

SUPPORTING INFORMATION

Pharmacokinetics of Mequindox and its Marker Residue 1,4-bisdesoxymequindox in Swine Following Multiple Oral Gavage and Intramuscular Administration: an Experimental Study Coupled with Population Physiologically Based Pharmacokinetic Modeling

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1. Mass balance differential equations for the flow-limited compartment (the kidney as an example)

$$RK1 = QK1 * (CAfree1 - CVK1) - Rurine1 \quad (S1)$$

$$d/dt(AK1) = RK1 \quad (S2)$$

$$\text{init } AK1 = 0 \quad (S3)$$

$$CK1 = AK1/VK1 \quad (S4)$$

$$CVK1 = AK1/(VK1*PK1) \quad (S5)$$

$$CK1\text{mg} = CK1 * MW1\text{mg} \quad (S6)$$

In these equations, RK1 is the rate of change in the amount of 1,4-bisdesoxy-mequindox (M1) in the kidney ($\mu\text{mol/h}$); QK1 is the blood flow to the kidney per hour (L/h); CAfree1 is the arterial blood unbounded concentration of M1 ($\mu\text{mol/L}$); CVK1 is the kidney venous blood concentration of M1 ($\mu\text{mol/L}$); Rurine1 is the urinary elimination rate of M1 ($\mu\text{mol/h}$); AK1 is M1 amount in the kidney (μmol); CK1 is M1 concentration in the kidney ($\mu\text{mol/L}$); PK1 is the kidney:plasma partition coefficient (PC, unitless); CK1mg is M1 concentration in the kidney ($\mu\text{g/L}$); MW1mg is M1 chemical molecular weight conversion factor from μmol to mg ($\text{mg}/\mu\text{mol}$).

2. Equations describing repeated oral gavage and intramuscular injection

It was assumed that following oral gavage the drug was immediately available in the stomach, and distributed into the intestine by gastric emptying, with a gastric emptying rate constant (Kst, 1/h). Once in the intestine, drug absorption and elimination were controlled by intestinal absorption rate constant (Ka, 1/h) and intestinal transit rate constant (Kint, 1/h), respectively. These processes were assumed to be linear. As an example, the process of intestinal absorption was described with the following equation:

$$RAI = Kst * AST - Ka * AI - Kint * AI \quad (S7)$$

$$RAO = Ka * AI \quad (S8)$$

Where RAI is the rate of change in the amount of MEQ in the intestine ($\mu\text{mol/h}$); RAO is the intestinal absorption rate ($\mu\text{mol/h}$); AST and AI are the amounts of the drug in the stomach and intestine, respectively (μmol).

Intramuscular absorption was described with a two-compartment model (site1 and site2) with absorption occurring from site1. The rate constants for distribution of MEQ from the central (site1) to the peripheral (site2) and site2 to site1 are k_{12} and k_{21} , respectively.

$$R_{site1} = R_{DOSEim} - R_{im} - R_{12} + R_{21} \quad (S9)$$

$$R_{im} = K_{im} * A_{site1} \quad (S10)$$

Where R_{site1} is the rate of change in the amount of MEQ in the injection site1 ($\mu\text{mol/h}$); R_{DOSEim} is the injection rate of MEQ in the injection site1 ($\mu\text{mol/h}$); R_{im} is the absorption rate of MEQ from injection site 1 ($\mu\text{mol/h}$); R_{12} is the distribution rate of MEQ from site1 to site2 ($\mu\text{mol/h}$); R_{21} is the distribution rate of MEQ from site2 to site1 ($\mu\text{mol/h}$); K_{im} is the absorption rate constant of MEQ (1/h); A_{site1} is the amount of MEQ in the injection site1.

Repeated oral exposure paradigms were described with the REPEAT/EXPOSURE function and shown below:

$$\text{REPEAT}[1..T_{doses}] = \text{SQUAREPULSE}(0+(i-1)*T_{interval}, T_{len}) \quad (S11)$$

$$\text{EXPOSURE} = \text{ARRAYSUM}(\text{REPEAT}[*]) \quad (S12)$$

Where $T_{interval}$ is the dosing interval time (h); T_{len} is the length of each exposure (here, as oral gavage exposure duration is very short, T_{len} was set at 0.001 h, while T_{len} was set at 0.01 h for IM); T_{doses} is the number of injections for multiple exposure.

