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Population pharmacokinetics in veterinary medicine: Potential use for therapeutic drug monitoring and prediction of tissue residues

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Population pharmacokinetics can be defined as a study of the basic features of drug disposition in a population, accounting for the influence of diverse pathophysiological factors on pharmacokinetics, and explicitly estimating the magnitude of the interindividual and intraindividual variability. It is used to identify subpopulations of individuals that may present with differences in drug kinetics or in kinetic/dynamic responses. Rooted in procedures used in engineering systems, population pharmacokinetics methods were conceived as a means to determine the pharmacokinetic profile in populations in which a sparse number of samples were obtained per individual, such as those in late stage human clinical trials. This is the situation commonly encountered in all aspects of veterinary medicine. The exploratory nature of this technique allows one to probe relationships between clinical factors (such as age, gender, renal function, etc.) and drug disposition and/or effect. Similarly, the utilization of these techniques in the clinical research phases of drug development optimize the determination of efficacy and safety of drugs. Given the observational nature of most studies published so far, statistical methods to validate the population models are necessary. Simulation studies may be conducted to explore data collection designs that maximize information yield with a minimum expenditure of resources. The breadth of this approach has allowed population studies to be more commonly employed in all areas of drug therapy and clinical research. Finally, in veterinary medicine, there is an additional field in which population studies are potentially ideally suited: the application of this methodology to the study of tissue drug depletion and drug residues in production animals, and the establishment of withdrawal times tailored to the clinical or production conditions of populations or individuals. Such application would provide a major step toward assuring a safe food supply under a wide variety of dose and off-label clinical uses. Population pharmacokinetics is an ideal method for generating data in support of the implementation of flexible labelling policies and extralabel drug use recently approved under AMDUCA (Animal Medicinal Drug Use Clarification Act. 21 CFR Part 530).

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INTRODUCTION

Basic concepts in mixed effects population models

To understand the basis of population kinetics, one need only realize the common thread that runs through most scientific efforts: a desire to generate overall population predictions based on sampling a limited amount of individuals. For illustrative purposes, consider a study in which the relationship between the birthweight of a sample of piglets of the same breed and the increase in weight during the first 20 days of life is investigated. In this study, piglets are selected at random, each one from a different litter, and the data are analysed *via* a simple linear regression. If birthweight is normally distributed, the data can be

analysed using parametric procedures. The resulting model might be expressed algebraically as follows:

$$\text{Wt. Increase} = \theta_1 + (\theta_2 \times \text{Birthweight}) + \varepsilon$$

where θ_1 and θ_2 are the intercept and the regression coefficient (slope), respectively, and ε represents the error term, defined as the difference between predicted vs. observed weight increase for any birthweight. This uncertainty may be attributable to within subject variability in productivity (for example, day-to-day variation, misspecification of the regression model (lack of linearity), error in measuring weight, and unexplained fluctuations) as well as uncertainty due to failure to define other factors that affect the relationship between birthweight and weight increase (e.g. covariates such as gender, genetic background, sow parity, temperature, hours of light, ventilation systems, other management conditions, etc.).

After observing the data, the investigator notices that there is a very poor fit between observed and predicted values of weight increase. Possibly, a multimodal frequency distribution is observed for the outcome variable. Therefore, a decision is made to consider the inclusion of covariates in the regression model. For example, males may grow faster than females. Older sows may provide better nourishment than younger sows. Animals exposed to steady warm temperatures during the first few days may grow faster than those exposed to excessive or too low temperatures. The genetic background may also influence weight increase. By definition, these covariates are considered to be perfectly defined and without experimental error (for example, a given piglet will be classified as either male or female). Therefore, covariates are considered to be 'fixed' effects.

Up to this point, the investigator has made some basic predictions with respect to the relationship between birthweight and weight increase. While these relationships may be adequate to describe the sample of subjects included in the study, they may not accurately predict the relationship between birthweight and weight increase across the entire population of piglets. Assuming that the appropriate covariates were included in the model, prediction inaccuracies may be attributed to the random variation in weight increase that normally occurs within a piglet population.

To assess the types of variations that can affect these predictions, the investigator must consider the uncertainty associated both within an individual (ε) and between individuals (η). Furthermore, if an infinite number of observations were obtained both within and across individuals, the investigator would be able to characterize the distribution of these error terms. The investigator would obtain an estimate of the within-subject variance (σ^2) and intersubject (population) variance (ω^2).

Ultimately, to adequately predict the relationship between birthweight and weight increase across all piglets, we can define both a structural model relating birthweight plus covariates to weight increase (the 'fixed' effects) and a model describing the random variation within and between subjects that occurs in the population (the 'random' effects). If σ^2 and ω^2 are normally distributed, parametric statistical methods are appropriate. However, if these sources of variation are skewed or multimodal, nonparametric statistical methods would need to be employed. Regression equations would contain fixed and random effects.

By developing mixed-effect models that provide both a structural model and a variance model, the investigator enhances the ability to predict, with some degree of certainty, an individual piglet weight increase on the basis of birthweight. In other words, from a range of possible outcomes, there is a weight increase/birthweight relationship which is the most probable based upon the characteristics of the population. If we extend these concepts to the therapeutic arena, we can use similar processes to model the relationship between dose vs. the blood concentrations. Instead of the equation relating weight increase to birthweight and the covariates, we have a pharmacokinetic equation that relates blood concentration at different times to dose. These are the fundamental considerations upon which population pharmacokinetic models are built.

Application of population models to pharmacokinetics

Pharmacokinetics in veterinary medicine has been used to describe the processes of drug absorption, distribution and elimination (metabolism and excretion) in animals. Drugs administered intravascularly distribute to sites where they produce the intended effects. The rate and extent of access to these sites of action (therapeutic or toxic) in the presence of competing events such as metabolism, excretion and distribution to other tissues, or presence of other drugs will determine the therapeutic outcome. If a drug is administered extravascularly, the accessibility of the drug to the systemic circulation, or systemic availability, will also influence the clinical outcome. This process is especially complex when the extravascular route is oral, as the drug must gain access to the portal circulation from the enteric environment and 'survive' passage through the gut wall and liver with their respective ability to metabolize and inactivate drugs. Knowledge of the pharmacokinetic and pharmacodynamic parameters that describe drug disposition and effects in animals, as well as the interindividual variability associated with these parameters and the pathophysiological factors that contribute to this variability, are critical to the design of appropriate dosage regimens in animals.

The goal of drug administration consists of achieving the desired effect while minimizing the risks of toxicity, and in the case of food animals, avoiding the occurrence of violative tissue residue concentrations. The complex processes that determine the circulation of drugs through the body, schematically outlined in Fig. 1, are influenced by many different factors, a number of which arise from the physiology of the subject receiving the drug. In addition, pathologic processes can alter drug disposition by modifying the physiological functions that influence the circulation of drugs in the body. Altered pharmacokinetics may ultimately result in therapeutic failure or altered tissue distribution. Consequently, knowledge of the factors that modify the disposition of drugs and the extent of this modification is critical to properly describe the kinetic relationships between drugs and patients. In other words, one must determine the *sources of pharmacokinetic variability* in a patient population as well as the

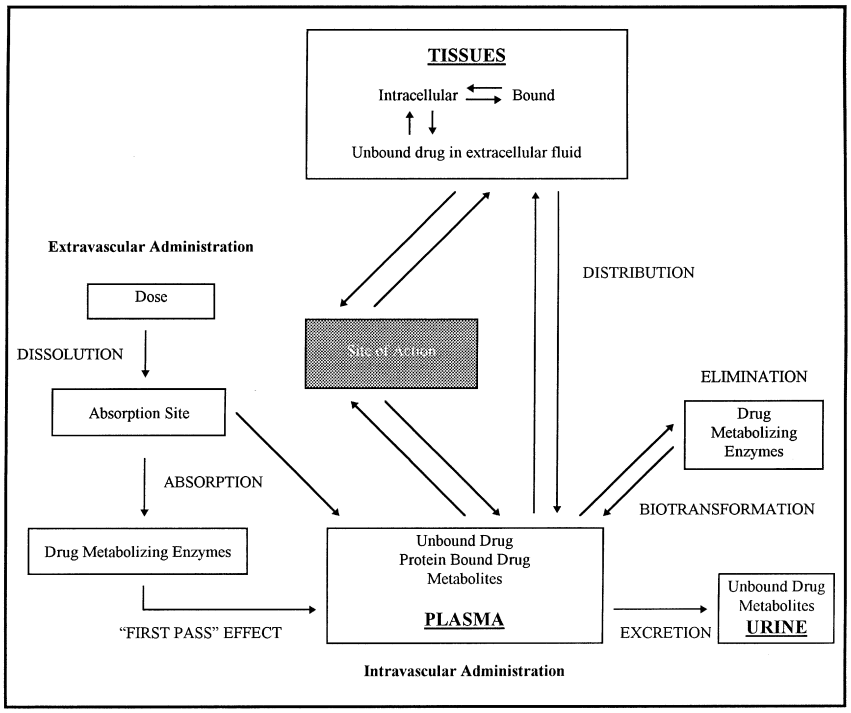

Fig. 1. Diagram of the interrelationships of the processes that determine the disposition of a drug in the body and the extent and duration of drug action.

magnitude of that variability, in order to design dosage regimens that *account for individual* patient characteristics. This knowledge may then be used to design dosage regimens tailored to different subsets of the population having these clinical characteristics. This approach is particularly important in the case of drugs with narrow therapeutic indices, especially those that show high interindividual variability even in apparently homogeneous patient populations. Potential sources of variability encountered in the clinical setting are age, weight, gender, breed, disease status, concomitant use of other drugs, altered physiological functions (such as renal or hepatic), hydration status, cardiac output, fever, nutritional status, genetic polymorphisms, etc. Although a detailed study of these and other variables is beyond the scope of this paper, it is important to keep in mind the enormous complexity of the processes that determine the disposition and effects of drugs in individuals, and the relationships of these processes with pathophysiological factors in individuals. In veterinary medicine this is further complicated by the variety of animal species to which therapeutic agents are administered. As defining the role of these factors in veterinary therapeutics has been neglected, a goal of this review is to present population pharmacokinetic techniques that can be used to account for the influence of these factors in the therapeutic outcome.

Customarily, dosage regimens are determined from studies conducted in a small number of generally healthy individuals which cannot account for all of the possible clinical factors that may be present in a patient and the relationship of these factors to drug disposition and effect. Dosage regimens designed in this way for drugs with high interindividual variability and narrow therapeutic indices have the potential to induce toxicity or lack of efficacy in diseased animals. Moreover, in veterinary medicine one deals not only with animals to be relieved from disease or suffering, but also with food animals whose tissues will enter into the human food supply. Therefore, concerns arise regarding not only toxicity or lack of efficacy, but also the appearance of violative tissue residues due to unaccounted variation in drug disposition. In order to design appropriate dosage regimens for drugs with narrow therapeutic indices and/or high interindividual variability in disposition, alternative modelling techniques must be used that account for the influence of pathophysiological factors on the pharmacokinetics and pharmacodynamics.

Various methods which establish relationships between parameter estimates and pathophysiological variables have been developed over the last two decades for prediction of serum drug concentrations and estimation of pharmacokinetic and pharmacodynamic parameters in heterogeneous populations of patients receiving drug treatment (Sheiner & Beal, 1980, 1981b, 1983; Mallet *et al.*, 1988; Schumitzky, 1991; Jelliffe *et al.*, 1994). The integration of pharmacokinetic and pharmacodynamic models in population studies allows the time course of a drug in the body to be linked with the extent and duration of drug effect while accounting for the influence of concomitant physiological variables. An important feature of these methods is the ability to make parameter estimates and valid predictions from sparse data obtained during routine monitoring of clinical patients rather than from carefully designed experimental studies. These data are usually insufficient to characterize each individual's pharmacokinetic profile, and obtained with analytical techniques of diverse qualities and limits of detection (Boeckmann *et al.*, 1994). Population pharmacokinetic methods are more efficient and practical than traditional methods for analysing such data (provided that the intraindividual variability is modelled accordingly). This does not mean that population studies cannot be conducted according to a carefully designed

protocol. In fact, whenever possible, this should be done. Simulation studies can be conducted before initiating actual study to determine the influence of different aspects of data collection designs (number of subjects, number of samples per subject and sampling times) on the efficiency of parameter estimation (D'Argenio, 1981; Al-Banna *et al.*, 1990; Ette *et al.*, 1995a).

Finally, the analysis and interpretation of clinical trial data is another area that benefits deeply from the ability of population models to handle less than perfectly designed sets of data. A common problem in the analysis of clinical trials for certain drugs is having to deal with censored data, such as those produced when subjects drop out of a study. In this type of situation, patient samples are biased toward those with a certain clinical outcome. For example, in analgesia trials some patients may be remedicated with an active agent if their pain relief is not sufficient. This would make the sample of patients at any given time to be biased toward those with better relief (Sheiner, 1994). Population methods can be used in situations such as this that model the time course of effect as a function of time, dose and individual random effects. This allows one to obtain unbiased predictions of the output of untested trial doses, as an aid to selecting recommended doses.

