

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

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ABSTRACT

Penicillin remains one of the most frequently identified violative drug residues in food-producing animals. The predominant violations of penicillin were found in cull dairy cows. In the United States, procaine penicillin G is approved to be used in dairy cows through intramuscular (IM) and intramammary (IMM) administrations. Physiologically based pharmacokinetic (PBPK) models are useful tools to predict withdrawal intervals and tissue residues of drugs in food animals to ensure food safety, especially for extralabel drug use due to the scarcity of experimental data after extralabel administrations. Currently, no PBPK model is available to predict penicillin concentrations in milk. A population PBPK model with a physiologically based compartment for the mammary gland was established for penicillin G in dairy cows. The model predicted the tissue and milk residues well based on comparison with data from previous pharmacokinetic studies. The predicted milk discard interval of procaine penicillin G administered at 10 times the label dose for 3 repeated IM administrations was 182 h, and 122 h at 4 times the label dose after 3 repeated IMM infusions. Predicted results showed that even 4 times label dose did not lead to violative tissue residues in healthy dairy cows with IMM infusions. The predominant violations found in cull dairy cows may be caused by altered pharmacokinetics due to mastitis, other diseases, and/or interactions with other drugs, which have impacts on penicillin distribution and elimination. The current PBPK model can help predict milk discard interval for penicillin following extralabel use through IM and IMM administrations.

Key words: PBPK modeling; dairy cows; food safety; drug residue; Food Animal Residue Avoidance Databank (FARAD); penicillin.

INTRODUCTION

Penicillin is one of the top 3 most commonly detected violative residues in animal-derived foods reported by the National Residue Program of United States Department of Agriculture (USDA) from 2014 to 2016 (USDA, 2015, 2017a,b). This is mainly because penicillin G is one of the most widely used

antimicrobials and tested regularly (FDA, 2013; Portis *et al.*, 2012; Vogel *et al.*, 2001). Extralabel use of penicillin G in food-producing animals is very common (Chiesa *et al.*, 2006) due to reduced sensitivity to penicillin in bacteria. The extralabel (also called off-label) use of veterinary drugs means to use a drug in a manner that is different from the approved labeling under the

supervision of a veterinarian, and extralabel use may only occur if prescribed by a veterinarian (FDA, 2017). Consumption of beef or pork products containing violative penicillin residues can lead to anaphylactic reactions for sensitive individuals (Dayan, 1993; Gomes and Demoly, 2005; Raison-Peyron et al., 2001). In addition, violative residues of penicillin G in milk could interfere with starter cultures for fermented dairy products (Payne et al., 2006; Suhren, 1996) and may result in suspension of the producer's permit or certification (NRC, 1999).

Drug residues above the regulatory safe level in animal-derived products challenge the global food safety (Baynes and Riviere, 2014; Baynes et al., 2016; Paige et al., 1997). The U.S. Food and Drug Administration (FDA) has established a zero tolerance for penicillin in milk products (FDA, 2017). In actual practice, FDA uses the "safe level" of 5 ng/ml for penicillin residues in milk. The USDA National Residue Program reported that 135 out of 213 (63%) and 153 out of 216 (71%) violative samples in cull dairy cows were found with penicillin G violations in 2015 and 2016, respectively (USDA, 2017a,b). The predominant violations of penicillin found in cull dairy cows lead to the concerns of potential drug residues in the cow-derived food products, including milk. In the United States, procaine penicillin G is approved to be used in dairy cows through intramuscular (IM) and intramammary (IMM) administrations. The approved doses of procaine penicillin G are at a daily dose of 6600 IU/kg of body weight (6.5 mg/kg) for no more than 7 consecutive days through the IM injection (Papich et al., 1993), and 100 000 IU (99 mg) into each affected quarter of udders every 12 h for no more than 3 doses (United States Pharmacopeia, 2003). The procaine penicillin G is slowly absorbed after IM administration (Papich, 1987). The major metabolite of penicillin G, penicilloic acid, does not have the antimicrobial activity and accounts for about 16%–30% of an IM dose of penicillin G in humans (DrugBank, 2018; Wishart et al., 2006). Unlike in humans, the metabolism of penicillin G in food-producing animals is not well characterized, and the elimination of penicillin G is mainly through renal secretion (Papich and Riviere, 2009).

The determination of withdrawal intervals (WDIs) for extralabel use of veterinary drugs is important to avoid the violations of tissue residues. The WDI or milk discard time is the time for drug residues in the edible tissues or milk to deplete below concentrations that are considered safe for human consumptions (FDA, 2012). The milk produced before the milk discard time must be discarded to avoid drug residues in milk (EMA, 2000). According to the Pasteurized Milk Ordinance (FDA, 2015a), milk violation tests carried out by FDA are based on the samples from bulk milk tank. In the United States, the 99th percentile tolerance limit with 95% confidence is used to determine the withdrawal period and milk discard time (FDA, 2006, 2016). In Europe, it is recommended to determine the withdrawal period with the upper one-sided 95% tolerance limit for the drug residue below the maximum residue limit with 95% confidence (EMA, 2016). Due to the lack of pharmacokinetic data after extralabel use of veterinary drugs, it is difficult to use statistical tolerance methods or classic pharmacokinetic methods to determine the WDIs after extralabel administrations. The physiologically based pharmacokinetic (PBPK) model is a useful tool to predict tissue residues and WDIs of veterinary drugs for food safety assessment (Henri et al., 2016a; Lin et al., 2016b). PBPK modeling is a mechanism-based approach to simulate the absorption, distribution, metabolism, and elimination of chemicals (Andersen, 2003; Lin et al., 2016a; WHO, 2010) and to predict the concentrations in the edible tissues and products beyond times when data are not available. By changing the related

parameter values, the PBPK model can be extrapolated to different species and therapeutic scenarios.

There is no whole-body PBPK model for predicting the milk discard intervals of drugs after extralabel use. Recently, we published a multiroute population PBPK model for penicillin G in beef cattle (Li et al., 2017). On the basis of cattle model, in the present study we developed a PBPK model with physiologically based compartment of mammary glands to predict milk concentrations and discard intervals after IM or IMM administration of penicillin G at extralabel doses in dairy cows. The systemic absorption from the mammary gland and the distribution of penicillin G were considered. The volume change by milk secretion and episodic milking process were also included. Probabilistic analysis using Monte Carlo simulation was applied to address the uncertainties of parameter values. This PBPK model contributes to the field of toxicological science and biological modeling by establishing a quantitative tool that allows to conduct drug residue safety assessment of cow-derived food products and creating a new physiologically based approach to simulate milk production and episodic milking processes, which can be applied to other species, including rodents, humans, and goats.

MATERIALS AND METHODS

Data source for model calibration. The pharmacokinetic data used in the calibration and evaluation of the PBPK model were acquired from the Comparative Pharmacokinetic Database from the Food Animal Residue Avoidance Databank (FARAD). FARAD is supported by USDA National Institute of Food and Agriculture with an overarching goal to provide veterinary practitioners the most current and accurate information to facilitate the production of safe foods of animal origin through the prevention and mitigation of violative chemical residues in food animal products (Craigmill et al., 2006; Riviere et al., 1986, 2017). Pharmacokinetic data in dairy cows after IM or IMM administration of procaine penicillin G were selected. The formulation of aqueous suspension was used for IM injection of procaine penicillin G. For IMM injection, the formulation of procaine penicillin G in peanut oil, which is more relevant to the current clinical formulations, was used. Brief description and key information of selected pharmacokinetic studies are summarized in Table 1. The graphic pharmacokinetic data were extracted from selected studies using WebPlotDigitizer (version 3.10, <https://automeris.io/WebPlotDigitizer/>; last accessed March 29, 2018).