3. The equation describing liver metabolism

$$R_{met} = K_m * CL * VL \quad (S13)$$

$$R_{met1} = R_{met} * \text{Frac} \quad (S14)$$

Where R_{met} is the total hepatic metabolic rate of MEQ ($\mu\text{mol/h}$); K_m is the metabolic rate constant (1/h); R_{met1} is the hepatic metabolic rate of MEQ to M1 ($\mu\text{mol/h}$); Frac is the fraction of MEQ metabolized to M1.

4. The equation describing urine clearance

$$R_{urine1} = K_{urine1} * C_{VK1} \quad (S15)$$

Where R_{urine1} is the urine elimination rate of M1 ($\mu\text{mol/h}$); K_{urine1} is the urine elimination rate constant of M1 (L/h); C_{VK1} is the kidney venous blood concentration of M1 ($\mu\text{mol/L}$)

5. Residue depletion experiments of MEQ in swine

Table S1. Residue levels of M1 in tissues and plasma following oral gavage of MEQ suspension at 10 mg/kg twice daily for 3 consecutive days in Group A.

| Animal number | Days post-dose | Tissues ($\mu\text{g}/\text{kg}$) | | | | Plasma ($\mu\text{g}/\text{kg}$) |
|---------------|----------------|-------------------------------------|--------|--------|-----|------------------------------------|
| | | liver | kidney | muscle | fat | |
| 1 | 0.16 | 283 | 225 | 22 | 49 | 66 |
| 2 | 0.16 | 383 | 280 | 35 | 69 | 98 |
| 3 | 0.16 | 447 | 389 | 73 | 128 | 188 |
| 4 | 0.16 | 690 | 467 | 91 | 198 | 254 |
| 5 | 0.16 | 664 | 582 | 130 | 268 | 348 |
| 6 | 0.25 | 264 | 254 | 18 | 57 | 64 |
| 7 | 0.25 | 378 | 268 | 39 | 74 | 89 |
| 8 | 0.25 | 480 | 390 | 75 | 142 | 195 |
| 9 | 0.25 | 645 | 441 | 98 | 184 | 247 |
| 10 | 0.25 | 730 | 625 | 128 | 275 | 374 |
| 11 | 0.5 | 256 | 272 | 35 | 44 | 55 |
| 12 | 0.5 | 334 | 289 | 49 | 83 | 81 |
| 13 | 0.5 | 490 | 384 | 69 | 135 | 193 |
| 14 | 0.5 | 623 | 425 | 83 | 178 | 265 |
| 15 | 0.5 | 743 | 586 | 118 | 289 | 364 |
| 16 | 1 | 223 | 205 | 20 | 55 | 60 |
| 17 | 1 | 312 | 254 | 43 | 78 | 78 |
| 18 | 1 | 399 | 340 | 67 | 124 | 145 |
| 19 | 1 | 547 | 389 | 83 | 143 | 225 |
| 20 | 1 | 721 | 579 | 108 | 258 | 354 |
| 21 | 3 | 125 | 75 | 9 | 22 | 22 |
| 22 | 3 | 157 | 98 | 19 | 39 | 51 |
| 23 | 3 | 187 | 168 | 28 | 57 | 66 |
| 24 | 3 | 234 | 225 | 39 | 78 | 105 |
| 25 | 3 | 368 | 295 | 64 | 127 | 178 |
| 26 | 5 | 45 | 24 | 6 | 7 | 8 |
| 27 | 5 | 65 | 34 | 9 | 15 | 16 |
| 28 | 5 | 78 | 74 | 14 | 22 | 28 |
| 29 | 5 | 105 | 107 | 18 | 41 | 54 |
| 30 | 5 | 168 | 137 | 22 | 55 | 78 |
| 31 | 7 | 15 | 12 | 5.2 | 7 | 7 |
| 32 | 7 | 25 | 22 | 6 | 9 | 11 |
| 33 | 7 | 41 | 36 | 8 | 12 | 14 |
| 34 | 7 | 62 | 48 | 10 | 15 | 22 |
| 35 | 7 | 84 | 66 | 14 | 25 | 36 |
| 36 | 9 | 10 | 8 | ND | 6 | 6 |
| 37 | 9 | 15 | 12 | ND | 7.9 | 7.9 |
| 38 | 9 | 25 | 25 | ND | 8.9 | 8.9 |
| 39 | 9 | 34 | 31 | ND | 10 | 9 |
| 40 | 9 | 59 | 47 | ND | 13 | 12 |
| 41 | 11 | 5.1 | 5 | ND | ND | ND |
| 42 | 11 | 9 | 6.2 | ND | ND | ND |
| 43 | 11 | 14 | 8 | ND | ND | ND |
| 44 | 11 | 18 | 15 | ND | ND | ND |
| 45 | 11 | 25 | 25 | ND | ND | ND |

