Pharmacokinetic/Pharmacodynamic Integration in Drug Development and Dosage-Regimen Optimization for Veterinary Medicine

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ABSTRACT

Pharmacokinetic (PK)/pharmacodynamic (PD) modeling is a scientific tool to help developers select a rational dosage regimen for confirmatory clinical testing. This article describes some of the limitations associated with traditional dose-titration designs (parallel and crossover designs) for determining an appropriate dosage regimen. It also explains how a PK/PD model integrates the PK model (describing the relationship between dose, systemic drug concentrations, and time) with the PD model (describing the relationship between systemic drug concentration and the effect vs time profile) and a statistical model (particularly, the intra- and interindividual variability of PK and/or PD origin). Of equal importance is the utility of these models for promoting rational drug selection on the basis of effectiveness and selectivity. PK/PD modeling can be executed using various approaches, such as direct versus indirect response models and parametric versus nonparametric models. PK/PD concepts can be applied to individual dose optimization. Examples of the application of PK/PD approaches in veterinary drug development are provided, with particular emphasis given to nonsteroidal anti-inflammatory drugs. The limits of PK/PD approaches include the development of appropriate models, the validity of surrogate endpoints, and the acceptance of these models in a regulatory environment.

KEYWORDS: Pharmacokinetic/Pharmacodynamic modeling, veterinary drug, dosage regimen, interspecies extrapolation, potency, efficacy

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INTRODUCTION

The 2 most important questions in drug development are "Has the right drug been selected?" and "Has the optimal dosage regimen been established?" Both questions can be addressed using an integrated pharmacokinetic (PK)/pharmacodynamic (PD) approach. Whereas PK and PD are traditionally considered in parallel during the drug development process, PK/PD models integrate the PK model (describing the relationship between dose and concentration vs time), the PD model (describing the relationship between concentration and effect vs time), a link model (bridging the PK and PD models), and, ideally, a statistical model (describing intra- and interindividual variability).

When a PK/PD model is employed, both the time course and variability in the effect versus time relationship can be predicted for different dosage-regimen scenarios. In this way, the PK/PD model helps developers select a rational dosage regimen for confirmatory clinical testing. Failure to determine a safe and effective dosage regimen for use in pivotal clinical trials has been acknowledged as a frequent flaw encountered during the development of many drugs for humans.[1] In veterinary medicine, the situation is even more complex because of potential interspecies differences in kinetics and dynamics. While dosage regimens of drugs with narrow therapeutic windows are often adjusted to account for PK/PD relationships (eg, 1 mg/kg xylazine in dog vs 0.075 mg/kg in cattle; 1 mg/kg succinylcholine in cat vs 0.02 mg/kg in cattle), they are largely ignored for drugs with less obvious dose-response relationships. Accordingly, the same dosage regimen is often pragmatically recommended for different species (eg, 2 mg/kg marbofloxacin in cattle, pig, dog, horse, and cat; 5 mg/kg enrofloxacin in cattle, dog, cat, and horse). An extreme case of "intercompany harmonization" in dosage regimen is observed with endectocides approved for use in cattle (abamectin, eprinomectin, ivermectin, doramectin, and moxidectin). In these cases, similar dosage regimens are recommended for both subcutaneous administration (200 µg/kg) and

pour-on application (500 µg/kg), despite differences in drug disposition and spectrum of activity.

The main impediments to implementation of a PK/PD approach in drug development include poor understanding of PK/PD concepts by developers[2] and, in veterinary medicine, the lack or inadequate recognition of its value by certain regulatory authorities (eg, The European Agency for the Evaluation of Medicinal Products (EMEA) guidelines for PK). Nevertheless, recent evidence clearly demonstrates that veterinary drug sponsors acknowledge PK/PD as a powerful tool that is well suited for multispecies development. In addition to its use for targeting safe and effective dosage regimens, other opportunities for integration of PK, PD, and toxicokinetics in rational drug development have been reported.[3,4]

In this review, the underlying concepts of PK/PD will be examined, with emphasis placed on its application in rational drug development for veterinary medicine. Technical aspects of PK/PD modeling have been widely discussed elsewhere.[5-13]

DOSE-EFFECT RELATIONSHIP, AND THE LIMITS THEREOF

The classical (regulatory) approach in dose selection is dose ranging based on a parallel dose-response design. With a parallel design, animals are randomly assigned to one of the different dose levels, and the effects are compared using a statistical test of hypotheses (Figure 1). Mathematically, this design is described by the equation:

$$Y_{ij} = \theta_j + \varepsilon_{ij} \qquad (1)$$

where:

Y_{ij} is the observed response with the jth dose in the ith subject

θ_j is the expected mean response for the jth dose

ξ_{ij} is the error about the observed versus expected response for the jth dose in the ith subject.

This design has 2 serious drawbacks. First, it is unable to provide information on the shape of the individual dose-response curve,[14] which, for reasons described later, is of great clinical value (Figure 2). Second, the "effective dose" is imposed by statistical analysis (ie, by testing the null hypothesis) and therefore is highly dependent on the power of the study. For this reason, studies with a small

sample size and large response variability ineluctably lead to selection of a high dose.[15,16] It is now recognized that pharmaceutical companies attempt to register excessively high doses,[17] which leads in turn to subsequent dosage adjustments.

An alternative approach is the use of a crossover design where each animal receives the various doses under consideration. In this way, individual dose-response curves can be generated. As shown in Equation 2, if an adequate number of animals are included in this investigation, the generated data can provide information on the distribution of individual animal dose-response parameters[14] (Figure 1).

$$E_{ij} = \frac{E\max_i \times Dose_{ij}}{ED_{50j} + Dose_{ij}} + \varepsilon_{ij} \qquad (2)$$

where:

E_{ij} is the observed effect with the jth dose for the ith subject

$E\max_i$ is the maximum effect for the ith subject

$Dose_{ij}$ is the jth dose administered to the ith subject

ED_{50i} is the dose producing half the Emax for the ith subject

ξ_{ij} is the difference between observed and expected effects when the ith subject is administered the jth dose.

The significance of each of these parameters is explained in detail below. Most significantly, it is important to note that when this design is used (as opposed to the parallel design), the decrease in parameter variability allows the resulting observations to provide a more powerful estimate of the dose-effect relationships.

Dose-effect predictions are generally achieved by fitting the study data to an appropriate PK/PD model. In this regard, one of the basic types of relationships can be described by an Emax model:

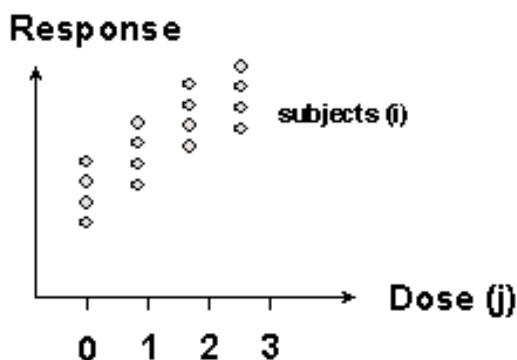$$Effect = E_0 + \frac{E\max \times Dose}{ED_{50} + Dose} \qquad (3)$$

where:

Effect (dependent variable) is the predicted effect for a given dose (independent variable)

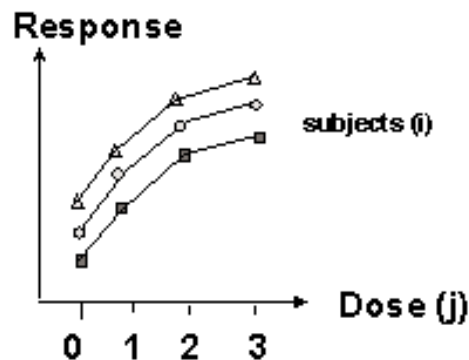E_0 is the effect with no drug (placebo effect)

Emax is the maximum effect

ED_{50} is the dose producing half Emax, a measure of drug potency

Parallel design

Response

Crossover design

Response

- **Hypothesis :** The null hypothesis (H0)
  placebo = D1 = D2 = D3

  Description of the alternative hypothesis (H1)

- **The model :** Linear model
  $$Y_{ij} = \theta_j + \varepsilon_{ij}$$

  Structural non-linear model
  $$E_{ij} = \frac{E_{max} \times Dose_{ij}}{Dose_{50i} + Dose_i} + \varepsilon_{ij}$$

- **Conclusion**
  e.g. D3 = D2 > D1 > Placebo

  Evaluation of 2 parameters for each subject : Emax : maximum response and Dose$_{50}$: dose producing half Emax

- **Information about population:** No

  Yes

Figure 1. Parallel versus crossover design for establishing dose-effect relationships. In a parallel design (left panel), animals (*i*) are randomly assigned to a dose level (*j*). Data analysis is performed using a statistical linear model to test the null hypothesis (lack of dose effect, ie, equality of mean effects). Rejection of the null hypothesis leads to selecting one of the tested doses. No interpolation is possible and, because of its lack of power, the parallel model encourages the selection of higher than necessary doses to achieve the desired therapeutic benefit. Advantages are simplicity and rapidity. In a crossover design (right panel), all animals typically receive each dose level, yielding information on individual dose-response curves. Data can be modeled using a nonlinear mixed-effect model (ie, modeling alternative hypotheses) to allow for intra- and interindividual variability, with covariates. Drawbacks of using a crossover design include implementation difficulties (eg, for antiparasitic drugs), risk of carryover, and long duration.

E_0, ED_{50}, and Emax are the parameters of interest

It should be realized that ED_{50} is not a pure PD parameter but a hybrid PK/PD variable. In this regard, ED_{50} reflects 3 different determinants, as described by the following relationship:

$$ED_{50} = \frac{Clearance \times EC_{50}}{Bioavailability} \qquad (4)$$

where:
Clearance is the plasma clearance
Bioavailability is the ratio of the area under the curve (AUC) of concentration versus time for a noninstanta-neous route of administration versus that effected following intravenous administration (where the bioavailability factor ranges from 0 to 1)

EC_{50} is the plasma concentration corresponding to Emax/2

Inspection of Equation 4 shows that 2 PK factors (clearance and bioavailability) and one PD factor (EC_{50}) can influence ED_{50}. For this reason, ED_{50} cannot be considered a drug property, and an ED_{50} must be determined for any new formulation or set of administration conditions. This point underscores the limitations associated with attempts to establish a dose-effect relationship. Confounded within any such evaluation is the underlying
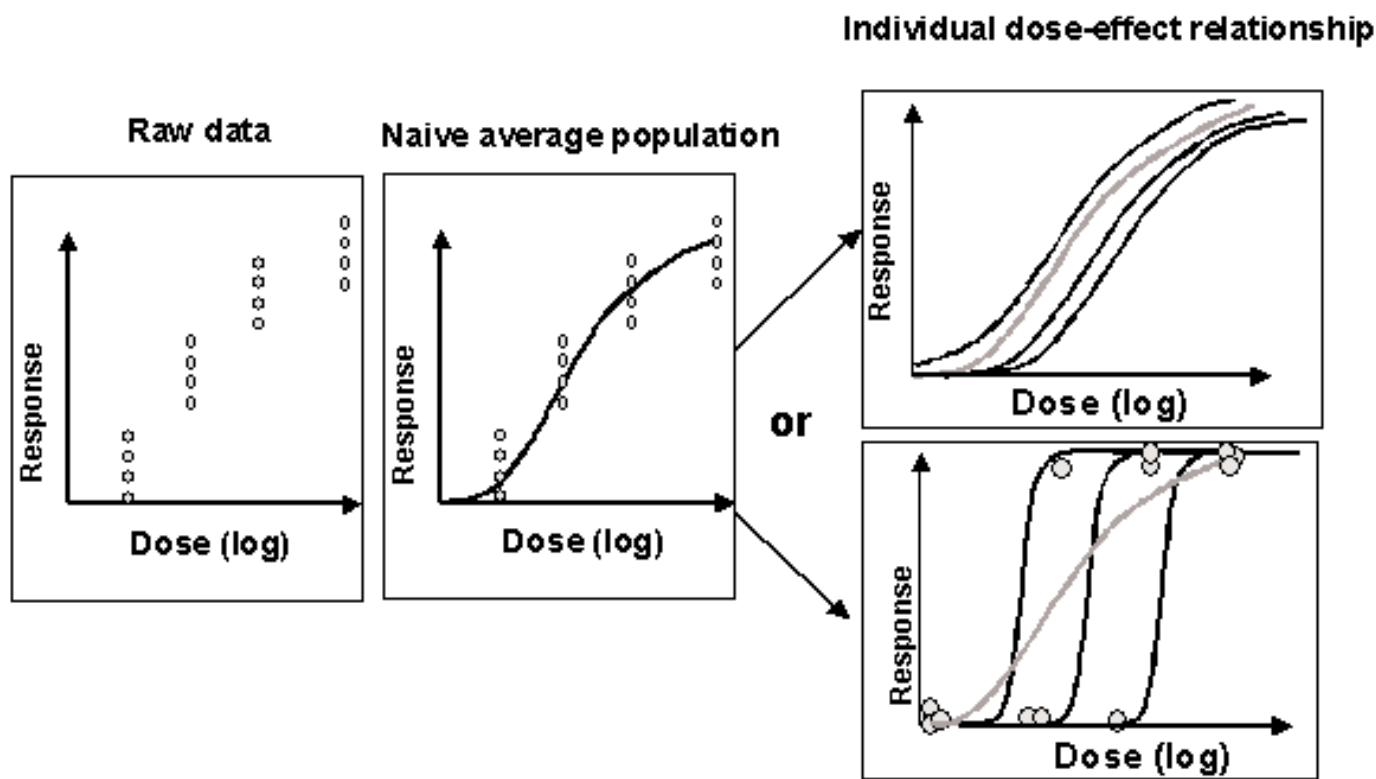
Figure 2. Parallel design for dose-effect relationship and individual dose-effect relationship. The parallel design recommended by regulatory authorities provides only a mean response to a given dose level (left panel). There is no information on individual dose-response relationships. Naive pooling of the raw data (middle panel) can be very misleading because it can result from very different individual dose-effect relationships (right panel). It cannot distinguish conditions where either all individuals have a shape similar to that of the naive average dose response (top right panel) or the existence of subpopulations that respond differently to the various drug concentrations (bottom right panel). See reference 14 for more explanation.

individual patient's correlation between dose and systemic drug exposure (ie, product and drug PK). This recognition has provided the impetus for developing more precise methods that focus on describing PK/PD relationships.

