SUPPLEMENTARY MATERIALS

Integration of Food Animal Residue Avoidance Databank (FARAD) empirical methods for drug withdrawal interval determination with a mechanistic population-based interactive physiologically based pharmacokinetic (iPBPK) modeling platform: example for flunixin meglumine administration

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Supplementary Method and Result

Model parameterization and calibration

The physiological parameters related to beef cattle and swine were obtained from previous experimental studies and published PBPK models [1-4]. The values of physicochemical parameters of flunixin and 5-OH flunixin, such as the molecular weight, octanol to water partition coefficients (logP), and acid dissociation constant (pKa), were acquired from PubChem and ChemSpider (**Table S6**). The values for tissue to plasma partition coefficients of flunixin and 5-OH flunixin used in the swine and cattle model were first estimated with different methods, including modules in PKsim, GastroPlus and Simcyp, and the well-accepted Rodgers and Rowland method [5] based on the tissue compositions of rats and swine [6]. The predicted values of tissue to plasma partition coefficients from commercial software are based on total chemical concentrations in plasma, while Rodgers and Rowland method uses unbound chemical concentrations in plasma. Taking into account plasma protein binding of flunixin [7,8], the values derived from commercial software can be converted to the values from Rodgers Rowland method by dividing the free drug ratio of flunixin (1%) in plasma. After converting all the predicted values of tissue to plasma partition coefficients to the ones based on unbound chemical concentrations in plasma, the results from these four methods are very similar (**Table S7**). The slight differences between these values from commercial software may be caused by different values of tissue components used. Due to the fact that tissue components were not well reported for swine and cattle, the predicted tissue to plasma partition coefficients may not be estimated correctly. Specifically, the active transporter-mediated drug distribution was not considered in the algorithm. These predicted values based on unbound chemical concentrations in plasma were used as initial estimates to further optimize partition coefficients by fitting to the selected

pharmacokinetic data (**Table 1**) using the Curve Fitting module in Berkeley Madonna (Version 8.3.23.0; University of California at Berkeley, CA, USA), and the final optimized values are provided in **Table 2** for cattle and swine.

Berkeley Madonna was used to develop the PBPK model and run all simulations. The model in Berkeley Madonna was also translated to R (version 3.5.0) using a published method [9] and all simulations were repeated in R. The model codes in Berkeley Madonna are provided in the Supplementary Materials and will be available at our center's website (http://iccm.k-state.edu/) upon publication. Since the model was constructed based on previous PBPK models, only key and new mathematical equations are described in detail in following paragraphs. For all the other equations, readers are directed to earlier publications for detailed explanations [3,10,11].

As the 5-OH flunixin metabolite submodel was included in the PBPK model, the input dose amount of flunixin was converted from milligrams into millimoles by dividing the molecular weight of flunixin. The 5-OH flunixin can be further metabolized to glucuronide conjugates [12], the conjugates can be hydrolyzed to free flunixin by enzymes [13] or under mild alkaline conditions [14]. The enterohepatic circulation of flunixin was simulated as a first-order kinetic process with the following equations:

$$Rehc = Kehc \times AI \quad (1)$$

$$RL = QL \times (CAfree - CVL) - Rmet - Rbile + Rehc \quad (2)$$

$$RAI = Rbile + Rbile1 - Kint \times AI - Rehc \quad (3)$$

where Rehc is the rate of flunixin reabsorbed through the enterohepatic circulation (mmol/h); Kehc is the rate constant of flunixin reabsorbed through the enterohepatic circulation (/h); AI is

the amount of flunixin in the intestine (mmol); RL is the rate of change in the concentration of flunixin in the liver (mmol/h); QL represents the volume of blood flow to the liver per hour (L/h); CAfree is the arterial blood concentration of flunixin not bound with plasma proteins (mmol/L); CVL is the concentration of flunixin in the liver venous blood (mmol/L); Rmet is the rate for the metabolism of flunixin into 5-OH flunixin (mmol/h); Rbile is the rate for flunixin excreted through bile (mmol/h); RAI is the rate of change for the amount of flunixin in intestine (mmol/h); Rbile1 is the rate for 5-OH flunixin excreted through bile (mmol/h), which can be hydrolyzed to free flunixin in the intestine [15]; Kint is the rate constant of flunixin absorbed from intestine (/h).

By using the modules of "Curve Fitting" and "Parameter Slider" in Berkeley Madonna, the enterohepatic circulation (KehcC) and biliary (KbileC) and urinary eliminations (KurineC) of flunixin were optimized by fitting the model prediction to the pharmacokinetic data from the FDA Freedom of Information for NADA 101-479 [16] and the study carried out by Odensvik and Johansson [17] for the cattle model. The rate constants of flunixin metabolism (KmetC), enterohepatic circulation (KehcC) and biliary (KbileC) and urinary eliminations (KurineC) of 5-OH flunixin in the cattle model were optimized by fitting with the data from the study by Shelver et al. [18], and the values of parameters related to IM (Kim) and SC (Ksc) administrations were fitted with pharmacokinetic data from IM [19] and SC administrations [18], respectively. For the swine model, the enterohepatic circulation (KehcC) and biliary (KbileC) and urinary eliminations (KurineC) of flunixin and 5-OH flunixin were optimized by fitting the pharmacokinetic data from the studies carried out by Howard et al. [20] and Pairis-Garcia et al. [21], and the absorption rate constant of IM administration was fitted with data from the study carried out by Pairis-Garcia et al. [21] and FDA Freedom of Information for NADA 101-479

[22]. The values of all physiological parameters and chemical-specific parameters used in the PBPK model for beef cattle and swine are shown in **Table 2**.

Translation of the PBPK Model into a User-Friendly iPBPK Interface (also called Extralabel Withdrawal Interval Simulator)

The PBPK model code in Berkeley Madonna format was converted into R. The "mrgsolve" package was chosen for coding the user-friendly iPBPK interface. The iPBPK interface was constructed with the "Shiny" package based on the R model code. In brief, the interface includes the following tabs on the left side, including "Main Plot", "Model Parameters", "Output Table", "Model Structure", "Code", "Tutorial", "Output Report", and "About". The "Main Plot" tab allows users to select the simulation scenario, including the drug (i.e., flunixin or other drugs to be included), species (i.e., swine, beef cattle, or others to be included), administration route, dose level, dose interval, number of administrations, number of animals in the Monte Carlo simulation, and terminal simulation time (i.e., simulation time after last administration). Once the simulation scenario is selected, click "Apply Changes", and then the simulation results will be displayed on this page. For advanced users, the "Model Parameters" tab provides the option to customize the physiological and chemical-specific parameters for a specific model simulation for a specific animal production class. On this page, users can also see the simulated concentrations of the drug in individual edible tissues. Once the simulation is done, the results can be downloaded as an Excel file through the "Output Table" tab for reanalysis or replotting. The simulation results can also be downloaded as a standard report in a PDF, Word, or HTML format. This report includes a summary of inputs for the simulation parameters, general information about the label use and label withdrawal period for the drug, and the predicted WDIs based on the simulation, as well as a plot showing the range of the simulated concentrations of

the drug in the target tissue from the selected population of animals. Finally, a very detailed tutorial illustrating how to use this interface step-by-step is provided and can be downloaded from the "Tutorial" tap. This "Tutorial" is also proved in this Supplementary Materials below.

Sensitivity Analysis

The sensitivity analysis was applied to screen the sensitive parameters in the PBPK model. Thirty-two model parameters in the PBPK model for beef cattle and swine were analyzed using the method of local sensitivity analysis based on 1% variation of the parameter values (**Table S8 and Fig. S6)**. Only parameters with at least one absolute value of NSC greater than 0.20 are shown in the table. For physiological parameters, the body weight (BW), the cardiac output (QCC), the tissue volume of liver (VLC) and the blood flow to liver (QLC) were the relatively sensitive parameters for the model. The AUC of 5-OH flunixin in plasma was highly sensitive to the kidney partition coefficient (PK) and protein binding in plasma (PB) for flunixin with NSC value of 0.61 and -0.46, respectively. The AUC of 5-OH flunixin in plasma was also sensitive to the rest of body partition coefficient (Prest1), protein binding in plasma (PB1) and the metabolism rate constant (KmetC1) with NSC values of -0.44, -0.46 and -0.24, respectively.

User-friendly interface establishment and improvement

The code for the PBPK model for flunixin in cattle and swine was translated into R using the "deSolve" and "mrgsolve" packages. The average simulation time for each iteration using "deSolve" was longer than using "mrgsolve". Especially for a larger number of iterations, the simulation time differences were considerable. For the 1000-iteration simulation, code in "deSolve" would take around 10 hours, and "mrgsolve" code only consumed around 6 seconds (**Table S5**). These results suggest that using "mrgsolve" for Monte Carlo analysis of PBPK

models can substantially decrease the simulation time, and is more suitable for the purpose of real-time simulation. The user-friendly interface was established with “Shiny” package based on the R code using “mrgsolve” due to the shorter simulation time. By using the user-friendly interface, non-modelers or regular users could easily use and apply the population PBPK model to predict tissue residues of flunixin and determine the model-predicted WDIs. Model simulation data could be exported to the spreadsheet, and reanalyzed by users. In addition, for the convenience to use, the simulation result can also be downloaded as a report, which includes relevant information for FARAD responders to recommend WDIs following extralabel use of flunixin.

Tutorial for the Extralabel Withdrawal Interval Simulator

Introduction

This Rshiny tool is designed to apply the physiologically based pharmacokinetic (PBPK) models for veterinary drugs to predict extended withdrawal intervals (WDIs) following extralabel use in food-producing animals.

Background

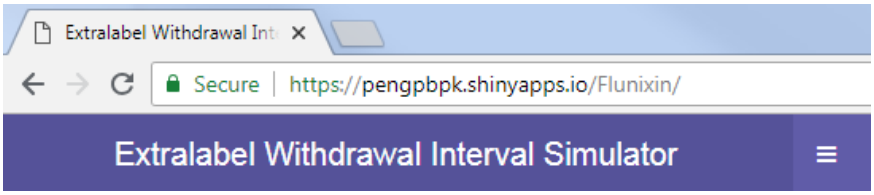
The current PBPK model interface for flunixin in swine and cattle is based on our currently developed model. The PBPK model is a mechanistic-based pharmacokinetic model. Due to the lack of pharmacokinetic data for extralabel use of veterinary drugs, traditional pharmacokinetic tools have limitations to predict pharmacokinetic profiles for drugs administrated with higher doses, by different routes or even to different animal species. The PBPK model can be a useful tool to overcome these difficulties. The withdrawal intervals were predicted using the population PBPK model with Monte Carlo simulation.

For FARAD Responders

1. Open the App

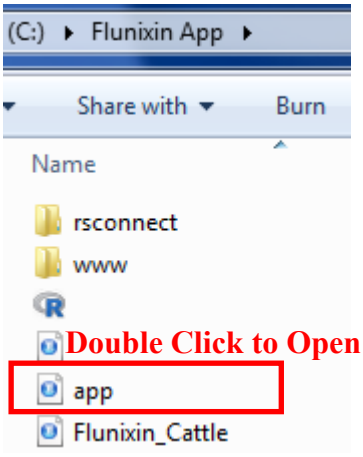
For the Rshiny app, there is no need to install each individual app if you have RStudio installed on your computer or have internet access. There are two ways to open the 'Extralabel Withdrawal Interval Simulator'.

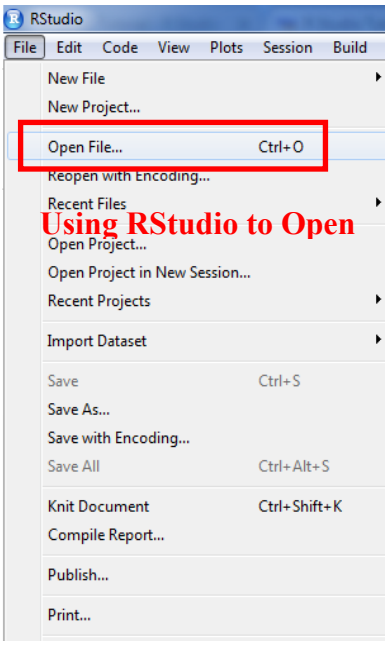
First option is to open the Rshiny app online. Currently, the app is available online through ShinyApps web server (http://www.shinyapps.io/). The app can be accessed through https://pengpbpk.shinyapps.io/Flunixin/ or by using a short name tiny.cc/flunixin

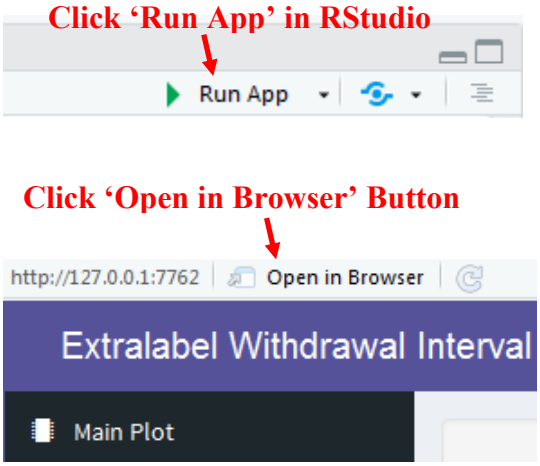


Second option is to open the Rshiny app locally. If the app files available on the local computer, the simulator can also be opened by using RStudio. Make sure you have RStudio installed on your local computer, before running the app. Please refer to RStudio Tutorial (http://web.cs.ucla.edu/~gulzar/rstudio/) for details of RStudio installation and some basic information.
Double click the 'app.R' in the app file to open the app in RStuido, or by 'File > Open File … > Flunixin App/app.R' in RStudio. Once you open the "app.R" file, you can click the "Run App" button to run the app on your local computer. We recommend to run the app on your local computer as Monte Carlo simulation for 1,000 animals is more stable to run on your local computer compared to the server online.

Note: If run the app on the local computer using RStudio, please click the 'Open in Browser' button to run the app in the web browser to avoid abnormal issues. If you click "Open in Browser", it looks like you are on a web page, but still it runs locally.

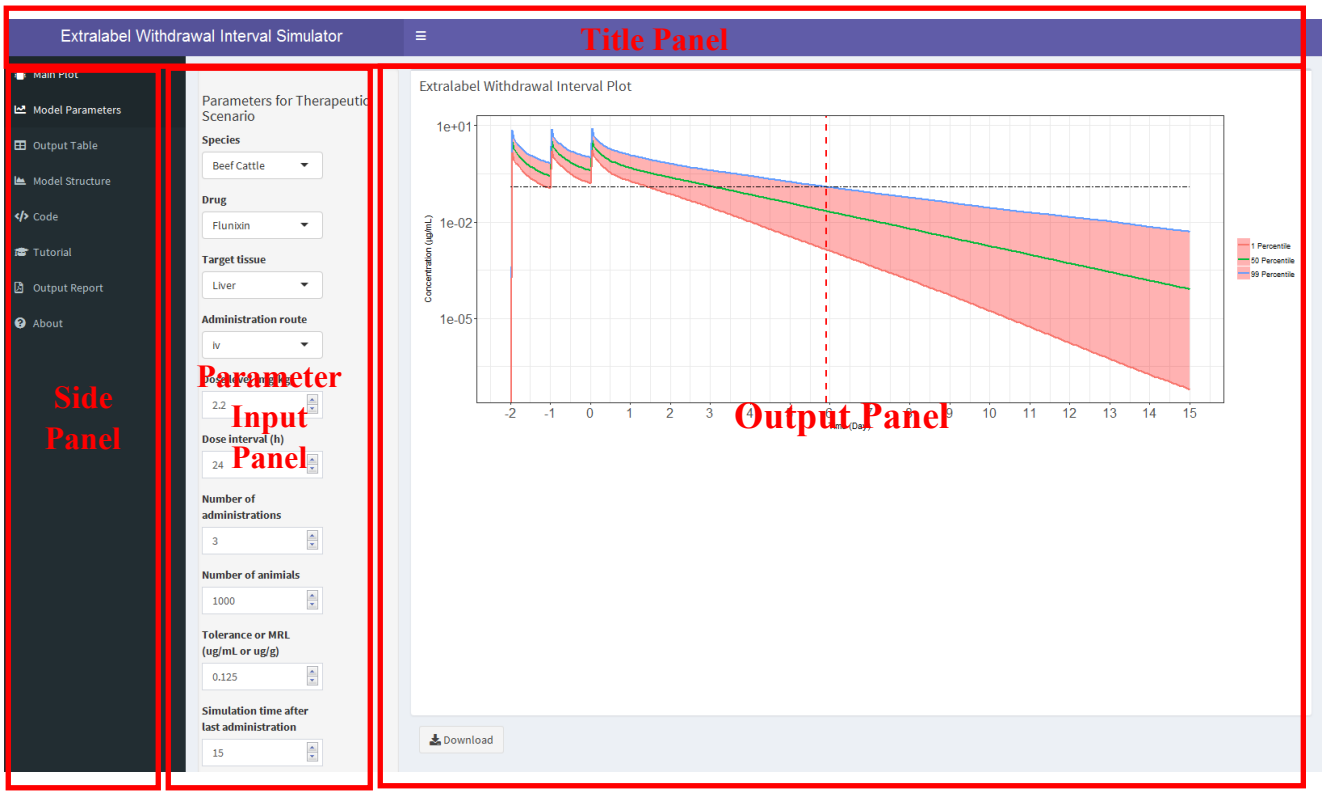






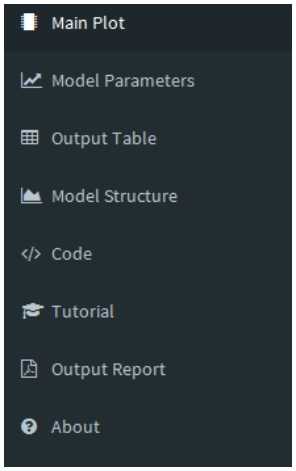
2. Interface Structure

The application interface contains four major parts including 'Title Panel', 'Side Panel', 'Parameter Input Panel', and 'Output Panel'.

