Archives of Toxicology (2019) 93:1865–1880
https://doi.org/10.1007/s00204-019-02464-z

TOXICOKINETICS AND METABOLISM

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

Miao Li[1] · Yi-Hsien Cheng[1] · Jason T. Chittenden[2] · Ronald E. Baynes[2] · Lisa A. Tell[3] · Jennifer L. Davis[4] · Thomas W. Vickroy[5] · Jim E. Riviere[1,2] · Zhoumeng Lin[1]

Received: 14 November 2018 / Accepted: 18 April 2019 / Published online: 25 April 2019

Abstract

Violative chemical residues in animal-derived food products affect food safety globally and have impact on the trade of international agricultural products. The Food Animal Residue Avoidance Databank program has been developing scientific tools to provide appropriate withdrawal interval (WDI) estimations after extralabel drug use in food animals for the past three decades. One of the tools is physiologically based pharmacokinetic (PBPK) modeling, which is a mechanistic-based approach that can be used to predict tissue residues and WDIs. However, PBPK models are complicated and difficult to use by non-modelers. Therefore, a user-friendly PBPK modeling framework is needed to move this field forward. Flunixin was one of the top five violative drug residues identified in the United States from 2010 to 2016. The objective of this study was to establish a web-based user-friendly framework for the development of new PBPK models for drugs administered to food animals. Specifically, a new PBPK model for both cattle and swine after administration of flunixin meglumine was developed. Population analysis using Monte Carlo simulations was incorporated into the model to predict WDIs following extralabel administration of flunixin meglumine. The population PBPK model was converted to a web-based interactive PBPK (iPBPK) framework to facilitate its application. This iPBPK framework serves as a proof-of-concept for further improvements in the future and it can be applied to develop new models for other drugs in other food animal species, thereby facilitating the application of PBPK modeling in WDI estimation and food safety assessment.

Keywords Flunixin · Interactive physiologically based pharmacokinetic (iPBPK) model · Food safety · Drug residues · Withdrawal intervals (WDIs) · Food Animal Residue Avoidance Databank (FARAD)

Introduction

Violative or potentially unsafe chemical residues, including drugs, pesticides, environmental contaminants, natural toxins and other harmful substances in animal-derived food products are an important consideration for global food safety (Baynes and Riviere 2014; NRC 1999a; Sundlof and Cooper 1996). Food products with illegal chemical residues could increase the risk of harming consumer health. The presence of illegal drug residues in animal-derived food products can result in the suspension of the producer's permit or certification, and affect the international trade of agricultural products (NRC 1999b). To mitigate drug residues and ensure animal-derived food safety, the Food Animal Residue Avoidance Databank (FARAD) program, a United States (US) congress-authorized and US Department of Agriculture (USDA)-supported national program, was established in 1981 to provide a portal for drug residue information and develop scientific tools to provide appropriate withdrawal interval (WDI) estimations for drugs in food

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00204-019-02464-z) contains supplementary material, which is available to authorized users.

✉ Zhoumeng Lin
zhoumeng@ksu.edu

Extended author information available on the last page of the article

animals (Craigmill et al. 2006; Riviere et al. 2017). A WDI is the time for drug residues in the edible tissues to deplete below concentrations that are considered safe for humans (FDA 2012). Estimation of drug WDIs after extralabel use using scientific approaches is important to avoid violations of drug residues.

Physiologically based pharmacokinetic (PBPK) modeling is a mechanism-based computational approach that simulates the absorption, distribution, metabolism and excretion (ADME) of chemicals in an organism (Lin et al. 2016a). PBPK modeling is a widely used scientific approach in the fields of drug discovery and development, as well as human health risk assessment of environmental chemicals (Rowland et al. 2011; Tan et al. 2018). FARAD has applied PBPK modeling to the field of food safety, and in the past two decades has developed multiple PBPK models for different drugs in food animals that helped to answer WDI inquires submitted to FARAD (Craigmill 2003; Li et al. 2017). Many independent research groups from different countries have developed PBPK models for various drugs in food animal species to support WDI determination for their respective regulatory legislations (Henri et al. 2017; Yang et al. 2012). However, one critical barrier in this field is that the published models are difficult to use because different modeling software is involved; some groups do not publish the model code; and risk assessors often do not have the programming skills to apply the published model codes. Therefore, a user-friendly PBPK modeling framework or methodology is needed to move this field forward.

Flunixin meglumine is the only nonsteroidal anti-inflammatory drug (NSAID) labeled in the US to use in food-producing animals, including cattle and swine (FDA 1998). It is labeled for intravenous (IV) use in beef and dairy cattle (2.2 mg/kg body weight once a day or divided into 2 daily doses for up to 3 days) as a treatment for pyrexia associated with respiratory diseases, endotoxemia and mastitis, and to modulate inflammation in endotoxemia (Kleinhenz et al. 2016). Recently, a transdermal formulation has also been approved by FDA for treatment of pyrexia associated with bovine respiratory disease and the control of pain associated with foot rot in beef cattle. FDA-approved uses in swine include intramuscular (IM) injection at the dose of 2.2 mg/kg body weight as a single injection for the control of pyrexia associated with swine respiratory disease (Table S1, Supplementary Materials) (FDA 1998, 2005).

Although extralabel drug use (ELDU) of flunixin meglumine is legal under the Animal Medicinal Drug Use Clarification Act (AMDUCA), it was still one of the top five violative drugs identified by USDA National Residue Program ("Red Book") from 2010 to 2016 (USDA 2018). The majority of violative residues have been attributed to non-compliant or extralabel drug use (Kissell et al. 2012; KuKanich et al. 2005). One frequent example of this is IM administration of flunixin meglumine to cattle which can cause tissue damage and inflammation, resulting in delayed or incomplete absorption, leading to violative drug residues (Sidhu et al. 2017).

Since flunixin meglumine is an important drug in food-producing animal, several different pharmacokinetic models have been developed in order to help predict WDIs (Leavens et al. 2014; Lin et al. 2016b; Wu et al. 2013). New pharmacokinetic studies (Kissell et al. 2016; Kleinhenz et al. 2016) have been carried out after the development of these pharmacokinetic models, necessitating that a new and more comprehensive PBPK model of flunixin for both cattle and swine be developed. Therefore, the objective of this study was to create a user-friendly interactive physiologically based pharmacokinetic (iPBPK) modeling framework that allows risk assessors, FARAD responders and other users to develop and apply PBPK models to predict drug WDIs in food animals. To illustrate the procedure of developing and applying a PBPK model using this framework, we chose to develop a population-based PBPK model for flunixin in cattle and swine as a case study. This iPBPK framework represents our first step of converting PBPK model codes into web-based interfaces and a significant advancement in the application of PBPK modeling in the field of toxicology. This framework still has some limitations that require further improvements, but it can be applied to develop new models for other drugs and environmental chemicals in other species, as well as to translate published models to user-friendly interfaces. The developed model itself can help to predict a WDI after extralabel use in cattle and swine, and can also be extrapolated to other food animal production classes or exposure routes.

Materials and methods

Workflow for the interactive physiologically based pharmacokinetic (iPBPK) model development

The workflow of the iPBPK framework from the collection of pharmacokinetic data to the development of the iPBPK interface is depicted in Fig. 1a. In brief, the first step of developing a PBPK model is to collect pharmacokinetic data for model calibration and evaluation. Pharmacokinetic data for drugs in food-producing animals can be acquired from PubMed, FARAD, or other biomedical databases. The present study collected relevant pharmacokinetic data from FARAD, because FARAD represents the largest and most comprehensive pharmacokinetic database for drugs in animals. With adequate pharmacokinetic data, a mechanistic PBPK model could be established and validated based on the method recently published (Li et al. 2017; Lin et al. 2016a). The model