Figure 3 shows the fundamental differences between a dose-ranging trial and a PK/PD trial. While both seek to document the relationship between dose (input) and response (output), dose ranging is simply a black-box approach, with no reference to the mechanisms linking dose and response. On the other hand, the PK/PD approach opens this black box, allowing the investigator to recognize the 2 primary processes that separate dose from response. The first step in this process (PK) concerns transformation of the dose into a plasma concentration profile. The second step in this process (PD) describes the relationship between an independent variable (the drug concentration profile) and the dependent variable (the intensity of drug action). In this way, this method of data analysis enables the investigator to estimate an EC_{50}, thereby providing an estimate of drug potency. Moreover, the relative value of EC_{50} and some measure of toxicity (eg, the concentration needed to reach 50% of some maximum toxic effect, TC_{50}), is an invaluable tool for understanding the cautions that must be exercised if this drug is used in a clinical setting.

Unlike ED_{50}, EC_{50} is a true PD parameter. For a given endpoint, there exists a single (steady-state) EC_{50} value that is not influenced by PK parameters, administration route (unless there is formation of active metabolite by a first-pass effect), or formulation. Moreover, because EC_{50} is a drug-dependent parameter (as opposed to ED_{50}, which is a formulation-dependent variable), use of a PK/PD approach precludes the need for replicating these trials if a drug sponsor elects to develop an alternative formulation. For this reason, EC_{50} is much more relevant
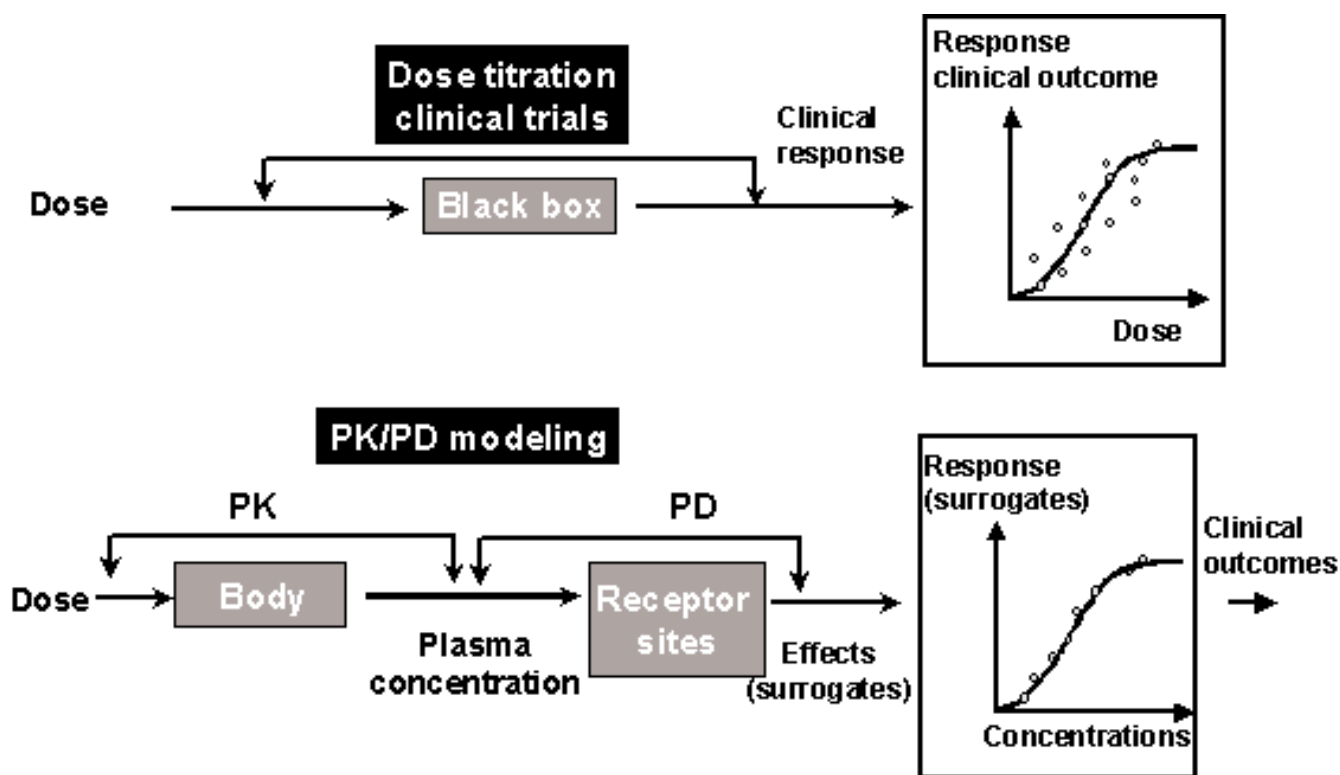
Figure 3. Dose-effect relationship versus PK/PD approach for establishing a clinically relevant dosage regimen. A dose-effect relationship is a black-box approach in which the independent variable is dose and the dependent variable is the clinical response of interest. Variability tends to be high (top right box) and may be of both PK and PD origin. In a PK/PD approach (bottom panel), the black box is opened and transformed into 2 "gray boxes" (for PK and PD, respectively). The plasma concentration profile becomes the independent variable explaining response. The variability (bottom right box) in the concentration-response relationship (which is lower than that associated with a dose-response relationship) is of purely PD origin. The advantage of a PK/PD trial is that an effect is explained in terms of a plasma concentration profile, the latter being an independent variable that is more informative with respect to time development of effect than is dose. The main drawback of the PK/PD approach concerns the clinical validity of the surrogate effects. These are generally used as endpoints, rather than actual clinical outcomes.

than ED_{50}, and its determination is one of the main goals of a PK/PD experiment.

Whereas dose is merely a nominal mass (with no intrinsic biological information), the concentration versus time profile reflects dose, formulation, and the major physiological processes affecting the distribution and residence of that drug within the animal (clearance, rate constant of absorption, etc). In addition, plasma concentration profiles provide information on temporal changes in response, allowing time to be an independent variable in PK/PD trials. For this reason, a PK/PD trial is the most suitable method for simultaneously determining the 2 main components of a dosage regimen: dose and dosage interval.

Drug action is mediated by the time course of free drug concentration that is available to bind to a targeted receptor. Since free drug plasma concentration equilibrates with the free drug at the biophase, plasma (total) concentrations are generally a suitable surrogate for assessing the active (free) drug concentrations. However, free concentrations must be considered whenever there is nonlinearity in plasma or tissue binding, as was observed during the PK/PD investigation of an angiotensin-converting enzyme inhibitor (ACEI) in dog.[18]

Another advantage of using the plasma concentration profile for these evaluations is that Emax and EC_{50} can be estimated following a single-dose administration. In this regard, measurement of the complete plasma concentration-time profile allows for single-sweep coverage of the entire concentration-effect relationship. This contrasts

sharply with dose-ranging designs, where at least 3 nonzero dose administrations are required. Comparison between these 2 approaches (dose-ranging vs PK/PD) for nimesulide use in dog showed that a PK/PD trial was able to determine both dose and dosage interval following a single oral-dose administration. The predictions obtained from these PK/PD models were consistent with those obtained through conventional dose titration (dose-ranging studies) and were validated in clinical trials.[19]

Although sources of PK variability in veterinary medicine (eg, species, breed, age, sex, dietary factors, kidney and liver function) have been widely discussed, sources of PD variability have been largely ignored. Nevertheless, it is recognized that PD variability can be far more pronounced than that associated with PK.[20] This is especially true in the case of antibiotics, where clinical response is affected not only by the ability of the drug to get to the site of infection but also by PD variability, such as the host response to the invading pathogen, the integrity of the host immune system, and the distribution of bacterial susceptibility as measured in vitro by the minimal inhibitory concentration (MIC).

One of the main advances facilitated by PK/PD approaches has been the application of computer-based methods for separating the 2 main sources (PK and PD) of variability through the use of population PK/PD approaches.[21] The conceptual framework behind population PK in veterinary medicine has been described by Martin-Jimenez and Riviere.[22] Through the use of mixed-effect models, population analysis provides an opportunity to explain variation between animals (or groups of animals) in terms of breed, age, disease state, level of production, and so forth. These models are capable of handling pooled (often sparse) data with allowance for fixed effects (eg, impact of weight, clinical score, age, breed, MIC) and random effects (eg, unexplained error). Improving the power of the PK and PD parameter estimation procedure will ultimately improve investigators' ability to develop optimal dosing regimens. Although widely used within the human pharmaceutical community, population PK/PD is still used only rarely in veterinary medicine.

Currently, there are 2 primary areas in veterinary medicine where population PK/PD tools are being considered: antimicrobial drugs and cancer chemotherapy. In terms of antibiotics, population PK/PD models can be invaluable because of the close interrelationship between the optimization of therapeutic effects and the minimization of emerging resistance. These models are well suited for breakpoint setting since PD variability, chiefly as regards bacterial susceptibility (MIC distribution), can easily be qualified by in vitro testing.[23]

In cancer therapy, population PK/PD should replace the outdated and debatable surface law as the best means of optimizing efficacy and minimizing toxicity.[24] With surface law, dosage regimen is adjusted on the basis of a power function to the measured body weight (BW). This is taken to represent skin surface area. This results in a disproportionately high dosage (mg/kg) being administered to small animals. For example, the recommended doxorobucin dose in dog is 30 mg/m^2, which results in an estimated dose of 1.74 mg/kg for a 5-kg dog versus 0.96 mg/kg for a 30-kg dog. This difference in mg/kg dose explains the frequent toxicity observed in small (<10 kg) but not in large (>10 kg) dogs.[25] Based upon this observed trend in drug toxicity, it was concluded that a 1 mg/kg dosing regimen provides more uniform therapeutic and toxic responses. With regard to the applicability of modeling tools in this situation, if size is responsible for the observed inter- and intra-animal variability in PK/PD, an experimental (population) relationship should be established. There exists no a priori reason for determining an optimum dose on the basis of some standardized dose proportionality to BW ($BW^{1.0}$), body surface area ($BW^{0.67}$), metabolic weight ($BW^{0.75}$), or any other power model. For this reason, antineoplasic drug development should include PK/PD studies that use fixed dosing regimens and apply population PK/PD models to examine the dose-response relationships as affected by such variables as surface area, BW, gender, or kidney and liver function.[26]

The PK/PD paradigm is consistent with a general objective whereby firms are encouraged to pursue new drug approval on the basis of a flexible labeling philosophy[27] accommodating adaptation of dosage regimens to PK and/or PD variabilities.

Within a given species, the intraindividual and interindividual variability in PD parameters is likely to be as high as or higher than that associated with the PK parameter estimates. However, the primary source of between-species variability is often attributable to variability that is mainly of PK origin. For example, it appears that the (free) drug plasma concentration required to elicit a given response is rather similar between species, whereas the

corresponding dose for eliciting the same effect can differ widely.[20] In this regard, the PK/PD approach offers a powerful general framework for interspecies extrapolation.

One of the best ways to extrapolate a dose from 1 species to another is to assume that drug potency is species independent—that is, that the same overall body exposure (AUC for plasma concentration) will produce the same effect in both species. For intravascular administration, the only determinant for AUC is plasma clearance. The following relation therefore holds:

$$AUC_{species\ 1} = AUC_{species\ 2} = \frac{Dose_{species\ 1}}{Cl_{species\ 1}} = \frac{Dose_{species\ 2}}{Cl_{species\ 2}} \quad (5)$$

Therefore, to estimate the dose for species 2 from an efficacious dose in species 1, the following equation can be applied:

$$Dose_{species\ 2} = \frac{Dose_{species\ 1} \times Cl_{species\ 2}}{Cl_{species\ 1}} \quad (6)$$

where $Cl_{species}$ and $Cl_{species2}$ are plasma clearances for species 1 and 2, respectively.

A refinement to Equation 6 consists of introducing a bioavailability factor, F, for extravascular administration. If plasma protein binding differs widely between the 2 species, the equation will also need to include an allowance for free fraction, fu, since it is only the free concentration that is responsible for the ultimate effect:

$$Dose_{species\ 2} = \frac{Dose_{species\ 1} \times fu_1 \times Cl_{species\ 2}}{fu_2 \times Cl_{species\ 1}} \quad (7)$$

Here, fu_1 and fu_2 are free fractions for species 1 and 2, respectively.

To illustrate the use of Equation 6, the most likely dose for morphine in dog and horse can be calculated from the dose recommended in human. The recommended dose in human is 10 mg in toto (ie, about 0.17 mg/kg), and plasma clearance in human is reported to be 14.7 mL/kg/min.[28] In dog, the morphine plasma clearance is higher (85 mL/kg/min),[29] which leads to an estimated dose of about 1 mg/kg for dog. In contrast, the morphine plasma clearance in horse is rather low (8.64 mL/kg/min),[30] which implies that a lower dose is needed

in horses (0.1 mg/kg). No correction for drug binding to plasma protein is required since the extent of plasma binding is similar across all 3 species.[31] The results obtained with this PK/PD approach to interspecies dose extrapolation is consistent with the doses currently recommended for use in horse and dog.

Extrapolation from in vitro to in vivo is another fruitful application of the PK/PD paradigm. If an efficacious concentration (*EC* for stimulation, *IC* for inhibition) is obtained on the basis of an in vitro or ex vivo assay, then a dose can be proposed by incorporating the in vitro *EC* directly into Equation 4. For example, using a membrane feeding system, the IC_{99} of lufenuron (a compound for control of flea infestation in dog and cat) for *Ctenocephalides felis* was found to be from 50 to 100 ng/mL. Assuming a lufenuron blood clearance in cat of about 0.56 L/kg/day,[32] Equation 4 predicts a minimal lufenuron dose of 0.028 to 0.056 mg/kg/day (or 5 to 10 mg/kg per 6 months), which matches the experimentally determined dose of subcutaneously injected lufenuron in cats.[33] The in vitro IC_{50} of carboplatin (an antineoplasic agent in the treatment of melanoma) is 6.1 µM (2.26 µg/mL).[34] The plasma clearance of carboplatin in dog is about 2.6 to 5 mL/kg/min for a 15-kg dog.[35] Thus, the estimated in vivo daily dose is within the range of 8.5 to 16.3 mg/kg. Converting this estimate to surface area, this equals an estimated dose of 200 to 389 mg/m², which is consistent with the recommended dose of 300 to 350 mg/m² for malignant melanoma.[36]

It should be noted that since in vitro concentrations are generally equivalent to free drug concentrations, corrections for drug binding to plasma protein might be needed to estimate the corresponding in vivo plasma *EC* or *IC*.

Direct in vivo estimation of *EC* is a major goal of PK/PD analysis. This necessitates the use of a modeling approach, both for accommodating the PK and PD data and for defining the kind of relationship by which the 2 are linked. While PK models are routinely used in veterinary medicine, PD models are less familiar. Therefore, the latter will be examined in greater detail.