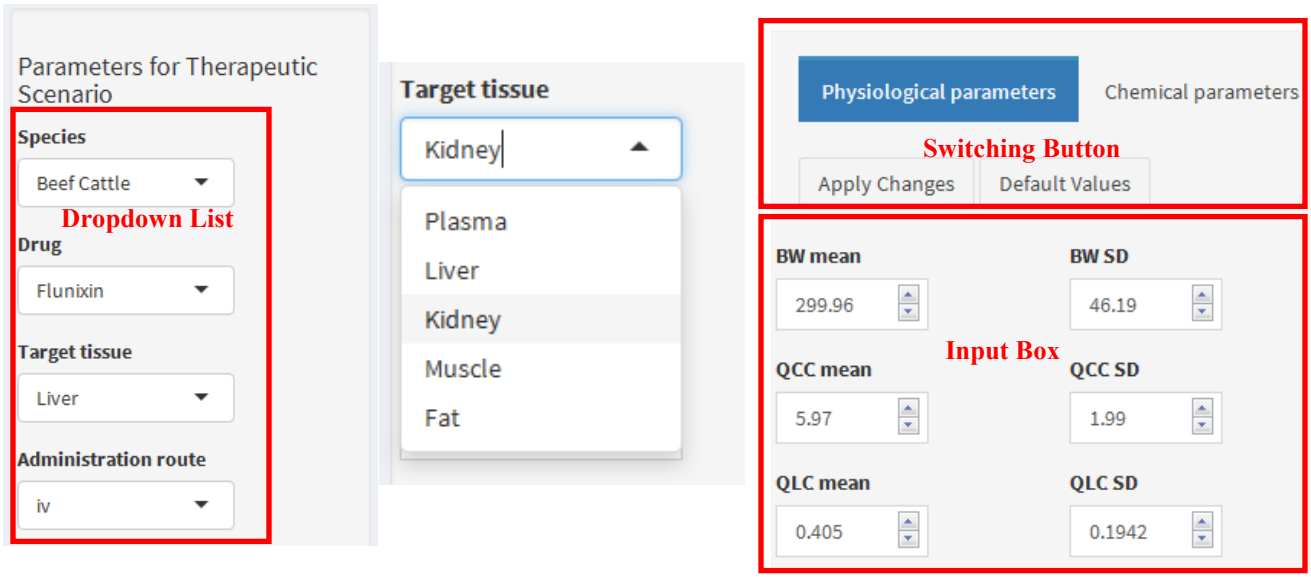


The ‘Title Panel’ includes the title ‘Extralabel Withdrawal Interval Simulator’ and the side panel controller. By clicking the side panel controller, you can choose to show up or hide the side panel.

The side panel contains ‘Main Plot’, ‘Model Parameters’, ‘Output Table’, ‘Model Structure’, ‘Code’, ‘Tutorial’, “Output Report”, and ‘About’ tabs. By clicking the tab name, you can switch among these tabs.



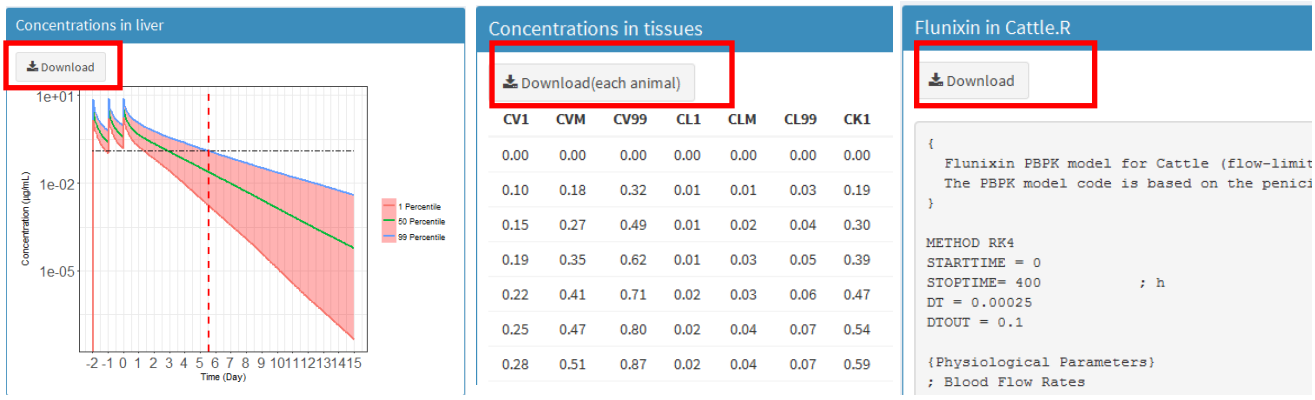
The ‘Main Plot’ and ‘Model Parameter’ tabs have parameter input panel. There are different types of controllers, such as switching buttons, dropdown lists, and input boxes. By clicking the



switching buttons, you can choose in-between different conditions. For dropdown lists, you can choose different choices by clicking the downwards arrow. For input boxes, you can directly type in the value, or use the arrow to increase or decrease the default value provided.

All the tabs contain the output panels. For 'Main Plot', 'Model Parameters', and 'Output Table', the output panels show the simulation results using the parameter values you choose. The output panels in 'Model Structure', 'Code', 'Tutorial', and 'About' tabs provide information about the simulator, and are independent from model simulation output. You can download figures, tables or related codes by using the 'Download' button.

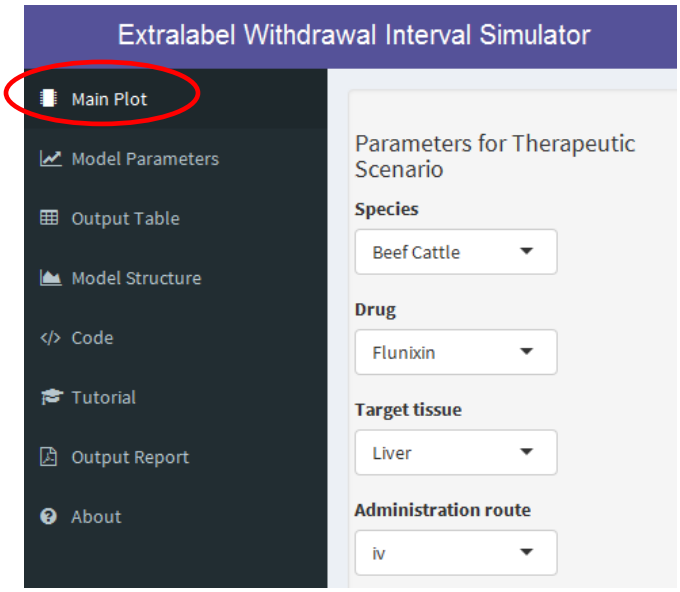
Download Buttons



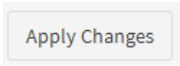
3. "Main Plot" Tab to Predict Extralabel WDIs

You can use the main tab ("Main Plot") to predict withdrawal intervals following extralabel use of veterinary drugs. By changing values of major parameters for therapeutic scenarios, the simulator will provide "Extralabel Withdrawal Interval Plot" as output.

Step One: Choose "Main Plot" tab (this is the default page, when you open the simulator)



Step Two: By clicking the 'Apply Changes' button, you can run the simulator using the default values for all parameters. The "Apply Changes" button is on the bottom of the Parameter Input Panel. Once you click the "Apply Changes" button, the model will start running and you will see a progress bar "Creating plot Please Wait" on the right bottom corner. The default setting includes 1000 animals in a Monte Carlo simulation, so the simulation will be completed within 1 minute.

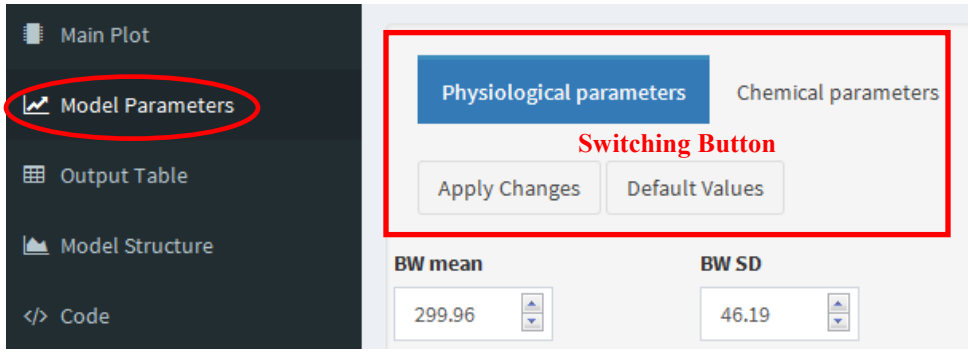


Step Three: You can also change the parameter values by using dropdown lists or input boxes. For example, if you want to know the WDI for m swine treated with 2.2 mg/kg flunixin for 3 times with 24 hour intervals, and you want the simulator create a population with 1000 animals, you can choose "Market-age Swine" in "Species", "Liver" in "Target tissue", "1000" in "Number of animals", and "0.03" in "FSIS action level" (0.03 ug/g is the tolerance for flunixin in swine liver). All the other values are the same as default. After clicking the 'Apply Changes' button, the simulator starts to run simulations with the parameter values you input. When the simulations finish, the simulator will provide the pharmacokinetic profile of flunixin in liver in the output panel.

4. "Model Parameters" Tab to Change Values for Physiological or Chemical Parameters

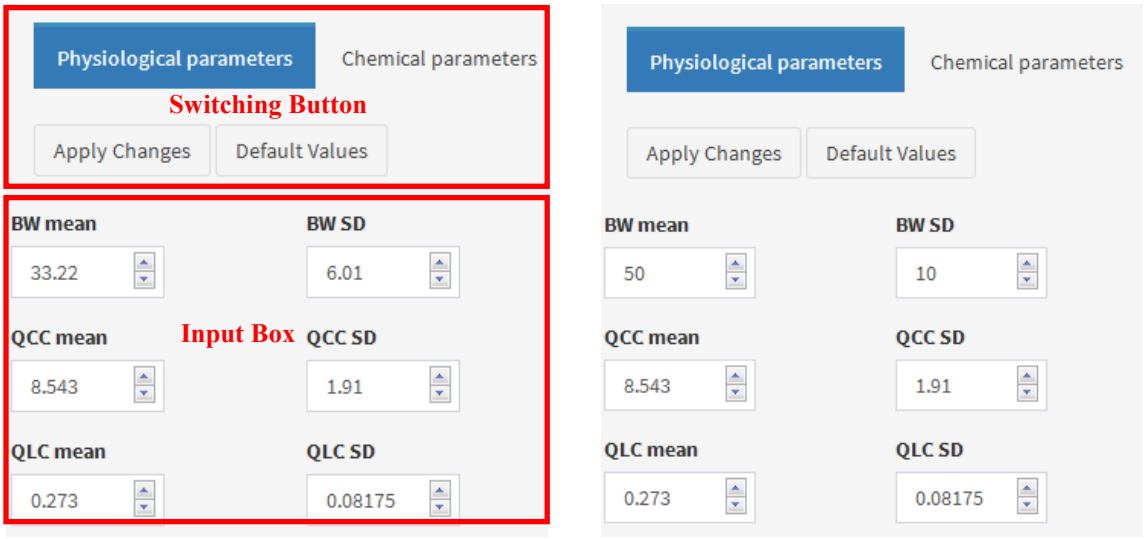
In the PBPK model, there are two types of parameters involved, including physiological parameters and chemical-specific parameters. Generally, the simulation results generated based on the default parameter values are sufficient to answer most of FARAD calls. We recommend you directly use the default physiological/chemical-specific parameters to run the simulations. For advanced users, these values could also be revised to make the simulations more relevant to a particular scenario they want to simulate. If you need to use this function and have any questions, please feel free to contact the interface development team.

Step One: Choose "Model Parameters" tab



Step Two: In the parameter input panel, you can choose to use your customized values in 'Physiological parameters' or 'Chemical parameters' by using the switching buttons.

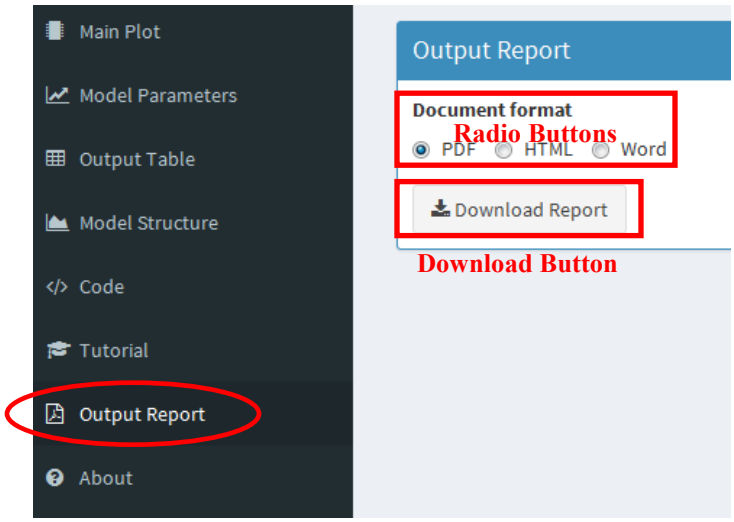
Step Three: By using input boxes, you can put your customized values for the parameters you are interested in. For example, if you want to simulate a group of swine with mean body weight as 50 kg, and standard deviation as 10. On the "Model Parameters" tab, you change the BW mean value from the default value to 50, and change the BW SD value from the default value to 10 (see the figure below). Then you go back to 'Main Plot' tab, and click apply changes. The results will be generated after all simulation finishes.



5. Download Figures and Tables from "Model Parameters" and "Output Table" Tabs

After the desired simulations, there are output figures and tables you can save to files in your local computer. In the "Model Parameters" tab, you can download figures of pharmacokinetic profiles in major edible tissues, including liver, kidney, muscle, and fat. You can also download the table of pharmacokinetic data for the drug in different edible tissues. Note that the generated table only contains the 1th, 50th, and 99th percentiles of the simulated concentrations of the drug in different tissues among the simulated population of animals. Due to the large number of animals that are typically included in a population simulation (e.g., 1,000 animals), the individual animal concentration data are not included in the output table. If individual animal concentration data are needed, please contact the interface development team.

The output report can be downloaded from the "Output Report" tab. By choosing the "Output Report" tab in the "Side Panel", the output report based on your input parameters could be generated from the simulator. Three different output formats, including PDF, HTML and WORD, could be applied to the output report by click the corresponding radio button. You can use the download button to save the report on your local computer.



