

ATTACHMENT 2

Challenges associated with the evaluation of veterinary product bioequivalence: an AAVPT perspective

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The Generic Animal Drug Patent Term Restoration Act (GADPTRA) enacted in 1988 provided the same benefits to animal drug products that were granted to human generic products. It has been over 13 years since the GADPTRA was enacted, and veterinary drug sponsors and regulators have gained enormous insight and experience into some of the unique challenges associated with the determination of product bioequivalence for veterinary dosage forms. Moreover, advances in information and technology have opened both new issues that must be addressed and new mechanisms for demonstrating product bioequivalence. While many aspects of the existing Center for Veterinary Medicine Bioequivalence Guidance continue to provide invaluable guidance to the animal drug industry, there are also aspects of this guidance that are being called into question. Therefore, during the 2001 annual meeting of the American Academy of Veterinary Pharmacology and Therapeutics, participants were asked to address issues and concerns associated with the evaluation of veterinary product bioequivalence. This manuscript provides a summary of the concerns and discussions that transpired.

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INTRODUCTION

In 1984, President Reagan signed into law the Drug Price Competition and Patent Term Restoration Act (Waxman Hatch Act). This historic piece of legislation created a system for the review and approval of generic versions of post-1962 human 'pioneer' drug products through the use of abbreviated new drug applications (ANDAs). It also provided the opportunity for patent holders to extend the duration of their patent to 17 years following the date of patent issue. In 1988, Congress enacted the Generic Animal Drug Patent Term Restoration Act (GADPTRA) to create a system for review and approval of generic versions of new animal drug products. Thus, abbreviated new animal drug applications (ANADAs) could be filed for any animal drug product approved after 1962, so long as the product or its active ingredient was not derived from recombinant DNA technology, protected by patent or pioneer marketing exclusivity (5 years for original approvals, 3 years for new uses approved on the basis of clinical or field investigation), withdrawn from market because of safety or effectiveness concerns,¹ or the subject of a notice of opportunity for hearing.

Following the enactment of the GADPTRA, the Center for Veterinary Medicine (CVM), US Food and Drug Administration (FDA) was responsible for developing and implementing veterinary generic drug regulatory policies that were consistent with the Act. Between 1988 and 1990, CVM issued nine policy letters and an initial Bioequivalence Guidance. However, it soon became apparent that certain modifications in the existing Bioequivalence Guidance were necessary. Therefore, in 1993, CVM, the American Academy of Veterinary Pharmacology and Therapeutics (AAVPT), Animal Drug Alliance, and Animal Health Institute cosponsored the landmark Veterinary Drug Bioequivalence Workshop (Martinez & Riviere, 1994). This resulted in the 1996 Revised CVM Bioequivalence Guidance that is in effect today. Although CVM issued a new version of this guidance in 2000, the update differed only in the addition of a section describing the algorithm for calculating confidence intervals for Ln-transformed data.

Generic products contain the identical active ingredient as that associated with the approved product. No active ingredient of a single active ingredient animal drug product may be substituted for another. However, some differences between innovator and generic products may be considered suitable for

ANADAs. Accordingly, Suitability Petitions may be filed if the proposed generic product differs from the listed product in one of the following ways (Section 512(n)(3) of the Food Drug and Cosmetic Act):

- 1 a different dosage form;
- 2 a different strength;
- 3 a different route of administration;
- 4 it contains more than one active ingredient and one of the active ingredients is different than that of the listed drug product;
- 5 it is a product intended for use in combination with another product in animal feed and the active ingredient of one of the products is different from the active ingredient of one of the listed products approved in combination.

For information regarding generic animal drugs and requirements for Suitability Petitions, refer to <http://www.fda.gov/cvm/index/memos/cvmm50.html>.

The labeling for the proposed generic product must contain all of the same indications, warnings, cautions, directions for use, etc., that are associated with the approved pioneer product [except as deemed appropriate on the basis of the Suitability Petition: 21 CFR 512(n)(1)(F)]. The finished product must also be of the same strength, dosage form, and route of administration as the approved product (unless it was the subject of an

approved Suitability Petition). In general, all the technical sections applicable to new animal drug applications (NADAs) are also associated with an ANADA. Both innovator and generic products must be manufactured in accordance with FDA's Good Manufacturing Practice regulations (GMPs), and an FDA inspection for compliance with GMP's is required (21 CFR Parts 211 and 226). Every application must contain either an environmental assessment or a request for a categorical exclusion [21 CFR 25.15(a)].

In its fifth policy statement, CVM stated the importance of the goals detailed in the generic legislation, including the need to avoid duplicate research, provide incentive for generic sponsors to innovate, and to make the conditions of use of the pioneer and generic drugs the same to the maximum extent possible. Flow diagrams of the technical sections associated with NADAs and ANADAs are provided in Figs 1 and 2. In general, the fundamental differences between the two types of applications are the components of the human food safety package and the kinds of data needed to support target animal safety and effectiveness. For example, while the safety and effectiveness of a new animal drug product is demonstrated through extensive clinical trials, safety and effectiveness of abbreviated applications may be confirmed through the demonstration of product bioequivalence. Moreover, if an innovator product is associated with a withdrawal time and

¹The following historical background on the Food, Drug and Cosmetic Act (FD&C) is excerpted from, http://www.fda.gov/ora/science_ref/lpm/lpchr16.html#16.2 Historical Background and Law

The food and Drug Act of June 30, 1936, prohibited adulteration and misbranding of drugs in interstate commerce. Following an adverse ruling (United States vs. Johnson) by the Supreme Court of the United States in 1911, Congress passed the Sherley Amendment the same year, which added to the Act a prohibition against claims of curative or therapeutic effects being placed on the package label with intent to defraud the purchaser. The burden of proof of adequacy of claims, however, was on the government. The question of safety was not raised.

In early 1938, the Wheeler-Lea Amendment to the Federal Trade Commission (FTC) Act, which clarified the jurisdiction of the FTC and the FDA with regard to advertising, reserved control of drug advertising for the FTC. This weakening of FDA authority, in conjunction with a drug-related disaster, paved the way for passage of a completely new law.

The disaster was the 'Elixir of Sulfanilimide' tragedy of 1937 in which 107 people were killed as the result of the use of a solvent, diethylene glycol, in a product. As the law did not require prior testing of drugs for safety, there was no way to anticipate, or prevent, the marketing of this lethal mixture. The Federal Food, Drug and Cosmetic Act was passed on June 25, 1938. The new law prohibited the marketing of 'new drugs', a term that is specifically defined in the FD&C Act, unless the new drug had been tested and found to be safe for use under the prescribed conditions. The new law also required that the names and amounts of active drug ingredients be declared, and that labels contain warnings against habituation in the case of certain drugs. The requirement that intent be demonstrated in the case of false or fraudulent claims was dropped.

The Durham-Humphrey Amendment of 1951 defined the term 'prescription drug', and proscribed the dispensing of such drugs without a legal prescription. Over-the-counter (OTC) drugs were required to bear labeling that contained adequate directions for use and specific warnings against misuse.

