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Mathematical modeling and simulation in animal health. Part III: Using nonlinear mixed-effects to characterize and quantify variability in drug pharmacokinetics

C. Bon
Roche Innovation Center, Basel, Switzerland

P. L. Toutain
Royal Veterinary College, Hatfield, UK

D. Concordet
Toxalim, Research Centre in Food Toxicology, Toulouse, France

R. Gehring
Kansas State University

A. Martin-Jimenez
University of Tennessee, Knoxville

See next page for additional authors

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Abstract

A common feature of human and veterinary pharmacokinetics is the importance of identifying and quantifying the key determinants of between-patient variability in drug disposition and effects. Some of these attributes are already well known to the field of human pharmacology such as bodyweight, age, or sex, while others are more specific to veterinary medicine, such as species, breed, and social behavior. Identification of these attributes has the potential to allow a better and more tailored use of therapeutic drugs both in companion and food-producing animals. Nonlinear mixed effects (NLME) have been purposely designed to characterize the sources of variability in drug disposition and response. The NLME approach can be used to explore the impact of population-associated variables on the relationship between drug administration, systemic exposure, and the levels of drug residues in tissues. The latter, while different from the method used by the US Food and Drug Administration for setting official withdrawal times (WT) can also be beneficial for estimating WT of approved animal drug products when used in an extralabel manner. Finally, NLME can also prove useful to optimize dosing schedules, or to analyze sparse data collected in situations where intensive blood collection is technically challenging, as in small animal species presenting limited blood volume such as poultry and fish.

Disciplines

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Comments

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Authors

C. Bon, P. L. Toutain, D. Concordet, R. Gehring, T. Martin-Jimenez, J. Smith, L. Pelligand, M. Martinez, T. Whittem, J. E. Riviere, and J. P. Mochel

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REVIEW ARTICLE

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Mathematical modeling and simulation in animal health. Part III: Using nonlinear mixed-effects to characterize and quantify variability in drug pharmacokinetics

C. Bon[1] | P. L. Toutain[2] | D. Concordet[3,4,5] | R. Gehring[6] | T. Martin-Jimenez[7] | J. Smith[8] | L. Pelligand[2] | M. Martinez[9] | T. Whittem[10] | J. E. Riviere[6] | J. P. Mochel[11]

[1]Roche Pharmaceutical Research and Early Development, Roche Innovation Center, Basel, Switzerland

[2]Department of Veterinary Basic Sciences, Royal Veterinary College, Hatfield, UK

[3]Toxalim, Research Centre in Food Toxicology, Toulouse, France

[4]Université de Toulouse, ENVT, INP, Toxalim, Toulouse, France

[5]Laboratoire de Physiologie et Thérapeutique, École Nationale Vétérinaire de Toulouse INRA, UMR 1331, Toulouse, France

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

[7]Department of Comparative Medicine, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA

[8]Veterinary Diagnostic and Production Animal Medicine, Iowa State University College of Veterinary Medicine, Ames, IA, USA

[9]Center for Veterinary Medicine, US Food and Drug Administration, Rockville, MD, USA

[10]Translational Research and Animal Clinical Trials (TRACTs) Group, Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Werribee, Vic., Australia

[11]Biomedical Sciences, Iowa State University College of Veterinary Medicine, Ames, IA, USA

Correspondence
Jonathan P. Mochel, Biomedical Sciences, Iowa State University College of Veterinary Medicine, Ames, IA, USA.
Email: jmochel@iastate.edu

A common feature of human and veterinary pharmacokinetics is the importance of identifying and quantifying the key determinants of between-patient variability in drug disposition and effects. Some of these attributes are already well known to the field of human pharmacology such as bodyweight, age, or sex, while others are more specific to veterinary medicine, such as species, breed, and social behavior. Identification of these attributes has the potential to allow a better and more tailored use of therapeutic drugs both in companion and food-producing animals. Nonlinear mixed effects (NLME) have been purposely designed to characterize the sources of variability in drug disposition and response. The NLME approach can be used to explore the impact of population-associated variables on the relationship between drug administration, systemic exposure, and the levels of drug residues in tissues. The latter, while different from the method used by the US Food and Drug Administration for setting official withdrawal times (WT) can also be beneficial for estimating WT of approved animal drug products when used in an extralabel manner. Finally, NLME can also prove useful to optimize dosing schedules, or to analyze sparse data collected in situations where intensive blood collection is technically challenging, as in small animal species presenting limited blood volume such as poultry and fish.

1 | INTRODUCTION

The primary objective of population pharmacokinetics (PK) and PK/pharmacodynamic (PD) studies is to help veterinary care providers understand the impact of factors (covariates) such as age, sex, breed, and disease on the drug dose–exposure–response (Lees, Landoni, Giraudel, & Toutain, 2004). As in human medicine, there is a need to identify the key variables that influence drug disposition and response, and to quantify the magnitude of their effects in veterinary species. In addition, the very nature of collective mass treatment in large animals can be a source of variability in the dose–exposure–response relationship, reflecting interactions between animal characteristics and husbandry practices (Lees & Toutain, 2012). In so doing, an appreciation of the sources of population variability will support efforts to optimize clinical efficacy, minimize safety risks, support dose and drug selection, as well as to identify potential concerns for safety and/or effectiveness in companion or food-producing animals.

Nonlinear mixed-effects (NLME) models are a versatile tool for quantifying variability in drug disposition and response as a function of significant patient characteristics (i.e., covariates) (Sheiner, Rosenberg, & Melmon, 1972). NLME can be used for analyzing either dense or sparse data, including observations from unbalanced study designs (FDA/CDER/CBER, 1999). The utility of NLME ranges from the analysis of data generated in preclinical studies to applications targeting recommendations for the individualization of dosage regimens (Holford & Buclin, 2012; Liefaard & Chen, 2015). NLME models are also being used by advising groups such as the Food Animal Drug Residue Avoidance Databank (FARAD) to provide a robust estimate of the withdrawal time for edible tissues in situations when a drug or drug product has been used in an extralabel manner (i.e., using a route, dosage, frequency, or in species other than that indicated on the approved drug product label).

