, 0.9*milking_time),delay(Vmilking, milking_time))        ; making a
confirmed number for Vmilking during milking_time
Vmaxmilk = max(Vmilksp,delay(Vmilksp, 0.1*milking_time),delay(Vmilksp, 0.2*milking_time),delay(Vmilksp,
0.3*milking_time),delay(Vmilksp, 0.4*milking_time),delay(Vmilksp, 0.5*milking_time),delay(Vmilksp,
0.6*milking_time),delay(Vmilksp, 0.7*milking_time),delay(Vmilksp, 0.8*milking_time),delay(Vmilksp,
0.9*milking_time),delay(Vmilksp, milking_time))                                            ; making a confirmed
number for Vmilksp during milking_time

; Penicillin in milk
Rmilk = Rinputimm+Rmilkex-Kab*Amilk-Rmilking                ; Rmilk the rate of change of the amount of
drug in milk (mg/h)
d/dt(Amilk) = Rmilk                                         ; Amilk amount of  the drug in milk (mg)
init Amilk = 0
Cmilka[1] = if time< t0 then Amilk/Vmaxmilk else 0
Cmilka[2..n+1] = if MOD(tm[i],Tempus[i,1]) <= milking_time then Amilk/Vmaxmilk*Stop[i]*StopFM[i] else
Amilk/Vmilksp*Stop[i]*StopFM[i]              ; Cmilk drug concentration in milk (mg/L)
Cmilk = arraysum(Cmilka[*])
d/dt(AUCCmilk) = Cmilk                       ; AUCCmilk AUC of drug concentration in milk (mg*h/L)
init AUCCmilk = 0
init Aab = 0
d/dt(Aab) = Kab*Amilk                        ; Aab amount of the drug absorbed from milk (mg)

; Penicillin in mammary gland
RU = QU*(CAfree-CVU)-Rmilkex+Kab*Amilk       ; RU the rate of change of the amount of drug in the udder
(mg/h)
d/dt(AU) = RU                                ; AU amount of  the drug in the udder tissue
init AU = 0
CU = AU/VU                                   ; CU drug concentration in the udder tissue (mg/L)
CVU = AU/(VU*PU)
d/dt(AUCCU) = CU                             ; AUCCU AUC of drug concentration in the udder tissue
(mg*h/L)
init AUCCU = 0

init Amilkex = 0
```

```
d/dt(Amilkex) = Rmilkex                          ; Amilkex amount of  the drug secreted to milk (mg)

VmaxC = 0.0022                          ; mg/L/kg BW, initiate based on Ziv et al. 1973 data from ewes, then
calibrate with PK data
Km = 0.7                                ; mg/L, initiate based on Ziv et al. 1973 data from ewes, then calibrate
with PK data
Vmax = VmaxC*BW
Rmilkex = Vmax*CVU/(CVU+Km)             ; mg/h, Rate of penicillin secretion from udder tissue
KabC = 1e-4                             ; L/h/kg BW
Kab = KabC*BW                           ; /h, Rate of penicillin absorption from milk

; dosing using schedule
Rimm = DOSEimm/Timm                                                    ; the rate of dosing
through IMM infusion for each administrations (mg/h)
IMM1 = if MOD(time, Tinterval) < 1.1*milking_time then 0 else 1        ; IMM injection start after
milking
IMM2 = if MOD(delay(time,1.1*milking_time), Tinterval)>=Timm Then 0 Else 1   ; IMM injection duration
control factor, DELAY used here to create the scenario that IMM injection is after milking
IMMstop = if time>Tdoses*Tinterval Then 0 Else 1                       ; IMM injection end
control factor
Rinputimm = Rimm*IMM1*IMM2*IMMstop
d/dt(AimmPen) = Rinputimm
init AimmPen = 0

{Mass balance equations}
Qbal = QC-QM-Qrest-QF-QK-QL-QU
Tmass = ABlood+AM+ALu+Arest+AF+AK+AL+Aurine+Amet+AU+Amilkex
Input = Absorbim+Aab
Bal = Input-Tmass

