Supplementary Materials: Application of a Physiologically Based Pharmacokinetic Model to Develop a Veterinary Amorphous Enrofloxacin Solid Dispersion

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Table S1. Physiological parameters used in the PBPK model for enrofloxacin in swine.

Parameter	Abbreviation	Model value	Published value
Body weight (kg)	BW	20.0	92.5 ± 4.0[c]
Cardiac output (L/h/kg)	QCC	5.0	8.70 ± 1.62[c]
Tissue volume[a]			
Small intestine	VSiC	0.036	0.0126 ± 0.0023[c]
Arterial blood	VartC	0.0156	0.0156[d]
Venous blood	VvenC	0.0444	0.0444[d]
Liver	VLC	0.0247	0.0201 ± 0.0025[c]
Kidney	VKC	0.004	0.0037 ± 0.001[c]
Muscle	VMC	0.4	0.426 ± 0.039[c]
Fat	VFC	0.32	0.32[d]
Lung	VLuC	0.01	0.0077 ± 0.0014[c]
Rest of body	VrestC	0.1813	0.1813[d]
Blood flow[b]			
Liver	QLC	0.2725	0.243 ± 0.075[c]
Kidney	QKC	0.12	0.114 ± 3.2[c]
Muscle	QMC	0.251	0.342 ± 0.346[c]
Fat	QFC	0.1275	0.1275[d]
Rest of body	QrestC	0.229	0.229[d]

Note: [a]fraction of body weight, unitless; [b]fraction of cardiac output, unitless.
[c]Lin et al, 2020 (the experimental measured data); [d]Lin et al, 2016.

Table S2. Chemical-specific parameters used in the PBPK model for enrofloxacin in swine.

Parameter	Abbreviation	Model value	Published value[a]
Absorption rate constant (/h)			
Gastric emptying	Kst	2.0	1.0
Intestinal absorption	Ka	0.55	0.55
Intramuscular	Kim	0.0	0.5
Subcutaneous	Ksc	0.0	0.1
Tissue: plasma partition coefficient (unitless)			
Liver	PL	4.3	4.3
Kidney	PK	5.5	5.5
Muscle	PM	3.0	3.0
Fat	PF	0.53	0.53
Lung	Plu	4.3	4.3
Hepatic metabolic rate [(/h*kg)]	KmC	0.045	0.045
Plasma protein binding rate	PB	0.46	0.46
Fecal elimination rate constant (/h)	Kfeces	0.01	0.01
Urinary elimination rate constant (L/h/kg)	KurineC	0.12	0.12

Note: [a]Lin et al, 2016.

Enrofloxacin PBPK model code in AcslX format

PROGRAM

INITIAL

! code that is executed once at the beginning of a simulation run goes here

!! Physiological parameters

! Blood flow rates (fraction of cardiac output)

CONSTANT QCC = 5 ! Cardiac output index (L/h/kg), also blood flow of lung, from Upton (2008)

CONSTANT QLC = 0.2725 ! liver, average from Buur et al. (2005) and Upton (2008)

CONSTANT QKC = 0.12 ! kidney, average from Buur et al. (2005) and Upton (2008)

CONSTANT QMC = 0.251 ! Muscle, average from Buur et al. (2005) and Upton (2008)

CONSTANT QFC = 0.1275 ! Fat, average from Buur et al. (2005) and Upton (2008)

! Tissue volumes (fraction of body weight)

CONSTANT BW = 55 ! Kg, body weight was study-specific; The actual value in present study

CONSTANT VLC = 0.0247 ! liver, average from Buur et al. (2005) and Upton (2008)

CONSTANT VKC = 0.004 ! Kidneys, average from Buur et al. (2005) and Upton (2008)

CONSTANT VMC = 0.40 ! Muscle, average from Buur et al. (2005) and Upton (2008)

CONSTANT VFC = 0.32 ! Fat, adipose tissue, average from Buur et al. (2005) and Upton (2008)

CONSTANT VLuC = 0.01 ! Lungs, average from Buur et al. (2005) and Upton (2008)

CONSTANT VBloodC = 0.06 ! Blood, average from Buur et al. (2005) and Upton (2008)

CONSTANT VSiC = 0.036 ! Small intestine, from Lautz et al. (2020).