The Animal Health Modeling & Simulation (AHM&S) Society was founded in 2012 to create an international forum promoting the use of in silico tools to support veterinary drug development (Mochel, Gabrielsson et al., 2013). This review article is part of a series of white papers designed to illustrate the value and applications of mathematical models in veterinary pharmacology and toxicology. In previous reviews, an introduction to modeling and simulation (Riviere, Gabrielsson, Fink, & Mochel, 2016), and the principles of physiologically based pharmacokinetics (Lin, Gehring, Mochel, Lave, & Riviere, 2016) have been presented. The focus of this study was on the concept, definition, and application of NLME models in veterinary sciences.

2 | MOTIVATION AND RATIONALE FOR USING NLME

The statistical methodology that supports NLME was described by Lewis Sheiner in a seminal paper entitled "*Modelling of individual pharmacokinetics for computer-aided drug dosage*" (Sheiner et al., 1972). Sheiner proposed a quantitative approach for analyzing sparse clinical data and recognized that the relationship between observed data and model parameters was nonlinear by nature. Therapeutic drug monitoring for individualized digoxin dosing in heart disease patients later crystallized the first human clinical application of NLME by Sheiner in the early 1970s (Peck, Sheiner, Martin, Combs, & Melmon, 1973; Sheiner, Halkin, Peck, Rosenberg, & Melmon, 1975). Subsequently, Sheiner and Beal (1983) contributed to the dissemination of the NLME approach by developing the computer software NONMEM® (nonlinear mixed-effect modeling, ICON Development Solutions), providing a unique analytical platform for pharmacokineticists (Mould & Upton, 2012).

Prior to the availability of the NLME approach, population parameters were expressed as either means or medians based upon a pooling of individual data into a single concentration–time course (i.e., the naïve pooling approach, which largely ignores between-subject differences), or by fitting a model to individual concentrations vs. time profiles, and subsequently generating average parameter values (the so-called "two-stage approach"). However, as summarized by Mould and Upton (Mould & Upton, 2012), both methods have inherent problems that are magnified in the presence of dosing noncompliance or missing data, potentially resulting in biased population estimates.

Alternatively, NLME models have the ability to leverage information: (i) using data from informative subjects (e.g., individuals presenting a rich and consistent pharmacokinetic time course, with only few "aberrant" data points) to estimate model parameters from apparent "outliers," while (ii) simultaneously considering drug effect and baseline and (iii) identifying relevant covariates that significantly affect drug disposition and/or response (Mochel & Danhof, 2015). NLME models can also be used to combine data from individual plasma and tissue PK experiments, or to conduct meta-analyses across published studies (Li, Gehring, Lin, & Riviere, 2015; Li et al., 2014; Ogungbenro & Aarons, 2014; Rey et al., 2014). While mixed-effects models efficiently function with only three or four samples per individual, a larger size of the target population is often required as compared to the naïve or two-stage approach.

To date, NLME models have been underutilized in veterinary sciences. However, and as discussed below, they can provide a highly efficient method for addressing some of the unique challenges encountered in veterinary medicine.

3 | VARIABILITY IS NOT NOISE

Between-individual variability is often perceived as noise that should be controlled through complex study designs and restrictive inclusion criteria (Ette & Williams, 2004). However, from a population perspective, this variability is in fact relevant biological information that should be quantified and not ignored. In essence, between-individual variability comprises *predictable* and *random* (i.e., not explained) variance components. The predictable component can be explained by *covariates* (i.e., by population characteristics, or external determinants such as husbandry or clinical practices). In human medicine, covariates typically comprise constitutional factors (e.g., bodyweight, age, genotype), and physiological parameters (e.g., markers of renal and/

or hepatic function). In veterinary medicine, additional factors need to be considered, such as hierarchy in pigs (Soraci, Amanto, Tapia, de la Torre, & Toutain, 2014), which influences the ingested amount of drug in medicated feeds, or social behavior manifested by allo- and hetero-licking described in cattle, which is known to affect the drug disposition of pour-on formulations (Toutain, Modric, Bousquet-Mélou, Sallovitz, & Lanusse, 2012). An overview of potential sources of variability in drug disposition kinetics in veterinary medicine is presented in Table 1.

In contrast to variability, sources of random error (i.e., the residual error, or noise) should be minimized to the extent possible. Noise is often described as a function of one of three categories: (i) pre-analytical error, (ii) analytical error, and (iii) postanalytical error.

Pre-analytical error is related to uncertainties associated with procedures carried out during the animal phase (e.g., drug administration, blood sampling, blood collection times, handling and processing, plasma storage).

On the other hand, *analytical error* is more broadly associated with the uncertainty of the bioanalytical technique (e.g., immunoassays, mass spectrometry) used to quantify drug concentrations. In this regard, a source of recurrent uncertainty in PK modeling pertains to the analysis of "below the limit of quantification" (BLQ) data (Ahn, Karlsson, Dunne, & Ludden, 2008). Ignoring BLQ data (referred to as the "M1 method" in Beal's original paper [2001]) typically leads to severe bias in parameters estimates, especially when the range of concentrations of interest is in the vicinity of the lower limit of quantification (LLOQ), as is the case in withdrawal time determination for depletion residues studies. This form of data censoring results in the generation of biased maximum-likelihood estimates. Censored data are referred to as left-censored when concentrations less than the LLOQ are missing. Conversely, right-censoring occurs when the high drug concentrations are ignored. The challenge imposed by right-censored data is that there is no set upper limit, making the imputation process highly subjective. An alternative to ignoring BLQ data is to use likelihood-based principles for inferring the value of left-censored information ("M3 and M4 methods" in Beal's description [2001]). M3 estimates the likelihood for measurements below the LLOQ, but allows for these values to be negative (on an assumption that these values follow a normal distribution). M4, on the other hand, constrains the data to values higher than 0. Bergstrand and Karlsson (2009) suggested an alternative to using M4, using a log-transformation of the dependent variable and then applying the simpler M3 approach. The authors found the latter approach to provide the least biased parameter predictions.

