ABSTRACT

MASON, SHARON ELIZABETH. Comparative Pharmacokinetic Approaches to Estimating the Pharmacokinetics of Water Medications in Swine. (Under the direction of Ronald E. Baynes).

Although water medications have been used in the United States for over 40 years, the pharmacokinetics of these drugs have not been clearly discerned using compartmental pharmacokinetics techniques. No repeated dosing pharmacokinetic studies have been published on water medications in swine. Therefore we proposed to use three proven techniques in veterinary medicine to model water medications in swine.

Non-compartmental modeling, physiologically based pharmacokinetic modeling and population based pharmacokinetic modeling have improved veterinary medicine pharmacology over the last decade. The applications of these techniques to the pharmacokinetics of water medications, however, have not been published. Therefore two *in vivo* studies were performed to collect pharmacokinetic information on these drugs and then pharmacokinetic modeling was performed to test these techniques and their applications to characterize the disposition characteristics of water medications in swine production settings.

In vivo experiments were performed in pigs based on the age and dosing schedules of those treated in commercial production units with tetracycline and sulfamethazine. Non-compartmental analysis techniques were initially applied to these populations. PBPK and population pharmacokinetic techniques were also applied where relevant to these medications to provide insight into situations that traditional modeling techniques were unable to elucidate. For sulfamethazine, the use of PBPK modeling

proved useful in characterizing the potentially small exposure concentrations that have been documented in the literature for over 25 years.

In contrast to sulfamethazine which is chemically very stable, tetracycline has been shown to degrade over time with exposure to high temperatures and sunlight. Ancillary experiments were performed to characterize the bioactivity of tetracycline water medication as dosed in a production setting. Furthermore, basic pharmacokinetic information on tetracycline administered in water was collected and analyzed.

Finally population based modeling was applied to data collected from commercial farm settings to determine factors that may apply to all water medication administration in the swine industry. This mixed effect modeling technique was able to provide increased support for the non-compartmental pharmacokinetic findings and to identify factors important to plasma concentrations of medications administered in water.

PBPK and population based modeling techniques can be effectively used in modeling water medications in swine. Furthermore, they were able to determine dosing amounts and schedules as well as other factors that affect the concentration of water medications in swine where traditional pharmacokinetic modeling is inadequate.

Comparative Pharmacokinetic Approaches to Estimating the Pharmacokinetics of Water Medications in Swine

by
Sharon E. Mason

A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy

Comparative Biomedical Sciences

Raleigh, North Carolina

2010

APPROVED BY:

Dr. Ronald E. Baynes Chairman of Advisory Committee	Dr. Glen W. Almond
Dr. Jim E. Riviere	Dr. Charles Eugene Smith

DEDICATION

L’cœur a raison que la raison ne comprend pas.

À ma familia.

Toujours.

The heart has reason that reason does not understand.

To my family.

Always.

BIOGRAPHY

Sharon Mason is a North Carolina native. She completed her bachelors of science at the University of North Carolina at Chapel, a master's of science degree in Forensic Toxicology at the University of Florida in Gainesville and in 2006 she completed her Doctor of Veterinary Medicine. Sharon has an interest in food safety and the application of pharmacokinetics to protecting the food supply.

ACKNOWLEDGEMENTS

I would like to thank countless friends and family for support. I cannot begin to list all of your names. Your ideas and inspiration have kept me going. This dissertation is just the end of one stage.

I would like to thank Drs. Ronald Baynes and Glen Almond for believing in me and helping me to shape and work these projects into reality; Dr. Jim Riviere for being a sounding board for all of the observations that did not seem typical; Dr. C.E. Smith for his unique insights into the data, the staff at CCTRP and the CVM community for their assistance to the research performed in this dissertation.

Finally, I would like to acknowledge my fellow graduate students for their input, support, and presence on a daily basis. FARAD funding supported me during this degree process and contributed to some of these findings. The College of Veterinary Medicine Animal Health Fund provided the initial funding for the first study. Finally Dr. Alan Scheidt and Pfizer Animal Health helped to support the in vivo research monetarily and provided ways for it to be publicized.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Food producing animals are often given medications in formulations or routes that are not typically used in other animal species or in humans. Although these formulations and dosing methods were approved by FDA, available published pharmacokinetic profiles have been of a more observational nature. Over the last few decades, public policy changes have resulted in a shift in the current process for drug approval being significantly different than the methods employed over 30 years ago. However, during this evolution, formulations previously approved have remained the same and bioequivalence methods have allowed the use of these previously approved formulations to serve as guidelines for more recent approvals. For example, chlortetracycline was approved for use in animal feeds in 1963 and sulfamethazine was approved for use in 1975 (Vetgram, www.farad.org/vetgram/). There have been many generic versions of these same medications, approved years after the first drug approval, without having to undergo a full NADA (New Animal Drug Application) process. These medications must meet bioequivalence requirements of the previously approved drugs in order to receive approval (Jackson et al. 2004). However, this does not require that they meet the requirements of the current NADA process. In addition to NADA processes changing, withdrawal time procedures have expanded to better protect the human population; however, older formulations and their approved withdrawal times may not meet the safety level required by newer drugs, but, nonetheless, are still reaching the market today.

Some antibiotic medications, referred to above, were developed and implemented for use in feed and water sources over 40 years ago. Many of the policy changes from FDA in more recent years reflect an increased understanding and implementation of population pharmacokinetics, but do not require scrutiny of previously approved drugs to meet those standards (FDA 1999; FDA 2003; FDA 2005). As such, some currently approved medications would not be granted approval in today's climate. This includes very common drugs like aspirin, which has never been official approved for food animal use.

These policy changes have been aided by our improved computational capabilities, which allow more complicated methods of modeling to be applied to both new human and new animal drug approvals, including drug approvals for food producing animals (Martinez et al. 2000). One area that has received minimal scrutiny in the United States, but which has harbored interest in Europe, is the use of pharmacotherapy via water. With the ban in 2006 of all non-therapeutic antimicrobial use in Europe (EC 2003), understanding drugs that are used and assumed to be therapeutic is of greater importance. However, compared to other methods of treating disease, little research has been performed on water medications compared to feed additives and parenteral dosing of these medications. A Pubmed search (on October 27, 2009) for "water medication pharmacokinetics swine" revealed 93 papers, of which only 4 were on water medications given to swine. Overall, only a handful of papers have directly looked at water medications in swine (McCaughey et al. 1991; Agerso et al. 1998; Mason et al. 2008; Dorr et al. 2009; Mason et al. 2009), whereas quite a few more have involved observational studies of these medications and reported plasma levels. Therefore research in the area of water medications is of great importance; however despite the lack of data, much of the pharmacokinetics of these medications was considered axiomatic.

Many of the limitations to analysis of water medications has related to the computational abilities of pharmacokinetic programs using traditional, readily available PK analyses, when other methods are necessary. One major limitation that is met with water, and feed additives for that matter, is the lack of a known dose of medication taken by animals. This is not a problem often encountered in human drug studies or in small animal medicine because these patients are often dosed directly by clinicians or technical staff, often using parenteral medications. Furthermore, individual animal and client compliance in domestic animals is often good (over 50%) as reported by the American Animal Hospital Association (http://www.aahanet.org/protected/ComplianceExecutiveSummary0309.pdf) summary in 2008. However, in the case of herd health or food animal medicine, an individual animal cannot be medicated several times a day due to labor and animal handling constraints. Therefore, the technique of medicating a whole herd, barn, or pen is commonly employed to avoid individual animal treatments. This ease of treatment, however, may quickly become a pharmacokinetic nightmare. Limitations to pharmacokinetic analysis stem from the way in which many pharmacokinetic programs (WinNonLin, WinBugs, etc) are written. Because most PK programs were originally designed to facilitate quick and easy PK analysis with a limited user interface, typically considered user friendly, these traditional PK programs are exactly the opposite of what is necessary to analyze the data that are acquired in a herd health situation. The largest problem presented by a herd health approach to pharmacokinetics is the unknown dose of the drug. All traditional PK programs the author has encountered allow for multiple dosing regimens; however, a known dose given at a known frequency is required for the program to calculate PK parameters. Even if an approximate dose can be provided, if there is large variability in dosing frequency, as with water medications, the programs are typically unable to account for the large inter-subject variability, which invariably occurs with water medication dosing. There are other sources of variability including body weights (which cannot always be measured), competition among animals, metabolic differences and sometimes other variables that are drug specific. Fortunately, these populations of food animals, most often are also more homogenous in other ways than clinically presented human or veterinary populations. Some of the relatively uniform variables include clearance, weight ranges, and illness status. Due to the complexity of modeling herds in a traditional manner, we have explored other pharmacokinetic methods of modeling water medications. Three PK approaches (traditional, physiologically based and population based modeling) were employed in this work. Hopefully, their applications to water medications in food animals, which are the focus of this work, will assist in addressing other problems faced by pharmacokineticists and toxicologists. These approaches may be applicable to other animal species, such as wildlife and pet birds (often medicated with water soluble drugs). There is also the potential that toxicologists may be able to use these techniques to model exposure even when the doses are unknown.

PHARMACOKINETIC BASICS

Pharmacokinetics (PK) is the science that quantifies and characterizes the way in which drugs are absorbed, distributed, metabolized and finally excreted from the body (referred to as ADME) (Riviere 1997). The effects of a drug on the body, at either the organism or molecular level, are known as pharmacodynamics (PD) (Lees et al. 2004; Rybak 2006). The PK of a drug applies the physiochemical properties of the drug, including (but not limited to) polarity and ionic charges, pH, molecular weight, and stereochemistry, in the context of absorption, distribution, metabolism and excretion (ADME). PK further defines the duration of a drug's effect on the body. A drug's physiochemical properties are defined outside of the body; therefore, the art and interpretation of pharmacokinetics begins when that drug is administered to a subject.

With all PK modeling, the basis is rates of drug moved or cleared from the body over time. Primary PK parameters are based on the idea of a mass of drug (i.e. the dose in mg, typically) that moves around the body and is removed from the body over a certain period of time (Riviere 1997). This mass over time is the rate and with most drugs at currently approved doses, the clearance remains constant over time. These rates of change can occur between compartments known as micro-constants, as with compartmental PK modeling, or they can refer to the whole body elimination rates or macro-constants. These rates of change within the body are best explained using the mathematical language of calculus through differential equations. Basic pharmacokinetic parameters can be calculated for an individual animal without a physiologic understanding of the processes that are ongoing; this is common with traditional PK modeling. However, because this is a mathematical explanation of physiologic processes, a good understanding of the body as well as the mathematics provides the best situation for application of PK models. This is most important in that PK models can be used to explain drug behavior to clinicians, scientists and pharmacists. And when done properly, PK modeling can both explain and predict what happens to a drug within the body. In the clinical setting, the most useful applications of PK are through models that incorporate drug dosing regimens into the prediction of therapeutic drug levels, with less invasive monitoring of patients (Martin-Jimenez & Riviere, 1998; Wahlby et al.

2004). For research scientists, PK models provide a greater understanding of the drug's action, disposition and toxic target tissues, as well as providing a method that requires use of fewer experimental subjects (FDA 1999).

Pharmacokinetic models can be, and frequently are, applied to many species; however, the most extensive information available is in humans. Much of the preliminary pharmacokinetic information obtained on drugs is from basic PK studies in animals (FDA 1999). Therefore PK models are key in extrapolating safety in animals to humans prior to testing (Caldwell et al. 1995).

The following review will touch on basic pharmacokinetic modeling principles and design before discussing modeling differences among traditional compartmental models, physiologic-based pharmacokinetic (PBPK) models and population pharmacokinetic models. Understanding PK modeling techniques, their applications and limitations is needed to best understand ADME and then predict drug deposition and elimination in vivo. A full review of ADME is beyond the scope of this work and is best addressed by other reviews (Caldwell et al. 1995; Grossman 2009). Finally, understanding pharmacokinetics (often via modeling) and being able to apply that information to predict dosing regimens, species extrapolations, and toxicity may assist with understanding how targeting a drug's movement to a certain application will or will not work (Theil et al. 2003; De Buck and Mackie 2007; Yengi et al. 2007; Hou and Wang 2008).

MODELING AND IDENTIFIABILITY

Modeling is a very important aspect of pharmacokinetic analysis. If experiments are crafted in a meaningful and biologically sound way, PK models designed from the measured data provide explanations and predictions for therapeutic levels of drugs and/or clearance rates (Wahlby et al. 2004; Buur et al. 2005). However, models are never one hundred percent correct. In order for that to happen, the model would be infinitely complex to account for every possible outcome (Merle et al. 2004). Parameters (or independent variables) are usually determined from experimental data, but they can be determined by other experiments including ex vivo or in vitro ADME experiments and chemical properties of the drug (Theil et al. 2003; De Buck and Mackie 2007).

Identifiability in modeling is the ability to determine a value to a parameter (Godfrey et al. 1980; Williams 1990). In order for the model to be identifiable, there have to be enough experimental data points from one or more experiment(s) that will help with determining parameters. These parameters can be traditional parameters, population-based parameters with variability as in population PK modeling, or physiologic rates or partition coefficients for PBPK models. Therefore, careful experimental design will shape the type of parameters to be determined and the overall usefulness of the model.

One aspect often overlooked is model structure which plays perhaps the most important role in determining identifiability. Models with unidentifiable parameters or parameters that may be quasi-identifiable (a range can be assigned), often lack enough data due to inability to measure compartments. This is often seen by using a measured compartment to link with unmeasured compartment(s) which leaves some micro-constants unknown. In this case some of the parameters may not be uniquely identifiable or identifiable only if one or more of the parameters in the measured compartment are known *a priori* (Williams 1990). In traditional PK modeling, the concentration time profile is used to determine values to parameters, but parameters may also be assigned values based on physiologic functions, covariates (inter-individual variability of one or more parameters) or other experimental methods mentioned above. This is important to population based PK modeling because a simple model is usually used in order to allow for identifiability of parameters (Wade et al. 1994). Unless external information is available, these simple models are often used for population PK studies due to the complexity and bias that may occur with more complex models (Ludden et al. 1994). Although models are unable to account for all biological variation, less if the parameters are identifiable, a model may be useful to prediction or explanation of a process (Godfrey et al. 1994; Wahlby et al. 2004). These parameters may be general PK parameters such as Vd (volume of distribution) common to compartmental modeling, or the parameters may be specific to a model, such as a metabolic pathway for a drug in a PBPK model. However, assigning a value to these parameters is necessary to solve the model based on the observed (plasma, tissue, urine concentrations) data (Wahlby et al. 2004).

The parameters discussed above are generally understood to be global parameters (variables). The parameters to which we typically refer are globally identifiable parameters, which have one unique value, or a very small range of values in the case of quasi-identifiable parameters or population models, when calculated under appropriate experimental conditions of low signal to noise ratios for analysis and good experimental design. In some models, in addition to the global variables, there are local parameters that are often within a programming routine (or compartment) of a model. These locally identifiable parameters can take on various values based on the data that is presented at that time in the program (Godfrey et al. 1994; Evans et al. 2001). These local or unidentifiable parameters lack any specific values and can take on an infinite number of values in the model (Evans et al. 2001). Therefore, when solving or quantifying a model, it is the global variables that we typically refer to needing solutions.

Identifiability in a model can only be determined if the model structure is valid and the experimental design has adequate controllability and observability (Godfrey et al. 1994; Evans et al. 2001). If the experimental designs do not adequately control for changes in response(s) due to parameters or do not allow (direct or indirect) observation of the response of interest, then there will be parameters in the model that are more likely to be unidentifiable or misspecified (Williams 1990). Although a model may have global identifiability, such as clearance in a compartmental PK model, the parameters predicted may be less specific or correct than another model that lacks global identifiability, such as a PBPK model where values are presumptively assigned. With PBPK models, because the physiologic processes are not completely understood or able to be controlled, often assumptions must be made for many of the parameters in the model. These values may be assigned to the model for various organ compartments, including blood flow, metabolic functions and volumes; however, the values may or may not be correct; and in reality we can never know their true values. PBPK models may also contain good local (compartment-based) identifiability. Models with unidentifiable parameters may be more useful or less useful than fully identifiable models

(Godfrey et al. 1994); however, the main assessment of a model is related to its usefulness, not its identifiability, as will be discussed below. If the parameters are not at least quasi-quantifiable, then the model is not solvable, and therefore, it is only a conceptual model.

ASSUMPTIONS AND MISSPECIFICATION

With all models, in order to solve the formulae for important parameters, certain assumptions must be made for the mathematical structure to be solved with available data. Generally, more complex models require more assumptions; however even if assumptions are not directly stated, the model type often has certain assumptions that are generally applied whether the modeler knows this or not. Some assumptions that are common to all traditional PK models are compartment homogeneity (Evans et al. 2001) and first order kinetics (Rescigno 2001). Most PBPK models assume a flow limited system (Kawai et al. 1994; Lee et al. 2009), while population based models assume normal distributions for most population-based parameters, unless a non-parametric algorithm is chosen (Lindstrom and Bates 1990; Davidian and Giltinan 1993; Park et al. 1997; Bustad et al. 2006).

Assumptions allow complicated mathematical formulae to be simplified into formulae that can be solved. Based on the number of data points and subjects in the model, modelers are left with a certain amount of room for predicting parameters and solving the proposed model, often discussed in statistics as degrees of freedom. Given an unlimited supply of data and infinite time and computational ability, all models could be solved. However, this would be unreasonable, and therefore, simpler models that explain something are often used in lieu of unwieldy giant models that explain everything, but cannot be mathematically applied to real world situations (Ludden et al. 1994).

Unfortunately, even with well developed models, based on good experimental design, there will always be model misspecifications (Merle et al. 2004). Misspecifications are errors in the model that affect parameter predictions. They arise from various sources, including falsely acquired variability in error terms, instrument errors and sometimes from bias in the reporting of data. However, major misspecifications occur from making assumptions that are not physiologically or chemically valid. Often this type of misspecification arises from model over-simplification. Unfortunately, many models would not be mathematically calculable without assumptions and simplifications to decrease the number of unknown variables (Evans et al. 2001). Therefore, models often use the fewest number of important parameters to account for the presumed pharmacokinetic processes (FDA 1999; Martin-Jimenez and Riviere 2001; Buur et al. 2005; Buur et al. 2005). These model simplifications are often made via assumptions, such as homogeneity in compartments and instantaneous absorption for intravenous doses (Evans et al. 2001; Merle et al. 2004; Buur et al. 2005; Buur et al. 2005), which may be physiologically equivocal.

For individual-based models, the variability in parameter estimations is assumed to be a random error factor, usually distributed around a mean of zero. This random error should account for both individual differences between subjects and the instrument error. However, when assumptions are made that are not physiologically valid or reasonable to a model or process, this random error term will become very large due to misspecification of the model. One goal in picking a model design or type is to decrease model misspecification in order to better predict parameter estimates. Also one must know what he is attempting to learn prior to designing an experiment, which should be based on the information needed. Table 1 has an overview of the three types of models to be discussed.

TRADITIONAL MODELING

Traditional pharmacokinetic modeling has typically relied upon intensive-sampling studies, with few to many subjects, with compartmental based results (FDA 1999). However, over the last decade non-compartmental modeling techniques have gained favor. Either of these conventional methods is based upon the idea (even with non-compartmental analysis) of at least a vascular compartment, necessary for the simulation of a concentration-time profile. The idea behind open compartmental analysis is that areas of the body which experience similar disposition and elimination of a drug are lumped together into compartments. These compartments are differentiated by curve fitting of an exponential equation for intravenous data, where drug concentrations are eliminated from the measured (central) compartment at constant rates per unit time (k_n) of the various exponential curves. For a two compartmental model, the following equation represents two compartments, labeled A and B:

$$Cp = A*e^{-k_a t} + B*e^{-k_b t} \qquad (1)$$

With compartmental models, there are several other assumptions of normality made about the population, including: typically constant, first order processes of elimination and partitioning, no saturation of tissues and absence of covalent/non-competitive binding in tissues or compartments. These assumptions, although broad, also mean that some drugs cannot be accurately modeled using these techniques because they violate one or more of these assumptions (Riond and Riviere 1988).

The most common PK modeling type is compartmental modeling, with one to three compartments (Godfrey et al. 1994; FDA 1999). Once the number of compartments exceeds three, modeling can become overly complex and often other mathematical techniques must be used. Traditional modeling is based on known, directly measured values, usually including the dose (D) and plasma concentrations time profile (Cp). These values are assumed to be precise with little to no variability or error. Primary parameters are calculated from these measured values. These fundamental parameters include the theoretical volume of distribution (Vd) and body clearance (Cl); however, they must be calculated from the concentration time profiles. The following equation illustrates the relationship between these two primary parameters (Riviere 1997):

$$Cp = D/Vd \tag{2}$$

Based on these primary parameters, volume of distribution and body clearance, secondary parameters, not based on physiology, are calculated to represent processes, occurring within the body. These parameters include half-life (T ½) and entry and exit rates for each compartment in multiple compartmental models.

Compared to compartmental models, non-compartmental models are usually constructed from sparse data which does not allow a differentiation of compartments from the semi-logarithmic curve or from situations in which inflection points in the elimination curves have inadequate sampling, which would lead to potentially gross inaccuracies in the compartmental rates and half-lives. Non-compartmental modeling is also used for groups of individuals where there is not one compartment model that fits all individuals (Riviere 1997). Non-compartmental model analysis uses a simple curve or regression line that is often calculated via a process called statistical moment theory (SMT) and referred by other names. SMT uses most of the same assumptions for compartmental analysis, but relies on moments of the concentration-time profile. In SMT, differential equations are often solved with calculus to provide AUC moments per unit time using the trapezoidal rule. Based on the AUC, as the primary moment, the secondary moment, i.e. the differential of the previous curve, reveals a peak, that represents the mean (average) residence time (MRT) for the average molecule. MRT is linked to the half-life of compartmental models by the equation:

$$T\,½ = \ln2 * MRT \qquad (3)$$

The MRT is the change in the concentration-time curve through time and its peak is therefore equal to the most frequent interval of time that molecules remain in the body. Therefore, non-compartmental analysis can be likened to compartmental analysis with fewer restraints on curve fitting. Traditional parameters can be calculated from SMT as explained elsewhere (Riviere 1997). Furthermore, the same drug over the same time frame may require a three compartment model in one person and a two compartment model in a different subject (Rescigno 2001; Merle et al. 2004). This physiologic variability makes a case for non-compartmental analysis over compartmental analysis and also provides a comparison between individual. Non-compartmental analysis can be used in cases where uncertainty in dose, limitations to modeling or increased variability exist; however, this modeling often requires pharmacokinetic or mathematical programs that do not require dosing schemes. One final benefit of SMT over compartmental modeling is the ability to calculate some primary PK parameters without a dosing schedule at all (see Chapter 3 for an example).

With all types of traditional modeling, the need to have adequate sample times is necessary, as can be seen in Figure 1. This figure exhibits the effects of sparse sampling compared to more intensive sampling techniques. Much more information is available from the top panel in Figure 1 than from the lower panel. From Figure 2, one can see the effect of performing logarithmic transformation of the plasma concentrations. The result is that a one-compartment model would fit data in Figure 2. The rate of elimination does not change, but semi-logarithmic transformation of data is best able to demonstrate when no change in the rate is present, compared to a non-transformed plot. The upper panel in Figure 2 shows typical plasma concentration versus time results, while the lower panel shows the transformation, which corresponds with a one compartment model.

For more than a one compartment, the true value of the distribution and elimination rates cannot be directly seen from the curve and data stripping is necessary to differentiate the elimination rates of the two compartments. The process of data stripping will not be discussed further in this review. There are other resources for this and most PK programs can perform data stripping (Riviere 1997; Rescigno 2001; Merle et al. 2004).

Traditional compartmental modeling techniques do not attempt to develop a physiologic rationale to explain the data or even to link the data to compartments if the model contains these. Rather traditional modeling relies upon the data for its constraints, using distribution and elimination rate constants from the data to best fit it to a number of compartments. It is often explained that compartments relate to physiologically related tissues or areas in the body; however, the time frame of experiments is often a better explanation as to why the same drug requires different structural models most of the time. The importance of the traditional approach is that data-intensive sampling is necessary to avoid missing changes in rate constants as seen in Figure 1 (Riviere 1997; FDA 1999; Green and Duffull 2003). From traditional models, physiology can be super-imposed upon the results to explain what may be happening in the body and assign physiologic functions and rationales to a mathematical process (Rescigno 2001). These approaches are often used for initial studies, when PK parameters are unknown.

Compartmental PK modeling techniques have been used to look at sulfamethazine and tetracycline in swine over the last few decades. However, no approaches used the SMT approach. Pharmacokinetic studies for sulfamethazine have been performed by Nouws et al (1986; 1989); and Sweeney et al (1993). Several compartmental studies have also been performed on tetracycline given intravenous and compared those levels with plasma concentrations attained by feed medicated with tetracycline (Kellaway and Marriot 1975; Kniffen et al. 1989; Nielsen and Gyrd-Hansen 1996). These data can serve as a comparative starting point for modeling these drugs dosed in water in commercially housed swine.

PHYSIOLOGICALLY-BASED PHARMACOKINETICS

Physiologic-based PK (PBPK) modeling requires an understanding of the above concepts in conjunction with specific information about ADME for the drug(s) being modeled. PBPK modeling is a data-intensive modeling system (FDA 1999; Buur et al. 2005; Buur et al. 2005) and is based on heavily sampled individuals. Unlike traditional PK models that are generally simplified to a log-linear regression model or a compartment model, PBPK modeling uses physiologically important parameters to develop a model that predicts known data (Kawai et al. 1994 (Buur et al. 2005; Buur et al. 2005). In many ways PBPK is similar to compartmental modeling; however, rather than allowing the drug profile to determine the model, in PBPK modeling, the modeler sets up compartments that correspond most often to tissues in the body with known volumes and blood flow rates (Emond et al. 2005). PBPK modeling is therefore an individual-based model. PBPK models attempt to more accurately predict the physiologic processes taking place than compartmental models. This is done by using actual organ or organ systems data, physiologically important to the drug in terms of drug distribution, drug targeting (PD), metabolism and elimination. Defining common parameters such as cardiac output (heart rate and stroke volume), respiration rates, blood flow rates, physiologic effectors (exercise, blood pressure) and metabolic processes are necessary for a functioning model. The major difficulty of PBPK models is appropriately applying the huge amount of physiologic data about organ volumes, blood flow rates and percentages of cardiac output to the model in a meaningful way. Many of these measured values are hard if not impossible to acquire in a valid and physiologically relevant way.

Furthermore, due to the complicated nature of PBPK models, there is a great need for simplification. One major simplification of PBPK modeling has been and remains the need for the model to be flow-limited (Kawai et al. 1994). Without the model being flow limited, the model would be completely unsolvable without protein-binding/dissolution rates and transporter processing rates. However, when making a PBPK model flow limited, one must assign partition or diffusion constants that determine the rate or portion of drug in the blood that will enter the compartment or organ being modeled. The partition coefficient is often very difficult to determine *in vivo* and model optimization is often performed to determine partition coefficients. Finally, PBPK models assume instantaneous diffusion with homogenous compartmental mixing. These simplifications ignore the effect of cells and various cell types within compartments that represent organs, but often work to make the model meaningful without being overly complex.

Because PBPK models are individual models, most variation in PBPK models is intra-individual error (Evans et al. 2001; Buur et al. 2005; Buur et al. 2005). Intra-individual error is found within an individual, often tied to physiologic processes discussed above. Due to their individual nature, PBPK models often lump intra-individual error with measurement error from variability in the analytical process which is up to 20% in some validated assays (Bressolle et al. 1996). Measurement error is often assumed to be larger than the physiologic error, and thus intra-individual error is often ignored. However, with some assays measurement error is very small and therefore, assuming measurement error is the total error in PBPK models can lead to misspecification of the model. This misspecification is especially troublesome when developing PBPK models for application to non-homogenous or genetically polymorphous populations, especially if differences in metabolic or elimination processes are unknown between the model population and a genetically different or diseased population. Model misspecification often cannot be differentiated from large intra-individual errors. Crucial to proper PBPK model development is a good understanding of ADME and its proper application. Based on the potential for misspecification, when variability is large, validation of PBPK models can be difficult.

The greatest limitation of PBPK modeling is its lack of certainty for many physiologic parameters. This unfortunately provides a sizeable and unquantifiable error, which makes model validation difficult. If two populations are known to be similar it may be safe to make population predictions with PBPK models. However, populations must be similar as the model may lack external validity in a target population due to variability not represented in the model (FDA 1999). One example of this type of variability occurs with genetic polymorphisms in populations. These polymorphisms result in a 'high' metabolic status and a 'low' metabolic status (Clewell and Andersen 1996). Some modeling programs allow optimization of models based on minimizing sum of squares of the residuals and allow comparison of the model output to the observed data. Model optimization algorithms predict the best parameter values based on the designed model, and they often allow other data sets to be included for model comparison. However, with PBPK models, due to the variability in sources of data for model development (including PK studies for comparable data, metabolism data, etc), methods of model comparison are not easily performed. It may be possible to use statistical programs to perform residual sum of squares comparisons between models for a single data set in addition to graphical methods.

PBPK models have been used in various ways in the last 2 decades. First, they can be used to extrapolate to situations of unknown dosing (e.g. (Mason et al. 2008), potential changes in pharmacokinetic parameters or rates of elimination (Emond et al. 2005; Emond et al. 2006) and extrapolation across species from those previously characterized (Kirman et al. 2003; De Buck and Mackie 2007). This techniques provides a way to scale physiologically rather than on traditional allometric (body weight), surface area or other scaling factors

(Kirman et al. 2003). This flexibility is a great asset and allows PK modeling of situations not feasibly modeled via traditional methods, especially where physiologic processes are known but there is limited data for that species (Emond et al. 2005). Chapter 2 of this work compares a traditional model to a previously validated PBPK model (Buur et al. 2005) to determine what might be happening in a production setting. The flexibility of PBPK modeling is the only technique discussed in this work that allows the aforementioned situation to be elucidated.

Finally, PBPK modeling can be combined with many other techniques including Monte Carlo analysis, covariate modeling, Bayesian forecasting and statistical techniques to provide a population approach to pharmacokinetics (Clewell and Andersen 1996; Sproule et al. 1997; Jonsson and Johanson 2003; Buur et al. 2006; Sprandel et al. 2006). Typically, since PBPK modeling is an individual-based modeling approach, the use of these population methods are applied on top of the developed model. These population modeling techniques are based on statistical analysis and typically apply normal distributions over parameter values to account for physiologic variability and error in the model. By running a PBPK model many hundreds of times using these distributions, one can develop a sense of the likelihood of what may be happening in a population of animals or humans exposed to a drug at a given route and dose. Buur et al (2006) applied this technique to residue analysis for veterinary medicine recently. The aforementioned techniques have been used in conjunction with PBPK models to determine population and pharmacodynamic effects (el-Masri et al. 1995; Gueorguieva et al. 2007) and should be reviewed for more information on PD.