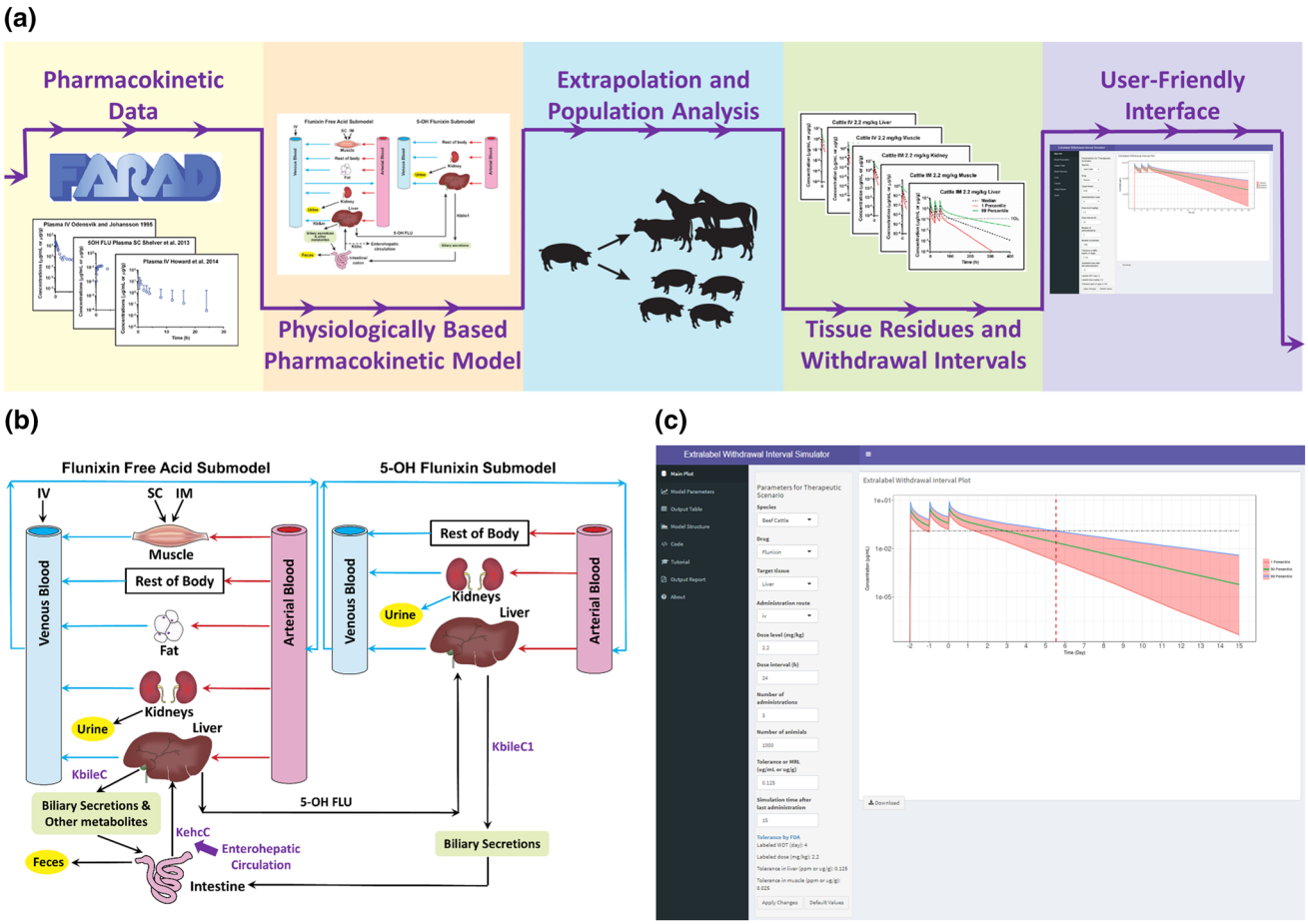

Fig. 1 Workflow for the interactive physiologically based pharmacokinetic (iPBPK) model development. **a** The diagram of the workflow for the current project. Pharmacokinetic data of food-producing animals were collected from the FARAD databank. Then, a mechanistic-based PBPK model was established and validated with these pharmacokinetic data. Based on the PBPK model, the population or probabilistic analysis with Monte Carlo method was applied to predict tissue residues and withdrawal intervals for the drug. Finally, the PBPK model was converted to a web-based user-friendly iPBPK interface. **b** Model structure of the PBPK model for flunixin in swine and cattle. The model contains both flunixin free acid and 5-OH flunixin submodels. The IV, IM and SC administrations are involved in the model. The enterohepatic circulation of flunixin is included in the model. The blood compartment was simulated as a mixed blood compartment (Lin et al. 2015). **c** A screenshot of the interactive physiologically based pharmacokinetic (iPBPK) interface for flunixin

structure of current model is shown in Fig. 1b. Next, the PBPK model in one species can be extrapolated to another species of interest and various statistical methods (e.g., Monte Carlo simulation) can be incorporated into the model to predict WDIs. PBPK models are typically developed using general programming software such as Berkeley Madonna and R language (Lin et al. 2017). The present model was developed and calibrated in Berkeley Madonna, and then converted into R language. In order for the model to be user-friendly, the final step is to convert the regular PBPK model into an iPBPK interface using the Shiny package in R. The screenshot of the iPBPK interface for flunixin is shown in Fig. 1c.

Data source for model establishment

All pharmacokinetic data used in the model calibration and evaluation were collected from FARAD Comparative Pharmacokinetic Database "BibKinFinder". Pharmacokinetic data in swine and beef cattle after IV, IM or SC administration of flunixin meglumine were acquired. The pharmacokinetic data in dairy cows were excluded due to the additional elimination route of flunixin through milk secretion, which is different from the elimination routes in swine and beef cattle. A brief description and key information of selected pharmacokinetic studies are summarized in Table 1. The graphic pharmacokinetic data were digitized from these

Table 1 The summary of pharmacokinetic studies used for the model calibration and evaluation

Use	Routes	Dose (mg/kg)	Repeat dose	Species	Sex	*n*	BW (kg)	Matrix	Compounds	Assay	References
Calibration	IV	2.2	3 days	Cattle	NA	3	82–127	L, M, K, F	FLU	Radioactive	FDA (1998)
Calibration	IV, SC	2.2	Single	Cattle	Male	8	288	P	FLU, 5OH FLU	LCMS	Shelver et al. (2013)
Calibration	IV, IM	2.2	Single	Cattle	Female	1	462	P	FLU	HPLC	Odensvik and Johansson (1995)
Calibration	IV	3	Single	Swine	Both	8–20	40.15	P	FLU, 5OH FLU	LCMS	Howard et al. (2014)
Calibration	IV, IM	2.2	Single	Swine	Female	6	121–168	P	FLU	LCMS	Pairis-Garcia et al. (2013)
Calibration	IM	2.2	3 days	Swine	Both	4	33–47	L, M, K, F	FLU	Radioactive	FDA (2005)
Evaluation	IV	2.2	3 days	Cattle	NA	4	53.3	P, L, M, K	FLU, 5OH FLU	LCMS	Kissell et al. (2016)
Evaluation	IV	2.2	4 days	Cattle	Female	6	410–570	P	FLU, 5OH FLU	HPLC	Jaroszewski et al. (2008)
Evaluation	IV	2.2	Single	Cattle	Male	8	60.2	P	FLU	LCMS	Kleinhenz et al. (2016)
Evaluation	IV	2.2	Single	Cattle	Female	6	407–562	P	FLU	HPLC	Odensvik (1995)
Evaluation	IV, IM	2.2, 1.1	Single	Swine	NA	7, 12	36–40	P	FLU	HPLC	Yu et al. (2007)
Evaluation	IV	2	Single	Swine	NA	5	18.6–26.5	P	FLU	LCMS	Buur et al. (2006a)
Evaluation	IM	2.4	3 days	Swine	NA	4	NA	L, M, K	FLU	Radioactive	EMEA (1999)

All data used for model calibration and evaluation were from healthy animals. The abbreviations for routes: *IV* intravascular injection, *IM* intramuscular injection, *SC* subcutaneous administration. The abbreviations for matrices: *P* plasma, *L* liver, *M* muscle, *K* kidneys, *F* fat. The abbreviations for assays: *LCMS* liquid chromatography mass spectrometry, *HPLC* high performance liquid chromatography. *NA* not available or not applicable. Only concentration data above limits of quantification in selected studies were used for model calibration or evaluation

pharmacokinetic studies using WebPlotDigitizer (version 4.1, https://automeris.io/WebPlotDigitizer/). All raw data are provided in the Supplementary Materials.

PBPK model structure

The present PBPK model for flunixin in swine and cattle was designed based on previous PBPK models for flunixin (Leavens et al. 2014; Lin et al. 2016b). The model structure is shown in Fig. 1b. Submodels for flunixin, the parent compound, and 5-OH flunixin, its major metabolite, were included in the model. The metabolite submodel was included so that this model structure can be readily extrapolated to dairy cows for which the marker residue in milk is 5-OH flunixin. The enterohepatic circulation of flunixin was also considered in the model based on published studies (Horii et al. 2004; Konigsson et al. 2003; Malik et al. 2016). The parent drug submodel consisted of six compartments corresponding to different tissues in the body, including liver, kidneys, muscle, fat, and the rest of body connected by the circulating blood system, and the metabolite submodel included blood, liver, kidney and the rest of body compartments. For food safety assessment purposes, all the major edible tissues, including liver, kidney, muscle and fat were included for the parent drug submodel. Each compartment was defined with a tissue weight and tissue blood flow rate calculated based on recently reported values (Li et al. 2017). The compartments for urine and feces were included as virtual excretory compartments without volume changes. The flow-limited model, which performed well for previous PBPK models of flunixin and other veterinary drugs (Lin et al. 2016a), was applied in the current model.

Model parameterization and calibration

The physiological parameters related to beef cattle and swine were obtained from previous experimental studies and published PBPK models (Buur et al. 2005; Leavens et al. 2014; Li et al. 2017; Lin et al. 2016b). The details for model parameterization and calibration are included in the Supplementary Materials. 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.

Model evaluation and sensitivity analysis

The evaluation of the PBPK model was performed by comparing model simulations with experimental pharmacokinetic data not used in the model calibration (Table 1). If the simulation results matched the reported pharmacokinetic profiles and were generally within the range of twofold of the measured values, the model was considered reasonable and validated on the basis of World Health Organization

Table 2 Parameter values used in the PBPK model for flunixin in beef cattle and swine

Parameter	Abbreviation	Beef Cattle	Swine	References
Cardiac output (L/h/kg)	QCC	5.97	8.543	Li et al. (2017)
Tissue volume (fraction of body weight, unitless)				
Arterial blood	VartC	0.010	0.016	Li et al. (2017)
Venous blood	VvenC	0.030	0.044	Li et al. (2017)
Liver	VLC	0.014	0.023	Li et al. (2017)
Kidney	VKC	0.0025	0.0045	Li et al. (2017)
Muscle	VMC	0.270	0.355	Li et al. (2017)
Fat	VFC	0.150	0.235	Li et al. (2017)
Rest of body	VrestC	0.5235	0.3225	Total adds to 1
Rest of body for 5OH flunixin	VrestC1	0.9435	0.9125	Total adds to 1
Blood flow (fraction of cardiac output, unitless)				
Liver	QLC	0.405	0.273	Li et al. (2017)
Kidney	QKC	0.09	0.116	Li et al. (2017)
Muscle	QMC	0.18	0.293	Li et al. (2017)
Fat	QFC	0.08	0.128	Li et al. (2017)
Rest of body	QrestC	0.245	0.19	Total adds to 1
Rest of body for 5OH flunixin	QrestC1	0.505	0.611	Total adds to 1
Absorption rate constant (/h)				
Absorption rate constant of IM administration (/h)	Kim	0.5	1	Model fitting
Absorption rate constant of SC administration (/h)	Ksc	0.4	0.4	Model fitting
Molecular weight for flunixin	MW	296.24	296.24	PubChem
Molecular weight for 5OH flunixin	MW1	312.24	312.24	PubChem
Tissue:plasma partition coefficient for the parent drug (unitless)				
Liver	PL	10.52	10.52	Model fitting
Kidney	PK	4	4	Model fitting
Muscle	PM	0.5	0.5	Model fitting
Fat	PF	0.6	0.6	Model fitting
Rest of body	Prest	8	8	Model fitting
Tissue:plasma partition coefficient for 5OH flunixin (unitless)				
Liver	PL1	9.26	9.26	Model fitting
Kidney	PK1	4	4	Model fitting
Rest of body	Prest1	5	5	Model fitting
Hepatic metabolic rate constant (5OH flunixin) (/h/kg)	KmetC	0.2	0.2	Model fitting
Rate constant for the regeneration of flunixin free acid from metabolites and enterohepatic circulation (/h/kg)	KehcC	0.05	0.15	Model fitting
Percentage of plasma protein binding for FLU (unitless)	PB	0.95	0.95	Model fitting
Percentage of plasma protein binding for 5OH FLU (unitless)	PB1	0.99	0.99	Model fitting
Biliary elimination rate for flunixin (L/h/kg)	KbileC	0.5	0.1	Model fitting
Biliary elimination rate for 5OH flunixin (L/h/kg)	KbileC1	0.1	0.1	Model fitting
Urinary elimination rate constant for FLU (L/h/kg)	KurineC	0.1	0.1	Model fitting
Urinary elimination rate constant for 5-OH FLU (L/h/kg)	KurineC1	0.2	0.1	Model fitting
Intestinal transit rate constant (/h)	Kint	0.4	0.4	Zeng et al. (2017)
Fecal elimination rate constant (/h)	Kfeces	0.5	0.5	Model fitting