Another issue arises when data from recent pharmacokinetic studies are merged together with those from older experiments to build a single population model. In fact, intrinsic differences in the LLOQ, accuracy, and precision of the various analytical methods used to derive these data can be a significant source of analytical variability. This can be handled by building a residual error function that includes assay performances as a covariate in its model structure (Bonate, 2011).

Finally, *postanalytical errors* are those associated with model misspecifications, reflecting approximations made through the mathematical description of the true underlying biology. Misspecifications can be related to the structural model itself (e.g., use of a one-compartment instead of a two-compartment model) or/and to the distribution of the random effects (e.g., normal vs. log-normal distribution).

In addition to between-subject and random variability, there is a need to recognize the potential impact of interoccasion variability on drug PK variance. This third level of randomness may be integrated with estimates of within-animal variability when only a single dosing event is considered. Alternatively, in the presence of repeated dose

TABLE 1 Possible sources of variability in drug pharmacokinetics in veterinary medicine

Origin of the variability	Reference
Uncertainty on dosage	
Actual individual dose when medication dispensed for a group in food or water	Soraci et al. (2014)
Interpatient variability	
Species	Riviere et al. (1997)
Age	Li et al. (2014)
Bodyweight	Fink et al. (2013) and Lee and Maxwell (2014)
Breed	Uney and Tras (2011)
Social behavior (allo- and hetero-licking in cattle)	Toutain et al. (2012)
Disease	Leavens et al. (2014), Kissell et al. (2015), Shelver et al. (2016), Sidhu et al. (2017) and Silber et al. (2010)
Lactation	Lin, Cuneo et al. (2016) and Lin, Gehring et al. (2016)
Interoccasion variability	
Time-dependent physiological changes	Lee et al. (2006) and Konturek et al. (2011)
Chronobiology	Mochel, Fink et al. (2013) and Mochel et al. (2014, 2015)
Loc(ation (owner's home vs. veterinary hospital)	Whittem et al. (2000)

administrations, fluctuations in systemic exposure over time can be captured by including an interoccasion variability term in the structural model (Kontny et al., 2013; Kristoffersson, Friberg, & Nyberg, 2015). Ignoring this "day-to-day" variability can lead to inflation of the within-subject variability term, which is lumped together with all other sources of unexplained variability (Karlsson & Sheiner, 1993). Although the biology underlying interoccasion variability is often poorly understood, physiological attributes known to exhibit circadian and interoccasion fluctuations such as gastrointestinal (GI) pH can affect the ionization and overall solubility of oral drugs (Erkekoglu & Baydar, 2012). Other factors, such as gastric emptying and GI blood flow, have been shown to have an impact on the time-dependent difference of drug absorption (Konturek, Brzozowski, & Konturek, 2011; Lee et al., 2006). Additionally, repeated dose administrations can be associated with changes in drug first-pass and/or metabolism in the context of (hepatic and/or intestinal) enzyme induction or inhibition. Overall, these changes can be described using time-dependent (e.g., cosine and sine) covariate functions in the model structure.

4 | STATISTICAL DEFINITION AND CONCEPTS OF NLME

The structure of the NLME model allows for some parameters to be fixed (i.e., considered to be constant within the population) while others are considered to be varied (termed random effects).

Random-effect parameters are used to represent variability in PK parameter estimates as a result of between- and within-individual variability. The individual statistical (or stochastic) model can be written as:

$$Y_{ij} = f(\text{Dose}, t_{ij}, \varphi_i) + g(\text{Dose}, t_{ij}, \varphi_i, \sigma) * \varepsilon_{ij} \quad \varepsilon_{ij} \sim N(0,1) \tag{1}$$

where Y_{ij} describes the drug concentration for the *i*th individual at time *j*. Note that within the framework of the NLME approach, the error structure is assumed to be parametric.

The function *f*() refers to the structural model while *g*() is the residual error model, with φ_i being the vector of individual PK (or PD) parameters, σ the standard deviation of the distribution of the residual error model, ε_{ij} the difference between the observed concentration and the concentration predicted by the model for the individual *i* at time *j*, and t_{ij} the *j*th sampling time of the *i*th individual.

The random-effect parameters are modeled as follows:

$$\varphi_i = \theta + \eta_i \quad \eta_i \sim N(0, \Omega)$$

where θ is the vector of population parameters (also referred to as fixed effects), η_i is the vector of random effects, assumed to be normally distributed, and characterizing the difference between the parameter value of the *i*th individual and the population (i.e., typical value) θ. Ω is the standard deviation of the distribution of the η_i corresponding to the interindividual variability of the model parameter.

Population models are referred to as *mixed effects* because they include both *fixed* and *random-effects* parameters. They are also *nonlinear* as they do not depend upon a linear relationship between the fixed and the random effects, for example, *f* is nonlinear with respect to η_i. As described in the previous section, the sources of variation between the individual parameters φ_i can be further explained by population characteristics that can be included additively or proportionally to θ. The core principles of NLME models are further outlined in Figures 1 and 2a,b.

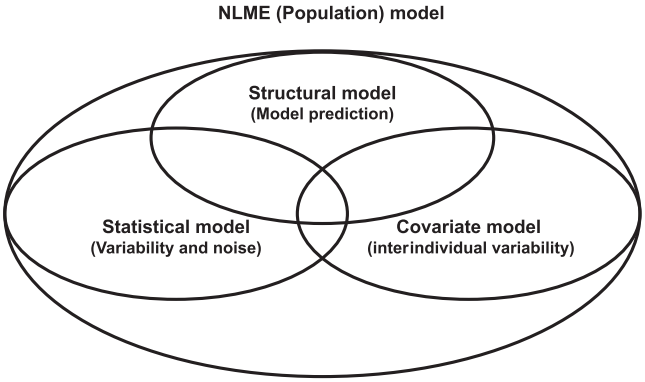

FIGURE 1 Structure of a NLME (Population) Model, including (i) a structural model, (ii) a statistical/stochastic model, and (iii) a covariate model to explain part of the between-subject variability. Figure adapted from Vučićević et al. (2011)

5 | APPLICATIONS IN VETERINARY MEDICINE

Initially, NLME modeling was developed in human medicine to support individualized dosing of drugs with narrow therapeutic indices. It proved to be particularly useful in the context of large interindividual variability, or where drug PK could be affected by the disease process or the drug itself (Sheiner, 1985). Because this modeling approach is amenable to sparsely sampled blood or tissue samples (Sheiner & Beal, 1983), it was later recognized to be of value in veterinary pharmacology to explore the impact of covariates such as age, sex, and disease status on drug PK (Martin-Jimenez & Riviere, 1998).