POPULATION PHARMACOKINETICS

Population PK modeling is a statistically based pharmacokinetic approach to modeling sparse data of a large numbers of subjects. This techniques uses the variability inherent to the population for better modeling sub-groups that cannot be addressed fully by traditional PK models (del Castillo et al. 2006). The covariate structures, or subject specific groupings, in a system of equations is overlaid on a traditional compartmental pharmacokinetic model. The statistical components of the modeling allow for a normal distribution for the error terms to account for intra-subject variability and variability in pharmacokinetic parameter distributions. These parameter distributions can be parametric, semi-parametric or non-parametric, but most modeling algorithms employ parametric distributions. These distributions are applied to the PK parameters population mean by error terms or equations based on multiplicative, additive or exponential relationships. The determination of the error modeling scheme is best determined by the nature of the data and is beyond the scope of this review (Davidian and Gallant 1992; Davidian and Giltinan 1993; Park et al. 1997; Parke et al. 1999).

The benefit of this type of modeling is evidenced when data is scarce for individuals and there are differences in pharmacokinetic parameters or measured outcomes across groups of individuals based on factors, typically referred to as covariates. In population PK models, it is useful to understand the physiology of the drug, as with PBPK (Karlsson and Sheiner 1994; Mager and Goller 1995; Martin-Jimenez and Riviere 2001; Martin-Jimenez and Riviere 2002; Vinks 2002; Wahlby et al. 2004). However, population PK modeling is not based on a physiologic model but rather statistical theory of population distributions with an assumed variability (set by the modeler). In order to help characterize or define the individual variability seen in PK models, covariates are used to address this inter-individual (or between subject) variability (FDA 1999; Merle et al. 2004; del Castillo et al. 2006). Emphasis is often placed on covariates that provide information on differences in pharmacokinetic parameters among subgroups. These differences typically relate to physiologic parameters of metabolism or clearance of a drug (such as enzyme polymorphisms or disease states), as well as other changes in basic physiologic parameters (Martin-Jimenez and Riviere 2001; Wahlby et al. 2004) including volume of distribution and protein binding. Population PK covariates may include physiologic functions, such as serum creatinine, liver-specific enzyme levels, age, blood pressure, or body condition score or weight; or they may be categorical variables, such as gender or health status (Martin-Jimenez and Riviere 2001; Mager 2004; Wahlby et al. 2004). These covariates are used to provide insight into the pharmacokinetics related to a drug by providing a physiologic basis for mimicking variability present in the population. Because this technique is based in statistics, not a physiologic-based technique, population PK experiments can use less intensive data collection than PBPK models while maintaining a robust ability to predict population parameters across subgroups (FDA 1999; Wahlby et al. 2004).

The strength to population PK modeling lies in its ability to account for both intra-individual and inter-individual errors (Martin-Jimenez and Riviere 2001; del Castillo et al. 2006). The intra-individual error has to be set before the inter-individual error is addressed. Once intra-individual variability is specified, often by repeated measures, the inter-subject error is set using the covariate distributions in the model. Finally, undefined ‘interoccasional’ or random error (ε) accounts for other variability (Wahlby et al. 2004). Therefore, we can say that the identifiable sources of variability (parameter variability, covariate effects, and measurement error) for the model are what allow this method to work. These are notated as follows:

ŋ for intersubject error (i.e. individual differences)

ω^2 for intersubject variability.

ε for random error for each observed ŋ, which may contain variability in intra-individual error depending upon how the model is specified.

Each of these types of errors is set for the parameters involved in the model in order to simulate the concentration-time profiles and other directly measured data. With population PK modeling, it is essential that differences among these error terms be specified. Without defining the error types, model covariates or bias may not be fully elucidated. It is possible to develop a model without defining each error type; however, the coefficient of variation across the PK parameters in the model would undoubtedly be very high or incalculable. Therefore the accuracy and precision of the PK parameters would be poor because of undefined error rates, which increase model uncertainty.

Because this is a statistical technique, one of the benefits of mixed effects population modeling is the ability to employ bootstrapping, re-sampling, and data splitting to the original data sets, if necessary, to increase sample population sizes. The above techniques along with varying the type of parameter distributions can help modelers avoid misspecification of parameter variability and improve model fit (Davidian and Gallant 1992; Park et al. 1997; Parke et al. 1999; Oberg and Davidian 2000; Bustad et al. 2006; Sprandel et al. 2006). Various studies have been performed in the literature in both human and veterinary medicine applying various programs including NonMEM, WinBugs and WinNonMix to population pharmacokinetic modeling. Further review is directed to the following sources (Davidian and Gallant 1992; Karlsson and Sheiner 1994; Yuh et al. 1994; Vinks et al. 1996; Concordet and Toutain 1997; Martin-Jimenez and Riviere 1998; Gupta et al. 2001; Martin-Jimenez and Riviere 2001; Martin-Jimenez and Riviere 2002; Peyrou et al. 2004; Anderson et al. 2006; del Castillo et al. 2006; Chervoneva et al. 2007).

MIXED EFFECTS MODELING OF WATER MEDICATIONS IN HERDS

The above population modeling techniques have been applied to veterinary medicine very effectively. However, when attempting to model medications administered via water, there are several unique concerns related to the method as well as to treating swine in commercial facilities. Therefore, many of the premises in previous studies may not be feasible in commercial settings. The largest aforementioned problem is the fact that there is a variable and unknown dose for each animal. Using deconvolution techniques may be laborious, unfeasible, or provide large ranges of doses based upon the structural PK model used. Deconvolution techniques also require prior information relative to the PK parameters for the model structure chosen as well. *A priori* information for PK parameters is helpful but not necessarily required for a population model. Whereas for a traditional deconvolution techniques, clearance or elimination rates must be known in order for a dose to be calculable. With some types of population pharmacokinetic modeling one can actually bypass calculating the actual dose if known covariates and fixed variables are available to help determine a dose. This technique is discussed further in Chapter 5.

Although the statistical portion of the model is defined by parameter distributions, a traditional PK structural (i.e. compartmental) model is still the basis of the design In determining which model type to use (compartmental type (micro vs. macro) and number, sampling schemes, etc.), there are many approaches in the literature and often involve different algorithms for crafting studies including D-optimal design (Tod et al. 1998; Green and Duffull 2003; Gueorguieva et al. 2006) and two stage model approaches (Yeap and Davidian 2001; Tam et al. 2003). There are also pharmacoepidemological (Clewell and Andersen 1996; Dorr et al. 2009) and pharmacogenetic approaches (Borges et al. 2006) that may be able to provide PK data for analysis. In Chapter 5, the final model chosen was an open one-compartment traditional PK model. However, a two compartment model was also considered as an option.

Due to the flexibility of pharmacostatistical models and the ability to use Bayesian forecasting, some of the limitations to traditional modeling, such as needing a dose, can be avoided. The model types chosen were based upon the data from the initial tetracycline study. The data was collected from a two-stage modeling scheme, with relatively data rich sampling in few animals over the dosing period. Much individual subject data was collected and calculated in this process in addition to plasma concentration-time profiles, including body weight, daily weight gain, water use, water concentration, and serum creatinine.

The two stage model allowed the data to be collected and interpreted by a traditional SMT technique that gave estimates of apparent bioavailability and clearance for the groups of animals (discussed further in Chapter 3). This two stage method allowed individual PK parameters to be calculated first and then group parameters were calculated. This initial data was applied to the population mixed effects model to determine factors important to plasma concentration variability for a larger population (Jonsson et al. 2000; Bustad et al. 2006). This method also provided *a priori* parameter estimates of the group PK parameters and served as a later comparison to the final model parameters.

In the initial model development, after this two-stage modeling was finished, a large amount of variability was left among individual animal concentrations. Furthermore, two of the treatment group's steady state concentrations were not able to be differentiated. A basic linear model was developed that related steady state plasma concentrations to the water concentrations to which the animals were exposed (Figure 3). The reasons behind this large variability were unknown in the initial study.

In creating the mixed effects model, potential covariates were selected from collected data as well as from published covariates commonly found in population modeling (Mager 2004). The major covariates considered were serum creatinine as a marker for clearance, body weight as a surrogate for volume of distribution and bioavailability. Other factors that were measured and considered for inclusion in the model were water consumption and the ambient temperature. The final covariates chosen for the model should account for inter-individual variability in the model and decrease the overall intra-subject and random error significantly.

However, not all covariates that seem important will be useful. Therefore a selection process is necessary. The basic ANOVA (linear) model above that characterizes concentration–time profiles related to the potential covariates was considered first. By graphically plotting residuals of this linear model as a function of the covariate values for each subject, a wealth of information on that covariate and its relationship to the data surfaced. The graphical method allows one to determine the effects of each variable to the results of a either a linear or compartmental model (Davidian and Gallant 1992). This is analogous to multiple linear regression analysis of data to compare model fits. The graphical technique, if used properly, can also reveal non-linear relationships of covariates that ANOVA methods cannot detect. The residuals' plot also helps explain the relationship of these covariates. When no relationship or bias to the model is present from the covariate, the residuals are evenly spaced above and below the estimated value for the model. However, if covariates skew the residuals in non-random patterns, then this covariate should be considered in the model design. This technique is often used with linear regression model evaluations. This highly useful tool provides some insight into variables of importance and can also suggest the type of relationship (linear, exponential, polynomial, etc.) that the covariate may have to the model.

In Figure 3, the ANOVA model for predicting tetracycline concentrations in swine was used as a predictor of data for Dorr et al (2009). Based on the plotted residuals, it is clear that there is some model misspecification that over-predicts most of the time points. This supported the need for a population model because the assumed linear model was inadequate to predict across the 2 populations.

Another method of selecting covariates is to use step-wise additions of suspected covariates to a population compartmental analysis (Gobburu and Lawrence 2002). Most modeling programs will show plot graphical relationships between PK parameters and the covariate being tested across the populations, so differences can be detected. This is another method for selecting covariates without performing statistical assessments prior to model inclusion (Phoenix WinNonMix 6.0, Pharsight Co., Mountain View, CA).

Once the basic model structure is selected, many programs include a list of different modeling algorithms. The algorithm selection allows one to compare the model results using various model algorithms to determine the best algorithm to use. However, there are also various published comparisons among algorithms and types of modeling methods to use. Different algorithms include differences in the assumptions on normality, independent of samples, and use of previous data in determining PK parameters. A few algorithms are implemented in most programs, including first order, first order conditional estimation and extended least squares (or other algorithm that is semi-parametric). With population studies, repeated measures from some individuals are present and therefore, intra-individual variability can be easily calculated with an algorithm that handles repeated measures.

The first order (FO) sum of squares is commonly used and typically assumes independence of samples with a normal (Gaussian) distribution of error terms (Tanigawara et al. 1994; Jonsson et al. 2000; Bustad et al. 2006). It may stray more for data samples with intensive sampling from fewer individuals than other modeling algorithms. The First Order Conditional Estimate (FOCE) model which includes Bayesian forecasting, does assume normality, but with a Lindstrom-Bates algorithm, it includes a correction for repeated measures (Lindstrom and Bates 1990; Yuh et al. 1994). Bayesian forecasting allows the use of previous data points and parameter estimates to help determine the next estimate for the model when used for each individual. This method can be more accurate for sparse population sampling where the individual parameters would normally contain greater error than the typical FO or least squares techniques (Tanigawara et al. 1994; Jonsson et al. 2000). Finally, the FOCE-Extended Least Squares or semi-parametric approach does not require normality and uses Bayesian forecasting as does FOCE to determine the parameters (Bustad et al. 2006). This method is best used to determine bimodal or populations with non-normal distributions of one or more parameters. However, when this type of algorithm is used for populations with normal parameter distributions, parameter skewing is more common (Bustad et al. 2006).

Differences in algorithms can only be appreciated by running a model with known parameters and comparing algorithm outputs directly. In general, for normal or fairly normal populations, FOCE –LB and FO work well (Gobburu and Lawrence 2002; Bustad et al. 2006). However, if there is a bimodal or non-normal population parameter, algorithms that do not assume normality and non-parametric techniques are better at fitting the data (Bustad et al. 2006). The algorithm may be chosen based on expected outcome or on model simulations. Evaluating the differences between models with covariate or PK structural differences can be very difficult. Some of the means to do this are discussed below.

VALIDATION AND COMPARISONS

Determining the validity of a model is quite subjective. Unfortunately when a model is developed, there is no definitive way to know that a model is valid or useful; and models can only be disproven, never proven true. However to determine a model's validity, it may be good to ask whether the model needs to be useful within only a limited context, such as in your laboratory among technicians, or whether validity is required in other contexts. This is a commonality in laboratory testing, where most sample ranges are laboratory specific.

This same concept can be applied to models. A model can be evaluated on several fronts. Firstly, a model that explains a process may not actually need to be validated. This would be a conceptual model that cannot be quantified. However, with pharmacokinetics, models need to often be applied to other similar situations. Applications of PK models to other situations is of greater importance than conceptual models because applications determine if the techniques used or methods employed are useful beyond the conditions of initial development.

A model's applicability beyond its development conditions is often referred to as its overall validity. There are two types of validity: internal and external. If experiments are designed and performed correctly, a model should have internal validity. Method validation is especially important for PBPK and population PK analyses because the method ensures internal validity (Bressolle et al. 1996; FDA 1999). However, external validity must be determined from a separate validation process. Validation of models has been discussed via FDA in its Guidance for Industry (2003). External validation is best done via at least two data sets. The first set is needed to develop the model while the second set is used to validate it. The validation set data are entered into the model and the model's predictions are compared to the response data. Model validation suggests that a model can predict data outside of its sample population but within some constraints such as population type or dosing range (FDA 1999; Wahlby et al. 2004; Buur et al. 2005; Buur et al. 2006).

There are several model selection criteria described in the literature, but only three will be mentioned here. Typically modeling programs provide at least one type of selection criterion with model runs. These criteria and the graphical results of nested models with progressive additions of covariates, including different relationships of the covariates, are a helpful way to develop a model. In developing population PK models, using two or more data sets collected from different studies provides for a more robust model design. However, this approach may omit certain covariate data for some inter-study comparisons, which may increase the total model score (show a decreased fit). Some of the typical model comparison tests for non-linear models are the loglikelihood ratio, Akaike's Information Criteria and Bayesian Information Criteria. There are many other methods that are employed, but these criteria are traditional recognized by many programs and are readily calculated.

The log likelihood ratio (LLR) is a test statistic that uses a value assigned to a model based on a chi square distribution. This distribution assumes statistically independent variables with a Gaussian distribution. If this assumption of independent variables or covariates is violated then the LLR may be invalid. This ratio is based on the comparison of two or more "nested" or similar models. When models are "nested", they contain the same basic structure and often additional variables are added one at a time to the model. When comparing the ratio of two LLR with different degrees of freedom (different numbers of parameters), the ratio is accessed based on the Type I error rate (alpha), which is set by the modeler. If the test statistic is significant, then the new model is better than the previous model. If the LLR is not significant then the model with fewer parameters is a better fit even thought the likelihood value is smaller. Whenever more parameters are added to any model, the fit of the model based on a sum of squares estimate will increase because the random error term of the model will decrease. This test provides a way to statistically compare models based on a p-value statistic. However, this statistic often exhibits a bias towards a simpler model and is considered biased when used with sparse sampling techniques (Ludden et al. 1994).

Akaike's information criterion (AIC) is one of the most popular and often used methods for both linear and non-linear model comparisons. The AIC is an algorithm that can also be used to compare similar models, but the models do not necessarily have to be nested for comparison. However, the use of AIC across datasets should be approached with care as this value tends to be affected by the sample size (Burnham and Anderson 2004). The AIC values can be directly compared, but the total value is affected by the data as well as the model itself which and therefore, changing only one at a time is the best practice for comparison. The lower the AIC value, the better the model fit. The following equation represents the AIC calculation:

$$AIC = 2k - 2\ln(L), \tag{5}$$

Where, k is the number of parameters and L is the maximized value of the likelihood function (or sum of squares) for the model in question (Ludden et al. 1994). This formula is based on the residual sum of squares for all observations (i.e. just the random error term variance). This value does not have to be compared to another model by a statistic like the LLR because it includes a small penalty, an increase in the AIC value, based on 2 times the number of parameters in the model. Therefore when AIC values are compared between two models, the modeler does not have to determine if over-fitting of the model is occurring but can rather directly compare the AIC values. The AIC is more predictive with complex models and with sparse sampling methodologies than the LLR or F-tests. This criterion is better for selecting complex models compared to either the LLR or the Bayesian information criterion (Ludden et al. 1994; Vonesh et al. 1996; Burnham and Anderson 2004). There is also a corrected AIC value, which has a modified equation to prevent bias when sample size is small. With large sample sizes it converges to the original AIC value, but some scholars suggest it always be used in place of the original AIC value (Burnham and Anderson 2004).

Bayesian information criterion (BIC), also called the Schwartz criterion, is another method of model comparison that has gained favor in the last decade as well. It is based on a similar equation as the AIC, residual sum of square (RSS) error with a penalty for over-specifying the model. The main difference between the BIC and the AIC, besides the philosophy of their bases, is the size of the penalty for adding parameters. The following equation describes the BIC:

$$-2 \ln p(x|k) = -2 \ln L + k \ln (n); \qquad (6)$$

where $p(x|k)$ is the likelihood of the observed data (x) given the number of parameters, k; L is the likelihood function for the estimated model and n is the number of observations. The value of $-2 \ln L$ is equal to RSS/(variance of error terms) and is therefore dependent on the number of observations (n). The statistics are more complicated with repeated dosing regimens, but the basic premise is that the model is based on decreasing the error term and avoiding an overfitted model. The BIC has been found to be less likely to correctly select more complex models compared to the AIC (Ludden et al. 1994; Burnham and Anderson 2004). However, overall, the BIC is good at selecting the correct model in sparse sampling situations (Markon and Krueger 2004; Link and Barker 2006). With the BIC, a smaller model number is a better fit, as with the AIC and LLR. It is likely that evaluating a model in light of these three criteria will give a better estimate than just using one alone. By using two or more, some of the biases will be removed and the models can be viewed in light of the limitations of each method.

Once a group of models is selected, one or more of the above criteria should then be used to assess an independently collected data set when run on the models. If one of the models represents a "good fit", then that model may be valid for multiple datasets. The model should then be applied to at least another population or dataset to determine its validity. With a robust enough model design and analysis, a model that appears valid for more than one dataset would appear to be potentially externally validated and should be tested across various populations.

However, one reported drawback for population modeling is the accuracy to individual patients (Vinks 2002). Using some traditional modeling techniques and known covariates, a tighter range for the individual may be conceivable; however, the goal of population pharmacokinetic modeling is not to model one person but to provide a way to determine what doses of a drug should be applied to a group of individuals with known disease (or other covariates), or conversely, to predict likely plasma levels of a drug when a person within a covariate group is in need of a specific treatment. The population model will determine a regimen that will work for a population of individuals; however, there are still 5 to 10 people per 100 that may not fit that population model. This lack of individuality is the reported drawback to population modeling. However, if the model is well designed and enough information is known about a subject, population PK models should provide a range into which most subjects will fall. In many respects this is better for most subjects as an individual PK model cannot be made for individuals during a treatment period due to the intensive sampling. This concept is beneficial in a clinical setting where ill patients cannot be monitored as closely as experimental subjects. Using population modeling techniques can also be applied as described above for water medication dosing because the average or common levels of a drug within the herd are more important than any individual animal's plasma concentration of that drug (FDA 1999; Wahlby et al. 2004).

VETERINARY MEDICAL APPLICATIONS OF THIS RESEARCH

The application of models in pharmacokinetic analysis is important for explanations and predictions of therapeutic drug concentrations in veterinary patients. Previous studies have applied population pharmacokinetic modeling to veterinary medicine and specifically to food animal medicine (Martin-Jimenez and Riviere 2001; Martin-Jimenez et al. 2002; Martin-Jimenez and Riviere 2002). Traditional modeling has been used extensively in veterinary medical research. PBPK models have used less frequently in veterinary medicine; however, this technique has been found useful in predicting population based drug elimination and withdrawal times in pigs (Buur et al. 2005). Finally, population based pharmacokinetics began being used in veterinary medicine in the 1990s and has been used by others (del Castillo et al. 2006) to better characterize observed concentrations in animal populations. Therefore these kinetic techniques have already been described and applied in veterinary medicine to oral and intravenous medications.

The previous comparisons of traditional, PBPK and population PK models illustrate that models can vary greatly and yet they can all be applied to veterinary medicine. Models are only as useful as their ability to complete the tasks for which they are employed. Any of the three model types can be used, but one model type may be better suited for one task over another. The usefulness of these models lies in their application to better explain real life circumstances. PBPK modeling used in Chapter 2 helps to better model a contamination situation which cannot be adequately addressed by traditional modeling. In Chapter 3, statistical moment theory is used to illuminate the pharmacokinetic parameters of tetracycline water medication, when an unknown dose is drunk by animals. However, these kinetics may only be dubiously applied to a population with various doses and situations. Therefore, Chapter 5 describes a non-linear mixed effects model that was developed to handle an unknown dose, with known exposure levels to three populations of animals. From this population based modeling technique, the role of covariates, both internal and external to the pig, are used to predict plasma levels that might be seen in swine operations. From this model, a 90% confidence interval for a population of animals with similar characteristics can be discerned. Finally Chapter 4 discusses the role of tetracycline water medication from the perspective of stability and it biological activity to round out the usefulness of this medication in industry settings.

These various PK techniques can add to the body of knowledge available on how to creatively approach problems that have been avoided due to their complexity and difficulty. These techniques may help with residue avoidance in populations of food animals (Martin-Jimenez and Riviere 1998; FDA 1999), toxicology dosing dilemmas in back-calculating potential exposure (el-Masri et al. 1995; Jonsson and Johanson 2003; Mason et al. 2008) and in other variable dosing situations such as in treating wildlife, pet birds and rabbits. The applications of these methods to veterinary medicine in general and to herd medicine specifically are the focus of this work.

Table 1: Generalized Characteristics of the Three Model Types

Model Type	Error	Approach	Direct Application	Sampling	Extended Application
Compartment	Random	Curve fitting	Individual	Few subjects, many time points	Averaging of PK parameters is used to apply to populations
PBPK	Random Intraindividual	Mechanistic	Individual	Many time points from few to many subjects	Monte Carlo, Bayesian and statistical techniques can be applied over model to extend it to a population
Population PK	Random Intraindividual Interindividual	Curve Fitting Statistical	Population	Few subjects with many time points to many subjects with few time points	Difficult to apply to individuals. May apply to covariate specific populations

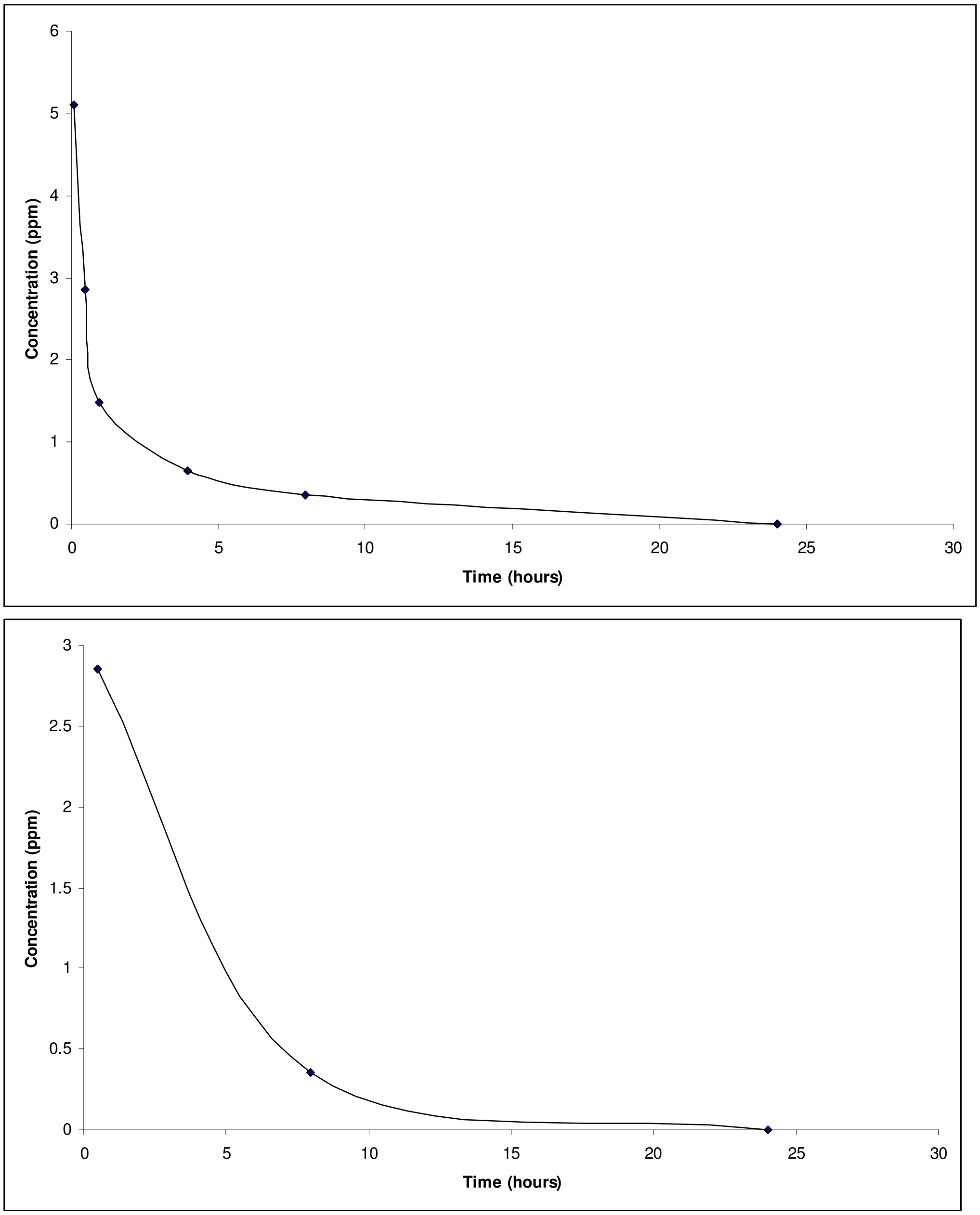


Figure 1: The top panel shows a more intensive sampling regimen of the plasma compartment than the lower panel. The compartmental PK analysis from the sparse sampling scheme will not detect the distribution phase from 0 to 5 hours in this example.

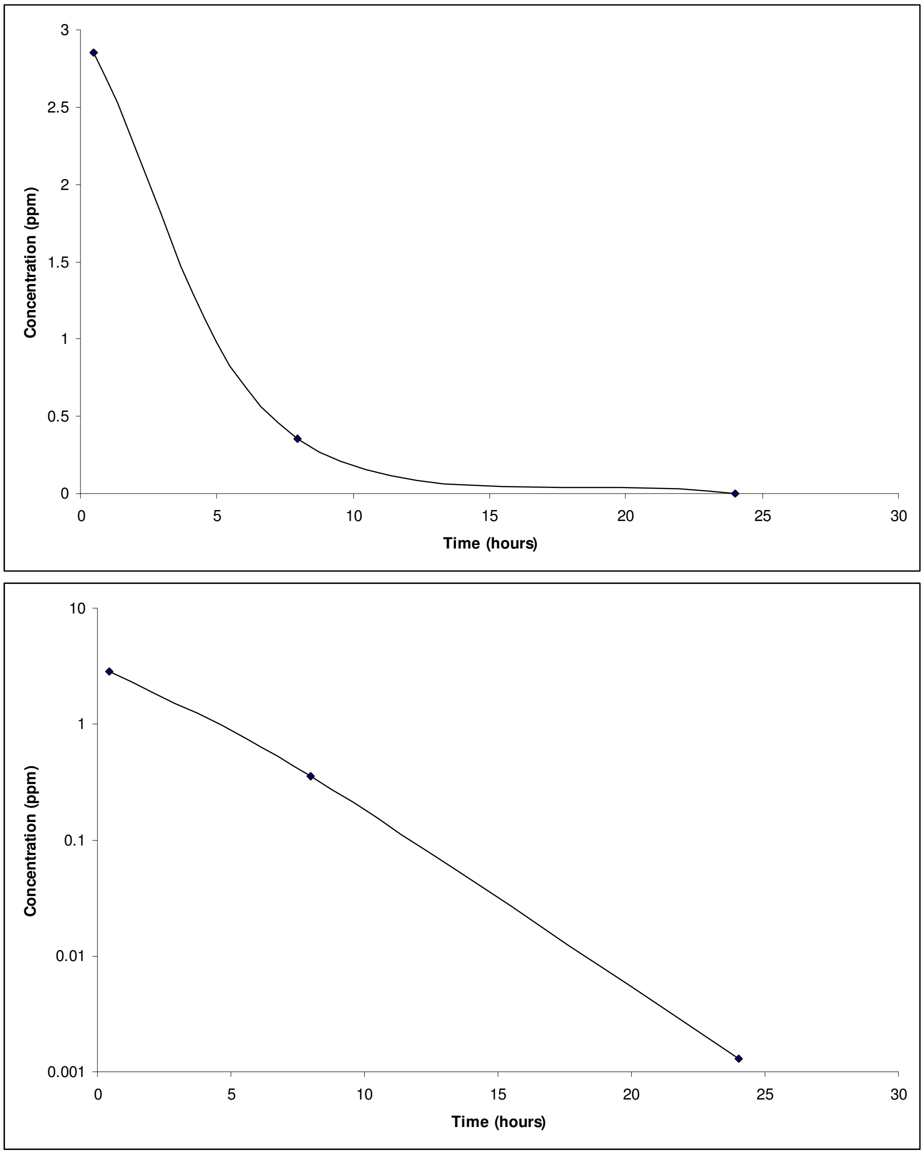


Figure 2: Data transformation of pharmacokinetic profiles by logarithmic transformation of the y-axis is frequently performed prior to compartmental PK analysis. The top panel exhibits untransformed data, while the lower panel shows the transformed data.