ND, below the LOD for M1 in the liver, kidney, muscle, fat, and plasma (5 $\mu\text{g}/\text{kg}$).

Table S2. Residue levels of M1 in tissues and plasma following oral gavage of MEQ suspension at 10 mg/kg twice daily for 3 consecutive days in Group B.

| Animal number | Days post-dose | Tissues (µg/kg) | | | | Plasma (µg/kg) |
|---------------|----------------|-----------------|--------|--------|-----|----------------|
| | | liver | kidney | muscle | fat | |
| 1 | 0.5 | 268 | 298 | 36 | 45 | 66 |
| 2 | 0.5 | 338 | 304 | 52 | 78 | 144 |
| 3 | 0.5 | 498 | 395 | 71 | 132 | 201 |
| 4 | 0.5 | 633 | 445 | 93 | 188 | 274 |
| 5 | 0.5 | 753 | 614 | 125 | 278 | 372 |
| 6 | 1 | 214 | 168 | 18 | 54 | 49 |
| 7 | 1 | 301 | 247 | 38 | 78 | 75 |
| 8 | 1 | 354 | 335 | 52 | 111 | 139 |
| 9 | 1 | 501 | 394 | 77 | 129 | 209 |
| 10 | 1 | 724 | 568 | 115 | 228 | 341 |
| 11 | 3 | 132 | 75 | 11 | 26 | 29 |
| 12 | 3 | 164 | 98 | 20 | 40 | 52 |
| 13 | 3 | 197 | 158 | 31 | 64 | 75 |
| 14 | 3 | 245 | 205 | 42 | 81 | 112 |
| 15 | 3 | 383 | 289 | 73 | 132 | 181 |
| 16 | 5 | 48 | 21 | 6 | 11 | 10 |
| 17 | 5 | 69 | 32 | 8 | 19 | 17 |
| 18 | 5 | 82 | 75 | 13 | 23 | 29 |
| 19 | 5 | 112 | 114 | 17 | 47 | 55 |
| 20 | 5 | 172 | 130 | 19 | 61 | 81 |
| 21 | 7 | 15 | 12 | 6 | 9 | 11 |
| 22 | 7 | 25 | 22 | 7 | 12 | 12 |
| 23 | 7 | 41 | 36 | 9 | 13 | 13 |
| 24 | 7 | 61 | 52 | 10 | 16 | 20 |
| 25 | 7 | 85 | 74 | 12 | 27 | 41 |
| 26 | 9 | 10 | 7 | ND | 6 | 6 |
| 27 | 9 | 12 | 11 | ND | 7 | 7 |
| 28 | 9 | 23 | 24 | ND | 8 | 8 |
| 29 | 9 | 32 | 29 | ND | 10 | 9 |
| 30 | 9 | 51 | 42 | ND | 12 | 12 |
| 31 | 11 | 6 | 5 | ND | ND | ND |
| 32 | 11 | 10 | 7 | ND | ND | ND |
| 33 | 11 | 13 | 9 | ND | ND | ND |
| 34 | 11 | 17 | 14 | ND | ND | ND |
| 35 | 11 | 24 | 26 | ND | ND | ND |

ND, below the LOD for M1 in the liver, kidney, muscle, fat, and plasma (5 µg/kg).