{
;;Milk volume (two-compartment model)
t0=6                            ; Start time for the first milking (first milking after IMM injection)
n = 10                          ; Times of milking
Tempus = 12                     ; Milking time interval
VMmilksp = 26.8                 ; Maximum volume of milk space
s = 23.2                        ; Time to get half of the total volume of milk space
F = 0.04                        ; Milk residue ratio after milking (estimated)
VMalv = 11.7                    ; total volume of alveoli
salv =  9.4                     ; time to get half of the total volume of alveoli
milking_time = 0.1
Timm = 0.01                     ; h, Time for IMM injection
; Milk volume
tm = time-t0-(i-1)*Tempus                                  ; tm, time after the preceding milking
i = if int((time-t0)/Tempus) < n then int((time-t0)/Tempus)+1 else n   ; the ith milking
Vcisresidue = 0.2                                          ; L
Vresidue = VMmilksp*F
Valvresidue = Vresidue - Vcisresidue
Vmilksp = Vmilking+Vresidue                                ; total milk volume in milk space
Vmilking = (tm*VMmilksp)/(tm+s)                            ; total volume of milk secreted after preceding milking
Valv = Vmilkingalv+Valvresidue                             ; total milk volume in alveoli
Vmilkingalv = (tm*VMalv)/(tm+salv)                         ; volume of milk secreted and in alveoli after preceding
milking
Vcis = Vmilksp-Valv
Vmilkingcis = Vcis-Vcisresidue
}
```

Population PBPK Model Code

Note: The Berkeley Madonna model code below is a population physiologically based pharmacokinetic (PBPK) model for procaine penicillin G in dairy cows. Parameter values used in the model code are for procaine penicillin G via IM injections. All parameter values used for the population model are summarized in **Tables 2 and 3.**

```
{
Penicillin PBPK milk model in dairy cows (flow-limited model, linear metabolism equation, plasma protein binding, milking model with Hill-Langmuir Equation, and systematic absorption from mammary gland)
The PBPK model code is based on the penicillin G model in cattle and swine by Li et al. (2017)
}

METHOD RK4

STARTTIME = 0
STOPTIME= 200                    ; h
DT = 0.00025
DTOUT = 0.1

{Physiological parameters of cattle and swine reported in Lin et al. (2016) only had an average value in each species. Later on, in our subsequent paper (Li et al., 2017), 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 the paper by Li et al. (2017) that describes a population PBPK model for penicillin G in cattle and swine. Overall, the value of each physiological parameter in Li et al. (2017) is still quite close to the value reported in Lin et al. (2016). Physiological parameters used in the present milk model is based on the updated values from Li et al. (2017).}
; The physiological parameters involve some parameters impacted by lactating status (VvenC, VartC)
; Blood Flow Rates
QCC = 5.97                       ; L/h/kg, Cardiac Output (Li et al. 2017; Doyle et al. 1960)

; Fraction of blood flow to organs (unitless)
init QLC =  0.405                ; Fraction of blood flow to the liver (Li et al. 2017; Lescoat et al. 1996; Doyle et al. 1960)
QKC = 0.090                      ; Fraction of blood flow to the kidneys (Li et al. 2017; Lin et al. 2016)
init QMC = 0.180                 ; Fraction of blood flow to the muscle (Li et al. 2017; Lin et al. 2016)
init QFC = 0.080                 ; Fraction of blood flow to the fat (Li et al. 2017; Lin et al. 2016)
QLuC = 1                         ; Fraction of blood flow to the lung (Li et al. 2017; Achenbach 2000)
init QUC = 0.081                 ; Fraction of blood flow to the udder (Campbell et al. 2016)
init QrestC = 1-QLC-QKC-QFC-QMC-QUC      ; Fraction of blood flow to the rest of body (total sum equals to 1)

