Drug Testing and Analysis

Published online in Wiley Online Library

(www.drugtestinganalysis.com) DOI 10.1002/dta.1374

The role of pharmacokinetics in veterinary drug residues

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This review provides a summary of those pharmacokinetic properties of veterinary drugs relevant to marker residues, marker tissues, and residue depletion rates. The scientific literature in this field is very extensive and there is also a wealth of data available on the websites of various regulatory jurisdictions. Therefore, this review is limited to selected examples, cited to illustrate general principles. The areas considered are: (1) the relationship of dose to plasma concentration through the pharmacokinetic properties, area under plasma concentration-time curve, bioavailability, and clearance; (2) the critical dependence of drug pharmacokinetics and residue depletion on product formulation; (3) disease state and population pharmacokinetics; and (4) the requirement for residue depletion studies for generic products shown to be bioequivalent to pioneer products.

Keywords: pharmacokinetics; formulation; marker residue; residue depletion; bioequivalence

Introduction

To ensure public confidence in foodstuffs obtained from animals treated with veterinary drugs, regulatory authorities set stringent standards and adopt conservative approaches on data requirements for pharmaceutical companies. Regulatory authorities require determination of no observable adverse effect levels (NOAELs) for toxicological and microbiological (and sometimes also pharmacological) effects. From these two or three NOAELs, the lowest (most sensitive) value is used to calculate the acceptable daily intake (ADI). This defines the amount of drug and/or drug metabolite that can be consumed by humans daily throughout a lifetime with only negligible risk to health. There is an in-built safety factor (SF) in determining ADI, usually of 100 or greater. Thus, ADI = NOAEL x SF. The ADI is typically based on an assumed body weight of 60 kg. The SF is the product of two separate 10-fold factors that allow for interspecies difference and human variability. These 10-fold factors allow for both toxicokinetic and toxicodynamic differences. From the ADI, the maximum residue limit (MRL) (tolerance in the USA) of the selected marker residue is calculated for each edible tissue. The marker residue is usually the parent drug but can be a drug metabolite or the total concentration of several metabolites. When companies seek a marketing authorization (MA) for a veterinary drug product, they are required to supply target species data on pharmacokinetics and metabolism when the product is administered to the target species at recommended dose rates. The pharmacokinetic studies generate quantitative data on the absorption, distribution, metabolism, and excretion of the drugs. Of particular importance are the plasma or blood concentration-time profiles, together with identification and quantitation of major metabolites because plasma concentration is the driving force controlling all tissue concentrations.

This review outlines some of the factors and circumstances which may alter the pharmacokinetic profiles of drugs (Table 1). Such alterations inevitably impinge on drug and drug metabolite depletion profiles from edible tissues in food-producing animals. It might therefore be argued that a whole range of depletion profiles should be conducted in the target species, each study matching a particular circumstance/factor, such as age, sex, breed, weight, and disease status. This would then lead to a whole series of drug withdrawal times, each linked to a particular circumstance. As this would be both impractical and very expensive, regulatory authorities build conservative assumptions into MRL determination and selection of the withdrawal time, designed (in theory) to ensure the safety of food, despite the variability in drug pharmacokinetics in differing circumstances. Nevertheless, it is important to recognize both the differences from healthy animals and the potentially very variable tissue depletion rates of drugs when they are used for disease prophylaxis, metaphylaxis, and therapeutics.

Dose/pharmacokinetic/tissue concentration relationships

Pharmacokinetics is the science which describes quantitative changes in drug concentration in the body over time as a function of administered dose. It is usually based on subjecting serum/plasma concentration-time data to mathematical models in order to determine quantitative terms which describe absorption, distribution, metabolism, and excretion of the drug and its metabolites. For detailed discussions, see the reviews of Toutain and Bousquet-Mélou.[1–4] Residue depletion profiles are linked to administered doses of drugs through their pharmacokinetic profiles.