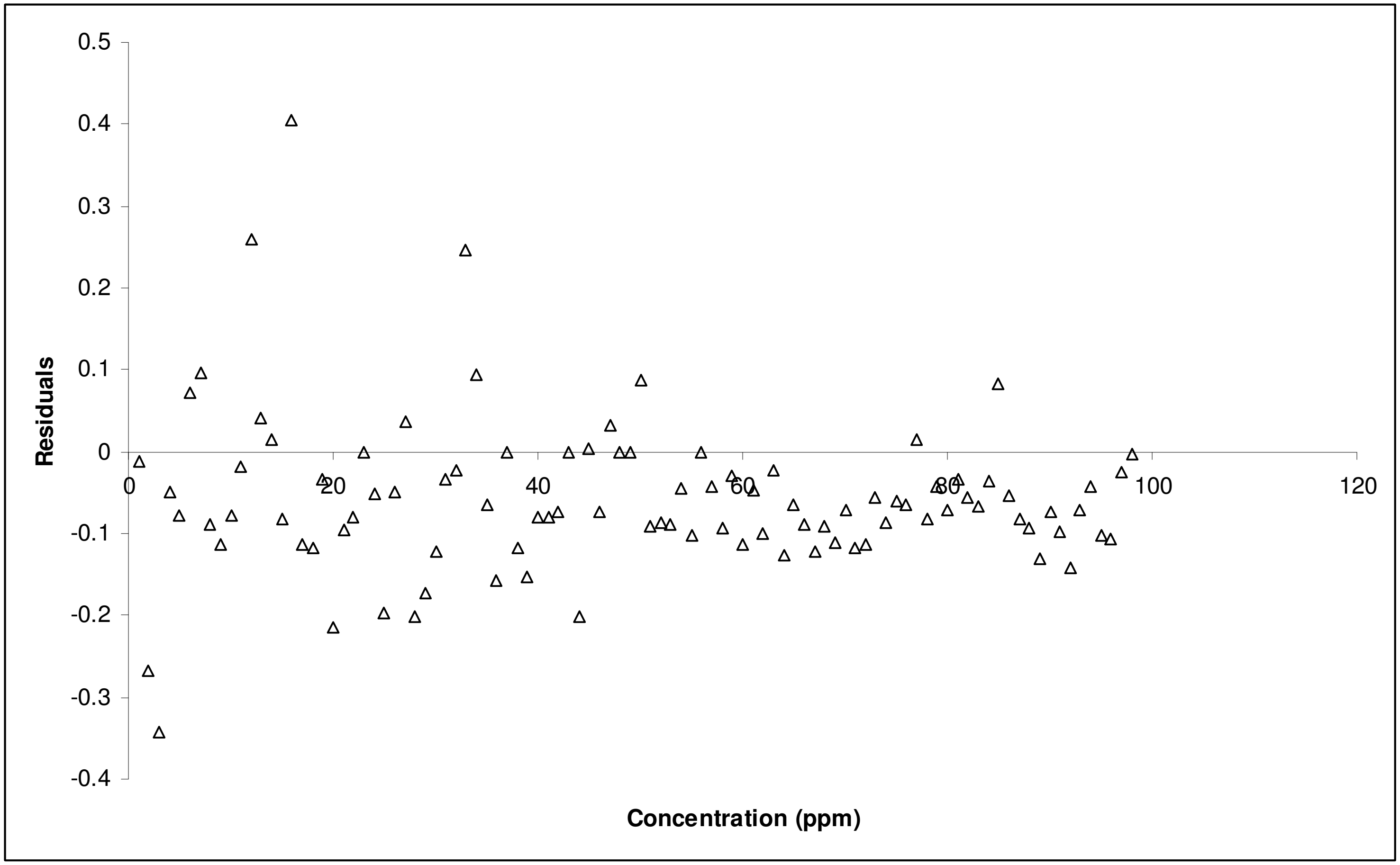


Figure 3: Plot of residuals versus time for a model applied to validation data. The residuals from this model are systematically too low for the data, suggesting that the model is overpredicting values, and is likely misspecified. As with many pharmacokinetic models, the early time points, where concentrations are higher, tend to exhibit greater variation than later time points. This bias can be corrected by weighting the terminal time points.

References

Agerso, H., C. Friis and J. Haugegaard (1998). "Water medication of a swine herd with amoxycillin." J Vet Pharmacol Ther 21(3): 199-202.

Anderson, B. J., K. Allegaert and N. H. Holford (2006). "Population clinical pharmacology of children: modelling covariate effects." Eur J Pediatr 165(12): 819-29.

Borges, S., Z. Desta, L. Li, T. C. Skaar, B. A. Ward, A. Nguyen, Y. Jin, A. M. Storniolo, D. M. Nikoloff, L. Wu, G. Hillman, D. F. Hayes, V. Stearns and D. A. Flockhart (2006). "Quantitative effect of CYP2D6 genotype and inhibitors on tamoxifen metabolism: implication for optimization of breast cancer treatment." Clin Pharmacol Ther 80(1): 61-74.

Bressolle, F., M. Bromet-Petit and M. Audran (1996). "Validation of liquid chromatographic and gas chromatographic methods. Applications to pharmacokinetics." J Chromatogr B Biomed Appl 686(1): 3-10.

Burnham, K. P. and D. R. Anderson (2004). "Multimodel Inference: Understanding AIC and BIC in Model Selection." Sociologica methods & research 33(2): 261-304.

Bustad, A., D. Terziivanov, R. Leary, R. Port, A. Schumitzky and R. Jelliffe (2006). "Parametric and nonparametric population methods: their comparative performance in analysing a clinical dataset and two Monte Carlo simulation studies." Clin Pharmacokinet 45(4): 365-83.

Buur, J., R. Baynes, G. Smith and J. Riviere (2005). "Use of probabilistic modeling within a physiologically based pharmacokinetic model to predict sulfamethazine residue withdrawal times in edible tissues in swine." Antimicrob Agents Chemother 50(7): 2344-2351.

Buur, J., R. Baynes, G. Smith and J. Riviere (2006). "Use of probabilistic modeling within a physiologically based pharmacokinetic model to predict sulfamethazine residue withdrawal times in edible tissues in swine." Antimicrob Agents Chemother 50(7): 2344-51.

Buur, J. L., R. E. Baynes, A. L. Craigmill and J. E. Riviere (2005). "Development of a physiologic-based pharmacokinetic model for estimating sulfamethazine concentrations in swine and application to prediction of violative residues in edible tissues." Am J Vet Res 66(10): 1686-93.

Caldwell, J., I. Gardner and N. Swales (1995). "An introduction to drug disposition: the basic principles of absorption, distribution, metabolism, and excretion." Toxicol Pathol 23(2): 102-14.

Chervoneva, I., T. Hyslop and W. W. Hauck (2007). "A multivariate test for population bioequivalence." Stat Med 26(6): 1208-23.

Clewell, H. J., 3rd and M. E. Andersen (1996). "Use of physiologically based pharmacokinetic modeling to investigate individual versus population risk." Toxicology 111(1-3): 315-29.

Concordet, D. and P. L. Toutain (1997). "The withdrawal time estimation of veterinary drugs revisited." J Vet Pharmacol Ther 20(5): 380-6.

Davidian, M. and A. R. Gallant (1992). "Smooth nonparametric maximum likelihood estimation for population pharmacokinetics, with application to quinidine." J Pharmacokinet Biopharm 20(5): 529-56.

Davidian, M. and D. M. Giltinan (1993). "Some general estimation methods for nonlinear mixed-effects models." J Biopharm Stat 3(1): 23-55.

De Buck, S. S. and C. E. Mackie (2007). "Physiologically based approaches towards the prediction of pharmacokinetics: in vitro-in vivo extrapolation." Expert Opin Drug Metab Toxicol 3(6): 865-78.

del Castillo, J. R., V. Laroute, P. Pommier, C. Zemirline, A. Keita, D. Concordet and P. L. Toutain (2006). "Interindividual variability in plasma concentrations after systemic exposure of swine to dietary doxycycline supplied with and without paracetamol: a population pharmacokinetic approach." J Anim Sci 84(11): 3155-66.

Dorr, P. M., M. S. Nemechek, A. B. Scheidt, R. E. Baynes, W. A. Gebreyes and G. W. Almond (2009). "Water-flow variation and pharmacoepidemiology of tetracycline hydrochloride administration via drinking water in swine finishing farms." J Am Vet Med Assoc 235(3): 299-304.

EC (2003). On additives for use in animal nutrition. Regulation (EC) No 1831/2003**:** 0029 - 0043.

el-Masri, H. A., R. S. Thomas, S. A. Benjamin and R. S. Yang (1995). "Physiologically based pharmacokinetic/pharmacodynamic modeling of chemical mixtures and possible applications in risk assessment." Toxicology 105(2-3): 275-82.

Emond, C., L. S. Birnbaum and M. J. DeVito (2006). "Use of a physiologically based pharmacokinetic model for rats to study the influence of body fat mass and induction of CYP1A2 on the pharmacokinetics of TCDD." Environ Health Perspect 114(9): 1394-400.

Emond, C., J. E. Michalek, L. S. Birnbaum and M. J. DeVito (2005). "Comparison of the use of a physiologically based pharmacokinetic model and a classical pharmacokinetic model for dioxin exposure assessments." Environ Health Perspect 113(12): 1666-8.

Evans, N. D., K. R. Godfrey, M. J. Chapman, M. J. Chappell, L. Aarons and S. B. Duffull (2001). "An identifiability analysis of a parent-metabolite pharmacokinetic model for ivabradine." J Pharmacokinet Pharmacodyn 28(1): 93-105.

FDA (1999). "Guidance for industry on Population Pharmacokinetics; availability. Food and Drug Administration, HHS. Notice." Fed Regist 64(27): 6663-4.

FDA (2003). Guidance for Industry #152. Guidance Document, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Veterinary Medicine. 2008.

FDA (2005). Actions Taken by FDA Center for Veterinary Medicine. 2008.

Gobburu, J. V. and J. Lawrence (2002). "Application of resampling techniques to estimate exact significance levels for covariate selection during nonlinear mixed effects model building: some inferences." Pharm Res 19(1): 92-8.

Godfrey, K. R., M. J. Chapman and S. Vajda (1994). "Identifiability and indistinguishability of nonlinear pharmacokinetic models." J Pharmacokinet Biopharm 22(3): 229-51.

Godfrey, K. R., R. P. Jones and R. F. Brown (1980). "Identifiable pharmacokinetic models: the role of extra inputs and measurements." J Pharmacokinet Biopharm 8(6): 633-48.

Green, B. and S. B. Duffull (2003). "Prospective evaluation of a D-optimal designed population pharmacokinetic study." J Pharmacokinet Pharmacodyn 30(2): 145-61.

Grossman, I. (2009). "ADME pharmacogenetics: current practices and future outlook." Expert Opin Drug Metab Toxicol 5(5): 449-62.

Gueorguieva, I., L. Aarons, K. Ogungbenro, K. M. Jorga, T. Rodgers and M. Rowland (2006). "Optimal design for multivariate response pharmacokinetic models." J Pharmacokinet Pharmacodyn 33(2): 97-124.

Gueorguieva, I., K. Ogungbenro, G. Graham, S. Glatt and L. Aarons (2007). "A program for individual and population optimal design for univariate and multivariate response pharmacokinetic-pharmacodynamic models." Comput Methods Programs Biomed 86(1): 51-61.

Gupta, S., C. Banfield, B. Kantesaria, M. Marino, R. Clement, M. Affrime and V. Batra (2001). "Pharmacokinetic and safety profile of desloratadine and fexofenadine when coadministered with azithromycin: a randomized, placebo-controlled, parallel-group study." Clin Ther 23(3): 451-66.

Hou, T. and J. Wang (2008). "Structure-ADME relationship: still a long way to go?" Expert Opin Drug Metab Toxicol 4(6): 759-70.

Jackson, A. J., G. Robbie and P. Marroum (2004). "Metabolites and bioequivalence: past and present." Clin Pharmacokinet 43(10): 655-72.

Jonsson, E. N., J. R. Wade and M. O. Karlsson (2000). "Nonlinearity detection: advantages of nonlinear mixed-effects modeling." AAPS PharmSci 2(3): E32.

Jonsson, F. and G. Johanson (2003). "The Bayesian population approach to physiological toxicokinetic-toxicodynamic models--an example using the MCSim software." Toxicol Lett 138(1-2): 143-50.

Karlsson, M. O. and L. B. Sheiner (1994). "Estimating bioavailability when clearance varies with time." Clin Pharmacol Ther 55(6): 623-37.

Kawai, R., M. Lemaire, J. L. Steimer, A. Bruelisauer, W. Niederberger and M. Rowland (1994). "Physiologically based pharmacokinetic study on a cyclosporin derivative, SDZ IMM 125." J Pharmacokinet Biopharm 22(5): 327-65.

Kellaway, I. W. and C. Marriot (1975). "The influence of mucin on the bioavailability of tetracycline." J Pharm Pharmacol 27(4): 281-3.

Kirman, C. R., L. M. Sweeney, M. E. Meek and M. L. Gargas (2003). "Assessing the dose-dependency of allometric scaling performance using physiologically based pharmacokinetic modeling." Regul Toxicol Pharmacol 38(3): 345-67.

Kniffen, T. S., D. P. Bane, W. F. Hall, G. D. Koritz and R. F. Bevill (1989). "Bioavailability, pharmacokinetics, and plasma concentration of tetracycline hydrochloride fed to swine." Am J Vet Res 50(4): 518-21.

Lee, H. A., T. L. Leavens, S. E. Mason, N. A. Monteiro-Riviere and J. E. Riviere (2009). "Comparison of quantum dot biodistribution with a blood-flow-limited physiologically based pharmacokinetic model." Nano Lett 9(2): 794-9.

Lees, P., F. M. Cunningham and J. Elliott (2004). "Principles of pharmacodynamics and their applications in veterinary pharmacology." J Vet Pharmacol Ther 27(6): 397-414.

Lindstrom, M. L. and D. M. Bates (1990). "Nonlinear mixed effects models for repeated measures data." Biometrics 46(3): 673-87.

Link, W. A. and R. J. Barker (2006). "Model weights and the foundations of multimodel inference." Ecology 87(10): 2626-35.

Ludden, T. M., S. L. Beal and L. B. Sheiner (1994). "Comparison of the Akaike Information Criterion, the Schwarz criterion and the F test as guides to model selection." J Pharmacokinet Biopharm 22(5): 431-45.

Mager, H. (2004). "Covariate selection in population pharmacokinetics/pharmacodynamics." Int J Clin Pharmacol Ther 42(3): 187-8.

Mager, H. and G. Goller (1995). "Analysis of pseudo-profiles in organ pharmacokinetics and toxicokinetics." Stat Med 14(9-10): 1009-24.

Markon, K. E. and R. F. Krueger (2004). "An empirical comparison of information-theoretic selection criteria for multivariate behavior genetic models." Behav Genet 34(6): 593-610.

Martinez, M., L. Friedlander, R. Condon, J. Meneses, J. O'Rangers, N. Weber and M. Miller (2000). "Response to criticisms of the US FDA parametric approach for withdrawal time estimation: rebuttal and comparison to the nonparametric method proposed by Concordet and Toutain." J Vet Pharmacol Ther 23(1): 21-35.

Martin-Jimenez, T., R. E. Baynes, A. Craigmill and J. E. Riviere (2002). "Extrapolated withdrawal-interval estimator (EWE) algorithm: a quantitative approach to establishing extralabel withdrawal times." Regul Toxicol Pharmacol 36(1): 131-7.

Martin-Jimenez, T. and J. E. Riviere (1998). "Population pharmacokinetics in veterinary medicine: potential use for therapeutic drug monitoring and prediction of tissue residues." J Vet Pharmacol Ther 21(3): 167-89.

Martin-Jimenez, T. and J. E. Riviere (2001). "Mixed effects modeling of the disposition of gentamicin across domestic animal species." J Vet Pharmacol Ther 24(5): 321-32.

Martin-Jimenez, T. and J. E. Riviere (2002). "Mixed-effects modeling of the interspecies pharmacokinetic scaling of oxytetracycline." J Pharm Sci 91(2): 331-41.

Mason, S. E., R. E. Baynes, G. W. Almond, J. E. Riviere and A. B. Scheidt (2009). "Pharmacology of tetracycline water medication in swine." J Anim Sci.

Mason, S. E., R. E. Baynes, J. L. Buur, J. E. Riviere and G. W. Almond (2008). "Sulfamethazine water medication pharmacokinetics and contamination in a commercial pig production unit." J Food Prot 71(3): 584-9.

McCaughey, W. J., C. T. Elliott and S. R. Crooks (1991). "Sulphadimidine in drinking troughs at bacon factories." Vet Rec 128(6): 125-6.

Merle, Y., A. Aouimer and M. Tod (2004). "Impact of model misspecification at design (and/or) estimation step in population pharmacokinetic studies." J Biopharm Stat 14(1): 213-27.

Nielsen, P. and N. Gyrd-Hansen (1996). "Bioavailability of oxytetracycline, tetracycline and chlortetracycline after oral administration to fed and fasted pigs." J Vet Pharmacol Ther 19(4): 305-11.

Nouws, J. F., D. Mevius, T. B. Vree and M. Degen (1989). "Pharmacokinetics and renal clearance of sulphadimidine, sulphamerazine and sulphadiazine and their N4-acetyl and hydroxy metabolites in pigs." Vet Q 11(2): 78-86.

Nouws, J. F., T. B. Vree, M. Baakman, F. Driessens, L. Vellenga and D. J. Mevius (1986). "Pharmacokinetics, renal clearance, tissue distribution, and residue aspects of sulphadimidine and its N4-acetyl metabolite in pigs." Vet Q 8(2): 123-35.

Oberg, A. and M. Davidian (2000). "Estimating data transformations in nonlinear mixed effects models." Biometrics 56(1): 65-72.

Park, K., D. Verotta, T. F. Blaschke and L. B. Sheiner (1997). "A semiparametric method for describing noisy population pharmacokinetic data." J Pharmacokinet Biopharm 25(5): 615-42.

Parke, J., N. H. Holford and B. G. Charles (1999). "A procedure for generating bootstrap samples for the validation of nonlinear mixed-effects population models." Comput Methods Programs Biomed 59(1): 19-29.

Peyrou, M., M. Y. Doucet, A. Vrins, D. Concordet, M. Schneider and A. Bousquet-Melou (2004). "Population pharmacokinetics of marbofloxacin in horses: preliminary analysis." J Vet Pharmacol Ther 27(5): 283-8.

Rescigno, A. (2001). "The rise and fall of compartmental analysis." Pharmacol Res 44(4): 337-42.

Riond, J. L. and J. E. Riviere (1988). "Multiple intravenous dose pharmacokinetics and residue depletion profile of gentamicin in pigs." J Vet Pharmacol Ther 11(2): 210-4.

Riviere, J. E. (1997). "Basic principles and techniques of pharmacokinetic modeling." J Zoo Wildl Med 28(1): 3-19.

Rybak, M. J. (2006). "Pharmacodynamics: Relation to Antimicrobial Resistance." The American Journal of Medicine 119(6A): 537-544.

Sprandel, K. A., G. L. Drusano, D. W. Hecht, J. C. Rotschafer, L. H. Danziger and K. A. Rodvold (2006). "Population pharmacokinetic modeling and Monte Carlo simulation of varying doses of intravenous metronidazole." Diagn Microbiol Infect Dis 55(4): 303-9.

Sproule, B. A., M. Bazoon, K. I. Shulman, I. B. Turksen and C. A. Naranjo (1997). "Fuzzy logic pharmacokinetic modeling: application to lithium concentration prediction." Clin Pharmacol Ther 62(1): 29-40.

Sweeney, R. W., P. C. Bardalaye, C. M. Smith, L. R. Soma and C. E. Uboh (1993). "Pharmacokinetic model for predicting sulfamethazine disposition in pigs." Am J Vet Res 54(5): 750-4.

Tam, V. H., S. L. Preston and G. L. Drusano (2003). "Comparative pharmacokinetic analysis by standard two-stage method versus nonparametric population modeling." Pharmacotherapy 23(12): 1545-9.

Tanigawara, Y., I. Yano, K. Kawakatsu, K. Nishimura, M. Yasuhara and R. Hori (1994). "Predictive performance of the Bayesian analysis: effects of blood sampling time, population parameters, and pharmacostatistical model." J Pharmacokinet Biopharm 22(1): 59-71.

Theil, F. P., T. W. Guentert, S. Haddad and P. Poulin (2003). "Utility of physiologically based pharmacokinetic models to drug development and rational drug discovery candidate selection." Toxicol Lett 138(1-2): 29-49.

Tod, M., F. Mentre, Y. Merle and A. Mallet (1998). "Robust optimal design for the estimation of hyperparameters in population pharmacokinetics." J Pharmacokinet Biopharm 26(6): 689-716.

Vinks, A. A. (2002). "The application of population pharmacokinetic modeling to individualized antibiotic therapy." Int J Antimicrob Agents 19(4): 313-22.

Vinks, A. A., J. W. Mouton, D. J. Touw, H. G. Heijerman, M. Danhof and W. Bakker (1996). "Population pharmacokinetics of ceftazidime in cystic fibrosis patients analyzed by using a nonparametric algorithm and optimal sampling strategy." Antimicrob Agents Chemother 40(5): 1091-7.

Vonesh, E. F., V. M. Chinchilli and K. Pu (1996). "Goodness-of-fit in generalized nonlinear mixed-effects models." Biometrics 52(2): 572-87.

Wade, J. R., S. L. Beal and N. C. Sambol (1994). "Interaction between structural, statistical, and covariate models in population pharmacokinetic analysis." J Pharmacokinet Biopharm 22(2): 165-77.

Wahlby, U., A. H. Thomson, P. A. Milligan and M. O. Karlsson (2004). "Models for time-varying covariates in population pharmacokinetic-pharmacodynamic analysis." Br J Clin Pharmacol 58(4): 367-77.

Williams, P. L. (1990). "Structural identifiability of pharmacokinetic models--compartments and experimental design." J Vet Pharmacol Ther 13(2): 121-31.

Yeap, B. Y. and M. Davidian (2001). "Robust two-stage estimation in hierarchical nonlinear models." Biometrics 57(1): 266-72.

Yengi, L. G., L. Leung and J. Kao (2007). "The evolving role of drug metabolism in drug discovery and development." Pharm Res 24(5): 842-58.

Yuh, L., S. Beal, M. Davidian, F. Harrison, A. Hester, K. Kowalski, E. Vonesh and R. Wolfinger (1994). "Population pharmacokinetic/pharmacodynamic methodology and applications: a bibliography." Biometrics 50(2): 566-75.

Sulfamethazine Water Medication Pharmacokinetics and Contamination in a Commercial Pig Production Unit

SHARON E. MASON*§, RONALD E. BAYNES*§, JENNIFER L. BUUR†, JIM E. RIVIERE*§, AND GLEN W. ALMOND*

*Department of Population Health and Pathobiology

§Center for Chemical Toxicology and Research Pharmacokinetics, North Carolina State University, College of Veterinary Medicine, 4700 Hillsborough Road, Raleigh, North Carolina 27606, USA

†Western University of Health Sciences, College of Veterinary Medicine, 309 E. Second St. Pomona, CA 91766

Journal of Food Protection, Vol. 71, No. 3, 2008, Pages 584–589

Sulfamethazine Water Medication Pharmacokinetics and Contamination in a Commercial Pig Production Unit

SHARON E. MASON, RONALD E. BAYNES,* JENNIFER L. BUUR,† JIM E. RIVIERE, AND GLEN W. ALMOND

Department of Population Health and Pathobiology, Center for Chemical Toxicology and Research Pharmacokinetics, North Carolina State University, College of Veterinary Medicine, 4700 Hillsborough Road, Raleigh, North Carolina 27606, USA

MS 07-409: Received 2 August 2007/Accepted 22 October 2007

ABSTRACT

Sulfamethazine is often used to treat disease in the swine industry. Sulfamethazine is available as water or feed medication and historically (over the past 40 years) has been associated with residue violations in both the United States and Europe. Despite sulfamethazine's approval for use as a water medication, little research on the pharmacokinetics of the water formulation is available. Therefore, a pilot study was performed to determine the plasma levels of an approved sulfamethazine water medication. Plasma levels in pigs treated with an oral bolus (250 mg/kg), which is equivalent to the total drug consumed within a 24-h period, achieved therapeutic concentrations (50 μg/ml). Noncompartmental-based pharmacokinetic model parameters for clearance, half-life, and volume of distribution were consistent with previously published values in swine. However, the above treatment resulted in exposure of pen mates to sulfamethazine at levels currently above tolerance (0.1 ppm). Using a physiologically based pharmacokinetic model, the treatment dose simulation was compared with observed plasma levels of treated pigs. Flexibility of the physiologically based pharmacokinetic model also allowed simulation of control-pig plasma levels to estimate contamination exposure. A simulated exposure to 0.15 mg/kg twice within approximately 8 h resulted in detectable levels of sulfamethazine in the control pigs. After initial exposure, a much lower dose of 0.059 mg/kg maintained the contamination levels above tolerance for at least 3 days. These results are of concern for producers and veterinarians, because in commercial farms, the entire barn is often treated, and environmental contamination could result in residues of an unknown duration.

Over the past decade, the concern of antimicrobial resistance has increased the scrutiny on veterinary medications, routes of administration, and dosages administered to food animals *(1–3, 19, 17)*. In 1996, as a direct result of the Animal Medicinal Drug Use Clarification Act of 1994 *(20)*, the prescribing power of veterinarians increased dramatically. The Act also included stringent criteria concerning the administration of antibiotics to food-producing animals. Despite this step to increase veterinarians' abilities to combat animal disease, human health advocacy and regulatory groups have become more vigilant in monitoring animal antibiotic use, emerging resistance, and its potential impact on public health. In the last decade, there have been five federal notices concerning antibiotic resistance that restricted the use of certain classes of antibiotics in food animals *(3)*. In order to protect human health, withdrawal times must be provided for all medications prescribed to food animals. Withdrawal times are set by the U.S. Food and Drug Administration (FDA), based on the 95th confidence interval of the 99th percentile of the population, as described in their *Guidance for Industry (2)*.

U.S. Department of Agriculture surveillance programs monitor carcasses for drug residues. Over the past several years, sulfamethazine use has resulted in multiple residue violations in swine. These violations may be due to an inaccurate withdrawal time *(5)*, inaccurate medication of animals, or from environmental contamination of the drug *(7, 10–12)*. There is evidence that the current FDA withdrawal time of 15 days (set in 1982) is inadequate for an oral loading dose of (247 mg/kg) and daily dose of 123 mg/kg for 4 consecutive days *(5, 14)*. In order to prevent violations, due to increased Japanese import restrictions, many swine production units implement an 80-day withdrawal time for sulfamethazine water medication in treated barns *(22)*.

With heightened scrutiny on food animal antimicrobial use and legislators encouraging a ban on all nontherapeutic use of antimicrobials, the food production industry needs effective and easily administered medications. Since no extra-label medicated feeds can be prescribed *(20)*, drugs are often administered by water. To our knowledge, there are no published reports on therapeutic concentrations of sulfamethazine water medications and their potential effect on the animal environment.

This study used both traditional noncompartmental pharmacokinetic (PK) modeling analysis and a previously validated physiologic-based pharmacokinetic (PBPK) model *(5, 6)* to validate this oral gavage study and to predict exposure in pen mates. PBPK has been used most in toxicology to explain dosing ranges in humans. Compared with

* Author for correspondence. Tel: 919-513-6261; Fax: 919-513-6358. E-mail: ronald_baynes@ncsu.edu.

† Present address: Western University of Health Sciences, College of Veterinary Medicine, 309 East Second Street, Pomona, CA 91766, USA.

traditional PK modeling. PBPK modeling uses parameters based on animal physiology to model drug deposition. Some parameters used are organ perfusion, metabolic pathways, and excretion mechanisms. These parameters allow a validated model to use sparse time points, from which a traditional model would not be able to predict. Buur et al. *(5, 6)* have shown that PBPK modeling can predict withdrawal times for drugs. The U.S. Food Animal Residue Avoidance Databank program has used PBPK models to calculate withdrawal intervals for tetracycline in sheep and sulfamethazine in swine. Therefore, we extended this model to predict oral exposure in nursery age pigs and pen mate exposure to sulfamethazine.

Because sick pigs do not drink as much as healthy pigs *(8, 9, 16)*, this pilot study modeled the pharmacokinetic PK parameters when healthy pigs were orally gavaged with a currently approved sulfamethazine product (Sulmet, Fort Dodge Animal Health, Madison, N.J.). This provides maximum plasma concentration levels that can be attained by oral sulfamethazine. Furthermore, we compared traditional PK modeling with a PBPK model to mimic the treated-pig levels. The purpose of this study was to validate our PBPK model for oral data, by comparing the data obtained from a known dose of sulfamethazine in an all-in–all-out production system to previously obtained research data *(5)*. Furthermore, we used this information to model the contamination seen in the control pigs housed with the treated pigs, an unexpected finding, and to make predictions on the magnitude of contamination that was seen.

MATERIALS AND METHODS

High-performance liquid chromatography methods. Sample preparation and processing were based on the method published by Buur et al. *(5, 6)*. Nine standard solutions containing sulfamethazine (Spectrum Chemical Manufacturing Company, New Brunswick, N.J.) were prepared (0.01, 0.05, 0.1, 0.5, 1, 2.5, 5, 10, and 20 ppm or μg/ml from a 1,000-ppm stock solution) in both double deionized water (ddH_2O) and plasma, using no more than 5% (volume) stock. Plasma and water standards produced calibration curves consistent and accurate, with variability less than 20% and plasma:water recovery of 93 to 96%. Plasma samples were filtered with a 3-cm MCX solid-phase extraction column (Waters, Mildford, Mass.) before injection *(5)*.

All samples were run on an Atlantis dC18 column (C18 column, 4.6 by 150 mm, 5 μl; Waters) in 77% ammonium acetate buffer, pH adjusted to 4.5, and in 23% acetonitrile on a liquid chromatograph (Waters 2696 with autosampler) at 1-ml/min flow rate. Detection and quantification were performed on a Waters 996 photodiode array detector at 267 nm. Prior to analysis, samples were held at 24°C before each run while the column was heated to 30°C for 6.5-min run times, with peak elution occurring at 5.1 min. Peaks were symmetrical, sharp, and crisp. Limit of quantification was at the current FDA tolerance limit of 0.1 μg/ml and limit of detection was set at 0.05 μg/ml.

Animals. Pigs at 4 to 5 weeks of age were housed at the North Carolina State University Teaching Animal Unit (TAU) swine facility nursery barn on tribar floors. The waste is removed via a wash system that runs under the pens every hour. The TAU acts as a commercial all-in–all-out one-site production facility. University Institutional Animal Care and Use Committee approval was obtained for the animal study. Fourteen pigs between 11.5 and 15 kg from the TAU nursery were chosen in a complete randomized block design (see Table 1). At 21 days of age, pigs were weaned and moved to the nursery. They were allowed 12 days of acclimation to the facility, during which they were fed a medicated starter ration containing penicillin for 4 days, and were provided a 21% protein, nonmedicated diet for the remainder of the study. On the first day of the study, animals were weighed, had initial blood samples obtained, and were ear tagged for identification. Six animals were used as controls. Of the 14 pigs, 8 were chosen at random of both male and female sex for the gavage treatment. A metal speculum was used as a gag and a polypropylene Foley catheter was inserted into the esophagus of each pig. Sulfamethazine (Sulmet, Fort Dodge Animal Health) was given orally at approximately 247.5 mg/kg (actual amount as dosed was 250 mg/kg), as per the labeled initial-day water medication dose. Due to the volume that had to be administered, the medication could not be diluted. No gastrointestinal or other side effects were observed from the treatment. Approximately 5 ml of whole blood was drawn from each animal at 0, 2, 4, 6, 8, 12, 24, 48, and 72 h postgavage. Blood was centrifuged at 1,500 rpm for 15 min, and the plasma was siphoned off and frozen at −80°C until samples were processed, within 6 weeks of the study being completed.