There are 2 main types of PD models: that which describe a graded concentration-effect relationship and that associated with a quantal concentration-response relationship. A graded model is used when a physiological system is able to respond quantitatively to different drug concentrations (eg, blood marker concentration,

survival time, reduction in number of parasites). On the other hand, in a quantal model (also known as a fixed-effect model) the described effects are discrete (eg, dead or alive, presence or absence of extrasystole, parasitic cure or not, appearance of unwanted effects or not). For this reason, a quantal response is measured using a categorical or ordinal scale. For quantal dose-response (or exposure-response) relationships, it is assumed that the animals respond maximally or not at all, and therefore dose or exposure is not related to intensity of effect but rather to the frequency of an all-or-none effect. Quantal responses are often clinical endpoint outcomes, whereas graded responses are often surrogates (see later).

The most general model for a graded effect relationship is the Hill model, also known as the sigmoidal *E*max model:

$$E(t) = E_0 + \frac{E\max \times C^h(t)}{EC_{50}^h + C^h(t)} \qquad (8)$$

where:
E(t) is the effect observed for a given concentration at time *t (C(t))*
*E*max is the maximal effect attributable to the drug
EC_{50} is the plasma concentration producing 50% of *E*max
h is the Hill coefficient, which adjusts the degree of sigmoidicity in the curve. When *h* = 1, the Hill model reduces to the *E*max model, which corresponds to a hyperbolic function (Figure 4).

The *E*max model originates from classical receptor theory as a description of ligand-receptor interaction. However, in PK/PD modeling it serves primarily as an empirical model.

Many drug effects involve modulation of a physiological variable (eg, blood pressure). In this case, the inclusion of the term E_0 in Equation 8 provides for the presence of a baseline effect. E_0 can also be used to traduce any placebo effect or a background effect that may vary depending upon the physiologic status of the patient (eg, the impact of normal vs compromised immune function on recovery from a microbial infection).

When drug effect corresponds to the inhibition of a biological process, the drug effect is subtracted from the baseline (E_0) and Equation 8 can be rewritten:

$$E(t) = E_0 - \frac{E\max \times C^h(t)}{IC_{50}^h + C^h(t)} \qquad (9)$$

where IC_{50} is the concentration producing 50% of the maximum inhibition effect (*E*max).

If the drug is capable of fully suppressing the measured effect, *E*max takes the value E_0, and after factorization, Equation 9 can be rewritten:

$$E(t) = E\max\left[1 - \frac{C^h(t)}{IC_{50}^h + C^h(t)}\right] \qquad (10)$$

where $C^h(t)\Big/\left[IC_{50}^h + C^h(t)\right]$ is the "fractional" *E*max ratio (from 0 to 1), which describes the fraction of maximal effect that can be reached by a given concentration of the drug. This model is also termed the fractional Hill model.

For simpler graded PD models (linear, log-linear), see Holford and Sheiner[5,7] for details.

Equations 8 and 9 contain an independent variable (concentration), a dependent variable (*E*), and several parameters (E_0, *E*max, EC_{50} or IC_{50}, and *h*). The ultimate goal of PK/PD modeling is to evaluate the means and variances of these parameters from the observations of *E* obtained over a range of *C(t)* values (the independent variable). Given the importance of understanding the precise meaning and significance of these variables and parameters, they are described in detail below.

The dependent variable *E* is named after the generic term "effect." However, according to Holford,[37] it can be useful to distinguish between drug action, drug effect, and drug response. For example, the action of a nonsteroidal anti-inflammatory drug (NSAID) is to inhibit cyclo-oxygenase (Cox). The effect of this inhibition is suppression of the production of proinflammatory prostaglandins. The clinical response corresponding to decreased production of proinflammatory prostaglandins is a reduction or suppression of lameness. When a PK/PD trial is mechanistically oriented, the drug action at a primary site (eg, at the enzyme receptor) should be measured (eg, the use of ex vivo studies designed to compare the potency of different NSAIDs in selectively inhibiting Cox_1 or Cox_2 isoenzymes). In contrast, if the purpose of a PK/PD trial
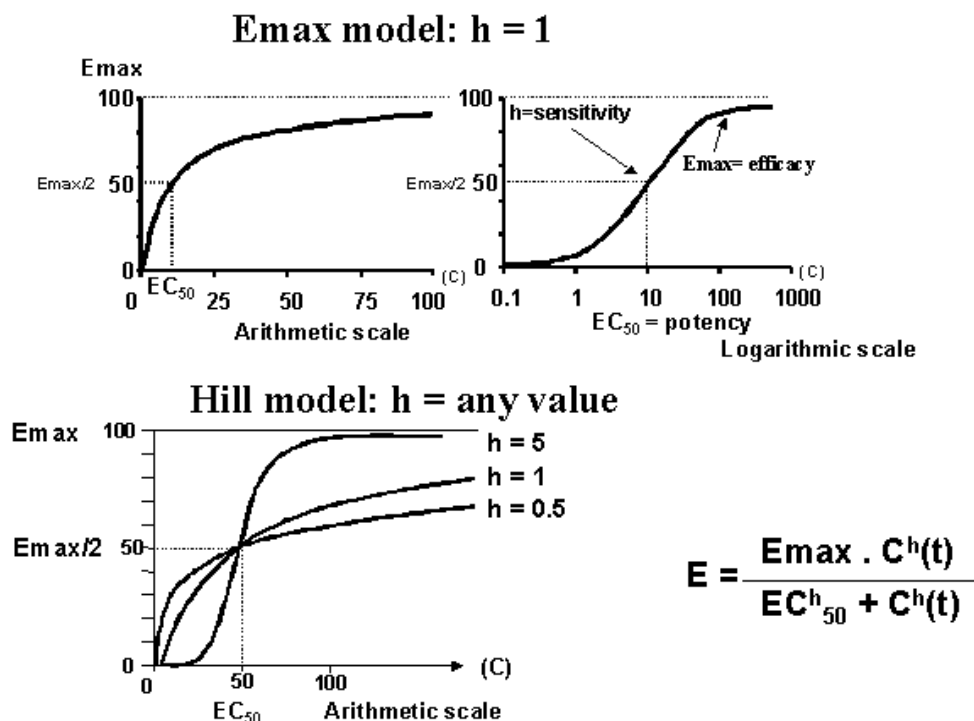
Figure 4. *E*max versus sigmoid model. Top: Graphical representations of the basic *E* max model using arithmetic scale (left) or logarithmic scale (right). With an arithmetic scale, the concentration-response curve is hyperbolic and asymptotically approaches the maximal response (*E* max), a measure of clinical efficacy. EC_{50}, the concentration corresponding to *E* max/2, is a measure of drug potency. When the same data are plotted using a logarithmic transformation of the concentration axis, a typical S-shaped log concentration-effect curve is obtained. This presentation, useful when there is a wide range of concentrations, facilitates comparative examination of different concentration-effect curves (eg, desirable vs undesirable effects). Bottom: A sigmoid model is an *E* max model for which *h* (the Hill coefficient) can be other than 1. The 3 curves were obtained using the same *E* max and EC_{50} but with different values for *h* (5, 1, and 0.5). The slope of the curve changes as a function of *h*. For *h* >1 the curve becomes sigmoid, with a steeper slope in the middle. For *h* >5 the curve becomes an all-or-none response curve. For *h* <1 the initial portion is above the hyperbolic curve at low concentrations but shallower after reaching EC_{50}. Inset: Equation for *E* max (*h* = 1) or Hill (*h* ≠ 1) model.

is to determine a dosage regimen for an NSAID, then the response of clinical interest (eg, lameness) should be measured.

Very often, the drug response of interest will be difficult to obtain (eg, bacterial cure for an antibiotic), difficult to quantitatively measure (eg, mood elevation elicited by an antidepressant), delayed in time (eg, survival time for cancer therapy), or unethical to measure (eg, necropsy score for safety evaluation). Therefore, the effect of ultimate interest in a PK/PD trial may be replaced by a surrogate endpoint. This will be a biomarker—that is, a characteristic (physical sign, blood analyte, physiological measurement, etc) that is objectively measured and validated as an indicator of a normal biological process, a pathogenic process, or a pharmacological response to a therapeutic intervention. In these studies, the surrogate

endpoint is intended to substitute for a clinical endpoint. A number of recent reviews[38-40] discuss the definition, characterization, validity, advantages, and limits of surrogates in the context of clinical trials. The clinical validity (relevance) of a surrogate is determined by its statistical association and mechanistic links with a clinical outcome (surrogate accuracy). In addition, the surrogate should have desirable metrological properties, namely reproducibility (of measurement), continuity (for a graded quantitative measurement), objectivity, specificity, and linearity.[41]

Examples of surrogates used in veterinary medicine include the PK/PD indices that have been proposed for predicting clinical success and bacteriological cure of antibiotics such as the inhibitory AUC, peak concentration versus MIC (*C*max/MIC), and time above MIC

(t>MIC). Prospective and retrospective trials in human medicine have demonstrated statistical correlations between the surrogate markers and either clinical success or prevention of resistance emergence. These indices are mechanistically related to clinical outcome since they are all constructed using the MIC value.[23] For ACEIs that help prevent heart failure (such as benazepril and enalapril), PK/PD relationships have been investigated using plasma and tissue angiotensin converting enzyme (ACE) inhibition. Based upon these relationships, canine dosage regimens have been for doses that totally inhibit ACE activity.[18,42] As with the case of AUC/MIC or Cmax/MIC, ACE inhibition is only a surrogate endpoint. Nevertheless, its utility has been documented, given that it is a more rapid method for estimating effect than is the traditional approach of estimating survival time and is easier to quantify than improvement in quality of life, the latter 2 endpoints being the ultimate goals of ACE inhibition therapy.

In Equations 8 and 9, $C(t)$ is the independent variable. In most PK/PD trials, $C(t)$ represents plasma drug concentrations. However, concentrations in other biological matrices are equally valid (eg, urinary concentrations of furosemide[43] or tissue-cage transudate or exudate concentrations for NSAID and antimicrobial drug studies[44,45]).

In the simplest situation, the biophase is plasma or a site in nearly instantaneous equilibration with plasma. Here, the drug concentrations can be directly incorporated into the Hill model. This is the case with ACEI, for which the biophase is both the circulating ACE and the ACE located at the luminal surface of blood vessels[18] and for inhibition of Cox_1 in circulating platelets by NSAIDs.[44] For instance, Equation 11 shows how Equation 8 can be rewritten for a drug obeying a monocompartmental PK model:

$$E(t) = E_0 + \frac{E\max \cdot \left[\frac{Dose}{Vc} \exp\left(-\frac{Cl}{Vc} \times time\right)\right]^h}{EC_{50}^h + \left[\frac{Dose}{Vc} \exp\left(-\frac{Cl}{Vc} \times time\right)\right]^h} \quad (11)$$

where the term $C(t)$ from Equation 8 is $\frac{Dose}{Vc}\exp\left(-\frac{Cl}{Vc} \times time\right)$, the monoexponential equation describing the behavior of plasma drug concentration.

Comparison of Equation 11 with the corresponding equation for a dose-effect relationship (see Equation 3) highlights the difference between a PK/PD model and a classic dose-effect model. The PK/PD model includes and distinguishes between PK parameters plasma clearance (Cl) and volume of distribution (Vc) and PD parameters Emax, EC_{50}, and h. In addition, inspection of Equation 11 shows that time appears as a second independent variable. For this reason, use of a PK/PD analysis enables us to determine both the optimal dose and the optimal dosage interval. This is important for drugs requiring multiple administrations for reasons of efficacy and/or safety (eg, antiepileptic drugs, antiarrhythmic drugs, time-dependent antibiotics).

For some drugs, the precise time development of drug concentration profile appears to be of secondary interest, owing to the long delay between drug exposure and an observed effect (eg, hematological toxicity in cancer therapy). This is also the case with avermectin drugs, where killing of the targeted parasites is achieved only after several weeks of drug exposure (2-3 weeks for microfilaria of *Dirofilaria immitis* and 5-6 weeks to remove larvae of *Strongylus vulgaris* from the arteries of horse[46]). With avermectins, what is observed is not the direct action of the drug on the parasites but rather the time integral of the direct action—that is, the delayed eradication of the parasites is monitored. Few attempts have been made to model a PK/PD relationship for these drugs. Rather, effects have been modeled with summary PK exposure variables such as AUC or the time above some threshold concentration.[47] Indeed, the Hill model can be simplified by integrating the independent variables (ie, the analytical expression giving rise to $C(t)$). When this is done, Equation 11 becomes:

$$E = E_0 + \frac{E\max \times AUC^h}{AUC_{50}^h + AUC^h} \quad (12)$$

where AUC is now the independent variable. This equation looks like Equation 3, but does, nevertheless, describe a PK/PD relationship. In this case, AUC is given by

F x *Dose* x *Clearance*[-1] and AUC_{50} is the level of exposure giving an effect of Emax/2.

Recently, Karlsson et al[47] presented a more general form of the AUC model, together with a model for time-dependent drugs. These models assume that concentrations elicit a direct but unobserved action, A_{direct}. The

observed clinical response (secondary to drug action), $E_{observed}$, is related to the cumulative action of drug exposure through this direct primary action. $E_{observed}$ is linked to the area under the A_{direct} time curve ($AUCA_{direct}$) according to the following relationship:

$$E_{observed} = \frac{E_{observed\ max} \times AUCA_{direct}}{AUCA_{direct,50} + AUCA_{direct}} \quad (13)$$

where $AUCA_{direct,50}$ is the duration of maximal direct action needed to produce half the maximal observed response. It should be noted that the AUC model described in Equation 12 is a special case of the model described by Equation 13 where $EC_{50} >> C(t)$, with:

$$E_{observed} = \frac{E_{observed,max} \times AUC/EC_{50}}{\left(\frac{AUC}{EC_{50}}\right)_{50} + AUC/EC_{50}} \quad (14)$$

In Equation 14, the independent variable (AUC/EC_{50}) and the potency parameter (AUC/EC_{50})$_{50}$ have time dimensions. It is also important to note that in Equation 14, the independent variable (AUC/EC_{50}) matches one of the indices empirically advanced to predict the efficacy of concentration-dependent antibiotics (ie, AUC/MIC, where the effective concentration, EC_{50}, is replaced by either MIC_{50} or MIC_{90}). Aliabadi and Lees,[48] in recent studies of the antimicrobial activity of fluoroquinolone antimicrobial drugs, have used this form of the Hill equation in studies of ex vivo antimicrobial activity. They used AUC/MIC ratios as the concentration term and, along with establishing the slope of the AUC/MIC bacterial inhibition relationship, they determined from the sigmoid curve AUC/MIC ratios required to produce 3 levels of inhibition: bacteriostasis, bactericidal activity, and elimination of bacteria.

Drug potency, maximal efficacy, and sensitivity are the 3 PD parameters of the Hill model.

Potency (EC_{50}). Potency expresses the intensity of drug activity in terms of concentration. EC_{50} (for stimulation) and IC_{50} (for inhibition) are estimated directly by the Hill model, but other percentage values (eg, EC_{80}) can also be easily computed.

