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The construction and application of a population physiologically based pharmacokinetic model for methadone in Beagles and Greyhounds

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Abstract

Methadone is an opioid analgesic in veterinary and human medicine. To help develop appropriate pain management practices and to develop a quantitative model for predicting methadone dosimetry, a flow-limited multiroute physiologically based pharmacokinetic (PBPK) model for methadone in dogs constructed with Berkeley Madonna™ was developed. The model accounts for intravenous (IV), subcutaneous (SC), and oral administrations, and compartmentalizes the body into different components. This model was calibrated from plasma pharmacokinetic data after IV administration of methadone in Beagles and Greyhounds. The calibrated model was evaluated with independent data in both breeds of dogs. One advantage of this model is that most physiological parameter values for Greyhounds were taken directly from the original literature. The developed model simulates available pharmacokinetic data for plasma concentrations well for both breeds. After conducting regression analysis, all simulated datasets produced an $R^2 > 0.80$ when compared to the measured plasma concentrations. Comparative analysis of the dosimetry of methadone between the breeds suggested that Greyhounds had ~50% lower 24-hr area under the curve (AUC) of plasma or brain concentrations than in Beagles. Furthermore, population analysis was conducted with this study. This model can be used to predict methadone concentrations in multiple dog breeds using breed-specific parameters.

KEYWORDS
Beagles, Greyhounds, methadone, opioids, physiologically based pharmacokinetic model

1 | INTRODUCTION

Methadone is an opioid used in veterinary and human medicine primarily for analgesic purposes, but also sedation in dogs. Methadone is known as a synthetic agonist at μ-opiate receptors, an antagonist on N-methyl-d-aspartate (NMDA) receptors, and as a norepinephrine and serotonin reuptake inhibitor (Clarke & Trim, 2014; KuKanich & Borum, 2008; KuKanich, KuKanich & Rodriguez, 2011). Methadone is often administered as a racemic mixture, with the R(−) enantiomer (levo-methadone) responsible for most of the μ-opiate

receptor agonist interactions (Clarke & Trim, 2014). Higher doses of methadone are required to antagonize NMDA receptors than doses that act as an agonist at μ-opiate receptors (Clarke & Trim, 2014).

In human medicine, 86% of methadone is plasma protein bound, predominantly to alpha-1-acid glycoprotein, and methadone is metabolized primarily by cytochrome P450 3A4 (Kapur, Hutson, Chibber, Luk & Selby, 2011). In dogs, the average plasma protein binding percentage is 64.8% (Derendorf & Garrett, 1983). Methadone is approved for usage in humans in the United States and has proven to be effective in treating some forms of pain that

are otherwise poorly managed by other opioids (Ingvast-Larsson, Holgersson, Bondesson, Lagerstedt & Olsson, 2010; Rettig & Yarmolinsky, 1995). However, methadone is not approved for canine usage in the USA and is used off-label (the use of a drug under the supervision of a veterinarian that does not align with the approved labeling) as a racemic mixture (FDA, 2016, 2017). In Europe, methadone (not including oral administrations containing povidone with a high molecular weight) is approved for human use (EMA, 2014). Canine usage of methadone is also approved in Europe and is available commercially in some countries (Clarke & Trim, 2014; Papich, 2015). In addition to being used for analgesia, methadone has also seen clinical usage in the form of a maintenance treatment for opioid-dependent human patients as an opioid replacement (Ward, Mattick & Hall, 1994). Methadone pharmacokinetics can be variable, and both humans and dogs have exhibited side effects of overexposure such as sedation, respiratory depression, and cardiac arrhythmias which can occur from a combination of high doses and/or slow metabolism (Carlquist et al., 2015; Lu, Zhou, Kreutz & Flockhart, 2011; Maiante, Teixeira Neto, Beier, Corrente & Pedroso, 2009; Modesto-Lowe, Brooks & Petry, 2010; Schlitt, Schroeter, Wilson & Olsen, 1978). Therefore, a robust predictive model is needed to evaluate the concentrations of methadone in physiologically relevant tissues.

Physiologically based pharmacokinetic (PBPK) modeling is a mathematical, mechanism-based technique that uses a variety of parameters (physiological- and chemical-specific) in order to predict the absorption, distribution, metabolism, and elimination of a chemical within an organism (Andersen, Clewell, Gargas, Smith & Reitz, 1987; Lin, Gehring, Mochel, Lave & Riviere, 2016). This modeling method is a quantitative simulation tool that has evolved within the last thirty years that can accomplish a variety of tasks such as predicting target tissue chemical/drug exposure concentrations and residues, helping construct therapeutic regimens, and allowing the evaluation of what impact the changing of physiological parameters has on tissue drug concentrations (Lin, Gehring, et al., 2016; Rowland, Peck & Tucker, 2011). PBPK models are a powerful tool for extrapolation across species, breeds, and exposure paradigms, as well as from in vitro to in vivo, and the models can integrate pharmacokinetic and toxicity mechanisms with many different compound properties to develop mechanistic models. Some setbacks also exist with this modeling method, such as the fact that model parameters may not be available outside of nondomestic species (Lin, Gehring, et al., 2016). PBPK modeling and other comparative pharmacometric methods such as population pharmacokinetic modeling have been used for a variety of veterinary drugs (Henri, Carrez, Meda, Laurentie & Sanders, 2017; Li et al., 2015; Yang et al., 2014; Zeng et al., 2017), although no PBPK model is available yet for methadone in dogs as well as a general lack of PBPK models for pain management in canines. This lack of PBPK modeling for opioids does not translate to humans; however, as multiple human models are available (Guo, Zhou, Li & Khanh, 2015; Shankaran, Adeshina & Teeguarden, 2013; T'Jollyn et al., 2015; Yang, Tong, McCarver, Hines & Beard, 2006). In particular, a PBPK model for methadone in humans that has been developed (Yang et al., 2006) was used to construct the model in this manuscript.

The objective of this study was to develop a population PBPK model for methadone in dogs, particularly the Beagle and Greyhound breeds, to predict tissue concentrations. This model is desired to be robust enough to enable model extrapolation to other breeds and species. Through the development of this model, the goal is to help design therapeutic regimens of methadone and provide a basis to improve our understanding of the pharmacokinetics after prolonged infusion of methadone and its metabolites in dogs. This study can also help to illustrate the utility of PBPK models to be able to extrapolate drug disposition from separate studies across different breeds. This study is necessary, because as stated before currently, no methadone PBPK model exists for dogs. The Greyhound breed was included in the model as data were available in the Greyhound to help construct a PBPK model to help use pharmacokinetics to understand the differences in methadone efficiency clinically observed between the two breeds (Robinson, Sams & Muir, 1986). Through this model, tissue concentrations of methadone can be approximated for the dog, an important ability as tissue samples cannot be harvested from dogs for methadone concentration measurements due to ethical reasons. Furthermore, this model can greatly help the understanding of methadone pharmacokinetics within Greyhounds as physiological parameters for Greyhounds are scarce and dispersed throughout the available literature. In effect, this study acts as a collection point for the data contained and dispersed throughout several publications and therefore allows the compilation of knowledge of the pharmacokinetic effects of methadone in Greyhounds within one study.