TABLE 1. *Spatial layout of animal pens*[a]

Pen A		Pen B		Pen C	
Animal	T or C	Animal	T or C	Animal	T or C
A1	C	B6	T	C12	T
A2	T	B7	C	C13	C
A3	T	B8	C	C14	T
A4	T	B9	T		
A5	C	B10	T		
		B11	C		

[a] T, treatment pigs; C, control.

PK analysis. Conventional PK analysis was performed using WinNonLin (Pharsight, Mountain View, Calif.), which included a noncompartmental analysis on data from each pig. The terminal slope from plotting the plasma concentrations over time was used to calculate half-life, λz, clearance, volume of distribution (V) and area under the curve (AUC) for each of the treatment pigs. The above parameters except for V and AUC were calculated for the control pigs as well. We compared these values with the literature to validate the study. A previously validated PBPK model *(5, 6)* allowed analysis of both sets of pig data, using AcslXtreme (Aegis Technologies, Huntsville, Ala.). The model was optimized using physiologic parameters from previously published data *(14, 18)*. Treatment-pig data were modeled and compared to this optimization. In order to analyze the control-pig data, a dosing portal was written to include an adjustable lag time and flexible dosing schedule (in both time and dose). This optimized model, including the new dosing portal with lag time, was used to determine the control-pig exposure. These data were compiled with averages and standard deviations using Excel (Microsoft Corp., Redmond, Wash.).

RESULTS

All treated pig plasma sulfamethazine concentrations rose during the first 2 to 12 h after oral gavage, and then began to drop from peak levels between 400 and 700 μg/ml (ppm) (Fig. 1A). Within 24 h of the gavage dose, sulfamethazine was detectable in the plasma of untreated pigs

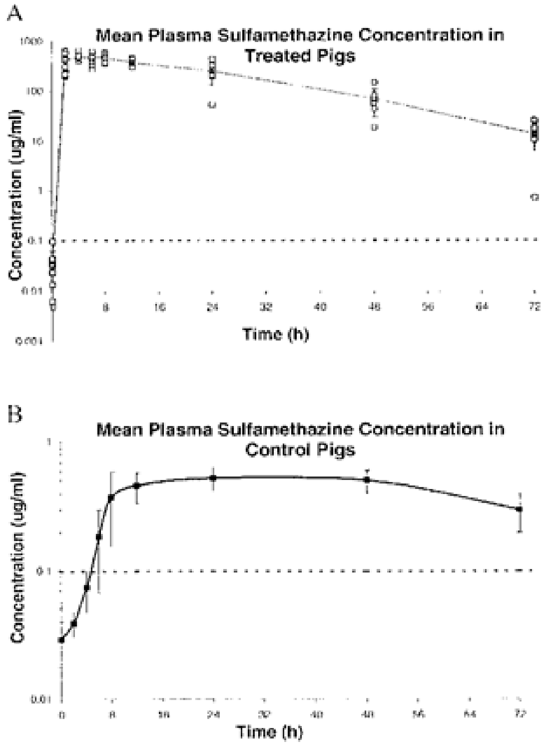


FIGURE 1. *(A) Mean concentration of the treatment pigs (n = 8) plasma levels with standard deviations present to show the spread of data. Note pig 2's last four time points were removed due to large deviation from the other data. (B) Mean plasma concentrations for the control pigs (n = 6) in the same pens as the treatment pigs. The straight dotted lines on each graph represent the tolerance limit for plasma.*

and varied between 0.20 to 0.71 μg/ml. The average apparent maximal concentration (C_{max}) in untreated control pigs was 0.573 μg/ml (Fig. 1B). Note that plasma levels were above tolerance in untreated pigs by 8 h after gavage of treatment pigs.

Noncompartmental analysis using WinNonLin of the eight treated pigs and six control pigs led to calculation of conventional PK parameters (Tables 2 and 3). Average half-life for the treated pigs was 11.4 h, with an apparent average time to maximal concentration of 5.75 h. Because no intravenous data are available, bioavailability could not be measured and therefore, PK parameters are confounded by unknown bioavailability.

In addition to performing a traditional PK analysis, we also used a previously validated PBPK model *(5, 6)* in an attempt to improve modeling of observed data. Using the original PBPK system, we optimized the model using published data, extending past 72 h *(18)*. Using the optimized model at a dosage of 250 mg/kg, the simulation predicted actual treated pig plasma concentrations (Fig. 2A). A regression line of observed data versus predicted data yields a correlation coefficient of 0.71 (Fig. 2B), and the residuals suggest no systematic bias in the results (Fig. 2C).

We modeled control-pig exposure and determined an estimated oral exposure dose and consistent time interval of exposure. The data in Figure 3A is a PBPK model simulation, which is a more accurate representation of continual oral drug exposure than is the model in Figure 1B. Figure 3 shows the simulation model compared with the control-pig data. The residual graph indicates constant variance. From PBPK simulation of the control-pig data, it appears that pigs were exposed to the equivalent of two initial doses of 0.15 mg/kg at hours 4 and 8 on day 1. Every 4 h subsequently, the pigs had only to consume approximately 0.059 mg/kg sulfamethazine to maintain plasma levels above tolerance. The model suggests this approximate exposure level until 56 h after the initial treatment.

DISCUSSION

Traditional PK values obtained from this animal study are consistent with PK data previously reported in the literature for administration of sulfamethazine *(13–15, 18, 21)*. Available papers typically report plasma concentrations of greater than 50 μg/ml for conventional therapeutic dosages *(13–15, 21)*. The treatment-pig data verify that therapeutic concentrations of sulfamethazine were achieved through water medication. This study did not investigate the ability of sulfamethazine as dosed in drinking water to achieve therapeutic concentrations. Instead, a total daily dose was given in one bolus in order to determine the highest plasma concentrations of oral sulfamethazine and to obtain relevant PK parameters. Appropriate dosing of water medications to achieve therapeutic concentrations is a subject of future research.

All pigs, except for pig 12 (treatment group), had initial sulfamethazine levels below the detection limit. Although pig 12 had 0.09 μg/ml sulfamethazine at the start of the study, this value is below the tolerance limit. This does bring into question if the pigs were previously exposed to sulfamethazine. Exposure could have occurred in the farrowing barn through milk or creep feed; however, the 12-day washout period should have been adequate to remove any sulfamethazine contamination, as the pigs were not intentionally exposed to sulfamethazine. Studies have reported plasma samples containing trace amounts of sulfamethazine in pigs that were not previously treated *(14)*. Interpig variability in metabolism is another possible cause of the trace amounts of sulfamethazine present *(12)*. There may also be unrecognized age differences in pig acetylation metabolism *(18)*. Finally, there was a wide range of C_{max} values (413 to 677 μg/ml), which were not associated with sex, age, or size. Acetylation differences (although never documented in pigs) could explain the variability of peak plasma levels in treated pigs. Due to the large dose of sulfamethazine given to pig 12 (a treatment pig), this trace level would not significantly alter the peak plasma levels.

Environmental contamination was shown to be a significant concern in this study. Control pigs showed plasma sulfamethazine levels above tolerance by 8 h after gavage treatment of pen mates. Thus, it is possible to contaminate

TABLE 2. *Pharmacokinetic parameter estimates for treatment pigs*[a]

Pig no.	λz (liter/h)	Oral half-life (h)	C_{max} (μg/ml)	T_{max} (h)	Clearance (ml/h/kg)[b]	V (liter/kg)[b]	AUC (mg·h/ml)
10	0.0548	12.651	641	2	18.862	0.34	11.57
12	0.0614	11.288	471	4	13.782	0.22	15.84
14	0.063	10.997	618	6	16.573	0.26	13.18
2	0.0637	10.883	677	4	39.738	0.62	5.5
3	0.0562	12.336	635	6	18.715	0.33	11.67
4	0.0964	7.19	496	8	28.693	0.3	7.61
6	0.0778	8.909	468	12	14.396	0.19	15.17
9	0.0411	16.879	413	4	20.107	0.49	10.86
Avg	0.0643	11.392	552	5.75	21.36	0.34	11.43
SD	0.0165	2.849	100	3.11	8.74	0.05	3.52

[a] Pharmacokinetic parameters for treated pigs given a 250-mg/kg dose of sulfamethazine by oral gavage. All parameters were calculated in WinNonLin (Pharsight) and reflect predictions until the plasma levels are no longer detectable. λz, elimination constant (also known as k_{el}); half-life, the time (h) to clear one-half of the concentration of the drug from the body; C_{max}, the maximum plasma concentration; T_{max}, the observed time of peak plasma concentration; V, the volume of distribution and can be related to the level of sulfamethazine within body compartments; clearance, total body clearance; AUC, area under the curve.

[b] Both clearance and V have been corrected for bioavailability when the noncompartmental values were calculated.

the pig enclosure and achieve plasma levels of antibiotic in nontreated animals that are of regulatory concern. To study the effects of this contamination, a PBPK model was adapted and used to estimate possible oral exposure scenarios. The model simulation (Fig. 3A), developed to represent the control pigs, uses physiology consistent with pig behavior. Pigs between 1 and 2 weeks of age suckle once every hour *(13)*. Their frequency of drinking decreases gradually from weaning at 21 days until it mirrors adult drinking patterns. Adults drink approximately 75% of their water between 8:00 a.m. and 5:00 p.m. Based on the age of these pigs, the model is consistent with pig behavior *(13)*.

Based on the PBPK simulation, we hypothesize that control pigs were being exposed from the elimination waste of the treated pigs until approximately 56 h after initial exposure of the treated pigs (Fig. 3A). This is of concern because kidney and muscle levels of the antibiotic are at least one-third of the peak plasma levels. Therefore, kidney and possibly muscle could be contaminated by sulfamethazine. After approximately 56 h, the amount of excretion was low enough that it could be eliminated as fast as it entered the body. Therefore, traditional PK parameters for control pigs, including clearance and half-life, are not accurate for sulfamethazine because of flip-flop kinetic, where kinetics absorption of the drug dictates the plasma concentration. Our model is based on intermittent exposure from eating or drinking, although pigs may be continuously exposed to low levels of sulfamethazine from urine and/or feces *(7, 10–12)*.

We do not know how much contamination is now occurring in large production systems from water medications. However, the apparent ease in transfer of antibiotic from treated to untreated pig can become an issue. The method of housing has been drawn into question with other studies *(7, 10, 11)*. McCaughey *(10–12)* demonstrated pen, food, and water contamination of pig housing at production and slaughter facilities when floors were solid or bedding was not changed daily. However, our observations are significant because there are no published reports in the literature that have reported detectable levels of contamina-

TABLE 3. *Selected pharmacokinetic parameters for control pigs*[a]

Pig no.	λz (liter/h)	Oral half life (h)	C_{max} (μg/ml)	T_{max} (h)	Clearance (ml/h/kg)[b]
1	0.0172	40.216	0.4751	24	6,584
11	0.0233	29.6853	0.7102	24	5,826
13	0.0157	44.0294	0.6063	48	3,984
7	0.0175	39.5412	0.657	48	4,192
8	0.0086	80.9163	0.5445	8	3,972
5[c]	0.0293	47.4412	0.4463	48	6,226
Control avg	0.0186	46.972	0.573	33.33	5,131
SD	0.007	17.668	0.103	17.1	1,211

[a] The pharmacokinetic parameters for control pigs exposed to environmental contamination of a 250-mg/kg dose of sulfamethazine by oral gavage. All parameters were calculated in WinNonLin (Pharsight) and reflect predictions to until the plasma levels are no longer detectable. λz, elimination constant (also known as k_{el}); half-life, the time (h) to clear one-half of the concentration of the drug from the body; C_{max}, the maximum plasma concentration; T_{max}, the observed time of peak plasma concentration; clearance, total body clearance.

[b] Clearance was corrected for bioavailability by WinNonLin.

[c] One data point was removed because of inconsistency with the rest of the data and inability to verify that it had not been mislabeled

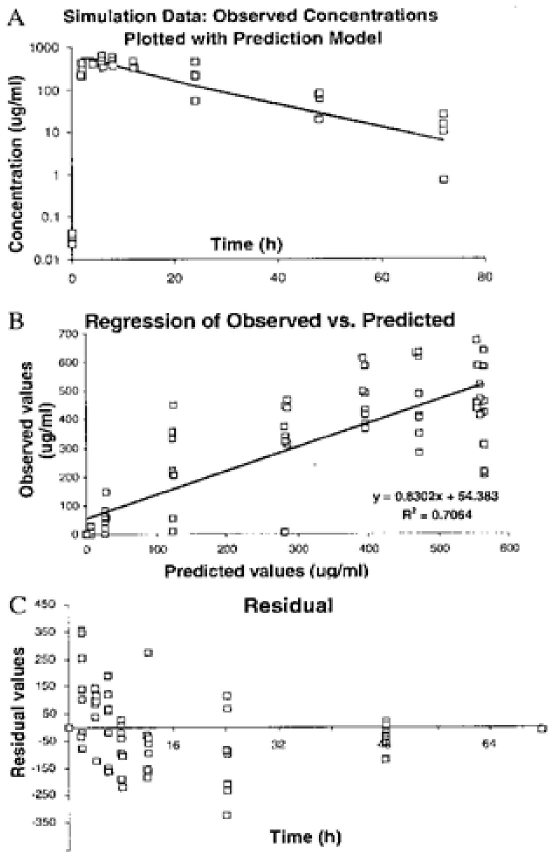


FIGURE 2. *(A) Simulation data shows the physiologic-based pharmacokinetic model (line) is a good representation of the individual points, which are treatment-pig plasma levels. (B) Regression line is a good fit given that this model is optimized to intravenous instead of oral data. (C) Residuals show that the model predicts better at later time points than at earlier time point.*

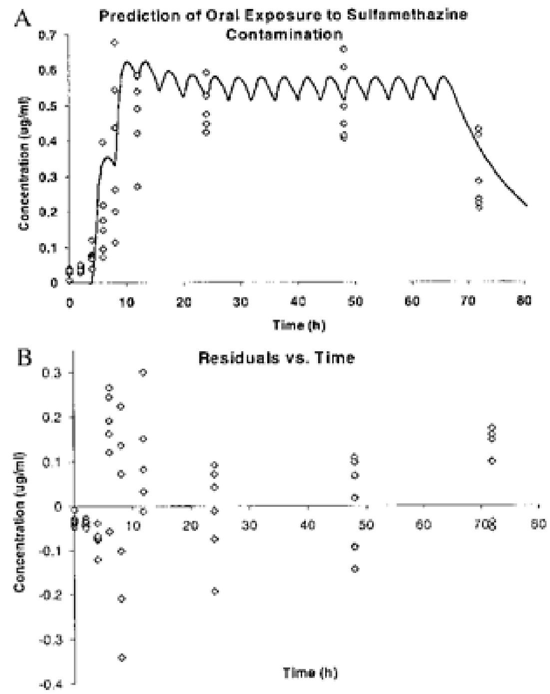


FIGURE 3. *(A) The optimized model predicts the dosing regimen for the pigs in the study. This model cannot be validated at this time due to lack of data points. (B) Residual values show that the model is not biased and has constant variance.*

tion in tribar-floor barns. Therefore, despite following an FDA-approved withdrawal time, violations may be seen in untreated pigs housed in the same environment as treated pigs, days after the medication was originally given.

Some residues may occur because sulfamethazine is stable in some environments. Ground water and soil studies have found it present more than 60 days since its last application *(4)*. In many commercial pig systems, the lagoon water is recycled, unlike at TAU. In this situation, the risk of contamination from a lagoon wash source, for instance, could result in extended residues. In this case, fecal contamination is a likely way in which the medication was available for consumption to the pigs. However, sulfamethazine's stability combined with more permanent environmental contamination could result in unexpected residues and fines to producers and veterinarians. This may also explain why despite measures put in place by the FDA Center for Veterinary Medicine as well as industry, sulfamethazine residue violations have continued to occur.

From this study, we recommend that procedures be put in place to protect untreated animals from contaminant exposure. Techniques need to be developed to ensure contamination is removed from the environment. Plasma levels of approximately 1 μg/ml from contamination are significant when compared to feed-additive studies, with plasma sulfamethazine levels of 9.8 to 16 μg/ml *(14)*. The PBPK model suggests that 0.059 mg/kg is capable of causing plasma drug levels to exceed tolerance. These blood levels are consistent with an 11-kg pig receiving less than 4 mg of sulfamethazine orally per day. With such low sulfamethazine levels contaminating pig enclosures, violative residues may continue to occur despite producer and veterinary compliance. Animal housing and husbandry throughout the entire production process remains important. Better approaches to model antibiotic use in populations of animals are needed in addition to research on the efficacy of water medications.

ACKNOWLEDGMENTS

The authors thank Patty Routh for assistance with pig handling, study logistics, and supplies. Beth Barlow was key in troubleshooting the high-performance liquid chromatography techniques. This research was made possible by the North Carolina State University College of Veterinary Medicine Animal Health Formula Fund.

REFERENCES

1. Anonymous. 2004. Draft risk assessment of streptogramin resistance in *Enterococcus faecium* attributable to the use of streptogramins in animals. *Fed. Regist.* 69:68384–68385. Available at: http://www.fda.gov/OHRMS/DOCKETS/98fr/04-25979.htm. Accessed 5 June 2007.
2. Anonymous. 2005. Guidance for industry: general principles for evaluating the safety of compounds used in food-producing animals. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Veterinary Medicine, Rockville, Md.
3. Anonymous. 2007. Antimicrobial resistance. U.S. Food and Drug Administration, Center for Veterinary Medicine. Available at: http://www.fda.gov/cvm/antimicrobial.html. Accessed 4 June 2007.
4. Blackwell, P. A., P. Kay, and A. B. Boxall. 2007. The dissipation and transport of veterinary antibiotics in a sandy loam soil. *Chemosphere* 67:292–299.
5. Buur, J., R. Baynes, G. Smith, and J. Riviere. 2005. Use of probabilistic modeling within a physiologically based pharmacokinetic model to predict sulfamethazine residue withdrawal times in edible tissues in swine. *Antimicrob. Agents Chemother.* 50:2344–2351.
6. Buur, J. L., R. E. Baynes, A. L. Craigmill, and J. E. Riviere. 2005. Development of a pharmacokinetic model for estimating sulfamethazine concentrations in swine and application to prediction of violative residues in edible tissues. *Am. J. Vet. Res.* 66:1689–1693.
7. Elliott, C. T., W. J. McCaughey, S. R. Crooks, and J. D. McEvoy. 1994. Effects of short-term exposure of unmedicated pigs to sulphadimidine contaminated housing. *Vet. Rec.* 134:450–451.
8. Harvey, R. E. 1994. Water consumption in pigs. *Pig J.* 32:95–98.
9. Harvey, R. E. 1998. Observation of the drinking habits of weaned growing pigs in relation to the rationale of water medication. *Pig J.* 42:137–140.
10. McCaughey, W. J., C. T. Elliott, and S. R. Crooks. 1990. Carry-over of sulphadimidine in the faeces and urine of pigs fed medicated feed. *Vet. Rec.* 126:351–354.
11. McCaughey, W. J., C. T. Elliott, and S. R. Crooks. 1990. Sulphadimidine in the urine of experimentally fed pigs. *Vet. Rec.* 126:323–326.
12. McCaughey, W. J., C. T. Elliott, and S. R. Crooks. 1991. Sulphadimidine in drinking troughs at bacon factories. *Vet. Rec.* 128:125–126.
13. Nouws, J. F., D. Mevius, T. B. Vree, and M. Degen. 1989. Pharmacokinetics and renal clearance of sulphadimidine, sulphamerazine and sulphadiazine and their *n*4-acetyl and hydroxy metabolites in pigs. *Vet. Q.* 11:78–86.
14. Nouws, J. F., T. B. Vree, M. Baakman, F. Driessens, L. Vellenga, and D. J. Mevius. 1986. Pharmacokinetics, renal clearance, tissue distribution, and residue aspects of sulphadimidine and its *n*4-acetyl metabolite in pigs. *Vet. Q.* 8:123–135.
15. Nouws, J. F., T. B. Vree, A. S. Breukink, A. S. J. P. A. M. Van Miert, and J. Grondel. 1986. Pharmacokinetics, hydroxylation and acetylation of sulphadimidine in mammals, birds, fish, reptiles and molluscs. Third Congress of European Association for Veterinary Pharmacology and Toxicology, Lancaster, UK.
16. Reeve-Johnson, R. 1998. Water medication and the treatment of respiratory diseases in pigs. *Pig J.* 42:74–86.
17. Sundlof, S. F. 1998. Letter on fluroquinolone drug products for animals. Available at: http://www.fda.gov/cvm/FOI/fluorquin2.htm. Accessed 4 June 2007.
18. Sweeney, R. W., P. C. Bardalaye, C. M. Smith, L. R. Soma, and C. E. Uboh. 1993. Pharmacokinetic model for predicting sulfamethazine disposition in pigs. *Am. J. Vet. Res.* 54:750–754.
19. U.S. Food and Drug Administration. 2007. Survey of antimicrobial-resistant enterococci in animals. Available at: http://www.fda.gov/cvm/antimicrobial.htm. Accessed 4 June 2007.
20. U.S. Food and Drug Administration, Center for Veterinary Medicine. 1994. Animal medicinal drug use clarification act. 21 CFR 530, 103-396. Accessed 7 November 1996.
21. Vree, T. B., Y. A. Hekster, E. F. Termond, M. W. Tijhuis, J. F. Nouws, and G. Dorrestein. 1985. Pharmacokinetics of sulfonamide revisited. *Antibiot. Chemother.* 34:1–208.
22. Wright, D. 1 October 1989. What's new in pork; pork that is pinker and purer appeals to the Japanese [press release]. Available at: http://query.nytimes.com/gst/fullpage.html?res=950DEFDE1631F932A35753C1A96F948260. Accessed 4 June 2007.

JOURNAL OF ANIMAL SCIENCE

The Premier Journal and Leading Source of New Knowledge and Perspective in Animal Science

Pharmacology of tetracycline water medication in swine

S. E. Mason, R. E. Baynes, G. W. Almond, J. E. Riviere and A. B. Scheidt

J Anim Sci 2009.87:3179-3186.
doi: 10.2527/jas.2009-1877 originally published online Jul 31, 2009;

Pharmacology of tetracycline water medication in swine[1]

S. E. Mason,*† R. E. Baynes,*†[2] G. W. Almond,† J. E. Riviere,*† and A. B. Scheidt‡

*Center for Chemical Toxicology and Research Pharmacology; †Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh 27606; and ‡Pfizer Animal Health, Hampstead, NC 28443

ABSTRACT: Medicating drinking water with tetracycline is commonly used in swine production systems to treat and prevent disease outbreaks. However, little information is known of the pharmacokinetics of this medication in water formulations. Twenty-four barrows, divided into 1 control group (of nontreated animals) and 3 equally sized treatments groups ($n = 6$/group), were treated with tetracycline water medication for 5 d at 125, 250, and 500 mg/L. Blood samples were collected at 0 (prestudy), 4, 8, 12, 24, 32, 48, 56, 72, 80, 96, and 104 h after exposure. Data analyses consisted of a noncompartmental pharmacokinetic analysis and statistical analysis of steady state concentrations with repeated measures ANOVA and multiple-comparison testing to determine whether plasma concentrations differed among groups. Derived pharmacokinetic parameters were consistent with previously published feed and intravenous data. Plasma tetracycline concentrations at steady state were 0, 0.33, 0.47, and 0.77 μg/mL for 0-, 125-, 250-, and 500-mg/L exposures, respectively. Treatment group steady-state plasma concentrations were significantly different from plasma concentrations in control animals ($P < 0.0001$); however, whereas the 125- and 250-mg/L groups were significantly different from the 500-mg/L group ($P < 0.0001$), their mean plasma tetracycline concentrations did not differ from one another. Furthermore, the study showed that tetracycline oral bioavailability is very small. The dose response curve also shows that concentrations of plasma tetracycline increase linearly, yet not in a 1 to 1 ratio, to the direct increase in water medication dose.

Key words: pharmacokinetics, plasma concentration, swine, tetracycline, water medication

J. Anim. Sci. 2009. 87:3179–3186
doi:10.2527/jas.2009-1877

INTRODUCTION

In commercial swine production, antimicrobial medication often is used after weaning, transport, or during disease outbreaks. Concern has grown over the last decade between increased antimicrobial resistance in human pathogens and food animal antimicrobial use (FDA, 2003). In Europe, this policy led to the ban of feed additives (EU, 2003) and a concurrent increase in the use of water medications (personal observation in European countries). Currently, there is only limited published pharmacokinetic (**PK**) data on water medication formulations, including amoxicillin (Agerso et al., 1998), sulfamethazine (Mason et al., 2008), and tetracycline (Nielsen and Gyrd-Hansen, 1996).

Although a ban on feed additives is not in effect in the United States, PK studies to evaluate antibiotic treatment and its effects should be conducted before adopting blanket policies prohibiting drug use. Pharmacokinetic studies would characterize therapeutic potential and PK parameters unique to water antimicrobials. In addition to traditional PK parameters, PK-pharmacodynamic studies can potentially relate the impact of antimicrobial use on the food supply and public health (CDC, 2006).

Tetracycline, an antibiotic labeled for the control and treatment of salmonellosis, some enteric bacteria, and

[1]The authors thank Beth Barlow (Center for Chemical Toxicology and Research Pharmacology; Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University) for HPLC assistance, Missy Lloyd (Animal Science Department, North Carolina State University) for performing atomic absorption analysis to verify carboy mineral content, Patty Routh (Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University) for her assistance in organization and facility set-up as well as bleeding and handling animals, Whitney Weeks (College of Veterinary Medicine, North Carolina State University) assisted with sample preparation, and Juliet Allen (Center for Chemical Toxicology and Research Pharmacology, College of Veterinary Medicine, North Carolina State University) supplied statistical expertise. All of the staff at NCSU Swine Education Unit who provided facility set-up and maintenance, animal care, handling, and movement were indispensible to this study. Finally, we thank Arato werk GmbH & Co. in Germany for the donation of Arato80 drinkers and Pfizer Animal Health for funding the entirety of this study.

[2]Corresponding author: ronald_baynes@ncsu.edu

Received February 9, 2009.
Accepted June 10, 2009.

susceptible respiratory diseases in pigs, was selected as a test compound because of its common use (Aarestrup, 2005) and lack of PK data from observational studies (Luthman et al., 1989; Pijpers et al., 1989; Reeve-Johnson, 1998). An experiment was designed to measure tetracycline concentrations in swine plasma. This study design allowed for group comparisons of 4 doses, including control, and to determine if the dose response of tetracycline is linear. These studies also assessed whether tetracycline concentrations in plasma consistently reached minimum inhibitory concentrations of quality control (sensitive) bacteria (Isenberg, 2004; CLSI, 2007; Qaiyumi, 2007).

MATERIALS AND METHODS

All animals used in this study were housed and treated in accordance with the North Carolina State University Internal Animal Care and Use Committee standards.

Animals, Treatment, and Facility

Twenty-four Yorkshire-Landrace cross barrows were housed at North Carolina State University's Swine Unit Facility in individual pens on concrete. Barrows, approximately 8 wk of age and weighing 16 to 18 kg at the initiation of the study, were assigned to 1 of 4 treatment groups (n = 6/group). One group received 0.5 times the label dose (125 mg/L) of tetracycline hydrochloride; the second, a label dose (250 mg/L); the third, 2 times the label dose (500 mg/L); and the control group received water without tetracycline. Treatments of 125, 250, and 500 mg/L were achieved by dissolving a preweighed amount of an approved tetracycline water medication (AmTech, IVX Animal Health Inc., Saint Joseph, MO) into 19.2 L of water in individual carboys for each animal. All animals were given 5 d to acclimate to the facility and adapt to the carboy drinking system before the start of the trial. The Nalgene carboys (Thermo Fisher Scientific, Waltham, MA) were suspended from the ceiling and attached to the barn plumbing via 0.9 to 1.4 m of 1.27-cm plastic tubing and plumbers fittings. Arato80 drinkers (Aratowerk GmbH & Co., Koln, Germany) were mounted in the individual pens to minimize waste and spillage of water used. Water flow rates varied between 500 and 800 mL/min, which is consistent with flow rates needed to prevent dehydration in adult pigs (Leibbrandt et al., 2001). Daily maximum and minimum temperatures within in the barn were collected with a thermometer and recorded daily.

Feed and Water Composition

Water was assessed the month before the trial by the North Carolina Agronomics department to determine water quality on the farm. A 17% protein antibiotic-free feed, ground on site, was fed to all animals ad libitum. Animals did not receive any antimicrobials or growth promotants at least 10 d before the start of the study. Carbadox, which did not interfere with HPLC, was the only medication these pigs received in the nursery. Water was changed and tetracycline was remixed every other day (i.e., d 1, 3, and 5) except for 2 pigs. One pig drank excessively, using most of 1 carboy every day; therefore, the water was replaced daily. One other carboy fell on d 2, and the water was replaced. Water use by each pig was measured with a graduated cylinder (to within 10 mL) on 2 separate days, the day before the study began, and on d 2, which was consistent with daily water use. Water samples, randomly chosen, were collected daily except for on d 2 and 5 when all carboys were collected.

Other Measurements

Free catch urine samples were attempted daily (AM only). Urine specific gravities were determined using a refractometer, and tetracycline concentrations were determined. All animals were weighed at the start of the acclimation period and on the final day of the study.

Plasma Sample Preparations and Chemistries

Blood was collected from each pig via venipuncture of the anterior vena cava into 10-mL sodium heparin tubes before administration of the tetracycline water medication (time 0, 800 h) and then at 4, 8, 12, 24, 32, 48, 56, 72, 80, 96, and 104 h after dosing. Whole blood (before centrifugation) was assayed for hematocrit with microcapillary tubes and total protein (via specific gravity) on d 1. Antech Diagnostic Labs (Ithaca, NY) performed urine creatinine (daily as collected) and serum creatinine on d 1 and 5 for all pigs. Plasma was harvested from all blood samples after centrifugation (1,110 × g; 15 min; 4°C) within 1 h of collection and split into 2 samples, for processing that week and for storage at −80°C.

HPLC Analysis

A Waters Alliance 2695 HPLC with vacuum pump and autosampler (Milford, MA) was used for tetracycline quantification. The assay was a modified version of Cheng et al. (1997) and Santosa et al. (1996). Two hundred microliters of plasma were added to an equivalent volume of releasing agent (78% water, 20% acetonitrile, 2% o-phosphoric acid) in a YM 10,000 Ultracel kit (Millipore Corporation, Milford, MA). The Ultracel apparatus was placed in an Eppendorf tube and centrifuged at 7,840 × g for 30 min at 22°C. Tetracycline was quantified using a Photodiode Array at 354.4-nm wavelength, with peak integration by Empower software (Waters Corporation). Samples were held away from light at 4°C for up to 7 d before a 4-min run on a Waters Atlantis 4.6- × 150-mm C18 column in 28% acetonitrile, 72%

0.05 M oxalic acid buffer at 35°C. Limit of quantification, the least concentration that can be reproducibly quantified, for plasma and distilled water was 0.1 μg/mL, and the limit of detection was consistently at or below 0.05 μg/mL. A linear curve for well water from the farm spiked with tetracycline from 80 to 800 mg/L was adequate for our water samples analyzed by HPLC. All values were within the accepted CV of 20%, based on daily standard curves.