Model structure. The PBPK model for penicillin G with the mammary gland compartment was established based on our previous generic PBPK model for penicillin G in beef cattle (Li et al., 2017). The model consisted of 8 compartments corresponding to different tissues in the body, including liver, kidney, muscle, fat, lung, the rest of body and udder connected by the circulating blood system (Figure 1A). For food safety assessment purposes, all the major edible tissues, including liver, kidney, muscle, and fat, were included. The udder was considered as a compartment, because the model considered the absorption of penicillin G from milk to the systemic circulation and the excretion of penicillin G into the milk. The lung was also included as a compartment as it is the therapeutic target for penicillin G. Each compartment was defined by a tissue weight and tissue blood flow rate. The compartments for urine and feces were established as virtual excretory compartments without volume changes. The flow-limited model, which performed well for the

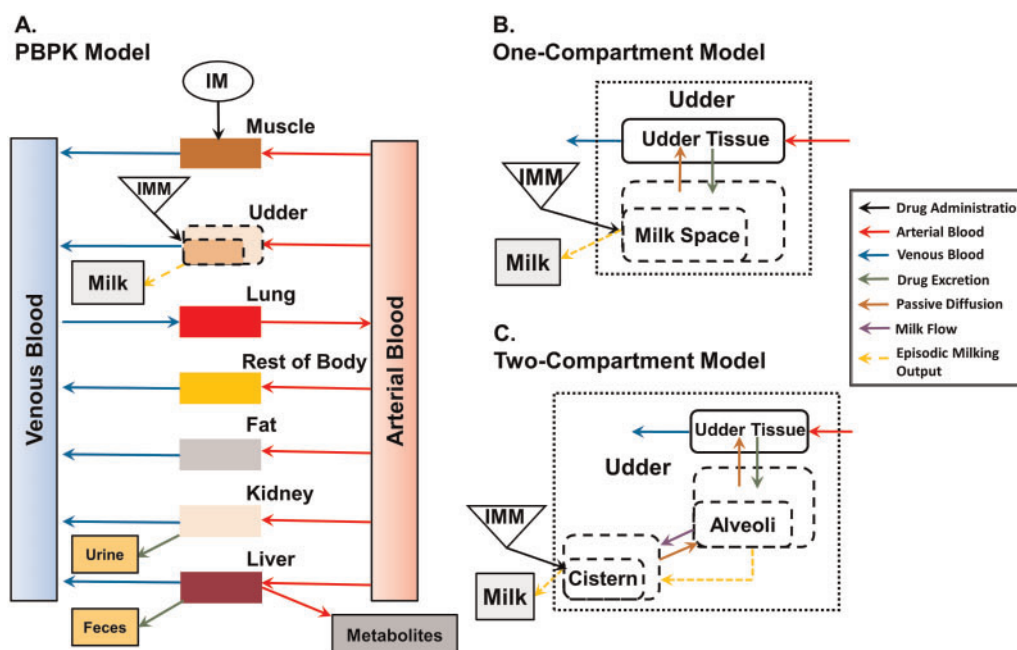


Figure 1. A schematic diagram of the physiologically based pharmacokinetic (PBPK) model for penicillin G in dairy cows and the 1- and 2-compartment udder models. A, The PBPK model for penicillin G in dairy cows. Two different administration routes including intramuscular (IM) and intramammary (IMM) administrations are presented in the model. The dash line for the compartment of udder indicates that the volume changes since the preceding milking and this could be simulated using the Hill-Langmuir equation. B, The 1-compartment model for the udder. The milk space represents the combined milk space including alveoli, ducts, and the cistern. C, The 2-compartment model for the udder.

Table 1. Summary of Pharmacokinetic Studies of Penicillin G in Dairy Cows Used for Calibration and Evaluation of the PBPK Model

PK Studies/Purposes	Routes	n	Matrices	Analytical Methods	Treated Quarters	Dose Regimen	Dose per Administration
Calibration							
Mercer et al. 1974	IMM	6	P, M	MM	All	One infusion	396.5 mg per quarter
Hogh and Rasmussen 1966	IM	13	P, M	NA	NA	One injection	10 mg/kg
Van OS et al. 1974	IM	4	P, M	MM	NA	One injection	40 mg/kg
Evaluation							
Vilim et al. 1979	IMM	6	M	MM	Two quarters	24-h interval 3 doses	100 mg per quarter
Vilim et al. 1980	IMM	6	M	MM	All	24-h interval 3 doses	100 mg per quarter
Knapstein et al. 2003	IMM	4 to 6	M	HPLC	All	24-h interval 3 doses	1898 mg per quarter
Randall et al. 1954	IM	2	P, M	MM	NA	One injection	11 mg/kg
Edwards 1966	IM	2	P, M	MM	NA	One injection	2.2, 4.4 or 11 mg/kg
FDA 2010 (NADA 065-010)	IM	20	M	HPLC	NA	24-h interval 4 doses	6.5 mg/kg

All data used for model calibration and evaluation were from healthy cows. The abbreviations for routes: IM, intramuscular injection; IMM, intramammary infusion. The abbreviations for matrices: P, plasma; M, milk. The abbreviations for assays: HPLC, high performance liquid chromatography; MM, microbiological methods. NA, not available or not applicable.

previous PBPK model of penicillin G, was applied in the current model.

Model calibration and parameterization. The current model was based on the published PBPK model for penicillin G in beef cattle, and the physiologically based mammary gland compartment and IMM infusion were added to the model. The physiological parameters related to dairy cows were acquired from previous research. The average values of blood flow to the udder (QUC), the tissue volume fractions of the udder (VUC), venous blood volume (VvenC), and arterial blood volume (VartC) were obtained or calculated based on previous experimental studies (Campbell and Marshall, 2016; Gionbelli et al., 2015). For chemical-specific parameters (eg, partition coefficients) of penicillin G, the original values used in the cattle model (Li et al.,

2017) were applied in the current dairy cow model. The parameters related to the udder compartment and IMM infusions, including V_{maxC} , K_m , and K_{abC} were fitted with pharmacokinetic data chosen for calibration (Table 1). V_{maxC} and K_m were optimized by fitting with calibration datasets following IM administrations (Hogh and Rasmussen, 1966; Van OS et al. 1974), and K_{abC} was estimated by fitting to the dataset after IMM administration (Mercer et al., 1974). The values of all physiological parameters and chemical-specific parameters used in the PBPK model are shown in Table 2.

Establishment and validation of the udder compartment. The compartments representing the milk and udder were established according to the physiologically based mathematical model described by Whittem et al. (2012). In brief, the Hill-Langmuir

Table 2. Parameter Values Used in the PBPK Model for Penicillin G in Milk From Dairy Cows

Parameter	Abbreviation	Mean	Reference
Cardiac output (l/h/kg)	QCC	5.97	Li et al. 2017
Tissue volume (fraction of body weight, unitless)			
Arterial blood (lactating cows)	VartC	0.012	Gionbelli et al. 2015
Venous blood (lactating cows)	VvenC	0.037	Gionbelli et al. 2015
Liver	VLC	0.014	Li et al. 2017
Kidney	VKC	0.002	Li et al. 2017
Muscle	VMC	0.27	Li et al. 2017
Fat	VFC	0.15	Li et al. 2017
Lung	VLuC	0.008	Li et al. 2017
Udder	VUC	0.008	Gionbelli et al. 2015
Rest of body	VrestC	0.507	Total adds to 1
Blood flow (fraction of cardiac output, unitless)			
Liver	QLC	0.405	Li et al. 2017
Kidney	QKC	0.09	Li et al. 2017
Muscle	QMC	0.18	Li et al. 2017
Fat	QFC	0.08	Li et al. 2017
Udder	QUC	0.081	Campbell and Marshall, 2016
Rest of body	QrestC	0.164	Total adds to 1
The maximum volume of milk space (l)	VMmilksp	26.8	Whittem et al., 2012
Time to reach half of the maximum volume of milk space (h)	S	23.2	Whittem et al., 2012
Milk residue ratio after milking (unitless)	F	0.04	Whittem et al., 2012
Absorption rate constant (/h)			
Intramuscular			
Absorption rate constant of IM administration (/h)	Kim	0.07	Li et al. 2017
Fraction of undissolved procaine penicillin G (unitless)	Frac	0.6	Li et al. 2017
Dissolution rate constant into penicillin G moieties (/h)	Kdiss	1.00E-05	Li et al. 2017
Intramammary			
Maximum velocity for penicillin G excretion in the mammary gland scaling to BW (mg/h/kg BW)	VmaxC	0.0022	Model fitted
Concentration of penicillin to reach half Vmax (mg/l)	Km	0.7	Model fitted
Rate constant of penicillin G absorbed from milk (/h)	KabC	1.00E-04	Model fitted
Tissue: plasma partition coefficient for the parent drug (unitless)			
Liver	PL	3	Li et al. 2017
Kidney	PK	2.5	Li et al. 2017
Muscle	PM	0.3	Li et al. 2017
Fat	PF	0.04	Li et al. 2017
Lung	PLu	0.18	Li et al. 2017
Udder	PU	0.2	Model fitted
Rest of body	Prest	0.479	Li et al. 2017
Hepatic metabolic rate constant (/h/kg)	KmetC	0.0025	Li et al. 2017
Percentage of plasma protein binding (unitless)	PB	0.483	Li et al. 2017
Urinary elimination rate constant (l/h/kg)	KurineC	0.45	Li et al. 2017

"Model fitted" indicates that the parameter values were determined by fitting the model with calibration pharmacokinetic data.

equation was used to describe the volume-time relationship for milk production. The concentration change of penicillin G due to milk production and episodic milk emptying was considered. Both the 1-compartment and 2-compartment models were created and validated using previously reported data (Davis et al., 1998). The 1-compartment model was established considering the milk space in the udder as 1 compartment with the function of milk secretion and storage (Figure 1B). The 2-compartment model divided the milk space into the alveoli, which serve the function of milk secretion and storage, and the cistern, which only has the function for milk storage (Figure 1C). For the 2-compartment model, the distribution of penicillin G between alveoli and cistern compartments was incorporated.