Potency varies inversely with the concentration required to produce the effect (Figure 5). However, provided the required dose can be given conveniently, potency as such is relatively unimportant from a therapeutic perspective.[49] In other words, a more potent drug is not necessarily a more valuable drug (Figure 5). Low potency becomes a disadvantage when the size of the effective dose renders it difficult to administer (eg, for a spot-on drug in pets) or when the plasma molar drug concentration reaches the same order of magnitude as the molar albumin concentration. When the latter occurs, there is a risk of nonlinear binding (eg, phenylbutazone) and an associated risk of nonlinear (ie, poorly predictable) disposition kinetics.

When primary drug action is measured, EC_{50} can tentatively be interpreted in terms of its mechanistic determinants. In both the operational model of drug receptor[50] and an Emax model, the EC_{50} of a full agonist drug represents a hybrid parameter that is governed by the drug's affinity for its receptors (measured by equilibrium dissociation constant, Kd) and by its efficacy. Accordingly, EC_{50} can be mathematically described as[50]:

$$EC_{50} = Kd/(1 + \tau) \quad (15)$$

where τ is a transducer constant measuring transduction efficiency as expressed by the amplification between drug binding (occupancy) and a physiological system response. t itself is a hybrid parameter that is defined as:

$$\tau = R_{total}/KE \quad (16)$$

where R_{total} is governed by the size of the receptor pool and KE is a dissociation constant for the agonist-receptor complex. In the presence of a large value for τ, a low KE can be interpreted as a system giving rise to a highly efficient stimulus-response relationship. We note here that EC_{50} (or IC_{50}) is a hybrid parameter that is linked to a specific drug property (Kd, drug affinity), a specific tissue property (size of receptor pool), and KE, which is both drug and tissue dependent.

At the early stages of drug development, it can be useful to compare drug affinity (Kd) with drug potency (EC_{50}) to determine whether a new compound is an efficacy-driven or affinity-driven agonist. If EC_{50} is much lower than Kd, the drug is said to be efficacy driven, since τ >>1 (Equation 15). In contrast, if EC_{50} is close to (but lower than) Kd, the drug is said to be affinity driven
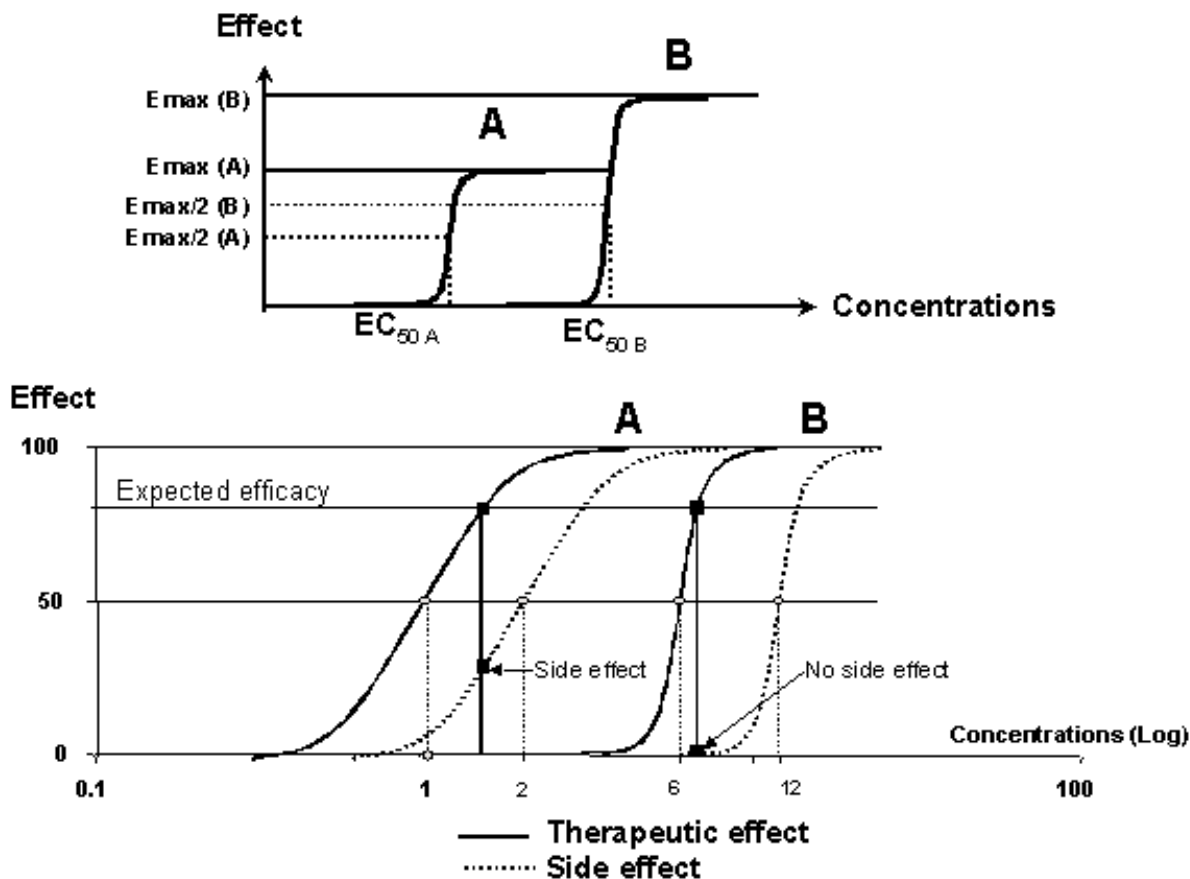
Figure 5. Efficacy, potency, sensitivity, and selectivity in rational drug selection. When a drug is selected from among several competitors, allowance should be made for the 3 parameters in the Hill model: efficacy (E max), potency (EC_{50}), and sensitivity (h). Top: Drug B should be preferred to drug A despite its lower potency (EC_{50B} > EC_{50A}) because B is more efficacious than A (E max$_B$ > E max$_A$). Bottom: Drug selectivity and slope (sensitivity) of the concentration-effect relationship. A is a drug with higher potency than B for both therapeutic (EC_{50A} = 1 vs EC_{50B} = 6) and side effects (EC_{50A} = 2 vs EC_{50B} = 12). A and B have the same clinical efficacy (same E max) and the same therapeutic index of 2 (same ratio of EC_{50} for side effects and therapeutic effect). The drugs differ in sensitivity (slope of concentration-effect curve), and B would probably be preferred to A despite its lower potency, because the steeper slope indicates better selectivity for desired versus undesired side effects. With drug A, significant side effects will be obtained whatever the selected dose is above the EC_{50A} of therapeutic effect.

because τ is close to unity. Efficacy-driven and affinity-driven drugs can be expected to differ in pharmacological profile: efficacy-driven drugs are minimally affected by a change in receptor density (change in potency but not in maximal response), whereas affinity-driven agonists are sensitive to change in receptor density (eg, down- and up-regulation), making the PK/PD relationship rather difficult to predict.[51] This is true across animal species and tissue types. High-efficacy agonists tend to produce full response in all tissues and all species, whereas low-efficacy agonists produce response in only well-coupled tissues. In addition, low-efficacy agonists display more absolute organ selectivity[52] and are more

prone to interspecies differences.[51]

Maximal Efficacy (Emax). Although *E*max is also known as the maximal (clinical) efficacy, this term can be confused with the definition of intrinsic efficacy mentioned in several drug-receptor theories. *E*max is the maximum pharmacological effect that can be generated by a particular system (eg, the maximal possible heart rate). It is the most important parameter for clinicians. However, it should be noted that *E*max is not linked to drug potency, and a less potent drug can develop a larger *E*max than one that is more potent (Figure 5).

In the operational model of drug action,[50] the observed

maximal drug effect is not necessarily the same as the maximum possible response for a given system. Accordingly, the following relationship holds for an Emax model:

$$(E\max, drug) = \frac{E\max, system \times}{\tau + 1} \qquad (17)$$

Often in clinical pharmacology, the Emax of a compound will be unknown owing to safety concerns, in which case alternative data analysis will be in order.[53]

Sensitivity. Within the Hill model, the shape coefficient (h) determines the (midpoint) slope of the concentration-effect relationship (Figure 4). It measures the sensitivity with which a particular system translates the concentration of an agonist into an effect. When $h = 1$, the curve describing a concentration-effect relationship takes on the shape of the classical rectangular hyperbola.

In receptor theory, h has a precise meaning. It measures system cooperativity, with h>1 interpreted as the binding of 1 agonist molecule facilitating the binding of subsequent molecules. In vivo, h should not be interpreted in mechanistic terms. However, consideration of h is of great relevance when examining selectivity and drug sensitivity—that is, the range of useful concentrations (doses) for achieving a desired effect or avoiding an unwanted effect (see Figure 5 for explanation).

An example of this can be seen with nimesulide, an NSAID for use in dog. Using a whole-blood assay, we found rather low Hill coefficients (0.7 for Cox_2 inhibition and 1.04 for Cox_1 inhibition).[54] Based upon these values of h, we determined that to fully distinguish between Cox_1 and Cox_2 inhibitory action (eg, Cox_2 inhibition of 90% with Cox_1 inhibition under 10%), a high potency ratio would have been needed (ie, $EC_{50,Cox1}/EC_{50,Cox2}$ of about 200!).

In vivo, for drugs with a low h, the PD profile is shallow, with only moderate changes in effect over a wide range of drug concentrations (several orders of magnitude). This type of relationship explains the very long lasting action of some low h compounds. The existence of measurable responses for low or very low plasma concentration (eg, beta-blockers) causes the length of the terminal half-life to be very important for predicting the duration of an effect.[8]

For drugs with a high slope, the steepness of the concen-

tration-response curve must be considered whenever the selected dose is close to EC_{50}. Minor variations in concentration around EC_{50} can produce effects ranging from null to nearly maximal. This is the case with phenylbutazone and flunixin in horses[55] (Figure 6). Drugs with high h but a low therapeutic index may require drug monitoring to guarantee efficacy without toxicity.

The antibacterial action of all antibiotics can be described using the same Hill model, provided different sets of parameters are used: high h for some antibiotics (eg, betalactams) and lower h for others (eg, aminoglycosides). According to Mattie,[56] it is erroneous, from a mechanistic perspective, to divide antibiotics into 2 classes, time dependent and concentration dependent. Rather, it is just that upon integrating the concentration-effect curve and PK properties of antibiotics, the overall effect becomes proportional either to time above a critical concentration or to AUC (Figure 7).

As h increases ($h>5$), the concentration range diminishes to become a simple threshold (ie, critical concentration just above EC_{50}) and the graded PD model becomes a quantal model, representing a limit of graded concentration when $h>>1$.

When an all-or-none effect is observed owing to the drug mechanism of action (anti-arrhythmic drug) or to the selected endpoint (cured or not cured, presence versus absence of side effects), the concentration-response curve represents the frequency with which a concentration of a drug produces the all-or-none effect. In this relationship, EC_{50} (or ED_{50}) is now a median effective concentration (dose) for which 50% of subjects are above the threshold, and the slope of the curve now represents the dispersion (variance) of the threshold in a population.

Another convenient graphic representation is the cumulative frequency distribution of individuals achieving the selected effect as a function of drug concentration. This is termed the concentration-percent curve, or the quantal concentration-effect curve (Figure 8). The ratio of median effective concentration for different endpoints must be considered when discussing drug selectivity. The slope should also be considered, because a shallow slope (indicating wide interindividual dispersion) leads to overlapping of desired and undesired effects among the population.

A quantal concentration dose-response relationship is analyzed using a logistic model of the form:
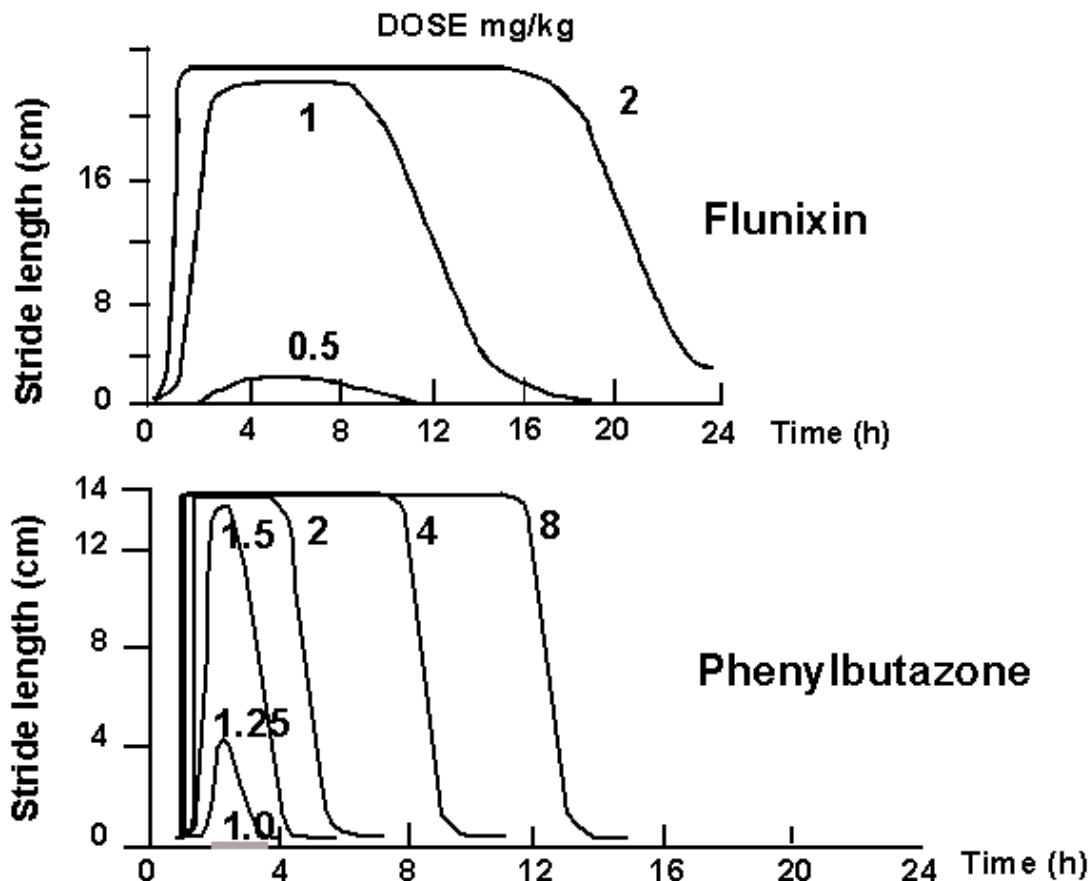
Figure 6. PK/PD simulation for dosage-regimen selection. Different scenarios can be simulated to help clinicians select a dosage regimen to be used in pivotal clinical trials. Dose-effect relationships were simulated for phenylbutazone (PBZ) and flunixin (FLU) in horse. PBZ and FLU were tested using an experimental arthritis induced by Freund's adjuvant in a carpal joint, and PK/PD parameters were determined from an IV study.[55] From these parameters, different dose levels were simulated. For FLU (top panel), minimal therapeutic effects are predicted for 0.5 mg/kg, whereas nearly maximum effect can be obtained with 1 mg/kg (the recommended dose). Increasing the dose to 2 mg/kg does not increase intensity of effect but does increase duration (to about 24 hours). For PBZ, the model also predicts a steep dose-effect relationship with no relevant effect under 1 mg/kg, maximum but short-lasting effects at 1.5 mg/kg, and maximum effect over 12 hours or longer at doses of 4 mg/kg (from Toutain et al 1994,[55] with permission).