Pharmacokinetic Analysis

Pharmacokinetic analysis was performed on each group with WinNonLin (Pharsight, Mountain View, CA) using a noncompartmental analysis to calculate mean residence time (**MRT**), area under the moment curve (**AUMC**), area under the curve (**AUC**), and the AUC between 32 and 48 h (**AUC$_{32-48}$**). Due to animal use limitations and the availability of previously published data, an apparent oral bioavailability (**F$_{oral}$**) was estimated using experimental oral data and intravenous data from Kniffen et al. (1989), including the intravenous dose and AUC. The following equation was used to calculate F_{oral} for each animal:

$$\frac{F_{oral} \times Dose_{oral}}{AUC_{oral}} = \frac{Dose_{IV}}{AUC_{IV}},$$

where F_{oral} is the apparent oral bioavailability of the tetracycline, $Dose_{oral}$ is the dose from the water (measured concentrations of the water medication multiplied by the amount of water consumed over 24 h) for the animal (Supplemental Table 1 in http://jas.fass.org/content/vol87/issue11/), AUC_{oral} is the AUC_{32-48} from WinNonLin for that animal, and AUC_{IV} and $Dose_{IV}$ are the AUC and corresponding intravenous dose from the Kniffen et al. (1989) study. The calculated F_{oral} was then used to estimate clearance and volume of distribution at steady state for each group based on the following equations:

$$Vd_{ss} = \frac{F_{oral} \times Dose_{24}}{C_{ss}}; Cl_{ss} = \frac{Dose_{24} \times F_{oral}}{C_{ss} \times 24},$$

where Vd_{ss} is the volume of distribution at steady state, Cl_{ss} is clearance for the group, F_{oral} is apparent bioavailability of the group, $Dose_{24}$ is the dose (water consumption multiplied by the concentration of water) over a 24-h period for the group, and C_{ss} is the concentration of the group at steady state. For each group, the individual animal peak plasma concentration (**C$_{max}$**) is reported with its corresponding time (**T$_{max}$**).

Statistical Analysis

A randomized block design was employed to test the hypotheses that tetracycline concentrations in plasma differed among 4 treatment groups and if steady-state concentrations of any group reached 1 μg/mL. Daily BW gain and group BW pre- and poststudy were calculated and compared via 1-way ANOVA. Daily maximum and minimum temperatures were used to calculate a mean maximum and minimum temperature during the study. Two-way repeated ANOVA was performed (SAS Inst. Inc., Cary, NC) using PROC MIXED with dose and time as independent variables for concentrations at steady state (from 32 to 104 h). Multiple comparison testing was performed with Scheffe to determine if treatment of tetracycline groups 125, 250, and 500 mg/L were different. The data were then graphed to determine if treatment groups displayed a linear exposure-concentration curve. One-way ANOVA, using dose as the independent variable with PROC MIXED, was also performed on group PK parameters. All errors were calculated as per error analysis methods by Taylor (1982).

The ANOVA compared plasma steady state concentration (32 to 104 h) points across all groups. Steady state is considered the time at which at least 5 half-lives have transpired. Previous data (Kniffen et al., 1989; Nielsen and Gyrd-Hansen, 1996) and our current analysis indicate that the half-life for tetracycline in swine ranges from 4.5 to 6 h. Therefore, steady state should be reached between 20 and 30 h; and the first time point after steady state was reached, 32 h, was used as a conservative initial time for steady-state PK analysis.

RESULTS

All animals remained healthy throughout the study irrespective of treatment group as evidenced by values for BW, hematocrit, serum creatinine, and urine specific gravity (except pig 13 discussed below) typical for pigs of this age (Supplemental Table 1 available online at http://jas.fass.org/content/vol87/issue11/). Daily peak temperatures were elevated at the beginning of the week, were less in the middle of the week, and then increased to above 32°C by the end of the week (Table 1). Plasma concentrations of tetracycline from each group increased from below the limit of detection to steady-state concentrations within a 32-h period (Figure 1). Steady-state concentrations between groups given 125 and 250 mg/L were not different from each other, but were different from control ($P < 0.0001$) and the 500 mg/L ($P < 0.0001$) treatment (Table 2). The AUC were also different between the control ($P < 0.0001$) and dosed groups, 125 and 500 mg/L ($P < 0.0001$) treatments, 250 and 500 mg/L ($P = 0.0025$) groups. A linear but indirect exposure-concentration relationship of tetracycline water concentration and plasma concentration is presented in Figure 2. This is consistent with reports in human literature and resources that indicate decreased absorption of larger doses of tetracycline (Chambers, 2006).

Pharmacokinetic parameters (Table 2) were calculated based on the HPLC data as explained in the Materials and Methods section. A water analysis on the farm

Table 1. Daily maximum and minimum ambient temperatures during the study

Day	Daily maximum,[1] °C	Daily minimum,[2] °C
1		21.5
2	31.1	20.1
3	28.4	20.7
4	29.1	21.7
5	32.9	23.3
6	33.2	
Average	31.0 ± 0.4	21.4 ± 0.2

[1]Daily maximum temperature is based on the peak temperatures reached within the pig facility each afternoon or early evening.

[2]Daily minimum temperature is the temperature reached within the pig facility in the early morning, listed as the minimum temperature from the previous day.

(Table 3) shows that the water quality was appropriate for the study and did not result in elevated levels of tetracycline cation-complexing. Water tetracycline concentrations were measured for each animal on 3 d or more, and freshly mixed tetracycline water concentrations were within the assay variability (20%) of the expected concentration (Table 4), which further supports water quality. Water consumption was not different across all groups of pigs. Based on linear scatter plots, there was no observable relationship between the water consumption and flow rates on tetracycline steady state or average plasma concentrations.

DISCUSSION

Pharmacokinetic parameters of MRT, AUC, and C_{max} were consistent with previously collected data (Kniffen et al., 1989; Nielsen and Gyrd-Hansen, 1996) from pigs given tetracycline orally. The main differences and intended test variable among the treatment groups are the concentration at steady state (C_{ss}). This is significant because the other parameters of half-life, MRT, F_{oral} and Vd_{ss}, are all similar to those previously reported. Based on statistical analysis, parameters with at least one difference were plasma concentrations at steady state, as discussed above, and the corresponding AUC_{total}. Calculated clearances are similar to previously reported values but should be interpreted carefully based on the age of the pigs in our study and our calculation of apparent bioavailability. An apparent bioavailability, or more correctly the ratio between the amount of tetracycline in water that was used by the pigs compared with the amount of an intravenous dose, was calculated at approximately 6% across all treatment groups. This ratio may be used in the future to help determine how much tetracycline in water would be needed to reach known concentrations in the blood. Essentially, this ratio theoretically means that a dose of tetracycline in water would have to be approximately 20 times the dose given intravenously to approach the AUC of that intravenous dose. This is based only on this study and may not hold for other scenarios; however, it provides an estimate of the magnitude of the difference between an oral dose in a production setting and an intravenous dose. This may not be a true bioavailability as is observed by the potential discrepancy in the clearances. Clearance rates were similar among the groups but slightly less (0.10 $\mu g \times mL^{-1} \times h^{-1}$) than those previously reported between 0.16 and 0.22 $\mu g \times mL^{-1} \times h^{-1}$ (Kniffen et al., 1989; Nielsen and Gyrd-Hansen, 1996), which adds credence to the apparent bioavailability not being a true bioavailability. Based on this F_{oral} value, volumes of distribution at steady state ($\boldsymbol{Vd_{ss}}$) also were calculated but cannot be directly compared with previous PK studies values of Vd_{area} or Vd_{actual} because they may not be equivalent (Williams, 1999).

It is interesting to note that a few of the animals appeared to overshoot steady state at 12 h, as seen by the plasma concentrations, but steady state is not reached for another day. Furthermore, the peak concentration for the 500 mg/L group is at 12 h. However, for some of these animals, the peak at 12 h was subsequently followed by a drop to steady-state concentrations by 32 h. This may be explained by the fact that some animals drank very little overnight and that the first day of the study was warm.

Furthermore, 2 animals on the dose response curve had greater than anticipated concentrations (greater than 1.5 SD from the average, with each animal included in the average). The first outlier, within the 125 mg/L group, used approximately 12 L of water within 1 d. The second outlier did not exhibit any measurable difference in BW, water consumption, or other factors that explain the high plasma concentrations. Exclusion of both animals increases the coefficient of determination for mean concentrations at steady-state from 0.75 to 0.91 for the dose-response relationship.

The high temperatures ranged from 28.4 to 33.2°C for the whole week. According to previous studies, the maximal estimated water consumption of 1 L of water per 10 kg of BW is expected each day for pigs up to 50 d of age (Harvey, 1994). Based on this estimated water consumption for pig BW of 15 to 20 kg, the expected water consumption for the pigs in our study was 1.5 to 2 L of water daily. However, the pigs in our study consumed a larger amount of water relative to their size. The increased water consumption for the animal size could be explained by increased ambient temperatures as compared with other studies. On average, the water consumption was 3 to 4 L per pig per day (Supplemental Table 1 available online at http://jas.fass.org/content/vol87/issue11/). Due to the warm ambient temperatures, it is anticipated that plasma concentrations of these animals are close to peak attainable concentrations based on the amount of water consumed (Harvey, 1998, 1994).

Only 1 animal appeared to use water excessively, up to 12 L over one 24-h period. This may be due to either psychogenic water drinking, which can occur in a small proportion of animals from multiple species or from the pig playing with the drinker. The urine-specific grav-

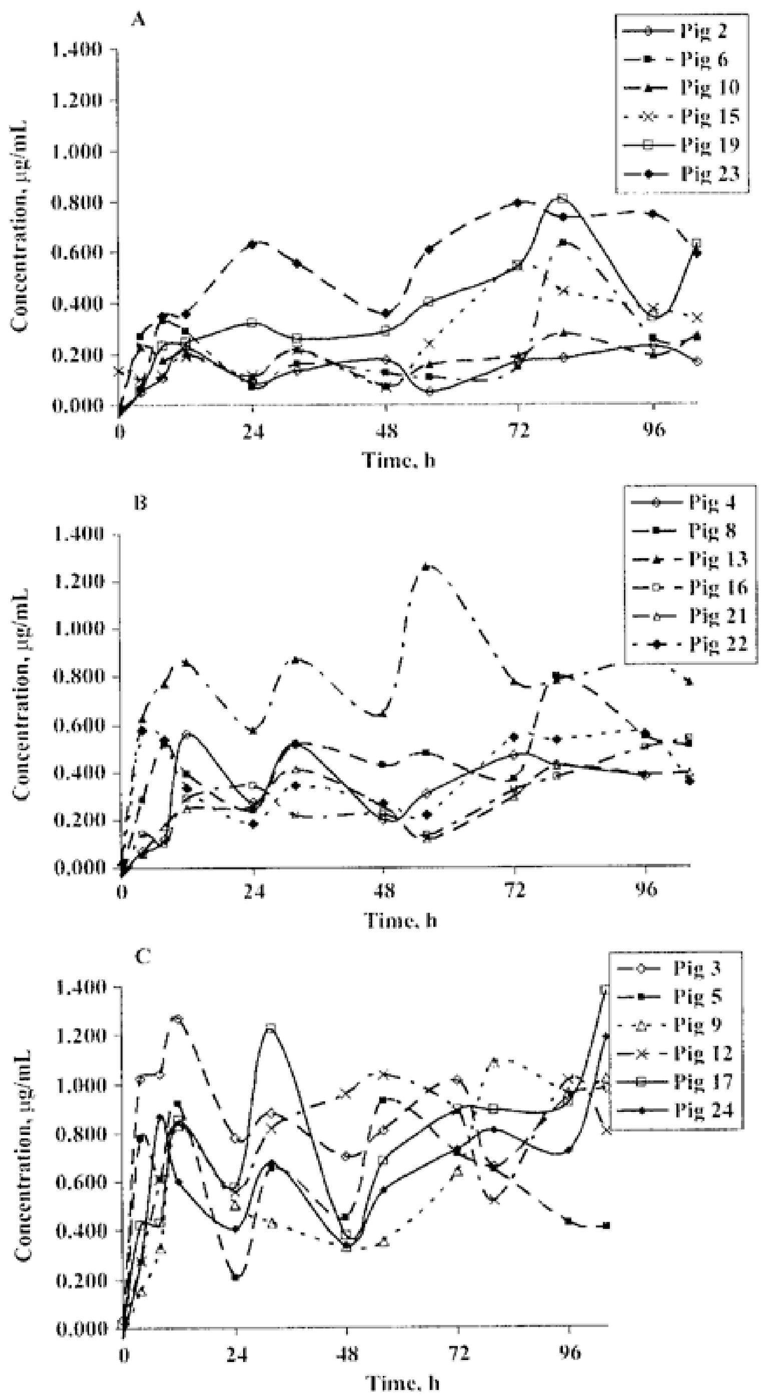


Figure 1. Individual animal plasma concentrations over time by treatment group. A) Plasma concentrations from pigs (n = 6) exposed to 125 mg/L of tetracycline in water. B) Plasma concentrations from pigs (n = 6) exposed to 250 mg/L of tetracycline in water. C) Plasma concentrations from pigs (n = 6) exposed to 500 mg/L. Note that for many animals, there was a slight overshoot of steady-state concentrations at 12 h on d 1 and a decline in plasma concentrations when the temperatures were less in the middle of the week. Then, at the end of the week as water consumption likely increased with increased ambient temperatures, the plasma concentrations rose again. All axes use the same units for easier comparison.

Table 2. Pharmacokinetic parameters for tetracycline water medication for each treatment group

Parameter/group	125. mg/L	250. mg/L	500. mg/L
C_{max},[1] μg/mL	0.80	1.26	1.29
T_{max},[2] h	80	56	12
C_{ss},[3] μg/mL	0.33 ± 0.03[a]	0.47 ± 0.04[a]	0.77 ± 0.04[b]
AUC_{total},[4] μg·h/mL	30.71 ± 6.61[c]	44.93 ± 8.26[c]	73.74 ± 4.88[d]
AUC_{32-48},[5] μg·h/mL	3.54 ± 0.82	6.55 ± 1.24	10.46 ± 1.31
MRT,[6] h	6.61 ± 0.25	6.61 ± 0.21	6.41 ± 0.15
Half-life, h	4.60 ± 0.25	4.58 ± 0.21	4.46 ± 0.15
Cl,[7] $L\cdot kg^{-1}\cdot h^{-1}$	0.083 ± 0.008	0.107 ± 0.006	0.106 ± 0.008
Vd_{ss},[8] L/kg	0.551 ± 0.169	0.646 ± 0.252	0.769 ± 0.209
F_{oral}[9]	0.059 ± 0.009	0.064 ± 0.014	0.057 ± 0.015

[a]125-mg/L and 250-mg/L group concentrations were different from control and 500-mg/L group ($P <$ 0.0001).

[b]500-mg/L group concentrations differed from all other groups ($P < 0.0001$).

[c]125-mg/L and 250-mg/L group area under the curve (AUC) differed from control ($P < 0.0001$) group AUC.

[d]500-mg/L group AUC differed from control and 125-mg/L group AUC ($P < 0.0001$) and 250-mg/L group AUC ($P = 0.0025$).

[1]The C_{max} is the peak value reported for one animal; no comparisons were performed for this value.

[2]The T_{max} is reported for the greatest individual value for an individual animal; no comparisons were performed for this value.

[3]C_{ss} is the mean concentration at steady state for the treatment group.

[4]AUC_{total} refers to the area under the concentration time curve from 0 to 104 h.

[5]AUC_{32-48} is the area under the concentration time curve from 32 to 48 h and was used to calculate clearance.

[6]MRT refers to mean residence time.

[7]Cl refers to the clearance at steady state.

[8]Vd_{ss} is the volume of distribution at steady state.

[9]F_{oral} refers to the apparent oral bioavailability of tetracycline compared with a typical 10-mg/kg intravenous dose.

ity of that pig on that same day was 1.003, which is consistent with medullary washout and excessive consumption of water. Regardless of this particular animal, increased water consumption is the only variable that appears correlated to increased temperatures.

It was also noted that the tetracycline from the 250-mg/L group began darkening in color by the late afternoon of d 2. This color change was also seen in the 125- and 500-mg/L-treated carboys at later times. The control water never changed color. This color change was presumed to be related to degradation from tetracycline exposure to light; however, a study by Wu and Fassihi (2005) suggested that increased temperatures and increased humidity cause degradation of tetracycline and not light exposure. The HPLC assay continued to detect the tetracycline present in the water even after the color change began. Follow-up studies are being performed to better characterize the antimicrobial

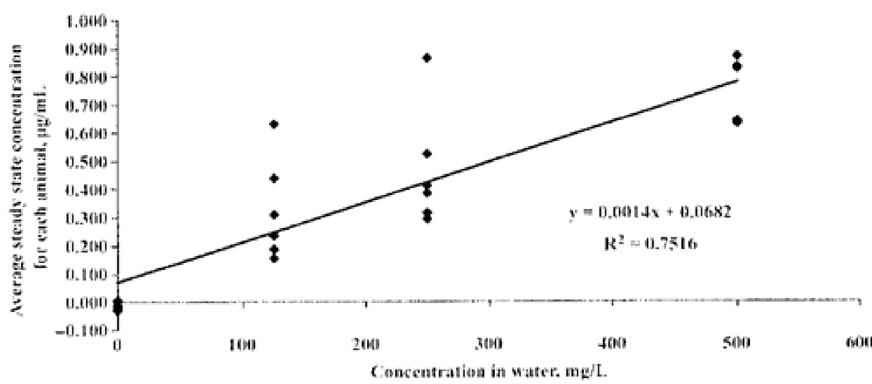


Figure 2. Exposure response relationship between tetracycline water medication concentration and individual animal steady-state plasma concentrations of tetracycline. The line represents the relationship, based on least squares best fit, between the average individual animal steady-state concentrations and the treatment exposure concentrations. Removal of 2 data points because each steady-state concentration was greater than 1.5 SD from the calculated mean, using all data points, increases R^2 to 0.91.

Table 3. Solution analysis report from the North Carolina Agronomics Department, Raleigh[1]

Measurement	Value
pH[2]	6.45
Electrical conductivity,[3] mho × 10^5/cm	0.00016
Sodium adsorption ratio	0.60
Total alkalinity ($CaCO_3$), mg/L	55
Hardness, mg/L	63
B, mg/L	0.02
Ca, mg/L	17.70
Cl, mg/L	4.53
Cu, mg/L	0.02
Mg, mg/L	4.63
Mn, mg/L	0.09
Inorganic N, mg/L	0.72
P, mg/L	0.18
K, mg/L	8.90
Na, mg/L	10.80
S, mg/L	1.58
Zn, mg/L	0.32

[1]Water from the farm well was assayed before the study. This assay tested for the suitability of the water for its intended purpose. Physical properties and concentrations of divalent and trivalent cations which could bind tetracycline were also reported. Recommendations from the report: "The general water quality… from this farm well looks good. Mineral nutrient and soluble salt levels are low and alkalinity is moderate."

[2]pH is defined as the $-\log_{10}(a_H)$, where a_H refers to the theoretical hydrogen ion activity.

[3]mho is 1 siemens, which is the SI unit of resistance; 1 mho is equal to 0.953 ohm.

effects of this color change, but the widely held assumption that tetracycline is deactivated by light appears incorrect.

One final potential source of concern for the tetracycline concentrations is cation-complexing due to concentrations of divalent and trivalent cations within the water. According to the North Carolina Agronomics Department report, the water was adequate and appropriate for farm animals. The concentrations of Ca, Mg, Mn, Zn, Fe, and Cu when added together are less than 26 mg/L. This implies that cation-complexing effects from water are relatively small, especially in respect to feed sources. The water analysis also reported that the water was slightly acidic, pH 6.45, which would tend to stabilize the tetracycline (Harvey, 1998; Wu and Fassihi, 2005). This stability is supported by HPLC analysis on water samples within 24 h of the start of the study and those measured right after mixing. Based on previous work, the medication should be stable for 48 h (Harvey, 1998).

Table 4. Tetracycline HPLC assay quantitation compared with the expected amount of tetracycline mixed into carboy water on d 3 of the study[1]

Treatment group	True dose/expected dose[2]
125	1.11
125	1.19
125	1.15
250	0.93
250	0.90
250	0.85
250	0.88
250	0.88
500	1.11
500	0.85
Average proportion of water dose to expected value	0.99 ± 0.01

[1]Each carboy was emptied, rinsed, and refilled with 19.2 L of water from the farm, and then preweighed tetracycline powder was added to the carboy. These samples were randomly collected on d 3.

[2]This equation relates the concentration of tetracycline measured by HPLC to the expected concentration of tetracycline and incorporates all errors, including measurement error in water volume and weighing of tetracycline, and variability in the HPLC method. A ratio of 1.00 shows that the measured water concentration matches the expected concentration.

After addressing the typical concerns (cation-complexing, water concentrations, and differences among the groups) and new problems noted (water color change and indirect linear exposure-concentration), tetracycline water medication concentrations as dosed were found to be consistent with tetracycline feed additive concentrations. In one feed additive study, Kniffen et al. (1989) dosed 4 gilts with 0.55 g of tetracycline per kilogram of BW. Tetracycline concentrations reached 0.4 to 0.6 μg/mL (peak). Another study administered a single water dose of tetracycline in fasted and fed pigs (Nielsen and Gyrd-Hansen, 1996). Animals received a dose of 45 mg/kg of tetracycline, and peak tetracycline concentrations of 4 μg/mL were seen in fasted pigs; however, when fed pigs were given this single oral dose, peak tetracycline concentrations only reached 0.8 μg/mL. These data are consistent with the data reported by Luthman et al. (1989) on oral tetracycline use. According to our study, if pigs consumed only 2 L of treated water per day, then they would have received a 25 or 50 mg/kg dose of tetracycline for the 250- and 500-mg/L treatment groups, respectively. Because it is impractical to fast pigs in a production facility, this information indicates that water medication values are in line with feed additive concentrations and will likely have similar efficacy. Furthermore, based on the Clinical and Laboratory Standards Institute guidelines, sensitive control bacteria used in antibiotic susceptibility assays need at least 1 μg/mL for growth inhibition (CLSI, 2007). Only in a few pigs is 1 μg/mL reached or exceeded, and even this level is not consistently met by any animal or group. Based on treatment group PK analyses, it is evident that all steady state plasma concentrations are inadequate for treatment of highly susceptible bacteria. Furthermore, tetracycline has been used more than any other medication in actual tons in Europe and the United States (Aarestrup, 2005). Bunner et al. (2007) showed that *Escherichia coli* are highly resistant to tetracycline with minimum inhibitory concentrations above 16 μg/mL, which is often considered the breakpoint of tetracycline.

As a reference for in vivo tetracycline concentrations, the Food and Drug Administration has set a tolerance of tetracycline at 2 μg/mL for muscle, 12 μg/mL for kidney and fat, and 6 μg/mL in liver (FDA, 2005).

Concentrations within the plasma from our study are not even 50% of the least muscle tolerance concentration for any time points. At this time no published tetracycline residue studies are available; therefore, the true tissue concentrations of tetracycline in vivo are unknown. However, based on oxytetracycline tissue concentrations in pigs and ruminants, the concentrations of tetracycline will likely be a maximum of 6 to 7 times the plasma concentrations in the kidney (Mercer et al., 1978). The lung, which is often the target of tetracycline treatment, only reaches or slightly exceeds the concentration in the plasma. Other tissues such as fat reach less than one-half of peak plasma concentrations (Mercer et al., 1978; Craigmill, 2003).

Injectable antibiotics are still used in livestock production; however, due to labor constraints, the possibility of needle breakage, and the expense of injectable medications, other methods of medicating animals are used first. Food producers need to be aware of the medications available and what they actually can treat. Based on this study and corroboration with previous work on tetracycline oral medications in pigs, tetracyclines should not be used enterally to treat salmonellosis and respiratory disease. There may still be positive gastrointestinal effects from using oral tetracycline for prevention of scours and other enteric disease, but this needs to be assessed further.

LITERATURE CITED

Aarestrup, F. M. 2005. Veterinary drug usage and antimicrobial resistance in bacteria of animal origin. Basic Clin. Pharmacol. Toxicol. 96:271–281.

Agerso, H., C. Friis, and J. Haugegaard. 1998. Water medication of a swine herd with amoxycillin. J. Vet. Pharmacol. Ther. 21:199–202.

Bunner, C. A., B. Norby, P. C. Bartlett, R. J. Erskine, F. P. Downes, and J. B. Kaneene. 2007. Prevalence and pattern of antimicrobial susceptibility in *Escherichia coli* isolated from pigs reared under antimicrobial-free and conventional production methods. J. Am. Vet. Med. Assoc. 231:275–283.

CDC. 2006. Action Plan: The Focus Areas: III. Research (Issue C). http://www.cdc.gov/drugresistance/actionplan/html/research2.htm Accessed Nov. 15, 2008.

Chambers, H. F. 2006. Protein Synthesis Inhibitors and miscellaneous antibacterial agents. Pages 1173–1179 in Goodman & Gilman's The Pharmacological Basis of Therapeutics. L. S. Goodman, A. Gilman, L. L. Brunton, J. S. Lazo, and K. L. Parker, ed. McGraw-Hill, New York, NY.

Cheng, Y., D. Phillips, and U. Neue. 1997. Simple and rugged SPE method for the determination of tetracycline antibiotics in serum by HPLC using a volatile mobile phase. Chromatographia 44:187–190.

CLSI. 2007. Performance Standards for Antimicrobial Susceptibility Testing; Seventeenth Informational Supplement. 1st ed. Clinical and Laboratory Standards Inst., Wayne, PA.

Craigmill, A. L. 2003. A physiologically based pharmacokinetic model for oxytetracycline residues in sheep. J. Vet. Pharmacol. Ther. 26:55–63.

EU. 2003. On additives for use in animal nutrition. Regulation (EC) No. 1831/2003. European Parliament and of the Council. 22 Sep. 2003. Pages 0029–0043.

FDA. 2003. Guidance for Industry #152. http://www.fda.gov/oc/antimicrobial/questions.html Accessed Oct. 17, 2008.

FDA. 2005. Actions Taken by FDA Center for Veterinary Medicine. www.fda.gov/cvm/Green Book/200512.pdf Accessed Nov. 20. 2008.

Harvey, R. E. 1994. Water consumption in pigs. The Pig Journal 32:95–98.

Harvey, R. E. 1998. Observation of the drinking habits of weaned growing pigs in relation to the rationale of water medication. The Pig Journal 42:137–140.

Isenberg, H. D. 2004. Clinical Microbiology Procedures Handbook No. 2. Section 2.1–2.14. ASM Press, Washington, DC.

Kniffen, T. S., D. P. Bane, W. F. Hall, G. D. Koritz, and R. F. Bevill. 1989. Bioavailability, pharmacokinetics, and plasma concentration of tetracycline hydrochloride fed to swine. Am. J. Vet. Res. 50:518–521.

Leibbrandt, V. D., L. J. Johnston, G. C. Shurson, J. D. Crenshaw, G. W. Libal, and R. D. Arthur.. 2001. Effect of nipple drinker water flow rate and season on performance of lactating swine. J. Anim. Sci. 79:2770–2775.

Luthman, J., S. O. Jacobsson, B. Bengtsson, and C. Korpe. 1989. Studies on the bioavailability of tetracycline chloride after oral administration to calves and pigs. Zentralbl. Veterinarmed. A 36:261–268.

Mason, S. E., R. E. Baynes, J. L. Buur, J. E. Riviere, and G. W. Almond. 2008. Sulfamethazine water medication pharmacokinetics and contamination in a commercial pig production unit. J. Food Prot. 71:584–589.

Mercer, H. D., R. H. Teske, P. E. Long, and D. H. Showalter. 1978. Drug residues in food animals. II. Plasma and tissue kinetics of oxytetracycline in young cross-bred swine. J. Vet. Pharmacol. Ther. 1:119–128.

Nielsen, P., and N. Gyrd-Hansen. 1996. Bioavailability of oxytetracycline, tetracycline and chlortetracycline after oral administration to fed and fasted pigs. J. Vet. Pharmacol. Ther. 19:305–311.

Pijpers, A., B. Van Klingeren, E. J. Schoevers, J. H. Verheijden, and A. S. Van Miert. 1989. In vitro activity of five tetracyclines and some other antimicrobial agents against four porcine respiratory tract pathogens. J. Vet. Pharmacol. Ther. 12:267–276.

Qaiyumi, S. 2007. Macro- and microdilution methods of antimicrobial susceptibility testing. Pages 76–79 in Antimicrobial Susceptibility Testing Protocols. A. C. Goodwin, R. Schwalbe, and L. Steele-Moore, ed. CRC Press, Boca Raton, LA.

Reeve-Johnson, R. 1998. Water medication and the treatment of respiratory diseases in pigs. The Pig Journal 42:74–86.

Santosa, M. D. F., H. Vermeersch, J. P. Remona, M. Schelkensa, P. De Backerb, R. Ducatellec, and F. Haesebrouck. 1996. Validation of a high-performance liquid chromatographic method for the determination of doxycycline in turkey plasma. J. Chromatogr. B Biomed. Sci. Appl. 682:301–308.

Taylor, J. R. 1982. An Introduction to Error Analysis: The Study of Uncertainties in Physical Measurements. 2nd ed. Univ. Sci. Books, Mill Valley, CA.

Williams, P. L. 1999. Non-compartmental models. Pages 159–160 in Comparative Pharmacokinetics: Principles, Techniques, and Applications. 1st ed. J. E. Riviere, ed. Iowa State Univ. Press, Ames.

Wu, Y., and R. Fassihi. 2005. Stability of metronidazole, tetracycline HCl and famotidine alone and in combination. Int. J. Pharm. 290:1–13.

Stability and Bioactivity of Tetracycline Water Medication in Swine Production Units

Sharon E. Mason DVM [†§], Mitsu Suyemoto MS[§], Ronald Baynes DVM PhD[†§], and Glen Almond DVM PhD[§]

[†]Center for Chemical Toxicology and Research Pharmacokinetics

[§]Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University

Submitted to the Journal of Swine Health and Production

Summary

Tetracycline hydrochloride-treated water on commercial farms may change color over time but its stability is unknown. Water samples containing tetracycline, darkened to yellow or brown, were assayed by HPLC and MIC micro-broth dilution for biological activity. Water samples were biologically active in the expected bioactivity range, but safety could not be determined.

Tetracycline hydrochloride (TcHCl) added to water is indicated for use in swine for the therapeutic treatment for *Escherichia coli* scours and susceptible respiratory pathogens. It was suggested that medications be mixed daily for use in swine facilities; however, mixing of water medications is often only performed when the batch of medication has been used. However, tetracycline hydrochloride medications may change color in water after mixing. During two recent studies, it was observed that the stock solution of tetracycline hydrochloride medicated water began to change color between 24 and 48 hours after mixing of the medication [1,2]. At least two reports state that tetracycline is deactivated by a combination of high humidity and temperatures over 100°F [3,4]. Tetracycline has also been shown to degrade after exposure to ultraviolet and fluorescent light [3].