Berkeley Madonna (Version 8.3.23.0; University of California at Berkeley, California) was used to develop the PBPK model with the mammary gland compartment and run all simulations. Codes of the model are provided in the [Supplementary](#)

[Data](#) and will be available on our website (<http://iccm.k-state.edu/>; last accessed March 29, 2018). As the model was constructed based on the previous PBPK model, only key and new mathematical equations are described in detail in following paragraphs. For all the other equations, details have been provided in the [Supplementary Data](#) and the previously published PBPK model for penicillin G in cattle and swine (Li et al., 2017).

For the 1-compartment udder model, the volume change of the milk space in udder was modeled the same as the volume change of the milk production. The milk volume in the udder compartment depends on the episodic milking and the milk production between milkings. The milk production was described using the Hill-Langmuir equation (equation 1) reported in the previous milk model (Whittem et al., 2012). According to the previous experimental data (Davis et al., 1998), the equation describes the milk production rises rapidly in a linear trend at the beginning, and the rate of milk secretion slows down with

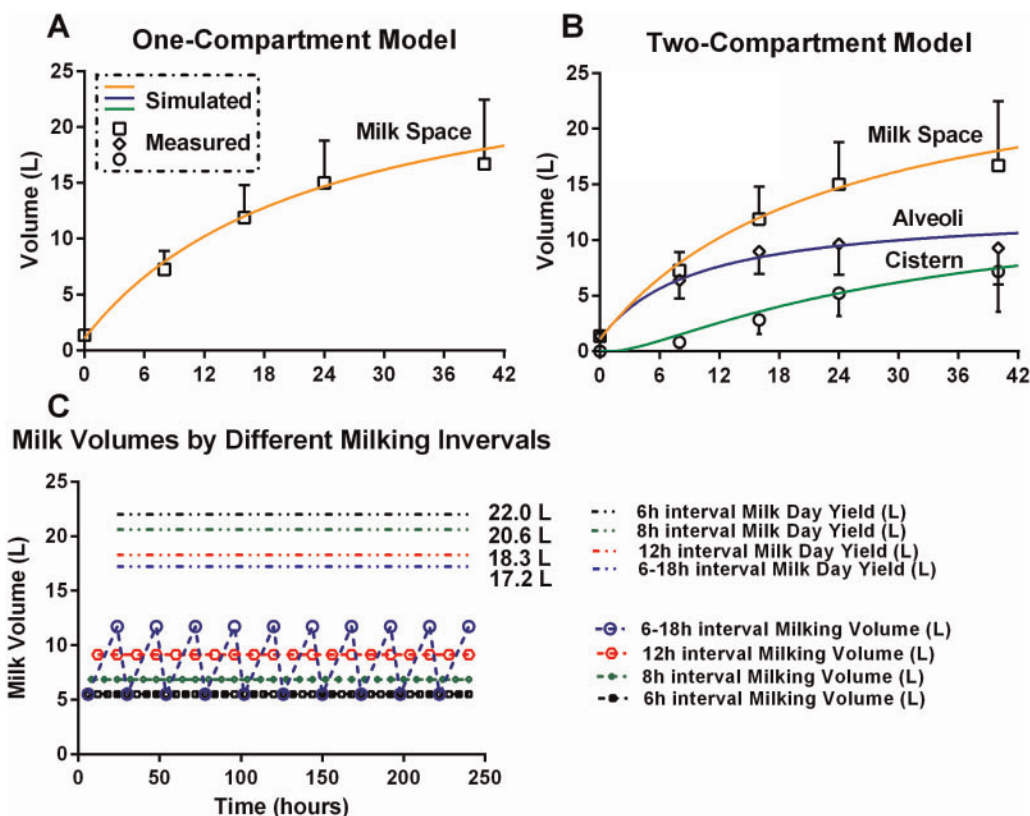


Figure 2. Validation and application of the milk models. Model predictions for milk volumes (solid line) and observed data (black squares, circles, and diamonds) are compared in panel A for the 1-compartment model and in panel B for the 2-compartment model. The observed data are from previous study by Davis et al. (1998). In panel C, the milk production volumes by different milking intervals were simulated using 1-compartment milking model based on the parameter values reported in Whittem et al. (2012) ($VM_{milksp} = 26.81$, $s = 23.2$ h, $F = 0.04$).

the increased hydrostatic pressure in the udder compartment. The volume of residual milk in udder can be determined by the fraction of maximum volume of the milk space (equation 2). Previous experimental research reported the residual milk fractions are in the range of 4%–14% (Carruthers et al., 1993; Isaksson and Arnarp, 1988; Knight et al., 1994), here 4% was used as the value for the residual fraction as reported in the previous model (Whittem et al., 2012). The total volume of milk space should account for the residual volume after milking and the volume of milk secreted after the preceding milking (equation 3).

$$V_{milk} = \frac{tm \times VM_{milksp}}{tm + s}, \quad (1)$$

$$V_{residue} = VM_{milksp} \times F, \quad (2)$$

$$VM_{milksp} = V_{residue} + V_{milk}, \quad (3)$$

where V_{milk} is the volume of milk produced since the preceding milking (l); tm is the time after the preceding milking (h); VM_{milksp} is the maximum volume of the milk space including spaces in alveoli, ducts, and the cistern (l); s is the time for V_{milk} reaching half of VM_{milksp} (h); V_{milksp} is the volume of the milk space (l); $V_{residue}$ is the residual volume of the milk after milking (l); F is the ratio of the residual volume to the maximum volume of the milk space (unitless).

Compared with the 1-compartment model, the 2-compartment model is more physiologically based, and can be used to simulate the drug distribution and movement in different parts of udder compartment. However, the 2-compartment model is

relatively complex, includes more parameters and requires robust datasets to validate. Briefly, the volume changes in the total milk space and the alveolar compartment were both simulated with the Hill-Langmuir equation (equation 4). The volume in cistern compartment was determined by subtracting the volume of alveoli from the total milk space (equation 5). The residual volume of milk in the milk space of udder was mostly retained in the alveoli (Vetharaniam et al., 2003), and the residual volume in cistern was assumed as zero immediately after milking (Knight et al., 1994).

$$Valv = Valv_{residue} + \frac{tm \times V_{Malv}}{tm + salv}, \quad (4)$$

$$V_{cis} = V_{milksp} - Valv, \quad (5)$$

where $Valv$ is the volume of milk in the alveoli (l); tm is the time after the preceding milking (h); V_{Malv} is the maximum volume of the alveoli (l); $salv$ is the time for the volume of milk in alveoli reaching half of V_{Malv} (h); $Valv_{residue}$ is the residual volume of the alveoli after milking (l); V_{cis} is the volume of milk in the cistern (l).

Both the 1-compartment and 2-compartment udder models were validated (Figs. 2A and 2B) based on published experimental data (Davis et al., 1998). As available pharmacokinetic data in milk are mostly sparse data and to avoid over parameterization, the 1-compartment udder model was used in the final PBPK model. The code describing the 2-compartment udder model is provided in the code in the Supplementary Data and can be used in the future when needed and when more robust datasets are available.