For parameters estimated through model fitting, please refer to the "Materials and methods" for further information on which datasets were used to estimate values for these parameters

PBPK modeling guidelines (WHO 2010). Considering that calibration data sets and evaluation data sets are obtained under different conditions, some level of discordance is to be expected (WHO 2010). The goodness of fit between log-transformed values of observed and predicted drug concentrations were further analyzed with linear regression

analyses and the determination coefficient (R^2) was calculated for both calibration and evaluation results. The R^2 is an indicator for the overall model simulation performance, and a model simulation with a R^2 value equal to or higher than 0.75 is typically considered acceptable (Li et al. 2018). The goodness of fit was also evaluated with the mean absolute percentage error (MAPE) value. The calculation of the MAPE values was carried out based on previously reported methods, and MAPE values lower than 50% were considered as an acceptable prediction (Cheng et al. 2016; Lin et al. 2017).

The sensitive parameters of the current PBPK model were determined using the sensitivity analysis. A local sensitivity analysis was performed for a discrete time point of 24 h to determine which parameters were most influential on the 24-h area under the curve (AUC) of plasma, liver, kidney concentrations of flunixin, and the 24-h AUC of plasma concentrations of 5-OH flunixin. Briefly, each parameter value was increased by 1% and the corresponding 24-h AUC of flunixin or 5-OH flunixin concentrations were simulated. Normalized sensitivity coefficient (NSC) was calculated using equations reported previously (Yoon et al. 2009; Lin et al. 2011).

Establishment of the population PBPK model

Once the PBPK model was validated, population-based PBPK model simulation of flunixin was conducted using the Monte Carlo method to obtain numerical results based on repeated random sampling of parameter values from specified distribution of each parameter. This method has been used in the applications of PBPK modeling to estimate drug tissue residues and WDIs in the area of food safety (Yang et al. 2012; Zeng et al. 2017). In the current PBPK model for flunixin, Monte Carlo simulations were applied to estimate the effects of parameter uncertainties and the intra-species variability of beef cattle and swine on flunixin concentrations in plasma and tissues. For these simulations, all parameters for cattle and swine models distributed randomly around the mean values are specified in Table S2 and Table S3, respectively. Coefficients of variance (CV) of some physiological parameters (i.e., the body weight, cardiac output, and tissue volume fractions of liver and kidneys, and the fractions of blood flows in liver for cattle in Table S2, as well as the body weight, cardiac output, and tissue volume fractions of liver, kidneys, muscle and fat and the fractions of blood flows in kidneys and muscle for swine in Table S3) were calculated based on the experimental data. For other physiological or chemical-specific parameters with no experimental data, their CVs were assigned as 20% for tissue to plasma partition coefficients and 30% for other physiological parameters, absorption, protein binding, and elimination rate constants based on the default assumptions used in other PBPK models (Clewell and Clewell 2008; Henri et al. 2017; Yang et al. 2015, 2016). Log-normal distributions of model parameters were assumed for all chemical-specific parameters such as partition coefficients, absorption rate constants, elimination rate constants, the enterohepatic circulation rate constant, etc. Physiological parameters, including body weights, cardiac outputs, and fractions of blood flows and tissue volumes were assumed to be normally distributed as reported in previous models (Li et al. 2017; Tan et al. 2006; Yang et al. 2015).

The detail of Monte Carlo simulation in Berkeley Madonna was introduced in a previous PBPK model for penicillin G in swine and cattle (Li et al. 2017) and the Monte Carlo codes of the current model in Berkeley Madonna are available in Supplementary Materials. Briefly, model parameters were randomly assigned around the mean values used or estimated in model calibration by their probabilistic distributions. The values of all physiological and chemical-specific parameters were randomly selected based on their distributions during each Monte Carlo simulation. The sum of tissue volume fractions and the sum of blood flow fractions should both be equal to one (i.e., 100%) in the PBPK model. Since the parameter values of tissue volume fractions and blood flow fractions were randomly selected in the Monte Carlo analysis, their sum may not be equal to one anymore. Therefore, the sum of tissue volume fractions and the sum of blood flow fractions after Monte Carlo simulation were adjusted/scaled to be equal to one (i.e., 100%) to ensure the plausibility and mass balance for the PBPK model (Covington et al. 2007). The Monte Carlo simulations of current PBPK models for flunixin were set up as batch runs for 1000 iterations per Monte Carlo simulation in Berkeley Madonna.

Application of the population PBPK model to predict withdrawal intervals

The population PBPK model can be used to predict the distributions and the variability of plasma and tissue flunixin concentrations after label or extralabel administration in a large and diverse population of swine and beef cattle. For cattle, IM injection is a common extralabel use. Therefore, IV and IM administrations at the IV label dosing regimen (2.2 mg/kg daily for 3 days) were simulated as representative label dose and extralabel dose, respectively, in beef cattle to predict WDIs. For swine, since there were no pharmacokinetic data available for SC injections, only the label dose and route with single IM injection and the three repeated IM injections at 24-h intervals were simulated using the population PBPK model. Each Monte Carlo simulation was run with 1000 iterations. The median value, 1st and 99th percentiles of the simulated plasma and tissue concentrations of flunixin were calculated and plotted. The predicted WDIs in edible tissues were determined as the time when

the predicted flunixin concentration in each target tissue fell below the tolerance of the corresponding tissue for the 99th percentile of the simulated animal population. The tolerance for flunixin in edible tissues of cattle is 0.125 μg/g for liver and 0.025 μg/g for muscle (FDA 1998) (Table S1). The tolerance for flunixin in pigs is 0.030 μg/g for liver tissue and 0.025 μg/g for muscle (FDA 2005). Due to the fact that the tolerance of flunixin for kidneys, fat and plasma is not defined, the tolerance for liver (0.125 μg/g for cattle, 0.030 μg/g for swine) was used for kidneys, fat and plasma.

Results from each Monte Carlo simulation can be used to calculate one WDI value. However, the calculated WDI based on one Monte Carlo simulation does not have 95% confidence interval, which is different from the regulatory definition of withdrawal period that has a 95% confidence interval (FDA 2012). To calculate the 95% confidence interval of the estimated WDI, the population PBPK model in Berkeley Madonna was converted into R program, and then 1000 Monte Carlo simulations with 1000 iterations per Monte Carlo simulation were performed. WDIs for each of these 1000 Monte Carlo simulations were calculated based on the 99 percentile of the simulated 1000 animals. The central tendencies and 95% confidence intervals of these 1000 WDIs were calculated for label and extralabel therapeutic regimens of flunixin in cattle and swine. In addition, we tested whether the calculated WDI based on a small sample size of 25 animals is representative of the calculated WDI based on a large population of 1000 animals. To achieve this, we calculated the WDI based on 25 samples bootstrapped from 1000 simulated animals in a Monte Carlo simulation, and we repeated this calculation 1000 times. And then, we compared the calculated WDIs based on small sample size of 25 animals to the WDI based on 1000 animals. The therapeutic scenario of cattle treated with 3 repeated IV injections of flunixin (2.2 mg/kg) was used for this simulation.

Translation of the PBPK model into a user-friendly iPBPK interface (also called extralabel withdrawal interval simulator)

Firstly, the PBPK model code in Berkeley Madonna format was converted into R using a published method for the PBPK model code conversion (Lin et al. 2017). Two different ODE solver packages, "deSolve" and "mrgsolve" (Baron et al. 2018), were evaluated to solve the differential equations in the R code. The simulation speed using the "mrgsolve" package was much faster than using the "deSolve" package (results described below), so 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. A screenshot of the design of the iPBPK interface is shown in Fig. 1c. Please refer to Supplementary Materials for more details about this interface. An example output report and the detailed tutorial are also provided in the Supplementary Materials.

Results

Model calibration

The PBPK models for flunixin in cattle and swine were used to simulate flunixin and 5-OH flunixin concentrations in plasma and edible tissues after different therapeutic regimens used in the previous pharmacokinetic studies. Model predictions were compared with observed concentrations in beef cattle exposed to flunixin meglumine through IV, IM or SC injections and in swine exposed through IV and IM administrations (representative results are shown in Fig. 2). Overall, the model well fitted the kinetic profiles of flunixin in plasma and edible tissues in cattle and swine. In particular, the model predicted the flunixin residues in the plasma and edible tissues accurately at the terminal time points, which are most important for residue monitoring and the determination of the time when concentrations of residues in the edible tissues fall at or below tolerance. The model only slightly under predicted the last time point for flunixin concentrations in liver (Fig. 2i) in swine. In addition, as shown on Fig. 2f, h, the model simulations for plasma concentrations of 5-OH flunixin corresponded to the observed data well. Some additional calibration results are shown in Supplementary Materials Fig. S1 and Fig. S2.