!! Mass transfer parameters (Chemical-specific parameters)

! Chemical molecular weights and unit conversion factors, from PubChem

CONSTANT MW = 359.4 ! g/mol, enrofloxacin

CONSTANT MWmol = 2.78 ! µmol/mg, enrofloxacin, from mg to µmol

CONSTANT MWmg = 0.36 ! mg/µmol, enrofloxacin, from µmol to mg

! Kinetic constants

! Oral absorption and fecal elimination rate constants for enrofloxacin

CONSTANT Kst = 2 ! /h, gastric emptying rate constant

CONSTANT Ka = 0.55 ! /h, intestinal absorption rate constant

CONSTANT Kfeces = 0.01 ! /h, intestinal transit rate constant (fecal elimination rate constant)

! IV infusion/injection rate constants

CONSTANT Timeiv = 0.01 ! h, IV infusion/injection time

! IM absorption rate constants (set parameter value equal to 0.0 when not used in a particular simulation)

CONSTANT Kim = 0.0 ! /h, intramuscular absorption rate constant

! SC absorption rate constants

CONSTANT Ksc = 0.0 ! /h, subcutenous absorption rate constant

! Partition coefficients for enrofloxacin (PC, unitless)

! The values from Buur et al. 2005 were used as initial values for further estimation.

CONSTANT PL = 4.3 ! Liver:plasma PC

CONSTANT PK = 5.5 ! Kidney:plasma PC

CONSTANT PM = 3 ! Muscle:plasma PC

CONSTANT PF = 0.53 ! Fat:plasma PC

CONSTANT PLu = 4.3 ! Lung:plasma PC

CONSTANT Prest = 8 ! Rest-of-body:plasma PC

! Partition coefficients for the ciprofloxacin (usually designated as the marker residue) (PC, unitless)

CONSTANT PL1 = 4.3 ! Liver:plasma PC

CONSTANT PK1 = 5.5 ! Kidney:plasma PC

CONSTANT PM1 = 4.3 ! Muscle:plasma PC

CONSTANT PF1 = 0.53 ! Fat:plasma PC

CONSTANT PLu1 = 4.3 ! Lung:plasma PC

CONSTANT Prest1 = 8 ! Rest-of-body:plasma PC

! Percentage plasma protein binding (unitless), Buur et al. (2005)

CONSTANT PB = 0.46 ! Percentage of enrofloxacin bound to plasma proteins

! Metabolic rate constants

CONSTANT KmC = 0.045 ! /(h*kg), liver metabolic rate constant of the enrofloxacin

! Urinary elimination rate constants

CONSTANT KurineC = 0.12 ! L/h/kg, for enrofloxacin

CONSTANT PDOSEoral = 5 ! mg/kg

CONSTANT PDOSEiv = 0 ! mg/kg

CONSTANT PDOSEim = 0 ! mg/kg

CONSTANT PDOSEsc = 0 ! mg/kg

END ! INITIAL

DYNAMIC

ALGORITHM IALG = 2

NSTEPS NSTP = 10

MAXTERVAL MAXT = 1.0e9

MINTERVAL MINT = 1.0e-9

CINTERVAL CINT = 0.1

DERIVATIVE

! code for calulating the derivative goes here

! Cardiac output and blood flows to tissues (L/h)

QC=QCC*BW ! Cardiac output

QL=QLC*QC ! Blood flow to the liver

QK=QKC*QC ! Blood flow to the kidney

QM=QMC*QC ! Blood flow to the muscle

QF=QFC*QC ! Blood flow to the fat

Qrest = QC-QL-QK-QM-QF ! Blood flow to the rest of body

! Tissue volumes (L)

VL=VLC*BW ! Liver

VK=VKC*BW ! Kidney

VM=VMC*BW ! Muscle

VF=VFC*BW ! Fat

VLu=VLuC*BW ! Lung

VBlood=VBloodC*BW ! Blood

Vven=VBlood*0.74 ! Venous blood

Vart=VBlood*0.26 ! Arterial blood

Vrest = BW-VL-VK-VM-VF-VLu-VBlood ! Rest of body

VSi=VSiC*BW ! Small intestine

! Dosing amounts (mg converted to µmol)

DOSEoral=PDOSEoral*BW*MWmol ! µmol

DOSEiv=PDOSEiv*BW*MWmol ! µmol

DOSEim=PDOSEim*BW*MWmol ! µmol

DOSEsc=PDOSEsc*BW*MWmol ! µmol

! Multiple oral dosing using the PULSE/EXPOSURE function

CONSTANT tlen = 0.001 ! Length of exposure, oral, iv, im, or sc (h/day)