PARAMETER ESTIMATION IN POPULATION PHARMACOKINETICS

Disease states may alter physiological processes which in turn may influence both the fate and effects of drugs administered to diseased individuals. Consequently, estimates of the basic characteristics of drug disposition in healthy individuals arising from 'average' demographic subsets of the population may, in many cases, only approximate the real characteristics of the disposition in individuals undergoing different disease processes and/or belonging to different demographic categories. The enormous complexity of the physiological processes involved in drug disposition and the dependency of these processes on individual features, precludes pharmacokinetic homogeneity even in healthy subjects. Hence, when disease is present and physiological mechanisms are altered, the degree of heterogeneity among individuals in drug disposition will be much greater as individual patients respond differently to the disease process. Therefore, designing drug dosage regimens under disease conditions requires estimating the pharmacokinetic parameters relative to the clinical factors that are present in an individual, as well as estimating the precision with which these pharmacokinetic parameters can be characterized. In other words, when a drug is administered to a diseased patient, the following two questions arise. First, what is the average value of a pharmacokinetic parameter (e.g. clearance) in a population in relation to a determined value of a similar physiological function (e.g. renal function) that can be directly measured or estimated by means of a surrogate marker (e.g. creatinine clearance)? Second, how well (with what degree of certainty) does this population estimate reflect the value of the pharmacokinetic parameter (e.g. clearance) in an individual, if we know for that individual the value of the physiological function (e.g. renal function) that is related to the pharmacokinetic parameter? Different methodologies have been described in the literature to address these two questions. Among them, the method known as standard two-stage, as well as the more recent 'true' population pharmacokinetic methods using both parametric and nonparametric approaches, have been by far the most commonly used. Only the 'true' population pharmacokinetic methods allow one to explicitly answer these questions based upon a sample of the entire patient population. Unfortunately, these have not been applied to problems in veterinary medicine. The traditional two-stage method provides information that is less generalizable and cannot handle sparse sampling per individual. Nevertheless, the latter is a valuable analytical tool when sufficient plasma samples can be obtained per individual.

Traditional standard two-stage method

The traditional approach to pharmacokinetic parameter estimation is referred to as the standard two-stage method (STS). In the first stage, the pharmacokinetic profile of each individual under study is estimated using traditional analysis to estimate the primary pharmacokinetic parameters. As most pharmacokinetic models are statistically nonlinear, usually nonlinear regression (weighted or unweighted) with the least-square criterion is used. In the second stage, the individual parameter estimates are pooled to obtain an average value (e.g. mean) that is representative of the study population, and a measure of an apparent interindividual variability (e.g. variance) that actually includes all sources of variability (both inter and intraindividual). In a further step, the relationship between pharmacokinetic parameters and clinical characteristics can be studied by classical regression techniques (e.g. clearance and renal function, or volume of distribution and age). Thus, the STS method proceeds first with the individual (stage 1) and then the use of statistics generates predictions regarding the patient population. Conversely, 'true' population approaches study the whole population at once, without first needing to examine the individuals.

Despite the widespread use of the STS method, it presents several disadvantages. STS studies are usually conducted in a small number of experimental individuals (usually healthy and with average demographic features) over a short period of time (often after single dose administration). On account of this, the mean parameter estimates obtained may not be representative of the patient population that will receive the drug to treat a particular clinical condition. STS studies require rigid experimental designs and extensive sampling per subject to allow for an accurate characterization of each individual's pharmacokinetic profile (the minimum amount of samples needed per individual is based in the number of parameters to be estimated). An insufficient number of samples per individual may lead to poor parameter estimates because identification of the pharmacokinetic model is not possible for a small number of individual measurements (even five samples per patient may yield poor estimates) (Kataria *et al.*, 1994). When these poor individual estimates are combined in the second stage of the process, biased

population estimates result. The main disadvantage of the STS method is that although it is generally adequate for obtaining mean estimates, it is not adequate for characterizing random effects (variance structure) which influence the pharmacokinetic profile of a population. This method does not provide accurate estimates of the interindividual and intraindividual variability in drug disposition because it pools together both sources of variability in a *common variance*. Even if the study is conducted with diseased individuals (provided enough data are available) and in a further step the pharmacokinetic parameter estimates are linked to disease status, the variance estimates will be inaccurate. Consequently, when pharmacokinetic parameter estimates obtained from a small number of individuals with the STS method are used to develop generalized dosage regimens, there may be a considerable risk of toxicity or lack of efficacy. This is specially true for drugs with a narrow therapeutic index and for patient subpopulations representing extreme demographic or health status categories (e.g. very young or very old, renal or hepatic impairment, etc.). To achieve the desired serum concentrations, the initial dosage regimen should reflect the likely requirements of individuals as determined by measurable clinical characteristics.

The main strength of the STS approach resides in its straightforwardness, experimental nature and the availability of user-friendly software that makes the process of fitting models to the data relatively simple. When a sufficient number of samples per individual are obtained, each individual's pharmacokinetic profile may be fully characterized. Consequently, in such 'data rich' situations, this method provides reliable individual estimates. Unfortunately, 'data rich' situations are not commonly encountered in the clinical setting. Obtaining numerous blood samples from patients is almost always a daunting task due to ethical and practical considerations (specially in very young, very old, or very ill patients). Studying the pharmacokinetic characteristics of patients with different degrees of disease arising from very different demographic subpopulations may result in unbalanced data arising from different data collection designs and obtained with analytical techniques of varied qualities and limits of detection. Such data are usually not sufficient to fully characterize each individual's pharmacokinetic profile. In this type of situation (by far the most common in the clinical setting), the traditional approach to estimating pharmacokinetic parameters would fail to provide reliable estimates of both the average parameter values in different patient subpopulations and its variability among and within individuals. It is in these scenarios, very common in veterinary medicine, where the 'true' population approaches with the ability to evaluate the whole population as a unit, using less than perfect data, become most valuable (Sheiner *et al*., 1977).

'True' population pharmacokinetic methods: parametric and nonparametric

What is usually referred to as population pharmacokinetic methods are a series of techniques that allow the study of the pharmacokinetic characteristics of a drug in a target population using sparse data obtained from the sampling of a few (in some cases only one) plasma concentrations per individual (from a large population) for routine clinical monitoring. Implementing this methodology not only allows one to estimate average values of pharmacokinetic parameters in a population with determined clinical features, but also provides information about the inter and intraindividual variability of those parameters and (depending on the specific method) may even allow one to estimate the joint probability distribution function of the pharmacokinetic parameters and covariates. The joint probability distribution reflects the frequency distribution associated with two variables, and consequently provides an indication of the variance of these two variables and their degree of correlation. In the typical clinical setting, there are not enough datapoints per subject to fully characterize each individual's pharmacokinetic profile. This limitation is overcome by studying a larger number (50–500 in most published studies) of clinical patients with each of them being sparsely sampled (1–4 samples per individual). Given the sampling and design restrictions, population pharmacokinetics methods were conceived to analyse observational (e.g. data not collected according to a rigid predetermined experimental design) rather than experimental data. In order to handle this type of data, population methods require an *a priori* thorough specification of the pharmacostatistical model. This includes specification of both the structural and regression pharmacokinetic models (containing the fixed effects), as well as a complete description of the statistical model (containing the random effects).

The object of a study is not the individual but the population as a whole. This makes population pharmacokinetic studies more representative of the population to which the drug is targeted. One of the most advantageous characteristics of population pharmacokinetic studies is that they can quantitatively express the influence of clinical conditions on the average pharmacokinetic characteristics of the population. Hence, this methodology allows one to explore possible relationships between pharmacokinetics and clinical features of patient populations. The second advantage of this type of methodology is that it provides estimates of the *interindividual and intraindividual* (residual) *variability* of the estimated pharmacokinetic parameters (parametric methods) or allows the direct estimation of the joint probability density function of the pharmacokinetic parameters in the population (nonparametric methods). This information allows for predictions in individuals according to their clinical features and further provides information about the degree of confidence that can be placed on those predictions. Additionally, (as will be later reviewed) these estimates can be included as *a priori* information in Bayesian forecasting techniques to further improve individual predictions.

In essence, there are three main components in any population assessment:

(a) Structural model - How drug moves in the body. Depending on the type of procedure, a structural model may or maybe not needed.

(b) Modelling of residuals.

(c) Estimation of the joint probability density function.

There are two general types of population pharmacokinetic methods, known as parametric and nonparametric. A third approach has been described (Davidian & Gallant, 1992b) that is midway between these two methods. It is called the semi-nonparametric or smoothed nonparametric approach. The differences between these methods can be summarized as follows:

Parametric methods: The pharmacokinetic variables and the error terms are assumed to come from a known distribution (normal or log-normal) with unknown parameters. Parameter estimation is restricted to some structural model. Confidence intervals and standard errors are based on parametric methods. These methods, as implemented by the computer program NONMEM can also handle some multimodal distributions if they are accounted for in the variance model.

Semi-nonparametric methods: Unlike parametric procedures, parameter estimation is not restricted to some structural model, and alternative fitting procedures (e.g. spline fits) can be employed. However, the estimate of uncertainty about these estimates is confined to parametric procedures.

Nonparametric methods: In this case, restrictions regarding structural models and distribution of inter and intraindividual error terms are relaxed. Accordingly, the uncertainty about parameter estimates are based upon nonparametric procedures, such as nonparametric confidence intervals. These methods compute the joint probability density function of the pharmacokinetic parameters, which measures the variance of two parameters and how well they are correlated.

Selecting the most appropriate method depends on the original assumptions about the underlying distribution. Parametric methods are usually easier to implement from a modelling standpoint, but lack the ability to identify situations of evident deviation from normality in the distribution of the pharmacokinetic parameters in the population, such as when bimodal (typical of drug polymorphisms) or very skewed distributions occur.

Parametric methods

Nonlinear mixed effects modelling (NONMEM)

This is the most representative parametric procedure and the first true population pharmacokinetics method ever to be developed and utilized in clinical medicine. It is implemented through a computer program known as NONMEM (Beal & Sheiner, 1980, 1989; Beal, 1984a, b). In this modelling strategy, the total residual variability is explained in terms of fixed and random effects (Boeckmann *et al*., 1994). This requires fitting a fully specified pharmacostatistical model to the population data from which estimates of the average population values of pharmacokinetic parameters and their variances as well as the residual variance are obtained. This is accomplished by using the method of extended least squares (ELS), as applied to a nonlinear mixed-effect statistical model (Sheiner & Beal, 1984). This method stems from the ordinary least squares method. In ordinary least squares, as applied to a subject's data, parameter values are sought that minimize the sum of squared deviations of the observations. The variances of the individual observations are assumed to be equal (homoscedasticity). If these variances differ but are known, then weighted least squares techniques can be used. When the differing variances are unknown, the ELS method can be used. This method models the variance as a function of the pharmacokinetic parameters, a vector of independent variables (fixed effects), and some random-effect parameters (inter and intraindividual). While the advantages of this method are many, there are also some important drawbacks to it. First, all data are fit to a single set of parameters, which may not be appropriate for all the individuals and some degree of model misspecification may arise from it. Second, repeated blood measures in an individual are treated as independent observations, which they are not. Finally, there may be some degree of confounding of the variance terms if there is not enough data to characterize them (i.e. too few η's).

For illustrative purposes, the full pharmacostatistical model can be divided into pharmacokinetic and statistical components (Fig. 2). In turn, the pharmacokinetic model can be further subdivided into structural and regression models, while the statistical model contains the two types of random effects, namely, inter and intraindividual.

Fixed effects are a series of variables and constants (e.g. dose, time, age, weight, serum creatinine, etc.) assumed to be measured without error. They are linked by a structural model (e.g. $Cp = D/Vd \times e^{-Cl/Vd \times t}$) with the dependent variable (plasma concentration) and by a regression model with the pharmacokinetic parameters (e.g. clearance = *f* (creatinine clearance) and volume of distribution = *f* (age)). The fixed effects of the structural model are dose and time. The proportionality constants (fixed-effect parameters) of the structural model are the pharmacokinetic parameters (e.g. clearance and volume of distribution). For example, for the one compartment open model with intravenous (i.v.) administration, the following expression applies:

$$Cp = D/Vd \times e^{-Cl/Vd \times t} \qquad \text{eqn 1}$$

where Cp is the observation (dependent variable), D is the dose, t is the time at which the observation takes place and Cl and Vd are, respectively, clearance and volume of distribution. Cl and Vd quantify the influence of the fixed effects (dose and time) on the dependent variable of the structural model.

This expression can be generalized as:

$$Cp_{ij} = f(p_j, D_j, t_{ij}) \qquad \text{eqn 2}$$

where Cp_{ij} is the ith serum concentration in the jth individual, p_j are the average population pharmacokinetic parameters (Cl_j, Vc_j, etc.), t_{ij} is the time of the ith measurement in the jth individual, D_j is the dosing history in the jth individual and *f* is the structural model.