Over the past 15 years, several peer-reviewed NLME analyses in veterinary pharmacology have been published. The most recent examples include the study of topiramate in epileptic dogs (Vuu et al., 2016); nonsteroidal anti-inflammatory drug (NSAIDs) in dogs and cats with osteoarthritis (Cox, Liao, Payne-Johnson, Zielinski, & Stegemann, 2011; Fink et al., 2013; Pelligand, Soubret, King, Elliott, & Mochel, 2016; Silber et al., 2010); tobramycin in horses (Haritova, Bakalov, Hubenov, & Lashev, 2012); valnemulin and cefquinome in pigs (Zhao et al., 2013, 2014); tulathromycin in lactating goats (Lin, Cuneo et al., 2016; Lin, Gehring et al., 2016); and penicillin G in cattle and swine (Li et al., 2014).

5.1 | In food-producing animals

5.1.1 | Regulatory determination of human food safety (HFS)

Determination of the withdrawal period is a key component of the HFS section of a new animal drug application to be used in food-producing

animals (FDA/CVM, 2015). This time period ensures that, at slaughter or harvesting, the residues levels in edible tissues or animal products (e.g., meat, milk, or eggs) are at or below a concentration determined to be safe for human consumption. Currently, the US FDA does not accept alternative approaches to those described in FDA Guidance documents for the regulatory determination of withdrawal times. While approaches such as NLME models may be applied in situations of extralabel drug use or for predicting violative residues in diseased animals (see discussion below), such assessments fall outside of the purview of the US FDA. Summary information on the determination of withdrawal times in the United States and the European Union is provided in Table 2.

5.1.2 | Importance of NLME for insuring HFS under conditions of extralabel drug use

Several NLME models have been published as tools for estimating the time when FDA-approved marker residues deplete below the tolerance or detectable levels in extralabel use situations. This is well exemplified by a study from Li et al. (2014) where the authors established a robust NLME model of penicillin for a large and diverse population of food-producing animals (Figure 3). The model was developed using published data from the literature and validated by comparing the concentrations predicted by the model with separate, both published and unpublished independent datasets.

Usage of drugs approved for major species in minor species requires that dosage regimens and withdrawal times are extrapolated between species. Allometric models of drug PK across animal species are usually formulated empirically, building equations that relate PK parameters to factors like bodyweight, brain weight, or maximum lifespan (Riviere, Martin-Jimenez, Sundlof, & Craigmill, 1997). Using retrospective data from the FARAD, Martin-Jimenez and Riviere (2001, 2002) applied NLME for analyzing the disposition of gentamicin and oxytetracycline across several mammalian species. In these studies, various covariates were incorporated into NLME PK models, using data from six and seven species for oxytetracycline and gentamicin, respectively. PK parameters were found to be exponentially related to bodyweight for both oxytetracycline and gentamicin. Interestingly, brain weight was also found to be a predictor of gentamicin clearance in the model.

An advantage of this approach is that it maximizes the information that can be gleaned from limited available data, and facilitates the estimation of population variability by combining data from several sources (e.g., literature and raw data from new studies). The models can be applied using Monte Carlo simulations to predict concentration–time profiles for theoretical populations and thereby determine when concentrations of the marker residue will fall below target levels (tolerance or levels of detection) in target tissues or secretions (e.g., milk) for a specified percentage of the population (e.g., 95th percentile).

5.1.3 | Future opportunities

Ultimately, the question is whether or not there may be situations in which the level of uncertainty already incorporated into regulatory methods, which are based upon data derived from a sample of healthy animals, is adequate to ensure the highly diverse sets of conditions that may be encountered with field use. For example, using retrospective data analysis, Whittem (1999) applied NLME to evaluate covariates that alter the necessary milk discard time after dosing of dairy cattle with pirlimycin. This first use of NLME for food safety evaluation confirmed the important impact of low milk production on