CONSTANT tinterval = 12 ! administration interval, varied dependent on the exposure paradigm (h)

CONSTANT Dstart = 0.0 ! Initiation day of exposure (day)

CONSTANT Dstop = 5 ! Termination day of exposure (day)

CONSTANT MAXT = 1.0 ! maximum comm. interval

CONSTANT CINTC = 0.1 ! Communication interval

CINT = CINTC ! Communication interval

Tsim=TSTOP*24 ! Tstop in hours

DS=Dstart*24 ! Initiation time point of exposure (h)

Doff=(Dstop-Dstart)*24 ! Exposure duration (h)

TimeOn=Dstart*24 ! Initiation time point of exposure (h)

TimeOff=Dstop*24+tlen ! Terminatison time point of exposure (h)

Exposure=PULSE(0,tinterval,tlen)*PULSE(DS,Tsim,Doff) ! Exposure paradigm

RDOSEoral=(DOSEoral/tlen)*Exposure ! Administrataion rate

RAST=RDOSEoral-Kst*AST ! Rate in the stomach

AST=Integ(RAST,0)!0.0 or Doseoral if the initial dose is twice as the subsequent dose.

RAI=Kst*AST-Ka*AI-Kfeces*AI ! Rate in the intestine

Rfeces=Kfeces*AI ! Fecal elimination rate

Afeces=Integ(Rfeces,0.0) ! Amount eliminated throμgh feces

AI=Integ(RAI,0.0) ! Amount in the intestine

CAI=AI/VSi ! Concentration of the enrofloxacin in small intestine, μmol/L

CAImg=AI*MWmg ! Concentration of the total enrofloxacin in small intestine, unit conversion from μmol/L to mg/L (μg/g)

RAO=Ka*AI ! Oral absorption rate

AAO=Integ(RAO,0.0) ! Amount absorbed

! Single IV dosing to the venous

IVR=DOSEiv/timeiv

RIV=IVR*(1.0-step(timeiv)) ! Intravenous injection rate

AIV=Integ(RIV,0.0) ! Amount injected

! Single IM exposure

Rim=Kim*Aimsite ! Intramuscular absorption rate

Aim=Integ(Rim,0.0) ! Amount absorbed via IM route

Rimsite=-Kim*Aimsite ! Rate of changes in the amount of the drug in the injection site

Aimsite=Integ(Rimsite,Doseim) ! Amount of the drug remained in the injection site

! Multiple IM exposure (if needed)

!RDOSEim=(DOSEim/tlen)*Exposure

!Rimsite=RDOSEim-Kim*Aimsite

!Aimsite=Integ(Rimsite,0.0)

!Rim=Kim*Aimsite

!Aim=Integ(Rim,0.0)

! Single SC exposure

Rsc=Ksc*Ascsite ! Subcutaneous absorption rate

Asc=Integ(Rsc,0.0) ! Amount absorbed via SC route

Rscsite=-Ksc*Ascsite ! Rate of changes in the amount of the drug in the injection site

Ascsite=Integ(Rscsite,Dosesc) ! Amount of the drug remained in the injection site

! Metabolic rate

Km=KmC*BW ! h-1

! Urinary elimination rates

Kurine=KurineC*BW ! L/h, for the enrofloxacin

Kurine1=Kurine1C*BW ! L/h, for the ciprofloxacin

! Venous blood/plasma

RV=QL*CVL+QK*CVK+QM*CVM+QF*CVF+Qrest*CVrest+Riv+Rim+Rsc-QC*CV ! Rate, μmol/h

AV=Integ(RV,0.0) ! Amount, μmol

CV=AV/Vven ! Concentration of the total enrofloxacin (free plus bound), μmol/L

CVfree=CV*(1-PB) ! Concentration of the enrofloxacin that is free, μmol/L

CVbound=CV*PB ! Concentration of the enrofloxacin that is bound, μmol/L

CVmg=CV*MWmg ! Concentration of the total enrofloxacin (free plus bound), unit conversion from μmol/L to mg/L (μg/g)