Table S3. Residue levels of M1 in tissues and plasma following IM administration of MEQ suspension at 5 mg/kg twice daily for 3 consecutive days in Group C.

| Animal number | Days post-dose | Tissues ($\mu\text{g}/\text{kg}$) | | | | Plasma ($\mu\text{g}/\text{kg}$) |
|---------------|----------------|-------------------------------------|--------|--------|-----|------------------------------------|
| | | liver | kidney | muscle | fat | |
| 1 | 7 | 66 | 55 | 11 | 22 | 33 |
| 2 | 7 | 128 | 95 | 16 | 42 | 55 |
| 3 | 7 | 188 | 138 | 25 | 49 | 65 |
| 4 | 7 | 261 | 193 | 35 | 62 | 85 |
| 5 | 7 | 352 | 279 | 55 | 70 | 125 |
| 6 | 9 | 22 | 22 | 6 | 12 | 22 |
| 7 | 9 | 51 | 47 | 9 | 21 | 42 |
| 8 | 9 | 65 | 60 | 12 | 31 | 49 |
| 9 | 9 | 98 | 70 | 16 | 46 | 62 |
| 10 | 9 | 168 | 104 | 32 | 65 | 70 |
| 11 | 11 | 12 | 9 | 5 | 9 | 9 |
| 12 | 11 | 21 | 15 | 7 | 11 | 11 |
| 13 | 11 | 32 | 20 | 7 | 15 | 15 |
| 14 | 11 | 56 | 25 | 10 | 20 | 20 |
| 15 | 11 | 81 | 35 | 11 | 25 | 40 |
| 16 | 14 | 5 | 5 | ND | 5 | 5 |
| 17 | 14 | 9 | 8 | ND | 7 | 6 |
| 18 | 14 | 12 | 10 | ND | 7 | 7 |
| 19 | 14 | 14 | 12 | ND | 10 | 9 |
| 20 | 14 | 20 | 15 | ND | 11 | 13 |

ND, below the LOD for M1 in the liver, kidney, muscle, fat, and plasma (5 $\mu\text{g}/\text{kg}$).

6. Physiological parameters distribution used in the Monte Carlo analysis

Table S4. Physiological parameter distributions used in the Monte Carlo analysis.

| Parameter ^a | Symbol | Mean | SD | CV | Lower bound | Upper bound |
|---|----------|--------|-----------|-------|-------------|-------------|
| <i>Body weight (kg)</i> | BW | 50.000 | 9.700E+00 | 0.194 | 3.099E+01 | 6.901E+01 |
| <i>Cardiac output (L/h/kg)</i> | QCC | 8.543 | 1.914E+00 | 0.224 | 4.792E+00 | 1.229E+01 |
| <i>Blood flow rates for MEQ(fraction of cardiac output, unitless)</i> | | | | | | |
| Liver | QLC | 0.273 | 8.190E-02 | 0.300 | 1.125E-01 | 4.335E-01 |
| Rest of body | QRC | 0.727 | 2.181E-01 | 0.300 | 2.995E-01 | 1.154E+00 |
| <i>Blood flow rates for M1(fraction of cardiac output, unitless)</i> | | | | | | |
| Liver | QLC1 | 0.273 | 8.190E-02 | 0.300 | 1.125E-01 | 4.335E-01 |
| Kidney | QKC1 | 0.116 | 1.728E-02 | 0.149 | 8.212E-02 | 1.499E-01 |
| Muscle | QMC1 | 0.293 | 4.219E-02 | 0.144 | 2.103E-01 | 3.757E-01 |
| Fat | QFC1 | 0.128 | 3.840E-02 | 0.300 | 5.274E-02 | 2.033E-01 |
| Rest of body | QRC1 | 0.190 | 5.700E-02 | 0.300 | 7.828E-02 | 3.017E-01 |
| <i>Tissue volumes for MEQ(fraction of body weight, unitless)</i> | | | | | | |
| Blood | VbloodC | 0.060 | 1.800E-02 | 0.300 | 2.472E-02 | 9.528E-02 |
| Liver | VLC | 0.023 | 3.450E-04 | 0.015 | 2.232E-02 | 2.368E-02 |
| Other of body | VRC | 0.917 | 2.751E-01 | 0.300 | 3.778E-01 | 1.456E+00 |
| <i>Tissue volumes for M1(fraction of body weight, unitless)</i> | | | | | | |
| Blood | VbloodC1 | 0.060 | 1.800E-02 | 0.300 | 2.472E-02 | 9.528E-02 |
| Liver | VLC1 | 0.023 | 3.450E-04 | 0.015 | 2.232E-02 | 2.368E-02 |
| Kidney | VKC1 | 0.005 | 1.900E-04 | 0.038 | 4.628E-03 | 5.372E-03 |
| Muscle | VMC1 | 0.355 | 2.485E-03 | 0.007 | 3.501E-01 | 3.599E-01 |
| Fat | VFC1 | 0.235 | 1.810E-02 | 0.077 | 1.995E-01 | 2.705E-01 |
| Rest of body | VRC1 | 0.322 | 9.660E-02 | 0.300 | 1.327E-01 | 5.113E-01 |