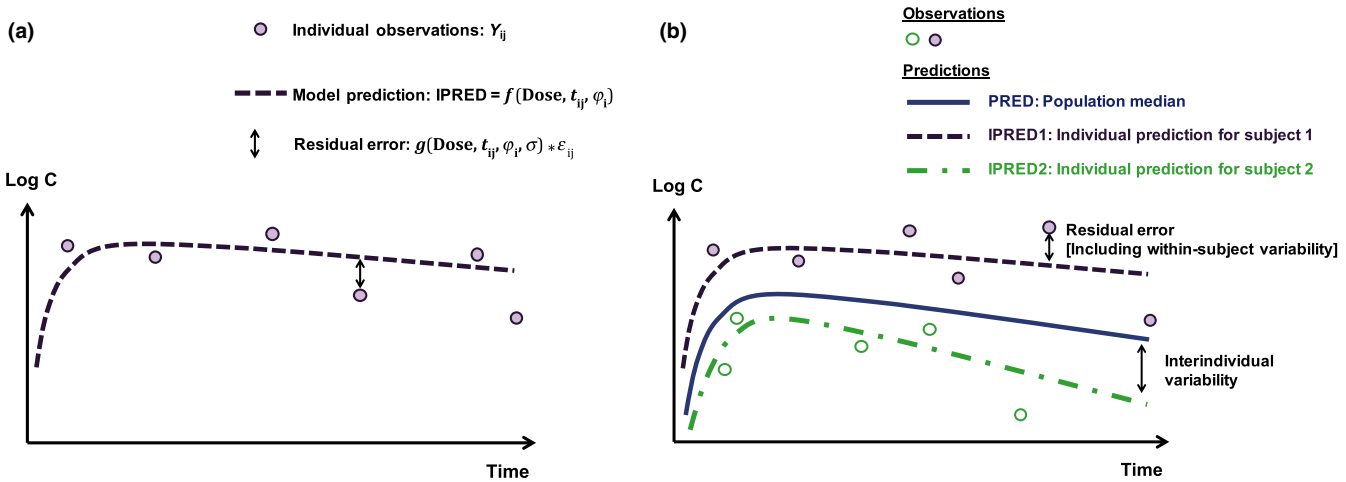

FIGURE 2 (a) Nonlinear mixed-effects models can be used to predict the individual time course of drug concentrations. The individual prediction (IPRED) is a function of a vector or individual parameters (φ_1), the input dose and time. Differences between model predictions and actual observations (Y_{ij}) can be quantified using a residual error model. More information about model parameters and nomenclature can be found in the body of the manuscript. (b) Nonlinear mixed effects account for variability and noise (i.e., residual error), and allow to predict the drug concentration–time course for a typical (i.e., median) animal patient (PRED), as well as individual subjects (IPRED). Differences between individual patients and the typical prediction are driven by interindividual variability, while deviations from individual observations are due to residual error

TABLE 2 Comparison of withdrawal period determination based upon the US FDA guidance versus EMA approach

	US FDA	EMA	Alternative population approaches explored by EMA
Subject population	Healthy animals representative of the commercial breed and target population	Animals should be healthy and, preferably, should not have been previously medicated. Study animals should be representative of the commercial breeds and representative of the target animal population that will be treated	Depending on covariates, WT could be also estimated for specific subpopulations (according to demography, clinical signs, production variables)
Number of sites	One	The benefits and drawbacks of combining studies are discussed in a general section of the "Guideline on statistical principles for clinical trials for veterinary medicinal products (pharmaceuticals)" (EMA/CVMP/EWP/81976/2010)	Could be multicentric (the center would be a discrete covariate)
Statistics	99% tolerance limit estimate with 95% confidence	Time when the upper one-sided 95% tolerance limit for the residue is below the MRL with 95% confidence	Time when the upper limit of the 98% prediction interval of the nonlinear mixed-effects model is below the MRL with 95% confidence
Target value	Tolerance (maximum concentration of the marker residue)	Maximum residue limit (MRL)	No change
Moiety being monitored	Marker residue (exits in a known relationship to total residue)	Marker residue is that residue whose concentration is in a known relationship to the concentration of total residue in an edible tissue	No change
Tissue	Typically the slowest depleting tissue	Typically the slowest depleting tissue	No change
Objective of WT determination	Insure consumer safety. Therefore, estimates are intentionally conservative	Ensure consumer safety	NLME modeling has many advantages that can help optimizing the information required to ensure consumer safety (but also to reduce costs for marketing authorization holders)
Conclusion	If marker residue is at or below tolerance, then the edible tissues will have a concentration of total residue that is at or below the concentration deemed safe for human consumption	The period necessary between the last administration of the veterinary medicinal product to animals, under normal conditions of use and in accordance with the provisions of the Directive 2001/82/EC, and the production of foodstuffs from such animals, in order to protect public health by ensuring that such foodstuffs do not contain residues in quantities in excess of the maximum residue limits for active substances	
Legal basis for HFS requirements	Sections 512 (d) (1) (A), (B), (D), and (F) of the Federal Food, Drug, and Cosmetic Act (http://biotech.law.lsu.edu/blaw/fda/fdcact5a.htm)	In line with article 12.3 of Directive 2001/82/EC, marketing authorization applications for veterinary medicinal products for use in food producing species must include an indication of the withdrawal period. Article 1.9 of the directive defines the withdrawal period	
Guidance documents available	FDA/CVM Guidance for Industry General principles for evaluating the Human Food Safety of new animal drugs used in food-producing animals #3. Guidance for Industry—Studies to evaluate the metabolism and residue kinetics of veterinary drugs in food-producing animals: Marker residue depletion studies to establish product withdrawal periods #207	VICH GL48 Studies to evaluate the metabolism and residue kinetics of veterinary drugs in food-producing animals: Marker-residue-depletion studies to establish product withdrawal periods AND EMEA/CVMP/036/1995 Approach towards harmonization of withdrawal periods	