2 | METHODS

2.1 | Data sources for model calibration and evaluation

The independent datasets found in KuKanich and Borum (2008) and Ingvast-Larsson et al. (2010) were used to calibrate the Greyhound and Beagle portions of the model, respectively. For model evaluation, the independent datasets found in KuKanich et al. (2011), Ingvast-Larsson et al. (2010), and KuKanich, Lascelles, Aman, Mealey and Papich (2005) were used for the Beagle and Greyhound portions of the model. Representative information of each study is listed in Table 1. The time–concentration data from the graphs of these selected pharmacokinetic studies were extracted using WebPlotDigitizer (version 3.10, http://arohatgi.info/WebPlotDigitizer/). Individual animal data from KuKanich et al. (2005), KuKanich and Borum (2008), and KuKanich et al. (2011) were also used in this study.

2.2 | Model structure

In designing the model structure (Figure 1), multiple sets of information were taken into account, such as what a similar PBPK model used in its structure (Yang et al., 2006), the known

TABLE 1 Summary of methadone pharmacokinetic studies used during PBPK model calibration/evaluation

Species/Purpose	Route	Sex	n	BW (kg)	Dose (mg/kg)	Limit of quantification	References
Beagle							
Calibration	Intravenous	4M/5F	9	17	0.4	0.6 ng/ml	Ingvast-Larsson et al. (2010)
Evaluation	Subcutaneous	4M/5F	9	17	0.4	0.6 ng/ml	Ingvast-Larsson et al. (2010)
	Intravenous	3M/3F	6	7.3–13.0 (avg. 10.15)	1	20 ng/ml	KuKanich et al. (2005)
Greyhound							
Calibration	Intravenous	3M/3F	6	26.1–38.8 (avg. 32.45)	0.45	2 ng/ml	KuKanich and Borum (2008)
Evaluation	Oral	3M/3F	6	26.0–41.6 (avg. 33.8)	2.1	1 ng/ml	KuKanich et al. (2011)

Notes. All administrations had the following parameters: Formulation, methadone hydrochloride; Matrix, plasma; Assay: high-pressure liquid chromatography (HPLC). However, different papers used different variants.

KuKanich and Borum (2008) and Ingvast-Larsson et al., 2010 used HPLC-electrospray ionization-tandem mass spectrometry; KuKanich et al. (2011) used HPLC with mass spectrometry; and KuKanich et al. (2005) used HPLC with ultraviolet (UV) detection or fluorescence polarization immunoassay (FPIA). All pharmacokinetic studies reported racemic total methadone and not the analysis on individual methadone enantiomers.

pharmacokinetics of methadone in dogs, and how methadone is currently administrated in dogs (Ingvast-Larsson et al., 2010; KuKanich & Borum, 2008; KuKanich et al., 2005, 2011). In particular, the current model sought to resemble a previous methadone PBPK model in humans in order to allow for comparisons of methadone pharmacokinetics between dogs and humans (Yang et al., 2006). The administration routes included within the model structure are intravenous (IV), subcutaneous (SC), and oral, and pharmacokinetic data of methadone after administration via these routes were selected for model evaluation and calibration. For compartments, the model includes the nine compartments of the blood, muscle, lung, rest of body, brain, heart, liver, gastrointestinal (GI) tract, and kidney. Each of these tissue compartments has a weight and a blood flow rate. As methadone is highly lipophilic with a log Kow (octanol:water partition coefficient) of 3.93 (Hansch, Leo & Hoekman, 1995), all compartments are assumed to be flow-limited and well-stirred based on the methadone PBPK model in humans (Hansch et al., 1995; Yang et al., 2006). Additional support for using a flow-limited model lies within the successful performance of other PBPK models for small molecular weights that were flow-limited (Lin, Gehring, et al., 2016). The elimination routes for methadone included in the model were urinary excretion and hepatic metabolism. Mass balanced differential equations were used to illustrate the rate of change in each of the designed tissue compartments, and only methadone not bound to plasma proteins was considered to be available for use by the body. Berkeley Madonna™ (Version 8.3.23.0; University of California, Berkeley, CA, USA) was utilized to construct the model and conduct simulations, a software that has been used successfully in other PBPK models (Lin et al., 2017). The model code is contained within the Appendix S1 and is also available on our center's website (http://iccm.k-state.edu/).
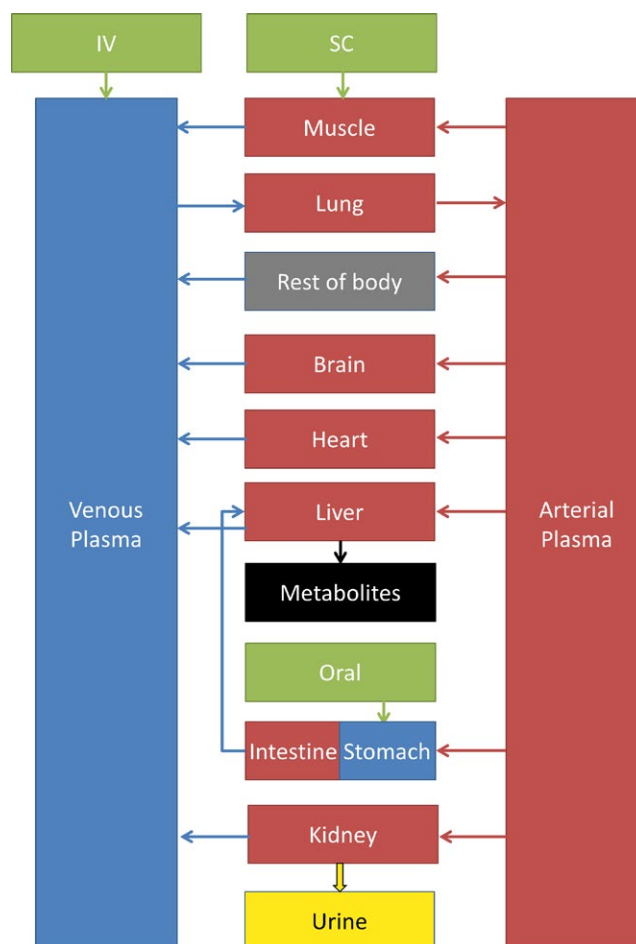