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

; Fractional organ tissue volumes (unitless)
VLC = 0.014                      ; Fractional liver tissue (Li et al. 2017; Swett et al. 1933)
VKC = 0.002                      ; Fractional kidney tissue (Li et al. 2017; Swett et al. 1933)
VFC = 0.150                      ; Fractional fat tissue (Li et al. 2017; Lin et al. 2016)
VMC = 0.270                      ; Fractional muscle tissue (Li et al. 2017; Lin et al. 2016)
VLuC = 0.008                     ; Fractional lung tissue (Li et al. 2017; Lin et al. 2016)
VvenC =  0.037                   ; 0.030 non-lactating; Venous blood volume, fraction of blood volume (Li et al. 2017; Lin et al. 2016) total blood increase from 3.8% to 4.9% of body weight (Campbell et al. 2016)
VartC =  0.012                   ; 0.010 non-lactating ; Arterial blood volume, fraction of blood volume (Li et al. 2017; Lin et al. 2016)
```

```
VUC = 0.008                                          ; Fractional udder tissue (Gionbelli et al. 2015)
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 (Li et al. 2017)
PM = 0.3                                             ; Muscle:plasma PC (Li et al. 2017)
PF = 0.04                                            ; Fat:plasma PC (Li et al. 2017)
PK = 2.5                                             ; Kidney:plasma PC (Li et al. 2017)
PLu = 0.18                                           ; Lung:plasma PC (Li et al. 2017)
PU = 0.2                                             ; Udder:plasma PC (estimated)
Prest = 0.479                                        ; Rest of body:plasma PC (Li et al. 2017)

{Kinetic Constants}
; IM Absorption Rate Constants
Kim = 0.070                                          ; /h, IM absorption rate constant
Frac = 0.600                                         ; the fraction of procaine penicillin G stayed undissolved (unitless)
Kdiss = 1e-5                                         ; /h

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

{Metabolic Rate Constant}
KmetC = 0.0025                                       ; L/h/kg

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

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

{Variances of Parameters}
limit BWm >=209.45
limit BWm <=390.464
limit QCCm >= 2.07
limit QCCm <= 9.87
limit QKCm >= 0.037
limit QKCm <= 0.143

limit PLm >= 1.824
limit PLm <= 4.176
limit PKm >= 1.52
limit PKm <= 3.48

limit Kimm >= 0.029
limit Kimm <= 0.111
limit Fracm >=0.576
limit Fracm <=0.624
limit VmaxCm >=0.0014
limit VmaxCm <=0.0026
limit Kmm >= 0.688
limit Kmm <= 0.712

limit KabCm >= 0.000053
limit KabCm <= 0.000147
```

```
limit KurineCm >= 0.185
limit KurineCm <= 0.715
limit VMmilkspm >= 11.042
limit VMmilkspm <= 42.558
limit sm >= 9.559
limit sm <= 36.841
limit Fm >= 0.016
limit Fm <= 0.064
limit KmetCm >= 0.001
limit KmetCm <=0.004

QCC_sd = 1.99                ; Standard Deviation of QCC
QKC_sd = 0.027               ; Standard Deviation of QKC
BW_sd = 46.180               ; Standard Deviation of Body Weight
PL_sd = 0.6                  ; Standard Deviation of PL
PK_sd = 0.5                  ; Standard Deviation of PK
Kim_sd = 0.021               ; Standard Deviation of Kim
Frac_sd = 0.012              ; Standard Deviation of Frac
VmaxC_sd = 0.0003            ; Standard Deviation of VmaxC
Km_sd = 0.006                ; Standard Deviation of Km
KabC_sd = 2.4e-5             ; Standard Deviation of KabC
KurineC_sd = 0.135           ; Standard Deviation of KurineC
VMmilksp_sd = 8.04           ; Standard Deviation of VMmilksp
s_sd = 6.96                  ; Standard Deviation of s
F_sd = 0.012                 ; Standard Deviation of F
KmetC_sd = 7.5e-4            ; Standard Deviation of KmetC

{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 QKCm = Normal(QKC, QKC_sd)          ; Generation of the QKCm based on normal distribution
init BWm = Normal(BW, BW_sd)             ; Generation of BWm 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 = QLC+QKCm+QFC+QMC+QUC+QrestC  ; Adjust factor to keep the sum of blood flow fractions to 1

; Creation of Adjusted Parameters
next QLC = QLC/AdjustF                   ; Adjustment of QLC based on the adjust factor
next QKCm = QKCm/AdjustF                 ; Adjustment of QKCm
next QFC = QFC/AdjustF                   ; Adjustment of QFC
next QMC = QMC/AdjustF                   ; Adjustment of QMC
next QUC = QUC/AdjustF                   ; Adjustment of QUC
next QrestC = QrestC/AdjustF             ; Adjustment of QrestC