Mason et al [2] took water samples from carboys from which 24 individual pigs were exposed to TcHCl medicated water at three different concentrations (the label dose (250 ug/mL or ppm, twice the label dose (500ppm) and one-half the label dose (125ppm). The medicated water sample concentrations of tetracycline were measured via HPLC. Those HPLC results are compared to MIC microdilution assays used to test the bioactivity of those same medicated water samples. The bacterial standard (*E. coli* ATCC25922) for the Clinical Laboratory Standards Institute (CLSI) was compared to both standard TcHCl MIC assays and the reported MIC. This study was performed because of changes in color of water medicated with tetracycline hydrochloride and the lack of knowledge of its stability and bioactivity once the color change has occurred. Therefore, we proposed that TcHCl in water is adequately stable and biologically active despite its color change.

Materials and Methods. HPLC analysis was performed on a Waters Alliance 2695 High Performance Liquid Chromatograph with autosampler (Waters Corporation, Milford, MA) coupled to a Photodiode Array. Peak integration at 354.4-nm wavelength by Empower software (Waters Corporation, Milford, MA) was used for tetracycline quantification for each run on a Waters Atlantis 4.6- × 150-mm C18 column held at 35°C during a 4 min run time in 28% acetonitrile, 72% 0.05 *M* oxalic acid buffer mobile phase. All samples were kept in darkness at 4°C and were initially run within 14 d of collection. Two hundred microliters of plasma were added to an equal volume of releasing agent (78% water, 20% acetonitrile, 2% o-phosphoric acid) in a YM 10,000 Ultracel kit (Millipore Corporation, Milford, MA), placed in an Eppendorf tube and centrifuged at 7,840 × *g* for 30 min at 22°C. The lower limit of quantification, the smallest concentration that can be reproducibly quantified, for all water samples was 0.1 µg/mL, and the limit of detection was consistently at or below 0.05 µg/mL. The standard curve for water sample standards from 80 to 800 µg/mL had an R^2 of 0.99 and intraday CV less than 15%. The clean up assay was based on Dorr et al [1] and the HPLC method was a modified version of Cheng et al. [5]and Santosa et al. [6]. This technique was identical to the procedure used by Mason et al[2].

Mueller Hinton broth (Sigma-Aldrich, St. Louis, MO) was made and cation adjusted to CLSI specifications [7,8] with $CaCl_2$ and $MgCl_2$ and then steam autoclaved. Two stock solutions of 250 µg/mL of tetracycline hydrochloride water medication (AmTech, IA) and tetracycline hydrochloride standard (Sigma, Thermo Fischer, Milford, MA) were made and filter sterilized (0.45 µm) under vacuum. These two standards were run on HPLC to confirm concentrations.

CLSI clinical isolate standards of *E. coli* ATCC 25922 were cultured at 37ºC on MacConkey agar. CLSI procedures [7-9] were followed for media preparation and for MIC testing. Briefly after 24h, a suspension of E. coli ATCC25922 was prepared by suspending growth in PBS to a McFarland turbidity standard of 0.5 with a nephelometer. This standard was diluted 1:100 into the above mentioned Mueller Hinton broth to give approximately 10^6 colony forming units (CFU)/mL. This suspension was used in the bacterial susceptibility testing. Two sets of serial dilutions of 10^{-2} to 10^{-6} were made in sterile glass vials for confirmation of the concentration of the standard suspension. An initial 1:100 dilution was made and serially diluted at 1:10 into vials to achieve the 10^{-2} to 10^{-6} concentration of the original standard suspension. Then 10 µL from each of the serial dilutions was plated onto two separate quadrant MacConkey agar plates, dried and then incubated at 37ºC. Plate counts were performed approximately 24 h later on quadrants containing 30-300 colonies. Sterile Costar round bottom 96 well plates with lids (Thermo Fischer Scientific, Milford, MA) were obtained for all experiments. Water samples from individual carboys collected from the study (Mason et al[2]) were thawed. A 0.01 mL aliquot of cation adjusted Mueller Hinton broth was dispensed into each well of the 96 well plates. An aliquot of 0.01 mL of each water sample and control was then dispensed into the first column of wells with an additional 0.01 mL of Mueller Hinton broth. One-hundred microliters from the first well was used to begin a serial dilution across the columns of the plates, as per the CLSI method. Plates were then covered with lids and cultured for 20-24 hours at 37ºC before being read for the lowest dilution that demonstrated no visible growth. All samples and controls were performed in duplicate. Any samples that varied more than one level of dilution were repeated.

Results. HPLC analyses of bothTcHCl standard and water medication solutions were within the expected AUC integration range. These controls exhibited the expected inhibition for the low end of the MIC range of *E. coli* ATCC 25922. HPLC analysis results (Table 1) show that the various treatment group levels from HPLC analysis were very close to the expected tetracycline concentrations. The HPLC assay showed that there is some degradation of tetracycline over time, but the grand mean for all concentrations was 84% of the expected levels based on mixing concentrations (i.e. not including the control samples). Figure 1 shows comparative chromatographs of TcHCl as it ages. The first chromatograph (A) shows the TcHCl standard, while chromatograph B represents the medicated water samples at ½ the concentration strength of chromatograph A. The final chromatograph (C) demonstrates the change in TcHCl after refrigeration for 6 months to exaggerate the changes discussed below. On the 3 chromatographs, the first peak changes markedly from chromatographs A&B to chromatograph C. These differences show that the second and tallest peak represents TcHCl while the smaller initial peak corresponds with an epimerization product. The small peak enlarges as tetracycline ages and becomes very large in the oldest tetracycline standard as seen in chromatograph C.

The MIC assay results (Table 1) show that TcHCl concentrations were within one microdilution of the expected value based on HPLC. All control samples (i.e. tetracycline stock solutions of both the water medication product and a chemical standard) inhibited bacterial growth at between 0.5 and 1 µg/mL, while bacteria grew in all of the untreated water samples. These results and those of the medicated water samples were consistent with the HPLC analysis run shortly after collection of water samples.

Discussion. The HPLC analysis shows that tetracycline, despite its change in color, was still biologically active against *E. coli* ATCC25922 at the CLSI reported susceptible concentrations. Tetracycline hydrochloride (TcHCl) inhibited bacteria at concentrations consistent with HPLC analysis despite freezing and thawing. There may be increased variability in the MIC dilution assay due to the delay in processing of samples and the thawing process. The control runs for TcHCl for both the chemical standard and water medication standard solutions were consistent across plates and days. The TcHCl standards results were consistent with the MIC values for *E. coli* ATCC25922 and thus should be able to inhibit the growth of susceptible bacteria. Table 1 shows that as tetracycline ages, there is no significant difference in the microbial inhibition for the first 24 to 48 hours. Based on this information, the stability of the drug is adequate for at least the first 24 hours after mixing in water. However, there is a slightly greater drop of the stability of the 500 ppm medicated water samples, compared to either the label dose or one-half label dose medicated solutions. This drop in stability may be consistent with the decreased buffering capacity of the water when a large amount of drug is present. It has been reported that tetracycline hydrochloride is more stable in acidic conditions than normal to alkaline pH[4]. This may be due to the ability of the increased number of hydrogen atoms to prevent epimerization of the drug.

In relation to this stability concern, it was noted on HPLC analysis that the chromatographs contained a small peak that increased in size when the samples were run 2 weeks after they had been thawed. This peak placement is consistent with peaks associated with tetracycline epimerization products called epitetracyclines [10,11]. One tetracycline called quatrimycin displays an increased UV spectral peak at approximately 270 nm to tetracycline's greater peak at 250nm [11]. The results of the chromatographic analysis correspond with this non-toxic epitetracycline peak, shown in detail on HPLC analyses by Naidong et al [10] and Stephens et al [11]. Although we did not run standards for tetracycline epimers, there are several papers that discuss the metabolism and formation of these products as well as their HPLC characteristics [3,10-12]. The epimerization product is not necessarily responsible for the color change of the medicated water but its placement is consistent with reported peaks on HPLC analysis. It is possible that a carrier or other molecule present in the TcHCl formulation is responsible for the color change.

It is well known that expired or aged tetracycline products may result in renal damage, a reversible Fanconi's syndrome of proximal tubular necrosis with proteinuria in mammals, of which anhydro-4-epitetracycline is the reported cause [13,14]. The enlarged initial chromatographic peak (Figure 1A) is not consistent with the chromatographic elution of peaks reported for epimers that result in renal damage. However, toxicology is beyond the scope of this study, but further evaluation of the safety of TcHCl should be performed in the future.

Implications. Our study showed that tetracycline water medication is effective in inhibiting the growth of susceptible bacteria, even after a mild to moderate color change. The stability of the product when used on label is within the margin of error of the assay for the first 24-48 hours after mixing for on-label preparations.

Acknowledgements. Authors would like to thank Paul Orndorff for the use of lab space; Meghan Fauls for the donation of bacterial strain *E. coli* ATCC25922 to complete this study.

The authors would also like to thank Dr. Sid Thakkur for the use of the MacFarland meter and suggestions for the MIC micro-dilution assay. This work was supported by Pfizer Animal Health.

Table 1: Comparison of HPLC Assay and MIC range for bacterial inhibition

Treatment Group (µg/mL)	*Average Concentration on HPLC (µg/mL)*	*Expected HPLC to Treatment Group (%)*	*CV[a] for HPLC Concentrations (%)*	*Water Concentration Ranges (µg/mL)[b]*
0[c]	0.00a	100	173.21	0
125	100.01	80	33.39	125-250
125	104.49	84	10.31	125
125	114.46	92	7.58	125-250
125	121.96	98	19.85	125-250
125	107.91	86	ND[d]	125-250
125	118.87	95	18.50	125-250
Average		**89±6.9**		
250	220.48	88	3.33	125-500
250	205.54	82	6.43	250-500
250	238.66	95	4.73	250-500
250	211.84	85	9.24	125-500
250	182.56	73	20.03	250-500

[a] CV is Coefficient of variation

[b]Water concentrations were back calculated from the MIC found for the bacteria based on control runs.

[c] All concentrations of tetracycline were below the limit of detection for unmedicated water samples. The rest of the results were from the 6 controls were identical for treatment concentration and Coefficient of Variation

[d] ND is not done. There was only one acceptable sample run and therefore CV was not calculated.

Table 1 Continued

Treatment Group (µg/mL)	*Average Concentration on HPLC (µg/mL)*	*Expected HPLC to Treatment Group (%)*	*CV for HPLC Concentrations (%)*	*Water Concentration Ranges (µg/mL)*
250	233.14	93	6.13	250-500
Average		**86±8.0**		
500	398.66	80	6.90	250-500
500	337.40	67	43.43	250-500
500	376.06	75	14.58	250-500
500	372.30	74	28.24	250-500
500	400.41	80	13.14	250-500
500	428.58	86	2.75	250-500
Average		**77±6.5**		
Grand Mean		**84**		

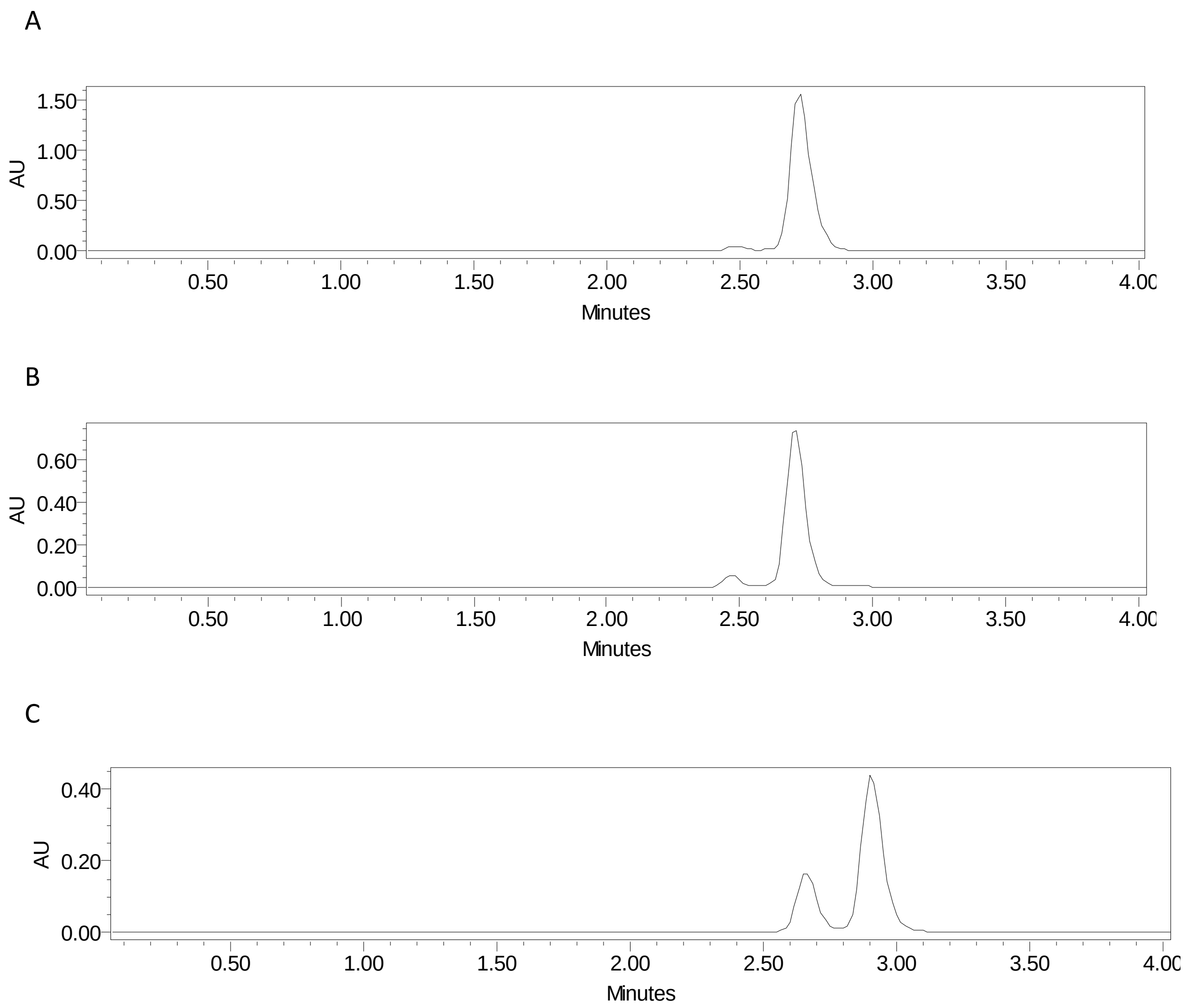


Figure 1: Chromatographs of three different aged water samples containing tetracycline. A.) Standard water sample containing 500 ppm tetracycline. B.) Water sample from farm containing 250 ppm of tetracycline. C.) A water sample containing 250 ppm of tetracycline after being stored for 6 months at 4ºC. As the tetracycline ages the first visible peak begins to enlarge in relation to the major (tetracycline) peak. This peak appears to correspond to the increase in 4-epitetracycline formation as described in the literature.

References

1. Dorr PM, Nemechek MS, Scheidt AB, Baynes RE, Gebreyes WA, Almond GW. Water-flow variation and pharmacoepidemiology of tetracycline hydrochloride administration via drinking water in swine finishing farms. J Am Vet Med Assoc 2009;235:299-304.

2. Mason SE, Baynes RE, Almond GW, Riviere JE, Scheidt AB. Pharmacology of tetracycline water medication in swine. J Anim Sci 2009.

3. Moreno-Cerezo JM, Cordoba-Diaz M, Cordoba-Diaz D, Cordoba-Borrego M. A stability study of tetracycline and tetracycline cyclodextrins in tablets using a new hplc method. J Pharm Biomed Anal 2001;26:417-426.

4. Wu Y, Fassihi R. Stability of metronidazole, tetracycline hcl and famotidine alone and in combination. Int J Pharm 2005;290:1-13.

5. Cheng Y, Phillips D, Neue U. Simple and rugged spe method for the determination of tetracycline antibiotics in serum by hplc using a volatile mobile phase. Chromatographia 1997;Volume 44:187-190.

6. Santosa MDF, Vermeerschb H, Remona JP, *et al.* Validation of a high-performance liquid chromatographic method for the determination of doxycycline in turkey plasma. J Chromatogr B, Biomed Sci Appl 1996;682:301-308.

7. Institute CLS. Performance standards for antimicrobial susceptibility testing; seventeenth informational supplement. 1 edClinical and Labortory Standards Institute, 2007.

8. Isenberg HD. Clinical microbiology procedures handbook. 2 ed. Washington, D.C: ASM press, 2004.

9. Goodwin AC, Schwalbe R, Steele-Moore L. Antimicrobial susceptibility testing protocols. Boca Raton: CRC press, 2007.

10. Naidong W, Roets E, Hoogmartens J. High performance liquid chromatography of chlortetracycline and related substances on poly(styrene-divinylbenzene)copolymer. Chromatographia 1990;30:105-109.

11. Stephens CR, Conover LH, Gordon PN, *et al.* Epitetracycline -the chemical relationship between tetracycline and "quatrimycin". Journal of the American Chemical Society 1956;78:1515-1516.

12. Fritz JW, Zuo Y. Simultaneous determination of tetracycline, oxytetracycline, and 4-epitetracycline in milk by high-performance liquid chromatography. Food Chemistry 2007;105:1297-1301.

13. Phillips ME, Eastwood JB, Curtis JR, Gower PC, De Wardener HE. Tetracycline poisoning in renal failure. Br Med J 1974;2:149-151.

14. Benitz KF, Diermeier HF. Renal toxicity of tetracycline degradation products. Proc Soc Exp Biol Med 1964;115:930-935.

Variables Important to the Population Pharmacokinetic Modeling of Tetracycline Water Medication in Swine

Sharon E. Mason, Glen W. Almond, Jim E. Riviere and Ronald E. Baynes

Submitted to the Journal of Antimicrobial Agents and Chemotherapy

ABSTRACT

Population based mixed effects pharmacokinetic modeling has not been applied to water medications despite there common use in swine productions units. We propose a non-linear mixed effects model to simulate and then predict factors that may determine plasma concentration levels of tetracycline water medication in swine. The model identified several covariates that may have an effect on the plasma concentration levels in populations of exposed animals. These covariates combined with knowledge of the pharmacokinetic and chemical properties of currently approved water medications may result in more prudent use of water medications in the future.

INTRODUCTION

Water medications have been used extensively in the livestock industries to treat populations of animals. However few pharmacokinetic studies have been conducted on water medications in general (2, 20, 21). This is likely related to concerns over determining the correct dosing levels and adequately modeling the pharmacokinetics of the drug (29). One potential technique that could provide insight and improved pharmacokinetic modeling is population based modeling using non-linear mixed effects (19). It appears that few population pharmacokinetic studies have been performed in veterinary medicine (3, 17, 18, 25, 27), and none on water medications. Only one study by del Castillo et. al.(3) looked at the population pharmacokinetics of doxycycline in a slurry (liquid) feed in swine. However, because this was slurry feed, the factors most important to plasma concentration levels in pigs from water medication treatments has not been investigated. Therefore this topic requires further investigation. Mason et al (20) determined the pharmacokinetics of three tetracycline water medication treatment levels in individually housed swine, while Dorr et al (4) investigated the pharmacoepidemiology of tetracycline water medication in an industrial setting. However, the predictability of plasma concentration levels was extremely variable and unable to be characterized by either of these methods alone.

Thus an understanding of water medication pharmacokinetics and the factors that contribute to *in vivo* plasma levels is important given the frequency and use of antibiotics in the swine industry (1). Therefore we used data collected by Mason et al (20) to develop a population pharmacokinetic model on tetracycline administered as a water medication to swine. This model was then externally validated with data independently collected by Dorr et al (4) to validate our model. This model can be used to predict plasma concentration levels in swine and more importantly has helped to identify production factors and pharmacokinetics factors that will affect plasma tetracycline levels in pigs.

MATERIALS AND METHODS

Study Design. A population pharmacokinetic program (Phoenix WinNonMix NLME, Pharsight Corp., Mountain View, CA) was used to conduct all of the population PK analyses. The model was a non-linear mixed effects model using written code (i.e. not a pre-coded model from the program database). Three data sets were input separately in individual spreadsheets as different populations of animals. The training set data were used to develop the initial model, which was written as a one compartment pharmacokinetic model. The tow other datasets, from two separate populations, were compared to the best models for analysis (150 ppm dataset) and validation (80 ppm dataset). Plasma concentrations were set as the dependent variable. The program was run using first order conditional estimate (FOCE) with the Lindstrom-Bates algorithm (11). This algorithm was used for early model selection due to the time to run the FOCE-Extended least squares algorithm (ELS) and independently compared to the FOCE-ELS models to determine the best fit (34). However, in the end, the final model predictions and selection for figures was run with the FOCE-LB due to no obvious model fit benefit from the extended least squares algorithm. A Gaussian curve to the data distributions based on the initial data set allowed for adequate model fit and required much less computational time to run.

A priori parameter estimates were used for model development (20) and to elucidate which potential covariates improved model fit (30, 35). The initial parameter estimates for the model validation datasets were initiated at the training data population average for each parameter. Covariates were included in the model based on statistical analysis (one-way ANOVA in SAS 9.1 Cary, NC) and graphical comparisons for residuals, before inclusion in the model. Some parameters, such as bioavailability, were set as fixed values because little variability existed in the initial parameter estimates and there was statistical and graphical support that they did not contribute to the model fit. By fixing this parameter, parameter degeneration was prevented for the rest of the model and a better fit was achieved for most of the data (31).

Structural model determination. Due to the type of information available from the previous study data collected on water medications and tetracycline pharmacokinetics (9, 24), both one and two compartment models were attempted for modeling the data. The three initial PK parameters included in the structural model were: volume (of the plasma compartment), absorption (bioavailability) and elimination (Kel) (16, 32).

Covariate Selection. The initial linear equation predicted by Mason et al (20) was a direct correlation between water concentration and plasma concentrations in treated pigs and was set as the default or null covariate model (which included only water concentration). Several parameters including volume of distribution at steady state, body clearance and apparent bioavailability were calculated in the study by Mason et al. The use of covariates (as surrogates) for bioavailability and clearance did not prove useful. Serum creatinine (often a covariate for clearance) was found to be non-predictive because all values for the population of animals were within 0.1 mmol/dl of each other. The urine specific gravity was also not predictive, due to highly concentrated urine in all animals. Bioavailability (F) was found to be consistent across all animals and when included in the structural model as a variable parameter. Model parameter estimates for F were very close to literature values of 0.06 or 6% (20, 24). Finally, body weight was linked to water use and volume of distribution and was therefore considered a covariate to two different factors, volume of the central compartment and tetracycline dose.

Based on both graphical and ANOVA comparisons, measured water use proved to be a useful parameter as did individual and group body weights. The water use in the training data set was high, compared to expected water use based on other investigators (7, 8). The Dorr data also fell significantly below the predicted linear relationship predicted by the null model (Figure 1). Based on this information and previous studies, water use was positively correlated with increased temperatures (23). Thus, temperature was included in the model covariate selection. Of all of variability in the proposed PK models, the most significant variable to data fit was water use rate for each animal. Therefore the various PK models included a variable drinking rate (called drate in the model code) that is the only difference represented by 5 different equations (Table 1) that included the above identified covariates and model structure.

Model Selection and Validation. These five models were run individually and compared to each other and the linear model based on graphical fit, Akaike's Information Criteria (AIC), Bayesian Information Criteria (BIC) and the loglikelihood ratios (LLV) (10, 12, 13, 15). The coefficients of variation, standard deviations of the observed values, and comparison of each model's parameter estimates to literature parameter values for animals of similar ages (9, 14, 20, 22, 24) were also taken into consideration of the model fits.

RESULTS

Initial model testing was used to select between a one and two compartment model. Although the two compartment model was able to fit the data, the coefficients of variation for all parameter estimates were high in relation to the coefficients of variation for the one-compartment model. Therefore, the two compartment model was eliminated.

The five model covariate relationships were then run under the structure of the one-compartment model. By comparing the five models using the training data, two models, Model 3 and 4, were found to fit the data poorly (Table 1). The other three models showed a similar fit with Model 1 exhibiting the lowest scores for each of the AIC, BIC and LLV tests (Table 1). The graphical fits of Models 1 and 2 appeared to mimic an asymptotic fit, while Model 5 exhibited an almost sine wave pattern, better following peak and trough levels, based on temperature variations. Using LLV, BIC and AIC, Model 5 exhibited a slightly lower fit than either Models 1 or 2. However, this model was included in subsequent comparisons due to the graphical fit and consideration of covariate selection.

The final model selection was then based on the effect of the Dorr data for 150 ppm exposure to tetracycline. This data set was run on the above three covariate models since this was an independently collected data set. Covariates are listed under each model with model-specific based parameter estimates (Tables 2 and 3). The performance of the models showed that Model 1 exhibited poor parameter estimates of drinking rate, due to high variability, and volume of distribution outside of physiologic ranges for tetracycline. The variability in estimates for Model 1 can be compared to Models 2 and 5 which also exhibit goodness of fit based on AIC, BIC and LV (Table 1).

The two more accurate models were compared using simulations compared to the Dorr 80 ppm validation dataset. Individual graphic results for the two final model predictions of Mason data are in Figure 2. The conditional weight residuals for each model (2 and 5) are shown graphically for both the 150 ppm and 80 ppm population values (Figure 3). The weighted residuals for the population etas represented an approximate Gaussian distribution for Model 5 data better than for Model 2 data (data not shown). Model 5 predictions for individual pigs from Dorr's 150 ppm dataset are in Figure 4. The predictive check data exhibits some values that fall below the expected distribution, but overall the data are encompassed by the model prediction of a 5% to 95% confidence interval (26). Finally, simulations using both models were run for the Dorr 80 ppm data to compare models (Figure 5). When the parameters are set to the model predictions from the initial training set predicted values (Table 2), the simulations exhibit a robust fit to the data for the 90% confidence interval.

DISCUSSION

Based on the tetracycline water medication study by Mason et al, there is an initial linear relationship between plasma concentration and the concentration of tetracycline in the water of pigs dosed in commercial settings (Figure 1). This relationship is predicted by the following equation:

$$\text{Plasma Concentration} = 0.0019 * \text{Water concentration} + 0.0435 \qquad (1)$$

This relationship appears to hold for the animals in the Mason study with a R^2 for approximately 0.79. The equation applied to a certain extent in the Dorr study but the average concentrations are less than those for the Mason et al data. This linear relationship does not address the variability in animals that is seen from the prospective of the population.

The major differences in animal plasma levels from the same treatment group (i.e. similar water concentration levels of tetracycline), when using the previously calculated population parameters, is not the apparent bioavailability or the values of the clearances. The obvious variability appears to arise mostly from water use in this modeling scheme. The water consumption is what determines the intake dose of the water medication, when compared to the base water concentration alone. With water medications, the typical pharmacokinetic programs cannot be easily used because they require a known dosing schedule. However, truly knowing how much an animal is dosed is not easily extrapolated (23, 33); therefore, by using an approximation based on calculated PK parameters and known water use, a theoretically determined dose of tetracycline that would be consumed can be related to a population of animals. This is what has been done with the model and the simulations with the ability to determine the factors that appear most important for the plasma levels in the study.

The model results show that in order to adequately model the dose of tetracycline with a true physiologic variability, not a random error from an average, we need to know several covariates. These covariates and fixed effects include daily water use (for each animal or the group); average body weights with a range or variance; temperature when available, and finally the anticipated or known concentration of the water medication. Based on the data and modeling, both Model 2 and Model 5 can adequately model the data. However, when more data is available, i.e. daily high and low ambient temperatures, it appears that Model 5 could potentially be more useful. Model 5 also exhibited less variability in the drinking rate than Model 2, which is most likely due to the temperature effect being removed from the primary drinking rate. This relationship is further supported by Figure 1 which shows that water concentration is not the only factor contributing to the water medication levels.

When the two models are compared, with and without the temperature variable, the validation dataset etas appear more skewed and random in Model 2 than when temperature is controlled in Model 5. Unfortunately the validation datasets did not contain temperature data and, therefore, Model 5 temperatures for validation datasets were based on a likely range. However, due to this artifact, the temperature accuracy is unknown, which prevents Model 2 and 5 from being accurately compared. This inaccuracy is due to unknown variability in temperature, which may mask its true effect on dosing in the three populations.

Unfortunately, we also do not have any information on water consumption in the barn from the comparison and validation datasets; therefore, the similarity in dosing rates between the training and validation data sets is likely due to the model converging on the initial parameter estimates. Furthermore, there is no published relationship between the temperature and water use for grower-finisher pigs in the literature. The only published relationship we found is based on the equation from Vandenheede and Nicks for 150 kg sows (23).

$$I = 0.92T - 1.52 \qquad (1),$$

where I is water intake, T is temperature and 1.52 is the y-intercept for baseline water consumption. When applied on a milligram per kilogram basis, water use is influenced by temperature. Based on the average drinking rate from our groups per day compared to the high temperature, drinking rate can be estimated by the following equation:

$$\text{Water use} = ((\text{Temperature}-20)+\text{Constant})*\text{Wt} \qquad (2),$$

where, temperature is in degrees Celcius, Wt is the body weight (kg) and Constant is a drink rate per hour within the thermoneutral zone for that age pig. This equation appears to hold pretty well for the relationship to water consumption and it is predictive in Model 5. We see similar values can be achieved for sows (150kg body weight) for temperature ranges over which the above equation is valid, however the low end of the thermoneutral zone temperature for sows would be considered 10ºC. One other major difference about these animals is that they are growing. Thus, their water use may be much higher (mg/kg) than a sow due to the fact that they have a higher concentration of body water (23).

Body weight fits into this model well because it directly affects the apparent volume of distribution and the dose (mg) of tetracycline. A large variability is present in the model volumes (of distribution) which can be assumed to be due to age variability or due to model misspecification. However, it is difficult to determine which. Data from the literature shows a wide range of volume values from less than 1 L/kg to 4.5 L/kg for tetracycline (9, 24). These differences may be affected by the plasma volume or total body water for various animals which is linked with age. In young animals, due to body water levels being very high, volumes of distribution may be higher than in older animals because as animals age, body fat percentage increases.

In addition to wide changes in Vd, total body clearance (Ke) is the other important factor to consider. This factor showed values of between 0.08 and 0.27 across the model. It is best to compare Ke across animals of the same age group, not across individual animals. The literature values reported by Kniffen et al (9) and Nielson and Gyrd (24) were based on 12 week and older aged pigs whereas in Mason et al (20) and Mevius et al (22), pigs were only 8-9 weeks old at the start of the study. Although this is only a four week difference at the start of the study, the clearance of young animals is often “impaired” up to 16 weeks of age in many of our domestic species that reach puberty at approximately 6 months of age (6). Therefore, the clearance for younger animals may in fact, as the model predicts, be much lower than for the validation set of 12 week old animals, which match the literature values of 0.16 to 0.25 ml/kg/h clearance rates.