The previous milk model assumed no absorption and mass transfer of drugs between the udder compartment and systemic circulation (Whittem *et al.*, 2012). In order to incorporate the udder compartment into a whole-body PBPK model, the absorption of penicillin G from the udder to the systemic circulation and the excretion of penicillin G from plasma to milk were considered so that the current model could be applied to both IMM and IM administrations. The absorption and excretion processes of penicillin G involve both the passive diffusion and active carrier-mediated transport (Rasmussen, 1959; Schadewinkel-Scherkl *et al.*, 1993; Ziv and Sulman, 1974). On the basis of previously reported experimental data (Schadewinkel-Scherkl *et al.*, 1993), the carrier-mediated transport accounts for around 25% of the absorption of penicillin G at mammary gland (Supplementary Figure 1A) and for over 75% of the penicillin G excretion (Supplementary Figure 1B). Due to very limited experimental data for both processes, the parameters related to the kinetics of the absorption and excretion need to be determined by fitting with pharmacokinetic data. In order to reduce uncertainties, the excretion process was considered as the active carrier-mediated transport only, and the absorption process was described as only through the passive diffusion (equation 6). The Michaelis-Menten equation was used for the carrier-mediated transport of penicillin excreted into milk (equation 7), and the first-order linear equation was applied for the passive diffusion. The rate of change of penicillin G in the udder compartment was described using mass balance differential equations as previously described (DeWoskin *et al.*, 2013; Leavens *et al.*, 2012; Lin *et al.*, 2016b). Only the penicillin G not bound to plasma proteins was considered as freely available for excretion. The mass balance equations of penicillin G in the udder compartment are described below.

$$RU = QU (CA_{free} - CVU) - R_{milkex} + Kab \times Amilk \quad (6)$$

$$R_{milkex} = \frac{V_{max} \times CVU}{CVU + K_m}, \quad (7)$$

$$CVU = CU/PU, \quad (8)$$

where RU is the rate of change in the concentration of penicillin G in the udder (mg/h); QU represents the volume of blood flow to the udder per hour (l/h); CA_{free} is the arterial blood concentration of penicillin G not bound with plasma proteins (mg/l); CVU is the concentration of penicillin G in the udder venous blood (mg/l); R_{milkex} is the rate for penicillin excreted into milk (mg/h); Kab is the rate constant of penicillin G absorbed from milk (/h); Amilk is the amount of penicillin G in milk (mg); V_{max} represents the maximum rate for penicillin G excretion from udder tissue to milk (mg/h); K_m is the concentration of penicillin G to achieve the half V_{max} (mg/l); CU is the penicillin G concentration in the udder tissue (mg/l); PU is the udder tissue: plasma partition coefficient (unitless).

The penicillin input through IMM administration, excretion from plasma to milk, penicillin absorption from milk, and elimination through the episodic milking process were considered for the change of penicillin concentrations in milk (equation 9). The concentration of penicillin in milk was simulated by dividing amount of penicillin in milk by the milk volume (equations 10 and 11).

$$R_{milk} = R_{inputimm} + R_{milkex} - Kab \times Amilk - R_{milking}, \quad (9)$$

$$d/dt(A_{milk}) = R_{milk}, \quad (10)$$

$$C_{milk} = A_{milk}/V_{milk}, \quad (11)$$

where R_{milk} is the rate of change in the concentration of penicillin G in milk (mg/h); R_{inputimm} stands for the input rate of penicillin through IMM administration (mg/h); R_{milkex} is the rate for penicillin excreted into milk (mg/h); Kab is the rate constant of penicillin G absorbed from milk (/h); Amilk is the amount of penicillin G in milk (mg); R_{milking} is the rate of change in the concentration of penicillin G by milking process (mg/h); V_{milk} is the volume of milk produced since the preceding milking (l); C_{milk} is the penicillin G concentration in milk (mg/l).

The milking intervals and frequencies have impacts on the milk production (Gehring and Smith, 2006) and lead to the change of drug concentrations in milk. Briefly, higher milk production could lead to further dilution of drug concentrations in milk. In pharmacokinetic studies for penicillin G in milk, many employed unequal intervals such as 10 + 14 h or 15 + 9 h intervals for milking instead of equal 12-h milking intervals. In order to better fit the experimental data, the array equations were used (Supplementary equations 1 and 2). The IM and IMM administrations were incorporated into this PBPK model because they are the label routes for procaine penicillin G in dairy cows. The IM administration was simulated using a 2-compartment dissolution model based on the approach used to simulate the absorption of long-acting oxytetracycline (Lin *et al.*, 2015). Detailed description of the dissolution model and the IM injection was included in our previous papers (Li *et al.*, 2017; Lin *et al.*, 2015). The multiple-dose therapeutic scenario was achieved by using the conditional operator of "IF...THEN...ELSE..." to create a control factor (Supplementary equations 3 and 7). The volume of milk was simulated using array functions, which helped to simulate the milking processes as the discrete steps. However, the amount of penicillin G in the milk and the udder was simulated using continuous functions. The DELAY functions were also applied here to help overcome the false peaks due to the function differences. For more details, please refer to the Supplementary Data.

Evaluation and sensitivity analysis. The performance of the PBPK model was evaluated by comparing model simulations with experimental pharmacokinetic data of milk and plasma not used in the model calibration (Table 1). On the basis of World Health Organization (WHO) guidelines (WHO, 2010), if the results from simulations matched the measured kinetic profiles and were generally within a factor of 2 of the measured values, the model was considered reasonable and validated. These criteria are based on the considerations that calibration datasets and evaluation datasets are obtained under different conditions (eg, different experimental animals/human subjects, laboratories, and detection methods), so some level of discordance is to be expected (WHO, 2010). The goodness of fit between log-transformed values of observed and predicted plasma and milk concentrations were further analyzed with linear regression and the determination coefficient (R²) was calculated for both calibration and evaluation results. The goodness of fit was evaluated using the determination coefficient (R²) for linear regression analysis and the mean absolute percentage error (MAPE) value. The R² is an indicator for the overall model simulation performance, and a model simulation with a R² value equal to or higher than 0.75 is considered acceptable. The analysis for MAPE was also carried out based on the previously reported methods (Cheng *et al.*, 2016; Lin *et al.*, 2017). The MAPE value lower than 50% was considered as the acceptable prediction.

Table 3. Values and Distributions of Parameters Used in the Monte Carlo Analysis for the PBPK Model of Dairy Cows

Parameter	Distribution	Mean	SD	CV	Lower Bound	Upper Bound
BW	Normal	299.96	46.180	0.15#	209.45	390.464
QCC	Normal	5.97	1.990	0.33#	2.07	9.87
QKC	Normal	0.09	0.027	0.30	0.037	0.143
VMmilksp	Lognormal	26.8	8.040	0.30	11.042	42.558
S	Lognormal	23.2	6.960	0.30	9.559	36.841
F	Lognormal	0.04	0.012	0.30	0.016	0.064
Kim	Lognormal	0.07	0.021	0.30	0.029	0.111
Frac	Lognormal	0.6	0.012	0.30	0.576	0.624
VmaxC	Lognormal	0.0022	0.0003	0.30	0.0014	0.0026
Km	Lognormal	0.7	0.006	0.30	0.688	0.712
KabC	Lognormal	1.00E-04	2.40E-05	0.30	5.30E-05	1.47E-04
PL	Lognormal	3	0.600	0.20	1.824	4.176
PK	Lognormal	2.5	0.500	0.20	1.52	3.48
KmetC	Lognormal	0.0025	7.50E-4	0.30	0.001	0.004
KurineC	Lognormal	0.45	0.135	0.30	0.185	0.715

A pound sign (#) indicates the CV was calculated based on previous experimental data. Default values reported by previous models were used for CVs of the other parameter values. The default values of CVs for physiological parameters were 0.3, and for chemical-specific parameters were 0.2. The 5th and 95th percentiles of each parameter were calculated as the lower and upper bounds to represent 95% confidence interval. Please refer to [Table 2](#) for the description of each parameter.

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 the plasma, liver, kidney, and milk concentrations of penicillin G. Briefly, each parameter was increased by 1% and the corresponding 24-h AUC of penicillin concentrations was computed. Normalized sensitivity coefficient (NSC) was calculated using equations reported previously ([Lin et al., 2011](#); [Yoon et al., 2009](#)). We conducted local sensitivity analysis rather than global sensitivity analysis because local sensitivity analysis is sufficient to identify highly sensitive parameters on our selected dose metrics of interest based on the multiple relevant studies ([Craigmill, 2003](#); [Leavens et al., 2014](#); [Zeng et al., 2017](#); [Zhu et al., 2017](#)). Global sensitivity analysis could be done in the future if the goal is to study interaction effects between parameters ([Lumen et al., 2015](#); [McNally et al., 2011](#)).