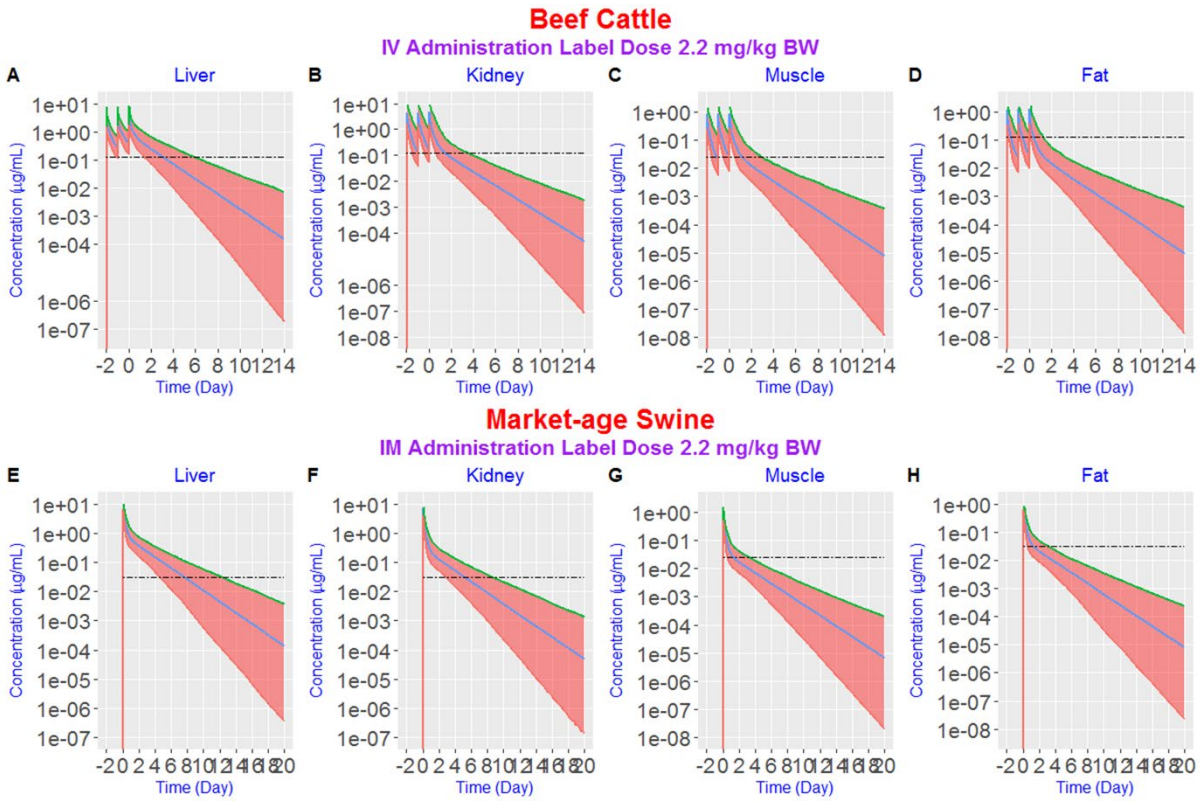
6. Common Simulation Results.

All the simulation results reported here are based on the simulations of 1,000 animals with 3 repeated intravascular (IV) doses for cattle and intramuscular (IM) single dose for swine. The predicted WDIs for the label use of flunixin are listed below in the table.

Dose	Beef Cattle (IV)			Market-age Swine (IM)		
	Label Withdrawal Time	Withdrawal Intervals (Days)		Label Withdrawal Time	Withdrawal Intervals (Days)	
		Exact Value	Round Value		Exact Value	Round Value
2.2 mg/kg (label dose)	4	5.92	6	12	12.38	13

Note: The labeled withdrawal time of flunixin is based on the formulation NADA 101-479. The labeled indications are 2.2 mg/kg daily IV injection for up to 3 days of treatment for cattle and 2.2 mg/kg through single IM injection for swine (all use classes).

Related simulation results are shown blow in figures.



Acknowledgement

The project is supported by the United States Department of Agriculture National Institute of Food and Agriculture for the Food Animal Residue Avoidance Databank (FARAD) Program (http://www.farad.org/).

Sample Output for iPBPK Interface

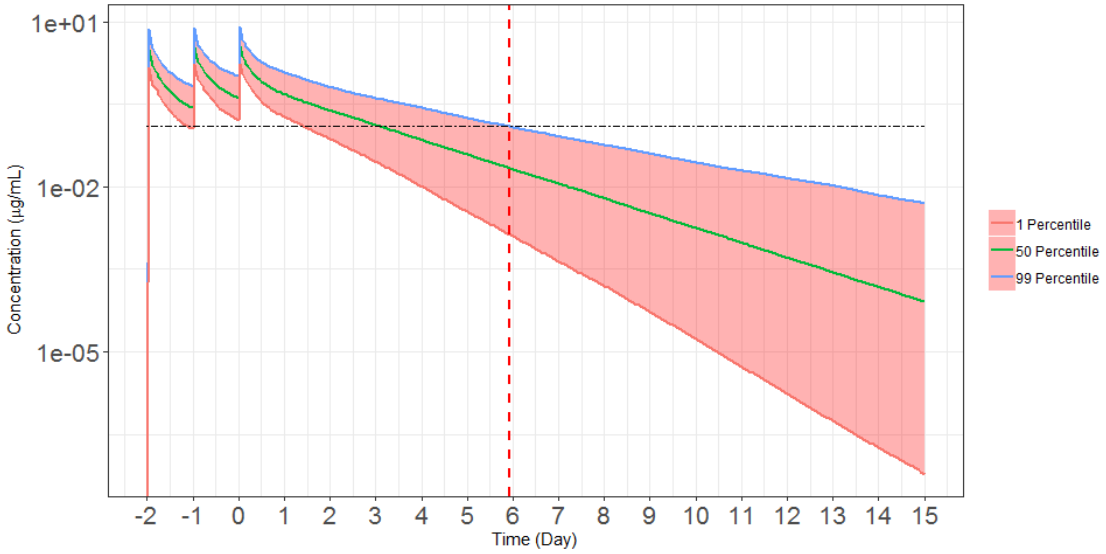
Flunixin PBPK Model Output

The results were achieved using our population PBPK model of Flunixin in Swine and Cattle. These results were based on the Monte Carlo simulation of 1000 animals after exposure to Flunixin via the iv administration at 2.2 mg/kg for 3 doses with 24-hour intervals in Beef Cattle.

Based on the VetGRAM database, the labeled use for Flunixin is 2.2 mg/kg daily or divided into 2 doses through iv injection for up to 3 days of treatment in Adult Cattle, and the labeled withdrawal period is 4 days for the formulation with NADA number of 101-479. The tolerance of Flunixin is 0.125 ppm or ug/g in the target tissue of liver in cattle.

Based on the parameters you have provided, the simulation results showed that at 5.92 days after the last administration, the concentration of flunixin in the liver was lower than the tolerance for the 99th percentile of the simulated population of animals. Thus, the recommended extended withdrawal interval (WDI) is 6 days. According to FDA guidelines, if the estimated WDI is a fraction of a day, it should be rounded up to the next whole day.

Liver as the target organ



Note: we would like to notify that by providing a withdrawal interval recommendation, it could be interpreted as advising/prescribing extra label drug use which requires a valid veterinary client patient relationship. In addition, the prescribing or advising veterinarian will be held responsible for any violative residues.

Supplementary Figures

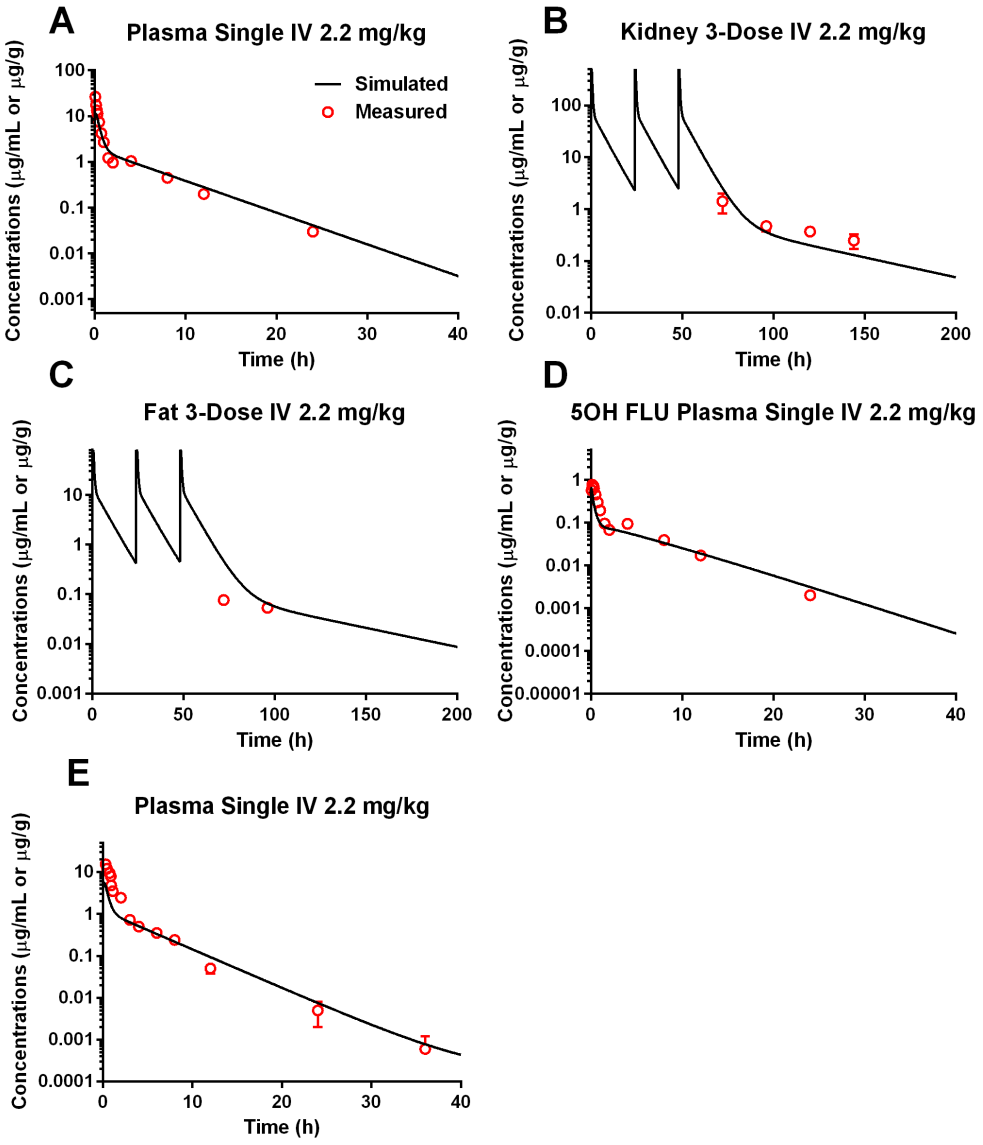


Fig. S1 Model calibration and evaluation of PBPK model for flunixin in cattle using pharmacokinetic data. Comparison of model predictions (solid line) and observed data (red circles) for concentrations of flunixin in the plasma, kidney and fat from beef cattle exposed to flunixin via single IV injection (A and E; 2.2 mg/kg; Shelver et al. 2013 [18] and Kleinhenz et al. 2016 [40]), 3 repeated IV administrations (B and C; 2.2 mg/kg; FDA, 1998 [16]). Model prediction for concentrations of 5-OH flunixin in plasma compared with observed data from single IV administration (D; 2.2 mg/kg; Shelver et al. 2013 [18]).

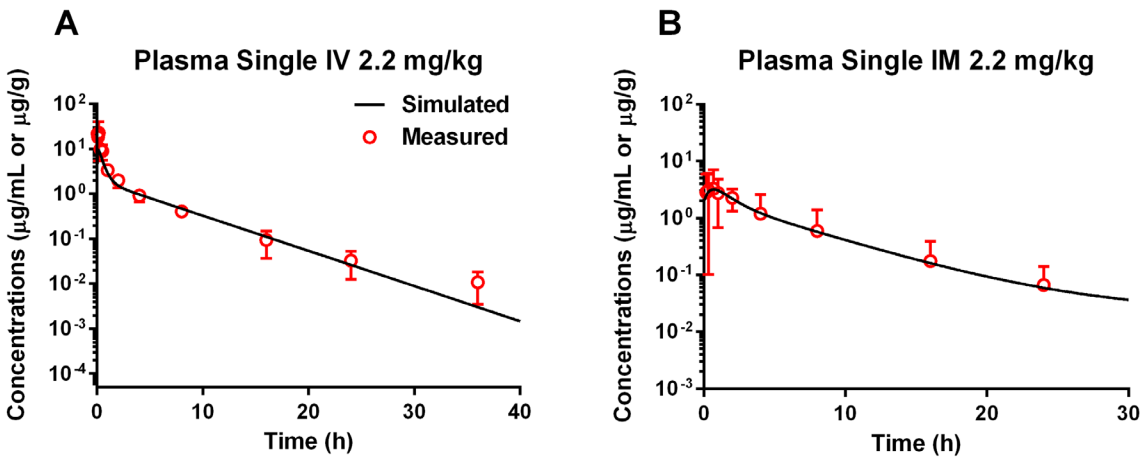


Fig. S2 Model calibration of PBPK model for flunixin in swine using pharmacokinetic data. Comparison of model predictions (solid line) and observed data (red circles) for concentrations of flunixin in the plasma from swine exposed to 2.2 mg/kg flunixin via single IV injection and single IM injection (A and B respectively; 2.2 mg/kg; Pairis-Garcia et al. 2013 [21]).

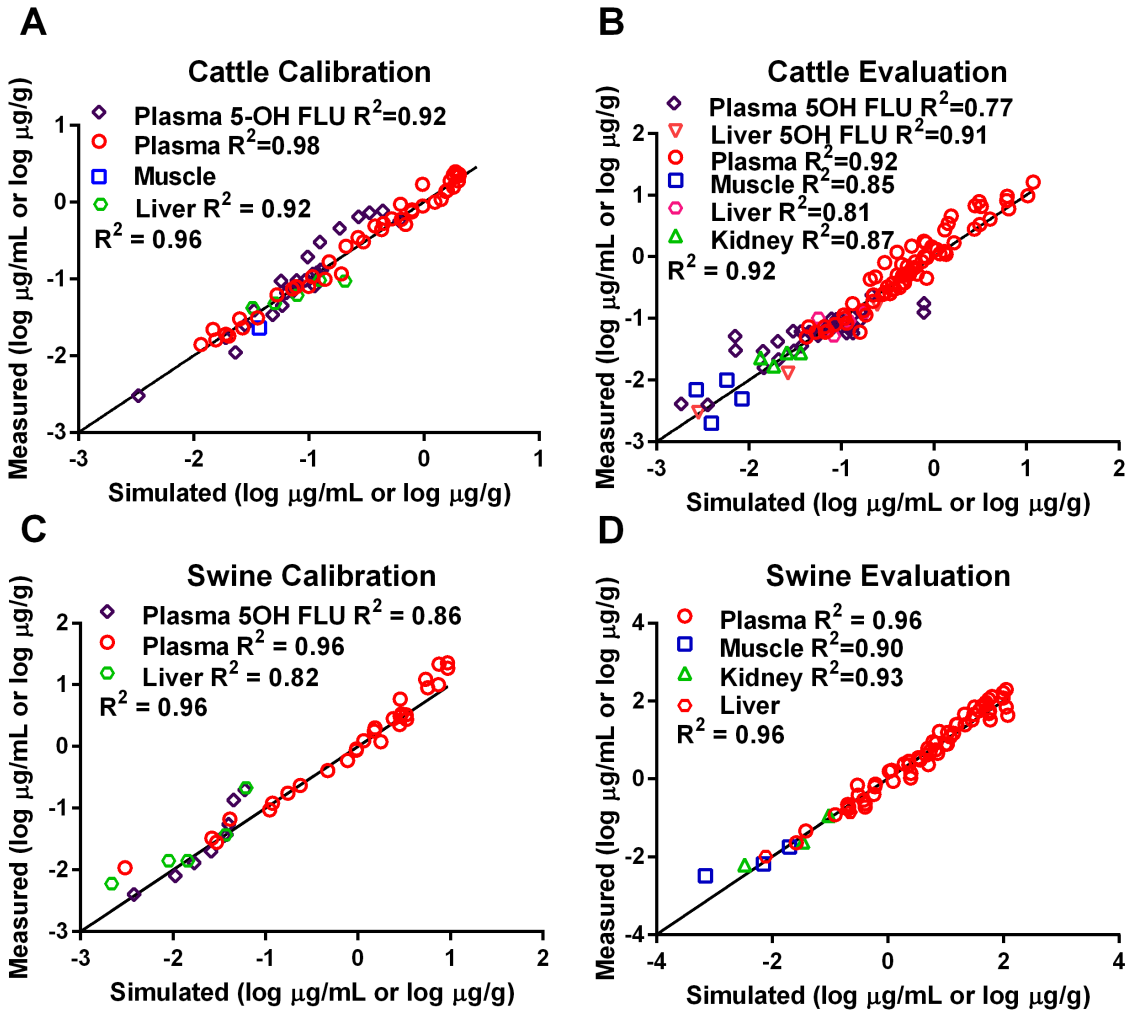


Fig. S3 Linear regression analysis for calibration and evaluation results in cattle and swine. A is the linear regression analysis for simulation results of cattle calibration. B is the linear regression analysis for simulation results of cattle evaluation. C is the linear regression analysis for simulated results of swine calibration. D is the linear regression analysis for simulated results of swine evaluation.