FIGURE 1 A schematic of the physiologically based pharmacokinetic model for methadone in dogs. IV, SC, and oral represent intravenous, subcutaneous, and oral administrations, respectively [Colour figure can be viewed at wileyonlinelibrary.com]

2.3 | Model parameterization

For the Greyhound, cardiac output was taken as an average between multiple studies (Frink et al., 1992; Hughes, Campbell & Fitch, 1980; Hughes, Mathie, Fitch & Campbell, 1979; Thomson, Fitch, Hughes, Campbell & Watson, 1986; Thomson, Hughes, Fitch & Campbell, 1982). The body weight obtained was study specific (KuKanich & Borum, 2008; KuKanich et al., 2011). The Greyhound blood flow rates were taken from a paper that addressed the way cardiac output in dogs is redistributed during heat stress after reporting the values for normal Greyhounds (Hales & Dampney, 1975). Tissue volumes were taken from a variety of different papers (Courtice, 1943; Crile & Quiring, 1940; Gunn, 1978; Hales & Dampney, 1975; Kesl, 1993).

For physiological parameters of the Beagle, almost all cardiac output, blood flow, and tissue volume parameters were from Brown, Delp, Lindstedt, Rhomberg and Beliles (1997), a paper well-known in the PBPK modeling community. The physiological parameter for the GI tract blood flow comes from a specific study on gastrointestinal blood flow in the mongrel dog (Delaney & Cluster, 1965). All physiological parameters for both species are described in Table 2.

For chemical-specific parameters, the tissue/plasma partition coefficients (PC) were obtained from averaging the partition coefficients of the two methadone enantiomers from experimental methadone data in humans (Yang et al., 2006). This model estimated the urine and metabolic elimination rate constants as well as the gastric emptying, intestinal absorption, intestinal transit, and subcutaneous absorption rate constants using the curve-fitting tool in Berkeley Madonna. These rate constants were estimated because no information regarding these parameters could be found in the literature on the topic. These estimated parameters were further optimized manually via iterative simulations if needed to better visually fit the experimentally measured data. During the curve-fitting process, zero was taken as the lower bound of possible values of the model parameters and the three oral administration parameters (gastric emptying, intestinal absorption, and intestinal transit rate constants) were not allowed to exceed five 1/hr. The construction of the model followed rules of parsimony to avoid unnecessary complexity. All chemical-specific parameter values are contained in Table 3.

TABLE 2 Physiological PBPK model parameters used in Beagle and Greyhound PBPK models for methadone

Parameter	Beagle	Source	Greyhound	Source
Body weight (kg)	17.0 (Cal/Evl)	1	32.45 (Cal)	5
	10.15 (Evl)	2	33.8 (Evl)	6
Cardiac output (L/hr/kg)	12.9	3	10.504	7
Tissue volumes (fraction of body weight, unitless)				
Liver	0.0329	3	0.0436	8
Kidney	0.0055	3	0.00645	8
Muscle	0.4565	3	0.571	9
Brain	0.0078	3	0.00432	8
Lung	0.0082	3	0.0148	8
Heart	0.0078	3	0.0126	8
GI tract	0.0368	3	0.0248	8
Blood	0.082	3	0.114	11
Blood flow rates (fraction of cardiac output, unitless)				
Liver	0.046	3	0.0515	10
Kidney	0.173	3	0.145	10
Muscle	0.217	3	0.4546	10
Brain	0.02	3	0.0134	10
Heart	0.046	3	0.0618	10
GI tract	0.1	4	0.1121	10

Notes. 1. Ingvast-Larsson et al. (2010); 2. KuKanich et al. (2005); 3. Brown et al. (1997); 4. Delaney and Cluster (1965); 5. KuKanich and Borum (2008); 6. KuKanich et al. (2011); 7. Average between Frink et al. (1992), Hughes et al. (1980), Thomson et al.(1986, 1982), and Hughes et al. (1979); 8. Crile and Quiring (1940); 9. Gunn (1978); 10. Hales and Dampney (1975); 11. Courtice (1943). Cal: Calibration. Evl: Evaluation.

TABLE 3 Chemical-specific parameters used in the PBPK model for methadone in Greyhounds and Beagles

Parameters	Description	Value	Source
PM	Muscle–plasma PC	3.852	1
PLu	Lungs–plasma PC	42.46	1
PBr	Brain–plasma PC	2.076	1
PH	Heart–plasma PC	9.233	1
PL	Liver–plasma PC	19.46	1
PG	GI Tract–plasma PC	7.922	1
PK	Kidney–plasma PC	10.61	1
PR	Rest of body–plasma PC	5.31[a] (5.44)[b]	2
$KmC\ (hr \times kg)^{-1}$	Metabolic elimination rate constant	0.1[a] (0.02)[b]	Estimated
$Ksc\ (hr^{-1})$	SC absorption rate constant	0.14[b]	Estimated
KurineC (L/hr/kg)	Urine elimination rate constant	2[a] (0.8)[b]	Estimated
$Kst\ (hr^{-1})$	Gastric emptying rate constant	5[a]	Estimated
$Ka\ (hr^{-1})$	Intestinal absorption rate constant	0.1[a]	Estimated
$Kint\ (hr^{-1})$	Intestinal transit rate constant	0.8[a]	Estimated
PB	Plasma protein binding percentage	64.8%	3

Notes. 1. Average of enantiomers (Yang et al., 2006); 2. Weighted arithmetic mean of other partition coefficients based on tissue volumes; 3. Derendorf & Garrett, 1983; a, represents value in Greyhounds; b, represents value in Beagles. PC, partition coefficient. Unless otherwise noted, values are assumed to be the same for both breeds. Unless otherwise noted, a parameter is unitless.

2.4 | Model calibration

The datasets used for model calibration were 0.4 IV mg/kg and 0.45 IV mg/kg administrations of methadone, the former for Beagles and the latter for Greyhounds (Ingvast-Larsson et al., 2010; KuKanich & Borum, 2008).

2.5 | Breed extrapolation

The Beagle was the first breed of the model that was calibrated as the needed parameters were more readily available for the Beagle, while the parameters needed for the Greyhound required a more exhaustive literature search. The Greyhound was extrapolated from the Beagle by changing physiological parameters to make them Greyhound-specific. In the Greyhound model, the metabolic rate and urine elimination rate constants were changed after model calibration. Other parameters (i.e., SC absorption rate constant, gastric emptying rate constant, intestinal absorption rate constant, intestinal transit rate constant, and plasma protein binding percentage) were held constant between the Beagle and the Greyhound. Chemical-specific parameters for both breeds are provided in Table 3.