In 1962, another drug disaster ensured the passage of sweeping drug control legislation. Thalidomide, a sedative, was found to cause deformities in children born to women who had taken the medication during pregnancy. The deformity, known as phocomelia, occurred in nine children born to women in the United States who had taken the drug while it was in investigational status. The drug had never been approved for commercial sale in the United States, but the tragedy had much greater proportions in Europe where thalidomide had been widely distributed. The danger of inadequate drug testing prior to clinical trials was amply demonstrated, and the need for tighter controls was unmistakable. On October 10, 1962, the Kefauver-Harris Drug Amendments were passed by Congress. These amendments included the following changes:

- FDA was authorized to establish current good manufacturing practices (CGMPs) under which drugs must be manufactured
- Drug manufacturers were required to register annually.
- A new drug could no longer be marketed prior to FDA approval based on convincing evidence of the drug's safety and effectiveness. The burden for this proof was placed on the manufacturer.
- Reasons were listed for which a previously approved drug could be removed from the market.
- New controls were placed on experimental and investigational drugs.
- Regulation of advertising of prescription drugs was returned to FDA.

In 1972, Congress passed the Drug Listing Act, which gave FDA the means to readily determine which drugs were actually being manufactured and commercially distributed.

Technical Sections of an NADA

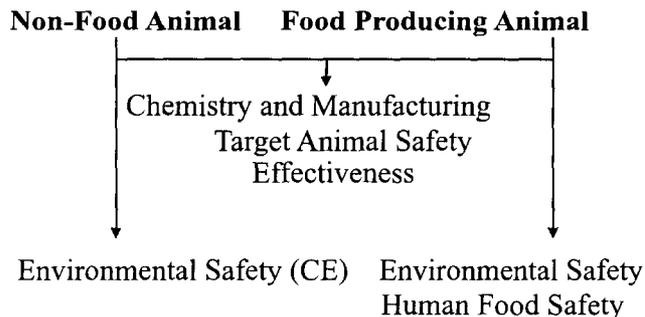


Fig. 1. Flow diagram of the technical sections associated with a new animal drug application (NADA). Components of the human food safety package include toxicology, total residue depletion and metabolism, comparative metabolism (toxicology species vs. target animal species), residue depletion, analytical method development, and (for antimicrobial agents) risk analysis (which may include an evaluation of the development of microbial resistance and the impact of the antibiotic on the integrity of human gut flora).

Technical Sections of an ANADA

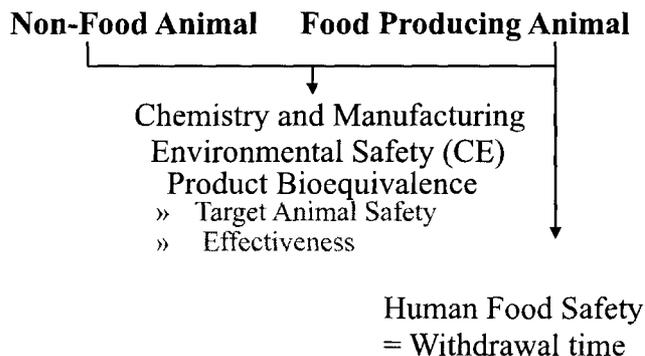


Fig. 2. Flow diagram of the technical sections associated with an abbreviated new animal drug application (ANADA). Note that in this case, the demonstration of product bioequivalence covers both the safety and effectiveness components of the application. Unlike that associated with human pharmaceuticals, the inclusion of the safety component necessitates that these studies be conducted under Good Laboratory Practices.

the demonstration of product bioequivalence is not waived, then the human food safety package will consist only of a study to assess the time to tolerance (tissue withdrawal time investigation).

Differentiation between the approval considerations associated with an NADA and an ANADA can be summarized as the evaluation of prescribability vs. switchability (with the reference and other therapeutically equivalent generic products). When

dealing with prescribability, the practitioner needs to know that a given dose, when administered to a particular patient, will result in a response consistent with that defined in the clinical safety and effectiveness trials associated with the original (pioneer) drug application. Targeting an appropriate dose is based both upon label information and, for dose ranged products, clinical judgment. Further individual dose adjustments may be necessary to meet the particular needs of the patient. Once that patient has been titrated to a particular dose, it is assumed that so long as that same dose is maintained, a predictable response will be achieved. Switchability refers to the ability to switch a patient to other therapeutically equivalent formulations without observing any change in the clinical response. In other words, these switches are invisible to the patient. Ensuring switchability is the goal of the generic drug approval process. This assurance applies not only to *in vivo* bioavailability but also to manufacturing controls and product quality.

More than 12 years have now passed since the GADPTRA was enacted, and today, the appropriateness of certain features of the CVM BE guidance are being challenged. This is because of the array of scientific and technological advances impacting the development of human and veterinary pharmaceuticals, the unique challenges encountered during the bioequivalence evaluation of animal drug products, and the evolution of animal husbandry practices and companion animal medicine. Accordingly, there is a concern that some of the recommendations initially forwarded within the CVM bioequivalence guidance may no longer be appropriate for certain types of veterinary products and that changes are needed to encourage submission of more veterinary generic drug applications.

During the Twelfth Biennial Symposium of AAVPT, a committee representing diverse perspectives developed a list of their most pressing questions associated with the evaluation of veterinary product bioequivalence. Because bioequivalence concepts are used not only to support the approval of generic drug products but also pre- and postapproval pioneer product changes, these issues have broad-reaching implications. In this meeting, an expert panel and Academy members were provided an opportunity to explore their perspectives on the design, criteria and statistical analysis of bioequivalence studies as currently recommended in the CVM 2000 Bioequivalence Guidance. Although not directly covered during this meeting of the AAVPT, these issues also have direct bearing on positions expressed in the European Agency for the Evaluation of Medicinal Products (EMA) Guidelines for the Conduct of Bioequivalence Studies for Veterinary Medicinal Products (www.emea.eu.int/pdfs/vet/ewp/001600en.pdf).

This paper provides a summary of the presentations and discussions occurring during this meeting. Positions stated in this paper reflect the authors' best attempt to capture the opinions expressed by the majority of workshop participants, and are not intended to reflect the position of the FDA or any specific individual or organization.

STUDY DESIGN

Clinical or physiological endpoint studies in lieu of in vivo blood level studies

There have been numerous occasions when sponsors have requested permission to perform clinical endpoint studies rather than blood level bioequivalence comparisons. Reasons for these requests have included highly variable plasma drug concentration/time profiles and product differences in rate and/or extent of absorption that a generic product manufacturer believes to be therapeutically insignificant. Therefore, the following questions were discussed:

- Other than those cases when blood drug concentrations are not quantifiable, when could clinical or physiological endpoint studies be used in lieu of *in vivo* blood level bioequivalence studies?
- What, if any, additional data would be needed to support clinical safety and/or effectiveness in these situations?
- If a clinical endpoint bioequivalence study is permitted, how do we select the indication for establishing bioequivalence if multiple indications appear on the product label? Are multiple studies needed if the different indications are associated with different biological mechanisms?
- If a clinical endpoint bioequivalence study is used for an antibiotic, will a clinical endpoint study be sufficient to ensure that the generic (or revised innovator) product will continue to be equivalent to the approved formulation in the face of decreasing pathogen susceptibility?
- If a clinical endpoint study is used to establish product bioequivalence, will the generic product need to conduct additional studies to obtain new pioneer indications once the exclusivity period has expired?