Model evaluation

The calibrated PBPK models for flunixin in swine and cattle were evaluated with pharmacokinetic data not applied for the model calibration. The model evaluation results for beef cattle and swine exposed to flunixin meglumin through IV or IM administrations are shown on Fig. 3. From these results, the model accurately predicted the observed independent pharmacokinetic data for model evaluation. The simulated results properly captured the pharmacokinetic characteristics for both single and repeated dose administrations of these pharmacokinetic data. The model performance for 5-OH flunixin was also evaluated by the comparison with plasma and liver concentrations of 5-OH flunixin in beef cattle (Fig. 3g, h). The goodness of fit for model calibration and evaluation results of cattle and swine were analyzed using the linear regression and the MAPE value. The overall R^2 values for these linear regression analyses were higher than 0.90, which indicates that the current PBPK model well simulates the pharmacokinetic data of flunixin both in cattle and swine (Fig. S3). All results of MAPE analyses for the calibration and evaluation results from the current model were lower than 50% (Fig. S4). Results of both analyses suggest that the

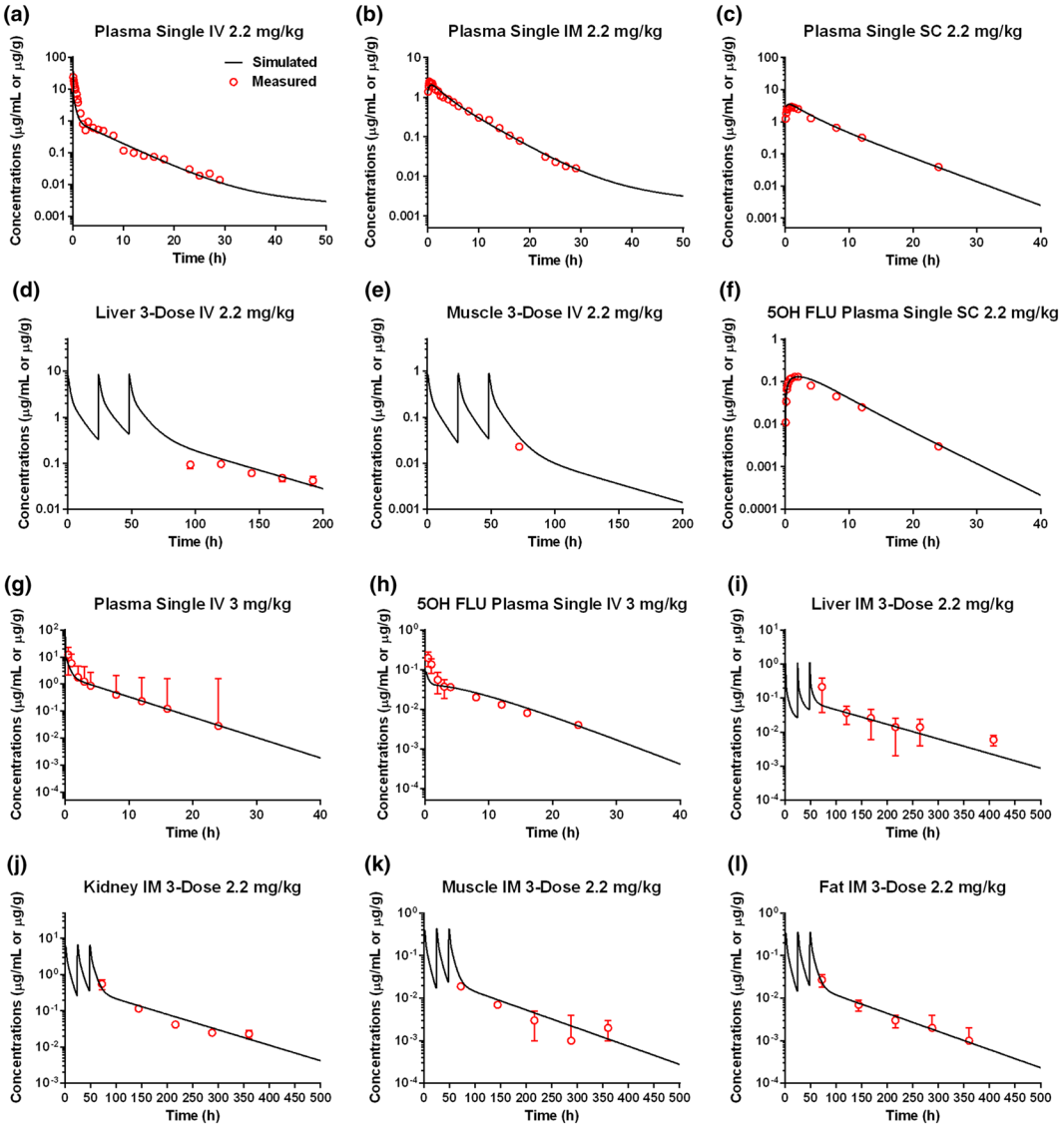

Fig. 2 Model calibration results of the PBPK model for flunixin in cattle (**a**–**f**) and swine (**g**–**l**). Comparisons of model predictions (solid lines) and observed data (red circles) are shown for concentrations of flunixin in the plasma, liver and muscle from beef cattle exposed to flunixin via single IV injection (**a**; 2.2 mg/kg; Odensvik and Johansson 1995), single IM injection (**b**; 2.2 mg/kg; Odensvik and Johansson 1995), single SC administration (**c**; 2.2 mg/kg; Shelver et al. 2013), 3 repeated IV administrations (**d** and **e**; 2.2 mg/kg; FDA 1998), and in the plasma, liver, kidneys, muscle and fat from swine exposed to flunixin via single IV injection (**g**; 3 mg/kg; Howard et al. 2014), and 3 repeated IM injection (**i**–**l**; 2.2 mg/kg; FDA 2005). Model predictions are shown for concentrations of 5-OH flunixin in plasma compared with observed data from single SC administration (**f**; 2.2 mg/kg; Shelver et al. 2013) and from single IV injection (**h**; 3 mg/kg; Howard et al. 2014)

model adequately simulates the observed data sets used for model calibration and evaluation.

Application of the population PBPK model

The population analysis for the current PBPK model of flunixin was performed using the Monte Carlo sampling technique. All physiological and chemical-specific parameters used in the PBPK model were involved in the population analysis. The tolerances of flunixin are only available in liver and muscle for cattle and swine. Based on the simulation results after label or extralabel use of flunixin meglumine in cattle and swine, the concentrations of flunixin in the liver were depleted slower than in muscle (Fig. 4a, e, i, m). Therefore, the liver was chosen as the tissue to determine the WDIs for label or extralabel use of flunixin meglumine in beef cattle and swine. The Monte Carlo simulations showed that the WDIs after three repeated IV injections and three repeated IM administrations with label dose 2.2 mg/kg in beef cattle were 5.76

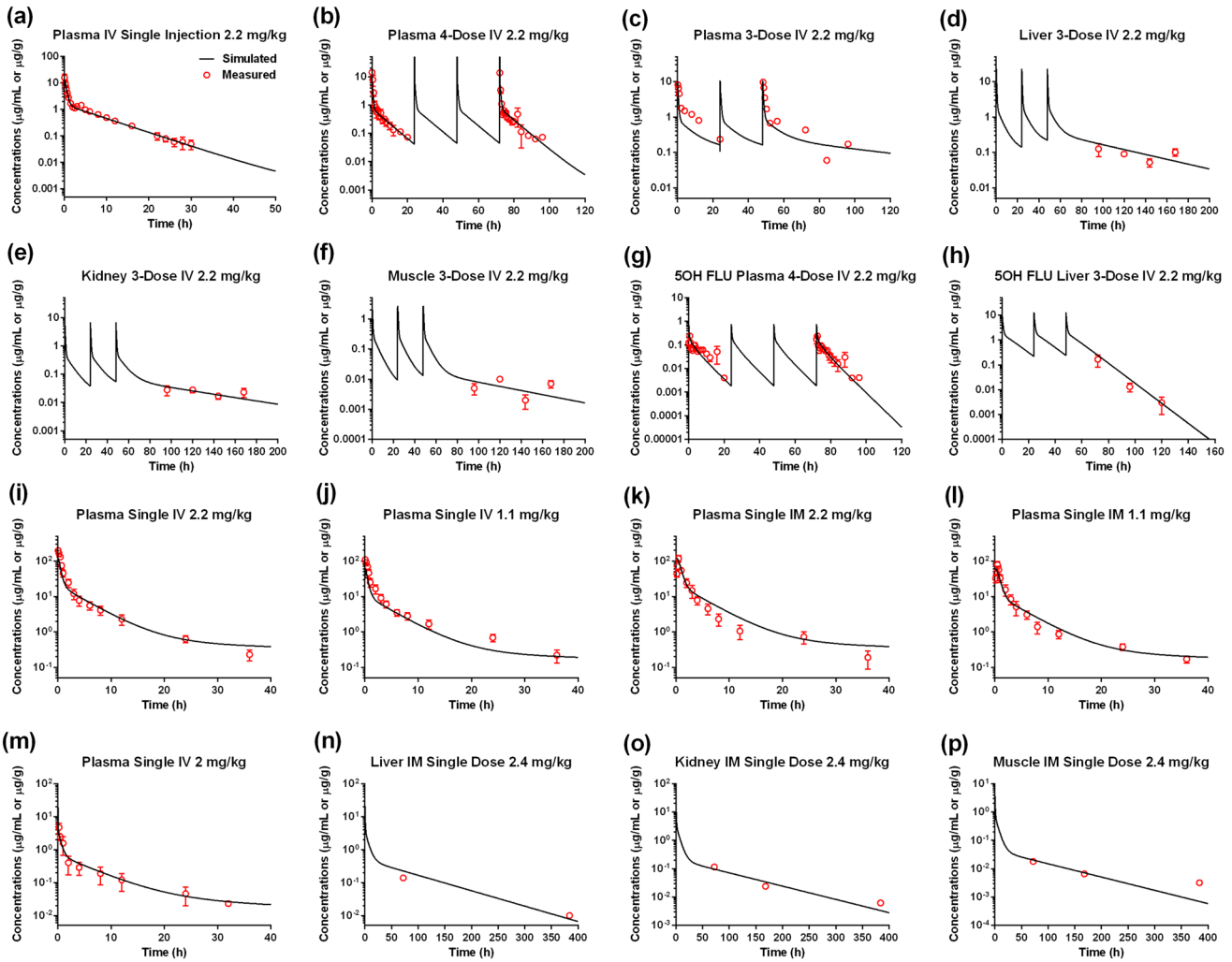

Fig. 3 Model evaluation results of the PBPK model for flunixin in beef cattle (**a**–**h**) and swine (**i**–**p**). Comparisons of model predictions (solid lines) and observed data (red circles) are shown for concentrations of flunixin in the plasma, liver, kidneys and muscle from beef cattle exposed to flunixin via single IV injection (**a**; 2.2 mg/kg; Odensvik 1995), 4 repeated IV injections (**b**; 2.2 mg/kg; Jaroszewski et al. 2008), 3 repeated IV administrations (**c**–**f**; 2.2 mg/kg; Kissell et al. 2016), and from swine exposed to flunixin via single IV injection (**i**, **j** and **m**; 2.2 mg/kg, 1.1 mg/kg and 2 mg/kg; Yu et al. 2007 and Buur et al. 2006a), single IM administration (**k**, **l**, **n**–**p**; 2.2 mg/kg, 1.1 mg/kg and 2.4 mg/kg; Yu et al. 2007 and EMEA 1999). Model predictions are shown for concentrations of 5-OH flunixin in plasma compared with observed data from 4 repeated IV injections (**g**; 2.2 mg/kg; Jaroszewski et al. 2008) and from 3 repeated IV administrations (**h**; 2.2 mg/kg; Kissell et al. 2016)

and 6.13 days, respectively (Fig. 4a, e). The predicted WDIs after single dose and three doses via IM injections with label dose 2.2 mg/kg in swine were 12.06 days and 15.45 days, respectively (Fig. 4i, m). The exact model-predicted WDIs are reported in this manuscript, but in practice, if the predicted WDI is a fraction of a day, the recommended WDI should be rounded up to the next whole day. The label withdrawal periods were obtained from the Veterinarian's Guide to Residue Avoidance Management (VetGRAM) of FARAD (Riviere et al. 2017). The labeled withdrawal period via IV injection is 4 days for edible tissues in beef cattle, and the labeled withdrawal period through IM administrations is 12 days for edible tissues in swine.