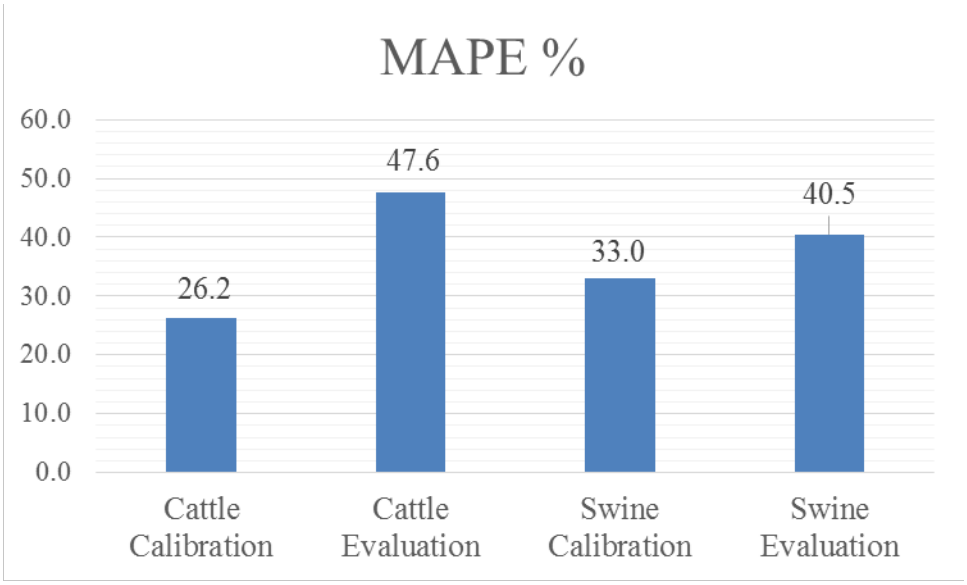


Fig. S4 The MAPE analysis for calibration and evaluation results in cattle and swine. All the MAPE values for model simulation results are lower than 50%.

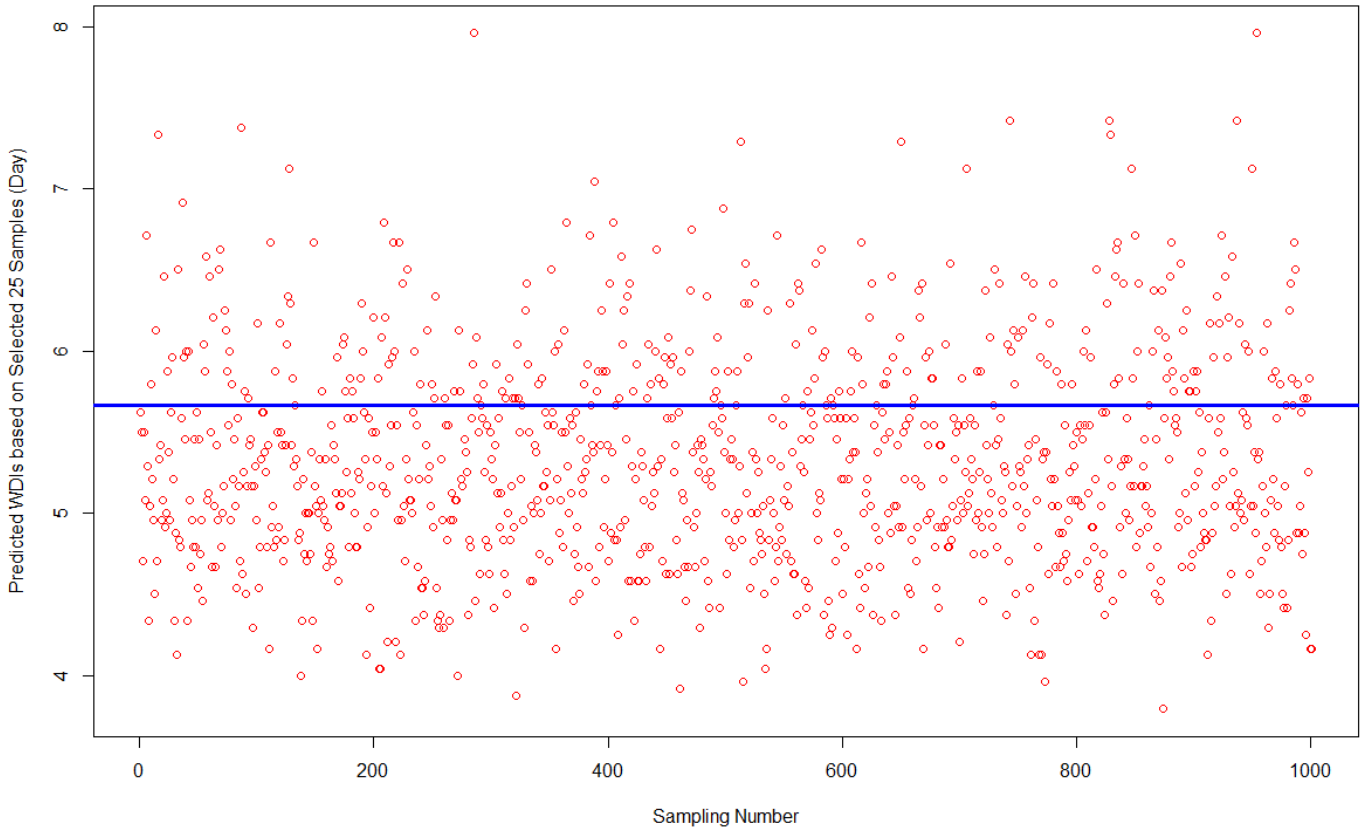


Fig. S5 The predicted WDIs calculated based on sampling of 25 samples from 1000 samples for 1000 times. The blue line stands for the predicted WDIs based on the 1000 samples. The red circles represent WDIs calculated based on 25 samples bootstrapped from the population with 1000 samples.

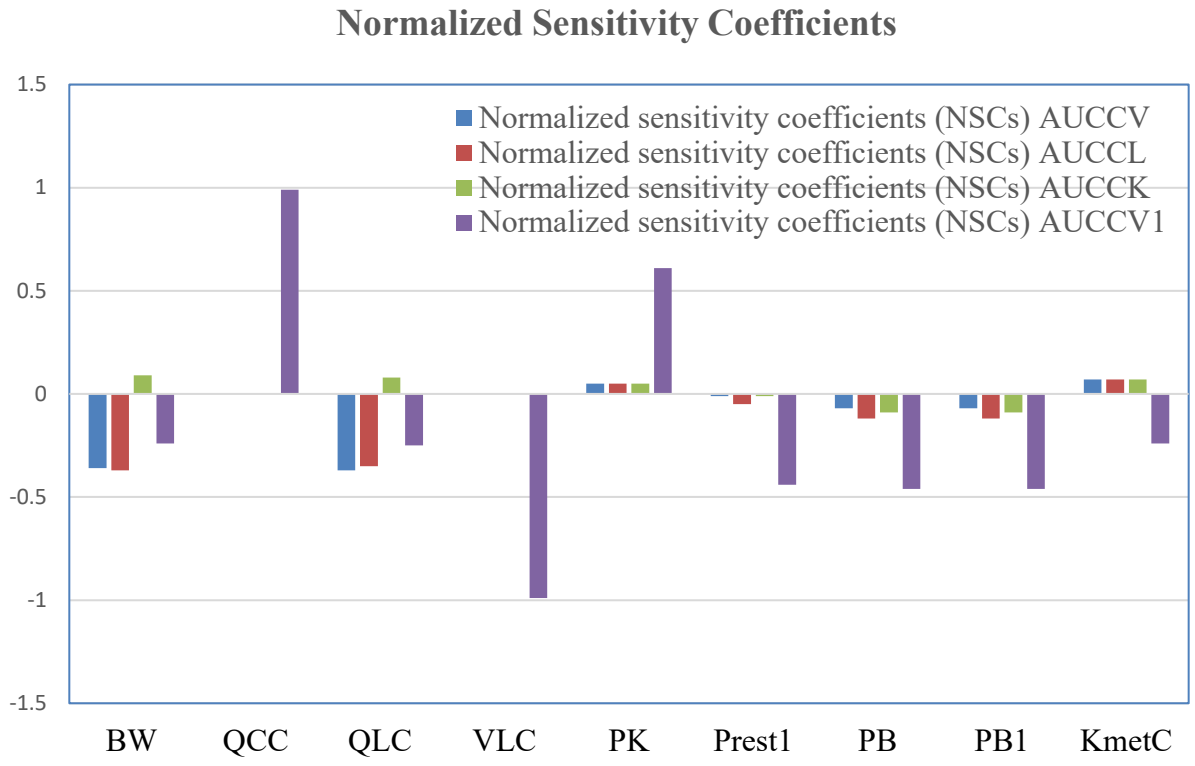


Fig. S6 The normalized senstitivity coefficients for sensitive parameters based on the sensitivity analysis. The non-sensitive parameters include: VartC, VvenC, VKC, VMC, VFC, VrestC, VrestC1, QKC, QMC, QFC, QrestC, QrestC1, Kim, Ksc, PL, PL1, PK1, PM, PF, Prest, KehcC, KbileC, KbileC1, KurineC, KurineC1, Kint and Kfeces.

Supplementary Tables

Table S1. Label use of flunixin in the form of flunixin meglumine

Label Species	Label Routes	Label Dose	Withdrawal Time	Tolerance	Indications for Use
Swine	IM	IM 2.2 mg/kg BW as a single injection	12 days	Liver: 30 ppb Muscle: 25 ppb	Control of pyrexia associated with respiratory disease
Cattle	IV, Transdermal	IV 1.1 to 2.2 mg/kg BW per day, as a single dose or divided into two doses at 12-hour intervals, for up to 3 days	For meat, 4 days (IV) and 8 days (Transdermal); For milk, 36 h	Liver: 125 ppb Muscle: 25 ppb Milk: 2 ppb	Control of pyrexia associated with respiratory disease and endotoxemia or for control of inflammation in endotoxemia.
Horse (Not for Meat Use)	IV, IM	1.1 mg/kg BW per day, IV or IM for up to 5 days	NA	NA	Alleviation of inflammation and pain associated with musculoskeletal disorders, and alleviation of visceral pain associated with colic.

Table S2. Values and distributions of parameters used in the population analysis for the PBPK model of beef cattle

Parameter	Abbreviation	Distribution	Mean	SD	CV	Lower bound	Upper bound	Reference
Body weight (kg)	BW	Normal	299.957	4.618E+01	0.154*	209.450	390.464	[23]
Cardiac output (L/h/kg)	QCC	Normal	5.970	1.990E+00	0.333*	2.070	9.870	[24]
Tissue volume (fraction of body weight, unitless)								
Arterial blood	VartC	Normal	0.010	3.120E-03	0.300	0.004	0.017	[4]
Venous blood	VvenC	Normal	0.030	8.880E-03	0.300	0.012	0.047	[4]
Liver	VLC	Normal	0.014	1.630E-03	0.120*	0.010	0.017	[25]
Kidney	VKC	Normal	0.0025	4.321E-04	0.174*	0.0016	0.0033	[25]
Muscle	VMC	Normal	0.270	8.100E-02	0.300	0.111	0.429	[2,4]
Fat	VFC	Normal	0.150	4.500E-02	0.300	0.062	0.238	[2,4]
Rest of body	VrestC	Normal	0.5235	1.572E-01	0.300	0.216	0.832	Total adds to 1
Rest of body for metabolite submodel	VrestC1	Normal	0.9435	2.832E-01	0.300	0.389	0.990	Total adds to 1
Blood flow (fraction of cardiac output, unitless)								
Liver	QLC	Normal	0.405	1.942E-01	0.480*	0.024	0.785	[24, 26]
Kidney	QKC	Normal	0.090	2.700E-02	0.300	0.037	0.143	[4]
Muscle	QMC	Normal	0.180	5.400E-02	0.300	0.074	0.286	[4]
Fat	QFC	Normal	0.080	2.400E-02	0.300	0.033	0.127	[4]
Rest of body	QrestC	Normal	0.245	7.360E-02	0.300	0.101	0.390	Total adds to 1
Rest of body for metabolite submodel	QrestC1	Normal	0.505	1.515E-01	0.300	0.208	0.802	Total adds to 1
Absorption rate constant (/h)								
Intramuscular	Kim	Lognormal	0.500	1.500E-01	0.300	0.269	0.851	Model fitted
Subcutaneous	Ksc	Lognormal	0.400	1.200E-01	0.300	0.216	0.681	Model fitted
Tissue:plasma partition coefficient for the parent drug (unitless)								
Liver	PL	Lognormal	10.520	2.104E+00	0.200	6.997	15.208	Model fitted
Liver (5OH FLU)	PL1	Lognormal	9.260	1.852E+00	0.200	6.159	13.387	Model fitted
Kidney	PK	Lognormal	4.000	8.000E-01	0.200	2.661	5.783	Model fitted
Kidney (5OH FLU)	PK1	Lognormal	4.000	8.000E-01	0.200	2.661	5.783	Model fitted
Muscle	PM	Lognormal	0.500	1.000E-01	0.200	0.333	0.723	Model fitted
Fat	PF	Lognormal	0.600	1.200E-01	0.200	0.399	0.867	Model fitted
Rest of body	Prest	Lognormal	8.000	1.600E+00	0.200	5.321	11.565	Model fitted
Rest of body (5OH FLU)	Prest1	Lognormal	5.000	1.000E+00	0.200	3.326	7.228	Model fitted
Hepatic metabolic rate (/h/kg)	KmetC	Lognormal	0.2000	4.000E-02	0.200	0.133	0.289	Model fitted
Rate constant for the regeneration of flunixin free acid from metabolites and enterohepatic circulation (/h/kg)	KehcC	Lognormal	0.0500	1.500E-02	0.300	0.027	0.085	Model fitted
Percentage of plasma protein binding (unitless)	PB	Lognormal	0.950	2.850E-01	0.300	0.5118	0.990#	Model fitted
Percentage of plasma protein binding (unitless) (5OH FLU)	PB1	Lognormal	0.990	2.970E-01	0.300	0.5334	0.990#	Model fitted
Biliary elimination rate constant (L/h/kg)	KbileC	Lognormal	0.500	1.500E-01	0.300	0.269	0.851	Model fitted
Biliary elimination rate constant (L/h/kg) (5OH FLU)	KbileC1	Lognormal	0.100	3.000E-02	0.300	0.054	0.170	Model fitted
Urinary elimination rate constant (L/h/kg)	KurineC	Lognormal	0.100	3.000E-02	0.300	0.054	0.170	Model fitted
Urinary elimination rate constant (L/h/kg) (5OH FLU)	KurineC1	Lognormal	0.200	6.000E-02	0.300	0.108	0.341	Model fitted
Intestinal transit rate constant (/h)	Kint	Lognormal	0.400	1.200E-01	0.300	0.216	0.681	[39]
Fecal elimination rate constant (/h)	Kfeces	Lognormal	0.500	1.500E-01	0.300	0.269	0.851	Model fitted

Notes: Some parameters were estimated by calibrating the PBPK model with pharmacokinetic data. These parameters were marked as 'model fitted'. The star sign (*) indicates the CV of that parameter was calculated based on previous experimental data. For the parameters without calculated CVs, the default values of CVs for physiological parameters were 0.3. For chemical-specific parameters such as tissue to plasma partition coefficients and hepatic metabolic rate, the default CVs were 0.2. For the other chemical-specific parameters such as percentage of plasma protein binding, and rate constants for enterohepatic circulation, biliary and urinary eliminations, the default CVs were 0.3. The pound sign (#) indicates the upper bound of plasma protein binding is lower than 1 based on the physiological plausibility. The 95% confidence interval of each parameter was calculated as the lower (i.e., 2.5^{th} percentile) and upper (i.e., 97.5^{th} percentile) bounds.