! Arterial blood/plasma

RA=QC*CVLu-QC*CAfree ! Rate, μmol/h

AA=Integ(RA,0.0) ! Amount, μmol

CA=AA/Vart ! Concentration of the total enrofloxacin (free plus bound), μmol/L

CAfree=CA*(1-PB) ! Concentration of the enrofloxacin that is free, μmol/L

CAbound=CA*PB ! Concentration of the enrofloxacin that is bound, μmol/L

ABlood=AV+AA ! Amount of the total drug in the blood, μmol

! Lung compartment

RALu=QC*(CV-CVLu) ! Rate, μmol/h

ALu=Integ(RALu,0.0) ! Amount, μmol

CLu=ALu/VLu ! Concentration of the total enrofloxacin in the lung, μmol/L

CVLu=CLu/PLu ! Concentration of the total enrofloxacin in venous blood drained from the lung, μmol/L

! Liver compartment

RL=QL*(CAfree-CVL)+RAO-Rmet ! Rate, μmol/h

AL=Integ(RL,0.0) ! Amount, μmol

CL=AL/VL ! Concentration of the total enrofloxacin in the liver, μmol/L

CVL=CL/PL ! Concentration of the total enrofloxacin in the venous blood drained from the liver, μmol/L

CLmg=CL*MWmg ! Concentration of the total enrofloxacin in the liver, mg/L (μg/g)

! Metabolism of the parent compound in the liver compartment

Rmet=Km*CL*VL ! Total hepatic metabolic rate, μmol/h

Rmet1=Rmet*Frac ! Hepatic metabolic rate to the ciprofloxacin, μmol/h

Rmet2=Rmet*(1-Frac) ! Hepatic metabolic rate to other minor metabolites, μmol/h

Amet=Integ(Rmet,0.0) ! Amount of the enrofloxacin that is metabolized in the liver, μmol

Amet1=Integ(Rmet1,0.0) ! Amount of the ciprofloxacin that is produced in the liver, μmol

Amet2=Integ(Rmet2,0.0) ! Amount of other minor metabolites that are produced in the liver, μmol

! Kidney compartment

RK=QK*(CAfree-CVK)-Rurine ! Rate, μmol/h

AK=Integ(RK,0.0) ! Amount, μmol

CK=AK/VK ! Concentration of the total enrofloxacin in the kidney, μmol/L

CVK=CK/PK ! Concentration of the total enrofloxacin in the venous blood drained from the kidney, μmol/L

Ckmg=Ck*MWmg ! Concentration of the total enrofloxacin in the kidney, mg/L (μg/g)

! Urinary excretion of the parent compound

Rurine=Kurine*CVK ! Rate, μmol/h

Aurine=Integ(Rurine,0.0) ! Amount, μmol

! Muscle compartment

RM=QM*(CAfree-CVM) ! Rate, µmol/h

AM=Integ(RM,0.0) ! Amount, µmol

CM=AM/VM ! Concentration of the total enrofloxacin in the muscle, µmol/L

CVM=CM/PM ! Concentration of the total enrofloxacin in the venous blood drained from the muscle, µmol/L

CMmg=CM*MWmg ! Concentration of the total enrofloxacin in the muscle, mg/L (µg/g)

! Fat compartment

RF=QF*(CAfree-CVF) ! Rate, µmol/h

AF=Integ(RF,0.0) ! Amount, µmol

CF=AF/VF ! Concentration of the total enrofloxacin in the fat, µmol/L

CVF=CF/PF ! Concentration of the total enrofloxacin in the venous blood drained from the fat, µmol/L

CFmg=CF*MWmg ! Concentration of the total enrofloxacin in the fat, mg/L (µg/g)

! Rest-of-body compartment

Rrest=Qrest*(CAfree-CVrest) ! Rate, µmol/h

Arest=Integ(Rrest,0.0) ! Amount, µmol

Crest=Arest/Vrest ! Concentration of the total enrofloxacin in the rest-of-body, µmol/L

CVrest=Crest/Prest ! Concentration of the total enrofloxacin in the venous blood drained from the rest-of-body, µmol/L

! Mass balance for the enrofloxacin

Qbal=QC-QL-QK-QM-QF-Qrest ! Blood flow balance

Tmass=ABlood+AL+AK+AM+AF+Arest+ALu+Aurine+Amet ! Total amount in the body, µmol

Bal=AAO+AIV+AIM+ASC-Tmass ! Mass balance, input minus output should be equal to zero at all time

END ! DERIVATIVE

! Add discrete events here as needed

! DISCRETE

! END

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

CONSTANT TSTOP = 230

TERMT (T .GE. TSTOP, 'checked on communication interval: REACHED TSTOP')

END ! DYNAMIC

TERMINAL

! code that is executed once at the end of a simulation run goes here

END ! TERMINAL

END ! PROGRAM