If, in turn, the pharmacokinetic parameters (p_j) can be further explained in terms of patient characteristics (including age, weight, serum creatinine, gender, breed), then a regression model is implemented in which the pharmacokinetic parameters become the dependent variables, the patient characteristics are the fixed effects and a set of fixed-effect parameters (θ_z) quantify the relationship between patient characteristics and pharmacokinetic parameters. NONMEM computes estimates of the fixed-

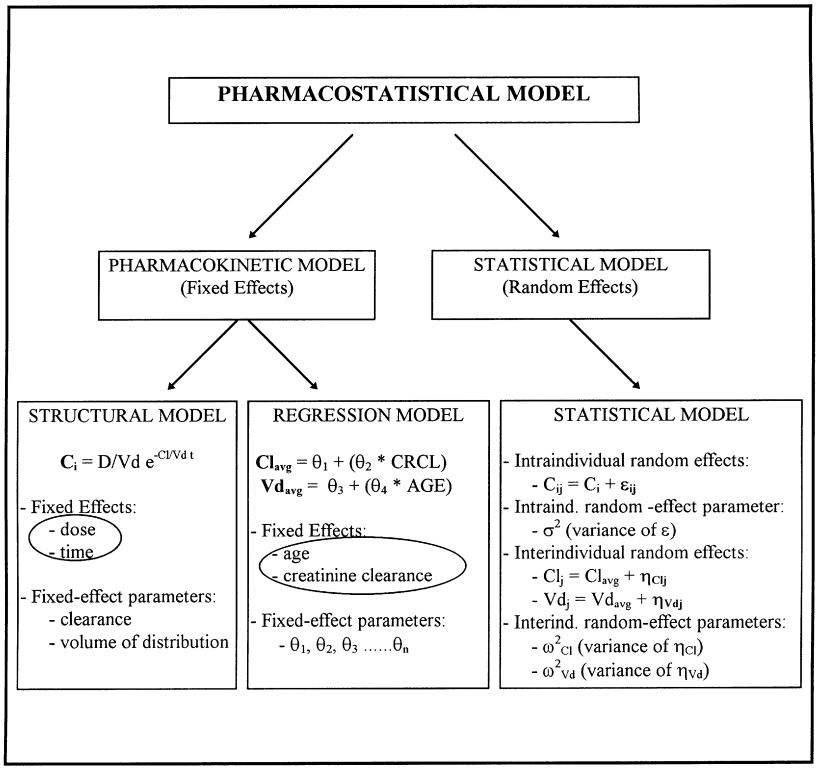

Fig. 2. Diagram of the different components of the pharmacostatistical model for a drug administered as an i.v. single dose. Pharmacokinetics are described by a one compartment open model with clearance and volume of distribution linearly correlated to creatinine clearance and age, respectively. The fixed effects (dose, time) are part of the structural model. The fixed-effect parameters of this model (pharmacokinetic parameters, *Cl* and *V*d) can be further broken down and explained in terms of another set of fixed effects (patient characteristics) through the regression model. The statistical model accounts for the influence of the interindividual (η) and intraindividual (ε) random variables. The random-effect parameters (quantifying the influence of η and ε on the model) are σ^2, ω^2_{Cl} and $\omega^2_{V_d}$ and correspond to the variances of the intraindividual, and interindividual (*Cl* and *V*d) random effects.

effect parameters of the regression model. The algebraic form of the equations of the regression model (excluding the random effects) is as follows:

$$Cl_{avg} = \theta_1 + (\theta_2 \cdot Cov_1) + (\theta_3 \cdot Cov_2) + \ldots\ldots + (\theta_n \cdot Cov_{n-1}) \quad \text{eqn 3}$$

$$Vd_{avg} = \theta_{n+1} + (\theta_{n+2} \cdot Cov_n) + (\theta_{n+3} \cdot Cov_{n+1}) + \ldots\ldots + (\theta_z \cdot Cov_{z-2}) \quad \text{eqn 4}$$

where Cov_z represent the fixed effects (covariates) and θ_z are the fixed-effect parameters. The intercepts of each regression equation represent the amount of the pharmacokinetic parameter value that is not due to the effect of the concomitant variables (i.e. each covariate value equal zero, for a linear relationship). For example, the term ω_1 in Fig. 2 represents the population average value of the nonrenal clearance. In the case that no concomitant variable is included in the model for a particular pharmacokinetic parameter, the latter becomes the fixed-effect parameter, not only of the structural but of the regression model as well (i.e. $\theta_1 = Cl_{avg}$). For simplicity of exposition, the relationships depicted above are linear, but other forms of relationships are also allowed (multiplicative, saturation) (Boeckmann *et al.*, 1994). So far we have discussed the relationships between average population pharmacokinetic parameters of the structural model and fixed effects (clinical variables), without considering any source of unknown variability either between or within individuals. This unknown variability is what is referred to as random effects.

Random effects are the unknown quantities that arise from a probability distribution whose shape is assumed in NONMEM to be normal or log-normal (Grasela & Sheiner, 1991). There are two kinds of random effects, namely, interindividual random effects and intraindividual random effects. Interindividual random effects are associated with the pharmacokinetic parameters of the structural model (*Cl*, *V*d) and reflect the between-subject variability in drug disposition. Each individual (j) has a particular value for their pharmacokinetic parameters that will differ from the average population value by an unknown quantity. This unknown quantity is assumed to arise from a normal or log-normal probability distribution, with a mean of zero and a certain variance ω^2 that is estimated by NONMEM. The interindividual random variable is represented in NONMEM by the Greek letter ETA (η) with a subscript relative to the pharmacokinetic parameter with which it is associated. The relationship between the random variable and the pharmacokinetic parameter is given by the statistical model.

For example:

$$Cl_j = Cl_{avg} + \eta_{Cl_j} \quad \text{eqn 5}$$

$$Vd_j = Vd_{avg} + \eta_{Vd_j} \quad \text{eqn 6}$$

where Cl_j and Vd_j represent the clearance and volume of distribution, respectively, in the jth individual, Cl_{avg} and Vd_{avg} are the population averages for clearance and volume of distribution, and η_{Cl_j} and η_{Vd_j} represent the deviations of the individual clearance and volume of distribution, respectively, from their population averages for the jth subject. The error model represented here has an additive form, but other types of models are also available (e.g. multiplicative: $Vd_j = Vd_{avg} \times (1 + \eta_{Vd_j})$, or exponential: $Vd_j = Vd_{avg} \times e^{Vd_j}$, etc.). In turn, we must also define the distribution of the η's within the population (assuming mean = 0 and variance = ω^2).

Intraindividual random effects represent the residual variability and arises from model misspecification (e.g. fitting a one-

compartment model to data that would be better described by a two-compartment model), analytical assay error, and time variation in pharmacokinetic parameters within the same individual. Formally expressed, the intraindividual random variable represents the deviation of the observed concentration from the value that would be expected were the true individual pharmacokinetic parameters known. Algebraically expressed:

$$C_{ij} = C_{ij,true} + \varepsilon_{ij} \qquad \text{eqn 7}$$

where C_{ij} is the observed concentration in individual j at time i, $C_{ij,true}$ is the true concentration for individual j at time i, and ε is the residual random error or difference between observation and true value for individual j at time i. As in the case of the interindividual random effect, the form of this relationship may be other than additive. The random variable ε is assumed to arise from a normal or log-normal probability distribution with mean zero and variance σ^2. NONMEM computes estimates of the variances of the interindividual and intraindividual random effects, namely, ω^2_{Cl}, $\omega^2_{V_d}$ and σ^2. Figure 3 represents the partitioning of the total residual variability that takes place with NONMEM.

The difference between the measured and predicted drug concentrations can be dissected into two components. First, residual variability, or the difference between the observed plasma concentration and the true concentration (expected concentration if the true pharmacokinetic parameter values of the individual were known). Second, interindividual variability, or the difference between expected concentrations using the true individual pharmacokinetic parameters and those expected using the pharmacokinetic parameter values estimated by the model.

If we recall the two main questions (formulated in an earlier paragraph) that we have in mind when conducting a population pharmacokinetic study, it is easy to see now how NONMEM addresses these. The pharmacokinetic model responds to the first question, i.e. the determination of the average pharmacokinetic parameter values in a population with determined values of a series of physiological functions upon which the pharmacokinetic parameters depend. The statistical model responds to the second question, i.e. the degree of uncertainty about which the pharmacokinetic parameters are estimated in an individual of known physiological functions.

NONMEM uses a variety of algorithms related to nonlinear regression and matrix algebra to obtain estimates of the fixed-effect parameters, the inter and intraindividual random-effect parameters (variances), and the standard errors of all these parameter estimates. The covariance and inverse covariance matrices are also computed to show if parameter values vary together. If the parameters of the model are not independent of each other the model should be reassessed. The correlation matrix of the parameter estimates is computed as an additional indication of the adequacy of the model, as highly correlated parameter estimates are indicative of model overparameterisation. Diverse scatterplots which show distribution of residuals for different levels of a covariate are also obtained. These kind of plots can be used to assess the relative importance of including the covariate under evaluation in the regression model. Other plots, such as that of predicted vs. observed concentrations can also provide hints as to the goodness of fit of the models tested. Finally, the minimum value of the ELS objective function is computed that will be used to compare different models during the search for the most adequate one.

The model building procedure (structural and regression model) is conducted in a stepwise fashion. The statistical significance of the reduction in the minimum value of the objective function (MVOF), and the decrease of the inter and intraindividual variability when adding a new covariate to the model (Boeckmann *et al.*, 1994) are assessed at every step. Each time NONMEM runs a model, it minimizes the value of the extended least squares (ELS) objective function. The minimum value of the ELS function is an indicator of the goodness of fit of the model. This value can be used to statistically compare full-reduced regression pairs. The full model is that whence the parameter of the added covariate is estimated. Alternatively, the reduced model is that whence the parameter in question is fixed to the null value. The difference between the MVOF of a full model and a reduced model approximates a chi-square (χ^2) distribution with degrees of freedom equal to the difference in the

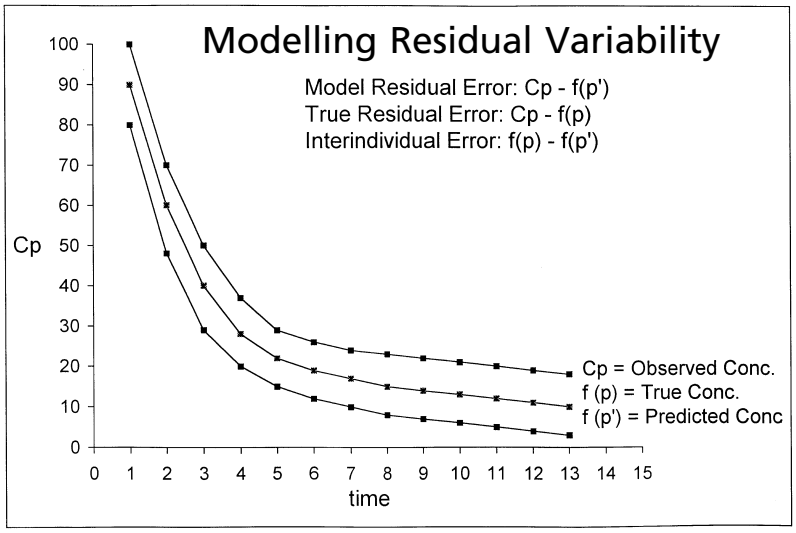

Fig. 3. Integration of pharmacokinetic model and statistical model in the prediction model. The *total residual variability* is partitioned with NONMEM in terms of the fixed and the random effects. The difference between the observed and the predicted drug concentrations can be dissected into two components. First, *true residual variability* (Cp–f(p)) arises from the difference between the observed plasma concentration and the true concentration (expected concentration if the true values of the individual PK parameters are known). Second, *interindividual variability* (f(p)–f(p′)) arises from the difference between expected concentrations using the true individual pharmacokinetic parameters and those expected using the values of pharmacokinetic parameters estimated by the regression model. NONMEM computes estimates of the pharmacokinetic model parameters and of the variances of the random variables.

number of parameters between the full and the reduced model (q). Its statistical significance can be determined by comparing the difference between both MVOF values, with the correspondent value of the χ^2 distribution for q degrees of freedom.

Nonparametric methods

Nonparametric methods provide the opportunity for analysis without implicit assumptions as to the population distribution of the random error terms for interindividual and residual variability. This allows one to visualize the data and determine the best function with which to represent the observed distribution. Due to this feature, nonparametric methods can handle bi- or multimodal populations, thereby discovering unsuspected clusters of patients such as that which occurs in genetic polymorphisms (e.g. slow and fast acetylators).

These techniques are based on the general method known as *maximum likelihood estimation* (Jennrich & Sampson, 1976). This method, as applied to regression, aims at identifying the relationships between outcome and independent variables that are the most reasonable. In other words, its objective is to obtain parameter values that provide the maximum probability of producing a sample in the neighbourhood of the one observed. The 'maximum likelihood' represents a family of statistical procedures used to determine when further iterations are no longer needed to improve the fit between the observed vs. the predicted values. Nonparametric methods compute the nonparametric maximum likelihood estimate of the unknown population density function (Mallet *et al.*, 1988). The differences between the two main types of nonparametric methods reside in the type of algorithm that they utilize. As for the relationships between covariates and structural parameters, nonparametric methods estimate the joint distribution of the parameters (both pharmacokinetic parameters and fixed-effect parameters that describe the relationships between pharmacokinetic parameters and covariates).

Nonparametric maximum likelihood (NPML)

This algorithm was first described by Mallet (1986) who showed that the joint probability distribution of parameter values in a population model is discrete as opposed to the continuous nature of a normally distributed parameter. Accordingly, it can be described by some frequency distribution (Jelliffe *et al.*, 1994). NPML computes the joint probability density function of the parameter estimates. A complete discussion of the mathematical basis of this method is beyond the scope of this paper. However, the general features of the algorithm and an example with simulated data, have been presented elsewhere (Mallet, 1986).