For a systemically acting drug, the equation linking dose to its pharmacokinetics is:

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Table 1. Factors influencing the pharmacokinetics and residue depletion rates of veterinary drugs

FACTOR	PHARMACOKINETICS / RESIDUE DEPLETION
Dose relationship to plasma concentration	Dose is related to plasma concentration by area under plasma concentration – time curve, bioavailability and clearance. Increased AUC increases tissue concentrations.
Formulation	As well as drug substance, the drug product formulation influences pharmacokinetic and residue depletion profiles.
Tissue uptake	For a given plasma concentration-time profile, the uptake of drugs and their metabolites by tissues depends on many factors, including blood flow and lipid solubility and acid/base characteristics of the drug.
Disease state and population pharmacokinetics	Drug pharmacokinetics (and hence residue depletion profile) is likely to differ between healthy and diseased animals.
Bioequivalence	When a generic product is bioequivalent to a pioneer product, the rate and extent of absorption are accepted by regulatory bodies as ensuring essential similarity in terms of efficacy and safety. Bioequivalence does NOT ensure that residue depletion profiles will be the same or even similar.

$$Dose = \frac{CLx\ AUC}{F} \quad (1)$$

where CL = whole body clearance, AUC = area under plasma or blood concentration-time curve and F = bioavailability (the proportion of the administered dose absorbed into the systemic circulation). Rearrangement of Equation (1) gives:

$$AUC = \frac{Dose\ x\ F}{CL} \quad (2)$$

AUC can be considered to represent overall exposure or 'internal dose', as opposed to the administered dose. AUC in blood or plasma contains both a concentration and time element; it therefore reflects the driving concentration over time for distribution of the drug to tissues. This equation indicates that the higher the dose and F, and the lower the CL, the greater will be the amount of drug in the plasma/blood over a measured time interval. If, in a dose-ranging study, the pharmacokinetics are linear, F and CL are constant, and the dose is doubled, then AUC increases 2-fold. However, if the pharmacokinetics is non-linear, CL and/or F are not PK parameters (i.e. intrinsic properties of the drug substance) but dose-dependent variables, AUC will not increase in simple proportion to the administered dose. For example, F may be reduced at higher dosages of orally administered drugs, which are highly lipid soluble but poorly water soluble, because drugs can be absorbed only from aqueous solution in the gastrointestinal fluids. With first-order pharmacokinetics, the terminal elimination half-life of a drug is independent of the administered dose, because elimination pathways are not saturated. In addition, drug elimination may be slower at high doses as a result of saturation of elimination pathways and elimination then becomes a zero order process. For most drugs administered at recommended dose rates, clearance is a parameter following first order pharmacokinetics, so that the rate of decrease in plasma concentration over time is exponential and proportional to concentration. There will always be a relationship between drug concentration in plasma and in tissues, because plasma concentration is the driving force for diffusion into tissues. However, the two will rarely be equal; tissue concentrations depend on a range of drug properties as well as animal characteristics. An example is presented in Table 2. This presents data for serum and tissue concentrations in calves after intramuscular administration of a long-acting formulation of oxytetracycline.[5] The zero time concentrations, terminal half-life, and time to depletion to a concentration of 0.1μg/g were similar for serum and muscle. Initially, concentrations were high in liver and very high in kidney, but the decline in concentration was slower from the liver, so that the time to depletion to 0.1μg/g was, in ascending order, muscle, kidney, and liver.

It is important to note differences in the interpretation and use of total tissue concentration from pharmacological and therapeutic perspectives on the one hand and for drug and metabolite depletion rates on the other. For tissue residues, it is the mean concentration rather than the separate concentrations in extracellular or intracellular fluids, which determines intake of meat and milk-products. On the other hand, tissue concentration has very limited value in relation to therapeutic requirements.

Tissue uptake

Tissue concentrations of veterinary drugs depend on a range of (mainly physicochemical) properties such as lipid solubility and acidic/basic characteristics, which influence the passive diffusion of drugs across cell membranes. In addition, for a few drugs, active uptake by, or extrusion from, tissues occurs. High lipid solubility drugs (fluoroquinolones, macrolides, phenicols and triamilides) cross cell membranes readily by passive diffusion to penetrate into intra- as well as extracellular compartments of tissues. Moderate to high lipid solubility drugs (diaminopyrimidines and tetracyclines) similarly generally enter readily into all water compartments of tissues. On the other hand, drugs of low lipophilicity (cephalosporins, penicillins, aminoglycosides, and polymyxins) generally do not readily enter cells, so that these drugs are located mainly or solely in the extracellular compartment of tissues.