$$\pi_{(outcome)} = \frac{e^{\alpha + \beta x}}{1 + e^{\alpha + \beta x}} \qquad (18)$$

where $\pi_{(outcome)}$ is the probability of an event for a given x (concentration, AUC, dose, etc), α is a location parameter, and ß is a scale (slope) parameter. The probability of no event is given by:

$$1 - \pi_{(outcome)} = \frac{1}{1 + e^{\alpha + \beta x}} \qquad (19)$$

The ratio of Equation 18 over Equation 19 is termed the odds (or likelihood) ratio and describes the relative risk of response:

$$\frac{\pi_{(outcome)}}{1 - \pi_{(outcome)}} = e^{\alpha + \beta x} \qquad (20)$$

The natural logarithm of the odds ratio is termed the logit (*L*) of π:

$$L = \alpha + \beta x \qquad (21)$$

L can be written as a more general linear function, allowing expansion of the model into a more advanced logistic model that includes several continuous or categorical
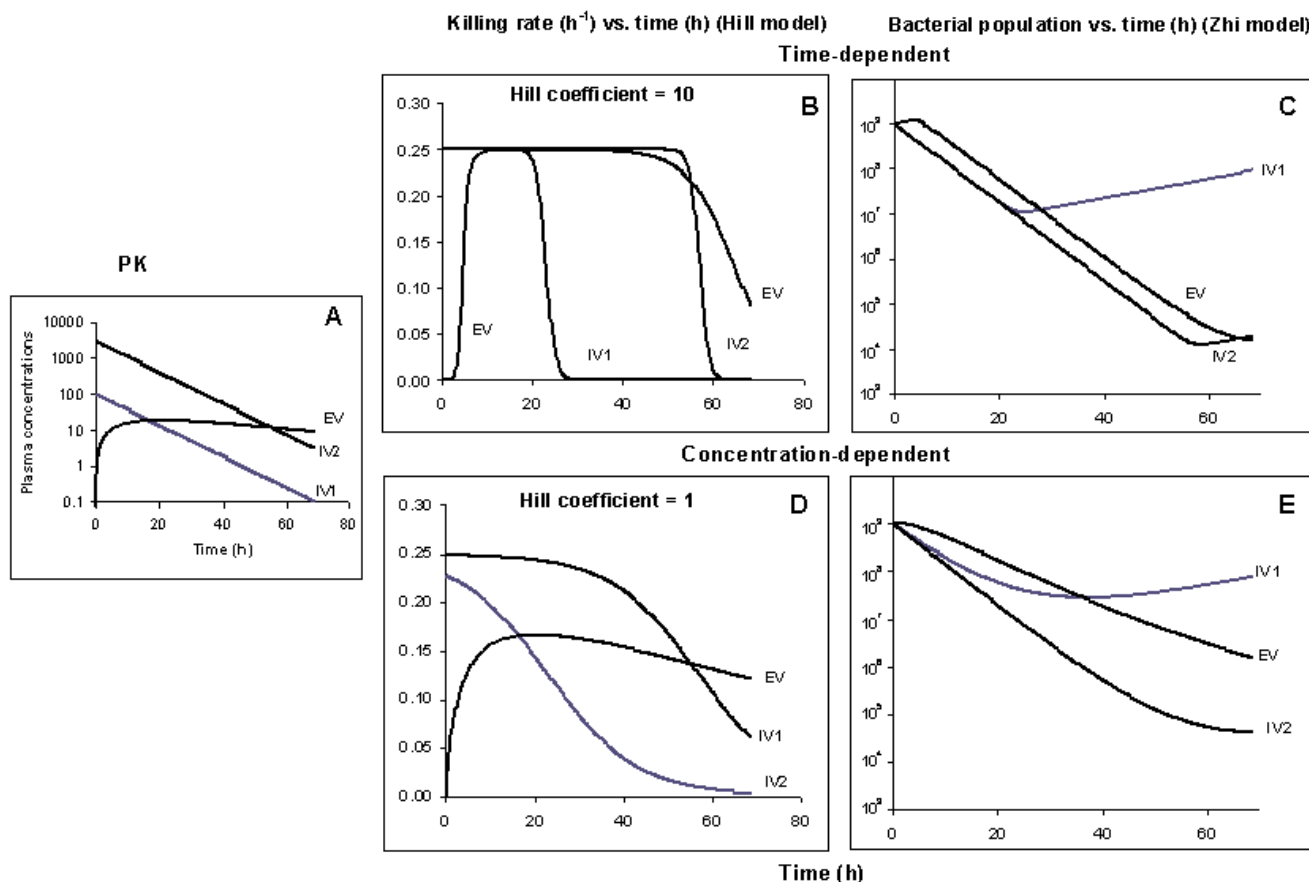
Figure 7. Time- versus concentration-dependent bactericidal action of antibiotic and the Hill coefficient. Antibiotics are often classified as time dependent or concentration dependent. However, antibiotic action can be described using the same Hill model with different sets of parameters. This simulation shows that the difference between time dependence and concentration dependence can be explained in terms of only the Hill coefficient (slope) (h) of the curve that predicts killing rate. Panel A: 3 PK curves were simulated using a monocompartmental model. For the IV route (IV1 dose = 100, IV2 dose = 3000), the elimination rate constant was 0.1 h^{-1}. Extravascular administration (EV) was simulated at the same rate constant of elimination but with a lower rate constant of absorption (Ka = 0.02 h^{-1}), thereby mimicking a long-acting formulation. The dose for EV was selected to provide the same total AUC as for IV1 from 0 to 72 hours. The 3 curves were used to predict the killing rate, with a Hill model (see equation 8) having an Emax of 0.25 h^{-1} and an EC_{50} of 10 (arbitrary units). For a time-dependent antibiotic (panel B), h is high (h = 10), whereas for a concentration-dependent antibiotic, h is fixed at 1 (panel D). The B and D panels show that killing rates behave very differently. For the time-dependent antibiotic, the resulting killing rate is maximum (>0.20 h^{-1}) for about 22 hours with IV1 but 57 hours with the EV route, despite the same overall systemic drug exposure. The influence of different killing rates on a bacterial population was simulated using the Zhi model[57] with a fixed growth rate (0.05 h^{-1}) and a killing rate as given by the Hill model. Initial bacterial population was 10^9 bacteria. For the time-dependent antibiotic (panel C), an EV route mimicking a long-acting formulation appears to perform best. Beyond 20 hours the low-dose IV (IV1) is unable to control bacterial population size, whereas for the same total exposure, bacterial population continues to decrease for up to 65 hours postadministration following the EV route. Only the high-dose IV (30 times overall EV exposure) gave a similar effect. For the concentration-dependent antibiotic (panel E), the killing rate is concentration dependent (IV2>IV1>EV) for about 17 hours.

independent variables (eg, sex, breed, immunity status) and their interactions.

What distinguishes a logistic model from a (nonlinear)

Hill model is that the dependent variable in a logistic regression is categorical. But both the Hill model and the logistic model are simply different parameterizations of
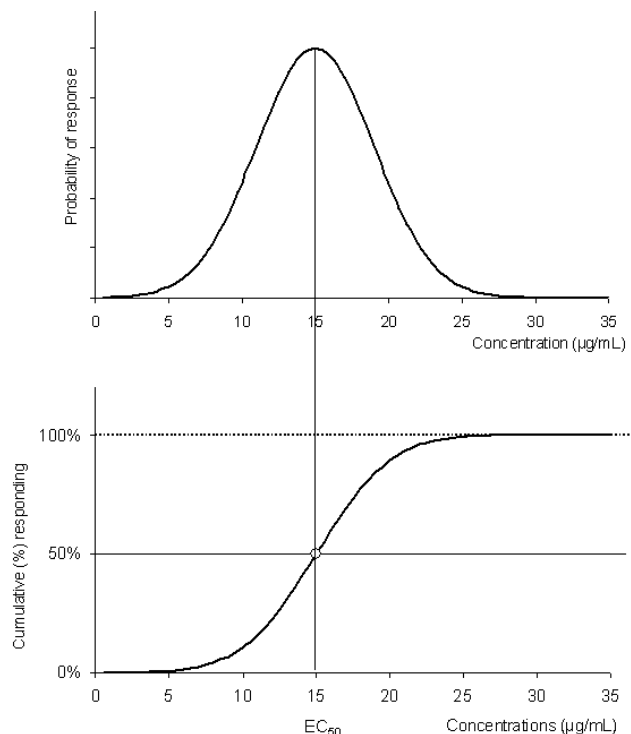
Figure 8. Quantal concentration (dose) effect relationship. Top panel: Curve representing the frequency of observation corresponding to the production of a given response (eg, occurrence of side effect) in individual animals over a range of plasma concentrations. Most frequently, individuals will require a concentration close to the mean concentration (15 μg/mL). Bottom panel: Cumulative percentage of animals responding to given concentration. At mean concentration of 15 μg/mL (EC_{50}), 50% of animals are responding. The slope of the curve represents the variance in response.

the same underlying model, and it can be more desirable to describe the probability of an outcome using the more traditional Hill model because this yields an expression containing parameters of more immediate interest. Representing the (unknown) median concentration by EC_{50} and the known concentration by C (with α defined as $\log_e EC_{50}{}^h$, x as $\log_e C$, and ß = h), we obtain the following by substitution into Equation 18:

$$\pi_{(outcome)} = \frac{C^h}{EC_{50}^h + C^h} \qquad (22)$$

where EC_{50} is the concentration at which the probability of drug effect is 50%, and h is the interpatient variability in response.

When effect is directly related to plasma concentration level, plasma concentrations can be directly incorporated into a PD model, as seen with Equation 11. Synchronization of the plasma concentration_time course and the effect-time course is fairly uncommon, and for most drugs, effects lag behind plasma concentrations (Figure 9). In this situation, PK models should be combined with PD models that can account for this delay. When delays exist, the same plasma concentration is associated with different responses, depending upon whether the plasma concentrations are increasing (input phase) or decreasing (elimination phase). This can be visualized by simply plotting effect (*y* axis) against plasma concentration (*x* axis). When the data points follow in chronological order, a loop (of greater or lesser width) is observed. This is known as a hysteresis loop (from the Greek word meaning "coming late"). This means that for any given plasma concentration, effect is more pronounced at a later time. The inverse situation (ie, a lesser effect at a later time for the same drug concentration) is termed proteresis (a neologism meaning "coming early")[8] (Figure 9). The old terminology, "clockwise" and "anticlockwise," is confusing and should be avoided (Figure 9).

When a hysteresis loop is observed, the cause of the delay must be identified to select a suitable modeling strategy. Examples include an effect compartment model to account for a delay in drug concentrations reaching the site of action, a physiological indirect response model to account for a PD delay due to interference with a physiological system, or a transit compartment model[58] for delay due to time-dependent transduction mechanisms (eg, involving the production of second messengers or protein synthesis). PK delay might arise from a slow rate of drug distribution to the biophase. The biophase is generally a poorly irrigated tissue or a site protected by a barrier. In this situation, the inclusion of an effect compartment allows for the synchronization of biophase drug concentrations and a direct drug effect (**Figure 10**). Another delay of PK origin arises from transformation of a prodrug into its active metabolite.

Sensitization—that is, increasing response with time when plasma concentration remains unchanged—produces hysteresis of PD origin. PD delays can also arise from alteration (inhibition, stimulation) of some physiological factors (eg, endogenous neuromediators) that control the input or dissipation of drug response. In this case, the drug response is said to be indirect and will be
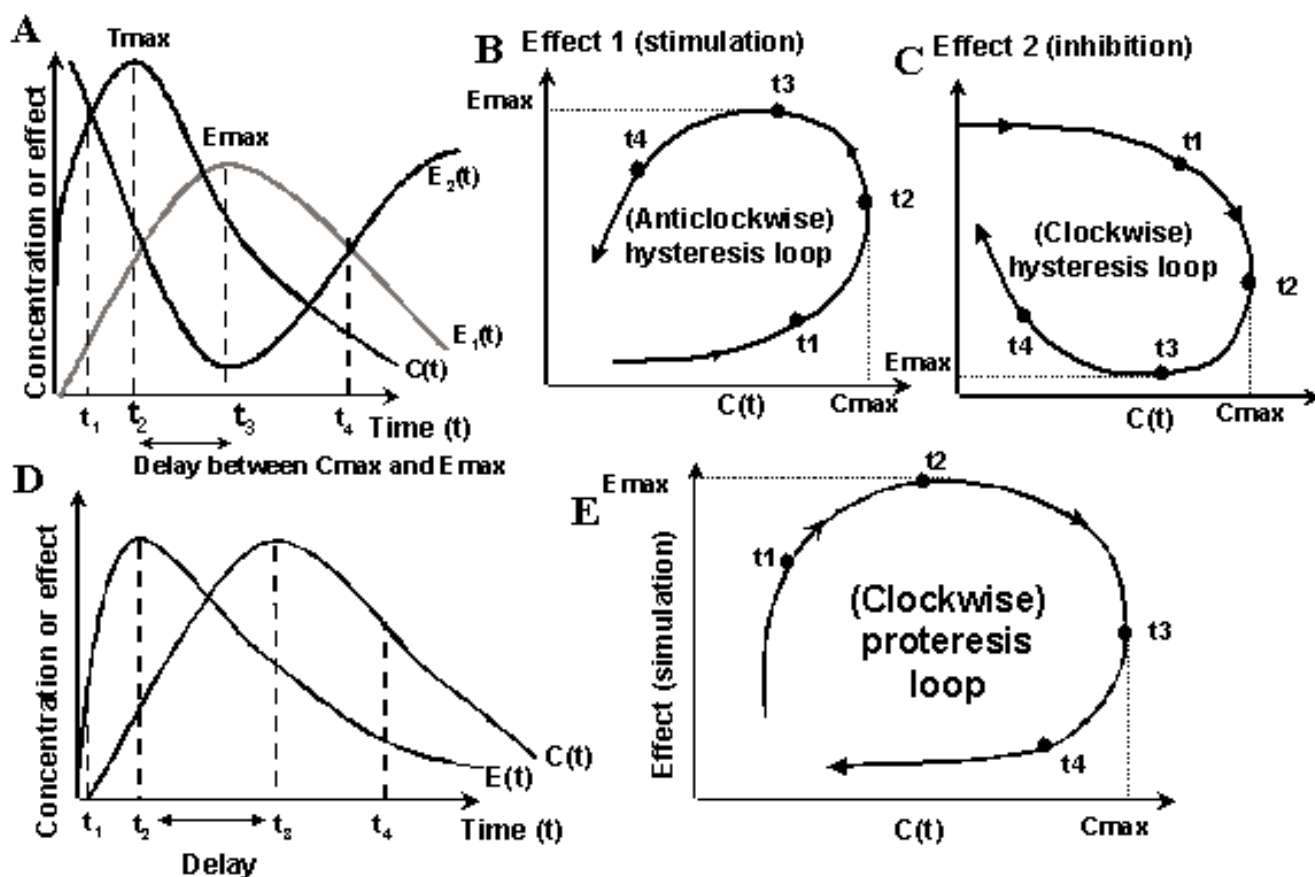
Figure 9. Hysteresis and proteresis. Time developments of effect and plasma concentration are not usually in phase. Panel A: The time development of plasma concentration (*C(t)*) and of 2 effects, corresponding to stimulation (E1) (panel B) and inhibition (E2) (panel C). For both effects, a hysteresis is observed with maximum effect (*E* max) at time 3 (t3) occurring after maximal plasma concentration (*C* max) had been achieved at time 2 (t2). The E1 curve is said to be anticlockwise and the E2 curve clockwise. Nevertheless, both curves represent a form of hysteresis and terms clockwise and anticlokwise should be avoided. Panel D: When effect *E(t)* develops faster than plasma *C(t)* (venous) concentration, a proteresis curve is observed (panel E), with maximal effect (t2) occurring prior to achieving maximal plasma drug concentrations (t3).

described by an indirect response model.