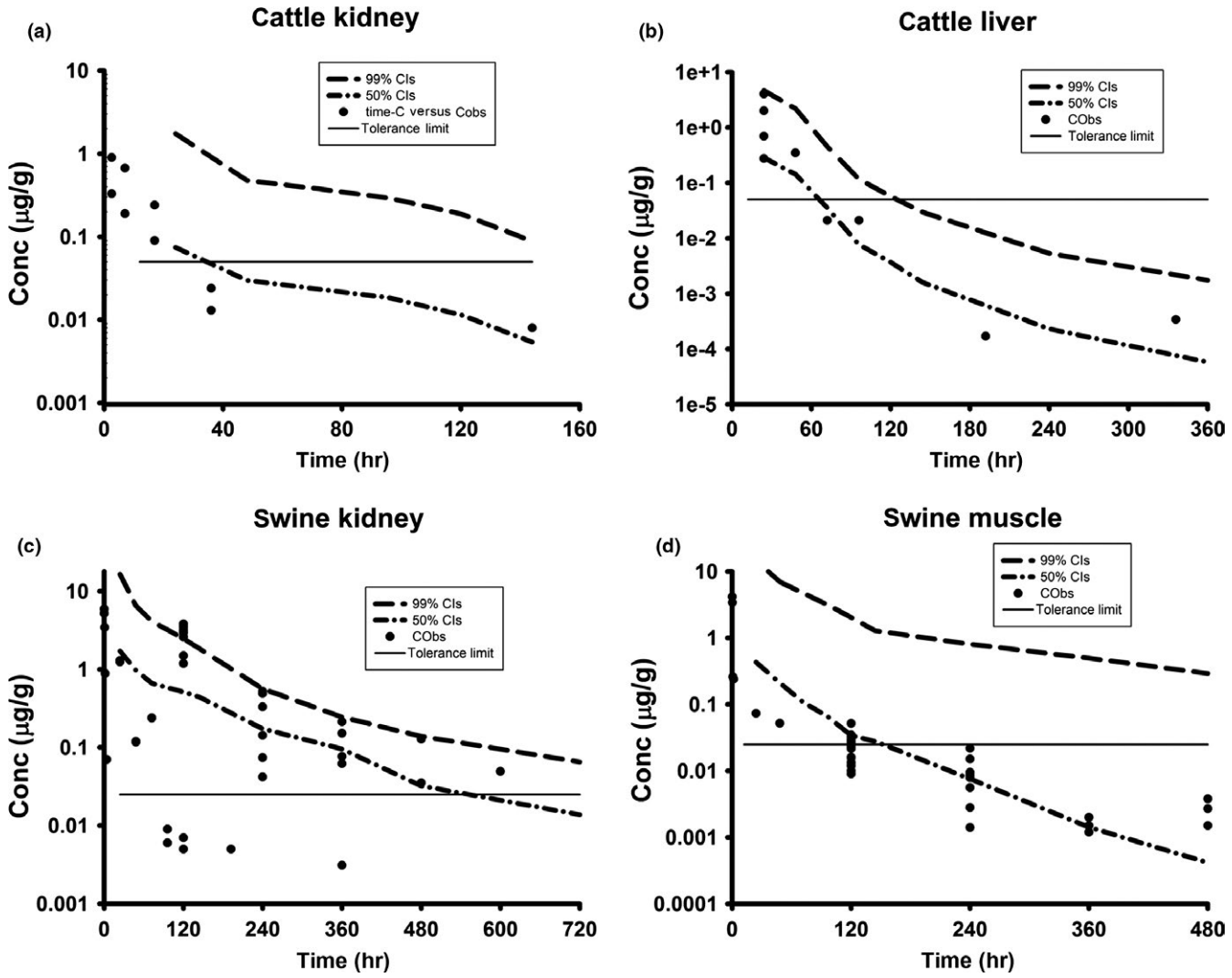

FIGURE 3 Simulated data for the tissue residues of penicillin G in cattle kidney (a), cattle liver (b), swine kidney (c), and swine muscle (d). The 99th percentiles of the simulated penicillin residues are represented by a dashed line. The 50th percentiles of the simulated penicillin residues are represented by a dash-dot line. The observed concentrations (CObs) of the tissue residues are represented by closed circles. The solid lines are the tolerance limits of penicillin G in cattle and swine tissues. Reproduced from Li et al. (2014) with permission from the publisher

persistence of drug residues in milk and demonstrated the application of NLME for meta-analysis of data derived from several separate studies. Although there are currently few examples of pharmacokinetic alterations in blood, milk, and tissues of food-producing animals, there is an overarching consensus that the disease status of an individual could impact the disposition kinetics of several drugs, potentially resulting in residue violations (Kissell, Leavens, Baynes, Riviere, & Smith, 2015; Leavens et al., 2014; Shelver et al., 2016; Sidhu et al., 2017). Changes in drug PK in the course of disease progression could easily be captured through NLME analyses, using only a few (blood, milk) samples per individual. Recently, the use of NLME for estimating milk withdrawal time in the European Union has been proposed by scientists from the French Agency for Veterinary Medicinal Products (Chevance, Jacques, Laurentie, Sanders, & Henri, 2017). Currently, NLME is not used by the US FDA for the determination of withdrawal periods of approved products, following the approved label directions. However, NLME would be of potential value for identifying those situations/conditions for which there may be a greater risk of marker residue concentrations exceeding the tolerance at the labeled withdrawal time. Overall, there is a growing interest from the regulators to explore whether or not NLME could be used to assess the magnitude of covariate-associated changes in drug PK susceptible to increase the risk of violative residues in edible tissues.

5.2 | In companion animals

Martin-Jimenez and Riviere (1998) published the very first NLME model applied to veterinary pharmacokinetics, using retrospective data from therapeutic drug monitoring of gentamicin in horses. The authors developed and validated a NLME model which was then used to design and adjust clinical doses according to individual horse characteristics. Bodyweight and serum creatinine concentration were found to explain 60%–70% of the variability in gentamicin PK in diseased horses. In fact, when interindividual PK variability is large (as in

this example), computation of antibiotic doses based on Monte Carlo simulations, integrating animal PK, bacterial susceptibility distribution, and target attainment rates, may result in under- or overexposure in some patients, unless patient subpopulations have been adequately characterized. Under these circumstances, dosage adjustments could be recommended on the basis of various covariates, such as bodyweight, hepatic, or renal function, thereby decreasing the overall risk of underexposure (leading to therapeutic failure) or overexposure (raising potential safety concerns) in some patients.

In 1977, Sheiner and co-workers used NLME to understand the factors affecting the clearance and volume of distribution of digoxin in humans (Sheiner, Rosenberg, & Marathe, 1977). Twenty years later, a similar retrospective analysis on the factors influencing digoxin clearance and volume of distribution in dogs was presented by Whittem, Hogan, Sisson, and Cooper (2000). Similar to humans, serum creatinine concentration was found to explain some of the PK variability of digoxin in dogs. However, an original finding in dogs was that serum potassium concentration was also a critical variable in the prediction of digoxin volume of distribution. The use of NLME modeling also led to the identification of two distinct canine subpopulations, with a tenfold difference in their respective absorption rates of digoxin. At the time, the authors hypothesized that oral dosing of dogs in teaching hospitals vs. at home caused the marked delay in digoxin absorption through known physiological impact of stress on gastric emptying. This has recently been confirmed in a study by Warrit et al. (2017), which showed that the gastric emptying time of hospitalized dogs (median: 71.8 hr) was significantly longer than that of dogs at home (median: 17.6 hr).