Monte Carlo analysis. Monte Carlo simulation obtains numerical results based on the 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 ([Buur et al., 2006](#); [Yang et al., 2012](#); [Zeng et al., 2017](#)). In the current PBPK model for penicillin G in milk, Monte Carlo simulation was also applied to estimate the effects of parameter uncertainties and intraspecies variability of dairy cows on milk penicillin G concentrations. For these simulations, the relatively sensitive parameters, which were identified from the local sensitivity analysis ([Supplementary Table 1](#)), distributed randomly around the mean values were specified in [Table 3](#). Log-normal distributions of model parameters were assumed for all chemical-specific parameters, such as partition coefficients, absorption rate constants, and elimination rate constants. The lognormal transformation for parameters in Monte Carlo simulations was achieved by using [Supplementary equations 8–10](#). Physiological parameters, including body weights, cardiac outputs, and fractions of blood flows and tissue volumes were assumed to be normally distributed ([Clewell III et al., 2000](#); [Li et al., 2017](#); [Shankaran et al., 2013](#); [Sterner et al., 2013](#); [Tan et al., 2006](#); [Yang et al., 2015](#)).

The detail of Monte Carlo simulation in Berkeley Madonna was introduced in our previous generic PBPK model for penicillin G in beef cattle ([Li et al., 2017](#)), and the code of the current model is available in the [Supplementary Data](#). Briefly, model parameters were varied randomly around the values (central tendencies) used or estimated in model calibration by their probabilistic distributions (variability). The values of sensitive physiological and chemical-specific parameters were randomly selected based on their distributions, it is necessary to use adjustment factors to ensure the plausibility and mass balance for the PBPK model ([Covington et al., 2007](#); [Gelman et al., 1996](#); [Sterner et al., 2013](#)). The Monte Carlo simulation was set up to batch run for 1000 times with model parameters randomly selected from the defined distributions.

Application of the model for milk production and tissue concentration prediction. The model of milk production can be used as a stand-alone program to predict the milk production based on the different milking intervals. The 12, 12-h interval, 8, 16-h interval, and 8, 8, 8-h interval were used to simulate and compare the milk productions with different milking intervals.

The PBPK model for penicillin G with the mammary gland compartment can be used to predict the systemic absorption and distribution of penicillin G after the IMM injection and to predict the milk concentration of penicillin G following IM injections. For IMM infusions, the label dose 100 000 IU (99 mg per quarter of udders) and 2 commonly used extralabel doses (2× label dose, 198 mg per quarter; 4× label dose, 396 mg per quarter) to 2 of the 4 quarters were simulated for 3 repeated doses with 12-h intervals. For IM administrations, the label dose 6000 IU/kg (6.5 mg/kg) and 2 commonly used extralabel doses (5× label dose, 32.5 mg/kg; 10× label dose, 65 mg/kg) were simulated for 3 repeated doses with 24-h intervals. A schematic illustrating the dosing regimens is provided in the [Supplementary Figure 2](#). The commonly used extralabel doses for IMM infusions were based on the phone call records from the FARAD call center, and for IM administrations were obtained from the previous report ([Payne et al., 2006](#)). Infusions of penicillin G for all 4 quarters are only for the worst scenario of mastitis, and infusions for 2 quarters are common ([Vilim et al., 1979](#)). Here, IMM infusions for 2 quarters were simulated. These doses were

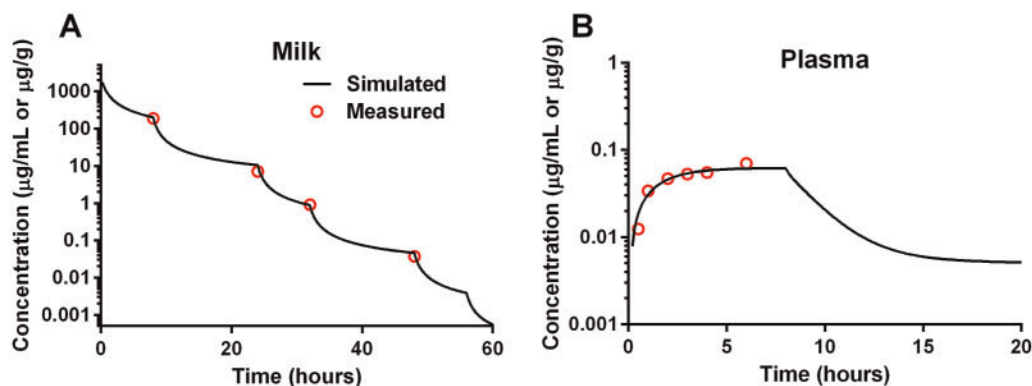


Figure 3. Calibration of the physiologically based pharmacokinetic model using pharmacokinetic data through the intramammary (IMM) infusion. Comparison of model predictions (solid line) and observed data (red circles) for concentrations of penicillin G in the plasma and milk from dairy cows exposed to procaine penicillin G via single IMM infusion (396 mg per quarter for all quarters). Experimental data are from the previous study (Mercer *et al.* 1974). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

applied as input for the current PBPK model to predict the concentrations of penicillin G in the milk, plasma, liver, and kidney.

The Monte Carlo analysis was also used to simulate the distribution of penicillin concentrations based on the distribution of parameter values. Different therapeutic scenarios were analyzed using Monte Carlo simulations. Each simulation was run 1000 times. The mean value, 1st and 99th percentile values of simulated results were calculated and plotted. The extended meat WDI and the milk discard interval were determined when the values of 99th percentiles of the target tissue and milk concentrations, respectively, fell below the tolerance or the safe level. The tolerance of penicillin G for edible tissues in cattle is 0.05 µg/g (FDA, 2013). The safe level of penicillin G for milk is 5 ng/ml (FDA, 2005, 2015b) and the tolerance is 0 by U.S. FDA (Brynes, 2005; FDA, 2017). The limits of detection (LODs) for penicillin G in the milk from current methods are lower than or equal to 1 ng/ml, such as 1 ng/ml (Holstege *et al.*, 2002), 0.25 ng/ml (Liu *et al.*, 2011), and 0.23 ng/ml (Huang *et al.*, 2013). In addition, we compared the model-predicted milk discard intervals from Monte Carlo simulations between 1000 and 10 000 iterations based on a 3 daily IM administration paradigm at the labeled dose level.

RESULTS

Validation and Application of the Milk Model

The 1- and 2-compartment milk models were used to simulate the volume of milk produced. Due to the lack of published data for the volumes of milk produced by different milking intervals, the model was only calibrated with experimental data from a study that determined the pattern of milk accumulation in the milk space of udder over time (Davis *et al.*, 1998), and the parameter values were based on the parameter values from the previous milk model (VMmilksp = 26.8 l, $s = 23.2$ h, $F = 0.04$) (Whittem *et al.*, 2012). There were no other data available for the model evaluation. The 1-compartment model predicted the total milk volume in the milk space well (Figure 2A). The simulated results from 2-compartment model were also in good agreement with milk volumes measured in alveoli, cistern, and total milk space in udder (Figure 2B). Both the 1- and 2-compartment milk models could be used as the udder compartment for the PBPK model. The milk model could also be applied to simulate the impact of different milking intervals on the daily milk production (Figure 2C represents the results from the 1-compartment model). The results showed that the 12-h milking intervals

produced slightly higher milk volumes compared with 6–18 h alternative intervals. Milking 4 times per day achieved the highest daily milk yields compared with milking 2 or 3 times a day, which is in accordance with previous reported research (Allen *et al.*, 1986; Erdman and Varner, 1995). However, milking more than twice a day does not fit the circadian rhythm and the real-life working shifts well and requires more labor (Lyons *et al.*, 2014).

Model Calibration

The PBPK model with the 1-compartment milk model was used to simulate penicillin G concentrations in plasma and milk after different therapeutic regimens. Model predictions were compared with observed concentrations in dairy cows exposed to penicillin G through IMM infusion or IM injection (results are shown in Figs. 3 and 4, respectively). Overall, the model well simulated the kinetic profiles of penicillin G in the milk and plasma in dairy cows through both IM and IMM administrations. In particular, the model can accurately predict the penicillin G residues in the milk and plasma even at the later time points, which are important for residue monitoring and the determination of times when the concentrations of residues in the milk fall below or near the tolerance of penicillin G.