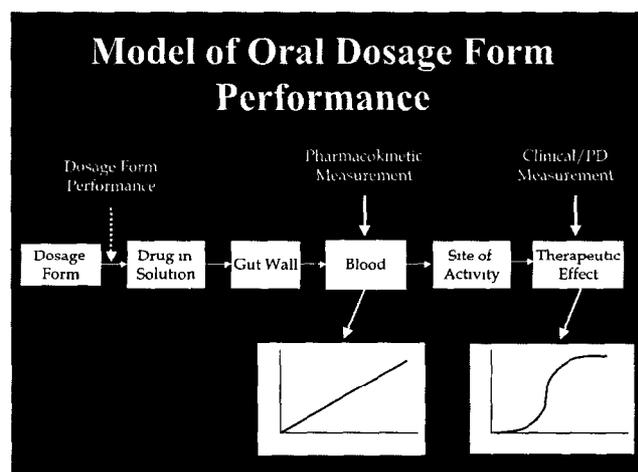


Fig. 3. Diagram illustrating the sequence of processes associated with the progression of drug performance from the point of administration to the ultimate therapeutic effect.

Expressed views

Situations where clinical endpoint studies are clearly appropriate include typically active drug products (including ophthalmic and otic preparations), and locally acting gastrointestinal (GI) products (including enteric parasiticides). Nevertheless, there are several fundamental difficulties associated with the use of clinical endpoints for demonstrating product bioequivalence:

- As illustrated in Fig. 3, the critical step in the comparative performance of two dosage forms is the drug leaving the formulation and dissolving into solution, at which point it is available for absorption into the body. Although this event generally cannot be measured directly, it is the most important formulation-related step in the assessment of bioequivalence. Additional steps that are outlined in Fig. 3 move from the point of drug absorption to the arrival of the compound at the site(s) of activity, leading to both desirable and undesirable therapeutic effects. The intervening steps are entirely patient or subject dependant processes, thereby resulting in progressively increasing levels of endpoint variability. Consequently, substantially greater variability can be anticipated in the dose-response relationship than in the corresponding relationship between dose and blood drug concentrations, which occurs earlier in the illustrated process. For this reason alone, fewer subjects will be needed to achieve the power to confirm blood level bioequivalence as compared with clinical endpoint bioequivalence. In addition, blood level measurements are 'closer' in the process to the critical formulation event.
- Assuming linear kinetics, the same relationship in product bioavailability can be expected, regardless of the dose employed (Fig. 4). In contrast, dose/effect relationships are often sigmoidal, with very little change in response occurring at both the initial and terminal portions of the curve (Fig. 5). Under this situation, two products can have significantly different bioavailability,

Plasma Concentration-Dose

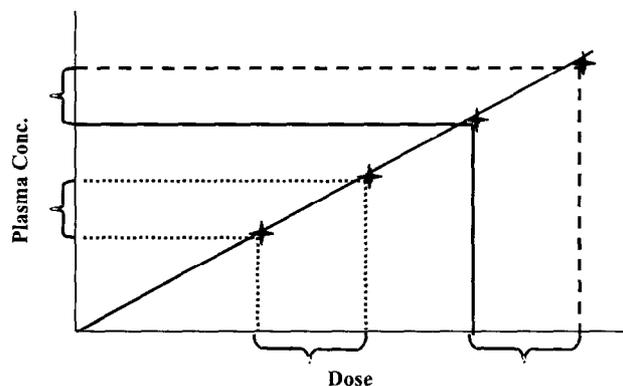
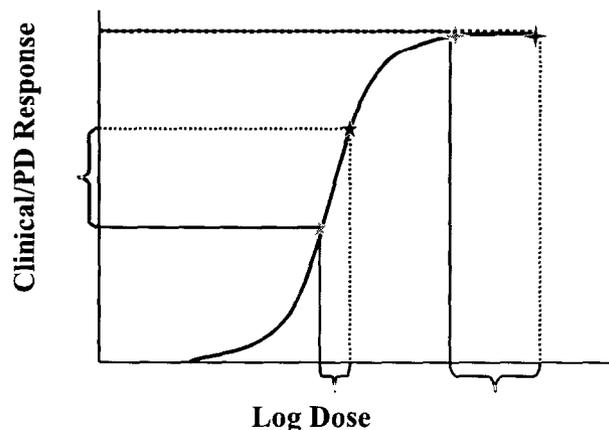


Fig. 4. Linearity of the relationship between dose and blood drug concentrations associated with well-behaved drugs.



Topical Dermatologic Corticosteroids:
In Vivo Bioequivalence (www.fda.gov/cder/guidance/old098fn.pdf)

Fig. 5. Example of the sigmoidal-type relationship frequently observed when evaluating dose-response profiles.

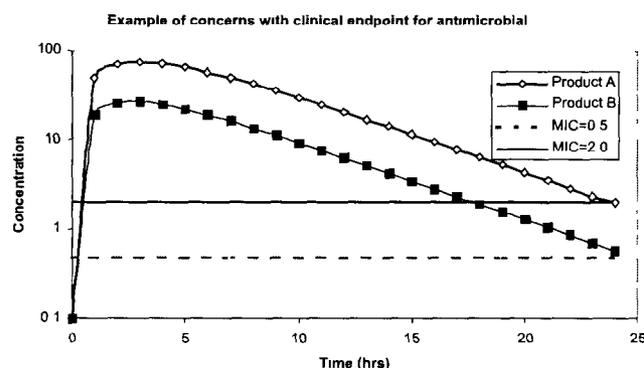


Fig. 6. Example of the potential consequences associated with using a single-point clinical endpoint bioequivalence trial for confirming the comparability of two formulations of an antimicrobial agent. In this case, the MIC of the pathogen has increased over time, rendering the products no longer comparable. Let us assume that at the time test product testing, the MIC of the targeted pathogen was $0.5 \mu\text{g/mL}$. However, over time the MIC increases to $2 \mu\text{g/mL}$. Let us also assume that the active drug substance exhibits time-dependent killing and is associated with little if any postantibiotic activity. In this case, we see that while the test and reference product would perform comparably when the pathogen MIC is $0.5 \mu\text{g/mL}$, only product A would be effective when the MIC of the pathogen increased to $2.0 \mu\text{g/mL}$.

leading to different blood concentrations, but effect identical clinical responses. For this reason, the Center for Drug Evaluation and Research (CDER) considers a clinical endpoint bioequivalence study valid when it has been demonstrated that the administered dose is being tested on the linear portion of the dose/response curve. Otherwise, the study may lack the sensitivity needed to distinguish between products with different formulation performance. An example of CDER's protocol recommendations for these types of studies can be found in

their guidance titled 'Topical Dermatologic Corticosteroids' (www.fda.gov/cder/guidance/old098fn.pdf). As seen in Fig. 6, failure to provide this confirmation can be particularly problematic with antimicrobial compounds where pathogen susceptibility may decrease over time. If the rate and extent of drug absorption are not equivalent, two products may perform comparably when the pathogen susceptibility is high [i.e. has a low minimum inhibitory concentration (MIC) value], but may not perform comparably if the MIC of the pathogen increases. In other words, the dose-response curve for that drug and pathogen shifts to the right so that the administered dose is now on the steep part of the response curve in which previously unperceived differences are now apparent.