^aThe values were adopted from Li et al ¹. All physiological parameters were assumed to be in normal distribution.

7. Evaluation of the oral gavage model with tissue and plasma data

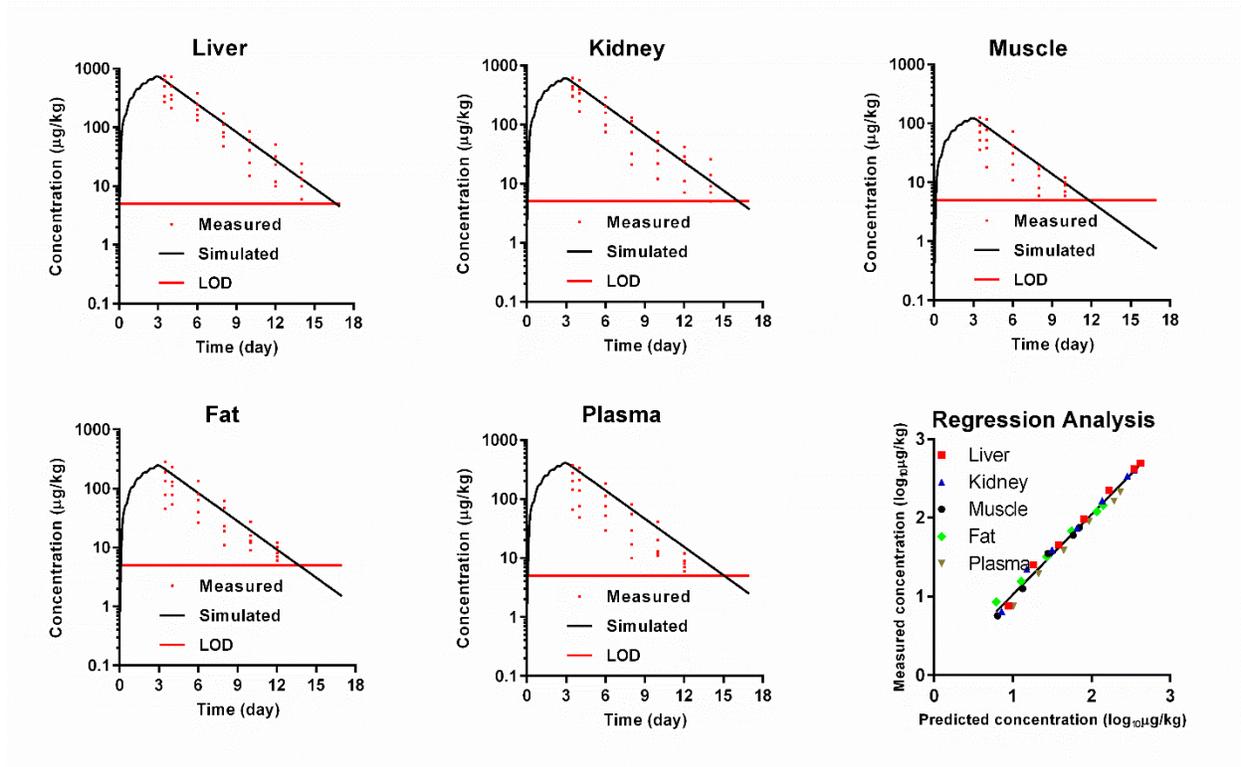


Figure S1 Evaluation of the oral gavage model with tissue and plasma data. Comparison of model predictions (solid line) and observed data (squares) for M1 concentrations in liver, kidney, muscle, fat, and plasma of swine exposed to MEQ via oral gavage at 10 mg/kg twice daily for 3 consecutive days. Result of regression analysis between model predictions and observed data is shown. The determination coefficient R^2 value is 0.98.