{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
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
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
VmaxC_ln = logn(VmaxC^2/(VmaxC_sd^2+VmaxC^2)^0.5)                ; Lognormal transformation of VmaxC
VmaxC_lnsd = (logn(1+VmaxC_sd^2/VmaxC^2))^0.5
Km_ln = logn(Km^2/(Km_sd^2+Km^2)^0.5)                            ; Lognormal transformation of Km
Km_lnsd = (logn(1+Km_sd^2/Km^2))^0.5
KabC_ln = logn(KabC^2/(KabC_sd^2+KabC^2)^0.5)                    ; Lognormal transformation of KabC
KabC_lnsd = (logn(1+KabC_sd^2/KabC^2))^0.5
VMmilksp_ln = logn(VMmilksp^2/(VMmilksp_sd^2+VMmilksp^2)^0.5); Lognormal transformation of VMmilksp
VMmilksp_lnsd = (logn(1+VMmilksp_sd^2/VMmilksp^2))^0.5
s_ln = logn(s^2/(s_sd^2+s^2)^0.5)                                ; Lognormal transformation of s
s_lnsd = (logn(1+s_sd^2/s^2))^0.5
F_ln = logn(F^2/(F_sd^2+F^2)^0.5)                                ; Lognormal transformation of F
F_lnsd = (logn(1+F_sd^2/F^2))^0.5
KmetC_ln = logn(KmetC^2/(KmetC_sd^2+KmetC^2)^0.5)                ; Lognormal transformation of KmetC
KmetC_lnsd = (logn(1+KmetC_sd^2/KmetC^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 PKm = exp(Normal(PK_ln, PK_lnsd)) next PKm = PKm            ; Generation of PKm
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 KurineCm = exp(Normal(KurineC_ln, KurineC_lnsd)) next KurineCm = KurineCm  ; Generation of KurineCm
init VmaxCm = exp(Normal(VmaxC_ln, VmaxC_lnsd)) next VmaxCm = VmaxCm            ; Generation of VmaxCm
init Kmm = exp(Normal(Km_ln, Km_lnsd)) next Kmm = Kmm                           ; Generation of Kmm
init KabCm = exp(Normal(KabC_ln, KabC_lnsd)) next KabCm = KabCm                 ; Generation of VmaxCm
init VMmilkspm = exp(Normal(VMmilksp_ln, VMmilksp_lnsd)) next VMmilkspm = VMmilkspm
                                                                  ; Generation of VMmilkspm
init sm = exp(Normal(s_ln, s_lnsd)) next sm = sm                  ; Generation of sm
init Fm = exp(Normal(F_ln, F_lnsd)) next Fm = Fm                  ; Generation of Fm
init KmetCm = exp(Normal(KmetC_ln, KmetC_lnsd)) next KmetCm = KmetCm  ; Generation of KmetCm

{Cardiac output and blood flow to tissues (L/h)}
QC = QCCm*BWm                ; Cardiac output
QL = QLC*QC                  ; Liver
QK = QKCm*QC                 ; Kidney
QF = QFC*QC                  ; Fat
QM = QMC*QC                  ; Muscle
QLu = QLuC*QC                ; Lung
QU = QUC*QC                  ; Udder
Qrest = QrestC*QC            ; Rest of body