The predicted WDIs and their 95% confidence intervals are summarized in Table S4. With 1000 simulations of 1000 samples each, the 95% confidence intervals in general cover a small range. Especially, when the WDI was rounded up to the next whole day, the upper and lower bounds of the 95% confidence intervals were the same as the mean value of the WDI. Our result is consistent with the result in a previously published PBPK model for sulfamethazine in swine (Buur et al. 2006b). This is not

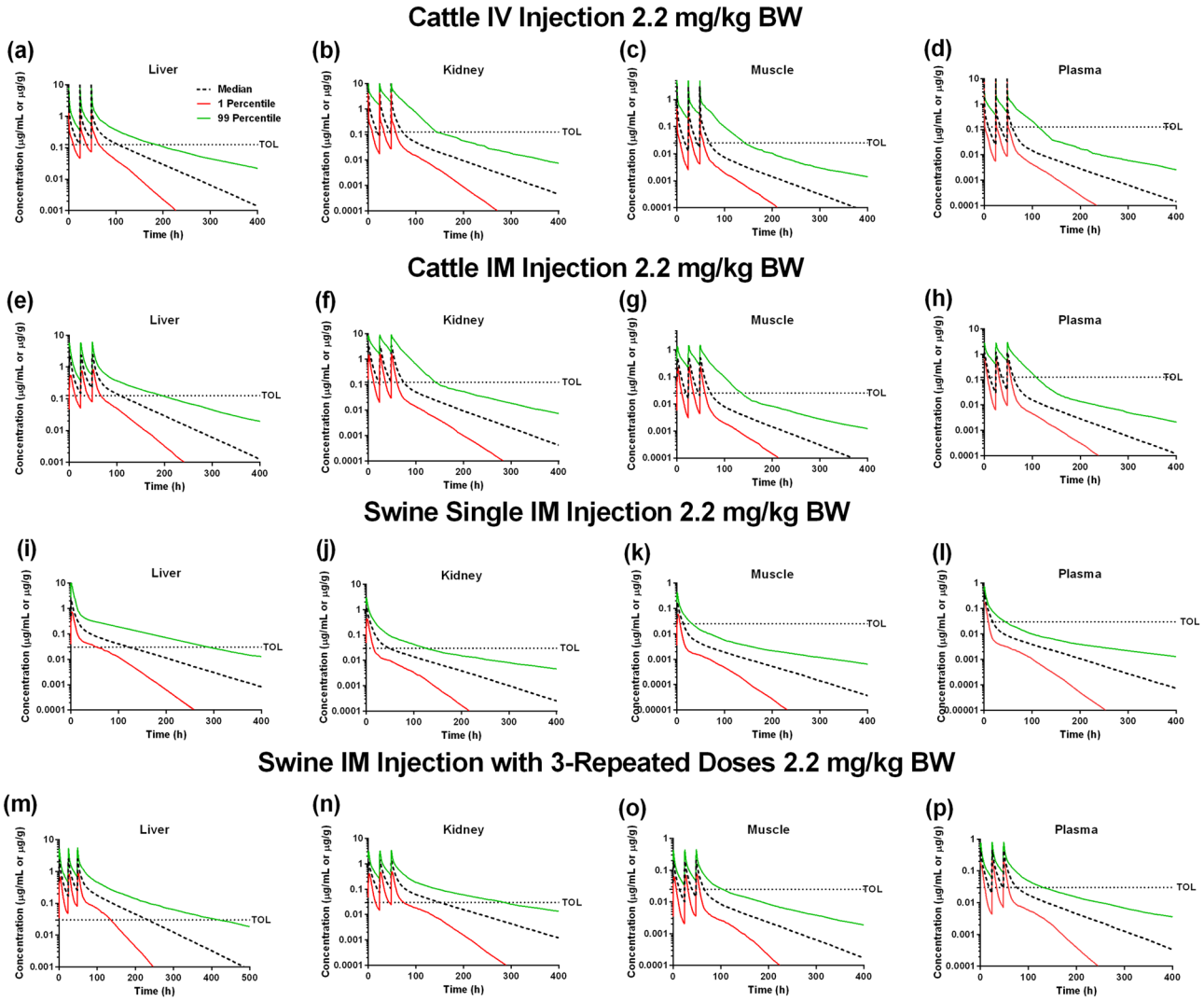

Fig. 4 Results of the population PBPK model for flunixin in beef cattle and swine. The label dose (2.2 mg/kg) of 3 repeated IV injections with 24-h intervals and the extralabel use of flunixin of 3 repeated IM injections with 24-h intervals were simulated for beef cattle, and the single IM injection of 2.2 mg/kg body weight or 3 repeated IM injections of 2.2 mg/kg body weight with 24-h intervals were simulated for swine. Each of the simulations was run for 1000 iterations. The median, 1st and 99th percentiles of simulated results were plotted. The tolerance is shown on each of panels using the dotted line. The extended withdrawal intervals were determined when the tissue concentrations of flunixin fall below tolerance for the 99th percentile of the population. Tolerance of flunixin for liver is 0.125 μg/g, and for muscle is 0.025 μg/g in cattle. The tolerance of flunixin in swine is 0.030 μg/g for liver, and 0.025 μg/g for muscle. As no defined tolerance is available for kidney and plasma, the tolerance for liver (0.125 μg/g in cattle and 0.030 μg/g in swine) was used for kidney and plasma

unexpected as the WDI was calculated based on the data from 1000 animals, which is representative of the result for the entire population; thus, the WID result is relatively stable and reliable. On the other hand, the WDIs calculated based on the randomly selected 25 samples may have values smaller or larger than the predicted WDI based on the 1000 samples (Fig. S5). Some extreme WDI values based on the data from 25 samples could be up to 2 days different from the predicted WDI based on 1000 animals.

User-friendly interface establishment and improvement

The code for the PBPK model for flunixin in cattle and swine was translated from Berkeley Madonna 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 differences of simulation time were considerable (Table S5). For the 1000-iteration simulation, code in

"deSolve" would take around 10 h, and "mrgsolve" code only consumed around 6 s. 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 in real time.

Discussion

In the present study, a comprehensive PBPK modeling framework from pharmacokinetic data collection to iPBPK interface development was constructed. A new iPBPK model for flunixin meglumine through IV, IM and SC administrations in beef cattle and through IV and IM administrations in swine was established to illustrate the detailed procedure of this framework. The flunixin model well predicted tissue residues of flunixin and 5-OH flunixin in plasma and/or edible tissues for both cattle and swine. Population simulations with Monte Carlo method were incorporated into the framework so that each iPBPK model can be applied to predict times needed for drug concentrations to fall below established tolerances following extralabel use. Thus, the new PBPK model of flunixin can be used to predict the tissue residues and estimate extralabel WDIs in beef cattle and swine. This iPBPK modeling framework can be extrapolated to other food animal species or other specific use classes. This new iPBPK modeling framework represents a significant advancement in the field of toxicology and it greatly improves the use of PBPK modeling approach in WDI estimation. This framework still has some limitations, but it lays a foundation to facilitate moving drug withdrawal time estimation from empirical methods to mechanistic PBPK approaches, which are now user-friendly and in real time.

PBPK modeling is a valuable tool for regulatory science in many different areas such as risk assessment (EPA 2006), exposure science (Cohen Hubal et al. 2018), and new drug development (FDA 2018). However, some existing issues limit the application of PBPK modeling by regulatory agencies. The shortage of individuals as modelers with sufficient trainings in modeling and simulation restricted the development and application of PBPK modeling in the area of risk assessment (Tan et al. 2018). One of the advantages of iPBPK platform is providing a user-friendly interface, which helps non-modelers to use the PBPK models. The commercial software, such as GastroPlus and Simcyp, are well accepted by FDA to waive the drug–drug interaction studies and bioequivalence studies (Shebley et al. 2018). The iPBPK platform is based on R, and is more flexible and more transparent compared to commercial software. R is a free software environment to use for programming, which makes it potential to be used across agencies and organizations. It is also one of the top three mostly used softwares for PBPK modeling (Paini et al. 2017), and PBPK model coding in other programming languages can be translated to R (Lin et al. 2017). Furthermore, the iPBPK interface is a web-based graphical user interface (GUI). The web-based GUI is not even requiring the installation of R, and can be used directly through the webpage or a server (Wojciechowski et al. 2015). It is similar as a standalone software but with the flexibility to be updated and revised according to different requirements. Especially with the development of R packages to do the statistical analysis for PBPK modeling (Hsieh et al. 2018; Carpenter et al. 2017) and to help in the analysis of high-throughput data (Pearce et al. 2017), all these functions achieved through these R packages can be incorporated into the iPBPK platform for further improvements. The iPBPK platform concept has the potential to fill the gap for the application of PBPK modeling by regulatory agencies. In summary, we anticipate that the concept of the iPBPK platform will be the direction of future PBPK models, which will facilitate the use of PBPK modeling by non-modelers and regulatory agencies. The present work represents a proof-of-concept and our first step towards this direction.