Table S3. Values and distributions of parameters used in the population analysis for the PBPK model of swine

Parameter	Abbreviation	Distribution	Mean	SD	CV	Lower bound	Upper bound	Reference
Body weight (kg)	BW	Normal	33.182	6.451E+00	0.194*	20.539	45.825	[27-30]
Cardiac output (L/h/kg)	QCC	Normal	8.543	1.910E+00	0.224*	4.800	12.287	[27, 28]
Tissue volume (fraction of body weight, unitless)								
Arterial blood	VartC	Normal	0.016	4.680E-03	0.300	0.006	0.025	[4]
Venous blood	VvenC	Normal	0.044	1.332E-02	0.300	0.018	0.071	[4]
Liver	VLC	Normal	0.023	3.609E-04	0.015*	0.023	0.024	[28, 29, 31]
Kidney	VKC	Normal	0.0045	1.738E-04	0.038*	0.0042	0.0049	[28, 39]
Muscle	VMC	Normal	0.355	2.494E-03	0.007*	0.351	0.360	[32]
Fat	VFC	Normal	0.235	1.802E-02	0.077*	0.200	0.270	[30]
Rest of body	VrestC	Normal	0.3225	9.346E-02	0.300	0.128	0.495	Total adds to 1
Rest of body for metabolite submodel	VrestC1	Normal	0.9125	2.736E-01	0.300	0.376	0.990	Total adds to 2
Blood flow (fraction of cardiac output, unitless)								
Liver	QLC	Normal	0.273	8.175E-02	0.300	0.112	0.433	[4, 33]
Kidney	QKC	Normal	0.116	1.733E-02	0.149*	0.082	0.150	[27, 28]
Muscle	QMC	Normal	0.293	4.216E-02	0.144*	0.211	0.376	[29]
Fat	QFC	Normal	0.128	3.825E-02	0.300	0.053	0.202	[4, 33]
Rest of body	QrestC	Normal	0.190	5.712E-02	0.300	0.078	0.302	Total adds to 1
Rest of body for metabolite submodel	QrestC1	Normal	0.611	1.833E-01	0.300	0.252	0.970	Total adds to 1
Absorption rate constant (/h)								
Intramuscular	Kim	Lognormal	1.000	3.000E-01	0.300	0.539	1.703	Model fitted
Subcutaneous	Ksc	Lognormal	0.400	1.200E-01	0.300	0.216	0.681	Model fitted
Tissue:plasma partition coefficient for the parent drug (unitless)								
Liver	PL	Lognormal	10.520	2.104E+00	0.200	6.997	15.208	Model fitted
Liver (5OH FLU)	PL1	Lognormal	9.260	1.852E+00	0.200	6.159	13.387	Model fitted
Kidney	PK	Lognormal	4.000	8.000E-01	0.200	2.661	5.783	Model fitted
Kidney (5OH FLU)	PK1	Lognormal	4.000	8.000E-01	0.200	2.661	5.783	Model fitted
Muscle	PM	Lognormal	0.500	1.000E-01	0.200	0.333	0.723	Model fitted
Fat	PF	Lognormal	0.600	1.200E-01	0.200	0.399	0.867	Model fitted
Rest of body	Prest	Lognormal	8.000	1.600E+00	0.200	5.321	11.565	Model fitted
Rest of body (5OH FLU)	Prest1	Lognormal	5.000	1.000E+00	0.200	3.326	7.228	Model fitted
Hepatic metabolic rate (/h/kg)	KmetC	Lognormal	0.200	4.000E-02	0.200	0.133	0.289	Model fitted
Rate constant for the regeneration of flunixin free acid from metabolites and enterohepatic circulation (/h/kg)	KehcC	Lognormal	0.150	4.500E-02	0.300	0.081	0.255	Model fitted
Percentage of plasma protein binding (unitless)	PB	Lognormal	0.950	2.850E-01	0.300	0.5118	0.990#	Model fitted
Percentage of plasma protein binding (unitless) (5OH FLU)	PB1	Lognormal	0.990	2.970E-01	0.300	0.5334	0.990#	Model fitted
Biliary elimination rate constant (L/h/kg)	KbileC	Lognormal	0.100	3.000E-02	0.300	0.054	0.170	Model fitted
Biliary elimination rate constant (L/h/kg) (5OH FLU)	KbileC1	Lognormal	0.100	3.000E-02	0.300	0.054	0.170	Model fitted
Urinary elimination rate constant (L/h/kg)	KurineC	Lognormal	0.100	3.000E-02	0.300	0.054	0.170	Model fitted
Urinary elimination rate constant (L/h/kg) (5OH FLU)	KurineC1	Lognormal	0.100	3.000E-02	0.300	0.054	0.170	Model fitted
Intestinal transit rate constant (/h)	Kint	Lognormal	0.400	1.200E-01	0.300	0.216	0.681	[39]
Fecal elimination rate constant (/h)	Kfeces	Lognormal	0.500	1.500E-01	0.300	0.269	0.851	Model fitted

Notes: Some parameters were estimated by calibrating the PBPK model with pharmacokinetic data. These parameters were marked as 'model fitted'. The star sign (*) indicates the CV of that parameter was calculated based on previous experimental data. For the parameters without calculated CVs, the default values of CVs for physiological parameters were 0.3. For chemical-specific parameters such as tissue to plasma partition coefficients and hepatic metabolic rate, the default CVs were 0.2. For the other chemical-specific parameters such as percentage of plasma protein binding, and rate constants for enterohepatic circulation, biliary and urinary eliminations, the default CVs were 0.3. The pound sign (#) indicates the upper bound of plasma protein binding is lower than 1 based on the physiological plausibility. The 95% confidence interval of each parameter was calculated as the lower (i.e., 2.5th percentile) and upper (i.e., 97.5th percentile) bounds.

Table S4. Withdrawal time predictions and 95% confidence intervals for the 99th percentile of the population for different therapeutic scenarios after 1000 Monte Carlo runs of 1000 iterations.

Species	Therapeutic Scenarios	WDI (days)	95% confidence interval (days)	
			Lower bound	Upper bound
Cattle	Label (IV 3 repeated doses)	5.87	5.86	5.88
	Exlabel (IM 3 repeated doses)	5.96	5.95	5.97
Swine	Label (IM single doses)	12.14	12.12	12.15
	Exlabel (IM 3 repeated doses)	15.29	15.27	15.31

Notes: The abbreviations: WDI, withdrawal interval; IV, intravascular injection; IM, intramuscular injection.

Table S5. Comparison of simulation time with different iterations between the "deSolve" and "mrgsolve" packages.

Iteration Number	Simulation Time (second)	
	deSolve (original)	mrgsolve (improved)
1	11.98	0.01
10	115.66	0.07
100	1482.59	0.61
1000	35790.92 (9.94 hours)	6.22
10000	~ (speed slows down significantly with increased simulation)	60.53

Notes: The configurations of the computer for these reported simulation times were listed as followings. Processor: Intel(R) Xeon(R) CPU E5-1620 @ 3.60GHz; RAM: 26.0 GB; System type: Windows 7 Professional 64-bit Operating System.

Table S6. Values of physicochemical parameters for flunixin and 5-OH flunixin.

	Flunixin	5-OH Flunixin
CAS#	38677-85-9	75369-61-8
MW	296.25	312.25
pKa	5.82	4.47*
logP	4.9*	3.89*
fup	0.01[8]	0.02#

Notes: CAS#, Chemical Abstracts Service number; MW, molecular weight; pKa, acid dissociation constant; logP, octanol to water partition coefficient; fup, fraction of unbound in plasma. Values were acquired from PubChem unless otherwise indicated. *, values acquired from ChemSpider. #, predicted value from Simcyp Animal Module (Version 16.0.113.0)

Table S7. Tissue to plasma partition coefficients for flunixin and 5-OH flunixin estimated using different methods

Flunixin

Parameters	GastroPlus	PK-Sim	Simcyp	RR Rat	RR Swine
Kidney	14 (0.14)	14 (0.14)	14.1 (0.141)	14.12	14.04
Liver	10 (0.1)	10 (0.1)	9.7 (0.097)	9.74	10.52
Muscle	7 (0.07)	7 (0.07)	7.2 (0.072)	7.2	7.41
Fat	7 (0.07)	9 (0.09)	8.6 (0.086)	6.10	8.57
Skin	29 (0.29)	30 (0.3)	30.4 (0.304)	30.4	31.77
Rest of body	13 (0.13)				
Lung	22 (0.22)	22 (0.22)	22.5 (0.225)	22.51	22.39
Heart	17 (0.17)	17 (0.17)	16.7 (0.167)	16.71	16.93
Spleen	11 (0.11)	10 (0.1)	10.4 (0.104)	10.46	10.73
Brain	7 (0.07)	7 (0.07)	6.7 (0.067)	6.77	9.86
Bone		11 (0.11)	10.8 (0.108)	10.86	
Gut		18 (0.18)	17.8 (0.178)	17.78	
Gonads		5 (0.05)			
poorly					
richly					
ReproOrg	15 (0.15)				
RedMarrow	17 (0.17)				
YellowMarrow	7 (0.07)				

5-OH Flunixin

Parameters	GastroPlus	PK-Sim	Simcyp	RR Rat	RR Swine
Kidney	13 (0.13)	13 (0.13)	13.7 (0.137)	13.509	13.492
Liver	9 (0.09)	9 (0.09)	9.3 (0.093)	9.089	9.264
Muscle	7 (0.07)	7 (0.07)	6.3 (0.063)	6.814	6.862
Fat	5 (0.05)	6 (0.06)	5.7 (0.057)	5.287	5.957
Skin	28 (0.28)	28 (0.28)	28.5 (0.285)	28.476	28.784
Rest of body	13 (0.13)				
Lung	22 (0.22)	22 (0.22)	21.7 (0.217)	21.726	21.699
Heart	16 (0.16)	16 (0.16)	16.3 (0.163)	16.193	16.242
Spleen	10 (0.1)	10 (0.1)	10.3 (0.103)	10.132	10.194
Brain	5 (0.05)	6 (0.06)	5.7 (0.057)	5.520	6.212
Bone		10 (0.1)	10.6 (0.106)	10.298	
Gut		16 (0.16)	16.6 (0.166)	16.485	
Gonads		5 (0.05)			
ReproOrg	15 (0.15)				
RedMarrow	16 (0.16)				
YellowMarrow	5 (0.05)				

Notes: The values of tissue to plasma partition coefficients for unbound drugs in plasma by different methods were listed in the table. The original predicted values based on total drugs in plasma by the selected commercial software were included in the table with parentheses. RproOrg is the abbreviation for reproductive organs. RR is the abbreviation for Rodgers and Rowland method. The predicted values of tissue to plasma partition coefficients from three commercial software are based on total chemical concentrations in plasma, while Rodgers and Rowland method uses free chemical concentrations in plasma in the calculation of partition coefficients.

Table S8. Sensitive parameters identified by the local sensitivity analysis

Sensitive Parameter	Normalized sensitivity coefficients (NSCs)			
	AUCCV	AUCCL	AUCCK	AUCCV1
BW	-0.36	-0.37	0.09	-0.24
QCC	0.00	0.00	0.00	0.99
QLC	-0.37	-0.35	0.08	-0.25
VLC	0.00	0.00	0.00	-0.99
PK	0.05	0.05	0.05	0.61
Prest1	-0.01	-0.05	-0.01	-0.44
PB	-0.07	-0.12	-0.09	-0.46
PB1	-0.07	-0.12	-0.09	-0.46
KmetC	0.07	0.07	0.07	-0.24

Additional Files and Instructions

1. Additional files

The "additional files" folder includes several separate folders: Codes, Datasets, and Results.

- **Codes folder: Berkeley Madonna codes for model calibration, evaluation and population analysis.**
  - **Cattle: The codes for average and population PBPK models in cattle.**
  - **Swine: The codes for average and population PBPK models in swine.**

- **Datasets folder**: All datasets used in model calibration and evaluation are included in this folder. Please refer to Table 1 for details.

"Calibration" folder (the following data file names are designed according to the figure numbers and figure panel names):

The following data files are for cattle:

- **2a: The dataset from Odensvik and Johansson 1995 [17]: Single IV (2.2 mg/kg); Matrix: Plasma.**
- **2b: The dataset from Odensvik and Johansson 1995 [17]: Single IM (2.2 mg/kg); Matrix: Plasma.**
- **2c: The dataset from Shelver et al. 2013 [18]: Single SC (2.2 mg/kg); Matrix: Plasma.**
- **2d: The dataset from FDA 1998 [16]: 3-Dose IV (2.2 mg/kg); Matrix: Liver.**
- **2e: The dataset from FDA 1998 [16]: 3-Dose IV (2.2 mg/kg); Matrix: Muscle.**
- **2f: The dataset from Shelver et al. 2013 [18]: Single SC (2.2 mg/kg); Matrix: Plasma (5-OH Flunixin).**

The following data files are for swine:

- **2g: The dataset from Howard et al. 2014 [20]: Single IV (3 mg/kg); Matrix: Plasma.**
- **2h: The dataset from Howard et al. 2014 [20]: Single IV (3 mg/kg); Matrix: Plasma (5-OH Flunixin).**
- **2i: The dataset from FDA 2005 [22]: 3-Dose IM (2.2 mg/kg); Matrix: Liver.**
- **2j: The dataset from FDA 2005 [22]: 3-Dose IM (2.2 mg/kg); Matrix: Kidney.**
- **2k: The dataset from FDA 2005 [22]: 3-Dose IM (2.2 mg/kg); Matrix: Muscle.**
- **2l: The dataset from FDA 2005 [22]: 3-Dose IM (2.2 mg/kg); Matrix: Fat.**

"Evaluation" folder:

The following data files are for cattle:

- **3a: The dataset from Odensvik 1995 [19]: Single IV (2.2 mg/kg); Matrix: Plasma.**
- **3b: The dataset from Jaroszewski et al. 2008 [34]: 4-Dose IV (2.2 mg/kg); Matrix: Plasma.**
- **3c: The dataset from Kissell et al. 2016 [35]: 3-Dose IV (2.2 mg/kg); Matrix: Plasma.**
- **3d: The dataset from Kissell et al. 2016 [35]: 3-Dose IV (2.2 mg/kg); Matrix: Liver.**
- **3e: The dataset from Kissell et al. 2016 [35]: 3-Dose IV (2.2 mg/kg); Matrix: Kidney.**
- **3f: The dataset from Kissell et al. 2016 [35]: 3-Dose IV (2.2 mg/kg); Matrix: Muscle.**

- **3g: The dataset from Jaroszewski et al. 2008 [34]: 4-Dose IV (2.2 mg/kg); Matrix: Plasma (5-OH Flunixin).**
- **3h: The dataset from Kissell et al. 2016 [35]: 3-Dose IV (2.2 mg/kg); Matrix: Plasma (5-OH Flunixin).**

The following data files are for swine:

- **3i: The dataset from Yu et al. 2007 [36]: Single IV (2.2 mg/kg); Matrix: Plasma.**
- **3j: The dataset from Yu et al. 2007 [36]: Single IV (1.1 mg/kg); Matrix: Plasma.**
- **3k: The dataset from Yu et al. 2007 [36]: Single IM (2.2 mg/kg); Matrix: Plasma.**
- **3l: The dataset from Yu et al. 2007 [36]: Single IM (1.1 mg/kg); Matrix: Plasma.**
- **3m: The dataset from Buur et al. 2006 [37]: Single IV (2 mg/kg); Matrix: Plasma.**
- **3n: The dataset from EMEA 1999 [38]: Single IM (2.4 mg/kg); Matrix: Liver.**
- **3o: The dataset from EMEA 1999 [38]: Single IM (2.4 mg/kg); Matrix: Kidney.**
- **3p: The dataset from EMEA 1999 [38]: Single IM (2.4 mg/kg); Matrix: Muscle.**

"Supplementary" folder:

The following data files are for cattle:

- **S1a: The dataset from Shelver et al. 2013 [18]: Single IV (2.2 mg/kg); Matrix: Plasma.**
- **S1b: The dataset from FDA 1998 [16]: 3-Dose IV (2.2 mg/kg); Matrix: Kidney.**
- **S1c: The dataset from FDA 1998 [16]: 3-Dose IV (2.2 mg/kg); Matrix: Fat.**
- **S1d: The dataset from Shelver et al. 2013 [18]: Single IV (2.2 mg/kg); Matrix: Plasma (5-OH Flunixin).**
- **S1e: The dataset from Kleinhenz et al. 2016 [40]: Single IV (2.2 mg/kg); Matrix: Plasma.**

The following data files are for swine:

- **S2a: The dataset from Pairis-Garcia et al. 2013 [21]: Single IV (2.2 mg/kg); Matrix: Plasma.**
- **S2b: The dataset from Pairis-Garcia et al. 2013 [21]: Single IM (2.2 mg/kg); Matrix: Plasma.**

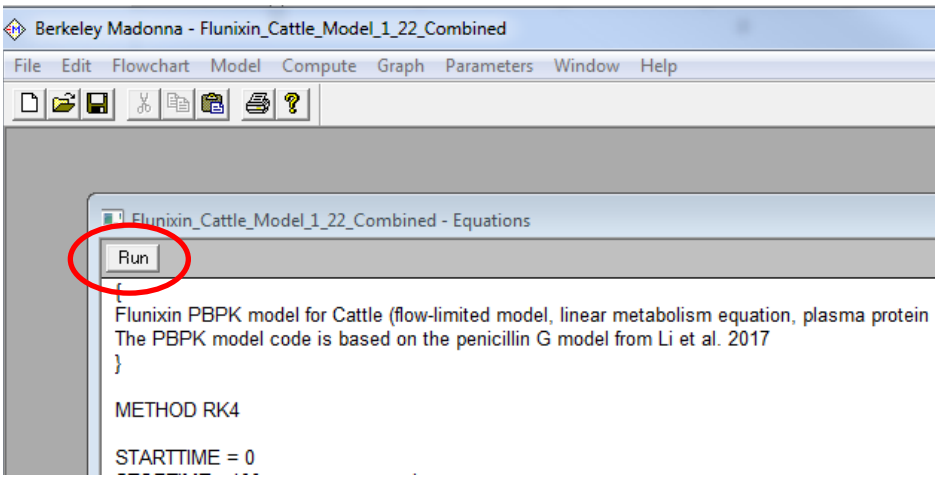
- **Results folder**: This folder contains the original data files used to generate all figures presented in the manuscript and in the Supplementary Materials.