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

```
; Urinary Glomerular Filtration Rate
Kurine = KurineCm*BWm          ; L/h

; Metabolism rate constants
Kmet = KmetCm*BWm              ; /h

{Dosing}
; Dosing calculation based on BW
DOSEim = PDOSEim*BWm           ; (mg)
DOSEimm = 0                    ; (mg)

; Dosing, repeated doses
Tinterval = 24                 ; Varied dependent on the exposure paradigm (h)
Tdoses = 3                     ; Number of injections for multiple administrations

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           ; Kimm is the randomly sampled value of Kim
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

{Penicillin distribution in each compartment}
; Penicillin in venous blood compartment
RV = (QL*CVL+QK*CVK+QF*CVF+QM*CVM+Qrest*CVrest+Rim+QU*CVU)-QC*CV; RV the rate of change
in the amount of the drug 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 rate of change 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 rate of change 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 rate of change 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 rate of change 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 rate of change 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
Rrest = Qrest*(CAfree-CVrest)  ; Rrest the rate of change of the amount of drug in the rest of the body (mg/h)
d/dt(Arest) = Rrest            ; Arest amount of the drug in the rest of the body (mg)
init Arest = 0
Crest = Arest/Vrest            ; Crest drug concentration in the rest of the body (mg/L)
CVrest = Arest/(Vrest*Prest)
d/dt(AUCCrest) = Crest         ; AUCCrest AUC of drug concentration in the rest of the body (mg*h/L)
init AUCCrest = 0

; Penicillin in lung compartment, flow-limited model
RLu = QLu*(CV-CVLu)            ; RLu the rate of change 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

;; Milk volume (one-compartment model)
```

```
; Milking constants (Whittem et al. 2012)
milking_time = 0.1              ; h, Time for milking process
Timm = 0.01                     ; h, Time for IMM injection
VMmilksp = 26.8                 ; L, maximum volume of milk space in udder
s = 23.2                        ; h, Time to get half of the maximum volume of milk space
F = 0.04                        ; unitless, residual milk ratio after milking
t0 = 0                          ; h, starting time for milking
n = 35                          ; number of milkings, varied depending on the study design

; milking time intervals and total time (h)
; n+1 by 2 (13 by 2 array in this code) array is created for milking time. Tempus[i, 1] stands for milking intervals,
Tempus[i, 2] stands for total time at the ith milking
; Tempus[i,2] is created to help reset time (tm) for milk secretion process
Tempus[1,1] = t0                                ; first milking time after IMM injection
Tempus[1,2]= t0                                 ; total time before first milking
Tempus[2..n,2] = Tempus[i-1,2]+Tempus[i,1]      ; total time before ith milking
Tempus[n+1, 2]=STOPTIME                         ; the total time before simulation stops
Tempus[2,1]=12                                  ; milking interval between the first and second milking
Tempus[3,1]=12                                  ; milking interval between the 2nd and 3rd milking
Tempus[4,1]=12                                  ; milking interval between the 3rd and 4th milking
Tempus[5,1]=12                                  ; milking interval between the 4th and 5th milking
Tempus[6,1]=12                                  ; milking interval between the 5th and 6th milking
Tempus[7,1]=12                                  ; milking interval between the 6th and 7th milking
Tempus[8,1]=12                                  ; milking interval between the 7th and 8th milking
Tempus[9,1]=12                                  ; milking interval between the 8th and 9th milking
Tempus[10,1]=12                                 ; milking interval between the 9th and 10th milking
Tempus[11,1]=12                                 ; milking interval between the 10th and 11th milking