Model Evaluation

The pharmacokinetic datasets not used for the model calibration were applied to evaluate the model performance. Measured concentrations of penicillin G in the milk and plasma of dairy cows after IMM and IM administrations for single or multiple doses were compared with the model predictions (Figs. 5 and 6). Overall, the model predicted the concentrations of penicillin G adequately at different time points. For the IMM administration, the evaluation results shown in Figure 5 indicate the model well simulated the penicillin G concentrations in milk following different milking intervals. The R^2 values of the regression analyses between log-transformed values of measured and simulated concentrations of penicillin G in the milk and plasma from both calibration and evaluation datasets were all higher than 0.75 (Supplementary Figure 3). For the evaluation datasets, the value of overall R^2 for the IMM administration was 0.99 (Supplementary Figure 3C), and for the IM administration was 0.92 (Supplementary Figure 3D). The R^2 for data in milk was 0.81, and for plasma was 0.97 for the IM administration. All the MAPE values for calibration and evaluation results were lower than 40% (Supplementary Figure 4), which indicates that the model predictions are acceptable. In particular,

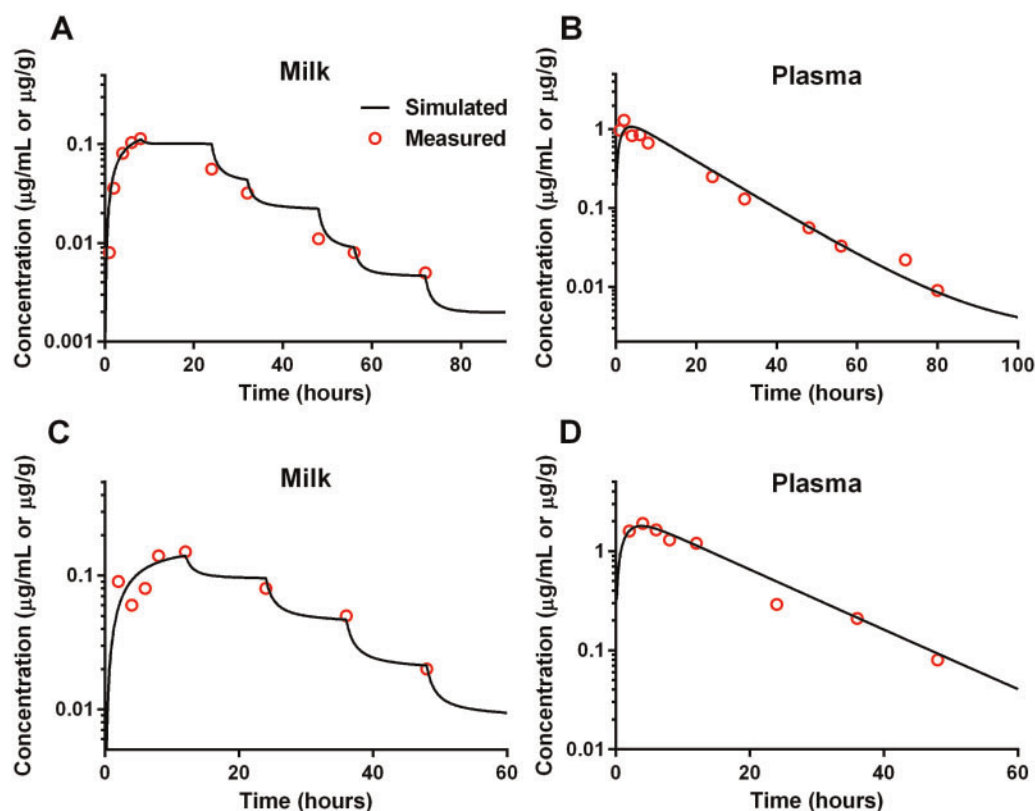


Figure 4. Calibration of the model using the pharmacokinetic data through the intramuscular (IM) injection. Comparison of model predictions (solid line) and observed data (red circles) for concentrations of penicillin G in the plasma and milk from daily cows exposed to procaine penicillin G via single IM injection (10 mg/kg, A, B; 40 mg/kg, C, D). Experimental data of panels A and B are from the study carried out by [Hogh and Rasmussen \(1966\)](#), and panels C and D are from the pharmacokinetic data reported by [Van OS et al. \(1974\)](#). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

the MAPE values for plasma for different simulations were generally lower than or near 20% indicating good predictions of plasma concentrations following either IM or IMM administrations. Overall, the PBPK model adequately simulates the measured results in the milk and plasma from the independent studies of dairy cows via both IMM and IM administrations.

Sensitivity Analysis

The sensitivity analysis was applied to screen the sensitive parameters in the PBPK model. Thirty-four model parameters in the PBPK model for dairy cows were analyzed using the method of local sensitivity analysis. Results of the local sensitivity analysis based on 1% variation of the parameter values are shown in [Supplementary Table 1](#). Only parameters with at least 1 absolute value of NSC > 0.15 are shown in the table. For physiological parameters, only the body weight (BW), the cardiac output (QCC), and the blood flow to kidney (QKC) were the relatively sensitive parameters for the model. The results indicate that the PBPK model was not that sensitive to physiological parameters. The rate constant of penicillin G absorbed from milk back into the udder tissue (K_{abC}) was the sensitive parameter for AUCs of the plasma, liver and kidney, and the fraction of undissolved procaine penicillin G (Frac) was sensitive for all AUCs. The AUC of liver was also highly sensitive to the liver partition coefficient (PL) with NSC value of 1.00. The AUC of kidney was highly sensitive to the urine elimination rate constant and the kidney partition coefficient with NSC values of -0.90 and 1.00 , respectively. For AUC of milk, it was sensitive to the body weight (BW), the maximum volume of the milk space in udder

($V_{Mmilksp}$), the time for V_{milk} reaching half of $V_{Mmilksp}$ (s), the ratio of the residual volume to the maximum volume of the milk space (F), and the maximum rate of penicillin G excretion in the mammary gland (V_{maxC} ; body weight-scaled) with NSC values of 0.99 , -0.99 , 0.61 , -0.24 , and 1.00 , respectively.

Probabilistic Analysis

The relatively sensitive parameters listed in [Supplementary Table 1](#) were subsequently used for probabilistic analysis based on the current PBPK model for penicillin G in dairy cows. The Monte Carlo sampling technique was applied for the probabilistic analysis. From the simulation results of the PBPK model after label and extralabel use of procaine penicillin G through IM and IMM administrations, the concentrations of penicillin G in the liver are higher than other edible tissues and plasma ([Supplementary Figs. 5B and 5D](#)). Therefore, the liver was chosen as the tissue to determine the WDIs for extralabel use of penicillin G in dairy cows. The Monte Carlo simulations showed that the WDIs after 3 repeated IMM infusions with label dose and $4\times$ label dose in dairy cows were 98 and 122 h for milk, and were both 0 h for edible tissues, respectively ([Figs. 7A–D](#)). The predicted WDIs after 3 doses via IM injections with label dose and $10\times$ label dose were 125 and 182 h for milk, and were 4 and 5 days for edible tissues ([Figs. 7E–H](#)). The exact model-predicted milk discard intervals are reported in this article, but in practice, the recommended milk discard intervals should be adjusted accordingly to fit within a milking schedule for a particular dairy farm. If the estimated WDI of edible tissues was a fraction of a day, it was rounded up to the next whole day. The results of the