Proteresis is more rare and can be of PK or PD origin. Proteresis is observed when the drug is delivered to the site of action via the arterial circulation and the effect site equilibrates more rapidly with arterial blood than with the venous blood used for plasma concentration measurement. Accumulation of antagonist metabolite also leads to proteresis. In addition, PD proteresis can result from a tolerance phenomenon due to down regulation or reduction of drug affinity.

When a hysteresis loop is observed for a drug associated with a direct PD response, there are 3 possible modeling approaches for coping with distributional hysteresis: (1) sample the effect site if possible (eg, synovial fluid for

NSAIDs); (2) perform multiple steady-state experiments, though this is cumbersome and does not allow quantification of a relevant equilibration delay (eg, anaesthetics); (3) model the effect site as a hypothetical kinetic compartment (the "effect compartment" model,[59] [Figure 10]). The latter involves incorporating concentrations at the effect site (*Ce(t)*) into a PD model:

$$E(t) = \frac{E \max \times Ce(t)}{EC_{50} + Ce(t)} \qquad (23)$$

Ce(t) is not actually measurable, and *E*max and *EC$_{50}$* (at the biophase) cannot be directly estimated. Nevertheless, the rate of drug exchange between the plasma and the effect site can be determined from the time course of the effect itself. Indeed, the rate of onset and offset of an
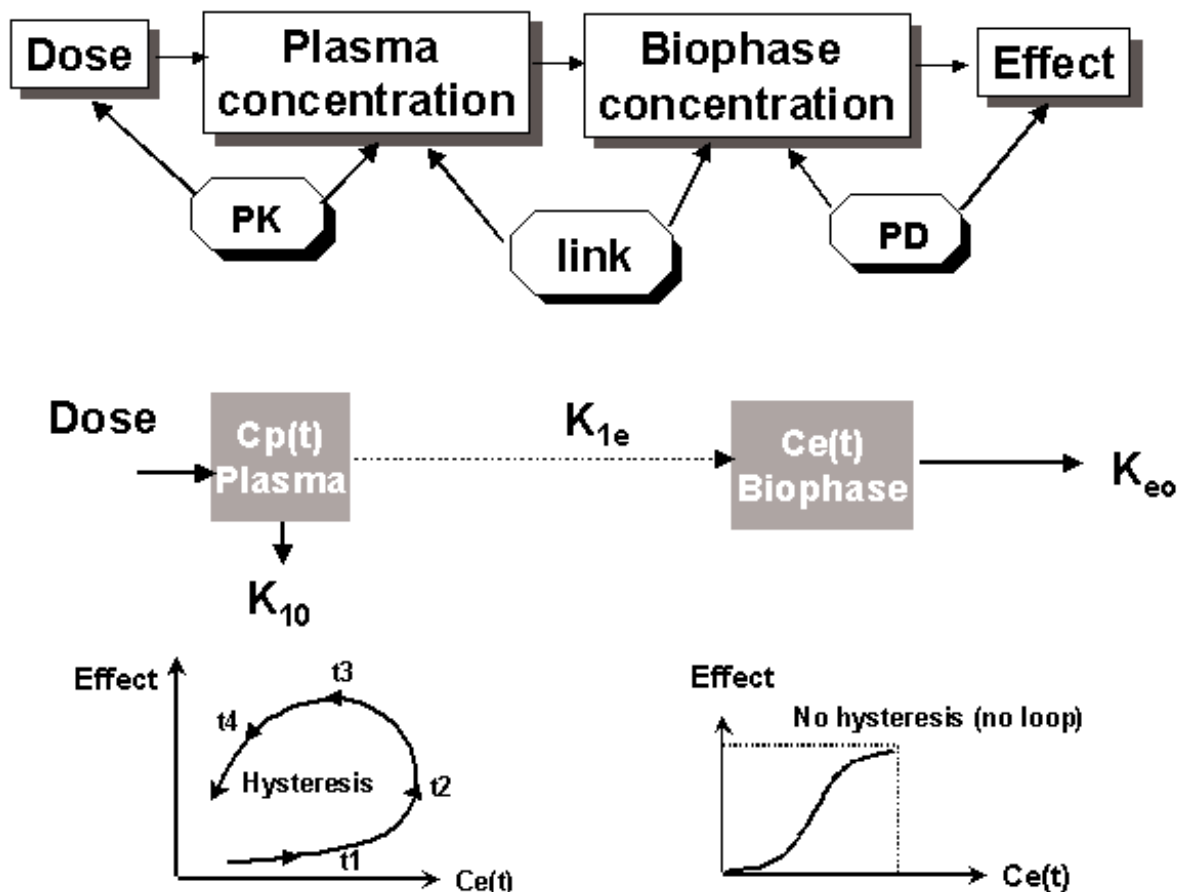
Figure 10. Effect compartment model. Top: Parametric modeling of direct-effect response curves involves 3 models: a PK model transforming dose into a concentration versus time profile, a link model describing transfer of the drug into the biophase, and a PD model relating biophase concentration to an effect. Middle: PK models are usually classic compartmental models (here, a monocompartmental model with rate constant of elimination $K10$). Drug transfer into the biophase is characterized by a first-order rate constant (K_{1e}), which is the parameter of the link model. Since the biophase is a hypothetical compartment and transfer of drug to the biophase is negligible, it can be assumed (without loss of generality) that $K_{1e} = Ke_0$. Ke_0 is the first-order rate constant of drug elimination from the hypothetical effect compartment and can be estimated from the time course of the effect (hence the link parameters). Bottom: Hysteresis at plasma level, with effect lagging behind plasma concentration. However, with the use of a link model, there is no hysteresis (no loop) at the biophase level.

effect is governed by only the rate of drug distribution to and from the effect compartment. In addition, the rate of equilibration between the plasma and effect compartments will account for the temporal delay between plasma concentrations and effects.

The effect compartment should have 2 main features:

The rate of change of drug amount in the effect compartment can be described by the following differential equation:

$$dAe/dt = K_{1e} \times A_1 - Ke_0 \times Ae \quad (24)$$

where:

A_1 is the amount of drug in the central compartment

Ae is the amount of drug in the effect compartment

K_{1e} is the first-order rate constant describing input into the effect compartment from plasma (driving force)

Ke_0 is the first-order rate constant for loss of drug from

the effect compartment

Since the amount of drug entering the effect compartment is assumed to be negligible, any drug entering the effect compartment can be eliminated directly from that compartment rather than returning to the central compartment for systemic elimination. This assumption greatly simplifies the model.

Drug concentration in the effect compartment (*Ce(t)*) can be described by an extension of the conventional compartmental PK model. Equations describing *Ce(t)* for a variety of classical PK models are given by Holford and Sheiner.[5] For a drug obeying a monocompartmental model, the concentration of drug in the effect compartment is given by:

$$Ce(t) = \frac{K_{1e} \times Dose}{Ve(Ke_0 - K_{10})}\left[\exp^{-K_{10}t} - \exp^{-Ke_0t}\right] \qquad (25)$$

With K_{1e} and Ke_0 as defined above, *Ve* is the volume of the effect compartment and K_{10} is the rate constant of drug elimination from the central compartment. Inspection of Equation 25 shows that K_{1e} and *Ve* are just proportionality factors relating *Ce(t)* to plasma concentration. The kinetic profile (shape) of *Ce(t)* (and therefore *E(t)*) is determined by only K_{10} and Ke_0.

In Equation 25 there are 3 unknown parameters: Ke_0, K_{1e}, and *Ve*. Without direct measurement of drug at the effect site, this model is not identifiable from the plasma concentration profile alone. Given this difficulty, K_{1e} and *Ve* should be eliminated considering the situation at equilibrium (5)—that is, with $K_{1e}V_1V_1C(t) = Ke_0VeCe(t)$. This represents a condition under which the ratio between *Ce(t)* and plasma concentration (*C(t)*) is constant and equal to *Kp*, with:

$$Kp = K_{1e}.V_1/Ke_0Ve \qquad (26)$$

Since *Kp* is a constant interpreted as a partition coefficient at equilibrium, under steady-state conditions, Equation 25 can be rewritten as:

$$Ce(t) = \frac{Ke_0 \times Dose \times Kp}{V_1(Ke_0 - K_{10})}\left[\exp^{-K_{10}t} - \exp^{-Ke_0t}\right] \qquad (27)$$

In Equation 27, V_1 (the known volume of the central compartment) has replaced the *Ve* of Equation 26. Nevertheless, *Kp* and Ke_0 still make the model unidentifiable. The method for resolving this problem consists of incorporating *Ce(t)/Kp* rather than *Ce(t)* into the *E*max model of Equation 23:

$$E(t) = \frac{E\max \times Ce(t)/Kp}{EC_{50}/Kp + Ce(t)/Kp} \qquad (28)$$

where EC_{50} is the EC_{50} for the effect compartment. Equation 28 can then be rewritten using the analytical expression for *Ce(t)*:

$$E(t) = \frac{E\max \cdot \dfrac{Ke_0 \times Dose}{V_1(Ke_0 - K_{10})}\left(e^{-K_{10}t} - e^{-Ke_0t}\right)}{(EC_{50}/Kp) + \dfrac{Ke_0 \times Dose}{V_1(Ke_0 - K_{10})}\left(e^{-K_{10}t} - e^{-Ke_0t}\right)} \qquad (29)$$

The parameters in Equation 29—that is, *E*max, EC_{50}/*Kp* (PD parameters), and Ke_0 (the link model parameter)—can now be estimated from PD versus time data using nonlinear regression techniques, with PK parameters (K_{10} and V_1) set at the values obtained by independently solving the PK model. In doing this, we should bear in mind the precise meaning of EC_{50}/*Kp*, which is *not* the drug concentration at the *effect site* giving *E*max/2 (this would be EC_{50}). Rather, it is the plasma EC_{50} (EC_{50}, plasma) that, under equilibrium conditions, would produce *E*max/2.

The actual value of EC_{50} at the receptor site remains unknown, but $EC_{50, plasma}$ is of greater practical value, as it enables establishment of a dosage regimen. Indeed, plasma concentration is the only readily measurable drug concentration on which the clinician can exercise direct control. However, if a more mechanistic investigation is warranted (eg, comparison of EC_{50} in vivo and in vitro), *Kp* factor can be of interest and should be estimated by sampling the biophase.

The process of equilibration between plasma and effect sites is determined by solely Ke_0, the parameter that controls the time development of effect with respect to the effect-site concentration. Equilibration half-time is determined from Ke_0 according to the relationship equilibration half-time = Ln2/Ke_0. $T_{1/2}Ke_0$ indicates the length of time it will take for plasma concentrations and effect-site concentrations to reach equilibrium. Equilibration half-

time can range from a few minutes for rapid-action drugs like meperidine in goat[60] to several hours for drugs with a slower onset of action, like phenylbutazone and flunixin when acting on articular inflammation in horse.[55]

If the biophase distribution process is rapid with respect to drug elimination and distribution into other body compartments, the biophase will reach rapid equilibrium with the plasma concentration. Observed plasma concentration will therefore be a good predictor of the concentration at the receptor site (and thus of the time development of effect). In contrast, if Ke_0 is lower than the terminal elimination rate constant (eg, as in a tissue cage), there will never be equilibration between the plasma and the amount of drug in the effect compartment. In this case, the effect will persist longer than drug concentration in plasma and plasma profile will be a poor predictor of the effect.

From a mechanistic point of view, Ke_0 is a hybrid parameter:

$$Ke_0 = \frac{Cld}{Ve} \qquad (30)$$

where Cld is the distribution clearance between the central and the effect-site compartment, and Ve the volume of the effect compartment. A short or long half-time of equilibration can be interpreted either in terms of flow diffusional clearance (eg, blood flow to the effect site) or as the extent of effect-compartment distribution space.[61]

Peak concentration in the effect compartment is reached at time Te,max, given by:

$$Te, \max = \frac{\ln\left(\frac{Ke_0}{K_{10}}\right)}{Ke_0 - K_{10}} \qquad (31)$$

An unconventional but valuable apparent volume of distribution can be calculated by dividing the administered dose by the plasma concentration at Te,max for predicting an effective initial dose (eg, for anesthesia). This volume (termed Ve,max) has been shown to be more useful than the generally underestimated dose calculated using Vc (initial volume of distribution) or the overestimated dose calculated from Vss (steady-state volume of distribution).[62]

The modeling approach discussed above is known as a full parametric approach because all 3 models (PK, link, and PD models) are fully parameterized. One simplification (the semiparametric approach) involves directly estimating Ke_0 as the value that collapses the hysteresis loop. This single curve now represents an empirically steady-state concentration-effect relationship.[63,64] Here, no a priori PD models are specified, and only the PK and link models remain. The advantage of this "semiparametric" approach is that it can facilitate direct visual inspection of the actual steady-state concentration-effect relationship prior to selecting the most appropriate PD model. This can then be followed by a second stage that involves the estimation of PD parameters from the effect versus time curve. This procedure guards against the risk of PD model misspecification (eg, erroneously setting Emax to 100%).