Tempus[12,1]=12                                 ; milking interval between the 11th and 12th milking
Tempus[13,1]=12                                 ; additional milking intervals for backup
Tempus[14,1]=12
Tempus[15,1]=12
Tempus[16,1]=12
Tempus[17,1]=12
Tempus[18,1]=12
Tempus[19,1]=12
Tempus[20,1]=12
Tempus[21,1]=12
Tempus[22,1]=12
Tempus[23,1]=12
Tempus[24,1]=12
Tempus[25,1]=12
Tempus[26,1]=12
Tempus[27,1]=12
Tempus[28,1]=12
Tempus[29,1]=12
Tempus[30,1]=12
Tempus[31,1]=12
Tempus[32,1]=12
Tempus[33,1]=12
Tempus[34,1]=12
Tempus[35,1]=12
Tempus[n+1, 1]=STOPTIME-Tempus[n,2]  ; time after the last milking

; milk secretion after each preceding milking
Control[2..n] = if time > Tempus[i,2] then 0 else 1                 ; milk secretion stop factor for each milking
interval after the first milking
```

```
tm[2..(n+1)] = if time< Tempus[i-1,2] then 0 else time-Tempus[i-1,2]          ; milk secretion start factor for each milking interval after the first milking
Vmilkinga[1] = if time <t0 then time*VMmilkspm/(time+sm) else 0      ; L, volume of milk secreted before the first milking
Vmilkinga[2..n] = if tm[i]>0 then Control[i]*(tm[i]*VMmilkspm)/(tm[i]+sm) else 0      ; L, volume of milk secreted before the ith milking
Vmilkinga[n+1] = if time< Tempus[n,2] then 0 else (tm[i]*VMmilkspm)/(tm[i]+sm)      ; L, volume of milk secreted after the last milking
Vmilking = arraysum(Vmilkinga[*])                                                  ; L, the sum of each milk secretion interval creates the volume of milking secreted throughout the study duration
Vresidue = VMmilkspm*Fm                                                            ; L, volume of residual milk after preceding milking
Vmilksp = arraysum(Vmilkinga[*])+Vresidue                                          ; L, volume of total milk in milk space

;; Penicillin G in milk
; milking using schedule (this is the important part where problems be), this part of code simulates the penicillin amount change in milk by episodic milking
; The amount of penicillin G eliminated by milking depends on the ratio of the volume of secreted milk and total milk in udders at each milking time point. But the dynamic of milk volume change is simulated separated from the dynamic of penicillin G amount change. During the milking_time, the dynamic changes of secreted milk volume and total milk volume have impact on penicillin G eliminated by milking. So the MAX combined with DELAY function was used to make the milking ratio a confirmed number

Rmilkingex = (max(Amilk, delay(Amilk, milking_time))*Vmaxmilking)/(milking_time*Vmaxmilk)
        ; Rmilkingex stands for the rate of penicillin G eliminated by milking
StartFM[2..n+1] = if MOD(tm[i], Tempus[i,1]) >= milking_time then 0 else 1
        ; Start factor for each milking process
StopFM[2..n+1] = if time-Tempus[i-1,2] > Tempus[i,1] then 0 else 1
        ; Stop factor for each milking process after each milking interval
Stop[2..n+1] = if time<Tempus[i-1,2] then 0 else 1
        ; Stop factor for each milking process before each milking interval
Rmilkinga[2..n+1] = Rmilkingex*StopFM[i]*StartFM[i]*Stop[i]
        ; Rmilkinga is the array of Rmilking
Rmilking = arraysum(Rmilkinga[*])
        ; Rmilking, the rate of change of the amount of drug via milk (mg/h)
; Vmaxmilking = max(Vmilking,delay(Vmilking, milking_time))
        ; It is OK to use only two numbers for getting the Vmaxmilking, using more in delay function is to reduce the step length
Vmaxmilking = max(Vmilking,delay(Vmilking, 0.1*milking_time),delay(Vmilking, 0.2*milking_time),delay(Vmilking, 0.3*milking_time),delay(Vmilking, 0.4*milking_time),delay(Vmilking, 0.5*milking_time),delay(Vmilking, 0.6*milking_time),delay(Vmilking, 0.7*milking_time),delay(Vmilking, 0.8*milking_time),delay(Vmilking, 0.9*milking_time),delay(Vmilking, milking_time))
        ; making a confirmed number for Vmilking during milking_time