Lately, NLME models have been applied to several therapeutic indications in companion animal species, including cardiovascular disease and pain. For example, Mochel, Fink et al. (2013) and Mochel et al. (2014, 2015) studied the chronobiology of the renin–angiotensin aldosterone system (RAAS), blood pressure, and urinary electrolytes in dogs to characterize the optimum time of dosing with angiotensin-converting enzyme inhibitors (ACEIs). In their mathematical description of the RAAS, NLME was used as a tool for utilizing densely sampled plasma variables (i.e., renin activity) to improve parameter estimation of (sparse) urinary endpoints. Subsequently, the authors developed a mechanism-based PK/PD NLME model that captured the disposition kinetics of the ACEI benazeprilat, and the time-varying changes of systemic RAAS biomarkers, without and with ACE inhibition therapy. Based on their results, the optimal efficacy of ACE inhibitors is expected with evening dosing in dogs, which is consistent with earlier observations in humans (Kuroda et al., 2004). Their data further showed that benazepril had a significant impact on the dynamics of the renin–angiotensin cascade, resulting in a profound decrease in angiotensin II (AII) and aldosterone (ALD) while increasing renin activity for about 24 hr. Extrapolating these results to recent investigations in humans (Guder et al., 2007), it is hypothesized that the reduction in AII and ALD is one of the drivers of increased survival and improved quality of life in dogs receiving ACEI therapy.

Another therapeutic indication where NLME has been used for dose selection and covariate identification of NSAIDs in companion animals is osteoarthritis (OA) (Cox et al., 2011; Fink et al., 2013; Pelligand et al., 2016; Silber et al., 2010).

The original analysis by Silber et al. (2010) showed that robenacoxib blood clearance was 75% higher in healthy dogs compared with OA patients. Synovial fluid concentrations were modeled using an effect-compartment-type approach predicting longer residence times in OA dogs compared to healthy Beagles (e.g., concentrations remaining above the IC50 of COX-2 for 16 hr vs. 10 hr in OA and healthy dogs, respectively), supporting the recommended 1–2 mg/kg once-a-day dosing of robenacoxib in OA dogs. This dosing frequency contributed to the favorable safety index of robenacoxib in field trials. Further to this work, and using a NLME modeling approach combining data from three prospective, multicenter field studies in 208 osteoarthritic dogs, Fink et al. (2013) showed that neither sex, age, breed, kidney, or liver function significantly influenced robenacoxib PK, ruling out the need for dose adjustments based on these covariates. Finally, Pelligand et al. (2016) used NLME to characterize feline robenacoxib disposition kinetics in 83 cats, pooling data from across seven preclinical (laboratory) studies and one field (client-owned cats, perioperative sampling) study, in order to evaluate the effect of anesthesia on robenacoxib PK. Using individual parameter estimates, robenacoxib concentration vs time profiles were generated to determine the time interval during which plasma levels remained above a target PD threshold, for example, IC_{80} COX_2 and IC_{20} COX_1 for efficacy and safety assessment, respectively. This exposure index will be further used as a covariate to support the perioperative clinical efficacy and safety evaluation of robenacoxib in cats.

For mavacoxib, simulations from a NLME PK model showed that a 2 mg/kg dose was sufficient to maintain trough concentrations above a threshold efficacy level in dogs. These results were key to reduce the recommended dose of mavacoxib from 4 to 2 mg/kg, which ultimately increased the therapeutic index of mavacoxib in dogs (Cox et al., 2011).

6 | SPECIFIC CHALLENGES TO VETERINARY MEDICINE

6.1 | Data analysis of sparse and/or unbalanced data in limited sample sizes

In human medicine and during development of a new chemical entity, phase III PK studies typically involve a large number of subjects and oftentimes (Rubino, Bhavnani, Moeck, Bellibas, & Ambrose, 2015), but not always (Desai et al., 2016), contain patient data derived from a sparse sampling schedule. In veterinary medicine, similarly large studies are uncommon often due to economic and practical constraints.

When using sparse data, accurate and precise estimation of PK parameters and their variability is heavily dependent on the experimental design (Ette, Williams, & Lane, 2004). As the sample size decreases, so does the ability to properly estimate model parameters. Therefore, when the sample size of a PK experiment is expected to be relatively low (as is often the case in veterinary medicine), one needs to carefully consider the design of the experimental protocol (Jones, Sun, & Ette, 1996; Jonsson, Wade, & Karlsson, 1996). Among relevant factors are

(i) the sampling times, (ii) a priori knowledge about the expected variability and (iii) the underlying PK model structure. For instance, when a one-compartment model is used to describe the PK of a drug administered intravenously (IV), the best sampling times are those early time points after dose administration, and the late time points when the concentrations are close (but above) the lower limit of quantification (Endrenyi, 1981; Ette, Howie, Kelman, & Whiting, 1995).

The relatively short period of time during which veterinary patients are available for sampling, and the limited number of samples that can be collected per animal are additional factors to be considered. Under production conditions, food animals tend to be available for extended periods of time, but the number of samples that can be collected from each animal varies depending on the animal size, restraining measures available, and the health status of the animal. For small animals, the length of stay in veterinary hospitals, the animal size, and other ethical or practical issues (e.g., owner consent) will affect not only the number of samples that can be collected per animal, but also the timing of sample collections. In practice, gathering information about the terminal phase of the PK profile will often be contingent upon the feasibility of hospitalizing the animal for more than a couple of hours after dosing.

In light of these challenges, the incorporation of optimum sampling strategies in the study design is critical. In theory, samples should be obtained at those times when the information on relevant PK parameters is maximal. In fact, Sheiner and Beal (1983) showed that parameters are estimated with increased accuracy when the number of subjects increases, but that increasing the number of samples does not improve parameters estimation if the drug is administered IV and exhibits a mono-exponential decline. Likewise, Al-Banna, Kelman, and Whiting (1990) used Monte Carlo simulation to compare several sampling strategies in 50 subjects for a test drug with one-compartment IV kinetics. Acceptable results were obtained with two samples per individual collected at random times, but improved precision and accuracy in the estimation of clearance was observed when the second sample was collected at later time points. In this analysis, increasing the number of subjects or the sampling frequency did not significantly improve the estimation of the model fixed effects, but significantly increased that of the random effects. This was later confirmed in a study by Ette, Sun, and Ludden (1998). More recently, Lee (2001) used Monte Carlo simulations to assess the effect of sampling schedules on the ability to identify a subpopulation with a 30% higher clearance than the average individual. Although increasing the number of subjects from 100 to 200 had little effect on the power of the test, the frequency and sampling times appeared to be critical to detect this patient subpopulation.