2.6 | Model evaluation

The model was used to compare model predictions to experimental data that were not used when calibrating the model. Model evaluation followed World Health Organization (WHO) PBPK modeling guidelines (WHO, 2010), where if simulated values are within a factor of two of the predicted values, the model is considered tolerable. The WHO guidelines are made with the knowledge that calibration and evaluation datasets are obtained under different experimental conditions, so a level of difference between these datasets is expected (WHO, 2010). Linear regression analysis was used to obtain determination coefficients (R^2) to measure how well the log-transformed simulated data fits the log-transformed measured data. The mean absolute percentage error (MAPE) analysis was performed for all data used in calibration and evaluation following previously reported methods (Cheng et al., 2016; Li, Gehring, Riviere & Lin, 2018). The values of MAPE lower than 50% were considered as acceptable predictions. The sensitivity analysis was conducted to determine what parameters the 24-hr area under the time–concentration curve (AUC) of plasma and brain are particularly sensitive to and therefore cause the greatest impacts on the pharmacokinetics of methadone.

2.7 | Sensitivity and uncertainty analysis

Sensitivity analysis was conducted to identify model parameters with a significant impact on the plasma and tissue AUCs of methadone. To accomplish this, every model parameter was increased by 1% and the model responses of the tissue and plasma concentration AUCs to these increases were calculated. Within this analysis,

normalized sensitivity coefficients (NSC) were calculated by the following equation (Lin, Fisher, Ross & Filipov, 2011):

$$NSC = \frac{\Delta r}{r} \times \frac{p}{\Delta p}$$

In this equation, p represents the original value of the altered parameter (e.g., PC of liver of methadone), Δp represents the change in the altered parameter (1% increase), r represents the value of the response variable (e.g., AUC brain concentration for methadone), and Δr represents the change in the response variable as the result of the change in the altered parameter. The larger the value of the NSC, the more sensitive a parameter is considered to be for this method. Furthermore, a positive NSC value indicates a direct correlation between the altered parameter and the response variable, and a negative value indicates an inverse relationship between the altered parameter and the response variable. An NSC value of one indicates a one-to-one relationship between the altered parameter and response variable. For the sake of this study, any parameter that produced a |NSC| ≥ 0.2 was considered sensitive enough to be significant. Uncertainty analysis was conducted based on the following criteria (Lin, Monteiro-Riviere, et al., 2016; Teeguarden & Riviere 2005):

- Low (L): data were available directly for the specific breed in question.
- Medium (M): parameter value scaled from another breed/species with a high likelihood that this scaling is valid between the breed/species.
- High (H): data were not directly available for this parameter, and therefore it had to be estimated based on fitting of the model.

2.8 | Monte Carlo analysis

Monte Carlo analysis was undertaken in order to analyze the uncertainty and interindividual variability of methadone pharmacokinetics by sampling sensitive parameter values within a plausible range with established limits of the 2.5th and 97.5th percentiles of each parameter. These simulations produce a hypothetical breed population with parameters that are distributed around the mean values used for calibration in Tables 2 and 3. For this analysis, a normal distribution was assumed for physiological-specific parameters (e.g., blood flows, tissue volumes, cardiac output) and a log-normal distribution was assumed for chemical-specific parameters (e.g., partition coefficients) (Clewell, Gentry, Covington & Gearhart, 2000; Li, Gehring, Riviere & Lin, 2017; Shankaran et al., 2013; Tan et al., 2006; Yang, Doerge, Teeguarden & Fisher, 2015). Within this analysis, model parameters are randomly varied around the model calibration values. The variability of parameters was constructed from variability in previous experiments, which if were not available were then calculated from previously reported interindividual variability. A thorough literature search was conducted to find studies that reported variance. The previously reported coefficients of variation are 20% for partition coefficients and 30% for the rest of the model parameters (Clewell et al., 2000; Shankaran et al.,

2013; Tan et al., 2006; Yang et al., 2015). The one exception for this is that the coefficient of variation for the liver was set at 0.3 instead of the calculated coefficient of variation because this calculated value produced a negative lower bound. The upper and lower bounds of variation distribution are the mean plus or minus 1.96 times the standard deviation (2.5th and 97.5th percentiles) in order to ensure physiological plausibility and model mass balance (Yang et al., 2015). The model was run 1,000 times with randomly sampled model parameters from the predefined distributions. As parameters were randomly chosen in this analysis, the model could be anatomically or physiologically inaccurate in that, for example, all blood flows may sum to be less or more than the cardiac output because the component flows are randomized. In order to ensure mass balance and physiological plausibility within the physiological model parameters (e.g., fractional blood flow summation equaling one), these parameters were selected in a fractional manner through the usage of adjustment factors. The implementation of normal and log-normal distributions of parameters in Berkeley Madonna™, was based on our recently reported method (Li

et al., 2017). The Monte Carlo analysis used the same therapeutic scenarios that were used in model calibration for both breeds. The sensitive parameters used in Monte Carlo analysis can be seen in Table 4.

2.9 | Model application

This model was used to predict the plasma and brain methadone 24-hr AUCs, which allows the direct comparison of the internal dose metrics of both plasma and tissue among different treatment regimens and between different breeds of dogs. These data can help veterinarians in constructing optimal therapeutic plans for methadone in dogs.

3 | RESULTS

3.1 | Model calibration

For model calibration, the simulated data predictions from the flow-limited model were compared to the observed experimental data that

TABLE 4 Sensitive parameters used for Monte Carlo analysis

Parameters	Abb.	Dist.	Mean	SD	CV	LB	UB	Ref.	UN.
Beagle									
Body weight (kg)	BW	Normal	17.00	3.100	0.182	10.92	23.08	1	L
Liver volume	VLC	Normal	0.033	0.002	0.073	0.028	0.038	2	L
Liver blood flow	QLC	Normal	0.297	0.089	0.300	0.122	0.472	2	L
GI Tract blood flow	QGC	Normal	0.100	0.030	0.300	0.041	0.159	3	L
Brain PC	PBr	Log-normal	2.076	0.415	0.200	1.381	3.001	5	M
Liver PC	PL	Log-normal	19.46	3.892	0.200	12.94	28.13	5	M
Hepatic metabolic rate (hr × kg)$^{-1}$	KmC	Log-normal	0.020	0.006	0.300	0.011	0.034	4	H
Urinary elimination rate constant (L/hr/kg)	KurineC	Log-normal	0.800	0.240	0.300	0.431	1.362	4	H
Greyhound									
Body weight (kg)	BW	Normal	32.45	5.278	0.163	22.11	42.79	6	L
Liver volume	VLC	Normal	0.044	0.013	0.300	0.018	0.069	7	L
Liver blood flow	QLC	Normal	0.052	0.015	0.300	0.021	0.082	8	L
GI Tract blood flow	QGC	Normal	0.112	0.034	0.300	0.046	0.178	8	L
Oral intestinal absorption	Ka	Log-normal	0.100	0.030	0.300	0.054	0.170	4	H
Oral intestinal transit	Kint	Log-normal	0.800	0.240	0.300	0.431	1.362	4	H
Brain PC	PBr	Log-normal	2.076	0.415	0.200	1.381	3.001	5	M
Liver PC	PL	Log-normal	19.46	3.892	0.200	12.94	28.13	5	M
Hepatic metabolic rate (hr × kg)$^{-1}$	KmC	Log-normal	0.100	0.030	0.300	0.054	0.170	4	H
Urinary elimination rate constant (L/hr/kg)	KurineC	Log-normal	2.000	0.600	0.300	1.078	3.406	4	H