- If validation of the dose-response relationship is required, the cost of conducting a clinical endpoint bioequivalence study will markedly exceed that associated with traditional *in vivo* blood level trials. Moreover, considering the difficulty associated with demonstrating improvement over placebo when conducting traditional effectiveness trials, it is easy to recognize the enormous difficulty associated with the demonstration of clinical endpoint bioequivalence. For this reason, there is a much greater risk of failure to perform a successful study with acceptable sensitivity associated with a clinical endpoint bioequivalence study than for a traditional blood level bioequivalence investigation.

There are other complications that should also be considered. For example, many approved products have multiple indications. In this situation, it would be economically prohibitive to conduct multiple clinical endpoint bioequivalence trials. Several participants expressed the opinion that multiple clinical endpoint studies should be conducted if multiple indications appear on the pioneer product label. However, the majority of participants agreed that if the dose-limiting indication is evaluated and if the study is conducted on the linear portion of the dose-response curve, only one indication needs to be evaluated. For example, in Fig. 7, let us assume that indication 1 = analgesia control and indication 2 = fever control. If fever control is shown to be proportional to product bioavailability, then this indication can be used as an end-point for establishing product bioequivalence. Accordingly, these two products would obtain identical indications, even though equivalence was confirmed only on the basis of indication #2. In fact, this is the type of situation encountered with the evaluation of human generic topical corticosteroid products.

Everyone agreed that given the number of critical variables that must be considered when developing these clinical endpoint bioequivalence study protocols, greater CVM guidance on protocol development is essential.

Concerns with use of parallel study designs

There are many occasions when it is difficult to conduct crossover studies in animal species. Examples include:

- studies in growing animals whose physiology can change significantly over time;

Level of response to the reference and test products for two different indications

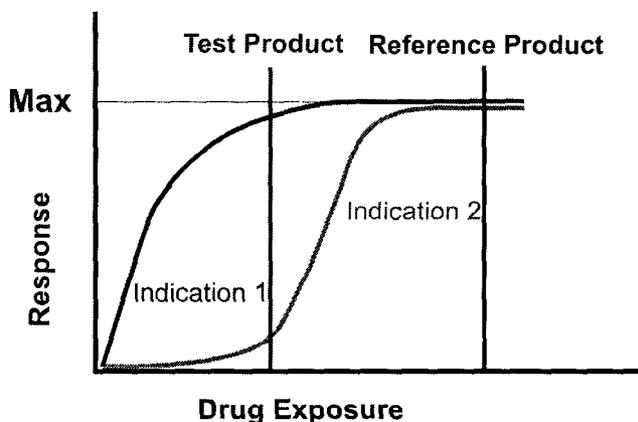


Fig. 7. Example of two very indication-specific dose-response relationships. In this situation, unless the two products were tested on the linear portion of the dose-response curve for one of the indications, verification of equivalence for one indication may not be adequate to confirm equivalence with respect to the other.

- drugs with very long elimination half-lives;
- sustained release products whose release continues over several months;
- very small animals (e.g. fish, poult, chicks).

The fundamental challenge related to the use of parallel study designs is the substantial increase in variability associated with the parameter estimates. Unlike crossover trials where clearance and volume are assumed to be constant within each pairwise (within-subject) comparison, such assumptions are not appropriate when rendering comparisons across study subjects (parallel study design). Accordingly, the power associated with the treatment comparison decreases, necessitating the inclusion of more study subjects. In addition, it is more important to balance treatment groups for factors that may influence bioavailability and pharmacokinetics, including age, weight and gender.

These concerns lead to the question: what are some reasonable alternatives for establishing product bioequivalence when it is difficult to conduct a crossover trial?

Expressed views

The impact of study design on estimates of variability and treatment means was demonstrated by the example of a parallel design pilot study that failed to identify product bioequivalence. This pilot study was conducted in four animals, three receiving the test product and one receiving the pioneer product. As shown in Fig. 8, based upon the pilot data, the two treatments were concluded to perform similarly and therefore the pivotal *in vivo* bioequivalence trial was undertaken. Using a crossover study design, the pivotal study clearly indicated that the test product was more bioavailable than the reference (Fig. 9). It was concluded that because of the marked intersub-

ject variability, there is a clear preference for conducting pilot studies using a crossover design. Alternately, parallel pilot studies, when appropriate, should employ enough subjects to accurately predict the likelihood of product inequivalence.

Unfortunately, no clear-cut alternatives were suggested to improve situations when crossover trials cannot be undertaken. Alternative designs that allow for scaling the bioequivalence criteria to accommodate larger reference product variability generally require the use of extended crossover designs (three or more study periods). The use of statistical population bioequivalence criteria, while allowing for reference scaling with parallel study designs, are appropriate for evaluating prescribability and not switchability. Other suggestions such as *post hoc* changes in confidence interval criteria based upon published literature were met with criticisms for reasons also discussed later. Each of these alternatives is discussed elsewhere in this review. Although the use of population approaches (such as nonlinear mixed effects models) were mentioned, it was agreed that such novel approaches need further evaluation and validation before being used as an alternative approach for confirming product bioequivalence within a regulatory environment.

Defining the moiety for the bioequivalence assessment

Protein binding

Protein binding is a significant concern when evaluating the pharmacokinetics of a new drug substance. In these situations, the relative proportion of free to bound molecule can markedly impact the accuracy of therapeutic predictions based upon total (free plus bound) drug moiety (Wise, 1986; Shargel & Yu, 1999; duSouich *et al.*, 1993). In contrast, as formulation does not impact drug pharmacokinetics beyond the absorption phase, it is unlikely that product free drug concentrations will differ if total concentrations are found to be similar. Therefore, we explored the issue of whether or not there are situations when something other than total drug concentrations should be considered in evaluating product bioequivalence.

Expressed views

Participants concurred that as the majority of drugs are dosed within a range associated with linear protein binding, differences in product bioavailability will rarely effect differences in the free concentration that are not adequately described by total drug comparisons (see Fig. 10). However, in those rare instances where nonlinear protein binding is of concern, alternative study designs and analyze measurements may be necessary. Examples of drugs known to exhibit nonlinear protein binding include valproic acid (Wong *et al.*, 2001), disopyramide (Piscitelli *et al.*, 1994), ceftriaxone (McNamara *et al.*, 1983), and a MK-826, a carbapenem antibiotic (Wong *et al.*, 1999). It should also be noted that protein binding characteristics may vary not only between species (e.g. Riond & Riviere, 1989; Riond & Riviere, 1990; Lin *et al.*, 1994), but also across maturity levels within a species (e.g. Wong *et al.*, 2001).