Translating these published simulations to real-life situations need to be tempered by an appreciation of the simplistic premise upon which they are based. In many instances, an IV formulation was used for generating the PK model in early preclinical studies. However, in the clinic, there are few marketed veterinary formulations that are approved for use as an IV injection. Rather, alternative routes (subcutaneous or intramuscular injections, oral formulations, transdermal systems) are associated with an absorption component that may confound the shape of the profile observed following drug administration via IV injection. One advantage of NLME models is that data from various sources, including IV experiments from early research and clinical extravascular studies, can be pooled together to allow the estimation of PK model parameters. The experimental design used to capture the individual variations in the absorption, distribution(s), and elimination phases needs to be considered when gathering the data used for characterizing PK variability across a patient population. Examples of approaches that have been used for characterizing population variability in PK include the use of an adaptive rather than a fixed study design (Drusano, Forrest, Snyder, Reed, & Blumer, 1988); the MAP-Bayesian (nonparametric) approach (Kashuba, Ballow, & Forrest, 1996), or the population bridging approach as discussed by Foo and Duffull (2012). Such methods involve interim analysis and fine-tuning of the design as subjects are accrued. Note that, from the perspective of the conditions under which the data will be gathered, it may be more appropriate to randomize individuals with respect to a time window rather than a rigidly constrained set of time points (Graham & Aarons, 2006).

6.2 | The input dose as a random variable

In collective therapy, the input dose can be greatly influenced by social hierarchy, as for instance reported in pigs after oral administration of fosfomycin (Soraci et al., 2014). In this study, variations in fosfomycin PK were largely explained by differences in feeding behavior, resulting in varying ingested amounts of drug between animals. Although several workarounds are described in the literature, the approach of Li and Nekka (2007), which consists of formally including a stochastic drug input function in the PK model, seems particularly relevant. An alternative is to model the nominal dose as an independent variable and introduce a random effect on oral bioavailability to account for the variability in drug intake between individuals. Part of this variability can then be explained by specific covariates such as social hierarchy and husbandry practices (e.g., pen size). Future directions in pig production will allow individualization of dosing through RFID (radiofrequency identification) technology. Under the NLME framework, covariates of interest (e.g., sex and bodyweight) could be used to individualize dosing schedules. Also, by monitoring water consumption via RFID (a fingerprint of early disease in pigs), one should be able to selectively treat diseased animals, which is the next step forward to the stewardship use of antimicrobials in livestock production systems (Ferran, Toutain, & Bousquet-Melou, 2011; Vasseur et al., 2014).

7 | REGULATORY ISSUES AND ACCEPTANCE

The regulatory requirements that can be instituted by the FDA are constrained by the existing laws that govern the activities of the Agency. Unlike that encountered in human medicine (FDA/CVM, 2016), there is no statutory requirement for PK data to be provided in support of the approval of an animal drug application. Accordingly, although the FDA reviewer may share perspectives with drug sponsors with respect to the potential benefits of collecting PK information,

the FDA typically cannot legally require sponsors to collect PK data in support of their new animal drug application. In other words, there is no requirement for sponsors to provide a description of the population PK variability that is expected when the product is used in the targeted animal patient population. The absence of regulatory requirement does not, however, prohibit a drug sponsor from submitting such information to support product development, or to provide a basic foundation of information that can address regulatory questions that may arise over the pre- and postapproval life of a product. An example of this is embodied in the recently published CVM guidance on parenteral dosage forms (FDA/CVM, 2016), which contains a section on how to establish in vitro–in vivo correlations for addressing formulation and manufacturing questions that can occur during product development, and as a consequence of postapproval product modifications.

In the EU, the situation is different. The last Committee for Medicinal Products for Veterinary Use (CVMP) guideline for the demonstration of efficacy for veterinary medicinal products containing antimicrobial substances explicitly mentions:

> *For group/flock medication via water or feed, the variability between animals in feed/water intake should be explored through appropriate sampling of the animals, with the purpose of ensuring that the dose selected will provide therapeutic exposure levels in all animals. In addition, population PK/PD models (such as Monte Carlo simulations) based on data from field trials could be used to bring support for a post-hoc analysis of the selected dose.*

This is in line with the CVMP Concept paper for the current revision on the guideline for the conduct of pharmacokinetic studies in target animal species (EMEA/CVMP/133/99-Final).

Irrespective of whether or not PK data are a necessary component of a regulatory application, the systemic availability of a parent compound and its metabolites can influence drug product safety and effectiveness. As such, disposition kinetic data can affect the design and interpretation of clinical trials, the extrapolation of target animal safety results to the intended animal patient population, or the assessment of postmarketing adverse event reports. Therefore, although not required by the US FDA, PK information is an invaluable component of product development in veterinary medicine.

8 | CONCLUSIONS

This review emphasizes the need for a concerted effort within the animal health community to enhance awareness and understanding of the potential applications of NLME in veterinary medicine. Identification of the key population characteristics that drive variability in the drug PK between animals is essential to the selection of a rational dosing strategy. This has broad implications to ensure optimum efficacy and safety of therapeutics in both companion and food-producing animals. However, caution must be exercised both in the execution and interpretation of these models. Equally critical are the statistical and sample (study design) assumptions within which these studies are analyzed. Hopefully, as veterinary pharmacologists appreciate the strength and efficiency of these tools, there will be an increase in the use of modeling and simulation approaches to support the future of animal health.

CONFLICT OF INTEREST

The authors report no conflict of interests.

AUTHORS' CONTRIBUTION

All authors (CB, PLT, DC, RG, TMJ, JS, LP, MM, TW, JER, JPM) have contributed to the writing of the manuscript. JPM was responsible for the final production of the white paper and the responses to the reviewers' comments. All authors have read and approved the final manuscript.

ORCID

P. L. Toutain http://orcid.org/0000-0002-8846-8892
R. Gehring http://orcid.org/0000-0002-1329-201X
M. Martinez http://orcid.org/0000-0002-4512-853X
J. P. Mochel http://orcid.org/0000-0002-0997-3111

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