Vmaxmilk = max(Vmilksp,delay(Vmilksp, 0.1*milking_time),delay(Vmilksp, 0.2*milking_time),delay(Vmilksp, 0.3*milking_time),delay(Vmilksp, 0.4*milking_time),delay(Vmilksp, 0.5*milking_time),delay(Vmilksp, 0.6*milking_time),delay(Vmilksp, 0.7*milking_time),delay(Vmilksp, 0.8*milking_time),delay(Vmilksp, 0.9*milking_time),delay(Vmilksp, milking_time))
        ; making a confirmed number for Vmilk during milking_time

; Penicillin in milk
Rmilk = Rinputimm+Rmilkex-Kab*Amilk-Rmilking          ; Rmilk the rate of change of the amount of drug in milk (mg/h)
d/dt(Amilk) = Rmilk                                   ; Amilk amount of  the drug in milk (mg)
init Amilk = 0
Cmilka[1] = if time< t0 then Amilk/Vmaxmilk else 0
```

```
Cmilka[2..n+1] = if MOD(tm[i],Tempus[i,1]) <= milking_time then Amilk/Vmaxmilk*Stop[i]*StopFM[i] else
Amilk/Vmilksp*Stop[i]*StopFM[i]; Cmilk drug concentration in milk (mg/L)
Cmilk = arraysum(Cmilka[*])
d/dt(AUCCmilk) = Cmilk                    ; AUCCmilk AUC of drug concentration in milk (mg*h/L)
init AUCCmilk = 0
init Aab = 0
d/dt(Aab) = Kab*Amilk                     ; Aab amount of  the drug absorpted in udder (mg)

; Penicillin in mammary gland
RU = QU*(CAfree-CVU)-Rmilkex+Kab*Amilk    ; RU the rate of change of the amount of drug in the udder
(mg/h)
d/dt(AU) = RU                             ; AU amount of  the drug in the udder tissue
init AU = 0
CU = AU/VU                                ; CU drug concentration in the udder tissue (mg/L)
CVU = AU/(VU*PU)
d/dt(AUCCU) = CU                          ; AUCCU AUC of drug concentration in the udder tissue
(mg*h/L)
init AUCCU = 0
init Amilkex = 0
d/dt(Amilkex) = Rmilkex                   ; Amilkex amount of  the drug secreted to milk (mg)

VmaxC = 0.0022                  ; mg/h/kg BW
Km = 0.7                        ; mg/L
Vmax = VmaxCm*BWm               ; mg/h, Rate of penicillin secretion from udder tissue
Rmilkex = Vmax*CVU/(CVU+Kmm)
KabC = 1e-4                     ; L/h/kg BW
Kab = KabCm*BWm                 ; /h, Rate of penicillin absorption in udder tissue

; dosing using schedule
Rimm= DOSEimm/Timm
IMM1 = if MOD(time, Tinterval) < 1.1*milking_time then 0 else 1                 ; IMM injection start after
milking
IMM2 = if MOD(delay(time,1.1*milking_time), Tinterval)>=Timm Then 0 Else 1      ; IMM injection duration
control factor, DELAY used here to create the scenario that IMM injection is after milking
IMMstop = if time>Tdoses*Tinterval Then 0 Else 1                                ; IMM injection end
control factor
Rinputimm = Rimm*IMM1*IMM2*IMMstop
d/dt(AimmPen) = Rinputimm
init AimmPen = 0

{Mass balance equations}
Qbal = QC-QM-Qrest-QF-QK-QL-QU
Tmass = ABlood+AM+ALu+Arest+AF+AK+AL+Aurine+Amet+AU+Amilkex
Input = Absorbim+Aab
Bal = Input-Tmass

{
;;Milk volume (two-compartment model)
t0=6                    ; Start time for the first milking (first milking after IMM injection)
n = 10                  ; Times of milking
Tempus = 12             ; Milking time interval
VMmilksp = 26.8         ; Maximum volume of milk space
s = 23.2                ; Time to get half of the total volume of milk space
F = 0.04                ; Milk residue ratio after milking (estimated)
VMalv = 11.7            ; total volume of alveoli
salv =  9.4             ; time to get half of the total volume of alveoli
```

```
milking_time = 0.1
Timm = 0.01                          ; h, Time for IMM injection
; Milk volume
tm = time-t0-(i-1)*Tempus                                          ; tm, time after the preceding milking
i = if int((time-t0)/Tempus) < n then int((time-t0)/Tempus)+1 else n      ; the ith milking
Vcisresidue = 0.2                                  ; L
Vresidue = VMmilksp*F
Valvresidue = Vresidue - Vcisresidue
Vmilksp = Vmilking+Vresidue                        ; total milk volume in milk space
Vmilking = (tm*VMmilksp)/(tm+s)                    ; total volume of milk secreted after preceding milking
Valv = Vmilkingalv+Valvresidue                     ; total milk volume in alveoli
Vmilkingalv = (tm*VMalv)/(tm+salv)                 ; volume of milk secreted and in alveoli after preceding
milking
Vcis = Vmilksp-Valv
Vmilkingcis = Vcis-Vcisresidue
}
```

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