8. Model code (MMD file)

METHOD RK4

STARTTIME = 0
STOPTIME = 408 ;24
DT = 0.0025
DTOUT = 1

; Physiological parameters; Physiological parameter values of swine reported in Lin et al. (2016) were for an average pig. 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 swine. As a result, some of the physiological parameters have been updated from the Li et al. (2017) and the present paper. Overall, the value of each physiological parameter is still quite close to the value reported in Lin et al. (2016).

; Blood flow rates
QCC = 8.543 ; Cardiac output (L/h/kg)

; Blood flow rates for MEQ, unitless
QLC = 0.273 ; Fraction of blood flow to the liver
QRC = 0.727; Fraction of blood flow to the rest of body for MEQ, QRC = 1-QLC

; Blood flow rates for M1, unitless
QLC1 = 0.273 ; Fraction of blood flow to the liver
QKC1 = 0.116 ; Fraction of blood flow to the kidneys
QMC1 = 0.293 ; Fraction of blood flow to the muscle
QFC1 = 0.128 ; Fraction of blood flow to the fat
QRC1 = 0.190; Fraction of blood flow to the rest of body, QRC1 = 1-QLC1-QKC1-QFC1-QMC1

; Tissue volumes
BW = 50 ; Body weight (kg)

; Tissue volumes for MEQ, unitless
VLC = 0.023 ; Fractional liver tissue
VbloodC = 0.060 ; Blood volume, fraction of BW
VRC = 0.917 ; Fractional other of body for MEQ, VRC = 1-VLC-VbloodC

; Tissue volumes for M1, unitless
VLC1 = 0.023 ; Fractional liver tissue
VKC1 = 0.005 ; Fractional kidney tissue
VMC1 = 0.355 ; Fractional muscle tissue
VFC1 = 0.235 ; Fractional fat tissue
VbloodC1 = 0.060 ; Blood volume, fraction of BW
VRC1 = 0.322; Fractional rest of body for M1, VRC1 = 1-VLC1-VKC1-VFC1-VMC1-VbloodC1

; Mass Transfer Parameters, Chemical-specific parameters

; Chemical molecular weights and unit conversion factors

MW = 218.21 ; g/mol, mequindox
MW1 = 186 ; g/mol, M1
MWmol = 4.58 ; umol/mg, mequindox, from mg to umol
MWmg = 0.22 ; mg/umol, mequindox, from umol to mg
MW1mol = 5.38 ; umol/mg, M1, from mg to umol
MW1mg = 0.19 ; mg/umol, M1, from umol to mg

; Partition coefficients for MEQ, PC, unitless
PL = 2.4 ; Liver:plasma PC, Assumed equal to the Liver:plasma PC of the major metabolite M1 based on structural similarity
PR = 0.4 ; Rest-of-body:plasma PC, Assumed equal to the Rest-of-body:plasma PC of the major metabolite M1 based on structural similarity

; Partition coefficients for M1, designated as the marker residue, PC, unitless
PL1 = 2.4 ; Liver:plasma PC, Measured in the present study
PK1 = 2 ; Kidney:plasma PC, Measured in the present study
PM1 = 0.4 ; Muscle:plasma PC, Measured in the present study
PF1 = 0.8 ; Fat:plasma PC, Measured in the present study
PR1 = 0.4 ; Rest-of-body:plasma PC, Estimated in the present study

; Kinetic constants

; Oral absorption and fecal elimination rate constants for MEQ
Kst = 0.5 ; /h, gastric emptying rate constant, estimated in the present study
Ka = 0.04 ; /h, intestinal absorption rate constant, estimated in the present study
Kint = 0.4 ; /h, intestinal transit rate constant, estimated in the present study

; IM absorption rate constants, set parameter value equal to 0.0 when not used in a particular simulation

; IM absorption was described using a two-compartment absorption model based on Leavens et al., 2014

Kim = 1 ; IM absorption rate constant (/h)

K12 = 0.1 ; The rate constants for distribution of MEQ from the absorption compartment 1 to the absorption compartment 2, /h

K21 = 0.05 ; The rate constants for distribution of MEQ from the absorption compartment 2 to the absorption compartment 1, /h