This age related change has been documented for gentamicin in very young pigs (newborn) which have a lower renal clearance and a larger volume of distribution than 42 day old pigs (5). This phenomenom is also seen for doxycycline in which pigs at 18 kg have a substantially lower clearance (28) than pigs weighing 25 kg (3). However, for tetracycline this is not explicitly documented in the literature, but the time line of development to reach adult renal and hepatic clearance for pigs has not been fully elucidated.

This potential immaturity in body clearance is also supported by the rapid drop in percent body water from approximately 80% to 50% between 28 and 180 days of age, with a plateau between 28 and 89 days at 65% (23). This drop in percent body water also appears to correspond to an inverse rate (increase) of renal clearance over time for young animals. Therefore, the clearance values reported by Mason et al appear to be potentially correct both physiologically and by the model in light of this interpretation. Unfortunately, there does not appear to be a lot of research conducted on the clearance of juvenile pigs, which is the age-range most dosed with antibiotics.

In conclusion, our non-linear mixed effects model relates plasma tetracycline levels in three groups of pigs to tetracycline concentrations in water and their water use. It appears based on the two best models that water concentration levels of medication and water use are the two biggest factors in determining the plasma concentrations of swine exposed to tetracycline dosed in water. These factors appear to effect plasma concentrations most as clearance and bioavailability exhibit tight estimates when age is considered. These two models were able to predict plasma concentrations for approximately 90% of the population. While the ability to predict plasma levels is necessary, the fact still remains that plasma concentration levels are very low for tetracycline as compared to bacterial MIC levels (14, 20).

The two factors may also apply to all other medications administered via water. Once bioavailability is known, water concentration levels and water use are the major factors that likely contribute to plasma concentration levels. Therefore, in selecting antibiotics for use in water, ideal candidates should have high bioavailability in the treated species and be readily dissolvable in water. If these two criteria are not met, then it appears, as with tetracycline, no matter what the water use and water concentrations of the medication, plasma levels will remain low. As always, prudent discrimination in the initial antibiotic selection should be performed prior to the criteria suggested above.

Table 1: Model types and Associated Model Selection Criteria for training data (Mason et al)

No.	Model Specification[a]	LLV	AIC	BIC
1	drate = average drate * exp (ndrate)	-286	588	616
2	drate = average drate *Wt*exp(ndrate)	-284	584	612
3	drate = average drate*(Temp-20)*exp(ndrate)	-310	635	664
4	drate = average drate*Wt*(Temp-20)*exp(ndrate)	-489	993	1022
5	drate = (teffect + average drate)*Wt*exp(ndrate),	-293	602	631

[a]Model specifications and their associated selection criteria. LLV is the loglikelihood value, AIC is the Akaike's Information Criteria, BIC is the Bayesian Information Criteria. Variables in the model include drate, the average population water use rate, calculated from known drinking rates; ndrate, the interindividual variability of the calculated drate; Wt the body weight of the animal; Temp, the ambient temperature in degrees Celcius, where 20ºC represents the low end of the thermoneutral zone of early grower pigs; teffect = Temp -20 in order to allow an additive drinking rate to the model instead of a multiplicative rate.

Table 2: Parameter Estimation for Models 1, 2 and 5 with CVs for Training Data (Mason et al)

Population Parameter†	Model 1 Mean	Model 1 CV%	Model 2 Mean	Model 2 CV%	Model 5 Mean	Model 5 CV%
Volume (ml/kg)	963	18.9	792	20	2577	15.5
Kel (1/h)	0.253	18.8	0.298	19.6	0.0876	16.1
Drate (var)*	203	9.8	8.32	10.1	5.22	27.6
Concentration st dev. (μg/ml)	0.192	5.0	0.189	5.0	0.198	5.5
Water use st dev. (ml)	1512	14.0	1604	13.3	1318.42	17.4

† CV% is the coefficient of variation of the parameter estimate.

* Units for Drate are ml/h for Model 1, ml/(h*kg) for Models 2 and 5

Table 3: Parameter Estimation of Dorr Data for Models 1,2 and 5 for Second Training dataset (Dorr et al)

Population Parameter†	Model 1 150ppm	Model 2 150ppm	Model 5 150ppm
Volume (ml/kg)	160	910	973
Kel (1/h)	0.278	0.278	0.279
Drate (var)*	41.73	8.05	6.54
Concentration St dev. (μg/ml)	0.121	0.121	0.121

- Units for Drate are ml/h for Model 1, ml/(h*kg) for Models 2 and 5
- † Water use data was not included because no water use was directly measured.
-

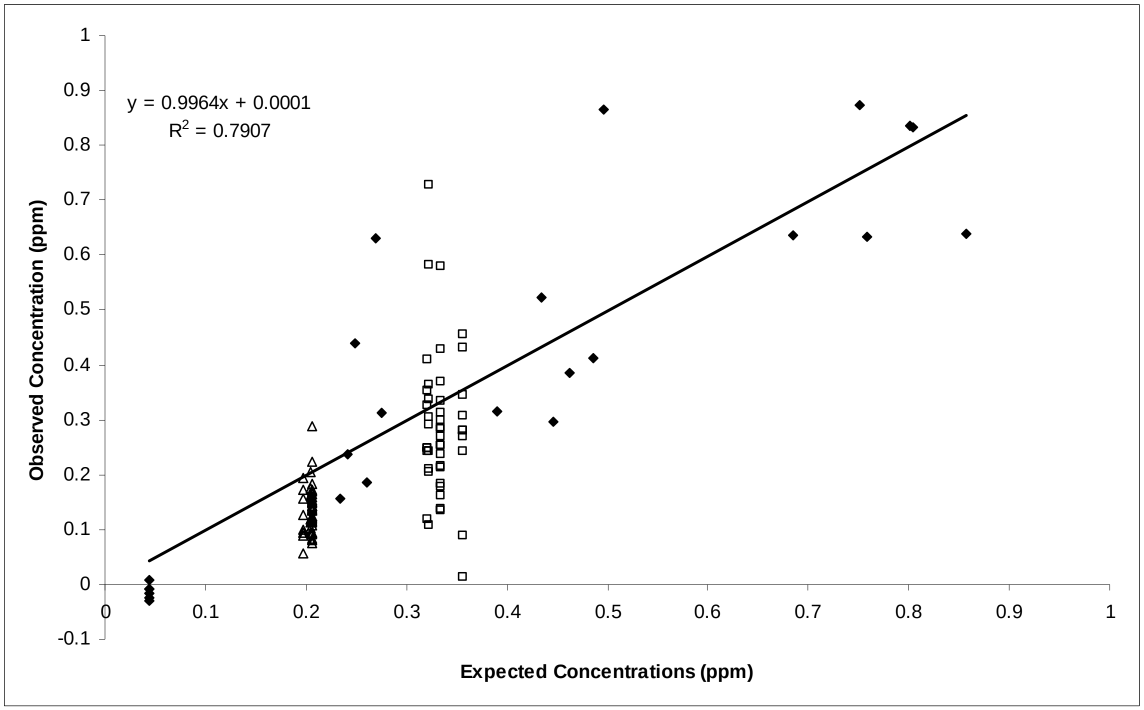
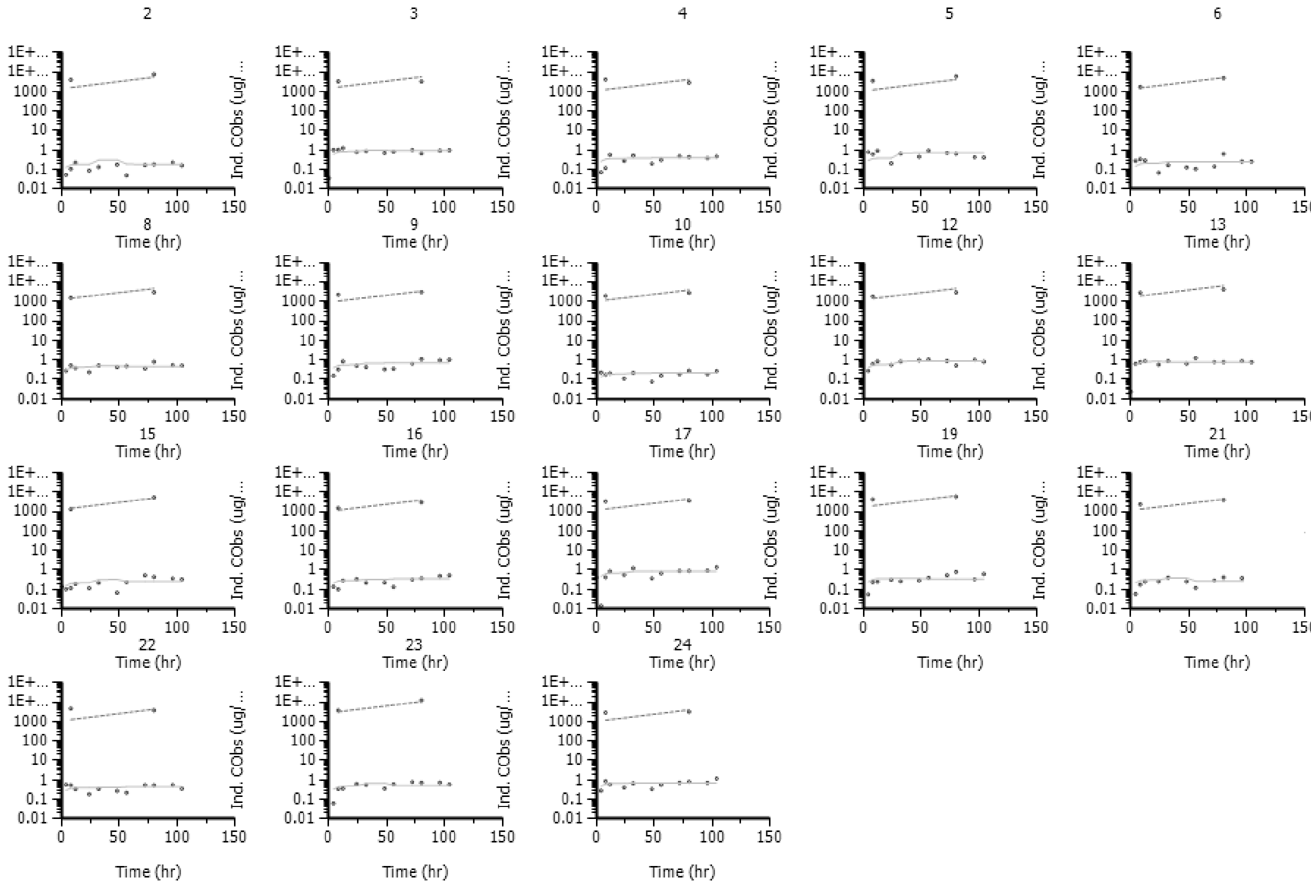


Figure 1. Relationship between water concentration and the plasma steady state concentrations of swine treated with tetracycline water medication. Diamonds represent plasma concentrations from Mason et al. Squares represent pigs exposed to approximately 150 ppm of tetracycline during the winter (Dorr et al); Triangles represent pigs exposed to 80 ppm tetracycline from an independent farm (Dorr et al). The trendline is for data from Mason et al. Notice that the data from Dorr's data is on average below the trendline for both farms. This is likely due to higher temperatures during the collection period for Mason et al.

Figure 2. Model Estimation of Mason data. The upper panel displays the model estimations for Model 2, which shows little daily variability compared to Model 5 (lower panel). This is because the water use rate varies throughout the day in Model 5 as a function of temperature, while Model 2 uses a constant daily water use rate. Circles are actual water use data (top points) and plasma concentration data (lower points); the complete line is predicted plasma concentrations; dotted line is predicted water use. Each lattice represents an individual animal.


2
3
4
5
6
8
9
10
12
13
15
16
17
19
21
22
23
24
1E+...
1000
100
10
1
0.1
0.01
0
50
100
150
Time (hr)
Ind. CObs (ug/...

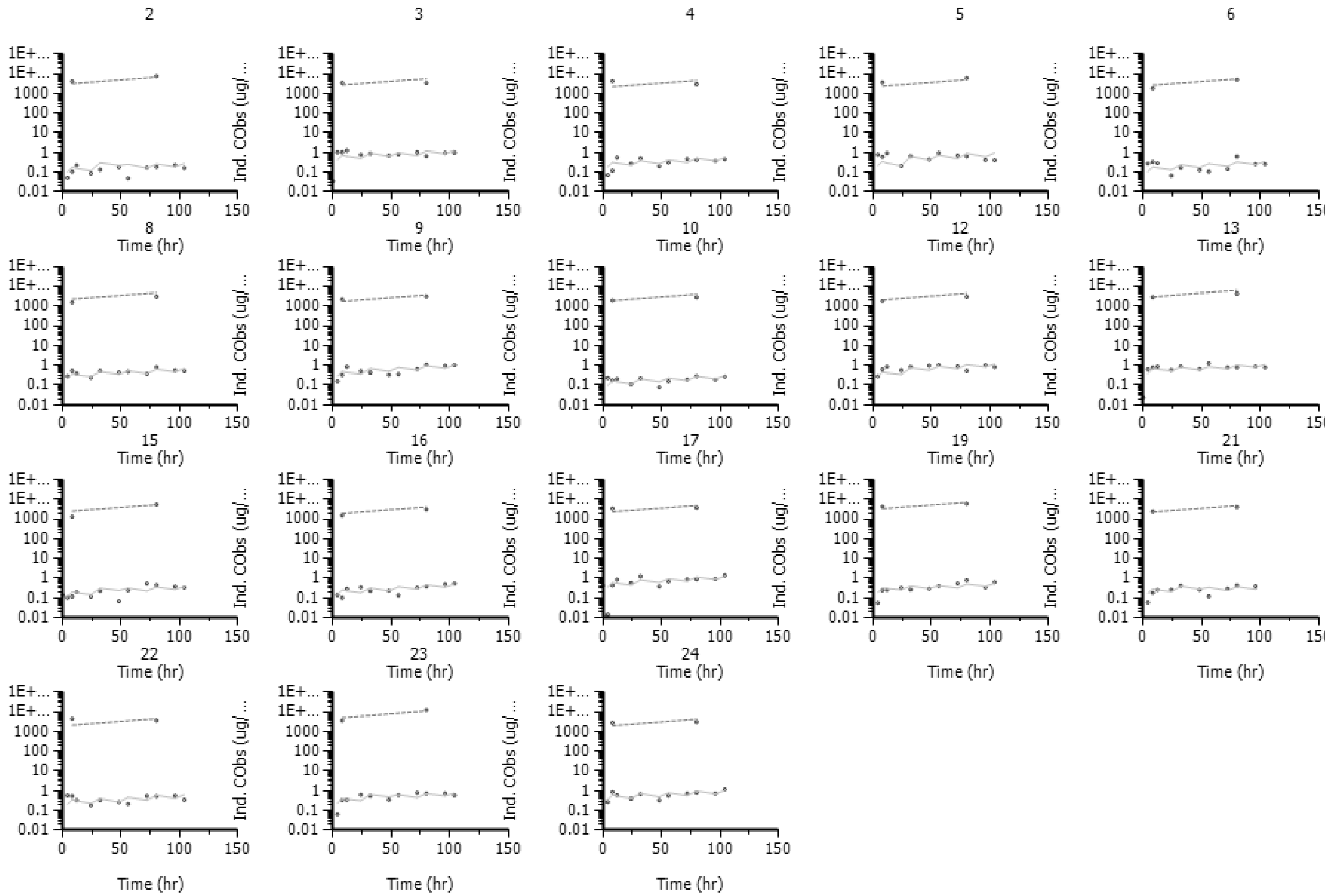
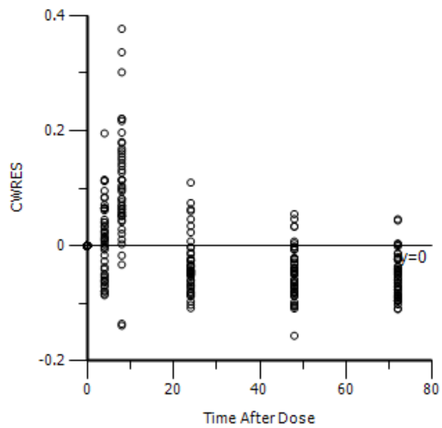

2
3
4
5
6
8
9
10
12
13
15
16
17
19
21
22
23
24
Ind. CObs (ug/...
Time (hr)

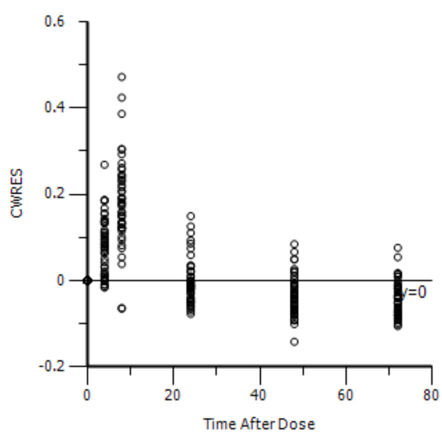
Figure 3: Conditional Weighted Residuals Comparison for Models 2 and 5. Dorr 80 ppm data is modeled by Model 2 and Model 5, respectively in upper A) and B) lower panels. Both models show a bias in the water concentration at 8 hours into treatment; however, Model 2 (A) tends to under-predict later time points, which is likely due to model misspecification. The second set of figures shows residuals for Model 2 (C) and Model 5 (D) for Dorr data at 150 ppm. Both models underestimate later time points and overestimate earlier time points. This may be due to degradation of tetracycline over time during the Dorr experiment.

A



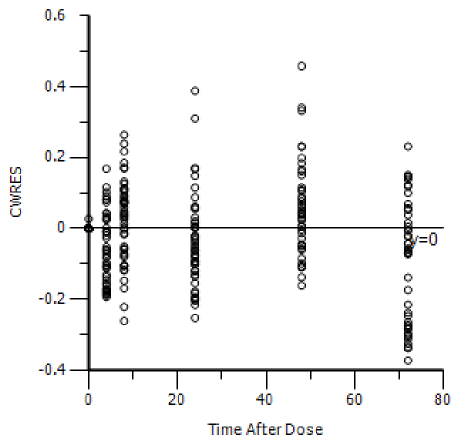
0.4
0.2
0
-0.2
CWRES
y=0
0
20
40
60
80
Time After Dose

B



0.6
0.4
0.2
0
-0.2
CWRES
y=0
0
20
40
60
80
Time After Dose

C



D

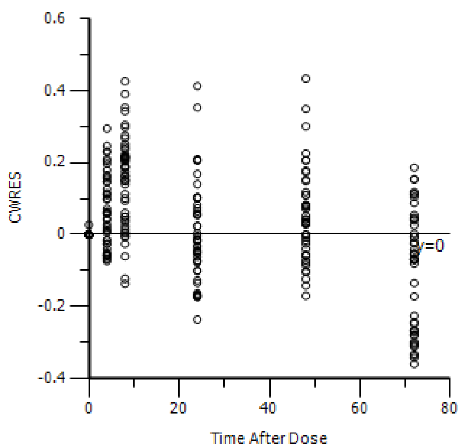
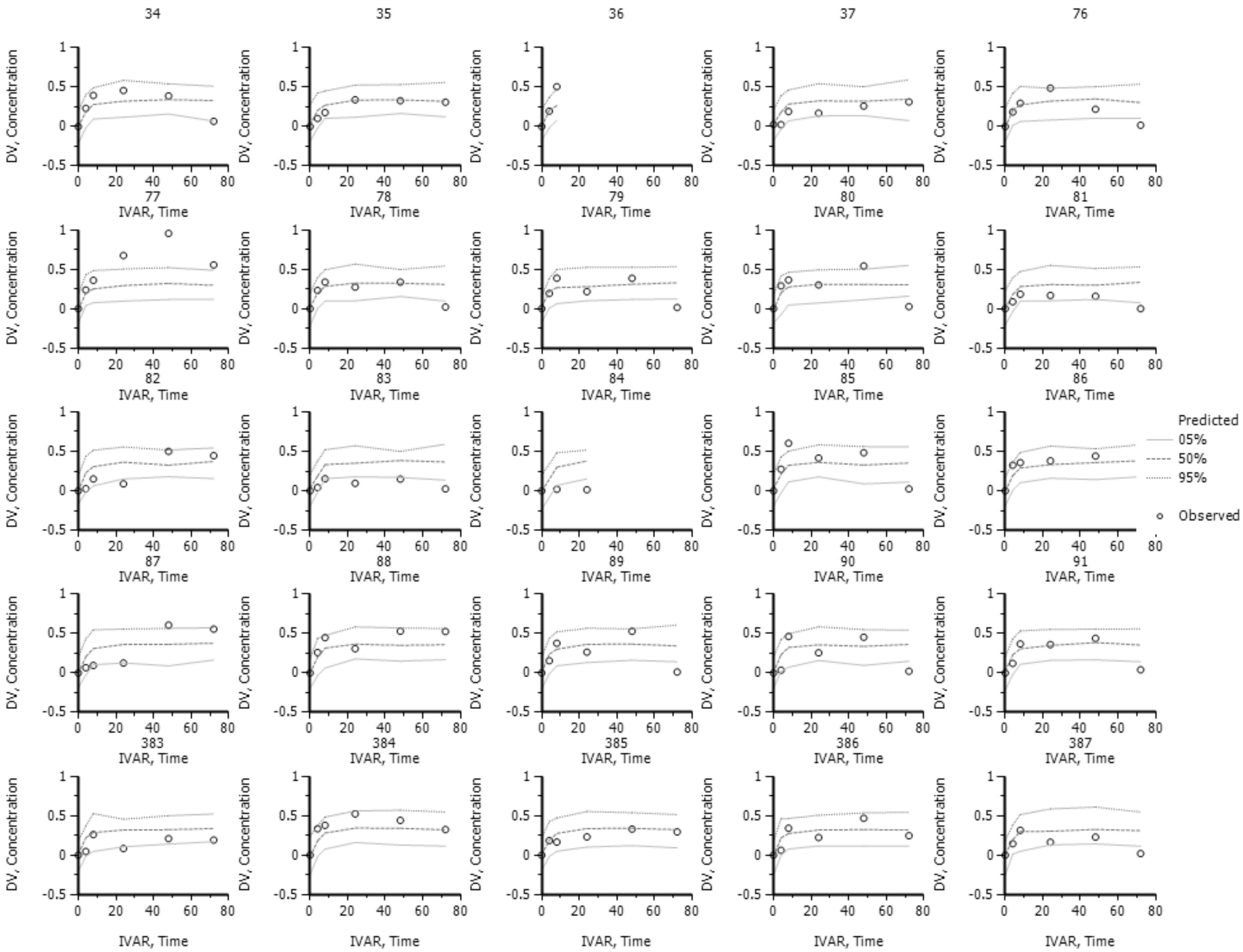


Figure 4: Model 5 Simulations of Dorr 150 ppm Data using Predictive Check Feature of PK program (n=50). It can be seen that the majority of data falls within a 90% confidence interval as predicted by the model. Circles are actual plasma concentration data; the complete lines show the predicted 5%, 50% and 95% plasma concentrations intervals by the model. Each lattice represents an individual animal.


34
35
36
37
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
383
384
385
386
387
DV, Concentration
IVAR, Time
Predicted
05%
50%
95%
Observed

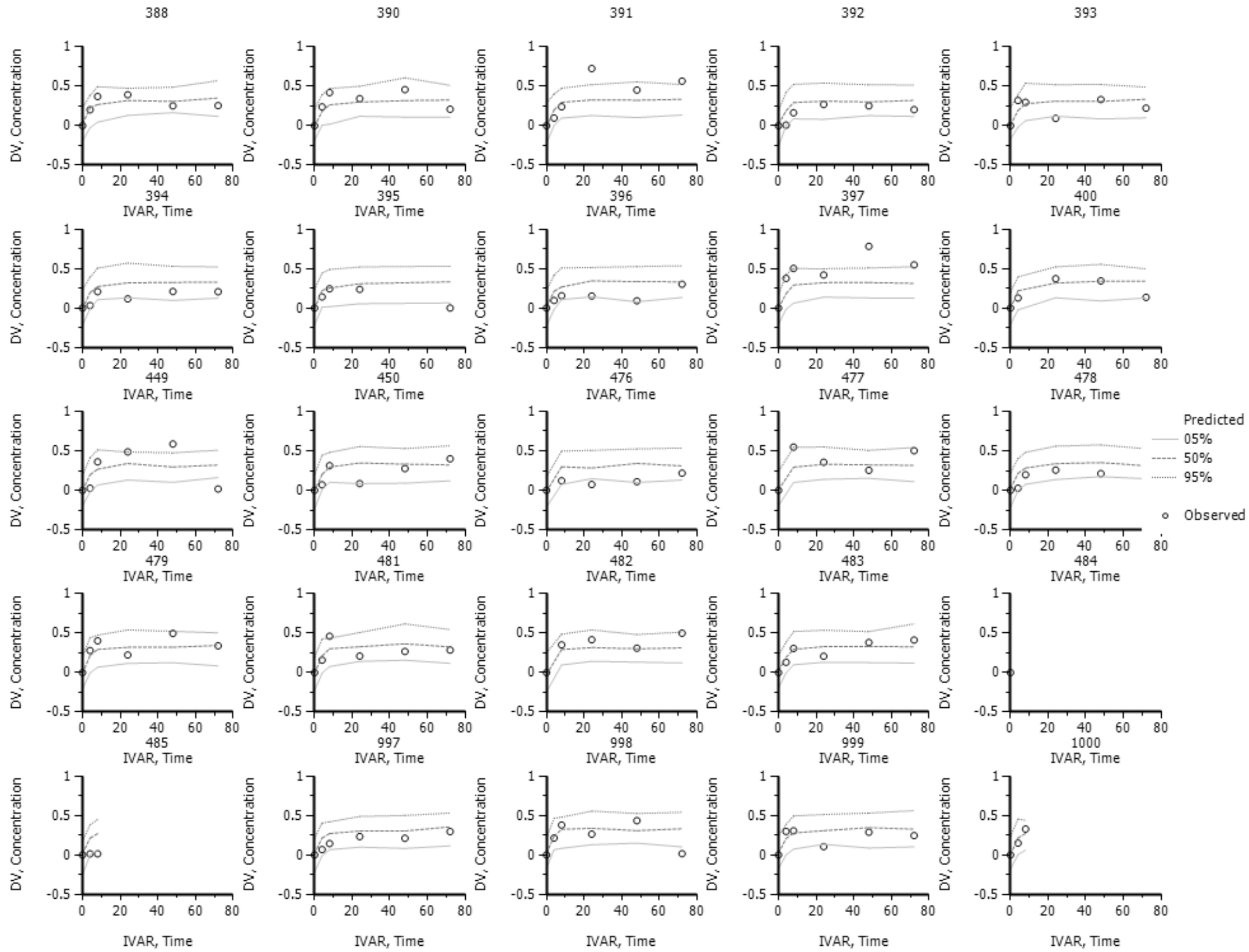
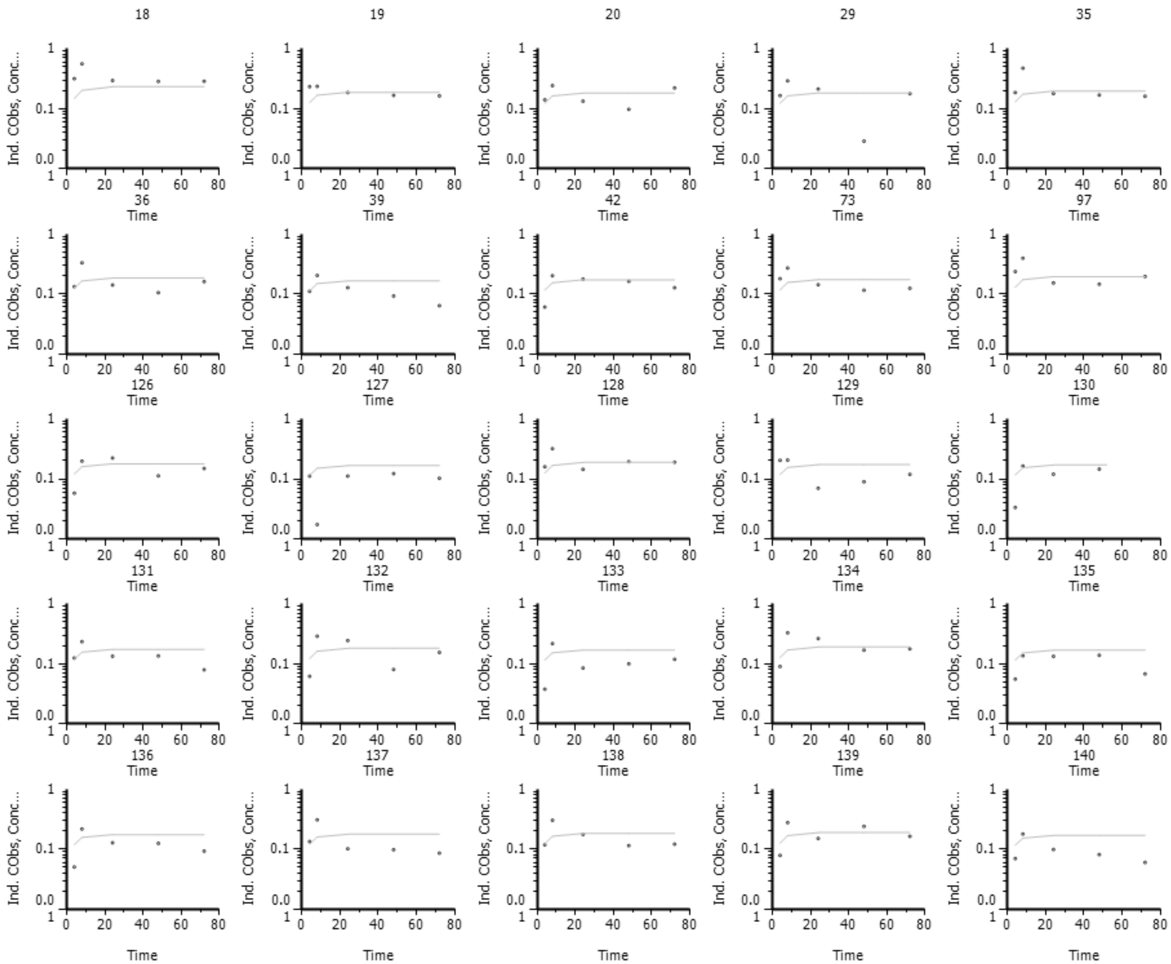
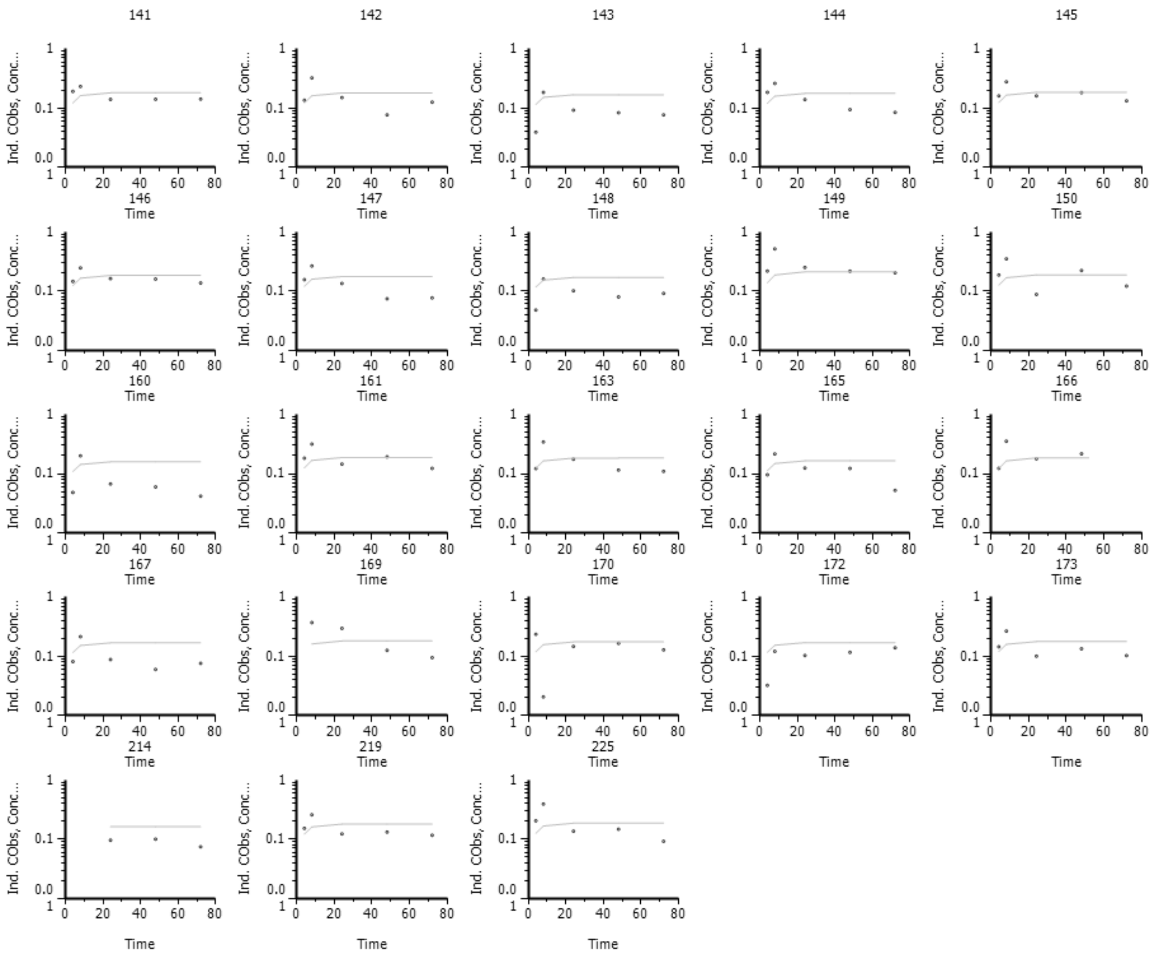

388
390
391
392
393
394
395
396
397
400
449
450
476
477
478
479
481
482
483
484
485
997
998
999
1000
DV, Concentration
IVAR, Time
Predicted
05%
50%
95%
Observed

Figure 5: Simulations of Dorr 80 ppm Data. Lattices in A and C can be compared directly for Models 5 and 2, respectively; while B and D compare the same group of pigs using Model 5 and 2, respectively. There are subtle differences between the outcomes of these two models but both models appear to simulate the data relatively well. Model 5 tends to predict slightly lower concentrations overall than model 2. Circles are actual plasma concentration data; the complete line is predicted plasma concentrations by the model. Each lattice represents an individual animal.