NPML states the problem of parameter estimation in terms of the probability of obtaining data similar to that actually observed. It relies on the maximum likelihood principle as applied to the estimation of pharmacokinetic parameters. In other words, given a set of unknown terms and a set of data related to unknowns, the best estimate of the unknowns consists of the values that render the set of data most probable (Mallet *et al.*, 1988). The most familiar situation is when the unknowns are the pharmacokinetic parameters of an individual and the set of data is the individual series of observations. The distribution of the pharmacokinetic parameters in the population can also be unknown in which case the data are the array of such series of observations within a sample of individuals. This method was first applied to the estimation of population pharmacokinetic parameters in patients treated with cyclosporine. The study included plasma levels obtained in 188 bone marrow transplant patients after intravenous infusion. The parameter estimates computed with the NPML method (both with 7.9 and 3 datapoints per individual) were in agreement with the results obtained with the STS method with a full set of data per individual. Systemic clearance and volume of distribution at steady-state were estimated without bias, although the volume of distribution of the central compartment and to a lesser extent the distribution and elimination half-lives were slightly biased. Since then, many studies have been published using this methodology. It has probably not been adopted more broadly because of the lack of user-friendly software, and the subsequent development of other nonparametric algorithms. Currently, software has been developed to run this algorithm in a PC Windows® environment. In general, nonparametric methods require more mathematical sophistication than the parametric methods, but they allow appropriate parameter estimates to be computed when departure from normality in the distribution of pharmacokinetic parameters in the population takes place.

Nonparametric expectation maximization (NPEM)

This nonparametric estimator uses an iterative expectation maximization (EM) algorithm with steps of both expectation and maximization. The numerical fundamentals of the method has been published elsewhere (Schumitzky, 1991). This algorithm computes the entire joint probability density function (PDF) of the parameters. During the initial phases of the estimation process, a continuous PDF is calculated. The population fit of the PDF improves with each iteration. With progressive iterations, the spikes of the joint density become narrower. At its limits, discrete distributions are obtained, approaching the Mallet solution discussed above (convergence).

Figure 4 depicts a graphical example of the joint PDF for a patient population. The joint PDF is projected as tridimensional spikes, the location and height of which represent the estimated values and probabilities of the pharmacokinetic parameters. Together with the joint probability density it also computes individual density functions for each parameter. This algorithm can operate with a single datapoint per patient and may be executed on a PC. It has been integrated as a segment of the USC*PACK software package (Jelliffe *et al.*, 1995). This software can handle one-, two- and three-compartment models with repeated oral and intravenous dosing, with a total of seven parameters being studied. Different studies (Dodge *et al.*, 1991; Kisor *et al.*, 1992; Gilman *et al.*, 1993) have shown similar results when either a nonparametric method or the STS method were used to model blood sample data from populations with normal distribution of the pharmacokinetic parameters. They

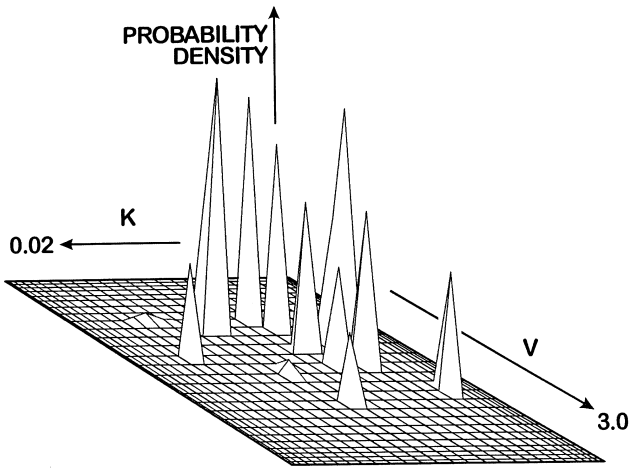

Fig. 4. Example of the three-dimensional plot of the joint PDF in a population of patients treated with a drug. K = elimination rate constant (range 0–0.02 h^{-1}). V = Volume of distribution (range 0–3.0 L/kg). Each spike represents the probability that a subject will have $V = x$ and $K = y$. If all true values for the population were known this would be reduced to a scatterplot with a dot per individual (or a spike in cases of two individuals with the same values of V and K). The number of dots would never exceed the number of subjects.

also produce estimates of the means, standard deviations, modes, medians, skewness, kurtosis, and correlations and covariances between parameters.

Semi-nonparametric methods:

Smooth nonparametric maximum likelihood (SNP)

This is a semi-nonparametric method, proposed for use in population pharmacokinetic analysis by Davidian and Gallant, (1992a). This modelling strategy is particularly relevant for population data that can be described with nonlinear mixed effects model (MEM) strategies. For this type of data, the SNP method simultaneously estimates the fixed effects (by maximum likelihood principles) and the entire random effects density. A description of the numerical basis of the method and an example of its application to the population pharmacokinetic analysis of quinidine in 136 patients has been published elsewhere (Davidian & Gallant, 1992b).

VALIDATION OF POPULATION PHARMACOKINETIC MODELS

The fact that many of the population pharmacokinetic studies that are conducted have an observational rather than an experimental nature has led to the necessity of establishing appropriate validation methods, to assure that the parameter estimates obtained in a population pharmacokinetics study are extrapolatable to the general population, and that the results are reasonable and independent of the analyst. Validation procedures are intended to assess how well a population model (obtained from a 'study' or an 'index' population) describes a set of data ('validation' set) that has not been used to develop the model itself. Whether validation of the population study is accomplished or not depends on the objective of the analysis. When a population model is developed for dosage recommendation, it must be adequately validated. Alternatively, when population models are developed for explaining variability or for providing some descriptive labelling information, validation may not be required.

It is beyond the scope of this review to discuss in detail the different validation methods that have been proposed, and the statistics involved in each of them. Selection of the validation method should be justified according to the ultimate goal of the population study. The issue of statistical validation is still open to discussion, and we will only present a brief description of some of the most commonly proposed alternatives. The interested reader should refer to the appropriate literature for more comprehensive information on each particular method.

Types of validation

The validation of a population model consists of the assessment of the stability and/or predictive performance of a population model on a 'validation' data set, different from that used to develop the model. Depending on the availability of validation data, we may distinguish two types of validation, namely, external and internal. In external validation, the validation set consists of an entirely new data set obtained from another study. Alternatively, internal methods use the original data set to derive both the 'index' and 'validation' data sets or use resampling techniques to validate the developed model. Internal validation techniques include data-splitting (Roecker, 1991), and resampling techniques such as cross-validation (Efron, 1983) and bootstrapping (Ette, 1997).

Data-splitting partitions the available dataset in two portions, the index data set (two thirds) and the validation data set (one third). As the predictive accuracy of the model is dependent on the sample size, it is recommended that after validation of the population model both sets are pooled together and the final model parameters are estimated using this overall data set.

Cross-validation consists of repeated data-splitting.

Bootstrapping consists of a resampling procedure that allows the evaluation of the stability and performance of a population model by repeatedly fitting the model to the bootstrap samples. The bootstrap samples consist of a large number (e.g. 200) of subsample replicates obtained by resampling the original data with replacement. Subsamples are distributed similar to the original sample and consequently the statistical inference of interest can be made as for the original sample. This method is computer-intensive and is an adequate alternative to external validation methods when original sample sizes are too small.

Methods of validation

Standardized prediction errors

This is one of the first validation methods ever proposed for population studies (Vozeh *et al.*, 1990). This method computes

the standardized mean prediction error (SMPE) and the variance for each patient. A *t*-test (actually a *z*-test) is performed to assess whether the average of $SMPE_j$'s across patients is different from zero, i.e. whether the prediction is on the average biased. Another *t*-test is conducted to test whether or not the model describes adequately the variability in the validation data set (within and between patients), by comparing the standard deviation of $SMPE_j$ (computed across patients) to 1. Although different population pharmacokinetic studies have used this approach (Aarons *et al*., 1989; Fattinger *et al*., 1991), the method has also received some criticism[a] regarding its inadequacy to test the latter hypothesis, the incorrect assumption of lack of error in the estimates of population parameters (Anon, 1997), and the very idea that testing this hypothesis is meaningful at all.

Concentration prediction error

This method (Sheiner & Beal, 1981a) is based on the prediction error, which is the difference between the predicted and the observed concentrations. This method assesses the predictive performance of a population model by using the mean squared prediction error (MSPE) as an indicator of precision, and the mean prediction error (MPE) as an indicator of bias. This method is inadequate when more than one observation is obtained per subject, because in that case prediction errors are not independent. Examples on the use of this approach for validation purposes can be found in the literature (Maitre *et al*., 1988; Lee *et al*., 1997; Schmitz *et al*., 1997).

Validation using model parameters

This method accomplishes validation with the parameters of the model, hence avoiding the problems encountered in the previous method (Bruno *et al*., 1996). Using the validation set, it assesses both qualitatively and quantitatively the model predictions of individual pharmacokinetic parameters, with or without covariates, and calculates the precision and bias for the predictions.

Graphical approach

For NONMEM modelling, a graphical approach[a] to the validation of a model may be initiated by plotting the model predicted vs. observed concentrations in the validation set. This plot provides one with a visual clue for the degree of agreement between model predictions and validation data. It has been argued that in judging this correlation from a clinical standpoint, rather than from an statistical one, the graphical approach may provide as much information, if not more, than is presented by standard statistical comparison approaches. Plots of the residuals (observed minus predicted concentrations) vs. some of the covariates provide additional information on the validity of the population predictions. Residuals should be conceptually viewed as the prediction error for every individual in the study. A plot of the residuals vs. age may provide an indication of the clinical adequacy of the model for different age groups. Such plots could uncover 'age clusters' for which the model fits the validation data with less accuracy and/or precision.

Weighted residuals can also be useful for validation purposes. Weighted residuals are obtained by normalizing the residuals by the standard deviation of the model. Use of weighted residuals is a potential source of bias if inappropriate weighting schemes are used. In computer programs such as NONMEM, the weighted residuals consist of the residuals expressed in population standard deviation units. Consequently, a plot of the weighted residuals vs. the individual patient identification number (ID) can be useful to assess whether the residuals follow the description established for them under the population model. If the model affords an appropriate description of the validation data, then the weighted residuals should be homogeneously scattered about the zero line on the weighted residuals axis. Similarly, plots of weighted residuals vs. some of the covariates included in the model (e.g. weight, breed, creatinine clearance, etc.) may uncover situations in which the influence of the covariate has not been adequately modelled. If a trend or lack of homogeneity can be observed in a plot of weighted residuals vs. the covariate, instead of a homogeneous scatter, this indicates that the model is not describing the variability adequately. In this case some changes are necessary regarding the relationship between the covariate and the pharmacokinetic parameter or parameters in the population model. Examples of these plots are presented in the next section (NONMEM simulation). Another plot suggested to be useful to examine the closeness of predictions to observations is that of the data vs. the covariate superimposed on a plot of predicted concentrations vs. the covariate (Fig. 5). These plots depict differences between predictions and observations, and clinically may be of interest to know whether these differences are within the margin of error described by the population model. Other sets of validation plots have been proposed that are applicable to population models obtained with NONMEM by means of Bayesian estimation[a].

AN EXAMPLE OF PARAMETRIC MODELLING: NONMEM SIMULATION

To illustrate the general procedure of building population models, a simple population pharmacokinetic analysis has been conducted with NONMEM on a simulated population dataset consisting of 100 young dogs. A certain drug was administered to all the animals as a single i.v. bolus dose. Doses ranged between 1 and 2 g. An average of 5.3 samples were obtained per individual and the sampling times ranged between 1 and 38 h postadministration. The simulated data was parameterized so that the covariates age, weight and creatinine clearance were linearly correlated to the pharmacokinetic parameters (volume of distribution and clearance). The values of these covariates for each individual were simulated according to a lognormal distribution. Age ranged between 0.3 and 7.2 months, weight ranged between 0.2 and 3.8 kg, and creatinine clearance ranged between 10 and 125 mL/min. The statistical structure for

[a]Validation of a Population Model. Comments by Dr Stuart Beal in the NONMEN Discussion List on the World Wide Web. NONMEN Topic 6. 1994.