2. Instructions on the model code

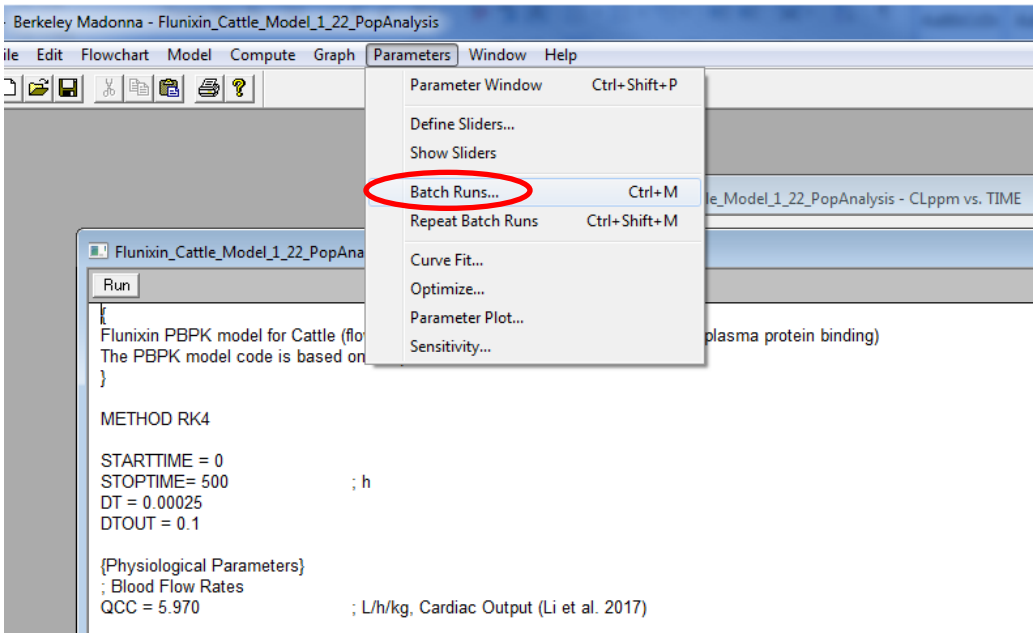
This instructions can be separated into two parts, including Part I: model calibration, evaluation and population analysis using Berkeley Madonna; Part II: raw data for all results presented in the manuscript.

Part I: Model calibration, evaluation and population analysis using Berkeley Madonna:

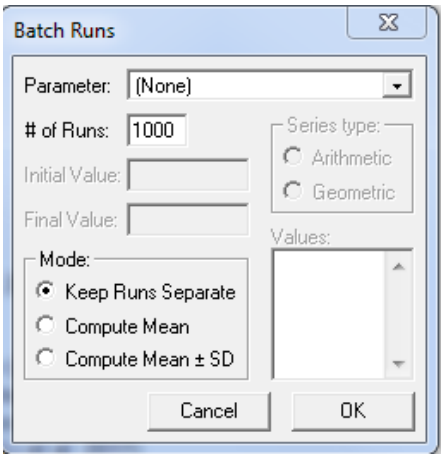
1. Open the code in Codes/Cattle.
2. Click the "Run" button, the single animal model takes about 10 seconds to run.



3. For the file with "PopAnalysis", choose "Batch run" from "Parameters" tab.



4. Set the number of runs to 1000, and click OK.



5. The 1000-time simulation may take from 2-12 hours to finish depending on the computer.

Part II: Raw data for all results presented in the manuscript

- Open the folder "**Results**". The folder include 10 sub-folder for figures in the manuscript and in the Supplementary Materials. Each of the folder contains relevant raw data and graphpad file for creating figures to reproduce the figures.

Model Codes

Average PBPK Model for Flunixin in Cattle using Berkeley Madonna

```
{
Flunixin PBPK model for Cattle (flow-limited model, linear metabolism equation, plasma protein binding)
The PBPK model code is based on the penicillin G model from Li et al. 2017
}

METHOD RK4

STARTTIME = 0
STOPTIME= 400                    ; h
DT = 0.00025
DTOUT = 0.1

{Physiological Parameters}
; Blood Flow Rates
QCC = 5.970                      ; L/h/kg, Cardiac Output (Li et al. 2017)

; Fraction of blood flow to organs (unitless)
QLC =  0.405                     ; Fraction of blood flow to the liver (Li et al. 2017)
QKC = 0.090                      ; Fraction of blood flow to the kidneys (Li et al. 2017)
QMC = 0.180                      ; Fraction of blood flow to the muscle (Li et al. 2017)
QFC = 0.080                      ; Fraction of blood flow to the fat (Li et al. 2017)
QrestC = 1-QLC-QKC-QFC-QMC       ; Fraction of blood flow to the rest of body (total sum equals to 1)
QrestC1 = 1-QLC-QKC              ; Fraction of blood flow to the rest of body for the metabolite submodel

; Tissue Volumes
BW = 300                         ; Body Weight (kg)

; Fractional organ tissue volumes (unitless)
VLC = 0.014                      ; Fractional liver tissue (Li et al. 2017)
VKC = 0.0025                     ; Fractional kidney tissue (Li et al. 2017)
VFC = 0.150                      ; Fractional fat tissue (Li et al. 2017)
VMC = 0.270                      ; Fractional muscle tissue (Li et al. 2017)
VvenC = 0.030                    ; Venous blood volume, fraction of blood volume (Li et al. 2017)
VartC = 0.010                    ; Arterial blood volume, fraction of blood volume (Li et al. 2017)
VbloodC = VvenC+VartC
VrestC = 1-VLC-VKC-VFC-VMC-VvenC-VartC; Fractional rest of body (total sum equals to 1)
VrestC1 = 1-VLC-VKC-VvenC-VartC; Fractional rest of body for the metabolite submodel

{Mass Transfer Parameters (Chemical-Specific Parameters)}
; Flunixin and 5OH Flunixin Molecular Weight
MW = 296.24                      ; mg/mmol
MW1 = 312.24                     ; mg/mmol

; Partition Coefficients for FLU (PC, tissue:plasma)
PL = 10.52                       ; Liver:plasma PC, model fitting
PK = 4                           ; Kidney:plasma PC, model fitting
PM = 0.5                         ; Muscle:plasma PC, model fitting
PF = 0.6                          ; Fat:plasma PC, model fitting
Prest = 8                         ; Rest of body:plasma PC, model fitting

; Partition Coefficients for 5OH FLU (PC, tissue:plasma)
PL1 = 9.26                       ; Liver:plasma PC , model fitting
PK1 = 4                          ; Kidney:plasma PC, model fitting
```

```
Prest1 = 5                      ; Rest of body:plasma PC, model fitting

{Kinetic Constants}
; Rate Constants in GI compartments
Kint = 0.4                      ; /h, intestinal transit rate constant, model fitting
Kfeces = 0.5                    ; /h, fecal elimination rate constant, model fitting

; IM Absorption Rate Constants
Kim = 0.50                      ; /h, IM absorption rate constant, model fitting

; SC Absorption Rate Constants
Ksc = 0.40                      ; /h, SC absorption rate constant, model fitting

; Percentage Plasma Protein Binding unitless
PB = 0.95                       ; Percentage of FLU bound to plasma proteins, Initial value of 0.99 from
Skidmore et al., 2008 and Thiry et al., 2017, then optimized by fitting to Odensvik and Johansson, 1995 dataset
Free = 1-PB

PB1 = 0.99                      ; Percentage of 5OH FLU bound to plasma proteins, Initial value of 0.99 by
structural similarity with FLU
Free1 = 1-PB1

{Metabolic Rate Constant}
KmetC = 0.200                   ; /h/kg, metabolic rate constant from FLU to 5OH FLU, model fitting
KehcC = 0.050                   ; /h/kg, rate constant for the enterohepatic circulation, model fitting

; Urinary Elimination Rate Constants
KurineC = 0.100                 ; L/h/kg, urine elimination rate constant for FLU, model fitting
KurineC1 = 0.200                ; L/h/kg, urine elimination rate constant for 5OH FLU, model fitting

; Biliary Elimination Rate Constats
KbileC = 0.500                  ; L/h/kg, biliary secretion rate constant for FLU, model fitting
KbileC1 = 0.100                 ; L/h/kg, biliary secretion rate constant for 5OH FLU, model fitting

{Parameters for Various Exposure Scenarios}
PDOSEiv = 0                     ; (mg/kg)
PDOSEsc = 0                     ; (mg/kg)
PDOSEim = 2.2                   ; (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
Qrest = QrestC*QC               ; Rest of body
Qrest1 = QrestC1*QC             ; Rest of body for the metabolite submodel

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

```
Vblood = VbloodC*BW             ; Total Blood
Vrest1 = VrestC1*BW             ; Rest of body for the metabolite submodel

; Metabolic rate constant
Kmet = KmetC*BW                 ; Metabolic rate constant from FLU to 5OH FLU
Kehc = KehcC*BW                 ; Enterohepatic circulation rate constant for FLU

; Urinary Elimination Rate Constants
Kurine = KurineC*BW             ; FLU
Kurine1 = KurineC1*BW           ; 5OH FLU

; Biliary Elimination Rate Constants
Kbile = KbileC*BW               ; FLU
Kbile1 = KbileC1*BW             ; 5OH FLU

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

; Dosing, repeated doses
tinterval = 24                  ; Varied dependent on the exposure paradigm (h)
Tdoses = 1                      ; times for multiple oral gavage
Timeim = 0.01                   ; IM injection time (h)
Timesc = 0.01                   ; SC injection time (h)
Timeiv = 0.005                  ; IV infusion time (h)

Fmultiple = if time < Tdoses*tinterval then 1 else 0

; Dosing, IM, intramuscular
IMR = DOSEim/(MW*Timeim)                                    ; mmol/h
Fim = IF MOD(time, tinterval) <= Timeim then 1 else 0
Rdoseim = IMR*Fim*Fmultiple                                 ; mmol/h
Rim = Kim*Amtsiteim             ; Rim, drug absorption rate of intramuscular route (mmol/h)
Rsiteim = RdoseIM -Rim          ; Rsiteim, changing rate of drug at the intramuscular injection site (mmol/h)
d/dt(Amtsiteim) = Rsiteim       ; Amtsiteim, amount of the drug at the intramuscular injection site (mmol)
init Amtsiteim = 0              ; DOSEim, amount of the drug injected through intramuscular injection (mmol)
d/dt(Absorbim) = Rim            ; Absorbim, amount of drug absorbed by intramuscular injection (mmol)
init Absorbim = 0

; Dosing, SC, subcutaneous
SCR = DOSEsc/(MW*Timesc)                                    ; mmol/h
Fsc = IF MOD(time, tinterval) <= Timesc then 1 else 0
Rdosesc = SCR*Fsc*Fmultiple                                 ; mmol/h
Rsc = Ksc*Amtsitesc             ; Rsc, drug absorption rate of subcutaneous route (mmol /h)
Rsitesc =Rdosesc -Rsc           ; Rsitesc, changing rate of drug at the subcutaneous injection site (mmol/h)
d/dt(Amtsitesc) = Rsitesc       ; Amtsitesc, amount of the drug at the subcutaneous injection site (mmol)
init Amtsitesc = 0              ; DOSEsc, amount of the drug injected through subcutaneous injection (mmol)
d/dt(Absorbsc) = Rsc            ; Absorbsc, amount of drug absorbed by subcutaneous injection (mmol)
init Absorbsc = 0

; Dosing, IV, injection to the venous
IVR = DOSEiv/(MW*Timeiv)            ; mmol/h, IVR rate of the drug through iv, Timeiv iv exposure time
Fiv = IF MOD(time, tinterval) <= Timeiv then 1 else 0
Riv = IVR*Fiv*Fmultiple
```

```
d/dt(Aiv) = Riv
init Aiv = 0

{Parent Drug (FLU) submodel}
{FLU distribution in each compartment}
; FLU in blood compartment
CV = ((QL*CVL+QK*CVK+QF*CVF+QM*CVM+Qrest*CVrest+Rsc+Rim+Riv)/QC) ; mmol/L
RA = QC*(CV-CAfree)
d/dt(AA) = RA
init AA = 0
CA = AA/Vblood
d/dt(AUCCV) = CV
init AUCCV = 0
CAfree = CA*Free
CVppm = CV*MW                                          ; mg/L

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

; Metabolism of FLU in liver compartment
Rmet = Kmet*CL*VL              ; Rmet, the metabolic rate from Flunixin to 5OH Flunixin in liver (mmol/h)
d/dt(Amet) = Rmet              ; Amet, the amount of 5OH FLU produced in liver (mmol)
init Amet = 0

; Biliary secretion of FLU
Rbile = Kbile*CVL              ; Rbile, the biliary secretion rate of FLU (mmol/h)
d/dt(Abile) = Rbile            ; Abile, the amount of FLU through biliary secretion (mmol)
init Abile = 0

; FLU in GI compartments and Enterohepatic Circulation
; Enterohepatic circulation of FLU
Rehc = Kehc*AI          ; Rehc, the changing rate of the amount of drug through enterohepatic circulation (mmol/h)
d/dt(Aehc) =  Rehc      ; Aehc, the amount of drug through enterohepatic circulation (mmol)
init Aehc = 0

; FLU in GI compartments
Rcolon = Kint*AI                       ; Rcolon, the changing rate of the amount drug into colon (mmol/h)
d/dt(Acolon) = Rcolon                  ; Acolon, amounts of the drug into the colon (mmol)
init Acolon = 0
RAI = Rbile+Rbile1-Kint*AI-Kehc*AI     ; AI, amount of the drug in the intestine (mmol)
d/dt(AI) = RAI                         ; RAI, the change rate of the amount of drug in intestine (mmol/h)
init AI = 0
Rfeces = Kfeces*Acolon
d/dt(Afeces) = Rfeces
init Afeces = 0

; FLU in kidney compartment, flow-limited model
RK = QK*(CAfree-CVK)-Rurine    ; RK, the changing rate of the amount of drug in kidney (mmol/h)
d/dt(AK) = RK                  ; AK, amount of drug in kidney (mmol)
```