Another simplification consists of suppressing the PK model and introducing drug concentrations into a PD model by means of a smoothing function (eg, linear or cubic spline). This avoids the need for a PK model and can be useful when the plasma-concentration driving effect (independent variable) cannot be described by a conventional PK model (eg, an endogenous substance with episodic release, slow release system).

In the model discussed previously, concentrations were directly related to drug effect. For this reason, the model was termed a direct PD model. But for most drugs, the measured response is not a primary drug action resulting from direct binding of the drug to its receptor. Rather, there is a cascade of time-consuming biological events that entail an indirect relationship between plasma rug concentration and the final observed response. Under these conditions, the observed delay between the kinetics of the plasma concentrations and the time development of a response is not of distributional origin but rather reflects the intrinsic temporal responsiveness of the system.

For this kind of response, 4 basic models were proposed,[65] based on Equation 32, which describes the rate of change of response over time with no drug present:

$$\frac{dR}{dt} = K_{in} - K_{out} R \qquad (32)$$

where dR/dt represents the rate of variation in response variable (R). R is assumed stationary with an initial value

of R_0. The model assumes that the measured response is being formed at a constant rate (K_{in}) but is eliminated in a first-order manner (K_{out}).

For modeling purposes, it is reasonable to assume that indirect drug action consists of inhibiting or stimulating physiological factors that control production or dissipation of the measured effect. Inhibition or stimulation of response production (or dissipation) can be described by allowing for inhibitory or stimulatory processes as described in Equation 33:

$$\frac{dR}{dt} = K_{in} \times \{1 + H_1(t)\} - K_{out} \times \{1 + H_2(t)\} \times R \quad (33)$$

where $H(t)$ is a function of time. An inhibitory process can be described by the function in Equation 34:

$$I(t) = -\frac{Imax \times C(t)}{IC_{50} + C(t)} \quad (34)$$

where:

IC_{50} is the drug (plasma) concentration that produces 50% of maximum inhibition

Imax is a number from 0 to 1 (1 for total inhibition)

$C(t)$ is the drug (plasma) concentration over time

By incorporating this function into Equation 33, we get 2 basic inhibitory PD models, as expressed in Equations 35 and 36:

$$\frac{dR}{dt} = K_{in}\left(1 - \frac{Imax \times C(t)}{IC_{50} + C(t)}\right) - K_{out} \times R \quad (35)$$

$$\frac{dR}{dt} = K_{in} - K_{out}\left(1 - \frac{Imax \times C(t)}{IC_{50} + C(t)}\right) \times R \quad (36)$$

The model shown in Equation 35 corresponds to inhibition of response production rate (eg, the action of synthetic glucocorticoid on the adrenal gland's secretion rate of cortisol). The model shown in Equation 36 corresponds to inhibition of response loss rate (eg, the inhibitory action of furosemide on Na^+ reabsorption

process at the Henle loop).

A process involving stimulation can be described by the function shown in Equation 37:

$$S(t) = \frac{Smax \times C(t)}{SC_{50} + C(t)} \quad (37)$$

where:

SC_{50} is the drug plasma concentration producing 50% of maximum simulation

Smax is a positive number

$C(t)$ is as described above

Incorporating the stimulatory function in Equation 33 gives 2 basic stimulatory PD models, as expressed in Equations 38 and 39:

$$\frac{dR}{dt} = K_{in}\left(1 + \frac{Smax \times C(t)}{SC_{50} + C(t)}\right) - K_{out} \times R \quad (38)$$

The model shown in Equation 38 corresponds to stimulation of response production rate (eg, production of cAMP by bronchodilatator beta 2-agonist).

$$\frac{dR}{dt} = K_{in} - K_{out}\left(1 + \frac{Smax \times C(t)}{SC_{50} + C(t)}\right) \times R \quad (39)$$

The model shown in Equation 39 corresponds to stimulation of response loss (eg, antipyretic effect of NSAIDs with stimulation of thermolysis).

These models have been used successfully for different classes of drugs (anticoagulants, corticosteroids, beta-adrenergics, antipyretics, etc).[66] Anti-inflammatory action of nimesulide in dog has been described using Equation 35 (assuming that the underlying mechanism involves mainly inhibition of inflammation mediator production rate), whereas nimesulide antipyretic action has been described using Equation 39 (since defervescence involves rapid increase in heat loss, with body temperature being the response proportional to R).[19] More advanced indirect models can be built to accommodate knowledge about the mechanism of drug action.[10]

For the 4 basic models, response to change in Imax (or Smax), IC_{50} (SC_{50}), and dose has been characterized,[67] and this work highlights a number of therapeutically meaningful model features. The time of maximal effect ($Rmax$) occurs later than Cmax because the drug causes incremental inhibition (or stimulation) for as long as drug plasma concentrations remain above IC_{50} or SC_{50}. After the response has reached its maximum (or minimum), the return to baseline is a function of both Kin (as defined in equation 32) and the drug elimination rate. This means that the response can persist largely beyond the presence of detectable drug levels because of the time needed for the system to return to equilibrium. In addition, it was shown that time for $Rmax$ was linearly proportional to the logarithm of dose over a wide range of doses and with a decrease in IC_{50} (or SC_{50}). This is at variance with the effect compartment model (Equation 31), where time for Emax is a dose-independent parameter.

The dependence of the shape of the concentration-effect relationship on dosage regimen has been evidenced by studies on simulation effects.[68] The conclusion from these investigations was that drug efficiency (ie, the effect per unit of drug concentration) is highly dependent on drug delivery rate, with sustained and targeted delivery maximizing efficacy and minimizing side effects. This has also been shown for the antipyretic effect of nimesulide in dog, where a 2.5 mg/kg dose twice a day is more efficient than a 5 mg/kg dose once per day.[19]

Indirect response model (IRM) has the advantage of accounting for the physiological components of drug action, which can be affected by disease, other drugs, gender, and other variables.[69] In addition, biological plausibility provides the model with good extrapolation capabilities. The main drawback with IRM is that several doses must be tested for simultaneous fitting. Indeed, for many drugs now considered to work via an indirect effect, classic fitting with the link model works well. However, when different doses are tested, Emax and EC_{50} are found to be dose dependent, thus indicating the poor suitability of the model. A simple way to distinguish between the effect compartment model and IRM is to simultaneously fit the PK/PD relationship obtained for different dose levels.[69]

PK/PD concepts hold promise for all phases of the veterinary medicine application process, including drug discovery, drug development, drug submission, and drug utilization.

Veterinary drug companies cannot disregard the genomic and proteomic revolution and the huge increase in new compound production. For these new compounds, initial drug potency (EC_{50}) is usually determined in vitro and the task is to select the most promising candidates for use in vivo (ie, those offering usability at a convenient and inexpensive dosage regimen). Only the PK/PD paradigm offers the necessary high-throughput framework for this preliminary stage of evaluation. If the clearance is known, the dose can be rapidly approximated using Equation 4. Only the AUC needs to be determined for this purpose, and for that, drug companies can consider "cassette analysis" (also known as cassette dosing or n-in-one dosing study) and "cocktail" approaches. Cassette analysis[70] consists of appropriately pooling several plasma samples obtained at different time points, to yield a single sample with concentration proportional to the AUC. In addition, analysis of pooled plasma samples requires only an abbreviated standard curve. The cocktail approach consists of administering several compounds to the same animal. This allows for a rank ordering of AUC estimates, thereby allowing compounds to be prioritized with respect to the most favorable potencies.[71]

It is important to eliminate unpromising candidates efficiently at the early phases of drug development. In human, a maximal clearance value of 4 mL/kg/min has been suggested for preliminary screening. Early data on plasma clearance are also desirable in veterinary medicine since they provide an approximate estimation of future dosage regimen, enabling developers to eliminate drugs with clearance values that are either too high or too low for the planned drug use. For instance, a relatively low clearance is desirable for a time-dependent antibiotic, a very low clearance is required for an avermectin, and a relatively high clearance would normally be preferred for a short-acting anesthetic.

It should be realized that the numerical value of plasma (blood) clearance reflects both cardiac output ($\dot{Q}$) (a species parameter) and overall body extraction ratio (ER) (a drug parameter). For plasma clearance, the minimal model is:

$$Body\ Clearance\ (Plasma,\ Blood) = \dot{Q} \times ER \quad (40)$$

where ER ranges from 0 to 1.
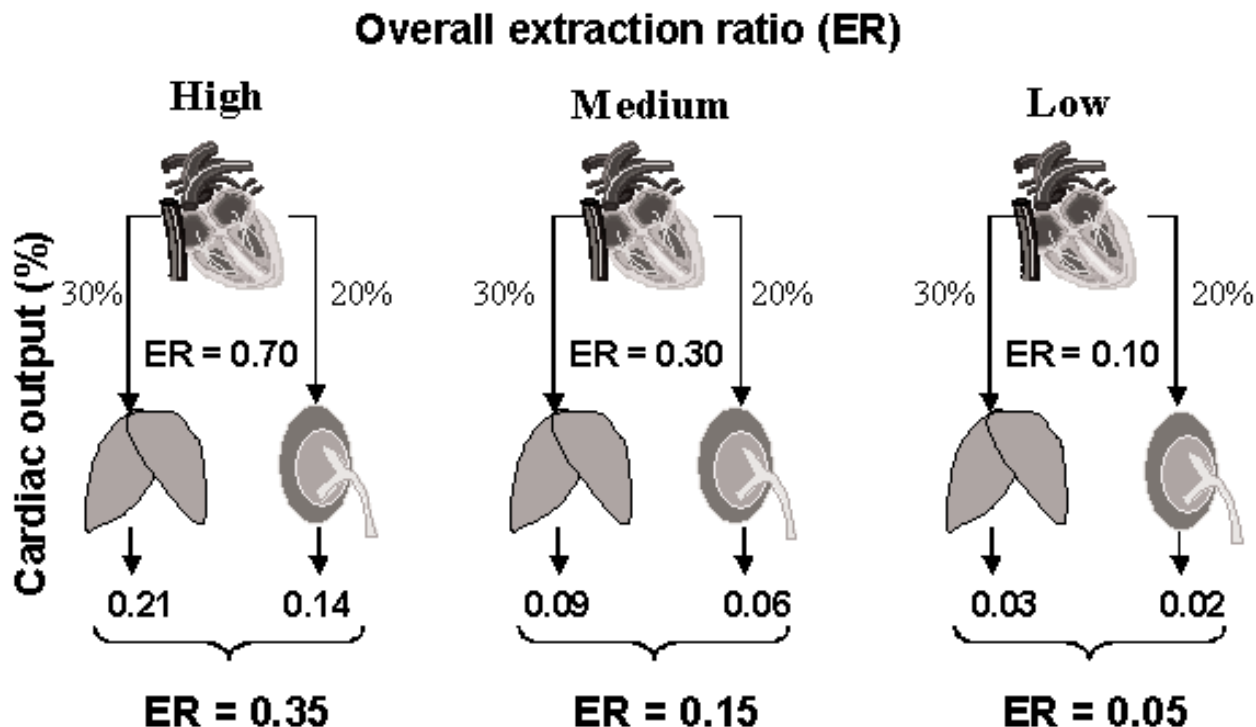
Overall extraction ratio (ER)

Figure 11. High, medium, and low overall body *ER* . A high, medium, or low overall body *ER* can be calculated bearing in mind that plasma (body) clearance is given by the relation: $Cl_{body} = Cl_{renal} + Cl_{hepatic} + Cl_{other}$ The kidney and liver are the 2 most important contributors to drug elimination, with Cl_{other} usually negligible. For liver and kidney, *ER* can be considered high if *ER* > 0.70, medium if *ER* = 0.30, and rather low if *ER* < 0.10.[72] Considering that kidney (20%) and liver (30%) blood flow represent about 50% of cardiac output, overall ER should be considered high if above 0.35 (0.70 × 0.50), medium if around 0.15 (0.30 × 0.50), and low if around 0.05 (0.10 × 0.50).

Cardiac output can be calculated by an allometric relationship:

$$\dot{Q}(\text{mL/kg/min }) = 180 \, BW \, (kg)^{-0.19} \qquad (41)$$

and clearance breakpoint (ie, 180 x *ER* x BW$^{-0.19}$) values across species can be calculated for high, medium, and low overall *ER*s. Figure 11 sets out the difference between high (>0.35), medium (0.15), and low (<0.05) *ER*s. Table 1 lists plasma (blood) clearance values in various domestic species where *ER* values are set to 0.35, 0.15, and 0.05, respectively. It will be noted that a given plasma clearance value (in mL/kg/min) can be considered high in a large species but low in a small species. Table 1 also shows typical daily dosage regimens among species for drugs with a targeted steady-state EC_{50} of 1 µg/mL (an *ER* of 0.05 being often selected as a breakpoint for drug screening).

The main application of PK/PD investigation is to document or suggest a dosage regimen for pivotal clinical trials. To examine the value of the PK/PD approach with respect to this therapeutic objective, the case of NSAIDs will be considered, since this is the only drug group to have been sufficiently investigated using the PK/PD approach in veterinary medicine.

Rational selection of a dosage regimen for an NSAID must take into account both beneficial and undesirable effects. Inhibition of Cox_1 and Cox_2 leading to suppression of synthesis of proinflammatory prostaglandin is currently assumed to be the major action of NSAIDs, determining both therapeutic and toxic effect. The desirable anti-inflammatory and analgesic effect of an NSAID arises largely and possibly entirely from inhibition of inducible Cox_2, whereas the unwanted effect is associated with inhibition of constitutive Cox_1.