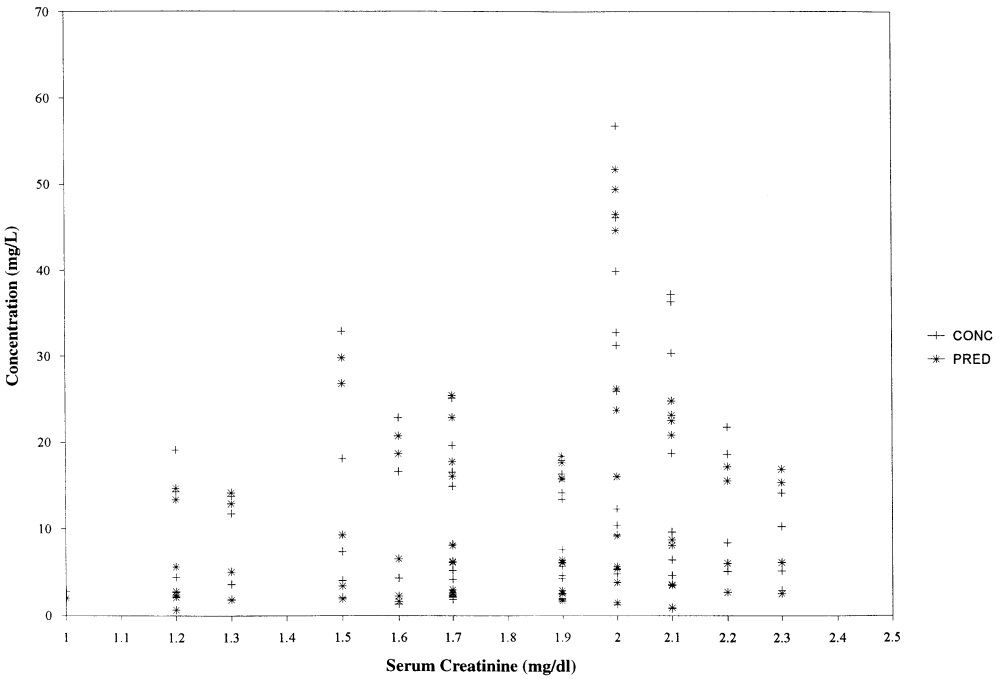

Fig. 5. A plot of the observed concentrations vs. a covariate of the model superimposed in another of the predicted concentrations vs. the same covariate, may reveal clinically important differences between predictions and observations, that are beyond the magnitude of error described by the model.

interindividual and intraindividual random effect was modelled according to a proportional error model (constant coefficient of variation). For simplicity of exposition, a 1-compartment model was adopted. In our simulated data, clearance was linearly related to weight and creatinine clearance, and volume of distribution was linearly related to age. Figure 6 shows the concentration-time profile of all the population plasma samples (normalized by dose). As we can see, the interindividual variability for the simulated drug disposition is quite high at almost every sampling time.

Initially, the simplest pharmacostatistical model (minimal model) was fitted to the data. This model consisted of a 1-compartment structural model in which clearance and volume of distribution were average population values not linked to any covariate or clinical variable. Consequently, the full pharmacostatistical model was:

Structural model: $Cp = Co \times e^{-kt}$

Regression model: $Cl_{avg} = \phi_1$

$$Vd_{avg} = \phi_2$$

Statistical model: $Cl_j = Cl_{avg} \times (1 + \eta_1)$ (interindividual)

$$Vd_j = Vd_{avg} \times (1 + \eta_2) \text{ (interindividual)}$$

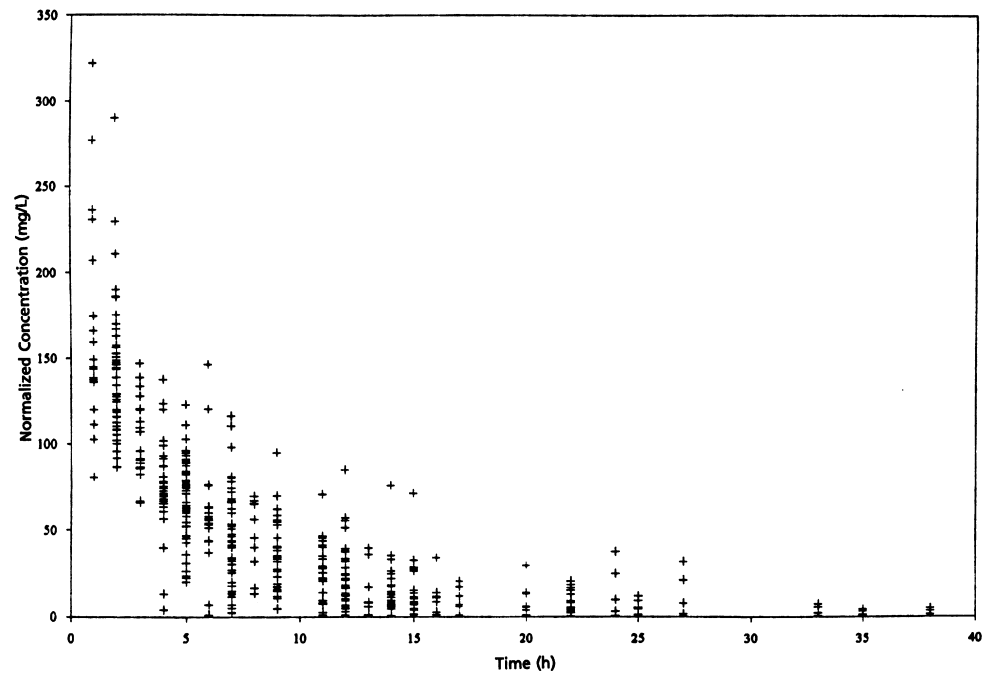

Fig. 6. The plot of concentration (normalized by dose) vs. time for all the individuals reveals a high degree of variability in disposition.

$$Cp_{ij} = F \times (1 + \varepsilon_1) \text{ (intraindividual)}$$

where Cp is the general expression for the observed concentration at time t, Co is the initial concentration, k is the elimination rate constant for the one-compartment model (Cl/Vd), Cl_{avg} and Vd_{avg} are the average population drug clearance and volume of distribution, respectively, ϕ_1 and ϕ_2 are the estimates of Cl_{avg} and Vd_{avg}, respectively, computed by NONMEM, $(1 + \eta_1)$ and $(1 + \eta_2)$ represent the interindividual random variable terms, F is the prediction of Cp_{ij} (ith time and jth individual) and $(1 + \varepsilon_1)$ represents the intraindividual random error (residual).

Figure 7a depicts the predicted vs. observed concentrations for the initial model (no relationship with clinical variables). This model estimates average values of Cl and Vd for the whole population without linking the pharmacokinetic parameters to any concomitant variable. This would be close to the results obtained by a traditional pharmacokinetic analysis with 'rich' data. The scatter of the datapoints is noticeable, especially for the higher observed values of drug concentration. A plot of the weighted residuals (weighted residuals are the observations minus the predictions expressed in units of population standard deviation) vs. the observed concentrations (Fig. 7b) indicates that the simple average population model fails to predict the plasma concentration values with the same degree of accuracy and precision for all the different levels of concentration. The lowest concentrations have the most negative residuals while the highest concentrations have the most positive. The MVOF for this model was 3453. The interindividual coefficients of variation for Cl and Vd were 39% and 30%, respectively. The intraindividual (residual) coefficient of variation for the initial model was 26%. Once the initial model is computed, the first step is to study potential relationships between the pharmacokinetic parameters and the covariates (clinical or physiological features) that are suspected to influence drug disposition. There are different procedures to study these relationships. One of the most common consists of studying the plots of residuals and weighted residuals of the initial model vs. the potential covariates of the model. A clear pattern in the scatter of the residuals in this plot would indicate a likely relationship between the covariate and some (or all) pharmacokinetic parameters. Even a simple lack of homogeneity in the scatter of the residuals may be indicative of the necessity to account for that particular covariate in the regression model. Accordingly, Fig. 7c–e shows the plots of weighted residuals vs. body weight, creatinine clearance and age, respectively. As can be seen in Fig. 7c there is a slight relationship between the sign of the residuals and patient body weight. The subjects with the largest weights tend to have the most negative residuals (predictions larger than observations) while those with lower weights show the opposite trend. An explanation for this effect is that one (or both) pharmacokinetic parameters may increase with weight. If weight is not accounted for in this model, the predictions are too low for the smaller animals and too high for the larger ones. Figure 7d does not depict a potential relationship as clearly as did Fig. 7c. Nonetheless the scatter of the residuals in Fig. 7d is far from homogeneous, and it is worthwhile to explore potential relationships between this covariate and the pharmacokinetic parameters. Figure 7e shows a clear pattern in the residuals when confronted with age. The magnitude of residuals (both negative and positive) seems to decrease with the age of the individuals. This may indicate that the average pharmacokinetic parameter values estimated by this model may be more adequate to describe the disposition of the drug in the oldest segments of the study population. Consequently, the covariate age should be included in the predictive model. In order to explore whether accounting for these clinical variables improves the fit, successive regression models would be run in which the different covariates are added in a stepwise fashion, statistically testing at every step whether

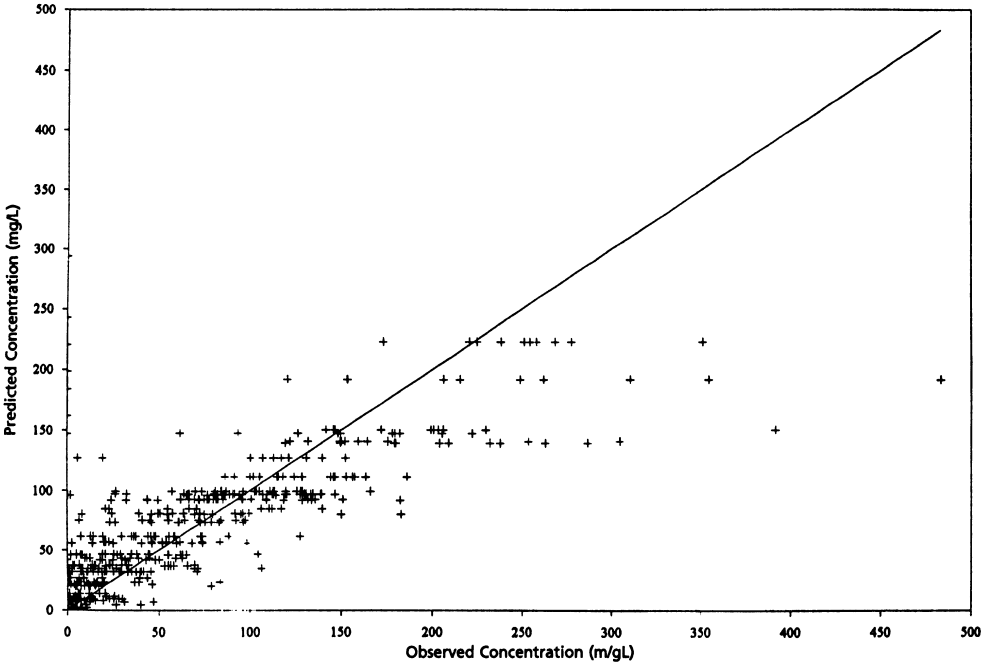

Fig. 7. (a) The plot of predicted vs. observed concentrations for the initial model reveals a considerable lack of precision in the predictions. A population pharmacokinetic model that does not account for patient characteristics will compute average values of pharmacokinetic parameters across the population. If the interindividual variability in disposition is high, the predictions derived from this model will be very imprecise.

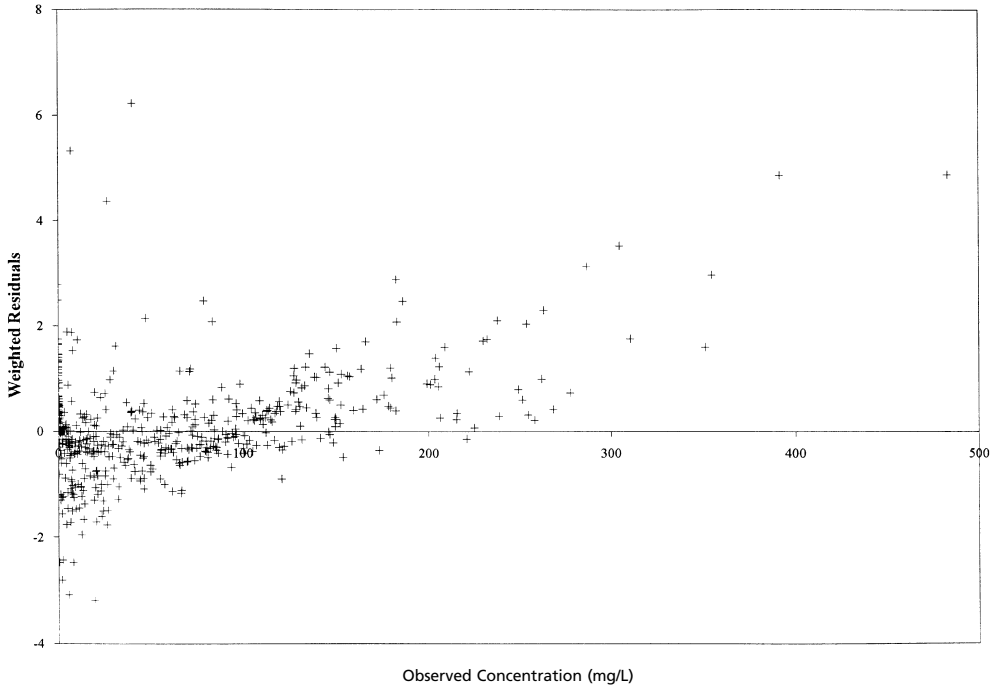

Fig. 7. (b) The plot of weighted residuals vs. observed concentrations shows a lack of homogeneity in the predictions. Lower concentrations are usually overpredicted while high concentrations are underpredicted.