Notes. Abb, abbreviation; Dist, distribution; LB, lower bound; Ref, references; UB, upper bound; UN, uncertainty analysis. L, M, and H stand for low, medium, and high uncertainty, respectively. 1. Ingvast-Larsson et al. (2010); 2. Brown et al. (1997); 3. Delaney and Cluster (1965); 4. Model fitted; 5. Yang et al. (2006); 6. KuKanich and Borum (2008); 7. Crile and Quiring (1940); 8. Hales and Dampney (1975).

measured serum methadone concentrations (Figure 2). The model prediction of an administration of 0.4 mg/kg via IV to an average Beagle fit the experimental data well. The model also adequately simulated the plasma kinetics of methadone in Greyhounds after 0.45 mg/kg IV administration. The accuracy of the IV calibration datasets was high, with R^2 values of 0.93 and 0.95, and MAPE values of 26% and 24% for the Beagle and Greyhound calibration datasets, respectively.

3.2 | Model evaluation

For model evaluation, datasets that were not used to calibrate the model were used to evaluate the accuracy of the parameters calibrated with the model calibration datasets (Figure 3). The datasets chosen for evaluation were 0.4 mg/kg SC, 1.0 mg/kg IV, and 2.1 mg/

FIGURE 2 Model calibration results. (a) compares model predictions to a 0.4 mg/kg IV administration dataset used for Beagle model calibration (Ingvast-Larsson et al., 2010), whereas (b) compares model predictions to a 0.45 mg/kg IV administration dataset used for Greyhound model calibration (KuKanich & Borum, 2008). R^2 stands for the determination coefficient of linear regression analysis, and MAPE is the value for the mean absolute percentage error analysis [Colour figure can be viewed at wileyonlinelibrary.com]
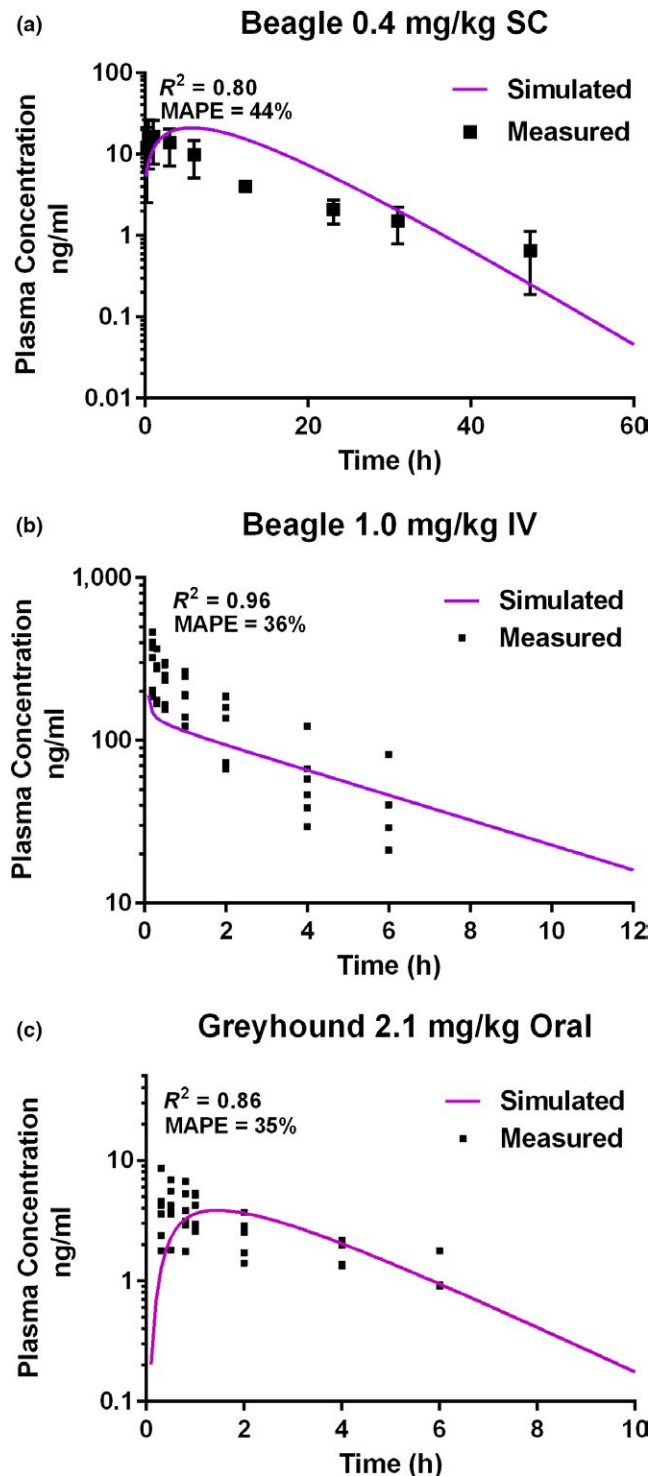
FIGURE 3 Model evaluation results. (a) compares model predictions to a 0.4 mg/kg SC administration dataset used for Beagle model route-extrapolation evaluation (Ingvast-Larsson et al., 2010), (b) compares model predictions to a 1.0 mg/kg IV administration dataset used for Beagle model evaluation (KuKanich et al., 2005), and (c) compares model predictions to a 2.1 mg/kg oral administration dataset used for Greyhound model evaluation (KuKanich et al., 2011). R^2 is the determination coefficient of linear regression analysis, and MAPE represents the value for the mean absolute percentage error analysis [Colour figure can be viewed at wileyonlinelibrary.com]

kg oral administrations, the former two in Beagles and the latter in Greyhounds, respectively. The predicted kinetic profiles match the measured plasma concentration data, and any differences are within the two-fold limits placed by the WHO guidelines. The regression analysis for the evaluation datasets indicated strong fits, with an R^2 value of 0.96 and MAPE value of 36% for the 1.0 mg/kg IV dataset and an R^2 value of 0.86 and MAPE value of 35% for the oral dataset (KuKanich et al., 2005, 2011). However, the accuracy of the model prediction of the SC evaluation dataset in Beagles was not as strong, with an R^2 value of 0.80 and MAPE value of 44%. While not a completely telling statistical test, the R^2 values indicate an adequate level of model performance. The final time point from the original study of the Beagle 1.0 mg/kg IV administration (KuKanich et al., 2005) was not included in the figure for this manuscript due to concerns of accuracy brought about from detection and quantification limits. Overall, the model adequately predicts concentrations as they simulate the measured experimental results from previous independent pharmacokinetic studies.