An additional factor is acid/base characteristics of the molecule. As mean intracellular fluid pH is somewhat lower than extracellular fluid pH, 7.0 versus 7.4, weak bases penetrate readily into cells, by the classical Henderson-Hasselbalch mechanism of ion/

Table 2. Oxytetracycline serum and tissue concentrations in calves after intramuscular administration of a long acting (20% $^{w}/_{v}$) formulation*

Tissue	Extrapolated zero time concentration (μg/g or μg/mL)	$t_{½\beta}$** (h)	Tissue:serum concentration ratio at zero time	Time to depletion to 0.1 μg/g (h)
Serum	4.47	26.2	-	143
Muscle	3.86	26.2	0.86:1	138
Liver	10.7	42.4	2.39:1	287
Kidney	28.86	23.6	6.45:1	193

* Dose = 20mg/kg.
** Terminal half-life.
Adapted from Toutain and Raynaud.[5]

diffusion trapping, whereas weak acids do not. However, within cells, drugs may be located predominantly within different subcellular compartments; for example, fluoroquinolones and betalactams in the cytosol and macrolides, lincosamides and pleuromutilins (weak organic bases) in phagolysosomes. The pH of the environment in phagolysosomes is very acid (pH = 4 to 6) compared to extracellular fluid, so that very high concentrations of weak bases can be achieved. A particular example is tulathromycin, which accumulates in lung tissue of calves and pigs in concentrations some 50- to 100-fold greater than in plasma. By far the greater proportion of the drug within the lung is located intracellularly. Conversely, for those drugs with low lipophilicity and also for lipophilic weak organic acids, the average/overall tissue concentration is made up of a high extracellular and low intracellular concentration.

The oxytetracycline example presented in Table 2 illustrates how drug uptake by and release from tissues varies between tissues. Tissue uptake may be passive (the normal case for most drugs), or active. Passive uptake involves passage from a high (plasma) concentration down a concentration gradient to a low (tissue) concentration. Subsequently, the gradients are reversed and drug is off-loaded from tissues into circulation. Whilst plasma is an extracellular fluid, tissues contain both intra- and extracellular compartments.

The classical example of high affinity of drugs for particular tissues is the propensity of aminoglycosides to accumulate and persist in renal tissue. First, it is necessary to consider the half-lives of drugs taking intravenous dosing as the simplest case. Half-life is related to the (terminal) slope of the decline in plasma concentration and is represented by Equation (3):

$$t_{1/2} = \frac{ln2}{Slope} = \frac{0.693}{\lambda} \quad (3)$$

For aminoglycosides, the decrease in plasma concentration is the sum of three separate exponential phases, each with its own slope and half-life. Thus, a semi-logarithmic plot of plasma concentration versus time reveals the three exponential phases (interpreted as a tricompartmental model). These are α, β, and γ phases and their pharmacokinetic significance is indicated in Table 4.[6] The α-phase is short and represents rapid distribution from plasma to other extracellular fluids. The β-phase is also short and represents the phase during which most of the drug is rapidly eliminated from the body, almost exclusively by excretion in urine in high concentrations. Important, in relation to residues of aminoglycosides, is the existence of a very late terminal phase. This final phase can be detected using a sensitive analytical technique. The plasma concentrations in this phase are less than those that are microbiologically effective and thus without therapeutic significance. This slow terminal phase reflects persistence of aminoglycoside residues in deep compartments. This terminal phase is controlled by the redistribution rate constant from tissue to plasma and accounts for the persistence of residues in renal tissue for weeks or even years.

Formulation

Many pharmacokinetic properties of drugs, including maximum plasma or blood concentration (C_{max}), time of achieving maximum concentration (T_{max}), AUC, absorption half-life, terminal elimination half-life, and bioavailability are dependent not only on the intrinsic properties the drug substance but also on a range of formulation factors, including composition, route of administration, and circumstances of administration (e.g. whether administered to fed or fasted animals). Therefore, drug withdrawal time is also product related, depending on route of administration and dosage regimen. On the other hand, the MRL is a drug substance property, intrinsically independent of any pharmacokinetic characteristics of the product. The MRL is thus fixed by regulatory authorities as a 'regulatory constant', with a universal meaning. It can therefore be used to achieve international harmonization, although in practice this ideal is rarely achieved.

A detailed discussion of this subject is outside the scope of this review, but it should be noted that the drug product excipients can profoundly influence both rate and extent of absorption of drugs. An example of particular importance to residue depletion is the use in food animal medicine of slow release/depot formulations. These are administered by intramuscular or subcutaneous injection to achieve slow sustained release of the active drug, maintaining plasma concentrations for prolonged periods, so that effective blood and tissue concentrations are maintained for periods of 2 days or even longer, thus achieving the advantage of single dose therapy.