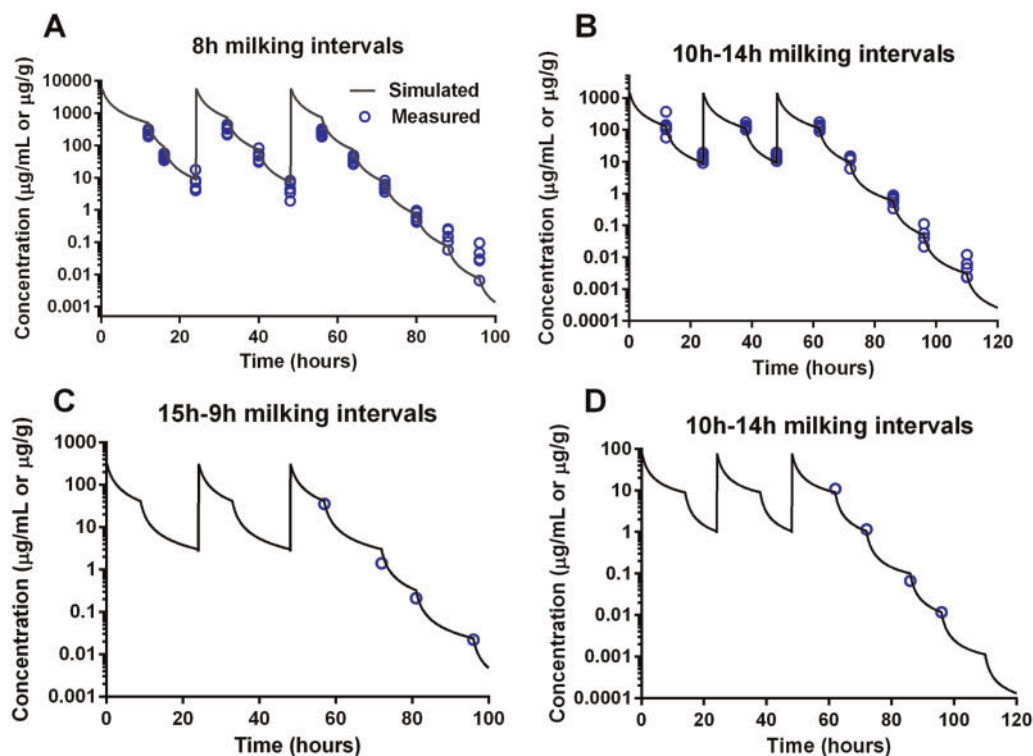


Figure 5. Evaluation of the model using intramammary (IMM) data with different milking intervals. Comparison of model predictions (solid line) and observed data (blue circles) for concentrations of penicillin G in the milk of dairy cows exposed to procaine penicillin G via IMM infusions for repeated 3 doses (1898 mg per quarter for all quarters, A, B; 100 mg per quarter for 2 quarters, C; 100 mg per quarter for all quarters, D) is shown. Experimental data for A, B (mean) are from the study reported by Knappstein et al. (2003), for panel C are from the previous pharmacokinetic study (Vilim et al. 1979), and for panel D are from the study performed by Vilim et al. (1980). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

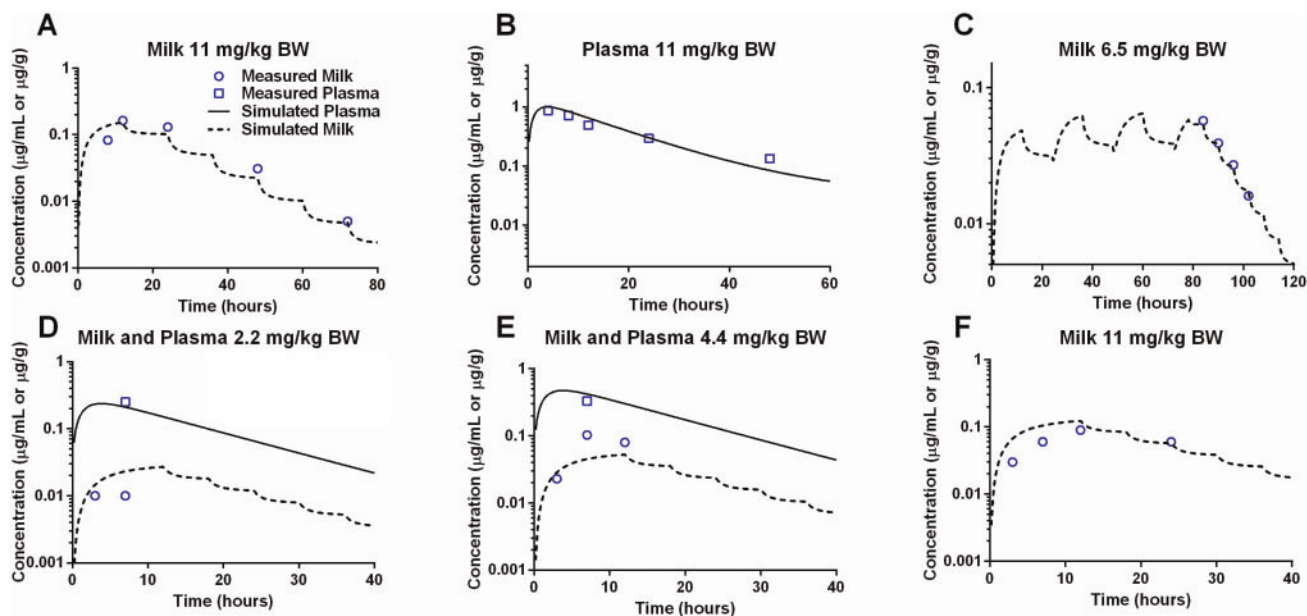


Figure 6. Evaluation of the model using intramuscular (IM) data with different milking intervals. Comparison of model predictions (solid line and/or dotted line) and observed data (blue squares or circles) for concentrations of penicillin G in the plasma and milk of dairy cows exposed to procaine penicillin G via 1 single IM injection (11 mg/kg, A, B; 2.2 mg/kg, D; 4.4 mg/kg, E; 11 mg/kg, F), and IM injection for repeated 4 doses (6.5 mg/kg, C) is shown. Experimental data for A, B (mean) are from the study reported by Randall et al. (1954), for panel C are from reported pharmacokinetic data (FDA, 2010), and for panels D-F are from the previous study by Edwards et al. (1966). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

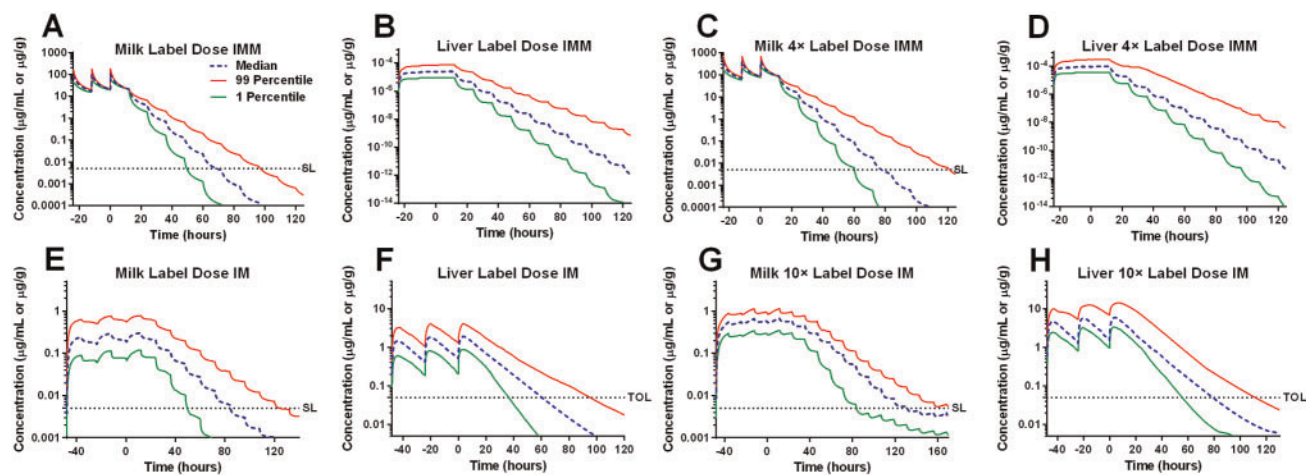


Figure 7. Probabilistic analysis for penicillin G concentrations in milk and liver via intramammary (IMM) and intramuscular (IM) administrations. IMM infusions with label dose (99 mg per quarter for 2 quarters) and 4× label dose (396 mg per quarter for 2 quarters) for 3 repeated doses were simulated as the therapeutic scenario for dairy cows (A–D). IM injections with label dose (6.5 mg/kg) and 10× label dose (65 mg/kg) for 3 repeated doses were simulated as the therapeutic scenario for dairy cows (E–H). The milking intervals were 12 h. Though zero tolerance was established for penicillin G in milk, the safe level (SL) of 5 µg/kg was used by FDA in actual practice. Tolerances (TOL) for penicillin G in edible tissues are 50 µg/kg.

probabilistic analysis of the PBPK model for 2× label dose through IMM infusions and 5× label dose following IM administrations are available in the [Supplementary Figure 6](#), and the results for probabilistic analysis in the plasma and kidney are provided in the [Supplementary Figure 7](#). No considerable differences were seen between the Monte Carlo simulation results between 1000 and 10 000 iterations ([Supplementary Figure 8](#)). The label withdrawal periods were obtained from the Veterinarian's Guide to Residue Avoidance Management (VetGRAM) of FARAD ([Riviere et al., 2017](#)). The labeled milk discard time via IMM infusions is 84 h, and for edible tissues the label withdrawal period is 4 days. The label withdrawal period of milk via IM injections is 48 h, and of edible tissues is 14 days in dairy cows.

DISCUSSION

In this study, a PBPK model for penicillin G in dairy cows was established for labeled routes of IM and IMM administrations. On the basis of model, probabilistic analysis was performed to predict the milk discard intervals and the extended WDIs for edible tissues after IM or IMM administration of procaine penicillin G at extralabel doses. The PBPK model for dairy cows was established and calibrated with pharmacokinetic data of penicillin G through IM and IMM administrations. As the systemic absorption and the drug excretion were both taken into account, the model can be used to predict the concentrations of penicillin G in milk and edible tissues through either the systemic or the local administration. Application of this PBPK model can help predict the extended milk discard intervals after extralabel use of penicillin G.