3.3 | Sensitivity analysis

The following parameters had a significant effect on either the AUCCV (plasma AUC) or the AUCCB (brain AUC) for any of the three administration routes: liver blood fraction, GI tract blood fraction, body weight, relative liver weight, brain:plasma partition coefficient, liver:plasma partition coefficient, intestinal absorption rate constant, intestinal transit rate constant, urine elimination rate constant, and the metabolic rate constant. Results from this analysis can be seen in Table 5 for any parameter with an |NSC| ≥ 0.2.

3.4 | Model application

After construction, calibration, and evaluation of the model, application was conducted by comparing the internal dosimetry of both Greyhounds and Beagles (Figure 4). This comparison was done by obtaining the 24-hr AUC tissue concentration values for the brain and venous plasma in both species. Beagles appeared to have higher concentrations in both the brain and venous plasma 24 hr after 1.0 mg/kg IV administration than Greyhounds, and IV administration appeared to result in slightly higher methadone concentrations in the brain and the venous plasma after 24 hr than SC administration in the Beagle. This comparison also indicates that a low amount of methadone concentration is noted in the brain and venous plasma after oral administration of methadone in Greyhounds compared to the IV administration within this breed.

3.5 | Monte Carlo analysis

Based on population PBPK simulation results shown in Figure 5, methadone concentrations in the brain tend to be higher than concentrations that were found in the plasma, which is expected based on the volume of distribution of methadone (Yang et al., 2006). The concentration of methadone in the plasma and brain tissue appears to show biphasic curves, with the biphasic nature of the curves most pronounced in the brain tissue concentrations of Greyhounds. Further results can be established with the inclusion of the minimum effective concentration (MEC) for therapeutic purposes as well as the concentrations of methadone where toxic effects begin to be seen. Based on human studies, the MEC of methadone (the lowest concentration required to produce a desired effect) is considered to be 29 ± 15 ng/ml for treating cancer pain and is graphically represented in Figure 5 (Foster, 2001; Gourlay, Cherry & Cousins, 1986). Graphing the MEC can help to show the population time range where methadone is considered to be effective, which from these data are 4.1–6.7 hr for Beagles after 0.4 mg/kg IV administration and 1.5–2.8 hr for Greyhounds after 0.45 mg/kg IV administration. Further studies can establish

TABLE 5 Normalized sensitivity coefficients (NSCs) of representative parameters produced during sensitivity analysis of the PBPK model

| Parameters | IV (0.4 mg/kg) | | SC (0.4 mg/kg) | | Oral (2.1 mg/kg) | |
	AUCCV	AUCCB	AUCCV	AUCCB	AUCCV	AUCCB
QLC	N/A	N/A	N/A	N/A	0.78	0.78
QGC	N/A	N/A	N/A	N/A	−0.45	−0.45
BW	−0.50	−0.50	−0.47	−0.47	−0.98	−0.98
VLC	−0.50	−0.50	−0.47	−0.47	−0.98	−0.98
PBr	N/A	1	N/A	1	N/A	1
PL	−0.51	−0.51	−0.48	−0.48	−0.98	−0.98
Ka	N/A	N/A	N/A	N/A	0.47	0.47
Kint	N/A	N/A	N/A	N/A	−0.46	−0.46
KurineC	−0.22	−0.22	−0.21	−0.21	N/A	N/A
KmC	−0.50	−0.50	−0.47	−0.47	−0.98	−0.98

Notes. Parameters are included in the table if the |NSC| ≥ 0.2. AUCCB: area under the concentration curve of methadone in the brain; AUCCV: area under the concentration curve of methadone in the plasma. IV, SC, and oral represent intravenous, subcutaneous, and oral administrations, respectively. The description of each parameter refers to Tables 2–4.
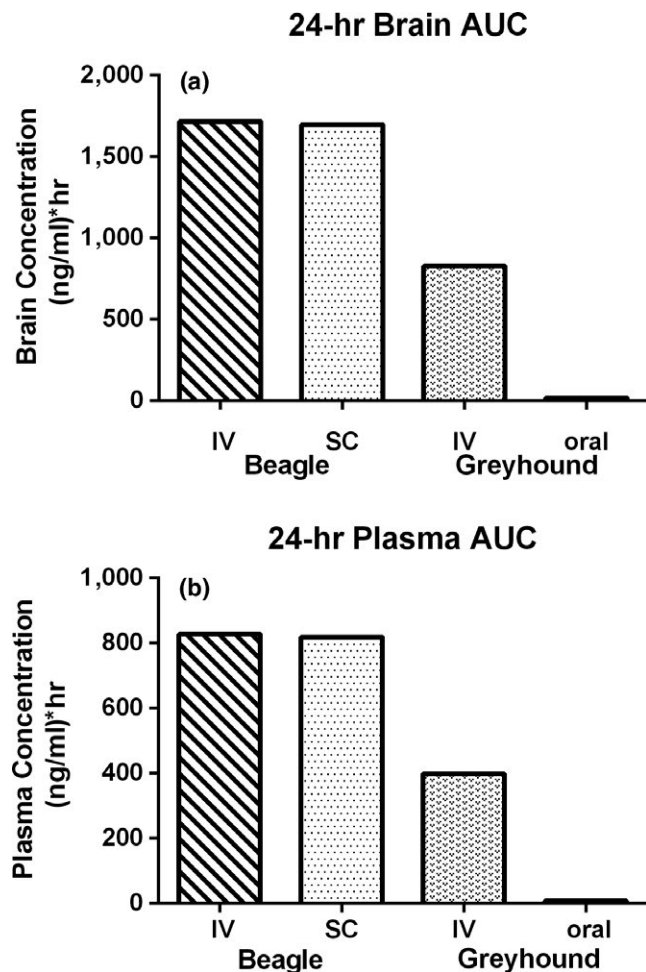
24-hr Brain AUC

24-hr Plasma AUC

FIGURE 4 Comparison of internal plasma and brain dosimetry of methadone between Beagles and Greyhounds. (a) shows the 24-hr area under the time–concentration (AUC, (ng/ml) × hr) of methadone in the brain, while (b) shows the 24-hr AUC ((ng/ml) × hr) of methadone in the plasma. Methadone at 1.0 mg/kg was given across administration routes and breeds

a MEC for plasma in dogs that would give ranges predicted by this model with more validity.