When the absorption half-life is slower than the elimination half-life as computed from an IV administration, 'flip-flop' pharmacokinetics occurs and the calculated terminal half-life now represents the slow absorption phase of the drug and bioavailability (Figure 1).

The pharmacokinetic profile for slow release products is illustrated by the study of Toutain and Raynaud[5] (Table 2). An initial rapid absorption phase was attributed to immediate availability of a fraction. The second much slower absorption phase was associated with a larger fraction of the administered dose, with half-lives of 18.1 and 26.2 h in two separate studies, and these were the terminal half-life. As the true elimination half-life after intravenous dosing was 9 h, the product displayed flip-flop pharmacokinetics. Two examples[5,7] of drug products displaying flip-flop pharmacokinetics are presented in Table 3.

Some depot products have given rise to injection site residue concerns, as discussed in a recent review[8] and in peer-reviewed articles.[9,10] The quantity of drug administered in most sustained release products is large, as it is required to provide an initially high and then well-maintained therapeutic concentrations over two to five days. For these products, depletion from injection sites is often erratic and non-exponential and therefore unpredictable.

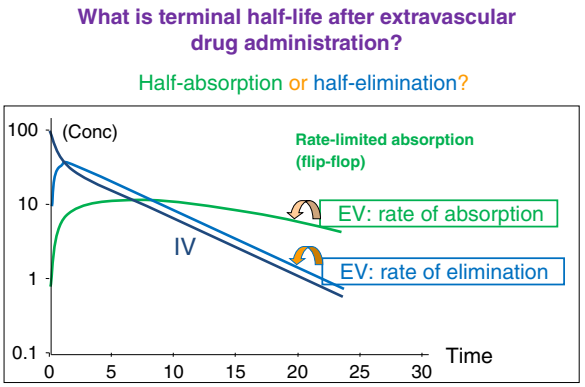

Figure 1. Flip-flop pharmacokinetics occurs when absorption rate is slower than elimination rate. The terminal phase then represents absorption half-life.

Table 3. Flip-flop pharmacokinetics of oxytetracycline and florfenicol in calves

Property	Oxytetracycline*	Florfenicol**
Concentration of drug in licensed product	20% $^{w}/_{v}$	40% $^{w}/_{v}$
Administered dose	20 mg/kg	40 mg/kg
Elimination half-life (terminal half-life after intravenous administration)	9.0 h	3.2 h
Terminal half-life after non-intravenous dosing	26.2 h (intramuscular)	41.1 h (subcutaneous)

* Data from Toutain and Raynaud.[5]

** Data from de Craene *et al.*[7] and unpublished findings from our laboratory.

This arises because, to achieve prolonged release, the products are formulated as suspensions in water (e.g. the poorly water soluble salts of benzathine and procaine benzylpenicillins) or suspensions in water repelling fixed oils (e.g. procaine benzylpenicillin) or as solutions containing organic solvents (e.g. 10%, 20% or 30% oxytetracycline). For products formulated in organic solvents, after intramuscular or subcutaneous dosing, the absorption of the solvents leads to precipitation of the drug. The drug then forms a depot and is slowly taken up into solution in interstitial fluid at the injection site. Because the suspension or precipitate may induce a local acute inflammatory response and/or act as a foreign body, a proportion may become walled off by granulation tissue. This fraction may be subject to very slow and erratic absorption.[10–12] The lack of consistency between regulatory authorities in addressing the issue of injection site residues has been discussed by Reeves.[13]

Disease state, population pharmacokinetics, and residue depletion

Anti-micicrobial and anti-inflammatory drugs are used extensively in farm animal species for prophylaxis (administration to prevent disease), metaphylaxis (treatment of animals not showing clinical signs of disease but in contact with those which are), and therapy (treatment of animals displaying clinical signs of disease). For regulatory purposes pharmacokinetic profiles are established in healthy animals; small numbers of animals, usually of a single breed, of similar age, and possibly of the same gender are used with an intensive sampling schedule. However, in the three clinical circumstances outlined above, the pharmacokinetic profiles are likely to differ in terms of mean values of clearance, terminal half-life, bioavailability, etc., and also possibly in respect of greater inter-animal variability.