The current PBPK model was designed for flunixin in both cattle and swine with the same model structure. Since the metabolite of flunixin, 5-OH flunixin, is the marker residue of flunixin in milk and the present model includes the metabolite submodel for 5-OH flunixin, this model can be extrapolated to dairy cows to predict 5-OH flunixin concentrations in milk following label and extralabel administrations. In addition, the enterohepatic circulation of flunixin was considered in the current model. As flunixin was reported to undergo enterohepatic circulation in different animal species such as cats and dairy goats (Horii et al. 2004; Konigsson et al. 2003; Malik et al. 2016), this process was considered in the current model and the model simulation was improved. Recently, population mixed-effects pharmacokinetic models of flunixin were developed to predict tissue residues and WDIs in cattle (Wu et al. 2013). Population pharmacokinetic modeling, which combines available pharmacokinetic data to make a population inference, is a useful approach in the area of pharmacology and toxicology (Bon et al. 2018; Li et al. 2015a; Martin-Jimenez and Riviere 1998). However, compared to PBPK models, the population pharmacokinetic model is not a physiologically based mechanistic method, which limits the extrapolation of the model beyond the inference range of experimental data (Lin et al. 2016a).

From the sensitivity analysis results, the uncertainties of a few parameters have influences on the predictions of flunixin residues. The plasma concentration and tissue residues of flunixin are less sensitive to parameter values compared to 5-OH flunixin. The partition coefficients of kidneys (PK) for flunixin, of the rest of body for 5-OH flunixin (Prest1), the plasma protein binding for both flunixin (PB) and 5-OH

flunixin (PB1), as well as the metabolic rate constant for 5-OH flunixin (KmetC1) were highly influential on the predictions of the plasma concentrations of 5-OH flunixin. The partition coefficients were estimated by fitting to the selected pharmacokinetic datasets due to lack of experimentally measured values. The plasma protein binding of flunixin was measured experimentally (Skidmore et al. 2008; Thiry et al. 2017), and our model-fitted value is close to the reported value. After an extensive literature search, limited information was available from experimentally measured PB1 for 5-OH flunixin. Since the sensitivity analysis has shown that these parameters are the most influential of the model, it would be very interesting to determine them experimentally to dissipate an important uncertainty. Additionally, global sensitivity analysis can be applied to the PBPK model to determine the influences of parameter interactions and non-linear processes on the sensitivity of model parameters (McNally et al. 2011). Global sensitivity analysis cannot be achieved in Berkeley Madonna. However, since the iPBPK platform is coded in R language, the global sensitivity analysis can be performed in R program in the future. There are different packages available in R to perform global sensitivity analysis for PBPK models, such as the packages "sensitivity" (Pujol et al. 2017) and "pksensi" (Hsieh et al. 2018).

Physiological parameters are a key component of a PBPK model. By changing physiological parameter values under different physiological conditions, PBPK models can be extrapolated to many other populations, including different age groups, different species, and diseased populations. PBPK models for pediatrics (Edginton et al. 2006) and elderly populations (Schlender et al. 2016) are widely used in the area of clinical pharmacology for dose determination (Templeton et al. 2018) and drug development (Rowland et al. 2011) for special subpopulations. PBPK models have also been applied to conduct human health risk assessment in vulnerable subpopulations, such as pregnancy women, children and elders (Clewell and Gearhart 2002; El-Masri et al. 2016). For food animals, some special production classes such as bob veal calves, heavy sows and culled dairy cows, which have different physiological conditions compared to market-age animals, can also be simulated with a PBPK model using corresponding physiological values. Disease conditions also affect the pharmacokinetic process of drugs, and some disease conditions, such as chronic kidney disease (Tan et al. 2019), heart failure (Rasool et al. 2015), and liver cirrhosis (Li et al. 2015b) have been well simulated using PBPK models in humans. PBPK models can also be used to study the ethnic differences (Zurlinden and Reisfeld 2017), gender differences (Kim et al. 2018), drug–drug interactions (Bois 2010), and lifetime exposures (Weijs et al. 2010).

In food animals, the breed differences, disease conditions and drug–drug interactions are also potential areas of applications of PBPK modeling. The current PBPK model for flunixin in swine and cattle can be extrapolated to dairy cows and dairy goats to predict the drug residues in milk, and similar extrapolation to dairy cows has been achieved by adding the mammary gland compartment to a PBPK model in beef cattle for penicillin G (Li et al. 2018). By adding the empirical (Chittenden and Riviere 2016) or mechanistic dermal administration compartments (Anissimov and Roberts 2014) to the current PBPK model, the model can be used for the transdermal administration of flunixin. The physiological parameters for humans and lab animals are well recorded and have been systematically reviewed (Brown et al. 1997). A systematic literature search and review of physiological parameters in food-producing animals is warranted and will facilitate the application of PBPK modeling tools in the field of food safety and risk assessment. In our ongoing literature search project on physiological parameters in different food animal species, we found that there are divergent values for physiological values in food animals. The variabilities for the physiological values may be due to the different experimental methods, different breeds, different ages and many other reasons. When calculating the mean, standard deviation, and range of a specific parameter, it is important to use physiological parameter values determined using the same or similar experimental methods from animals with similar ages and physiological conditions. Harmonization of the physiological databases for food animals remains to be a data gap and a challenge in the field of food safety and risk assessment.

This Monte Carlo sampling technique for population analysis has been applied for PBPK modeling in human drugs and environmental pollutants (Yang et al. 2016). This strategy was also applied to the population analysis in this PBPK model. Similar population PBPK models were recently reported (Elwell-Cuddy et al. 2018; Yang et al. 2016). For the current model, variations of all physiological and chemical-specific parameters were considered in the Monte Carlo analysis to better predict the wide range of tissue residue concentrations, and to simulate the diversity in the population of food animals. The distributions and variabilities of physiological parameters were based on previous reported values (Li et al. 2017). The default coefficients of variance were used only for parameters with no experimental data available. The present Monte Carlo analysis, considering variances of all parameters, may help to make the simulations more realistic for the diverse population of livestock being treated with flunixin meglumine.

The current population PBPK model can also be extrapolated to simulate the extralabel use scenarios of flunixin meglumine. The commonly seen extralabel administrations are IM injection in cattle and SC injection in swine. The label withdrawal period does not apply to extralabel use and violative residues may result if the withdrawal interval is

not extended. For on-label use, the model-predicted WDIs from Monte Carlo analyses for both swine and cattle based on respective tolerances were close to FDA label withdrawal periods (e.g., predicted 6 days vs. labeled 4 days in cattle; predicted 13 days vs. labeled 12 days in swine). For extralabel use of flunixin meglumine via the IM route in cattle, the WDI predicted by the current model (7 days) is one day more than the predicted WDI for the label use of flunixin meglumine via IV route. For extralabel use of flunixin meglumine with three-repeated doses through IM in swine, the WDI predicted by the current model (16 days) is 4 days more than the predicted WDI for the label use of flunixin meglumine with single IM dose. The predicted WDIs of extralabel doses from the current model are more protective to avoid violative tissue residues of flunixin in beef cattle and swine. Estimation of drug residue levels at the terminal phase using a well-validated PBPK model is important for reducing violations of illegal drug residues in edible tissues, as well as for food-producing animals destined for export to other countries with stringent residue criteria.

While the present study represents an advancement in the application of PBPK modeling in food animals, this study has several limitations. Specifically, the model was calibrated in Berkeley Madonna and there were several highly sensitive parameters that were estimated due to lack of experimentally measured values. To convert the model into a web-based interface and to perform more advanced Monte Carlo simulations, the model was later translated to R program. The parameter estimation module in Berkeley Madonna was less robust than the available parameter-fitting algorithms in R, resulting in uncertainties in the estimated parameters. Also, there were some uncertainties in the conversion of the codes from Berkeley Madonna into R due to different syntax, semantics, and different ordinary differential equation solvers between the two software programs. As a result, many new equations, conversion parameters, functions had to be created in order to make sure the simulation results in the R program are relatively comparative to the results from the originally created model in Berkeley Madonna. Therefore, it would be ideal if the model was calibrated directly from R program, which would eliminate all the uncertainties in the code conversion between software programs. This is the future direction of the continued improvement of the iPBPK framework. In this regard, our lab recently reported a PBPK model for perfluorooctane sulfonate in rodents and humans that was directly calibrated in R program and the entire R code was published in the Supplementary Materials (Chou and Lin 2019). In addition, the present iPBPK framework only contains one drug (flunixin) with two species and three administration routes (IV, IM and SC). Dermal administration of flunixin has also been approved in cattle by US FDA recently. Additional improvements are needed to extend the model to additional routes, other drugs, and other species. Overall, the present work serves as a proof-of-concept and a basis for future development in this field.

In summary, the present PBPK model of flunixin adequately simulates observed concentrations of flunixin residues in edible tissues of swine and cattle following label and extralabel routes of administration. The application of the population PBPK model via Monte Carlo simulations to estimate the WDIs for flunixin following label and extralabel use demonstrates the possibility to use PBPK modeling to provide more protective WDIs. The framework for a web-based and user-friendly iPBPK interface development provides an easy and convenient methodology to develop and apply population PBPK models to predict drug tissue residues and WDIs. This iPBPK framework can be extrapolated to other drugs and other food animal species. This study represents our first step of converting PBPK model codes into web-based interfaces to facilitate applications of PBPK modeling in food safety assessment. This iPBPK framework still has several limitations, but it represents a proof-of-concept and a significant improvement in the development and application of mechanistic and quantitative tools in the field of toxicology.

Acknowledgements The authors would like to acknowledge the help from Mal Hoover, the medical illustrator affiliated with College of Veterinary Medicine of Kansas State University, for creating Fig. 1. This work was supported by the United States Department of Agriculture (USDA) National Institute of Food and Agriculture (NIFA) for the Food Animal Residue Avoidance Databank (FARAD) Program (Award No. 2015-41480-23972, 2016-41480-25729, 2017-41480-27310, and 2018-41480-28805).