; Percentage of plasma protein binding (unitless), measured in the present study

PB = 0.25 ; Percentage of MEQ bound to plasma proteins

PB1 = 0.25 ; Percentage of M1 bound to plasma proteins

Free = 1-PB

Free1 = 1-PB1

; Metabolic rate constants

KmC = 0.05 ; /(h*kg), liver metabolic rate constant of MEQ, estimated in the present study

Frac = 0.10 ; Unitless, fraction of MEQ metabolized to M1, estimated in the present study

; Urinary elimination rate constant

KurineC = 0.1 ; L/h/kg, for MEQ, estimated in the present study

Kurine1C = 0.01 ; L/h/kg, for M1, measured in the present study

; Parameters for various exposure scenarios

PDOSEoral = 10 ; (mg/kg)

PDOSEim = 0 ; (mg/kg)

;code for calculating the derivative goes here.....

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

QC = QCC*BW ; Cardiac output

; Cardiac output and blood flows to tissues for MEQ (L/h)

QL = QLC*QC ; Liver

QR = QRC*QC ; Rest of body

; Cardiac output and blood flows to tissues for M1 (L/h)

QL1 = QLC1*QC ; Liver

QK1 = QKC1*QC ; Kidney

QM1 = QMC1*QC ; Muscle

QF1 = QFC1*QC ; Fat

QR1 = QRC1*QC ; Rest of body

; Tissue volumes (L)

; Tissue volumes for MEQ (L)

VL = VLC*BW ; Liver

Vblood = VbloodC*BW ; Blood

VR = VRC*BW ; Rest of body

; Tissue volumes for M1 (L)

VL1 = VLC1*BW ; Liver

VK1 = VKC1*BW ; Kidney

VM1 = VMC1*BW ; Muscle

VF1 = VFC1*BW ; Fat

Vblood1 = VbloodC1*BW ; Blood

VR1 = VRC1*BW ; Rest of body

; Dosing amounts (mg converted to umol)

DOSEoral = PDOSEoral*BW*MW/mol ; (umol)

DOSEim = PDOSEim*BW*MW/mol ; (umol)

; Multiple dosing using the REPEAT/EXPOSURE function

tlen = 0.01 ; Length of oral gavage exposure (h/day)

tinterval = 12 ; Varied dependent on the exposure paradigm (h)

Tdoses = 6 ; Number of injections for multiple oral gavage

REPEAT[1..Tdoses] = SQUAREPULSE(0+(i-1)*tinterval, tlen)

Exposure = ARRAYSUM(REPEAT[*])

; Dosing, multiple oral gavage

RDOSEoral = (DOSEoral/tlen)*Exposure

RAST = RDOSEoral-Kst*AST

d/dt(AST) = RAST

init AST = 0

$RAI = Kst \cdot AST - Ka \cdot AI - Kint \cdot AI$
 $Rcolon = Kint \cdot AI$
 $d/dt(Acolon) = Rcolon$
 $init\ Acolon = 0$
 $d/dt(AI) = RAI$
 $init\ AI = 0$
 $RAO = Ka \cdot AI$
 $d/dt(AAO) = RAO$
 $init\ AAO = 0$

; Dosing, multiple intramuscular exposure
 $RDOSEim = (DOSEim/tlen) \cdot Exposure$
 $Rim = Kim \cdot Asite1$
 $Rsite1 = RDOSEim - Rim - R12 + R21$
 $d/dt(Asite1) = Rsite1$
 $init\ Asite1 = 0$
 $R12 = Asite1 \cdot K12$
 $R21 = Asite2 \cdot K21$
 $d/dt(Asite2) = R12 - R21$
 $init\ Asite2 = 0$
 $d/dt(Aim) = Rim$
 $init\ Aim = 0$

; Metabolic rate
 $Km = KmC \cdot BW ; h^{-1}$

; Urinary elimination rates
 $Kurine = KurineC \cdot BW ; L/h, \text{ for MEQ}$
 $Kurine1 = Kurine1C \cdot BW ; L/h, \text{ for M1}$

;Sub-model for MEQ (parent drug).....