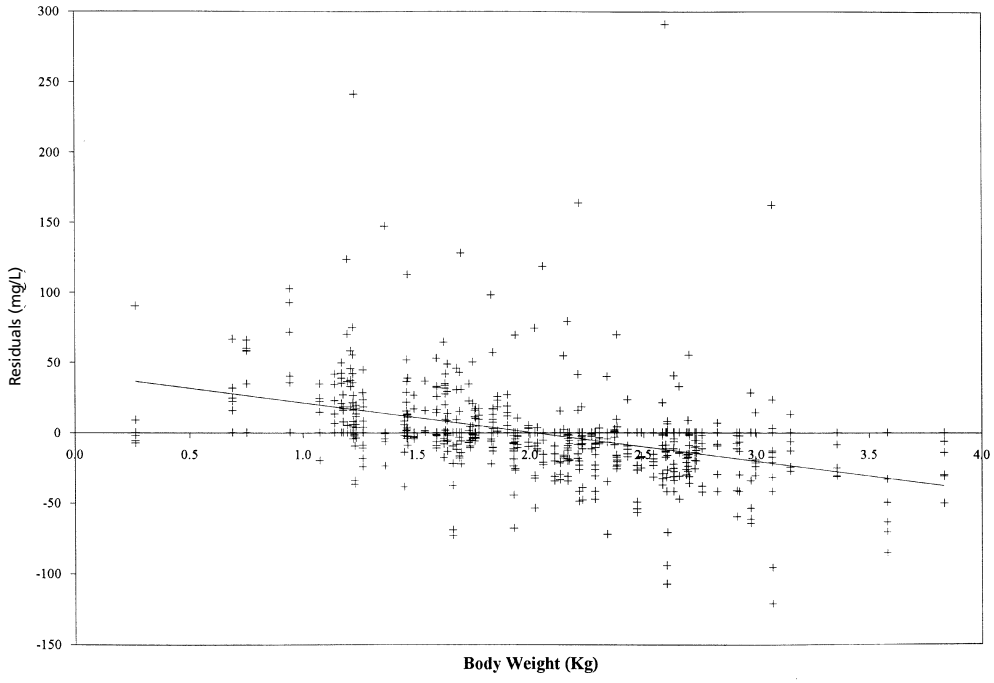

Fig. 7. (c) The plot of residuals vs. body weight shows a lack of homogeneity in the scatter of the residuals. This indicates that the covariate body weight probably has some type of correlation with clearance and/or volume of distribution.

the new covariate significantly improves the model or not (as mentioned earlier, the tests are conducted by assessing the statistical significance of the difference in the MVOF between the 'full' and the 'reduced' model).

Different models were tested following a stepwise procedure. For simplicity of exposition we will skip a detailed description of the model building procedure. In the final model average clearance was a linear function of weight and creatinine clearance, and average volume of distribution was a linear function of age. The complete model is expressed as:

$$\text{Structural model: } Cp = Co \times e^{-k \times t}$$

$$\text{Regression model: } Cl_{avg} = \phi_1 + (\phi_2 \times \text{WGT}) + (\phi_3 \times \text{CCR})$$

$$Vd_{avg} = \phi_4 + (\phi_5 \times \text{AGE})$$

$$\text{Statistical model: } Cl_j = Cl_{avg} \times (1 + \eta_1) \text{ (interindividual)}$$

$$Vd_j = Vd_{avg} \times (1 + \eta_2) \text{ (interindividual)}$$

$$Cp_{ij} = F \times (1 + \varepsilon_1) \text{ (intraindividual)}$$

Table 1 summarizes the fixed-effect parameter and random-effect

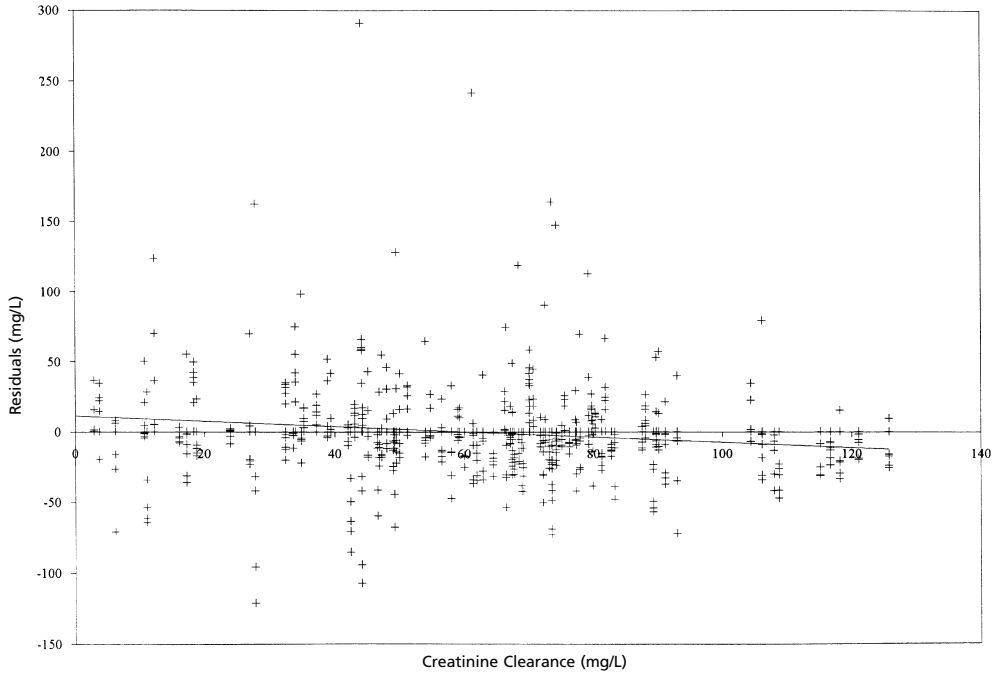

Fig. 7. (d) The plot of residuals vs. creatinine clearance also shows a certain lack of homogeneity that is indicative of some possible influence of this covariate (actually this is a surrogate marker of renal function) in the disposition of the drug.

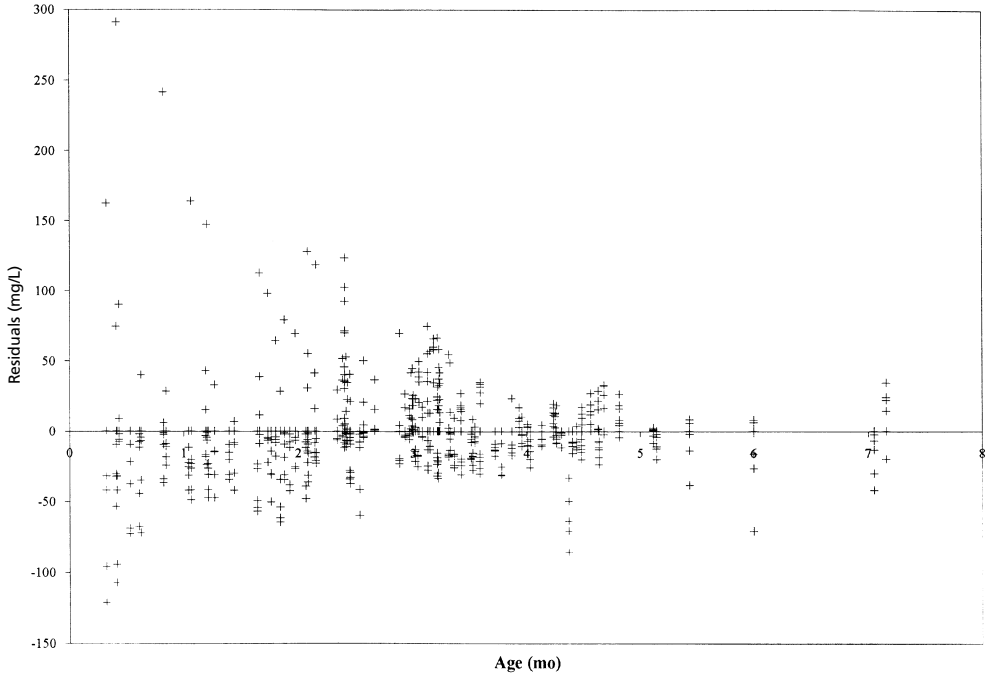

Fig. 7. (e) The plot of residuals vs. age shows a clear trend in the scatter relative to the age of the individuals. The predictions seem to be more precise in the older segments of the population. This suggests that the inclusion of the variable age in the regression model, correlated to one or both pharmacokinetic parameters, will improve the fit.

parameter estimated values for the different models. Note the improvement in the MVOF value at each model building step (it drops 2109 points from the initial to the final model). As was previously mentioned this is a measure of goodness of fit. The decrease in the value of the MVOF that takes place between the reduced and full model is used to formally decide which model is best. Note also the significant reduction in the estimates of interindividual variability once the covariates weight, creatinine clearance and age are included in the model. The coefficients of variation for clearance and volume of distribution for the initial model were 39% and 30%, respectively. The same coefficients for the final model were 10% in both cases. This indicates that once the covariates are included in the model there is less unexplained interindividual variability. The residual variability was reduced from 26% in the initial model to 7% in the final model.

Figure 8a depicts the plot of predicted vs. observed concentrations for the final model. Note the evident improvement in the fit, relative to the initial model (Fig. 7a). Figure 8b shows the plot of weighted residuals vs. observed concentrations for the final model. A comparison of this plot to its initial counterpart (Fig. 7b) shows that the scatter of the residuals is much more homogeneous at all values of plasma concentration in the final

Table 1. Stepwise model build-up for the simulation with the summary statistics. Notice the statistically significant reduction in MVOF as new covariates are added to the model. The random inter and intraindividual variability is also reduced when covariates are added to the model. The values of the fixed-effect parameters for the initial and final models, respectively, were: $\theta_1 = 0.942$, $\theta_2 = 6.8$ (model 1). $\theta_1 = 0.0666$, $\theta_2 = 0.38$, $\theta_3 = 0.0029$, $\theta_4 = 0.769$, $\theta_5 = 1.48$ (model 4)

Model No.	Pharmacostatistical model	C.V. (%)			Objective	Compared	
	Cp_{ij}: $(Co_j \times e^{-kt_i}) \times (1 + \varepsilon_{ij})$	Cl	Vc	Resid	Function	to Model	*P* Value
1	$Cl = \theta_1 \times (1 + \eta_1)$ $Vc = \theta_2 \times (1 + \eta_2)$	39	30	26	3452	—	$P < 0.0005$
2	$Cl = \theta_1 \times (1 + \eta_1)$ $Vc = (\theta_2 + \theta_3\ \mathrm{AGE}) \times (1 + \eta_2)$	39	24	18	3116	1	$P < 0.0005$
3	$Cl = (\theta_1 + \theta_2\ \mathrm{WT}) \times (1 + \eta_1)$ $Vc = (\theta_3 + \theta_4\ \mathrm{AGE}) \times (1 + \eta_2)$	15	11	8	1545	2	$P < 0.0005$
4	$Cl = (\theta_1 + \theta_2\ \mathrm{WT} + \theta_3\ \mathrm{CCR}) \times (1 + \eta_1)$ $Vc = (\theta_4 + \theta_5\ \mathrm{AGE}) \times (1 + \eta_2)$	10	10	7	1343	3	$P < 0.0005$

model, than in the initial model. This indicates that the final model does a much better job than the initial model at fitting to the data at all levels of concentration. Figure 8c–e shows the plots of weighted residuals vs. the covariates included in the final model (body weight, creatinine clearance and age). Note (by comparison to Fig. 7c–e, respectively) how the scatter of the residuals is much more homogeneous and the magnitude significantly reduced, when the aforementioned covariates are accounted for in the predictive model.

This very simple example of a population pharmacokinetic analysis illustrates the basic concepts and procedures involved in building the predictive model using a parametric method for population pharmacokinetics analysis. Much more sophisticated models can be built to analyse complicated datasets in which data collection design is lacking. Different types of error models can be implemented according to the specific features of the study dataset. As mentioned earlier, validation of the model is always necessary in the case of observational studies. More in depth information on the model building procedures for this and other methods have been published elsewhere (Boeckman *et al.*, 1994; Ette & Ludden, 1995b).

APPLICATION OF POPULATION PHARMACOKINETICS IN VETERINARY MEDICINE

Population pharmacokinetics strategies have been widely applied in human therapeutics and clinical research of human drugs, especially during the last decade. The potential benefits of applying this methodology to veterinary medicine have been previously discussed (Riviere, 1984, 1988) and a study has been recently completed on the population pharmacokinetics of gentamicin in horses using retrospective clinical data (Martín-Jiménez *et al.*, 1998). The key results of this study are summarized in Table 2. The potential for using population pharmacokinetic techniques in different areas of veterinary therapeutics is therefore evident.

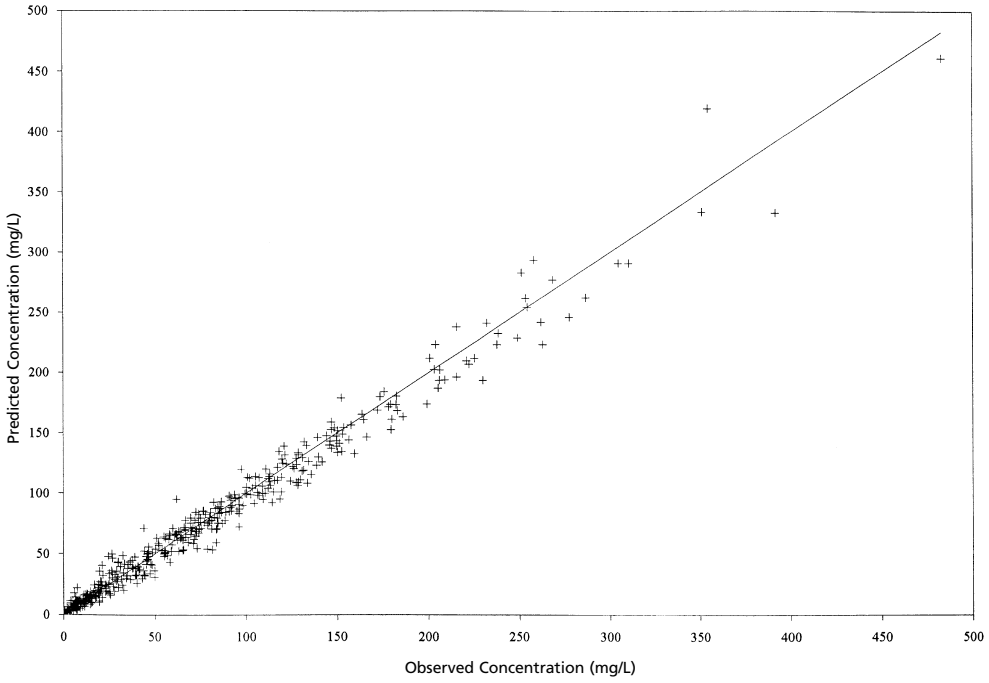

Fig. 8. (a) The plot of predicted vs. observed concentrations for the final model illustrates the dramatic improvement in the precision and accuracy of the predictions over Fig. 6(a), once the covariates age, weight and creatinine clearance are accounted for in the model.