In the field of toxicology, several PBPK models with a milk compartment are available, such as the lactational PBPK models for atrazine in rodents ([Lin et al., 2013](#)), perfluorooctanoic acid in humans ([Loccisano et al., 2013](#)), manganese in humans ([Yoon et al., 2011](#)), and flunixin in dairy cows ([Leavens et al., 2014](#)). The milk compartments in all these models were described as virtual compartments and were not physiologically based, which limits the models' capability to extrapolate to other species. In the present model, a physiologically based approach simulating

the mammary gland compartment was established based on the milk production and episodic milking processes to facilitate realistic prediction of drug concentrations in the milk and systemic distribution through the IMM infusion. The Hill-Langmuir equation was adapted from previous milk model ([Whittem et al., 2012](#)) to simulate the decrease of milk production due to the increased hydrostatic pressure ([Mercer et al., 1970](#); [Smith et al., 2004](#); [Whittem et al., 2012](#)). The 1- and 2-compartment milk models were both adapted to the current PBPK model for penicillin G. The 1-compartment is good enough for the sparse data available from existing pharmacokinetics studies. The 2-compartment model could help predict the drug movements within the 2 compartments of mammary glands, which is useful for the estimation of the drug concentration at the diseased state. The 2-compartment model would help design therapeutic regimens of penicillin G and other veterinary drugs for cows. Both 1- and 2-compartment models provide a basis to simulate pharmacokinetics of penicillin G in mastitis cows. The systemic and local routes of drug administrations, the milk production process including the milk producing rate and milking intervals, and the movement of penicillin G such as the episodic milking process and transfer of penicillin G between the plasma, udder tissue and milk were all considered in the current PBPK model. This model greatly improves our understanding of the pharmacokinetic property of penicillin G in dairy cows, especially in the mammary glands after both IM and IMM administrations. This physiologically based approach simulating the milk compartment can be extrapolated to other species, including rats, humans, and goats.

Currently, different statistical methods, such as the "Time To Safe Concentration," the "Safe Concentration based on Linear Regression" and the "Safe Concentration Per Milking" methods, were used to calculate the withdrawal period for milk ([Henri et al., 2017b](#)). However, these methods have various limitations, including the assumption of linearity of the log-transformed tissue/milk depletion data, which may not be true for all drugs ([Chevance et al., 2017](#); [Henri et al., 2017b](#)). The non-linear mixed-effects pharmacokinetic modeling has many advantages ([Martin-Jimenez and Riviere, 1998](#)) as a pharmacostatistical approach to estimate the withdrawal period for milk

(Chevance *et al.*, 2017). If the pharmacokinetic data are not available for extralabel use of veterinary drugs, the method of half-life multipliers could be applied to calculate the extended WDIs (Riviere *et al.*, 1998; Smith *et al.*, 2004). However, the nonlinear mixed-effects pharmacokinetic model and the half-life multipliers method are not based on the mechanisms of drug action and the physiology, and cannot be extrapolated to different therapeutic scenarios and animal species. The current PBPK model for milk is a physiologically- and mechanistic-based model and could be extrapolated to the different species, drugs and therapeutic scenarios to predict tissue residues and milk concentrations.

The current model is based on the assumption that the 4 quarters of the udder tissues are identical in dairy cows, and no milk production changes were considered for different lactating stages. As the model simulations are about 2 weeks, the potential impact of lactational stages on the milk production was not considered in this model. On the basis of available research (Novak *et al.*, 2009; Yoon *et al.*, 2004), the lactational stages could be incorporated into the model if needed. The parameters of the absorption and excretion processes were determined through model fitting with pharmacokinetics data. These parameters are related to the organic cation transporters and organic anion transporters expressed in the mammary glands of dairy cows (Al-Bataineh *et al.*, 2009). However, the information on the expression of transporters related to the drug transport in mammary glands of dairy cows is limited. In the future, with more research on transporters in food-producing animals, the strategy of *in vitro* to *in vivo* extrapolation can be used to calculate the relevant parameter values for drug distribution through mammary glands. For some sensitive parameters related to the milk secretion, such as VMmilksp, *s*, and *F*, although their values were validated in an earlier study (Whittem *et al.*, 2012), the experimentally measured values are not available and not commonly reported by pharmacokinetic studies for drugs in milk. These values, whenever available experimentally, would help reduce uncertainties for the current milk model.

The binding of penicillin G to milk proteins is not considered in the current model, due to the limited information on protein binding of penicillin G in milk. The current model could be improved when more experimental data on protein binding of penicillin G in milk become available in the future. Also note that the milk is a suspension of fat droplets in an aqueous phase containing proteins, carbohydrates, and electrolytes, in which proteins can extensively bind some antibiotics, resulting in uneven distribution of the drug in different components of the milk (Ziv and Rasmussen, 1975). It has been shown that drug distribution in different fractions of milk can be very different for a systemic route of administration versus a local infusion depending on the drug lipophilicity (Ziv and Rasmussen, 1975). Thus, drug concentrations can be different between different milk samples (whole milk vs skim milk; foremilk vs hind-milk) depending on the drug lipophilicity and sampling methods. At this stage, there are no sufficient data that are granular enough to predict drug concentrations in different components of milk. Therefore, the present model may need some refinements depending on the investigated drugs (lipophilic vs hydrophilic), the dairy products of interest (whole milk vs skim milk), and the sampling methods (cisternal milk vs total milk). Additional studies are needed in order to establish a milk model considering different milk components, which will help model extrapolation to different animal species and different drugs. This is important because the milk compositions are different among different species (eg, 3.3%–5.4% fat in cow milk vs

5.3%–9.0% in buffalo vs 10.2%–21.5% in deer milk) (Claeys *et al.*, 2014) and different drugs have different interactions with milk components.

The milk discard interval for penicillin G following labeled IMM infusion predicted by the current model was in accordance with the labeled milk discard time from FDA (98 vs 84 h). The model-predicted milk discard interval following labeled IM injection was longer than the labeled milk discard time by FDA (125 vs 48 h), suggesting that the model-predicted result after labeled IM administration is relatively conservative. The inconsistency between the model-predicted results and the labeled milk discard time after IM administration may be due to different pharmacokinetic studies used in our model compared with the FDA method. The current model was based on a number of datasets from different studies using different drug formulations in different animal populations, thus the results consider the variability of a large diverse population of animals. On the other hand, the labeled milk discard time is typically obtained based on the data from a small set of animals. Overall, the present model results support the recommendations for the appropriate time to harvest milk following the use of penicillin G.

On the basis of simulation results, the model-predicted WDIs in the target tissue liver after labeled IMM or IM exposure were consistently much lower than the labeled withdrawal periods. Also, the IMM infusion did not lead to the violations in edible tissues even at 4× label dose. However, major violations of penicillin G were identified in cull dairy cows by USDA. Also, a prolonged elimination of penicillin G in emergency-slaughter dairy cows after IM administration of penicillin G has been reported (Nouws and Ziv, 1978). These inconsistencies may be due to the pharmacokinetic data used for the model calibration of the current model were all from healthy dairy cows. Mastitis and other diseased conditions, as well as the interactions with other drugs in diseased animals may lead to the different distribution and elimination patterns of penicillin G. Due to the fact that pathophysiological data of mastitis in dairy cows are limited, the diseased model for dairy cows with mastitis is not available now. The diseased model is needed to more accurately simulate tissue distribution of penicillin G and to avoid tissue residue violations in cull dairy cows. Thus, the present model cannot be used to predict extended WDIs in target tissue after extralabel use of penicillin G in cull dairy cows. The PBPK model in dairy cows with mastitis would be a future direction for our research when additional data become available.

In conclusion, the PBPK model for penicillin G in dairy cows successfully predicts the drug concentrations in the milk and plasma following IM or IMM administration of procaine penicillin G. This is the first reported PBPK model with a physiologically based compartment of mammary glands. By utilizing probabilistic Monte Carlo analysis, the model could help estimate a conservative milk discard time after extralabel use of penicillin G, thereby facilitating food safety assessment of cow-derived milk products. This PBPK framework can be applied to different antibiotics used for dairy cows to help avoid residue violations in the milk and reduce the amount of waste milk. This modeling strategy could also be extrapolated to other species, such as rodents, humans, and goats.

SUPPLEMENTARY DATA

Supplementary data are available at *Toxicological Sciences* online.

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