The question therefore is when does the measurement of total drug concentrations fail to adequately identify inequivalent

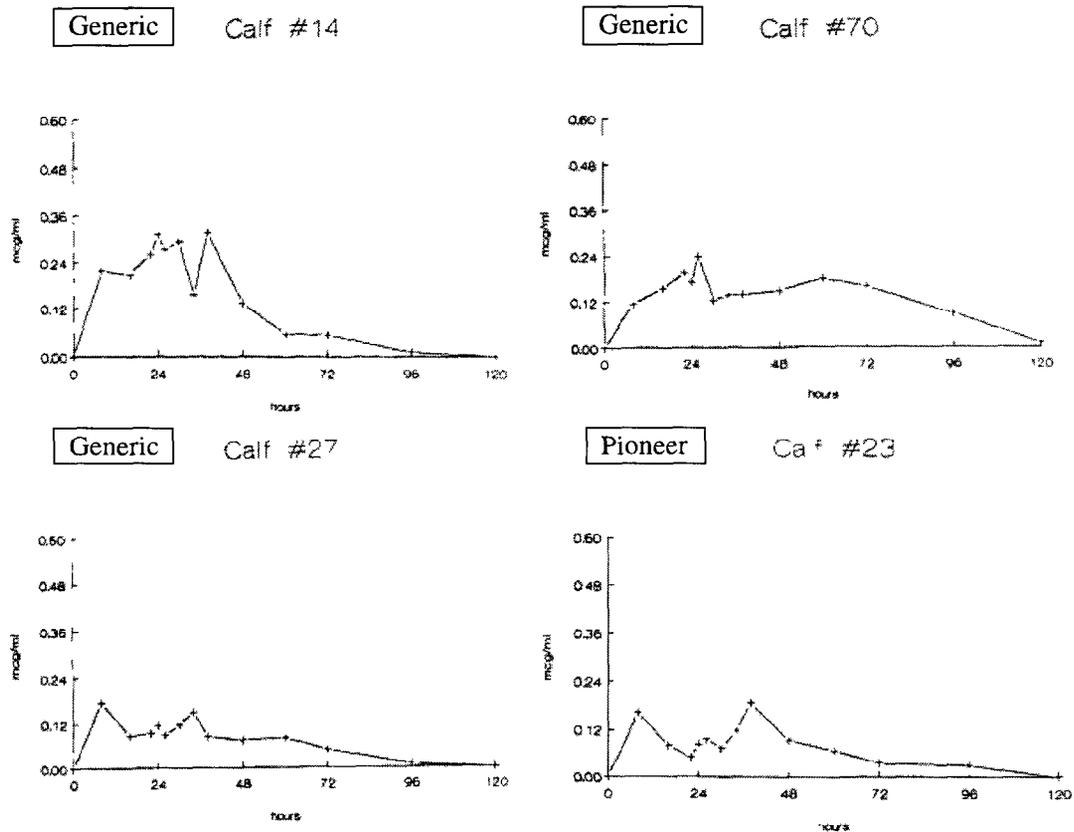


Fig. 8. A parallel design pilot study suggested that the pioneer and generic products are likely to be bioequivalent. On the basis of this observation, a crossover design pivotal bioequivalence study was undertaken. The results of the pivotal trial are provided in Fig. 9.

products? Generally, this concern is limited to the rate instance of a highly bound drug that exhibits nonlinear kinetics, high clearance rate and rapid absorption. Under these conditions, differences in absorption rate may lead to differences in peak free fraction concentrations that are not adequately detected on the basis of the total drug moiety (Rolan, 1994). This difference could be important if the drug has a narrow therapeutic window. However, for low clearance drugs, altering the fraction unbound (f_u) will simultaneously alter the total drug clearance (CL). As $CL = f_u \times CL_{u, int}$ (where $CL_{u, int}$ is the intrinsic drug clearance of the unbound drug), we can expect to see a decrease in elimination half-life and consequently a change in total drug concentration. Thus, a change in free fraction for these compounds is likely to also change CL [and therefore area under the curve (AUC)]. The corresponding extent to which elimination half-life will change depends upon the magnitude of change in the volume of distribution. Therefore, one can envision pharmacokinetic conditions under which total drug concentrations measured at steady state may be adequate to identify an inequivalent product even if the drug moiety is associated with nonlinear protein binding. Evaluation of total drug concentrations at steady state is suggested so that bioequivalence can be evaluated when plasma drug concentrations are raised to levels likely to occur when the products are administered under clinically relevant conditions.

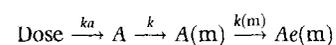
An example of the latter is disopyramide, which has a low CL (dependent upon free fraction) and is associated with nonlinear protein binding. When comparing single dose vs. steady state administration or when comparing immediate release vs. controlled release formulations, it was noted that marked inequivalence of total drug concentrations was observed (Piscitelli *et al.*, 1994). However, conclusions of equivalence were obtained when free drug concentrations were measured. Accordingly, as the pharmacodynamic effect (QTc interval) was associated with free drug concentrations, the maximum percent change in QTc interval from baseline was not significantly changed between preparations.

Metabolite profiles

The second issue pertained to those instances where the administered compound is a prodrug (and therefore the parent molecule is inactive) or when both parent and metabolite(s) are active. Discussions focused on identifying those situations when metabolites should be measured.

Expressed views

In general, the scheme for metabolite kinetics in the body can be described as follows (Houston, 1982):



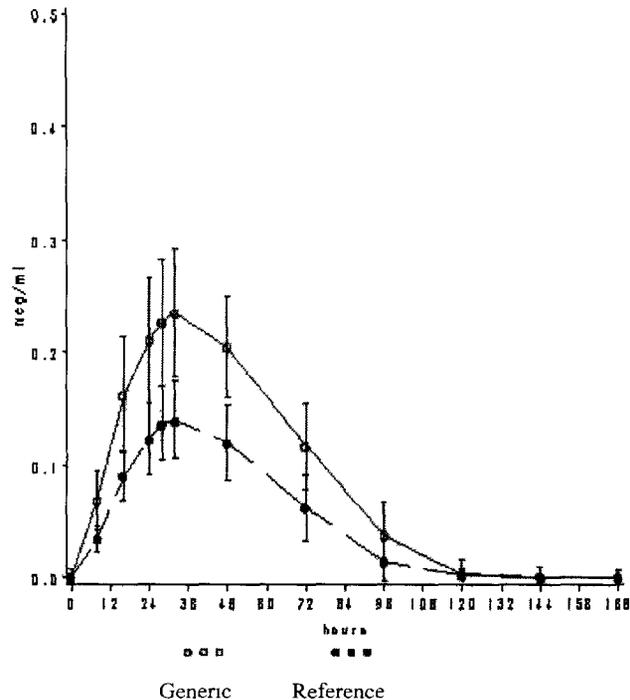


Fig. 9. In contrast to the relative bioavailability predictions derived from the pilot investigation (Fig. 8.), the pivotal data, the pivotal data clearly demonstrated that the test and reference products were bioequivalent.