There are a few literature studies conducted with large animal numbers and a sparse blood/plasma sampling schedule, which illustrate this variability. Such data are described as population pharmacokinetics as they are obtained from a sample of the clinical, usually diseased, population. Toutain *et al*. presented data on the population pharmacokinetics of doxycycline administered orally (in feed) to a large group (n = 273) of pigs.[14] Plasma concentrations exhibited marked inter-animal variability, such that plasma AUC for the drug ranged from 3 to 20 mg.h/ml.

The objective of population pharmacokinetics is to determine both pharmacokinetic variables which enable an optimal dose to be determined for the clinical population and also to explain inter- and intra-animal variability in terms of age, sex, breed, and health/disease status. As discussed by Martinez and Modric[15,16] in recent reviews, population pharmacokinetics is of relevance to tissue residue depletion rates. These authors emphasized that 'the implications of altered pharmacokinetics on human food safety and withdrawal times should be considered ..... human food safety implications are particularly pronounced when the covariant impact results in the prolonged residence or increased bioavailability of the drug'.[15,16]

In conducting residue studies for licensing purposes, the health status of the experimental animals is ignored, in that studies are conducted in groups of healthy animals. This is of concern for veterinary drug licensing purposes as, according to Nouws,[17] the disease state is an important factor affecting the withholding time. He determined tissue residue concentrations and persistence in tissues of a range of anti-microbial drugs, including beta-lactams, aminoglycosides, macrolides, tetracyclines, chloramphenicol, and sulfonamides in both normal and emergency-slaughtered ruminants after parenteral or intramammary dosing. He concluded that to predict withholding time in emergency slaughtered ruminants for muscle and kidney, it was necessary to multiply by a factor greater than 1 the values obtained in healthy cattle. There are no recent studies using current analytical methods to update these data. However, it is very likely that residues depletion of drugs is dissimilar in healthy and diseased animals. A possible solution would be to define the depletion profile in clinical subjects or in disease models which closely simulate clinical disease. However, there has been no major consideration of this possibility for a range of ethical, economic and scientific reasons.

Requirement for residues studies for bioequivalent products

Many of the veterinary drug products authorized for use in food producing species are generic drugs. They contain drugs developed initially as pioneer products, and which have subsequently been formulated in products containing the same drug, usually in the same concentration and usually, though not necessarily, in a similar formulation. For generic products, regulatory authorities require a pivotal study to establish average bioequivalence between the generic and pioneer product. Bioequivalence enables applicants for MAs for generic products to claim essential similarity, in respect to efficacy and safety, to the pioneer product.

This assessment of average bioequivalence is based on 90% confidence intervals for the ratio of the population geometric means (test/reference articles) for pivotal pharmacokinetic variables. The statistical method is two, one-sided *t* tests with the null hypothesis of bio-inequivalence at the 5% significance level. Generic and pioneer products are declared bioequivalent if upper and lower limits of the confidence intervals of the mean ratio of the log transformed variables AUC and C_{max} each fall within the *a priori* bioequivalence intervals of 0.80 to 1.25. It is then allowed that rate (represented by C_{max}) and extent (represented by AUC) of absorption are 'essentially similar'. Regulatory bodies generally