```
init AK = 0
CK = AK/VK                    ; CK, drug concentration in kidney (mmol/L)
CVK = AK/(VK*PK)
d/dt(AUCCK) = CK              ; AUCCK, AUC of drug concentration in kidney (mmol*h/L)
init AUCCK = 0
CKppm = CK*MW                 ; mg/L

; Urinary excretion of FLU
Rurine = Kurine*CVK           ; Rurine, the urinary secretion rate of FLU (mmol/h)
d/dt(Aurine) = Rurine         ; Aurine, the amount of FLU through urinary secretion (mmol)
init Aurine = 0

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

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

; FLU in the compartment of rest of body, flow-limited model
Rrest = Qrest*(CAfree-CVrest) ; Rrest, the changing rate of the amount of drug in the rest of the body (mmol/h)
d/dt(Arest) = Rrest           ; Arest, amount of the drug in the rest of the body (mmol)
init Arest = 0
Crest = Arest/Vrest           ; Crest, drug concentration in the rest of the body (mmol/L)
CVrest = Arest/(Vrest*Prest)
d/dt(AUCCrest) = Crest        ; AUCCrest, AUC of drug concentration in the rest of the body (mmol*h/L)
init AUCCrest = 0
Crestppm = Crest*MW           ; mg/L

{Metabolite (5OH FLU) submodel}
{5OH FLU distribution in each compartment}
; 5OH FLU in blood compartment
CV1 = ((QL*CVL1+QK*CVK1+Qrest1*CVrest1)/QC)
RA1 = QC*(CV1-CAfree1)
d/dt(AA1) = RA1
init AA1 = 0
CA1 = AA1/Vblood
d/dt(AUCCV1) = CV1
init AUCCV1 = 0
CAfree1 = CA1*Free1
CV1ppm = CV1*MW1                                    ; mg/L

; 5OH FLU in liver compartment, flow-limited model
```

```
RL1 = QL*(CAfree1-CVL1)-Rbile1+Rmet  ; RL1, the changing rate of the amount of 5OH FLU in liver (mmol/h)
d/dt(AL1) = RL1                      ; AL1, amount of 5OH FLU in liver (mmol)
init AL1 = 0
CL1 = AL1/VL                         ; CL1, 5OH FLU concentration in liver (mmol/L)
CVL1= AL1/(VL*PL1)                   ; CVL1, 5OH FLU concentration in venous blood from liver (mmol/L)
d/dt(AUCCL1) = CL1       ; AUCCL1, area under the curve of 5OH FLU concentration in liver (mmol*h/L)
init AUCCL1 = 0
CL1ppm = CL1*MW1                     ; mg/L

; Biliary secretion of 5OH FLU
Rbile1 = Kbile1*CVL1                 ; Rbile1, the biliary secretion rate of 5OH FLU (mmol/h)
d/dt(Abile1) = Rbile1                ; Abile1, the amount of 5OH FLU through biliary secretion (mmol)
init Abile1 = 0

; 5OH FLU in kidney compartment, flow-limited model
RK1 = QK*(CAfree1-CVK1)-Rurine1  ; RK1, the changing rate of the amount of 5OH FLU in kidney (mmol/h)
d/dt(AK1) = RK1                      ; AK1, amount of 5OH FLU in kidney (mmol)
init AK1 = 0
CK1 = AK1/VK                         ; CK1, concentration of 5OH FLU in kidney (mmol/L)
CVK1 = AK1/(VK*PK1)
d/dt(AUCCK1) = CK1                   ; AUCCK1, AUC of 5OH FLU concentration in kidney (mmol*h/L)
init AUCCK1 = 0
CK1ppm = CK1*MW1                     ; mg/L

; Urinary excretion of 5OH FLU
Rurine1 = Kurine1*CVK1               ; Rurine1, the urinary secretion rate of 5OH FLU (mmol/h)
d/dt(Aurine1) = Rurine1              ; Aurine1, the amount of 5OH FLU through urinary secretion (mmol)
init Aurine1 = 0

; 5OH FLU in the compartment of rest of body, flow-limited model
Rrest1 = Qrest1*(CAfree1-CVrest1) ; Rrest1, the changing rate of the amount of 5OH FLU in the rest of the body
(mmol/h)
d/dt(Arest1) = Rrest1                ; Arest1, amount of 5OH FLU in the rest of the body (mmol)
init Arest1 = 0
Crest1 = Arest1/Vrest1               ; Crest1, 5OH FLU concentration in the rest of the body (mmol/L)
CVrest1 = Arest1/(Vrest1*Prest1)
d/dt(AUCCrest1) = Crest1         ; AUCCrest1, AUC of 5OH FLU concentration in the rest of the body (mmol*h/L)
init AUCCrest1 = 0
Crest1ppm = Crest1*MW1                   ; mg/L

{Mass balance equations}
; Mass balance equations for FLU
Qbal = QC-QM-Qrest-QF-QK-QL
Tmass = AA+AM+Arest+AF+AK+AL+Aurine+Amet+Abile
Input = Aiv+Absorbim+Absorbsc+Aehc
Bal = Input-Tmass

; Mass balance equations for 5OH FLU
Qbal1 = QC-QL-QK-Qrest1
Tmass1 = AA1+Arest1+AK1+AL1+Aurine1+Abile1
Input1 = Amet
Bal1 = Input1-Tmass1
```

Population PBPK Model for Flunixin in Cattle using Berkeley Madonna

```
{
Flunixin PBPK model for Cattle (flow-limited model, linear metabolism equation, plasma protein binding)
The PBPK model code is based on the penicillin G model from Li et al. 2017
}

METHOD RK4

STARTTIME = 0
STOPTIME= 500                    ; h
DT = 0.00025
DTOUT = 0.1

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

; Fracion of blood flow to organs (unitless)
QLC =  0.405                     ; Fraction of blood flow to the liver (1996 Lescoat, 1960 Dlyle)
QKC = 0.090                      ; Fraction of blood flow to the kidneys (2016 Lin)
QMC = 0.180                      ; Fraction of blood flow to the muscle (2016 Lin)
QFC = 0.080                      ; Fraction of blood flow to the fat (2016 Lin)
QrestC = 1-QLC-QKC-QFC-QMC ; Fraction of blood flow to the rest of body (total sum equals to 1)
QrestC1 = 1-QLC-QKC

; Tissue Volumes
BW = 300                         ; Body Weight (kg)

; Fractional organ tissue volumes (unitless)
VLC = 0.014                      ; Fractional liver tissue (1933 Swett)
VKC = 0.002                      ; Fractional kidney tissue (1933 Swett)
VFC = 0.150                      ; Fractional fat tissue (2016 Lin, 2014 Leavens)
VMC = 0.270                      ; Fractional muscle tissue (2016 Lin, 2014 Leavens)
VvenC = 0.030                    ; Venous blood volume, fraction of blood volume (2016 Lin; 2008 Leavens)
VartC = 0.010                    ; Arterial blood volume, fraction of blood volume (2016 Lin; 2008 Leavens)
VbloodC = VvenC+VartC
VrestC = 1-VLC-VKC-VFC-VMC-VvenC-VartC; Fractional rest of body (total sum equals to 1)
VrestC1 = 1-VLC-VKC-VvenC-VartC

{Mass Transfer Parameters (Chemical-Specific Parameters) prediction}
; Flunixin and 5OH Flunixin Molecular Weight
MW = 296.24                      ; mg/mmol
MW1 = 312.24                     ; mg/mmol

; Partition Coefficients for FLU (PC, tissue:plasma)
PL = 10.52                       ; Liver:plasma PC
PK = 4                           ; Kidney:plasma PC
PM = 0.5                         ; Muscle:plasma PC
PF = 0.6                          ; Fat:plasma PC
Prest = 8                        ; Rest of body:plasma PC (average from all other partition coefficients)

; Partition Coefficients for 5OH FLU (PC, tissue:plasma)
PL1 = 9.26                       ; Liver:plasma PC
PK1 = 4                           ; Kidney:plasma PC
Prest1 = 5                       ; Rest of body:plasma PC
```

```
{Kinetic Constants}
; Rate Constants in GI compartments
Kint = 0.4                    ; /h, intestinal transit rate constant
Kfeces = 0.5                  ; /h, fecal elimination rate constant

; IM Absorption Rate Constants
Kim = 0.5                     ; /h, IM absorption rate constant

; SC Absorption Rate Constants
Ksc = 0.4                     ; /h, SC absorption rate constant

; Percentage Plasma Protein Binding unitless
PB = 0.95                     ; Percentage of drug bound to plasma proteins
PB1 = 0.99

{Metabolic Rate Constant}
KmetC = 0.2                   ; /h/kg, metabolic rate constant for 5OH FLU
KehcC = 0.05                  ; /h/kg, rate constant for the enterohepatic circulation

; Urinary Elimination Rate Constants
KurineC = 0.1                 ; L/h/kg
KurineC1 = 0.200              ; L/h/kg

; Biliary Elimination Rate Constats
KbileC = 0.500                ; L/h/kg
KbileC1 = 0.100               ; L/h/kg

{Parameters for Various Exposure Scenarios}
PDOSEiv = 2.2                 ; (mg/kg)
PDOSEsc = 0                   ; (mg/kg)
PDOSEim = 0                   ; (mg/kg)

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

limit VartCm >= 0.004
limit VartCm <= 0.017
limit VvenCm >= 0.012
limit VartCm <= 0.047
limit VLCm >= 0.010
limit VLCm <= 0.017
limit VKCm >= 0.002
limit VKCm <= 0.003
limit VMCm >= 0.111
limit VMCm <= 0.429
limit VFCm >= 0.062
limit VFCm <= 0.238
limit VrestCm >= 0.216
limit VrestCm <= 0.832

limit QLCm >= 0.024
limit QLCm <= 0.785
```

```
limit QKCm >= 0.037
limit QKCm <= 0.143
limit QMCm >= 0.074
limit QMCm <= 0.286
limit QFCm >= 0.033
limit QFCm <= 0.127
limit QrestCm >= 0.101
limit QrestCm <= 0.390

limit Kimm >= 0.269
limit Kimm <= 0.851
limit Kscm >= 0.216
limit Kscm <= 0.681

limit PLm >= 6.997
limit PLm <= 15.208
limit PKm >= 2.661
limit PKm <= 5.783
limit PMm >= 0.333
limit PMm <= 0.723
limit PFm >= 0.399
limit PFm <= 0.867
limit Prestm >= 5.321
limit Prestm <= 11.565
limit PL1m >= 6.159
limit PL1m <= 13.387
limit PK1m >= 2.661
limit PK1m <= 5.783
limit Prest1m >= 3.326
limit Prest1m <= 7.228

limit KmetCm >= 0.133
limit KmetCm <= 0.289
limit KehcCm >= 0.027
limit KehcCm <= 0.085
limit PBm >= 0.5118
limit PBm <= 0.99
limit PB1m >= 0.5334
limit PB1m <= 0.99

limit KbileCm >= 0.269
limit KbileCm <= 0.851
limit KbileC1m >= 0.054
limit KbileC1m <= 0.170
limit KurineCm >= 0.054
limit KurineCm <= 0.170
limit KurineC1m >= 0.108
limit KurineC1m <= 0.341
limit Kintm >= 0.216
limit Kintm <= 0.681
limit Kfecesm >= 0.269
limit Kfecesm <= 0.851

BW_sd = 46.180                  ; Standard Deviation of Body Weight
QCC_sd = 1.99                   ; Standard Deviation of QCC
VartC_sd = 3.12e-3
```

```
VvenC_sd = 8.88e-3
VLC_sd = 1.63e-3
VKC_sd = 4.321e-4
VMC_sd = 8.1e-2
VFC_sd = 4.5e-2
VrestC_sd = 0.1572
QLC_sd = 0.1942
QKC_sd = 0.027                    ; Standard Deviation of QKC
QMC_sd = 0.054
QFC_sd = 0.024
QrestC_sd = 0.0736
Kim_sd = 0.15                     ; Standard Deviation of Kim
Ksc_sd = 0.12
PL_sd = 2.104                     ; Standard Deviation of PL
PK_sd = 0.8                       ; Standard Deviation of PK
PM_sd = 0.1
PF_sd = 0.12
Prest_sd = 1.6
PL1_sd = 1.852                    ; Standard Deviation of PL1
PK1_sd = 0.8                      ; Standard Deviation of PK1
Prest1_sd = 1
KmetC_sd = 0.04
KehcC_sd = 0.015
PB_sd = 0.285
PB1_sd = 0.297
KbileC_sd = 0.15
KbileC1_sd = 0.03
KurineC_sd = 0.03
KurineC1_sd = 0.06
Kint_sd = 0.12
Kfeces_sd = 0.15

{Generation of Parameters based on Normal Distribution}
; Generation of Parameters based on Normal Distribution
init BWm = Normal(BW, BW_sd)                    ; Generation of BWm based on normal distribution
init QCCm = Normal(QCC, QCC_sd)                 ; Generation of the QCCm based on normal distribution

init VartCm = Normal(VartC, VartC_sd)
init VvenCm = Normal(VvenC, VvenC_sd)
init VLCm = Normal(VLC, VLC_sd)
init VKCm = Normal(VKC, VKC_sd)
init VMCm = Normal(VMC, VMC_sd)
init VFCm = Normal(VFC, VFC_sd)
init VrestCm = Normal(VrestC, VrestC_sd)

init QLCm = Normal(QLC, QLC_sd)
init QKCm = Normal(QKC, QKC_sd)                 ; Generation of the QKCm based on normal distribution
init QMCm = Normal(QMC, QMC_sd)
init QFCm = Normal(QFC, QFC_sd)
init QrestCm = Normal(QrestC, QrestC_sd)