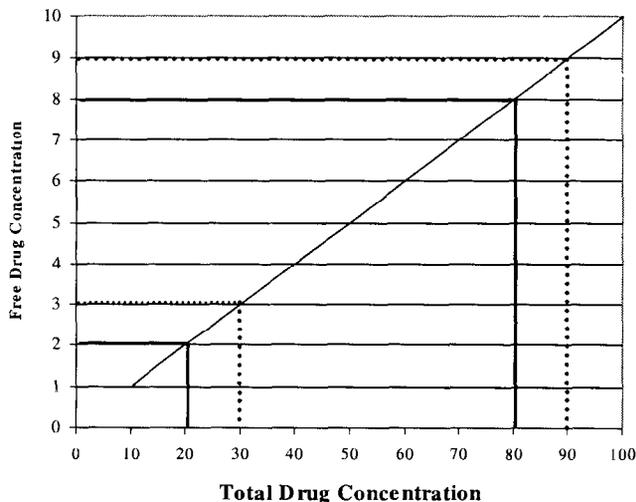


Fig. 10. Graphic demonstration of the linear relationship between total and free drug concentrations for well-behaved compounds.

where A is the amount of drug in the body; $A(m)$ and $Ae(m)$ are the amount of metabolite in the body and amount excreted, respectively; k_a is the first-order rate absorption rate constant for the parent compound, k is the first-order rate constant for elimination of the drug, leading to the formation of the metabolite; and $k(m)$ is the first-order elimination rate constant

of metabolite. As the metabolite is generally formed subsequent to drug absorption, the profile of the parent compound is a more sensitive index of absorption rate. This was born out in a study by Chen & Jackson (1991) when they evaluated both real and simulated datasets. In that study, the authors determined that the parent compound provides a much more sensitive measure of differences and variability in drug absorption than does the metabolite.

Participants agreed that there are, nevertheless, several situations when the metabolite rather than the prodrug may need to be measured. These include when the rate of metabolite formation is approximately equal to the rate of parent drug absorption ($k \sim k_a$) and when there are significant stability or analytical problems associated with the prodrug. Except for these relatively rare situations, only the parent compound needs to be included in the evaluation of product bioequivalence. Perspectives similar to this are contained in the CDER guidance titled 'Bioavailability and Bioequivalence Studies for Orally Administered Drug Products - General Considerations' (October 2000). That guidance states that the one situation when both parent and metabolite should be measured is when a metabolite is formed as result of gut wall or other presystemic metabolism and when that metabolite contributes to the safety and/or effectiveness of the product. In that case, the guidance states that the metabolite data is submitted to the Agency as supportive evidence of bioequivalence, but that the parent compound data is analyzed by the confidence interval approach and is considered the primary evidence of bioequivalence.

Defining the administered dose

Tablet strength. Many oral dosage forms are manufactured in multiple strengths. In human medicine, these differences usually translate into differences in the administered mg/kg dose. In veterinary medicine, in addition to dose-ranged products, a wide range of tablet strengths is needed to accommodate size differences among breeds. In human medicine, bioequivalence studies are generally conducted with the highest tablet strength to ensure against problems such as poor blend uniformity and dissolution characteristics, and to protect against potential nonlinear kinetics (that could magnify the clinical impact of inequivalent absorption characteristics). For consistency, these same recommendations were included in the 1996 CVM Bioequivalence Guidance. However, such recommendations do not account for differences in tablet size intended solely to compensate for the size of breed. Therefore, in veterinary medicine, such a policy could significantly encumber the effective execution of an *in vivo* bioequivalence trial. Accordingly the following questions were addressed:

- What alternatives to the current recommendations may be appropriate to veterinary medicine?
- If lower tablet strengths are employed, should dissolution or other supportive data be considered to support biowaivers for the higher strength tablets?

Table 1. Example of dose normalization of parameter estimates

	Dose correction	Observed AUC (units)	Corrected AUC (units)	Observed C _{MAX} (units)	Corrected C _{MAX} (units)
SUBJ A	10/8 = 1.25	150	187.5	40	50
SUBJ B	10/10 = 1	150	150	40	40
SUBJ C	10/12 = 0.83	150	125	40	33.3

Expressed views

Consensus opinion supported the need to demonstrate product bioequivalence on the basis of the highest mg/kg dose allowed on the pioneer product label. However, to avoid either overdose studies or the need to use very large breed animals, participants agreed that the tablet strength should be commensurate with the strength needed to achieve the maximum allowable mg/kg dose for whatever breed is included as the study population.

If the *in vitro* dissolution characteristics of the test and reference tablets undergoing *in vivo* bioequivalence testing are similar, then the higher and lower strength tablets would be compared against the corresponding strength of the reference tablet. In cases where the bio-batch *in vitro* dissolution characteristics differ, then the dissolution profiles associated with the various strengths of the test product would be compared with the lot of the test product that underwent *in vivo* bioequivalence testing. As indicated in the CVM Bioequivalence Guidance, the granting of a waiver for the various strengths of the test product will also depend upon the comparability of manufacturing methods and the presence of dose-proportionality in the relative amounts of active and inactive ingredients.

Differences in mg/kg dose

Concern was expressed regarding those instances when the mg/kg dose may differ across subjects, such as when comparing a paste to a tablet or when testing for tablet bioequivalence using a parallel study design. The mg/kg differences may also occur when subjects receive drug *ad libitum* in food or water. The question therefore was how best to accommodate these differences in dosage so that they do not increase to the statistical variability associated with the bioequivalence parameter estimates.

Expressed views

Nearly all participants agreed that shaving tablets is inappropriate because it can alter the disintegration and dissolution characteristics of the dosage form. When comparing tablet to tablet in a crossover study, differences in mg/kg dose are not of concern, assuming that the clearance is constant between study periods. This point is clearly demonstrated in the equation for AUC:

$$AUC = (\text{Dose} \times F) / CL$$

$$\frac{AUC_{\text{test}}}{AUC_{\text{ref}}} = \frac{[\text{Dose} \times F_{\text{test}}] / CL}{[\text{Dose} \times F_{\text{ref}}] / CL}$$

where *F* is the absolute bioavailability of the drug in the dosage form, CL the total systemic clearance of the drug from the body and AUC the area under the concentration/time curve, which reflects total systemic exposure.

If neither CL nor the mg/kg dose changes between periods 1 and 2, this equation reduces to:

$$\frac{AUC_{\text{test}}}{AUC_{\text{ref}}} = \frac{F_{\text{test}}}{F_{\text{ref}}}$$

In contrast, when conducting a parallel study, differences in the mg/kg dose will inflate the residual error, thereby contributing to a widening of the confidence interval. To avoid this problem, sponsors can mathematically correct the observed data via dose normalization. For example, let us say that for subject A, the administration of a single tablet resulted in a true dose received of 8 mg/kg. In subject B, the true dose received was 10 mg/kg. In subject C, the true dose received was 12 mg/kg. The dose-corrected AUC and C_{MAX} values would be corrected as shown in Table 1.

In this situation, it is the dose-normalized dataset that would be subjected to the statistical analysis. One caveat, however, is that for dose normalization to be appropriate, the drug must exhibit linear pharmacokinetics.