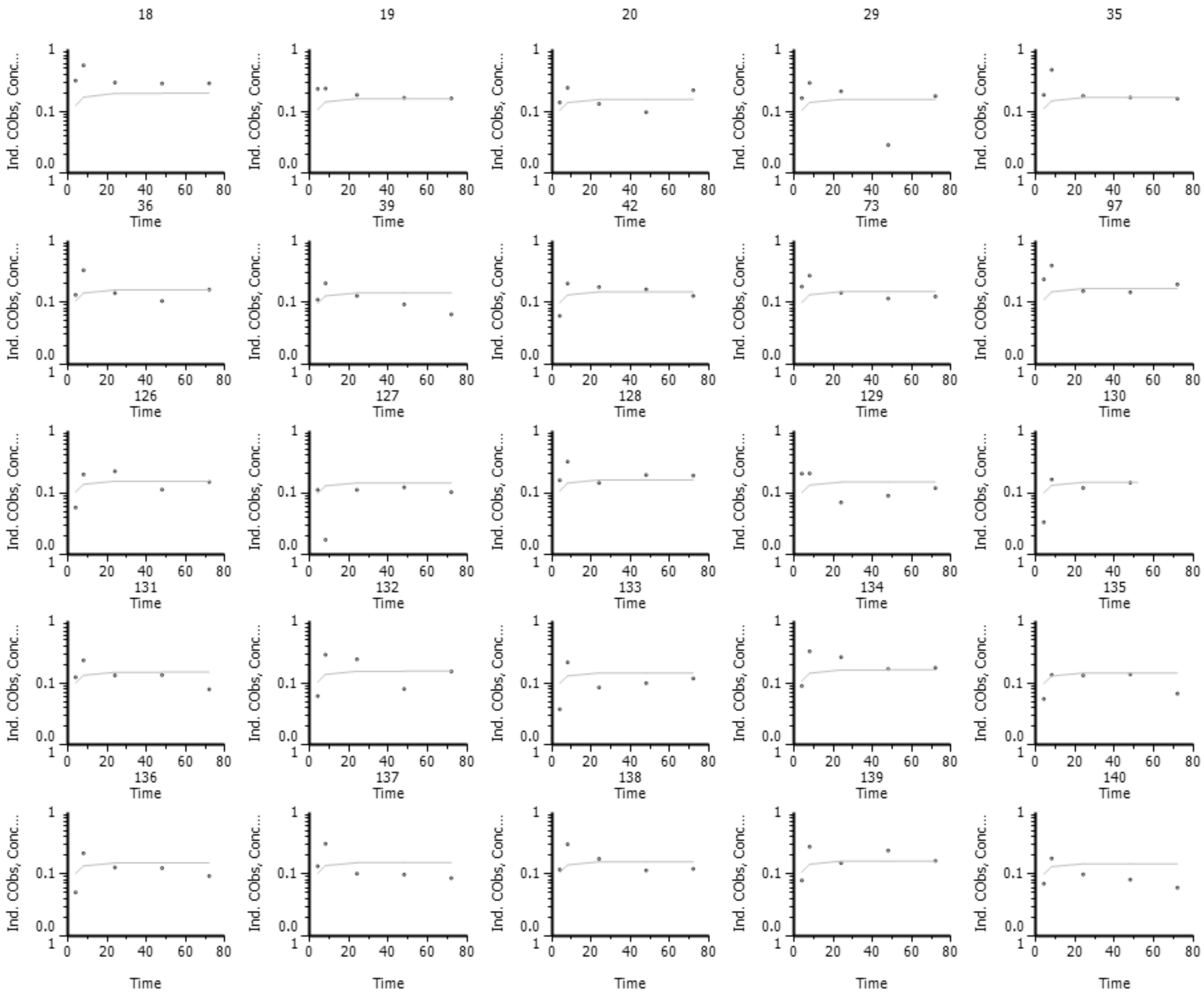
A


18
19
20
29
35
36
39
42
73
97
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
Ind. CObs, Conc...
Time

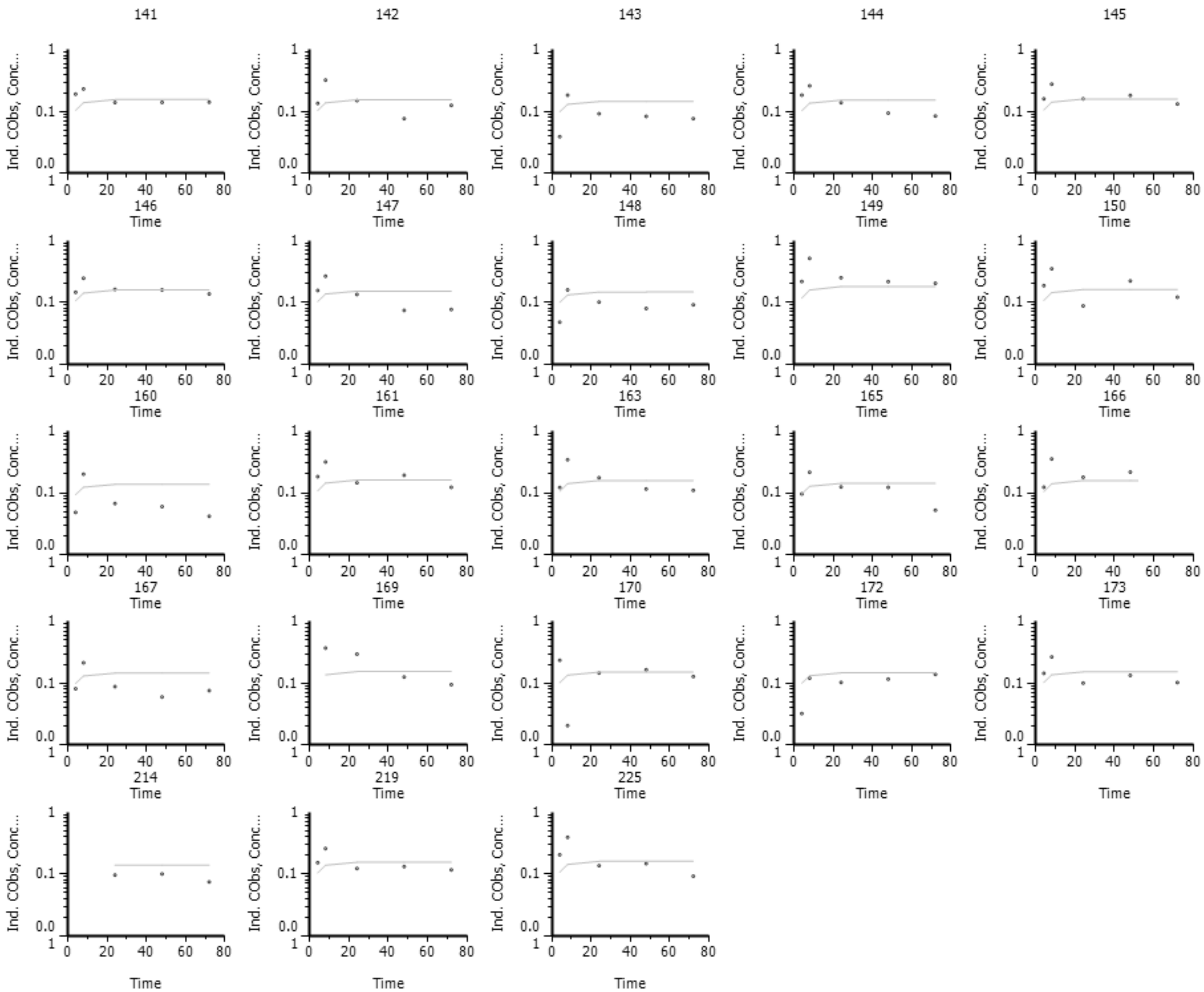
B


141
142
143
144
145
146
147
148
149
150
160
161
163
165
166
167
169
170
172
173
214
219
225
Ind. CObs, Conc...
Time

C


18
19
20
29
35
36
39
42
73
97
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
Ind. CObs, Conc...
Time
1
0.1
0.0
1
0
20
40
60
80

D


141
142
143
144
145
146
147
148
149
150
160
161
163
165
166
167
169
170
172
173
214
219
225
Ind. CObs, Conc...
Time

References

1. **Aarestrup, F. M.** 2005. Veterinary drug usage and antimicrobial resistance in bacteria of animal origin. Basic Clin. Pharmacol. Toxicol. **96:**271-81.

2. **Agerso, H., C. Friis, and J. Haugegaard.** 1998. Water medication of a swine herd with amoxycillin. J Vet Pharmacol Ther **21:**199-202.

3. **del Castillo, J. R., V. Laroute, P. Pommier, C. Zemirline, A. Keita, D. Concordet, and P. L. Toutain.** 2006. Interindividual variability in plasma concentrations after systemic exposure of swine to dietary doxycycline supplied with and without paracetamol: a population pharmacokinetic approach. J Anim Sci **84:**3155-66.

4. **Dorr, P. M., M. S. Nemechek, A. B. Scheidt, R. E. Baynes, W. A. Gebreyes, and G. W. Almond.** 2009. Water-flow variation and pharmacoepidemiology of tetracycline hydrochloride administration via drinking water in swine finishing farms. J Am Vet Med Assoc **235:**299-304.

5. **Giroux, D., G. Sirois, and G. P. Martineau.** 1995. Gentamicin pharmacokinetics in newborn and 42-day-old male piglets. J Vet Pharmacol Ther **18:**407-12.

6. **Hardy, R. M., and C. A. Osborne.** 1979. Water deprivation test in the dog: maximal normal values. J Am Vet Med Assoc **174:**479-83.

7. **Harvey, R. E.** 1998. Observation of the drinking habits of weaned growing pigs in relation to the rationale of water medication. The Pig Journal **42:**137-140.

8. **Harvey, R. E.** 1994. Water consumption in pigs. The Pig Journal **32:**95-98.

9. **Kniffen, T. S., D. P. Bane, W. F. Hall, G. D. Koritz, and R. F. Bevill.** 1989. Bioavailability, pharmacokinetics, and plasma concentration of tetracycline hydrochloride fed to swine. Am J Vet Res **50:**518-21.

10. **Lee, H., and S. K. Ghosh.** 2009. Performance of Information Criteria for Spatial Models. J Stat Comput Simul **79:**93-106.

11. **Lindstrom, M. L., and D. M. Bates.** 1990. Nonlinear mixed effects models for repeated measures data. Biometrics **46:**673-87.

12. **Link, W. A., and R. J. Barker.** 2006. Model weights and the foundations of multimodel inference. Ecology **87:**2626-35.

13. **Ludden, T. M., S. L. Beal, and L. B. Sheiner.** 1994. Comparison of the Akaike Information Criterion, the Schwarz criterion and the F test as guides to model selection. J Pharmacokinet Biopharm **22:**431-45.

14. **Luthman, J., S. O. Jacobsson, B. Bengtsson, and C. Korpe.** 1989. Studies on the bioavailability of tetracycline chloride after oral administration to calves and pigs. Zentralbl Veterinarmed A **36:**261-8.

15. **Markon, K. E., and R. F. Krueger.** 2004. An empirical comparison of information-theoretic selection criteria for multivariate behavior genetic models. Behav Genet **34:**593-610.

16. **Martinez, M. N.** 1998. Use of pharmacokinetics in veterinary medicine. Article. II: Volume, clearance, and half-life. J Am Vet Med Assoc **213:**1122-7.

17. **Martin-Jimenez, T., and J. E. Riviere.** 2001. Mixed effects modeling of the disposition of gentamicin across domestic animal species. J Vet Pharmacol Ther **24:**321-32.

18. **Martin-Jimenez, T., and J. E. Riviere.** 2002. Mixed-effects modeling of the interspecies pharmacokinetic scaling of oxytetracycline. J Pharm Sci **91:**331-41.

19. **Martin-Jimenez, T., and J. E. Riviere.** 1998. Population pharmacokinetics in veterinary medicine: potential use for therapeutic drug monitoring and prediction of tissue residues. J Vet Pharmacol Ther **21:**167-89.

20. **Mason, S. E., R. E. Baynes, G. W. Almond, J. E. Riviere, and A. B. Scheidt.** 2009. Pharmacology of tetracycline water medication in swine. J Anim Sci.

21. **Mason, S. E., R. E. Baynes, J. L. Buur, J. E. Riviere, and G. W. Almond.** 2008. Sulfamethazine water medication pharmacokinetics and contamination in a commercial pig production unit. J. Food Prot. **71:**584-9.

22. **Mevius, D. J., L. Vellenga, H. J. Breukink, J. F. Nouws, T. B. Vree, and F. Driessens.** 1986. Pharmacokinetics and renal clearance of oxytetracycline in piglets following intravenous and oral administration. Vet Q **8:**274-84.

23. **Mroz, Z., A. W. Jongbloed, N. P. Lenis, and K. Vreman.** 1995. Water in pig nutrition: physiology, allowances and environmental implications. Nutr Res Rev **8:**137-64.

24. **Nielsen, P., and N. Gyrd-Hansen.** 1996. Bioavailability of oxytetracycline, tetracycline and chlortetracycline after oral administration to fed and fasted pigs. J Vet Pharmacol Ther **19:**305-11.

25. **Peyrou, M., M. Y. Doucet, A. Vrins, D. Concordet, M. Schneider, and A. Bousquet-Melou.** 2004. Population pharmacokinetics of marbofloxacin in horses: preliminary analysis. J Vet Pharmacol Ther **27:**283-8.

26. **Post, T. M., J. I. Freijer, B. A. Ploeger, and M. Danhof.** 2008. Extensions to the visual predictive check to facilitate model performance evaluation. J Pharmacokinet Pharmacodyn **35:**185-202.

27. **Regnier, A., D. Concordet, M. Schneider, B. Boisrame, and P. L. Toutain.** 2003. Population pharmacokinetics of marbofloxacin in aqueous humor after intravenous administration in dogs. Am J Vet Res **64:**889-93.

28. **Riond, J. L., and J. E. Riviere.** 1990. Pharmacokinetics and metabolic inertness of doxycycline in young pigs. Am J Vet Res **51:**1271-5.

29. **Riviere, J. E.** 1997. Basic principles and techniques of pharmacokinetic modeling. J Zoo Wildl Med **28:**3-19.

30. **Tam, V. H., S. L. Preston, and G. L. Drusano.** 2003. Comparative pharmacokinetic analysis by standard two-stage method versus nonparametric population modeling. Pharmacotherapy **23:**1545-9.

31. **Vonesh, E. F., V. M. Chinchilli, and K. Pu.** 1996. Goodness-of-fit in generalized nonlinear mixed-effects models. Biometrics **52:**572-87.

32. **Williams, P. L.** 1990. Structural identifiability of pharmacokinetic models--compartments and experimental design. J Vet Pharmacol Ther **13:**121-31.

33. **Xin, H., and J. A. DeShazer.** 1991. Swine Response to constant and modified diurnal cyclic temperatures. American Society of Agricultural Engineers **34:**2533-2540.

34. **Yamaoka, K., H. Tanaka, K. Okumura, M. Yasuhara, and R. Hori.** 1986. An analysis program MULTI(ELS) based on extended nonlinear least squares method for microcomputers. J Pharmacobiodyn **9:**161-73.

35. **Yeap, B. Y., and M. Davidian.** 2001. Robust two-stage estimation in hierarchical nonlinear models. Biometrics **57:**266-72.

Summary of Results and Future Applications

Sharon E. Mason

For more than 40 years, water medications have been approved to control and treat disease in swine. Over the last two decades their use has increased and they have become a mainstay in the United States and in Europe for the treatment, control and prevention of bacterial diseases. However, during that same time period, few studies have looked at the pharmacokinetic disposition of these medications in swine when dosed via water. Due to the nature of the treatment regimens necessary to administer water medications, traditional pharmacokinetic techniques that can and have been used for individual animals are inadequate to address the dosing of a population. Furthermore, due to the lack of individual dosing and follow up, greater understanding of the consequences on the population as a whole are necessary for the best possible treatment in these herd situations.

In treating any animal with water medication (or other “at-will” dosing regimens), there is a need to know the plasma or tissue concentrations of the antimicrobials or other medications being used in the population. The largest challenge to this understanding is a dosing regimen or determining an amount of drug to which these animals are exposed. Sometimes, in traditional pharmacokinetic studies, this can be circumvented by using AUC ratios. Further research using other non-traditional pharmacokinetic techniques including physiologic based pharmacokinetic modeling (PBPK) and population pharmacokinetic modeling enables understanding of the mechanisms or factors that affect the dose and subsequent *in vivo* levels of water medications used in food animals. Using these pharmacokinetic modeling techniques in conjunction with assays that determine chemical properties of drugs are necessary to adequately model water medications and to explain the final pharmacokinetic behaviors of the drugs when used in a clinical or farm setting.

Two prominent medications dosed via water in food animals, namely swine, are sulfamethazine and tetracycline. There have been various studies on sulfamethazine in pigs that have described pharmacokinetic profiles with emphasis on its high bioavailability and metabolism. However, more papers have documented contamination of sulfamethazine within farms and abattoirs. In the early to mid-2000s, sulfamethazine had the highest number of residue violations in swine in the United States. Research by Buur in conjunction with FARAD used a PBPK model to show that the approved US label withdrawal time should be extended to prevent future tissue residues. In industry, for the last decade, there has been an industry wide increase to an 80-day withdrawal time of sulfonamide medications in response to residue violations, contamination concerns and exportation restrictions. With this extended withdrawal time, a pig must be younger than 3 months of age when treated with sulfamethazine to still meet the extended 80 day withdrawal time.

The initial study was conducted at the Teaching Animal Unit at North Carolina State University in nursery age pigs to determine the pharmacokinetic parameters in animals this young when orally dosed with sulfamethazine at product approved levels. Eight pigs aged 4 to 5 weeks were treated via oral gavage with an undiluted daily dose of sulfamethazine. Pharmacokinetic parameters for young animals were not directly compared to adult values for this medication, but contamination was seen in the control animals (6 pigs) who received no sulfamethazine in food or water. This study demonstrated contamination in a swine barn with oral sulfamethazine treatment of a water medication formulation. Secondly, the above mentioned PBPK model was employed to calculate a dose that would mimic the plasma profiles of the control pigs exposed to sulfamethazine via contamination. This PBPK model was validated by the oral data collected on the treated pigs and then showed that a validated PBPK model can be used to calculate a dose of water medication when the plasma profile is known.

The second drug that is used regularly in commercial swine production units is tetracycline. In the case of this drug, much less research has been performed in swine and none on water medication pharmacokinetics at the youngest age at which pigs are exposed. The study design was a two-stage model with rich sampling to determine the common pharmacokinetic parameters in these animals exposed to tetracycline at three different water concentration levels. Twenty-four 8 to 9 week old barrows were housed individually with separate carboy drinking systems. The animals all received tetracycline exclusively in the water and had never been exposed to tetracycline prior to the study. The results showed that bioavailability was extremely low in these pigs which matched reports in previous studies. The pharmacokinetic parameters appeared to be slightly different from those reported for older animals (22-33 kg), with clearance being approximately half that experienced in pigs 12 weeks and older. The plasma concentrations were similar to those observed in other animal studies. Non-compartmental modeling was employed in this situation and it revealed that the pharmacokinetics were slightly different under a repeated dosing regimen of water medications than typical dosing. This study confirmed observations that volume of distribution often decreases in animals as they age while clearance increases during maturation. The study also helped to show that a dosing rate could not be easily determined from traditional modeling techniques and therefore other pharmacokinetic techniques are necessary to explore water medications further. Namely, there remained a need to determine covariates and other factors that affect the plasma concentrations of tetracycline dosed via water.

During the course of this study, it was noticed that the treated water changed color over time, with the 250 ppm dose changing first, followed by the 500 ppm dose and finally by the 125 ppm dose. The control water did not experience this color change. There remained the question of whether the tetracycline was degrading in the water with this color change and if the medication would be effective against susceptible bacteria. These observations led to the following study that determined the antimicrobial bioactivity of the tetracycline. Initially, all water samples were assayed by HPLC and quantified with ultraviolet spectrum for tetracycline. A couple of control tetracycline compounds were also stored for 6 months and then rerun to determine the tetracycline peak concentrations to document peak changes over time. Samples were compared for bioactivity and HPLC assay quantification to see if they remained effective. Based on HPLC assay, the amount of tetracycline was approximately 77 to 85% of the initial concentration depending on the mixed concentration, while the bacterial assay determined that they were within the expected micro-dilution range for the compound. Therefore, tetracycline, although unstable, was still effective at close to the expected concentrations and would be expected to treat susceptible bacterial infections, if appropriate levels were acheived in vivo.

The final modeling study used data from the study on tetracycline and data from an independently collected study of three distinct populations to develop a model for plasma concentrations of tetracycline in commercial swine herds exposed to tetracycline in water. The model used the two-stage data to determine which covariates would contribute to the model. Based on the measured data, covariates that were found to be useful were body weight and water drinking rate. The measured effects that contributed to the model were temperature and water concentration of tetracycline. The only fixed effect not directly measured but determined *a priori*, was bioavailability. Based on the model selection process using both Akaike's Information Criteria and Schwarz Criteria (Bayesian Information Criteria) there were two models that could predict concentrations based on the circumstances. When temperatures were constant or close to the thermo-neutral zone, temperature was not important to the model and therefore, the use of drinking rate as a function of body weight was only necessary. During ambient known temperature fluctuations, values were better predicted using the temperature based model. Overall, population pharmacokinetic modeling was useful in determining factors both farm- and situation-dependent and specific to the pigs to better predict steady-state plasma tetracycline concentrations compared to a general linear model. More importantly, this model helped to elucidate the factors for selection of appropriate medication candidates for dosing in water over other available medications.

In order to validate the model for other water medications, it would be necessary to take a subset of commonly used drugs in swine production and test the above-mentioned model using known PK parameters and using sparse sampling designs to see if the model can be validated for drugs other than tetracycline. If the model were useful for several water medications, swine production could be positively affected and candidate drugs better selected than in the past.

The data from these studies show that various techniques are available and can be used to model drugs for which there is no known specific dose delivered. This is often the case in barn treatments of water medication or in medicating wild animals or pets such as psittacine birds. However, these modeling techniques could be used in phase III clinical trials, where patients take oral chemotherapeutics at home. Instead of treating a group as an intention to treat for PK analysis, the dose could actually be estimated and compared to the rest of the population to better characterize drugs and their efficacy.

Aftermarket surveillance of drugs by FDA is also a major undertaking. The use of population based techniques could predict doses and side-effects better on a per body weight basis than currently used techniques. Determining if doses are inappropriate for patients based on clinical presentations (which may not be overtly visible with pharmaco-epidemiological studies) may be one benefit. This surveillance could be used to educate health care professionals on at-risk populations who need dosing adjustments, based on simulations and population based modeling.

Finally toxicologists are often faced with situations in which there is doubt or no known dose for an exposure. With either PBPK or population based modeling techniques, the use of validated models in humans already exposed or exposed at variable levels, a dose might be determined when other factors are known, or when plasma concentrations in exposed individuals can be simulated with PBPK or population models. Population pharmacokinetic modeling techniques have also been shown to provide useful information not just from the clinical perspective that has been used with human population modeling, but in determining other fixed factors that are not patient-related, but nevertheless can contribute to pharmacokinetic outcomes.

In conclusion, the exploration of modeling techniques in unknown dosing situations is an area of toxicology and pharmacology that heretofore was either not addressed or involved risk factor terms, with dubious merit. In the future, the use of these modeling techniques may continue to provide potentially improved approaches to unknown exposure risks, both intentionally and unintentionally.

APPENDICES

Supplemental Data Published in the Journal of Animal Science
"Pharmacology of tetracycline water medication in swine"

Table 1: Individual pig data divided into treatment groups

Parameter/ Pig number	Animal ID ear notch	Hematocrit Day 1, %	Urine Specific Gravity[a]	Day 1 Serum Creatinine, mg/dL[b]	Day 2 Water Use, mL	Weight Gain, lb	Urine Creatinine, mg/dL
pig 1	11210	39	ND[c]	1.0	3380	10.0	ND
pig 2	11304	40	1.028	0.8	4000	14.9	116.6
pig 3	12208	42	ND	1.0	3320	12.6	ND
pig 4	12008	44	1.023	0.9	4160	11.7	147.8
pig 5	11613	38	1.030	0.7	3550	13.4	140.6
pig 6	12007	46	1.029	0.8	1750	13.4	93.7
pig 7	11605	34	1.030	0.7	3140	15.3	141.8
pig 8	12310	31	1.037	0.7	1540	9.3	197.8
pig 9	12111	36	ND	0.8	2240	9.7	198.7
pig 10	12005	37	1.033	0.7	1960	12.7	137.1
pig 11	11803	34	1.019	0.9	4160	12.9	89.8
pig 12	12311	37	1.031	0.8	1820	14.9	168.1
pig 13	11309	37	1.006	0.9	2850	15.2	65.6
pig 14	11709	33	1.026	1.0	4480	14.6	114
pig 15	11307	40	1.028	0.9	1320	14.2	Err[d]
pig 16	11102	35	1.026	0.8	1500	14.3	200.1
pig 17	11910	42	1.026	1.0	3340	11.6	194.2
pig 18	11207	30	1.029	1.0	2650	13.0	185.6
pig 19	12012	38	1.031	0.8	4180	12.0	219.3
pig 20	11410	37	1.030	0.8	5720	16.5	ND
pig 21	11105	37	1.030	0.9	2340	12.5	207
pig 22	11408	38	ND	0.8	4580	14.5	ND
pig 23	11907	37	1.010	0.9	3700	15.9	71.2
pig 24	11802	35	1.026	0.8	2840	16.0	Err

[a] A few animals had repeated water specific gravity measures, but none were more than 0.006 different from each other in the same animal
[b] Serum creatinine levels were performed on the last day of the study, but no changes were seen with these values
[c] ND no urine was collected on these animals on any day
[d] Err was reported by Antech lab when tried to run test, some urine samples had a very small volume

Code for Model for “Variables Important for Modeling Water Medications”

```
test(){
#Structural Model
        deriv(A1 = drate*Wconc*fa - Ke * A1)       # 1 compartment model
        deriv(W = drate)                           #mL/hr water use rate
        C = A1 / V              # ug/mL units
        error(CEps = 1)
        error(WEps=1)
        observe(CObs = C + CEps)                   # observed Conc read in
        observe(Water = W + WEps)                  # observed water use read in
#Statistical model parameter definitions
        stparm(V = (tvV*Wt)*exp(nV) )
        stparm(Ke = (tvKe)*exp(nKe))
        stparm(teffect = Wt*(Temp-20))
        stparm(fa = 0.06)
        stparm(drate = (teffect+tvdrate*Wt)*exp(ndrate))    # Model 5
        stparm(drate = (tvdrate*Wt)*exp(ndrate))            # Model 2
#Covariates
        covariate(Wconc)
        covariate(Wt)
        covariate(Temp)
#Error Structures
        fixef(tvV = c(0, 2600, ))
        fixef(tvKe = c(, 0.084, ))
        fixef(tvdrate = c(0, 3.856,))
        ranef(diag(ndrate,nV,nKe) = c(1,1,1))      # model error structure
        sequence{W=0;sleep(56);W=0}        # allows correct water use sampling
}
```

Additional features of the validation models to account for unmeasured variables

```
stparm(Wt = (tvWt))
stparm (Temp=(tvTemp))
fixef(tvWt = c( 25, 30, 35))
fixef(tvTemp = c(20, 22,24))
```