The MEC for plasma can also be applied to predict brain tissue concentrations at the corresponding plasma MEC, allowing these brain tissue concentrations to be estimated without taking tissue samples. For the Beagle, brain tissue concentrations at the plasma MEC ranged from 58.41–59.86 ng/g, whereas in Greyhounds these brain tissue concentrations ranged from 59.70–62.93 ng/g.

4 | DISCUSSION

A PBPK model for methadone in the Beagle and Greyhound breeds was developed and Monte Carlo population analysis considering sensitive parameter variances was applied. This model is the first PBPK model in dogs for methadone. The model successfully simulates the plasma and brain methadone dosimetry, an important

factor in considering target site-specific dose–response considerations and extrapolation. This model also allows for the simultaneous dose–response simulations of both plasma and tissues. Furthermore, this model offers an advantage because nearly all physiological parameter values for the Greyhound were directly taken from original literature. Therefore, this model can help greatly in the understanding of methadone pharmacokinetics with the Greyhound, which currently has little information. This model can be used for predicting tissue concentrations for different therapeutic regimens as well as providing an estimate of a population-level response to methadone.
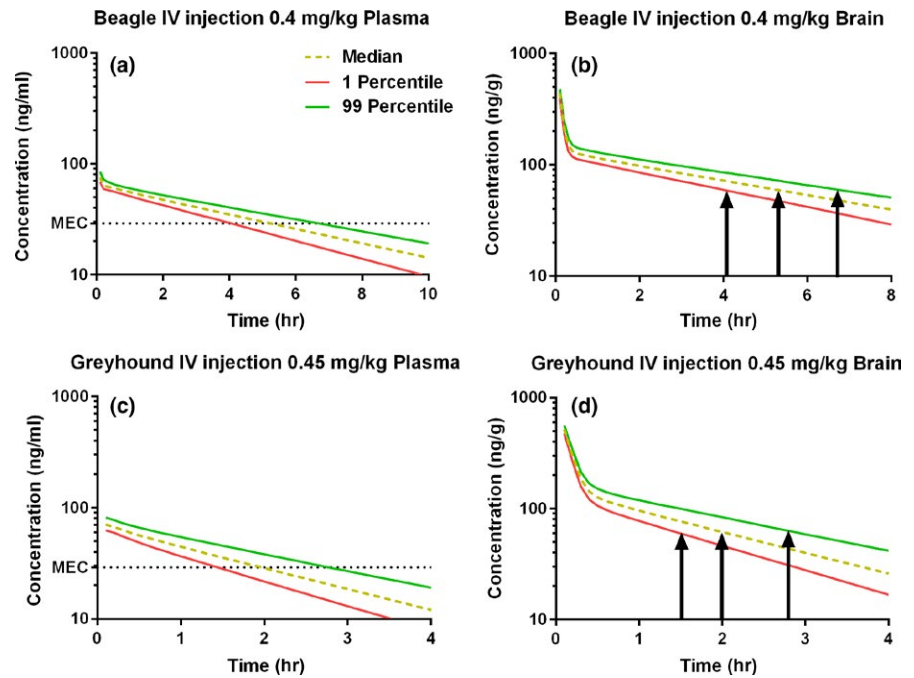
The success of this model also offers the opportunity for extrapolation. Model application predictions indicate that (a) IV administration will result in a slightly higher brain 24-hr AUC methadone concentration than SC administration, (b) IV administration will result in a substantially higher brain 24-hr AUC methadone concentration than an oral administration, and (c) Beagle 24-hr brain AUC of methadone appears to be slightly higher than Greyhound 24-hr brain AUC for the same administration route and dose.

This current model can offer several advantages over traditional pharmacokinetic models, such as real-world separate tissue compartments that help to estimate individual tissue concentrations as well as extrapolation beyond the range of given experimental pharmacokinetic data. PBPK models have multiple advantages in tissue concentration predictions, such as being based on the mechanistic action of a chemical in question (Lin, Gehring, et al., 2016). This technique allows for drug concentration predictions in any tissue of interest that has been incorporated into the model structure. Furthermore, PBPK modeling allows for the continuous introduction of new data which can reduce uncertainty even further as well as allowing for population analysis. Once in vivo metabolic capacity and breed-specific enzyme expression data become available, they can be incorporated into the model to provide a more physiologically based estimate of the metabolic elimination rate constant between the two species. PBPK modeling also allows for the analysis of altered tissue or organ function on the pharmacokinetics of a chemical by altering parameters and can therefore be used to evaluate the effects of disease and drug interaction on methadone pharmacokinetics.

This model was able to successfully predict datasets not used within calibration, and therefore has a strong degree of predictive ability. Therefore, the model can be extrapolated to other exposure routes. This model can help to lay the foundation for developing PBPK models in other breeds and species.

Since collection of methadone tissue concentration data can raise ethical concerns, the ability of this model to predict methadone tissue exposure is crucial. This model was able to make adequate predictions using PCs from humans (Yang et al., 2006). Therefore, this model further validates the fact that parameter values that exist in certain species but not in others can be used as surrogates as long as the organs have similar function and structure (Lin, Li, Gehring & Riviere, 2015). This surrogate ability is essential when trying to model data-poor species or breeds.

FIGURE 5 Monte Carlo simulation for methadone concentrations in the plasma and brain of Beagles and Greyhounds. The median, 1st, and 99th percentiles were calculated from the averages of 1,000 batch runs on Berkeley Madonna. The dashed line on the plasma graphs represents the minimum effective concentration (MEC) of methadone in plasma adapted from the human methadone study by Gourlay et al. (1986), a value of 29 ± 15 ng/ml. The arrows in the brain graphs represent the corresponding concentrations for methadone in the brain at the times when the simulated concentrations of methadone in plasma fell below the minimum effective concentration in the plasma graphs [Colour figure can be viewed at wileyonlinelibrary.com]

Furthermore, the use of accurate PCs is necessary due to how sensitive PCs are to methadone exposure. The results suggest that the model can predict plasma and tissue exposure over a wide range of therapeutic regimens. The simulated plasma and tissue data can help to design optimal therapeutic regimens for methadone in the Beagle and Greyhound, particularly when considering the model's ability to simulate total concentration of methadone. In this model, plasma protein binding was considered while analyzing the pharmacokinetics of methadone in dogs. As only total methadone concentration data are available in the dog, the present model does not simulate the two methadone enantiomers separately. However, once data on individual enantiomer concentrations become available, the present model can be revised to simulate the kinetic profiles of individual enantiomers. Other factors that affect pharmacokinetics can be considered if more data becomes available for these two breeds.