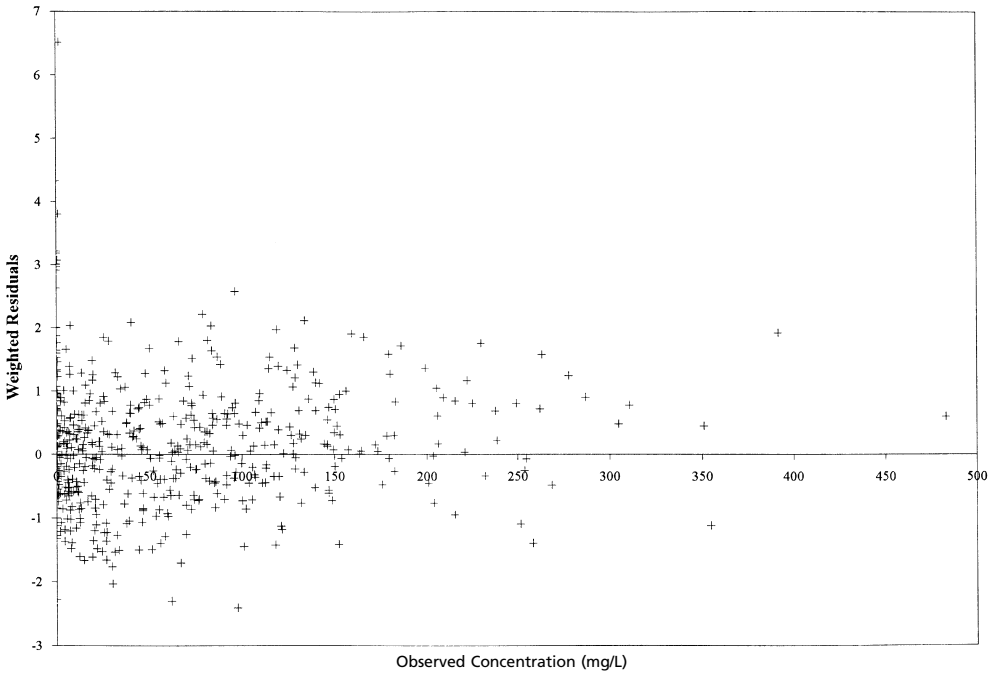

Fig. 8. (b) In contrast with Fig. 7(b), the plot of weighted residuals vs. observed concentrations for the final model shows a more even scatter of the residuals. This indicates that the predictive performance of the model is more homogeneous across all levels of concentration.

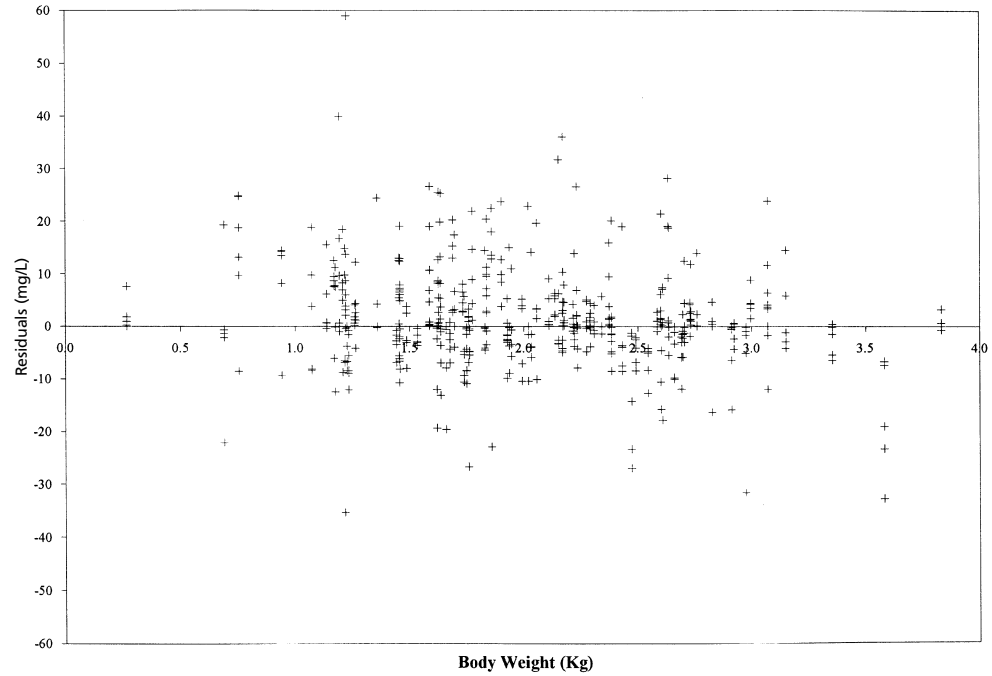

Fig. 8. (c) The plot of residuals vs. body weight shows homogeneous distribution of the residuals. This indicates that once the covariable body weight is accounted for in the regression model, the predictive performance of the population model is significantly improved.

Clinical use

Population pharmacokinetic modelling can be used in the clinical setting in two ways. First, it can be utilized to design dosage regimens for new individual patients or patient-clusters according to their clinical features. Second, population models can be used as prior information in Bayesian forecasting methods to further improve the accuracy of the predictions in a patient from which only a few plasma samples can be obtained.

When a drug is used to treat a pathologic condition in a patient (human or animal), the first objective is to optimize the dose for the individual patient. This is particularly the case when the drug has a narrow therapeutic index and/or a large interindividual variability in its disposition or effect. Variability in therapeutic outcome can be partitioned into pharmacokinetic and pharmacodynamic components. Consequently, pharmacokinetic and pharmacodynamic variability in a population will dictate how confidently the clinician will be able to administer

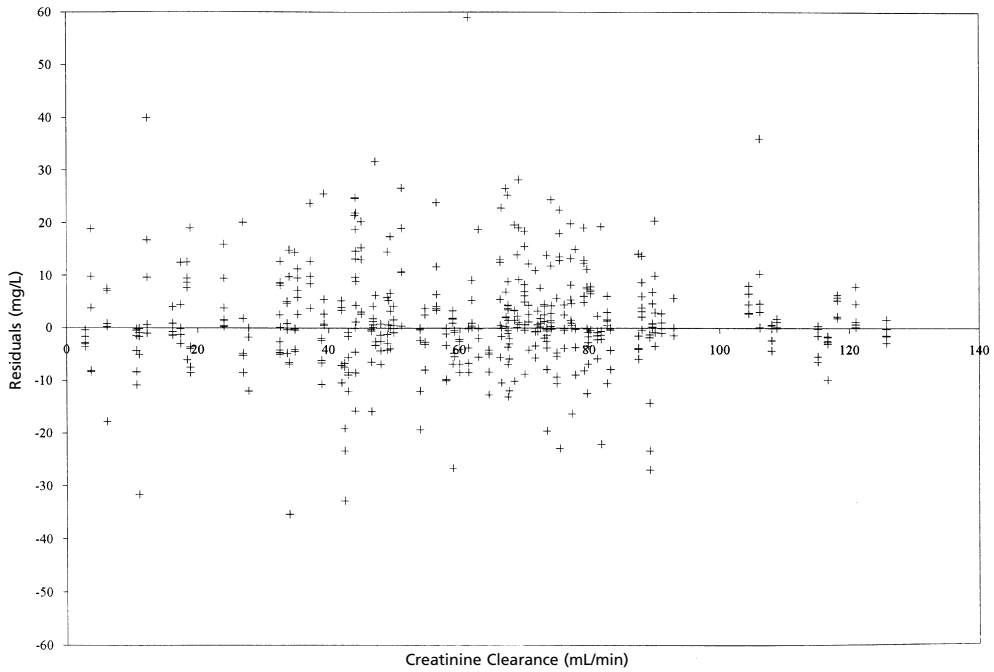

Fig. 8. (d) The plot of residuals vs. creatinine clearance shows a more homogeneous scatter of the residuals, as compared with that of Fig. 7(d). This and the reduction in random variability are evidence in favour of including this covariate in the final model. Creatinine clearance (and consequently, renal clearance) seem to have a significant influence on the disposition of this drug.

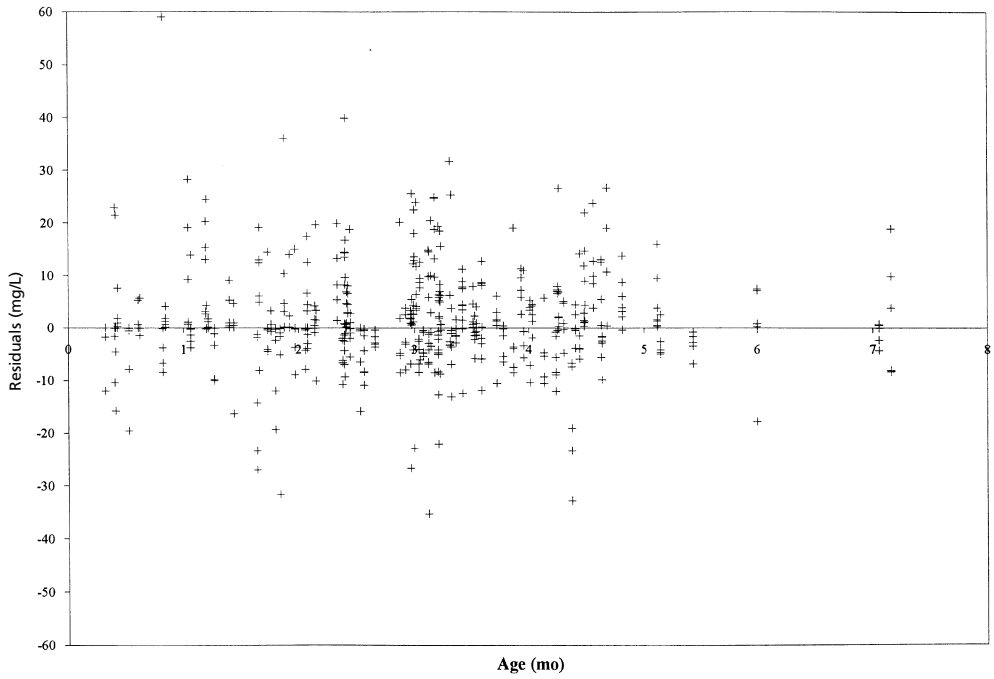

Fig. 8. (e) Plot of residuals vs. age for the final model. Note the dramatic reduction in the magnitude of the residuals and the disappearance of specific trends, when compared with the residual plot of Fig. 7(e).

an average population dose to an individual subject. The magnitude of this variability and the factors which contribute to it are the critical issues in dealing with dose individualization. When drugs exhibit a large variability in disposition across individuals, poor correlation between plasma concentrations and dose will exist. The consequence of this will depend on the pharmacodynamic characteristics of the drug for both the therapeutic and the toxic effects. By explaining part of this variability in terms of a series of pathophysiological variables (weight, age, renal function, etc.), dosage regimens can be designed, using this additional clinical data, that correlate well with serum concentrations for each particular subpopulation as the residual variability is greatly reduced. If the inclusion of pathophysiological variables in the model manages to reduce the interindividual variability to a relatively small magnitude and the pharmacodynamic variability is not large, we can design an optimum dose for each of these subpopulations derived from their average pharmacokinetic parameter estimated values. This is specially valuable for subpopulations that are more prone to deviate from the general population values (e.g. very young

Table 2. Stepwise model build-up for the population pharmacokinetics of gentamicin in horses. In the optimum model, body weight is linearly correlated to both clearance and volume of distribution, and serum creatinine is linearly correlated to clearance. Notice the decrease in the MVOF and the intra and interindividual variability when new covariates are added to the model

Model	Regression model	Coefficient of variation (%)						Compared	
		Cl	*Vc*	k12	k21	Res	MVOF	to model	*P* Value
1	$Cl = \theta_1 \times (1 + \eta_1)$ $Vc = \theta_2 \times (1 + \eta_2)$	59	50	—	—	17	733.3	1-Comp	$P < 0.0005$
2	$Cl = (\theta_1 \times (1 + \eta_1)$ $Vc = (\theta_2 + \theta_3\ \mathrm{WT}) \times (1 + \eta_2)$	60	16	—	—	17	684.8	1	$P < 0.0005$
3	$Cl = (\theta_1 + \theta_2\ \mathrm{WT}) \times (1 + \eta_1)$ $Vc = (\theta_3 + \theta_4\ \mathrm{WT}) \times (1 + \eta_2)$	40	18	—	—	16	583.1	2	$P < 0.0005$
4	$Cl = (\theta_1 + \theta_2\ \mathrm{SCr}) \times (1 + \eta_1)$ $Vc = \theta_3 \times (1 + \eta_2)$	58	40	—	—	17	690.2	1	$P < 0.0005$
5	$Cl = (\theta_1 + \theta_2\ \mathrm{SCr}) \times (1 + \eta_1)$ $Vc = (\theta_3 + \theta_4\ \mathrm{Age}) \times (1 + \eta_2)$	57	33	—	—	17	683.4	4	$P < 0.01$
6	$Cl = (\theta_1 + \theta_2\ \mathrm{SCr}) \times (1 + \eta_1)$ $Vc = (\theta_3 + \theta_4\ \mathrm{WT}) \times (1 + \eta_2)$	55	17	—	—	16	653.1	4	$P < 0.0005$
7	$Cl = (\theta_1 + \theta_2\ \mathrm{Scr} + \theta_4\ \mathrm{WT}) \times (1 + \eta_1)$ $Vc = (\theta_3 + \theta_4\ \mathrm{WT}) \times (1 + \eta_2)$	24	16	—	38	13	433.7	3, 6	$P < 0.0005$ (with both)

individuals, very old individuals, subjects with renal or hepatic impaired functions).