Similar types of dose normalization procedures can be applied to bioequivalence studies conducted with drug in feed (medicated articles) or water.² If two identical dosage forms are being compared, normalization may be an acceptable procedure and should be clearly stated within the study protocol as part of the data analysis. However, participants agreed that in some cases, an animal may consume very little of its food or need to be dropped from the study because of a failure to consume the feed. If this is a frequent event (where frequent would be defined in the protocol), or if it occurs with greater frequency for one formulation than the other, additional palatability data may be needed. However, most people agreed that the appropriate handling of outliers could not be addressed without knowing whether or not palatability is an issue. The question was raised as to whether or not some animals can simply be deleted as 'outliers' if they fail to consume most of the allotted food. Nevertheless, sponsors need to recognize the importance of ensuring sufficient subject numbers to maintain the necessary study power, and the particularly large problem associated with dropping treatment data when the trial is conducted as a crossover design.

²A somewhat different set of concerns may arise if the equivalence study is intended to compare two dosage forms (e.g. top dress vs. complete feed or complete feed vs. water delivery). These kinds of questions may occur in generic applications filed through an approved Suitability Petition, in certain hybrid NADAs, or in cases where an innovator product is looking to bridge between two types of formulations. These situations tend to be complicated by the intake process itself being an integral component of product equivalence/comparability. Therefore, sponsors are advised to discuss these issues with CVM early in the product application process.

In general, because of the complications arising from animal behavior, participants generally agreed that drug administered in feed and drinking water presents a unique challenge in the assessment of product bioequivalence. Furthermore, there remain many unresolved questions dealing with problems that may arise in the handling of these data.

Limited number of samples per individual

Although still fairly rare, there are occasions when product bioequivalence must be ascertained under conditions when only one blood sample can be obtained per subject. Examples include products targeted for use in fish, poult, and chicks. In this situation, it is uncertain as to how to best design a study for evaluating product bioequivalence.

Expressed views

Composite blood level profiles resulting from sparse sampling designs (including that associated with withdrawal time estimation) can be markedly altered by the randomization of animals to a specific sampling time, particularly when a limited number of observations are included at each time point. To illustrate this point, 50 curves were simulated from a single set of population parameters. One time point was used per animal (50 animals, 10 time points, and five animals per time-point). C_{MAX} was defined as the observed peak concentration associated with the composite profile (i.e. the highest average drug concentration observed of the 10 sampling times). The variability estimate [the percent coefficient of variation (CV)] was estimated as the standard deviation about C_{MAX} divided by C_{MAX} . The *AUC* for each profile was estimated using the linear trapezoidal rule. The variability about the *AUC* values (expressed as percentage CV) was estimated by a summation of the standard errors at each sampling time as described by Bailer (1988).

The 50 simulated profiles were randomly assigned to one of the 10 time points. Two of the resulting composite profiles are found in Fig. 11 (denoted as Attempts 1 and 2). Using the parameter estimation procedure described above, *AUC* and the corresponding percentage CV for Attempts 1 and 2 were found to

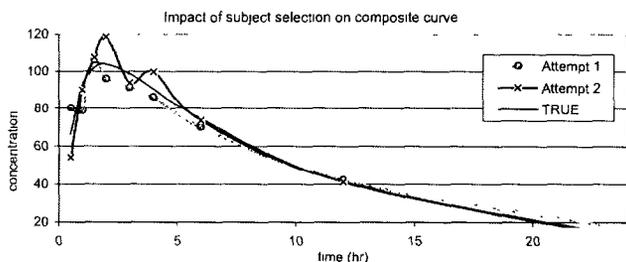


Fig. 11. Impact of randomization of sampling times in the development of composite concentration/time profiles. Because of the large between-subject variability defined in the population model used in the generation of this dataset, iterations of the randomization process resulted in profiles with very markedly different shapes, even though the identical subjects were contained in each of the curves. The true curve represents the summation of the complete blood level profile obtained with each subject.

be similar. However, marked differences were observed in both the mean and the percentage CV associated with C_{MAX} . Furthermore, although not shown, it was noted that the greater the pharmacokinetic variability of the drug in question, the greater would be the potential disparity between composite curve and their corresponding variability estimates.

These simulation results raise several questions pertaining to an appropriate algorithm and bioequivalence criteria for these types of studies, and alternative methods for optimizing the experimental design. While several methods of data analysis have been suggested, none have been adequately validated. Examples include:

- Bailer's method for estimating confidence intervals about treatment means (Bailer, 1988). This method employs the summation of standard errors about each sampling time to estimate an average error associated with the *AUC* estimate. The standard errors for the test and reference products can be used for conducting a Student's *t*-test or for estimating confidence intervals about the difference between treatment means (Jawien, 1992). Unfortunately, for reasons mentioned above, these suggestions fail to address the very different profiles that can be obtained for the same product. Therefore, selection of subject number and sampling times are critical to the population prediction of product comparability. Furthermore, there have been numerous debates regarding the appropriate assumptions underlying the confidence interval estimation. Points of concern include the definition for degrees of freedom, the distribution assumptions associated with parameter estimates, and the method of confidence interval construction (e.g. use of variance estimated directly from the data vs. using bootstrap or jackknife techniques for estimating the standard error of the estimate, Bonate, 1998). Moreover, confounded within this basic issue is the additional question of whether to pool data from multiple subjects to form a single datapoint (this is particularly prevalent in studies conducted on fingerling fish) or rather to use each animal as a unique datapoint.
- Use of a limited sampling method (LSM) that can be used to predict *AUC* and C_{MAX} values on the basis of a known linear relationship between these parameters and the observed blood concentrations at one or two samples times (Mahmood, 1997; Suarez-Kurtz *et al.*, 1999). With this method, all subjects are sampled at the same one or two time points. However, potential problems with this method include the need for *a priori* information on the pharmacokinetic model for that compound, the need for more than one sample per animal for highly variable drugs, and the likelihood that the relationship between the concentration at a specified sampling time vs. *AUC* and C_{MAX} may be formulation specific (Mahmood, 1997). The two-sample LSM may be more robust and therefore applicable across formulations, particularly with regard to the ability to accurately predict product differences in *AUC* (Suarez-Kutz *et al.*, 1999).
- The use of population pharmacokinetic methods have been suggested for assessing product comparability for rich datasets (Pentikis *et al.*, 1996), and appear to provide bioequivalence conclusions similar to those derived from the standard two-stage

approach. Population methods have also been proposed as a mechanism for dealing with sparse datasets (comparably with regard to *AUC* comparisons based upon sparse datasets, Kaniwa *et al.*, 1990). However, as previously mentioned, the *AUC* is far more robust than is C_{MAX} . Accordingly, opportunities for error in C_{MAX} comparisons are far more likely. This appears to be the case, even when population methods are employed (Wright & Fisher, 1998). The magnitude of this difficulty is proportional to the variability in the dataset and the rate of drug (Wright & Fisher, 1999). For this reason, population methods may be most helpful when used on sparse datasets obtained with low variability drug products that are slowly absorbed.

Clearly, each of these methods are associated with limitations that must be well understood to avoid potentially biasing the interpretation of the bioequivalence study results. Accordingly, additional work is needed on each of these methods before any one can be instituted as an alternative for confirming product bioequivalence in a regulatory environment.