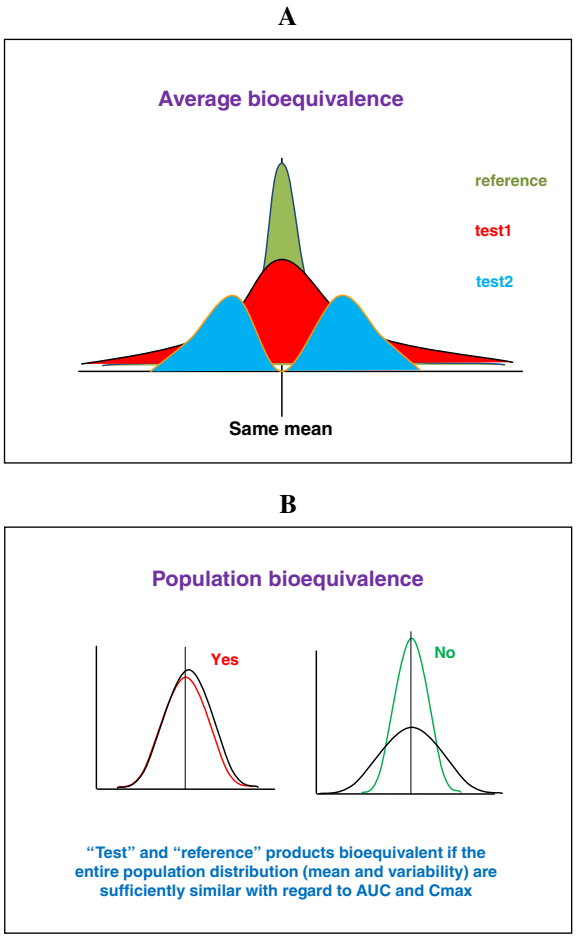

Figure 2. Distributions of values of bioequivalence variables (C_{max} or AUC) for test and reference products indicating bioequivalence: A, average bioequivalence; B, population bioequivalence.

accept that, although the pioneer and generic products are not identical in their pharmacokinetic profiles, they are considered to be *sufficiently similar* to be regarded as therapeutically equivalent, i.e. the products will have the same efficacy and safety profiles in clinical use. In general, the company applying for an MA for the generic product will not have to undertake extensive and expensive laboratory animal and target species safety studies and clinical trials to establish safety and efficacy in clinical use.

It is important to note that establishing average bioequivalence does not obviate the need to conduct residue depletion studies for each generic product for the following reasons:

(1) The definitions and statistical requirements of withdrawal time and average bioequivalence differ. Ensuring that 90% confidence intervals for the ratio of two treatment means for AUC and C_{max} are entirely contained within the limits 80% to 125% gives no guarantee that the upper 95% confidence limit of the 95th percentile of the population is below the MRL for a veterinary drug. It should be noted that bioequivalence can be determined at three levels, namely average, population, and individual. Average bioequivalence is the least stringent of the three in its requirements. Only establishing average bioequivalence is required by regulatory authorities (Figure 2A). Two formulations can be bioequivalent whilst their variances for AUC differ. This may have large implications for withholding times, which control a population percentage and not a mean parameter. Only establishing population bioequivalence guarantees equivalence of variance (Figure 2B).

(2) For two parenteral products (pioneer and generic), each administered intramuscularly or subcutaneously, depletion rates from the injection site may be sufficiently similar to provide AUC and C_{max} means that fall well within the preset limits for average bioequivalence. However, they may differ sufficiently to yield significant and even quite large differences in injection site concentration.[11,12]

(3) It is not only at the injection site that residue depletion from tissues may differ, however. The depletion rate is not guaranteed by average bioequivalence *for any edible tissues*. Average bioequivalence demonstrates only essential similarity in rate and extent of absorption between two products for a range of therapeutically useful plasma concentrations. It cannot ensure the same rate of decrease of concentration in the terminal phase. The terminal phase may have no therapeutic relevance, as discussed for aminoglycosides such as gentamicin. Table 4 reports for gentamicin in sheep a rapid elimination phase, followed by a much slower fall in concentration with $t_{½}$ values of 1.83 and 44.9 h, respectively, for exponential β and γ phases. The γ phase is attributable to unloading and elimination of drugs from tissues, including

Table 4. Aminoglycoside pharmacokinetics*

Phase	Description	Significance
α	Distribution half-life	Distribution from plasma to principally extracellular fluids.
β	Therapeutic elimination half-life	Determines interval between doses.
γ	Very late elimination half-life	Represents drug release from tissues, i.e. residue depletion**

Half-lives of gentamicin in sheep.

Half-life	Time (h)
$t_{½}α$	≤ 0.5h
$t_{½}β$	1.44 - 1.77h
$t_{½}γ$	42.6h at 3mg/kg dosage and 164.2h at 20mg/kg dosage

* Adapted from Papich and Riviere.[6]
** Firm binding to renal cortical tissue.

edible tissues, in this case the kidney. Clearly, it is not possible for average bioequivalence established over the first 12–24 h (or less) after dosing to give assurance on the same tissue exposure in the γ phase many days or even weeks later. In addition, a late terminal phase may arise due to flip-flop pharmacokinetics undetected by plasma concentration in a bioequivalence study.

Acknowledgement

The support of the Organisation for Economic Cooperation and Development is gratefully acknowledged.

Conflicts of interest

P. Lees received financial support from OECD to enable him to present a lecture on the topic of this article at a meeting, held in Saskatoon, Canada.

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