This model can be used as a quantitative tool to predict brain concentrations of methadone and can help to illustrate the contribution of methadone to therapeutic benefits as the model provides adequate simulations of methadone brain dosimetry. Studies have demonstrated methadone detection and effects in the nervous system, therefore, the amount of methadone the nervous system is exposed to in a therapeutic regimen is important in determining the effects on a patient (Johnson & Rosecrans, 1980; Pertschuk, Ford & Rainford, 1978). This model incorporates the plasma protein binding of methadone, which is important as the therapeutic effects of methadone protein binding are well noted (Abramson, 1982; Garrido et al., 2000; Mohamad et al., 2012; Szeto, Umans, Umans & McFarland, 1982; Wilkins et al., 1997). Brain tissue concentrations are also important when looking at potential respiratory depression due to methadone, an important side effect when constructing therapeutic regimens. However, the exact mode of action and mechanisms of methadone in the brain are unclear and need further

investigation. In addition, by incorporating the urine production data for dogs treated with methadone (Hellebrekers, Mol, Van den Brom & Van Wimersma Greidanus, 1987), the current model can be used to predict the concentration of methadone in urine. Due to no reported methadone concentration in urine of dogs available, the simulated urine concentrations were not included. This study can act as a foundation to incorporate drug–drug interactions with methadone and CPY3A inhibitors or inducers once more data become available, such as in vitro data of other drugs that associate with the same enzyme or receptors as methadone.

For this model, the range of partition coefficients is within 2–20, with the exception of the lung:plasma PC. As previously noted, methadone is a highly lipophilic compound, a property that greatly affects the PC values observed for methadone. The observed PC values can change with altered lipid composition of a tissue. The large PC difference for the lungs could be due to a different plasma membrane lipid composition when compared to other organs, although more research is needed.

With regards to oral bioavailability, the model predicted the intestinal absorption rate constant for methadone. This model can calculate the fecal content of methadone by subtracting the amount absorbed from oral exposure from the oral dose. The model can also be used to predict oral bioavailability by comparing the predicted plasma concentration profiles after oral and IV administrations with the same dose level. Both the intestinal absorption rate constant (Ka) and the intestinal transit rate constant (Kint) have a high impact on methadone tissue exposure and are both estimated; therefore, these parameters add uncertainty to predictions. Methadone oral bioavailability is low as part of the high first-pass metabolism of opioids in the intestines and liver with dogs, although bioavailability can be increased with the coadministration of CYP-inhibiting drugs (Clarke & Trim, 2014; KuKanich et al., 2005, 2011). Due to the uncertainty

with certain oral administration parameters, caution should be applied when using the oral model to predict plasma concentrations and tissue exposure. Further studies on the oral absorption and elimination characteristics of methadone are needed.

Monte Carlo analysis was conducted by looking at the variance of sensitive parameters. This technique has been used previously in PBPK modeling (Li et al., 2017; Shankaran et al., 2013; Yang et al., 2015). A benefit of this technique is that the variances of the parameters are obtained or calculated directly from previous experimental data, and default coefficients of variation were used only when variances could not be obtained or calculated from previous publications. This analysis helps to make the model simulations more realistic for a population when varying model parameters, as few individuals in vivo will have parameters that are the same (as the means) for those considered in this model. Monte Carlo analysis was used to look at the timeframe within the population when methadone would be effective based on the MEC in plasma. While the model might not be able to design optimal therapeutic regimens, especially considering that the total brain concentration considered by the model is not a good surrogate for methadone distribution in the brain, it can provide a prediction of unbound serum concentration of methadone, which can provide a good, but not conclusive, estimate of methadone distribution in the brain (Kalvass, Olson, Cassidy, Selley & Pollack, 2007). It is therefore a limitation of the model to only predict the plasma free fraction of methadone and not beyond the free brain concentration and to a more meaningful prediction of methadone in different brain regions, although once such data become available this model provides the framework to construct a semimechanistic model to incorporate new data for a more meaningful therapeutic optimal dose discussion.

Sensitivity analysis was conducted on the parameter values contained within the model. This analysis shows that many parameters have uncertainties that result in sizeable impacts upon the model. Sensitive parameters were introduced and the following details reasoning of why some of these parameters might have a significant impact:

> QLC: The liver blood fraction greatly affects oral administration due to the role of the hepatic portal vein, which carries blood from the GI Tract to the liver. Any orally administered drug has to enter the bloodstream after passing through the hepatic portal vein and the liver; therefore, the amount of blood being supplied to the liver greatly affects the concentration of the drug in the blood, particularly when considering the hepatic portal vein supplies 75%–80% of the blood to the liver.
>
> (Eipel, Abshagen & Vollmar, 2010)

> QGC: For flow-limited drugs, a higher GI tract blood fraction results in a higher absorption rate, resulting in a higher drug concentration available in the bloodstream. This parameter only had a significant effect during oral administration of methadone.
>
> (Khazaeinia, Ramsey & Tam, 2000)

5 | CONCLUSION

A PBPK model was successfully developed for methadone in dogs, specifically the Beagle and Greyhound breeds, as the model adequately simulated observed plasma methadone concentrations from multiple independent datasets within these two breeds. Therefore, this model can be utilized to predict tissue and plasma exposure of methadone following various therapeutic regimens within dogs. Model simulations can be useful in designing optimal therapeutic regimens for methadone in veterinary medicine. The model also provides a basis for extrapolation to other animal species and other opioids, as well as a foundation in using Berkeley Madonna in PBPK-based population analysis based on the current application of Monte Carlo simulations.

CONFLICT OF INTEREST

The authors of this study have no declarations of interest.

AUTHOR CONTRIBUTION

ZL and BK conceived the study. ZL and TEC designed the PBPK model structure. BK provided the original methadone pharmacokinetic data for model calibration and evaluation. TEC developed the model, ran all simulations, and analyzed the data under the mentorship of ZL and ML. ZL and ML double-checked the accuracy of the final model. TEC, ZL, ML, and BK contributed to data interpretation. TEC and ZL wrote the manuscript. All authors have read and approved the final manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.