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

Anissimov YG, Roberts MS (2014) Mathematical models for topical and transdermal drug products. In: Shah VP, Maibach HI, Jenner J (eds) Topical drug bioavailability, bioequivalence and penetration, 2nd edn. Springer, New York, pp 249–298

Baron KT, Hindmarsh AC, Petzold LR, Gillespie B, Margossian C, Metrum Research Group LLC (2018) mrgsolve: simulation from ODE-based population PK/PD and systems pharmacology models. R package version 0.8.12. https://metrumrg.com/try-open-source-tools/. Accessed 14 Nov 2018

Baynes R, Riviere JE (2014) Strategies for reducing drug and chemical residues in food animals: international approaches to residue avoidance, management, and testing. Wiley, Hoboken

Bois FY (2010) Physiologically based modelling and prediction of drug interactions. Basic Clin Pharmacol Toxicol 106(3):154–161

Bon C, Toutain PL, Concordet D, Gehring R, Martin-Jimenez T, Smith J, Pelligand L, Martinez M, Whittem T, Riviere JE, Mochel JP (2018) Mathematical modeling and simulation in animal health. Part III: using nonlinear mixed-effects to characterize and quantify

variability in drug pharmacokinetics. J Vet Pharmacol Ther 41(2):171–183
Brown RP, Delp MD, Lindstedt SL, Rhomberg LR, Beliles RP (1997) Physiological parameter values for physiologically based pharmacokinetic models. Toxicol Ind Health 13(4):407–484
Buur JL, Baynes RE, Craigmill AL, Riviere JE (2005) Development of a physiologic-based pharmacokinetic model for estimating sulfamethazine concentrations in swine and application to prediction of violative residues in edible tissues. Am J Vet Res 66(10):1686–1693
Buur JL, Baynes RE, Smith GW, Riviere JE (2006a) Pharmacokinetics of flunixin meglumine in swine after intravenous dosing. J Vet Pharmacol Ther 29(5):437–440
Buur JL, Baynes RE, Smith GW, Riviere JE (2006b) Use of probabilistic modeling within a physiologically based pharmacokinetic model to predict sulfamethazine residue withdrawal times in edible tissues in swine. Antimicrob Agents Chemother 50(7):2344–2351
Carpenter B, Gelman A, Hoffman M, Lee D, Goodrich B, Betancourt M, Brubaker M, Guo J, Li P, Riddell A (2017) Stan: a probabilistic programming language. J Stat Softw 76(1):1–32
Cheng Y-H, Lin Y-J, You S-H, Yang Y-F, How C-M, Tseng Y-T, Chen W-Y, Liao C-M (2016) Assessing exposure risks for freshwater tilapia species posed by mercury and methylmercury. Ecotoxicology 25(6):1181–1193
Chittenden JT, Riviere JE (2016) Assessment of penetrant and vehicle mixture properties on transdermal permeability using a mixed effect pharmacokinetic model of ex vivo porcine skin. Biopharm Drug Dispos 37(7):387–396
Chou WC, Lin Z (2019) Bayesian evaluation of a physiologically based pharmacokinetic (PBPK) model for perfluorooctane sulfonate (PFOS) to characterize the interspecies uncertainty between mice, rats, monkeys, and humans: development and performance verification. Environ Int (**in press**)
Clewell RA, Clewell HJ (2008) Development and specification of physiologically based pharmacokinetic models for use in risk assessment. Regul Toxicol Pharmacol 50:129e143
Clewell RA, Gearhart JM (2002) Pharmacokinetics of toxic chemicals in breast milk: use of PBPK models to predict infant exposure. Environ Health Perspect 110(6):A333–A337
Cohen Hubal EA, Wetmore BA, Wambaugh JF, El-Masri H, Sobus JR, Bahadori T (2018) Advancing internal exposure and physiologically-based toxicokinetic modeling for 21st-century risk assessments. J Expo Sci Environ Epidemiol 29:11–20
Covington TR, Robinan Gentry P, Van Landingham CB, Andersen ME, Kester JE, Clewell HJ (2007) The use of Markov chain Monte Carlo uncertainty analysis to support a Public Health Goal for perchloroethylene. Regul Toxicol Pharmacol 47(1):1–18
Craigmill A (2003) A physiologically based pharmacokinetic model for oxytetracycline residues in sheep. J Vet Pharmacol Ther 26(1):55–63
Craigmill AL, Riviere JE, Webb AI (2006) Tabulation of FARAD comparative and veterinary pharmacokinetic data. Blackwell Pub, Ames
Edginton AN, Schmitt W, Willmann S (2006) Development and evaluation of a generic physiologically based pharmacokinetic model for children. Clin Pharmacokinet 45(10):1013–1034
El-Masri H, Kleinstreuer N, Hines RN, Adams L, Tal T, Isaacs K, Wetmore BA, Tan YM (2016) Integration of life-stage physiologically based pharmacokinetic models with adverse outcome pathways and environmental exposure models to screen for environmental hazards. Toxicol Sci 152(1):230–243
Elwell-Cuddy T, Li M, KuKanich B, Lin Z (2018) The construction and application of a population physiologically based pharmacokinetic model for methadone in Beagles and Greyhounds. J Vet Pharmacol Ther 41(5):670–683
EMEA (1999) Committee for veterinary medicinal products flunixin summary reports (1). http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500014324.pdf. Accessed 14 Nov 2018
EPA (2006) Approaches for the application of physiologically based pharmacokinetic (PBPK) models and supporting data in risk assessment. Final Report EPA/600/R-05/043A, Washington, DC, USA, 2006. http://ofmpub.epa.gov/eims/eimscomm.getfile?p_download_id=458188. Accessed 25 Feb 2019
FDA (1998) Freedom of information. NADA 101-479. BANAMINE (flunixin meglumine) Injectable Solution. US Food and Drug Administration, Silver Spring, MD. https://wayback.archive-it.org/7993/20170406083138/https://www.fda.gov/downloads/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/FOIADrugSummaries/ucm064905.pdf. Accessed 14 Nov 2018
FDA (2005) Freedom of information. NADA 101-479. BANAMINE-S (flunixin meglumine) Injectable Solution. U.S. Food and Drug Administration, Silver Spring, MD. https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/356. Accessed 14 Nov 2018
FDA (2012) Regulation of carcinogenic compounds used in food-producing animals. Code of federal regulations title 21, Parts 500.82. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=500.82. Accessed 14 Nov 2018
FDA (2018) Physiologically based pharmacokinetic analyses—format and content. Guidance for Industry. https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM531207.pdf. Accessed 25 Feb 2019
Henri J, Carrez R, Meda B, Laurentie M, Sanders P (2017) A physiologically based pharmacokinetic model for chickens exposed to feed supplemented with monensin during their lifetime. J Vet Pharmacol Ther 40(4):370–382
Horii Y, Ikenaga M, Shimoda M, Kokue E (2004) Pharmacokinetics of flunixin in the cat: enterohepatic circulation and active transport mechanism in the liver. J Vet Pharmacol Ther 27(2):65–69
Howard JT, Baynes RE, Brooks JD, Yeatts JL, Bellis B, Ashwell MS, Routh P, O'Nan AT, Maltecca C (2014) The effect of breed and sex on sulfamethazine, enrofloxacin, fenbendazole and flunixin meglumine pharmacokinetic parameters in swine. J Vet Pharmacol Ther 37(6):531–541
Hsieh NH, Reisfeld B, Bois FY, Chiu WA (2018) Applying a global sensitivity analysis workflow to improve the computational efficiencies in physiologically-based pharmacokinetic modeling. Front Pharmacol 9:588
Jaroszewski J, Jedziniak P, Markiewicz W, Grabowski T, Chrostowska M, Szprengier-Juszkiewicz T (2008) Pharmacokinetics of flunixin in mature heifers following multiple intravenous administration. Pol J Vet Sci 11(3):199–203
Kim SJ, Shin H, Lee YB, Cho HY (2018) Sex-specific risk assessment of PFHxS using a physiologically based pharmacokinetic model. Arch Toxicol 92(3):1113–1131
Kissell LW, Smith GW, Leavens TL, Baynes RE, Wu H, Riviere JE (2012) Plasma pharmacokinetics and milk residues of flunixin and 5-hydroxy flunixin following different routes of administration in dairy cattle. J Dairy Sci 95(12):7151–7157
Kissell LW, Brinson PD, Gehring R, Tell LA, Wetzlich SE, Baynes RE, Smith GW (2016) Pharmacokinetics and tissue elimination of flunixin in veal calves. Am J Vet Res 77(6):634–640
Kleinhenz MD, Van Engen NK, Gorden PJ, KuKanich B, Rajewski SM, Walsh P, Coetzee JF (2016) The pharmacokinetics of transdermal flunixin meglumine in Holstein calves. J Vet Pharmacol Ther 39(6):612–615
Konigsson K, Torneke K, Engeland IV, Odensvik K, Kindahl H (2003) Pharmacokinetics and pharmacodynamic effects of flunixin after intravenous, intramuscular and oral administration to dairy goats. Acta Vet Scand 44(3–4):153–159

KuKanich B, Gehring R, Webb AI, Craigmill AL, Riviere JE (2005) Effect of formulation and route of administration on tissue residues and withdrawal times. J Am Vet Med Assoc 227(10):1574–1577

Leavens TL, Tell LA, Kissell LW, Smith GW, Smith DJ, Wagner SA, Shelver WL, Wu H, Baynes RE, Riviere JE (2014) Development of a physiologically based pharmacokinetic model for flunixin in cattle (Bos taurus). Food Addit Contam Part A Chem Anal Control Expo Risk Assess 31(9):1506–1521

Li M, Gehring R, Lin Z, Riviere J (2015a) A framework for meta-analysis of veterinary drug pharmacokinetic data using mixed effect modeling. J Pharm Sci 104(4):1230–1239

Li R, Barton HA, Maurer TS (2015b) A mechanistic pharmacokinetic model for liver transporter substrates under liver cirrhosis conditions. CPT Pharmacomet Syst Pharmacol 4(6):338–349

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

Li M, Gehring R, Riviere JE, Lin Z (2018) Probabilistic physiologically based pharmacokinetic model for penicillin G in milk from dairy cows following intramammary or intramuscular administrations. Toxicol Sci 164(1):85–100

Lin Z, Fisher JW, Ross MK, Filipov NM (2011) A physiologically based pharmacokinetic model for atrazine and its main metabolites in the adult male C57BL/6 mouse. Toxicol Appl Pharmacol 251(1):16–31

Lin Z, Li M, Gehring R, Riviere JE (2015) Development and application of a multiroute physiologically based pharmacokinetic model for oxytetracycline in dogs and humans. J Pharm Sci 104(1):233–243