; Blood compartment
 $CV = (QL \cdot CVL + QR \cdot CVR + Rim) / QC$
 $RA = QC \cdot (CV - CAfree)$
 $d/dt(AA) = RA$
 $init\ AA = 0$
 $CA = AA / Vblood$
 $CAfree = CA \cdot (1 - PB)$
 $d/dt(AUCCV) = CV$
 $init\ AUCCV = 0$

; Liver compartment
 $RL = QL \cdot (CAfree - CVL) + RAO - Rmet$
 $d/dt(AL) = RL$
 $init\ AL = 0$
 $CL = AL / VL$
 $CVL = AL / (VL \cdot PL)$
 $d/dt(AUCCL) = CL$
 $init\ AUCCL = 0$

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; Metabolism of MEQ in the liver compartment
Rmet=Km*CL*VL ; Total hepatic metabolic rate, umol/h
Rmet1=Rmet*Frac ; Hepatic metabolic rate to M1, umol/h
d/dt(Amet) = Rmet ; Amount of MEQ that is metabolized in the liver, umol
init Amet = 0
d/dt(Amet1) = Rmet1 ; Amount of M1 that is produced in the liver, umol
init Amet1 = 0

; Rest-of-body compartment
RR = QR*(CAfree-CVR)-Rurine
RurineC = Kurine*CVR
d/dt(AR) = RR
init AR = 0
CR = AR/VR
CVR = AR/(VR*PR)
d/dt(AUCCR) = CR
init AUCCR = 0
d/dt(Aurine) = Rurine
init Aurine = 0

; Mass balance for the parent drug
Qbal = QC-QL-QR
Tmass = AA+AL+AR+Aurine+Amet
Bal = AAO+Aim-Tmass

; .....Sub-model for M1 (the marker residue).....

; Blood compartment
CV1 = (QL1*CVL1+QK1*CVK1+QF1*CVF1+QM1*CVM1+QR1*CVR1)/QC
RA1 = QC*(CV1-CA1free)
d/dt(AA1) = RA1
init AA1 = 0
CA1 = AA1/Vblood1
CA1mg=CA1*MW1mg
CA1free = CA1*(1-PB1)
d/dt(AUCCV1) = CV1
init AUCCV1 = 0

; Liver compartment
RL1 = QL1*(CA1free-CVL1)+Rmet1
d/dt(AL1) = RL1
init AL1 = 0
CL1 = AL1/VL1
CVL1 = AL1/(VL1*PL1)
CL1mg=CL1*MW1mg
d/dt(AUCCL1) = CL1
init AUCCL1 = 0

; Kidney compartment
RK1 = QK1*(CA1free-CVK1)-Rurine1
d/dt(AK1) = RK1

```

```

init AK1 = 0
CK1 = AK1/VK1
CVK1 = AK1/(VK1*PK1)
CK1mg=CK1*MW1mg
d/dt(AUCCK1) = CK1
init AUCCK1 = 0

; Urinary excretion of the major metabolite
Rurine1 = Kurine1*CVK1
d/dt(Aurine1) = Rurine1
init Aurine1 = 0

; Muscle compartment
RM1 = QM1*(CA1free-CVM1)
d/dt(AM1) = RM1
init AM1 = 0
CM1 = AM1/VM1
CVM1 = AM1/(VM1*PM1)
CM1mg=CM1*MW1mg
d/dt(AUCCM1) = CM1
init AUCCM1 = 0

; Fat compartment
RF1 = QF1*(CA1free-CVF1)
d/dt(AF1) = RF1
init AF1 = 0
CF1 = AF1/VF1
CVF1 = AF1/(VF1*PF1)
CF1mg = CF1*MW1mg
d/dt(AUCCF1) = CF1
init AUCCF1 = 0

; Rest-of-body compartment
RR1 = QR1*(CA1free-CVR1)
d/dt(AR1) = RR1
init AR1 = 0
CR1 = AR1/VR1
CVR1 = AR1/(VR1*PR1)
CR1mg = CR1*MW1mg
d/dt(AUCCR1) = CR1
init AUCCR1 = 0

; Mass balance for the parent drug
Tmass1 = AA1+AL1+AK1+AF1+AM1+AR1+Aurine1
Bal1 = Amet1-Tmass1

```

References

(1) Li, M.; Gehring, R.; Riviere, J. E.; Lin, Z. Development and application of a population physiologically based pharmacokinetic model for penicillin G in swine and cattle for food safety assessment. *Food Chem. Toxicol.* **2017**, 107, 74-87.