The Landoni and Lees group evaluated Cox_1 and Cox_2 inhibition ex vivo using an experimental model of acute inflammation involving surgically implanted tissue cages.[73-75] EC_{50}, Emax t1/2, Ke_0, and Hill coefficient for different NSAIDs (flunixin, ketoprofen, tolfenamic acid,

Table 1. Interspecies Variation in Plasma Clearance (mL/kg/min) for High, Medium, and Low Overall ERs *

	Mouse	Rat	Cat	Dog	Sheep	Human	Pig	Cattle
BW (kg)	0.02	0.2	3	20	50	70	100	800
Clearance for								
$ER = 0.35$	132	85	51	41	30	28	26	17.7
$ER = 0.15$	57	37	22	17.4	13	12	11.3	7.6
$ER = 0.05$	19	12.2	7.3	5.8	4.3	4.0	3.76	2.52
Dose (mg/kg) for EC = 1 µg/mL and ER = 0.05	27.4	17.6	10.5	8.4	6.2	5.8	5.4	3.6

*BW indicates body weight; *EC*, efficacious average plasma concentration; *ER*, extraction ratio. Clearances were calculated using Equations 40 and 41. See also Figure 11 for the derivation of *ER*. An *ER* of 0.05 is generally considered a breakpoint value in drug development. Note that high clearance in cattle (17.7 mL/kg/min) (*ER*=0.35) can be considered medium clearance in dog and low clearance in mouse (*ER*=0.05). Dose is daily dose of drug with *ER*=0.05 to maintain an *EC* of 1 µg/mL.

etc) were computed using an effect compartment model to study the effect of these drugs on the synthesis of serum thromboxane (TxB_2 mediated by Cox_1) and exudate PGE_2 (tentatively mediated by Cox_2) across different species (sheep, calf, horse, goat, etc). As an example, tolfenamic acid in calf (plasma clearance 0.30 L/kg/h) had an EC_{50} of 0.077 µg/mL for PGE_2 inhibition and an EC_{50} of 0.137 µg/mL for TxB_2 inhibition. The Hill coefficient expressing the steepness of the concentration-effect relationship was relatively high for PGE_2 (2.38) and low for TxB_2 (about 0.6).[74] Using Equation 4 and these published data, we computed that it would require a plasma concentration of 0.245 µg/mL to achieve 95% PGE_2 inhibition, implying a tolfenamic acid dose of 1.76 mg/kg/day (roughly equal to the daily dose of 2 mg/kg currently recommended for this species). At the assumed 95% PGE_2 inhibition required for useful tolfenamic anti-inflammatory action, we calculated a corresponding Cox_1 inhibition (TxB_2) of 59%. Similar consistent results are obtained for the use of ketoprofen in calf.[73]

For flunixin in calf, EC_{50} was higher for PGE_2 (0.074 µg/mL) than for TxB_2 (0.024 µg/mL).[75] We calculated that it would require a plasma concentration of 0.22 µg/mL to inhibit 95% of PGE_2 production. With a plasma clearance of 0.20 L/kg/h in calf, this would necessitate a flunixin dose of 1.0 mg/kg/day, which is equal to half the recommended daily dose (2 mg/kg). In addition, at doses needed to achieve 95% PGE_2 inhibition, we would obtain nearly total TxB_2 inhibition (99.7%), suggesting that flunixin has unfavorable Cox_2/Cox_1 selectivity. Accordingly, it is likely that some side effects will be triggered at doses needed to achieve a desired therapeutic effect.

Within this approach to dose determination, the selected

endpoints are surrogates rather than actual outcomes of direct clinical interest. Indeed, PGE_2 in inflammatory exudate and serum TxB_2 are measures of drug action rather than clinical response, and the clinical validity of surrogate markers depends upon the contribution of Cox_2 and Cox_1 inhibition to the overall clinical effect. For NSAIDs, actions other than Cox_2 inhibition may also contribute to an anti-inflammatory effect, and a low potency for PGE_2 inhibition is not necessarily predictive of a drug with poor clinical efficacy. This is well exemplified by carprofen, an NSAID of the 2-aryl-propionate class. At clinically recommended dose rates in horse (0.7 mg/kg), this drug reduces inflammatory swelling but inhibits serum TxB_2 and exudate PGE_2 only moderately. In dog, recommended dosage (4 mg/kg) is also weakly inhibitory to serum TxB_2 and does not inhibit exudate PGE_2, but the dosage is clinically effective.[76,77] This drug has, therefore, been described as a prostaglandin-sparing NSAID.

If the main objective of a PK/PD trial is to screen for dosage regimen, the best strategies should relate plasma NSAID concentration to an outcome of direct clinical interest. Examples include body temperature for fever, or lameness for locomotive inflammation. In horse, the stride length of animals subjected to a Freund's adjuvant carpitis has been used to determine the potency and efficacy of flunixin and phenylbutazone.[55] For phenylbutazone (plasma clearance 41.3 mL/kg/h), EC_{50} was 3.6 µg/mL and the Hill coefficient was very high (23.3). These results suggest that the dose required to inhibit by half the lameness (Equation 4) would be 3.6 mg/kg/day (which is consistent with the currently recommended dosage regimen of 4.4 mg/kg/day). Due to a very high Hill coefficient, the EC_{50} of phenylbutazone can be considered a threshold value above which near-maximal
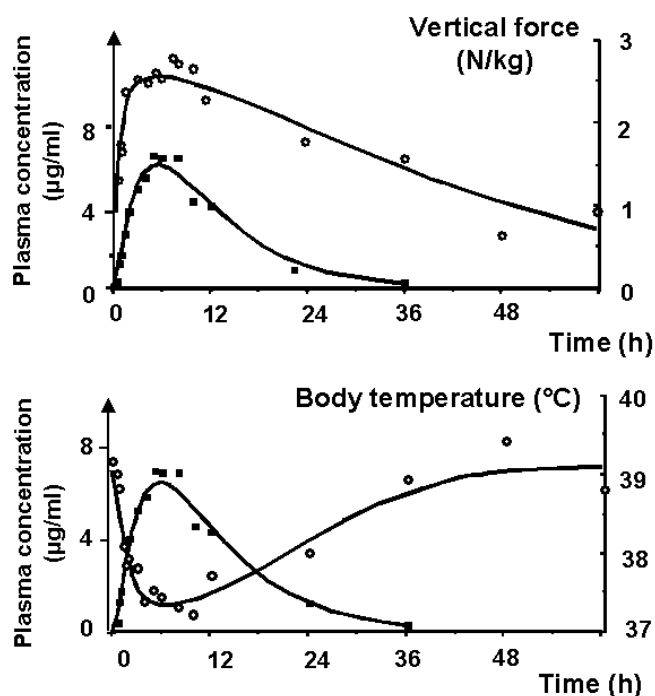
Figure 12. Concentration-effect relationship on lameness and fever for nimesulide in dog. PK/PD relationships for nimesulide (an NSAID with some Cox2 selectivity in dog) were obtained using a Freund adjuvant arthritis model characterized by permanent hyperthermia and lameness.[19] Lameness was measured using a forceplate and expressed in terms of vertical force applied on the ground. Nimesulide was administered as a single oral dose (5 mg/kg). Data were analyzed using an indirect effect model as described by Dayneka et al.[65] The upper panel shows the development of fitted and observed plasma concentrations (n) and the corresponding fitted and observed effect on vertical force ($\bigcirc$), which gradually increase to maximum, traducing a reduction in lameness. EC_{50} for this effect was 6.26 μg/mL. Return to control values was observed within about 48 hours. For body temperature (bottom panel), nimesulide administration was associated with suppression of hyperthermia (●), with EC_{50} = 2.72 μg/mL.

effect is observed (eg, maximal increase in stride length of 13.9 cm in this trial).

In vivo, using a Freund's adjuvant model in dog,[78] the IC_{50} of nimesulide for lameness and antipyretic effect were found to be 6.26 μg/mL and 2.72 μg/mL, respectively (Figure 12). Using Equation 4 and considering the plasma clearance of nimesulide (15.3 mL/kg/h) and its oral bioavailability (47%),[54] the ED_{50} of oral nimesulide

administration for the treatment of lameness can be estimated as 4.9 mg/kg/day. This is nearly equal to the dose marketed in France for many years (5 mg/kg). Here we note with interest that for an EC_{50} of 6 μg/mL, ex vivo Cox_2 inhibition was 86%, which supports the observation that nimesulide has relevant mechanisms of anti-inflammatory action other than simply total Cox_2 inhibition.

The second parameter to be determined in a rational multiple-dose regimen is the time interval between administrations. Using the PK/PD model, a large number of dose and dosage interval scenarios can be simulated to screen for dosage regimens having the best efficiency or safety margin. Such analysis requires no additional time or cost during drug development. For example, we have shown that the model predicts better antipyretic efficacy for nimesulide at a dosage regimen of 2.5 mg/kg twice a day rather than at 5 mg/kg per day, although both dosage regimens are equivalent in terms of lameness suppression.[19]

Through simulation, PK/PD techniques can speed up drug development, thereby helping to plan pivotal clinical trials that limit testing to the dosing regimens most likely to demonstrate the best efficacy. More generally, simulation is useful in predicting the consequences of changes in dose regimen, formulation, noncompliance, and so forth.

The bioequivalence of 2 drug formulations is generally determined by the use of PK criteria (AUC, Cmax, Tmax). For some drugs, it would be of interest to examine bioequivalence in terms of PD rather than PK parameters, considering the AUC for pharmacological effect-time. This approach might be valid if there was no analytical assay, or if plasma concentration was too low to be adequately quantified. However, it must be emphasized that the use of a PD approach should not be generalized. Whereas equivalence of a PK parameter (AUC) does guarantee equivalence of all plasma-driven PD effects, the inverse is not true. Equivalence of a given effect does not guarantee equivalence of drug exposure. Therefore, there could be inequivalence with regard to the other effects of interest. This applies especially to drugs for which efficacy involves a 100% cure (eg, antiparasitic drugs, antibiotics). In this case, different formulations can provide 100% cures with very different bioavailabilities, and testing a lower dose or testing for an endpoint requiring a high concentration (eg, MIC_{90} for an antibiotic) can reveal major differences between formulations (Figure 13).
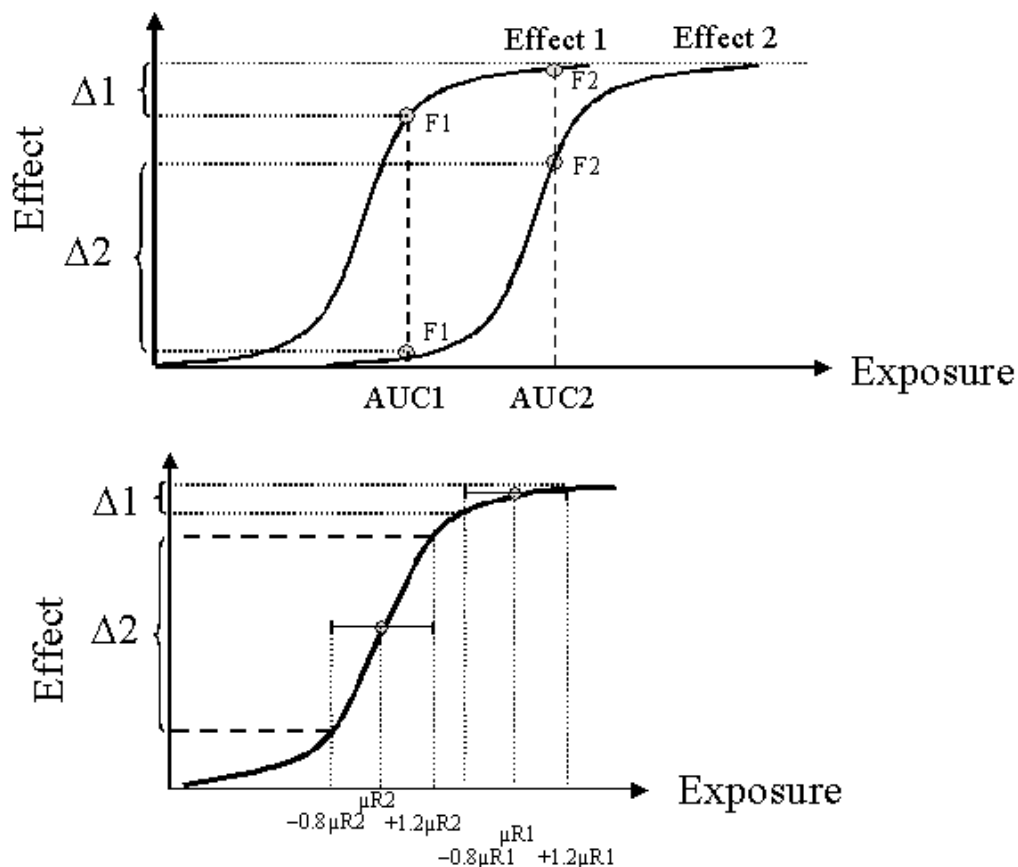
Figure 13. PK/PD and bioequivalence. PK/PD relationships should be taken into account when establishing bioequivalence using PD parameters (top) or when defining (or discussing) the a priori equivalence interval (bottom). The top panel illustrates a lack of generality in PD or clinical bioequivalence trial. Here, 2 formulations (F1 and F2) having 2 very different exposure levels (AUC1 and AUC2 for F1 and F2, respectively) are assessed for effect 1 and effect 2 (AUC-effect curves 1 and 2). Judging by effect 1, both formulations can be considered therapeutically equivalent (which is true because $\Delta 1$ is small). However, they cannot be considered bioequivalent because major differences are seen for effect 2 ($\Delta 2$). This can be observed, for example, when comparing antibiotic formulations using a clinical endpoint corresponding to pathogens having very different MIC values. The bottom panel illustrates the difficulty involved in selecting an a priori interval of equivalence. If the reference formulation is marketed at a dose giving close to Emax, a 20% exposure difference (-0.8 μR1 to 1.2 μR1) relative to the reference formulation will have minimal impact on effect ($\Delta 1$). In contrast, if the marketed dose (exposure) of the reference formulation provides concentration approaching EC_{50} (zone of steepest slope for exposure-effect relationship), a 20% difference in exposure (-0.8 μR2 to 1.2 μR2) can produce a very substantial difference in effect between the 2 formulations ($\Delta 2$); μR and (-0.8-1.2 μR): expected mean and a priori equivalence interval.

PK/PD information should also be considered when establishing therapeutic equivalence limits. If 2 drug products are equivalent in terms of drug exposure (rate and extent), it is assumed that they will be therapeutically equivalent. From a practical point of view, bioequivalence is granted if the average bioavailability of the test formulation is within 20% of that of the reference formulation, with 5% risk of a type I error. In other words, the 20% regulatory decision rule allows for a test formulation to theoretically exhibit up to a 20% difference in relative bioavailability compared to a test formulation. It

should be noted, however, that in practice, the allowable difference between the means is much smaller than 20%. Bioequivalence criteria require that not only differences between the means but also the inherent PK variability in the population be considered. Nevertheless, it is important to recognize that for certain compounds, a 20% variation in drug exposure can result in a substantial therapeutic impact. The magnitude of this impact will depend upon the shape of the concentration-effect curve and the position on the curve of the approved dose for the reference formulation (Figure 13 contains explanation).

CONCLUSION

PK/PD modeling is a scientific approach that addresses applications in screening and dosage-regimen selection (dose, interval of administration). Because it separates the 2 main sources of interspecies variation (PK vs PD), it is well suited to multispecies drug development. PK/PD modeling offers a general framework for extrapolation between species and from in vitro or ex vivo to in vivo. Moreover, it is useful for selecting new relevant endpoints capable of predicting drug efficacy or side effects (eg, resistance for antibiotics).

The main impediments to implementation of PK/PD modeling are regulatory understanding and hence acceptability and the reluctance of drug companies to adopt a new approach, for fear of adding another burden to drug development work. Hopefully, with a better understanding of these techniques, they can be more efficiently incorporated into the processes of veterinary drug development and product regulation.

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