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

```
; Creation of Adjust Factor
AdjustF = QLCm+QKCm+QFCm+QMCm+QrestCm     ; Adjust factor to keep the sum of blood flow fractions to 1
AdjustF1 = VartCm+VvenCm+VLCm+VKCm+VFCm+VMCm+VrestCm

; Creation of Adjusted Parameters
next QLCm = QLCm/AdjustF                  ; Adjustment of QLCm based on the adjust factor
next QKCm = QKCm/AdjustF                  ; Adjustment of QKCm
next QFCm = QFCm/AdjustF                  ; Adjustment of QFCm
next QMCm = QMCm/AdjustF                  ; Adjustment of QMCm
next QrestCm = QrestCm/AdjustF            ; Adjustment of QrestCm
QrestC1m = 1-QLCm-QKCm

next VartCm = VartCm/AdjustF1
next VvenCm = VvenCm/AdjustF1
next VLCm = VLCm/AdjustF1
next VKCm = VKCm/AdjustF1
next VFCm = VFCm/AdjustF1
next VMCm = VMCm/AdjustF1
next VrestCm = VrestCm/AdjustF1
VbloodCm = VartCm+VvenCm
VrestC1m = 1-VbloodCm-VLCm-VKCm

{Lognormal Transformation of Parameters}
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
Ksc_ln = logn(Ksc^2/(Ksc_sd^2+Ksc^2)^0.5)                    ; Lognormal transformation of Ksc value
Ksc_lnsd = (logn(1+Ksc_sd^2/Ksc^2))^0.5
PL_ln = logn(PL^2/(PL_sd^2+PL^2)^0.5)                        ; Lognormal transformation of PL values
PL_lnsd = (logn(1+PL_sd^2/PL^2))^0.5
PK_ln = logn(PK^2/(PK_sd^2+PK^2)^0.5)                        ; Lognormal transformation of PK values
PK_lnsd = (logn(1+PK_sd^2/PK^2))^0.5
PM_ln = logn(PM^2/(PM_sd^2+PM^2)^0.5)                        ; Lognormal transformation of PM values
PM_lnsd = (logn(1+PM_sd^2/PM^2))^0.5
PF_ln = logn(PF^2/(PF_sd^2+PF^2)^0.5)                        ; Lognormal transformation of PF values
PF_lnsd = (logn(1+PF_sd^2/PF^2))^0.5
Prest_ln = logn(Prest^2/(Prest_sd^2+Prest^2)^0.5)            ; Lognormal transformation of Prest values
Prest_lnsd = (logn(1+Prest_sd^2/Prest^2))^0.5
PL1_ln = logn(PL1^2/(PL1_sd^2+PL1^2)^0.5)                    ; Lognormal transformation of PL1 values
PL1_lnsd = (logn(1+PL1_sd^2/PL1^2))^0.5
PK1_ln = logn(PK1^2/(PK1_sd^2+PK1^2)^0.5)                    ; Lognormal transformation of PK1 values
PK1_lnsd = (logn(1+PK1_sd^2/PK1^2))^0.5
Prest1_ln = logn(Prest1^2/(Prest1_sd^2+Prest1^2)^0.5)        ; Lognormal transformation of Prest1 values
Prest1_lnsd = (logn(1+Prest1_sd^2/Prest1^2))^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
KehcC_ln = logn(KehcC^2/(KehcC_sd^2+KehcC^2)^0.5)            ; Lognormal transformation of KehcC
KehcC_lnsd = (logn(1+KehcC_sd^2/KehcC^2))^0.5
PB_ln = logn(PB^2/(PB_sd^2+PB^2)^0.5)                        ; Lognormal transformation of PB
PB_lnsd = (logn(1+PB_sd^2/PB^2))^0.5
PB1_ln = logn(PB1^2/(PB1_sd^2+PB1^2)^0.5)                    ; Lognormal transformation of PB1
PB1_lnsd = (logn(1+PB1_sd^2/PB1^2))^0.5
KbileC_ln = logn(KbileC^2/(KbileC_sd^2+KbileC^2)^0.5)        ; Lognormal transformation of KbileC
KbileC_lnsd = (logn(1+KbileC_sd^2/KbileC^2))^0.5
KbileC1_ln = logn(KbileC1^2/(KbileC1_sd^2+KbileC1^2)^0.5)    ; Lognormal transformation of KbileC1
KbileC1_lnsd = (logn(1+KbileC1_sd^2/KbileC1^2))^0.5
KurineC_ln = logn(KurineC^2/(KurineC_sd^2+KurineC^2)^0.5)    ; Lognormal transformation of KurineC
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KurineC_lnsd = (logn(1+KurineC_sd^2/KurineC^2))^0.5
KurineC1_ln = logn(KurineC1^2/(KurineC1_sd^2+KurineC1^2)^0.5)   ; Lognormal transformation of KurineC1
KurineC1_lnsd = (logn(1+KurineC1_sd^2/KurineC1^2))^0.5
Kint_ln = logn(Kint^2/(Kint_sd^2+Kint^2)^0.5)                   ; Lognormal transformation of Kint
Kint_lnsd = (logn(1+Kint_sd^2/Kint^2))^0.5
Kfeces_ln = logn(Kfeces^2/(Kfeces_sd^2+Kfeces^2)^0.5)           ; Lognormal transformation of Kfeces
Kfeces_lnsd = (logn(1+Kfeces_sd^2/Kfeces^2))^0.5

{Creation of Parameters based on Lognormal Distribution}
init Kimm = exp(Normal(Kim_ln, Kim_lnsd)) next Kimm = Kimm              ; Generation of Kimm
init Kscm = exp(Normal(Ksc_ln, Ksc_lnsd)) next Kscm = Kscm              ; Generation of Kscm
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 PMm = exp(Normal(PM_ln, PM_lnsd)) next PMm = PMm                   ; Generation of PMm
init PFm = exp(Normal(PF_ln, PF_lnsd)) next PFm = PFm                   ; Generation of PFm
init Prestm = exp(Normal(Prest_ln, Prest_lnsd)) next Prestm = Prestm     ; Generation of Prestm
init PL1m = exp(Normal(PL1_ln, PL1_lnsd)) next PL1m = PL1m              ; Generation of PL1m based on
lognormal distribution
init PK1m = exp(Normal(PK1_ln, PK1_lnsd)) next PK1m = PK1m              ; Generation of PK1m
init Prest1m = exp(Normal(Prest1_ln, Prest1_lnsd)) next Prest1m = Prest1m  ; Generation of Prest1m
init KmetCm = exp(Normal(KmetC_ln, KmetC_lnsd)) next KmetCm = KmetCm ; Generation of KmetCm
init KehcCm = exp(Normal(KehcC_ln, KehcC_lnsd)) next KehcCm = KehcCm   ; Generation of KehcCm
init PBm = exp(Normal(PB_ln, PB_lnsd)) next PBm = PBm                   ; Generation of PBm
init PB1m = exp(Normal(PB1_ln, PB1_lnsd)) next PB1m = PB1m              ; Generation of PB1m
init KbileCm = exp(Normal(KbileC_ln, KbileC_lnsd)) next KbileCm = KbileCm ; Generation of KbileCm
init KbileC1m = exp(Normal(KbileC1_ln, KbileC1_lnsd)) next KbileC1m = KbileC1m    ; Generation of KbileC1m
init KurineCm = exp(Normal(KurineC_ln, KurineC_lnsd)) next KurineCm = KurineCm   ; Generation of KurineCm
init KurineC1m = exp(Normal(KurineC1_ln, KurineC1_lnsd)) next KurineC1m = KurineC1m ; Generation of
KurineC1m
init Kintm =  exp(Normal(Kint_ln, Kint_lnsd)) next Kintm = Kintm                ; Generation of Kintm
init Kfecesm =  exp(Normal(Kfeces_ln, Kfeces_lnsd)) next Kfecesm = Kfecesm      ; Generation of Kfecesm
Free = 1- PBm
Free1 = 1- PB1m

{Cardiac output and blood flow to tissues (L/h)}
QC = QCCm*BWm                   ; Cardiac output
QL = QLCm*QC                    ; Liver
QK = QKCm*QC                    ; Kidney
QF = QFCm*QC                    ; Fat
QM = QMCm*QC                    ; Muscle
Qrest = QrestCm*QC              ; Rest of body
Qrest1 = QrestC1m*QC

{Tissue volumes (L)}
VL = VLCm*BWm                   ; Liver
VK = VKCm*BWm                   ; Kidney
VF = VFCm*BWm                   ; Fat
VM = VMCm*BWm                   ; Muscle
Vrest = VrestCm*BWm             ; Rest of body
Vven = VvenCm*BWm               ; Venous Blood
Vart = VartCm*BWm               ; Arterial Blood
Vblood = VbloodCm*BWm
Vrest1 = VrestC1m*BWm

; Metabolic rate constant
Kmet = KmetCm*BWm               ; Metabolic rate constant for 5OH FLU
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```
Kehc = KehcCm*BWm

; Urinary Elimination Rate Constants
Kurine = KurineCm*BWm
Kurine1 = KurineC1m*BWm

; Biliary Elimination Rate Constants
Kbile = KbileCm*BWm
Kbile1 = KbileC1m*BWm

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

; Dosing, repeated doses
tinterval = 24                ; Varied dependent on the exposure paradigm (h)
Tdoses = 3                    ; times for multiple oral gavage
Timeim = 0.01                 ; IM injection time (h)
Timesc = 0.01                 ; SC injection time (h)
Timeiv = 0.005                ; IV infusion time (h)

Fmultiple = if time < Tdoses*tinterval then 1 else 0

; Dosing, IM, intramuscular
IMR = DOSEim/(MW*Timeim)                                    ; mmol/h
Fim = IF MOD(time, tinterval) <= Timeim then 1 else 0
Rdoseim = IMR*Fim*Fmultiple                                 ; mmol/h
Rim = Kim*Amtsiteim           ; Rim drug absorption rate of intramuscular route (mg/h)
Rsiteim = RdoseIM -Rim        ; Rsiteim changing rate of drug at the intramuscular injection site (mg/h)
d/dt(Amtsiteim) = Rsiteim     ; Amtsiteim amounts of the drug at the intramuscular injection site (mg)
init Amtsiteim = 0            ; DOSEim amounts of the drug injected through intramuscular injection (mg)
d/dt(Absorbim) = Rim          ; Absorbim amount of drug absorbed by intramuscular injection (mg)
init Absorbim = 0


; Dosing, SC, subcutaneous
SCR = DOSEsc/(MW*Timesc)                                    ; mmol/h
Fsc = IF MOD(time, tinterval) <= Timesc then 1 else 0
Rdosesc = SCR*Fsc*Fmultiple                                 ; mmol/h
Rsc = Ksc*Amtsitesc           ; Rsc drug absorption rate of subcutaneous route (mg/h)
Rsitesc =Rdosesc -Rsc         ; Rsitesc changing rate of drug at the subcutaneous injection site (mg/h)
d/dt(Amtsitesc) = Rsitesc     ; Amtsitesc amounts of the drug at the subcutaneous injection site (mg)
init Amtsitesc = 0            ; DOSEsc amounts of the drug injected through subcutaneous injection (mg)
d/dt(Absorbsc) = Rsc          ; Absorbsc amount of drug absorbed by subcutaneous injection (mg)
init Absorbsc = 0

; Dosing, IV, injection to the venous
IVR = DOSEiv/(MW*Timeiv)      ; mmol/h, IVR rate of the drug through iv, Timeiv iv exposure time
Fiv = IF MOD(time, tinterval) <= Timeiv then 1 else 0
Riv = IVR*Fiv*Fmultiple
d/dt(Aiv) = Riv
init Aiv = 0

{FLU and 5OH FLU distribution in each compartment}
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```
; FLU and 5OH FLU in venous blood compartment
CV = ((QL*CVL+QK*CVK+QF*CVF+QM*CVM+Qrest*CVrest+Rsc+Rim+Riv)/QC) ; mmol/L
RA = QC*(CV-CAfree)
d/dt(AA) = RA
init AA = 0
CA = AA/Vblood
d/dt(AUCCV) = CV
init AUCCV = 0
CAfree = CA*Free
CVppm = CV*MW                                          ; mg/L

CV1 = ((QL*CVL1+QK*CVK1+Qrest1*CVrest1)/QC)
RA1 = QC*(CV1-CAfree1)
d/dt(AA1) = RA1
init AA1 = 0
CA1 = AA1/Vblood
d/dt(AUCCV1) = CV1
init AUCCV1 = 0
CAfree1 = CA1*Free1
CV1ppm = CV1*MW1                                       ; mg/L

; FLU and 5OH FLU in liver compartment, flow-limited model
RL = QL*(CAfree-CVL)-Rmet1-Rbile+Rehc          ; RL the changing rate of the amount of drug in liver (mmol/h)
d/dt(AL) = RL                                  ; AL amount of drug in liver (mmol)
init AL = 0
CL = AL/VL                                     ; CL drug concentration in liver (mmol/L)
CVL= AL/(VL*PLm)                               ; CVL drug concentration in venous blood from liver
(mmol/L)
d/dt(AUCCL) = CL                               ; AUCCL area under the curve of drug concentration in liver
(mmol*h/L)
init AUCCL = 0
CLppm = CL*MW                                  ; mg/L

RL1 = QL*(CAfree1-CVL1)-Rbile +Rmet1           ; RL the changing rate of the amount of drug in liver (mmol/h)
d/dt(AL1) = RL1                                ; AL amount of drug in liver (mmol)
init AL1 = 0
CL1 = AL1/VL                          ; CL drug concentration in liver (mmol/L)
CVL1= AL1/(VL*PL1m)                   ; CVL drug concentration in venous blood from liver (mmol/L)
d/dt(AUCCL1) = CL1                    ; AUCCL area under the curve of drug concentration in liver
(mmol*h/L)
init AUCCL1 = 0
CL1ppm = CL1*MW1                      ; mg/L

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

; GI compartments and Enterohepatic Circulation
Rcolon = Kintm*AI                     ; Rcolon the changing rate of the amount drug into colon (mg/h)
d/dt(Acolon) = Rcolon                 ; Acolon amounts of the drug into the colon (mg)
init Acolon = 0
RAI = Rbile+Rbile1-Kintm*AI-Kehc*AI   ; AI amount of the drug in the intestine (mg)
d/dt(AI) = RAI                        ; RAI the change rate of the amount of drug in intestine (mg/h)
init AI = 0
Rfeces = Kfecesm*Acolon
d/dt(Afeces) = Rfeces
```

```
init Afeces = 0

; Metabolism of FLU in liver compartment
Rmet1 = Kmet*CL*VL              ; Rmet the metabolic rate of 5OH Flunixin in liver (mmol/h)
d/dt(Amet1) = Rmet1             ; Amet the amount of 5OH FLU metabolized in liver (mmol)
init Amet1 = 0

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

Rbile1 = Kbile1*CVL1
d/dt(Abile1) = Rbile1
init Abile1 = 0

; FLU and 5OH FLU in kidney compartment, flow-limited model
RK = QK*(CAfree-CVK)-Rurine     ; RK the changing rate of the amount of drug in kidney (mmol/h)
d/dt(AK) = RK                   ; AK amount of drug in kidney (mmol)
init AK = 0
CK = AK/VK                      ; CK drug concentration in kidney (mmol/L)
CVK = AK/(VK*PKm)
d/dt(AUCCK) = CK                ; AUCCK AUC of drug concentration in kidney (mmol*h/L)
init AUCCK = 0
CKppm = CK*MW                   ; mg/L

RK1 = QK*(CAfree1-CVK1)-Rurine1     ; RK the changing rate of the amount of drug in kidney (mmol/h)
d/dt(AK1) = RK1                     ; AK amount of drug in kidney (mmol)
init AK1 = 0
CK1 = AK1/VK                        ; CK drug concentration in kidney (mmol/L)
CVK1 = AK1/(VK*PK1m)
d/dt(AUCCK1) = CK1                  ; AUCCK AUC of drug concentration in kidney (mmol*h/L)
init AUCCK1 = 0
CK1ppm = CK1*MW1                    ; mg/L

; FLU and 5OH FLU urinary excretion
Rurine = Kurine*CVK
d/dt(Aurine) = Rurine
init Aurine = 0

Rurine1 = Kurine1*CVK1
d/dt(Aurine1) = Rurine1
init Aurine1 = 0

; FLU and 5OH FLU in muscle compartment, flow-limited model
RM = QM*(CAfree-CVM)            ; RM the changing rate of the amount of drug in muscle (mmol/h)
d/dt(AM) = RM                   ; AM amount of the drug in muscle (mmol)
init AM = 0
CM = AM/VM                      ; CM drug concentration in muscle (mmol/L)
CVM = AM/(VM*PMm)
d/dt(AUCCM) = CM
init AUCCM = 0
CMppm = CM*MW                   ; mg/L

; FLU and 5OH FLU in fat compartment, flow-limited model
RF = QF*(CAfree-CVF)            ; RF the changing rate of the amount of drug in fat (mmol/h)
d/dt(AF) = RF                   ; AF amount of the drug in fat (mmol)
```

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init AF = 0
CF = AF/VF                    ; CF drug concentration in fat (mmol/L)
CVF = AF/(VF*PFm)
d/dt(AUCCF) = CF              ; AUCCF AUC of drug concentration in fat (mmol*h/L)
init AUCCF = 0
CFppm = CF*MW                 ; mg/L

; FLU and 5OH FLU in the compartment of rest of body, flow-limited model
Rrest = Qrest*(CAfree-CVrest)  ; Rrest the changing rate of the amount of drug in the rest of the body (mmol/h)
d/dt(Arest) = Rrest           ; Arest amount of the drug in the rest of the body (mmol)
init Arest = 0
Crest = Arest/Vrest           ; Crest drug concentration in the rest of the body (mmol/L)
CVrest = Arest/(Vrest*Prestm)
d/dt(AUCCrest) = Crest        ; AUCCrest AUC of drug concentration in the rest of the body (mmol*h/L)
init AUCCrest = 0
Crestppm = Crest*MW           ; mg/L

Rrest1 = Qrest1*(CAfree1-CVrest1)      ; Rrest the changing rate of the amount of drug in the rest of the body
(mmol/h)
d/dt(Arest1) = Rrest1                  ; Arest amount of the drug in the rest of the body (mmol)
init Arest1 = 0
Crest1 = Arest1/Vrest1                 ; Crest drug concentration in the rest of the body (mmol/L)
CVrest1 = Arest1/(Vrest1*Prest1m)
d/dt(AUCCrest1) = Crest1               ; AUCCrest AUC of drug concentration in the rest of the body (mmol*h/L)
init AUCCrest1 = 0
Crest1ppm = Crest1*MW         ; mg/L

{Mass balance equations}
; Mass balance equations for FLU
Qbal = QC-QM-Qrest-QF-QK-QL
Tmass = AA+AM+Arest+AF+AK+AL+Aurine+Amet1+Abile
Input = Aiv+Absorbim+Absorbsc+Aehc
Bal = Input-Tmass

; Mass balance equations for 5OH FLU
Qbal1 = QC-QL-QK-Qrest1
Tmass1 = AA1+Arest1+AK1+AL1+Aurine1+Abile1
Input1 = Amet1
Bal1 = Input1-Tmass1
```

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