Lin Z, Gehring R, Mochel JP, Lave T, Riviere JE (2016a) Mathematical modeling and simulation in animal health—part II: principles, methods, applications, and value of physiologically based pharmacokinetic modeling in veterinary medicine and food safety assessment. J Vet Pharmacol Ther 39(5):421–438

Lin Z, Vahl CI, Riviere JE (2016b) Human food safety implications of variation in food animal drug metabolism. Sci Rep 6:27907

Lin Z, Jaberi-Douraki M, He C, Jin S, Yang RS, Fisher JW, Riviere JE (2017) Performance assessment and translation of physiologically based pharmacokinetic models from acslX to Berkeley Madonna, MATLAB, and R language: oxytetracycline and gold nanoparticles as case examples. Toxicol Sci 158(1):23–35

Malik MY, Jaiswal S, Sharma A, Shukla M, Lal J (2016) Role of enterohepatic recirculation in drug disposition: cooperation and complications. Drug Metab Rev 48(2):281–327

Martin-Jimenez T, Riviere JE (1998) Population pharmacokinetics in veterinary medicine: potential use for therapeutic drug monitoring and prediction of tissue residues. J Vet Pharmacol Ther 21(3):167–189

McNally K, Cotton R, Loizou GD (2011) A workflow for global sensitivity analysis of PBPK models. Front Pharmacol 2:31

NRC (1999a) Drug Residues and microbial contamination in food: monitoring and enforcement. National Academy Press, Washington, DC

NRC (1999b) The use of drugs in food animals. In: Council NR (ed) The use of drugs in food animals: benefits and risks. National Academy Press, Washington, DC, pp 110–141

Odensvik K (1995) Pharmacokinetics of flunixin and its effect on prostaglandin F2 alpha metabolite concentrations after oral and intravenous administration in heifers. J Vet Pharmacol Ther 18(4):254–259

Odensvik K, Johansson IM (1995) High-performance liquid chromatography method for determination of flunixin in bovine plasma and pharmacokinetics after single and repeated doses of the drug. Am J Vet Res 56(4):489–495

Paini A, Leonard JA, Kliment T, Tan YM, Worth A (2017) Investigating the state of physiologically based kinetic modelling practices and challenges associated with gaining regulatory acceptance of model applications. Regul Toxicol Pharmacol 90:104–115

Pairis-Garcia MD, Karriker LA, Johnson AK, Kukanich B, Wulf L, Sander S, Stalder KJ, Coetzee JF (2013) Pharmacokinetics of flunixin meglumine in mature swine after intravenous, intramuscular and oral administration. BMC Vet Res 9(1):165

Pearce RG, Setzer RW, Strope CL, Wambaugh JF, Sipes NS (2017) Httk: R package for high-throughput toxicokinetics. J Stat Softw 79(4):1

Pujol G, Iooss B, Boumhaout AJ with contributions from Veiga SD, Delage T, Fruth J, et al (2017) Sensitivity: global sensitivity analysis of model outputs. https://cran.r-project.org/web/packages/sensitivity/index.html. Accessed 4 Feb 2019

Rasool MF, Khalil F, Läer S (2015) A physiologically based pharmacokinetic drug-disease model to predict carvedilol exposure in adult and paediatric heart failure patients by incorporating pathophysiological changes in hepatic and renal blood flows. Clin Pharmacokinet 54(9):943–962

Riviere JE, Tell LA, Baynes RE, Vickroy TW, Gehring R (2017) Guide to FARAD resources: historical and future perspectives. J Am Vet Med Assoc 250(10):1131–1139

Rowland M, Peck C, Tucker G (2011) Physiologically-based pharmacokinetics in drug development and regulatory science. Annu Rev Pharmacol Toxicol 51:45–73

Schlender JF, Meyer M, Thelen K, Krauss M, Willmann S, Eissing T, Jaehde U (2016) Development of a whole-body physiologically based pharmacokinetic approach to assess the pharmacokinetics of drugs in elderly individuals. Clin Pharmacokinet 55(12):1573–1589

Shebley M, Sandhu P, Emami Riedmaier A et al (2018) Physiologically based pharmacokinetic model qualification and reporting procedures for regulatory submissions: a consortium perspective. Clin Pharmacol Ther 104(1):88–110

Shelver WL, Tell LA, Wagner S, Wetzlich SE, Baynes RE, Riviere JE, Smith DJ (2013) Comparison of ELISA and LC–MS/MS for the measurement of flunixin plasma concentrations in beef cattle after intravenous and subcutaneous administration. J Agric Food Chem 61(11):2679–2686

Sidhu PK, Gehring R, Mzyk DA, Marmulak T, Tell LA, Baynes RE, Vickroy TW, Riviere JE (2017) Avoiding violative flunixin meglumine residues in cattle and swine. J Am Vet Med Assoc 250(2):182–189

Skidmore A, Roder J, Van De Ven J, Cloet P, Montgomery A (2008) Flunixin meglumine (Finadyne®, Banamine®) in pain management of cattle: pharmacology and applications, proceedings XXV World Buiatrics Congress, July 6–11, 2008, Budapest, Hungary, p 293

Sundlof SF, Cooper J (1996) Human health risks associated with drug residues in animal-derived foods veterinary drug residues, vol 636. American Chemical Society, Washington, pp 5–17

Tan YM, Liao KH, Conolly RB, Blount BC, Mason AM, Clewell HJ (2006) Use of a physiologically based pharmacokinetic model to identify exposures consistent with human biomonitoring data for chloroform. J Toxicol Environ Health Part A 69(18):1727–1756

Tan YM, Worley RR, Leonard JA, Fisher JW (2018) Challenges associated with applying physiologically based pharmacokinetic modeling for public health decision-making. Toxicol Sci 162(2):341–348

Tan ML, Zhao P, Zhang L, Ho YF, Varma MVS, Neuhoff S, Nolin TD, Galetin A, Huang SM (2019) Use of physiologically-based pharmacokinetic (PBPK) modeling to evaluate the effect of chronic kidney disease on the disposition of hepatic CYP2C8 and OATP1B drug substrates. Clin Pharmacol Ther 105(3):719–729

Templeton IE, Jones NS, Musib L (2018) Pediatric dose selection and utility of PBPK in determining dose. AAPS J 20(2):31

Thiry J, Fournier R, Roy O, Catala M (2017) Evaluation of flunixin meglumine pour-on administration on prostaglandin E2 concentration in inflammatory exudate after induction of inflammation in cattle. Res Vet Sci 114:294–296

USDA (2018) US National Residue Program: residue sample results—"Red Book". https://www.fsis.usda.gov/wps/portal/fsis/topics/data-collection-and-reports/chemistry/red-books/red-book. Accessed 14 Nov 2018

Weijs L, Yang RS, Covaci A, Das K, Blust R (2010) Physiologically based pharmacokinetic (PBPK) models for lifetime exposure to PCB 153 in male and female harbor porpoises (Phocoena phocoena): model development and evaluation. Environ Sci Technol 44(18):7023–7030

WHO (2010) Characterization and application of physiologically based pharmacokinetic models in risk assessment. World Health Organization, International Programme on Chemical Safety (WHO/IPCS), Geneva, Switzerland. https://apps.who.int/iris/bitstream/handle/10665/44495/9789241500906_eng.pdf. Accessed 22 Apr 2019

Wojciechowski J, Hopkins AM, Upton RN (2015) Interactive pharmacometric applications using r and the shiny package. CPT Pharmacomet Syst Pharmacol 4(3):146–159

Wu H, Baynes RE, Leavens T, Tell LA, Riviere JE (2013) Use of population pharmacokinetic modeling and Monte Carlo simulation to capture individual animal variability in the prediction of flunixin withdrawal times in cattle. J Vet Pharmacol Ther 36(3):248–257

Yang F, Liu HW, Li M, Ding HZ, Huang XH, Zeng ZL (2012) Use of a Monte Carlo analysis within a physiologically based pharmacokinetic model to predict doxycycline residue withdrawal time in edible tissues in swine. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 29(1):73–84

Yang X, Doerge DR, Teeguarden JG, Fisher JW (2015) Development of a physiologically based pharmacokinetic model for assessment of human exposure to bisphenol A. Toxicol Appl Pharmacol 289(3):442–456

Yang X, Duan J, Fisher J (2016) Application of physiologically based absorption modeling to characterize the pharmacokinetic profiles of oral extended release methylphenidate products in adults. PLoS One 11(10):e0164641

Yoon M, Nong A, Clewell HJ 3rd, Taylor MD, Dorman DC, Andersen ME (2009) Evaluating placental transfer and tissue concentrations of manganese in the pregnant rat and fetuses after inhalation exposures with a PBPK model. Toxicol Sci 112(1):44–58

Yu Z, Jiang C, Guo Y, Hu Y, Chen D (2007) Pharmacokinetics of flunixin meglumine after intravenous and intramuscular administration in pigs. Agric Sci China 6(11):1396–1401

Zeng D, Lin Z, Fang B, Li M, Gehring R, Riviere JE, Zeng Z (2017) Pharmacokinetics of mequindox and its marker residue 1, 4-bis-desoxymequindox in swine following multiple oral gavage and intramuscular administration: an experimental study coupled with population physiologically based pharmacokinetic modeling. J Agric Food Chem 65(28):5768–5777

Zurlinden TJ, Reisfeld B (2017) Characterizing the effects of race/ethnicity on acetaminophen pharmacokinetics using physiologically based pharmacokinetic modeling. Eur J Drug Metab Pharmacokinet 42(1):143–153

Affiliations

Miao Li[1] · Yi-Hsien Cheng[1] · Jason T. Chittenden[2] · Ronald E. Baynes[2] · Lisa A. Tell[3] · Jennifer L. Davis[4] · Thomas W. Vickroy[5] · Jim E. Riviere[1,2] · Zhoumeng Lin[1]

Miao Li
miaoli@ksu.edu

Yi-Hsien Cheng
yhcheng1987@ksu.edu

Jason T. Chittenden
jchittenden1010@outlook.com

Ronald E. Baynes
ronald_baynes@ncsu.edu

Lisa A. Tell
latell@ucdavis.edu

Jennifer L. Davis
jdavis4@vt.edu

Thomas W. Vickroy
vickroy@ufl.edu

Jim E. Riviere
jriviere@ksu.edu

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

[2] Center for Chemical Toxicology Research and Pharmacokinetics, Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606, USA

[3] Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California-Davis, Davis, CA 95616, USA

[4] Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA 24060, USA

[5] Department of Physiological Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610, USA