The Bayesian approach to the estimation of pharmacokinetic parameters in an individual takes advantage of both the prior information derived from the population, as well as the scarce information obtained from the actual patient treated with the drug. First, a population model (accounting for patient clinical conditions) is developed and validated. This model (prior probability) is used to develop an initial dosage regimen. This initial regimen will be based on the average population parameter values of the subpopulation to which the patient belongs (for example, 2-year-old beagle dogs with 13 kg of body weight and a serum creatinine of 1.9). The model is reassessed (Bayesian feedback) with new data obtained from a few blood samples from the patient. Finally, the probability distribution of the individual parameters is adjusted (posterior probability) in light of the observed patient's plasma concentrations. Iterative fitting procedures continue, selecting those values of individual pharmacokinetic parameters (Bayesian posterior) that minimize the Bayesian objective function (Rao, 1965; Sheiner *et al.*, 1979):

$$\sum \frac{(P_{\mathrm{pop}} - P_{\mathrm{ind}})^2}{\sigma^2 P_{\mathrm{pop}}} + \sum \frac{(C_{\mathrm{obs}} - C_{\mathrm{ind}})^2}{\sigma^2 C_{\mathrm{obs}}} \qquad \text{eqn 11}$$

where P_{pop} and P_{ind} represent the parameter values of the population pharmacokinetic model and of the patient's individualized model, respectively. C_{obs} and C_{ind} represent the various observed plasma drug concentrations and the estimates of those concentrations made with the patients individualized pharmacokinetic model (for each observation), respectively. $\sigma^2 P_{\mathrm{pop}}$ represents the variance for the different population pharmacokinetic parameter values. $\sigma^2 C_{\mathrm{obs}}$ represents the variance of the observed plasma concentrations. Different studies have determined the validity of this approach in making individualized pharmacokinetic models of drugs in patients (Kisor *et al.*, 1992; Thomson & Whiting, 1992; Jelliffe *et al.*, 1993) and have shown how predictive performance is improved (future serum drug concentrations) relative to the traditional methods of linear regression (Hurst *et al.*, 1990; Jelliffe *et al.*, 1991), in particular when the number of samples available from each patient is small. As the number of individual samples increase, the Bayesian solution approaches that obtained by the traditional least squares method (Higuchi *et al.*, 1988).

Amid the different methods of population analysis that use Bayesian techniques is one called the *Iterative two-stage method* (IT2S). This method uses the IT2S algorithm, that was first proposed by Prévost (1977) and later described by Steimer *et al.* (1984). This method can use rich data, rich and sparse data, or sparse data only, where rich and sparse refer to the number of samples collected within any individual. In this method an initial estimate of the pharmacokinetic (or pharmacodynamic) model is derived by the user (average values of model parameters, standard deviations of those values and model for residual variance). This can be accomplished with data from the literature or using a standard pharmacokinetic analysis (with rich data). Using these initial estimates as prior information in a Bayesian forecasting procedure, a maximum *a posteriori* Bayesian estimator is developed. This estimator allows one to obtain the values of the pharmacokinetic parameters for each individual subject, no matter whether they have rich or sparse data (stage 1). These individual parameter values are used to produce a second population model based on their mean values and standard deviations (stage 2). This second population model is re-entered as prior information in a new Bayesian estimation step and more accurate estimates of the individual parameters are obtained. The process is repeated until stable population parameter means and standard deviations are obtained according to some convergence criterion (basically until the difference between the new and old prior distributions is zero).

This method yields a description of the population (means of pharmacokinetic parameters, variance and correlation, and the residual variance) as well as individual estimates (point estimates of pharmacokinetic parameters and covariance matrices) for

each subject. This approach can be implemented with any kind of software supporting Bayesian estimation and least-squares regression. A computer program (Forrest *et al.*, 1991a,b) using this algorithm has been developed with modules of the ADAPT II package of programs (D'Argenio & Schumitzky, 1979, 1997).

Production medicine

The use of population pharmacokinetic/pharmacodynamic methods in food animals will most likely improve the conditions of herd drug usage in the near future. The ability of these methods to obtain valuable information from large populations in which each individual is sparsely sampled seems ideal to study drug therapeutics in food animals. Differences in drug disposition across individuals could be related to disease conditions, management practices, lactation status, or breed. This know-ledge, together with a better assessment of the sources and magnitude of variance will allow a more reasonable use of drugs in these animals. Differences in disposition can be related to individual characteristics and also to subpopulation characteristics, such as breed of animals or crop groups in fish. Consequently population pharmacokinetics in production medicine could be applied both to individual and subgroup therapeutics.

Food animal residue avoidance

As pointed out previously, veterinary medicine deals not only with companion species, but also with animal species that will ultimately serve as source for human food products. In this latter case, the importance of accurately describing the disposition of drugs in animals according to clinical or production variables without designing extensive individual pharmacokinetic studies, is clearly evident, especially considering the influence that these variables may bear in the deposition of drug residues in those animal's tissues or food products (milk, eggs).

Although there is great potential for the population approach to address drug tissue disposition and residue avoidance, adequate strategies for its implementation have yet to be explored. One of the obvious limitations of tissue residue studies is the lack of sufficient tissue samples per individual (unless biopsies are performed) to characterize tissue depletion kinetics individually (only one sample per animal and time point is usually available). To overcome these limitations, population pharmacokinetic studies could be conducted according to a multicompartment experimental protocol. Adequate multicompartmental or hybrid physiological-compartmental models could be analysed in order to define relationships between plasma and tissue concentrations, taking into account the influence of concomitant pathophysiological or production variables. If such plasma-tissue relationships are found during the decay phase, it will be necessary to explore the stability of these relationships. In other words, one must ascertain whether the amount of drug in the tissue compartment divided by the amount of drug in the plasma compartment is constant or equal to some definable function throughout the depletion (e.g. β-phase). Presumably a larger number of experimental individuals than those used for regular population studies would be necessary, as well as a large number of plasma samples per individual ('data rich') to 'compensate' for the scarcity of tissue samples. The outcome would be a model quantifying the relationship between plasma and tissue levels with concomitant variables. Such a model should be able to predict with a high degree of confidence tissue concentrations for determined doses and clinical or production conditions. Likewise, withdrawal times (and appropriate confidence intervals) could be determined for specific individuals or subpopulations, attending at the magnitude of their clinical or production parameters.

The ultimate application of this methodology would be the implementation of a Bayesian type of approach to define therapeutic models useful in the field. Although the initial development of the model would require a relatively large number of animals, these could be the same individuals involved in clinical trials. The strength of the population approach is that data collected from a wide variety of experimental protocols (efficacy, safety, residues) can be pooled into a single model for the drug. The final objective would be to estimate the probability of violative tissue residue levels in a herd undergoing drug therapy by considering the concomitant production variables (e.g. weight, daily gain, etc.) and screening a reduced number of animals in the production unit.

The situation for animal food products other than those derived from animal tissues (e.g. milk or eggs) should be more straightforward. Serial samples can be obtained from these 'compartments' and consequently more accurate pharmacokinetic profiles can be determined for the depletion of drug from these compartments.

Another area worthy of exploration using the population approach is that of allometric interspecies scaling of pharmacokinetic parameters, given the ability of this methodology to directly model large pools of data (often unbalanced) from many individuals (Riviere *et al.*, 1997). Population analysis of data from a single species may be incapable of detecting many covariates that influence the pharmacokinetic-pharmacodynamic (PK-PD) profile of a drug or its tissue depletion characteristics. In contrast, population analysis of data from several species, with body weight and enzymatic composition as covariates, has the potential to unveil allometric relationships which cannot be easily detected by other methods (Vozeh *et al.*, 1996). Studies of this kind would provide veterinarians and drug developers with the ability to extrapolate serum and tissue data across species, taking into account the influence of some important intra and interspecies clinical factors. Mixed effects modelling strategies have proved useful for interspecies allometric scaling using preclinical data (Cosson *et al.*, 1995).

Drug development

Much can be gained from the application of population pharmacokinetic and pharmacodynamic modelling methods during the process of drug development in veterinary medicine. One of the main goals of drug development is obtaining knowledge about the PK-PD characteristics of the drug in

populations. First, the dose-blood concentration relationship is defined. Then, the relationship between blood concentration and effect (some clinical output of interest often modelled as an effect compartment) is identified. The resulting PK-PD model allows for description of dose–response relationships across a population. The ability of this approach to build PK-PD models under diverse experimental and nonexperimental conditions, and tie them to measurable clinical conditions, can be of great advantage in the development of veterinary drugs. In humans, strategies involving the population approach have been advocated for assessing pharmacokinetic and pharmacodynamic variability as well as dose-concentration-effect relationships during the drug development process (Sheiner, 1992). Combined pharmacokinetic-pharmacodynamic models are used to optimize the completion of Phase III studies in humans (clinical trials) (Peck *et al.*, 1992; Steimer *et al.*, 1993; Van Peer *et al.*, 1993).

Although the clinical trials phase of the drug development process seems to be the best suited to population studies, very valuable information can be derived from the implementation of this approach at earlier stages during drug development. At these early stages, population kinetics can be very useful in targeting the appropriate dose to be used in clinical trials. The interest of the sponsor that develops a drug is to minimize the cost, the time, and the number of experimental subjects and patients that are necessary for completion of these studies, i.e. to minimize the amount of data that has to be obtained to demonstrate safety and efficacy of a drug. Well defined population PK-PD models would assist sponsors to avoid drug titration studies where the selected dosages lie on the flat (maximum) portion of the dose–response curve. It would also help the Food and Drug Administration (FDA) identify situations when lower doses may provide comparable efficacy but lower potential for toxicity.

The main goal of population pharmacokinetics is to identify subpopulations of patients whose response differs either with respect to location (mean) or variability, and to tie those differences to some measurable covariate. During clinical trials, population pharmacokinetic-pharmacodynamic models would allow identifying subpopulations which may require a different dosage regimen. This would provide a more efficient way to determine dose ranges. Nowadays, dose ranges are still based upon data collected in healthy animals with homogeneous physiological characteristics. Well defined population PK-PD models would be useful to support supplemental applications (e.g. different dosage regimens, alternative indications, new routes of administration). In the light of the upcoming flexible labelling policies (Martinez *et al.*, 1995) population PK-PD can be a very useful tool for sponsors to provide the required information with a minimum expenditure of resources.

Once a drug reaches the market, continued monitoring of the drug and completion of new population studies would provide additional information that, when compiled with previous information in an integrated database system, would help to define even more precisely the pharmacokinetic-pharmacodynamic characteristics of drugs in every clinical situation. In the case of drugs administered to food animals, this database would provide valuable information to adapt withdrawal times to specific clinical conditions. The extralabel use of drugs in food animals, as implemented by Animal Medical Drug Use Clarification Act (AMDUCA), would especially benefit from this approach, as information from different sources regarding the pharmacokinetics of the drug in edible tissues for different doses and clinical conditions, would allow computation of better estimates of preslaughter withdrawal times. Overall, this would improve the safety of animal products destined for human consumption.

In summary, the use of population modelling approaches during drug development would be optimal as it provides pharmacokinetic information linked to clinical variables using very few samples from each individual animal, and clinical data which is already collected may be used to construct a better defined model.

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APPENDIX I. LIST OF USEFUL TERMS

Bootstrapping

A computer-intensive resampling method for estimating confidence intervals, sampling variances and stability of regression models. In PPK it is used for internal validation of a developed population model.

Jackknife

A resampling technique for reducing bias in parameter estimates.

Model validation

The analysis of the predictive performance and/or stability of the population model.

Bayesian prediction

Predictive technique based on estimating the posterior probability of a set of parameters in an individual based on the prior probability of these parameters (population model) and a small number of individual measurements.

Mixed effect models

A statistical technique that accounts for fixed effects (variables measured without error) and random effects (random variables).