BIOEQUIVALENCE CRITERIA

'Inactive' ingredients

Generally, excipients are considered inert components of a dosage form, affecting only the physico-chemical properties of the product (e.g. dissolution and drug stability). However, there is mounting evidence to suggest that some excipients are capable of exerting their own direct physiological effects. Examples include mannitol, which decreases GI transit time via its osmotic activity (Adkin *et al.*, 1995), surfactants, which can alter membrane characteristics (Lee & Yamamoto, 1990; Lee *et al.*, 1991), and nutrients such as vitamin E, which can alter the activity of multidrug resistance proteins thereby affecting drug bioavailability (Yu *et al.*, 1999). In the majority of instances, these effects alter drug absorption characteristics and therefore affect *in vivo* product bioavailability. However, the question is whether or not there are circumstances when an *in vivo* bioequivalence trial may fail to detect excipient-related clinical effects?

Expressed view

The fundamental issue is defining what constitutes an inactive ingredient. If we include compounds that can affect the solubility or permeability of the active drug moiety, then the assessment of *in vivo* bioequivalence should be adequate for confirming product comparability. If the excipient affects the elimination of the active ingredient, this also can be detected with blood level bioequivalence studies. However, if the excipient has the potential to alter the safety and effectiveness of the drug product via mechanisms other than altering the systemic exposure of the 'active' ingredient, then we are moving outside the realm of bioequivalence and are actually describing different drug products. For example, it has been reported that surfactants such as polysorbate 80 and Cremophor can reverse the transporter pumps (multidrug resistant proteins) that block the

entry of certain therapeutic agents, such as paclitaxel, into resistant tumors (Woodcock *et al.*, 1992; Webster *et al.*, 1997).

Another example, while not involving an inactive ingredient, points to the potential for two substances to interact in a somewhat unpredicted manner. It was observed that antithrombotic agents such as aspirin can increase the effectiveness of antimicrobial therapy in the treatment of endocarditis (Nicolau *et al.*, 1995, 1998). While antiplatelet compounds would clearly be classified as active rather than inactive ingredients, this example does point to the potential for therapeutically important changes in drug activity that may occur without any observable changes in the serum drug concentrations. Therefore, whether the effect is because of a physiological mechanism (such as changes in the activity of transporter proteins, altered microbial colonization) or some other dynamic effect (such as the inclusion of an excipient that exhibits species-specific sensitivity reactions), the clinical safety and effectiveness profiles may be altered. Accordingly, we are no longer dealing with the issue of switchability but rather prescribability.

When an 'inert' ingredient has not been previously used in a particular animal species (including considerations for both route and administered amount), there may be target animal safety concerns. In these cases, there should be a clinical evaluation of the safety of that inactive ingredient. Species-specific excipient effects are known to occur. For example:

- Methanol: exhibits a sensitization reaction in guinea pigs, although very infrequent allergic reactions are noted in humans (Sharp, 1978).
- Polyethylene glycol (low molecular weight): associated with teratogenic effects in mice, but is not teratogenic in humans. These adverse effects are not considered to be relevant to human use (Vannier *et al.*, 1989; Gupta *et al.*, 1997).
- Polysorbate 80: results in toxic effects in a variety of neonatal species, including humans (Alade *et al.*, 1986; Farkas *et al.*, 1991). It has also been associated with histamine release and hypotension in a variety of species, although the concentration associated with this response appears to vary across species (Masini *et al.*, 1985).

The potential for species-specific excipient effects underscores the need for an inactive ingredient guide that provides information on excipients with respect to dose, route, amount administered and target animal species.

Biowaivers for medicated articles

It is usually assumed that medicated premixes will be added to various feeds, resulting in many different 'formulations'. However, once the Type A medicated article is approved, Type C formulations usually do not need to undergo *in vivo* bioequivalence testing. If formulation is irrelevant for Type C medicated feeds, it is unclear as to why bioequivalence study requirements are imposed for generic versions of Type A medicated articles, particularly when many premixes are simple dry mixtures of a feed ingredient plus the active pharmaceutical entity. Therefore, participants were asked to explore the question of whether or not Type A Medicated

Articles should be granted waivers, regardless of whether or not there are differences in the 'inactive' ingredients?

Expressed views

Concern was expressed over the automatic granting of waivers because of issues such as particle size, polymorphs, and excipient effects. With regard to the latter, excipients and manufacturing processes could impact not only drug dissolution but also the uniformity of drug dispersion within the feed. While physico-chemical tests could potentially support product approvals, *in vitro* dissolution testing would be difficult to conduct because of the clumping behavior of food substances. Possible use of Biopharmaceutics Classification System (BCS)-type considerations to support waivers for highly soluble compounds might be one alternative for identifying those medicated articles that can be waived.

Locally acting GI tract products

Given the difficulty associated with establishing product bioequivalence for products that act locally within the GI tract (such as pyrantel pamoate and neomycin), participants were asked to consider viable options existing for establishing product bioequivalence in these situations.

Expressed views

Center for Drug Evaluation and Research experiences similar problems in the evaluation of products such as inhalation aerosols and locally acting antiulcer compounds such as sucralofate. Although physico-chemical characterization of the product may be useful for manufacturing control purposes, CDER does not consider it adequate to ensure *in vivo* product bioequivalence. Therefore, a clinical endpoint bioequivalence study is likely to be requested to support product approval. However, if we also consider the need to demonstrate a dose-response relationship in these situations, bioequivalence requirements could become prohibitive.

For certain locally acting products (e.g. cholestyramine³), there does exist an *in vitro* endpoint that can be easily measured to support product bioequivalence. For topical corticosteroids,⁴ CDER allows for the use of a physiological endpoint bioequivalence study (skin blanching). Another suggested example of a physiological endpoint is the effect of lactulose on the pH and ammonia content in the colon and its subsequent impact on the ammonia content of the blood. Either of these effects could be used as a physiological endpoint for evaluating the comparability of lactulose preparations, even though these effects do not impact the therapeutic endpoint of this compound, which is the regression of central nervous system (CNS) signs in dogs with hepatoencephalopathy treated with lactulose (Booth, 2001).⁵

³Refer to CDER's Interim Biopharmaceutics Guidance titled 'Cholestyramine Powder In-vitro Bioequivalence', 15 July, 1993, <http://www.fda.gov/cder/guidance/cholesty.pdf>

⁴Refer to CDER's Guidance titled 'Topical Dermatologic Corticosteroids: In vivo Bioequivalence', <http://www.fda.gov/cder/guidance/old098fn.pdf>

⁵This example, although considered a standard therapy in dogs with hepatoencephalopathy, is an extra-label use of a human drug

Multiple species

A major obstacle facing manufacturers of animal health care products is the need to demonstrate *in vivo* bioequivalence for each major target animal species included on the pioneer product label. Based upon a survey of the 1999 Code of Federal Regulations, at least 25% of veterinary products approved for oral administration are indicated for use in two or more target animal species. For parenteral products, about 45% of the products are approved in two or more target animal species and approximately 25% are approved for use in three or more target animal species (note, these estimates exclude products that are eligible for waivers of *in vivo* bioequivalence study requirements).

Population simulations were conducted to explore the potential consequences of multiple study requirements. A typical 24 subject crossover study was simulated 100 times to ascertain the likelihood of meeting *in vivo* bioequivalence study requirements (Fig. 12). Simulated datasets were generated across a range of parameter variance estimates and ratios in mean product bioavailability (*F*). From this simulated study, we see that if treatment means differ by no more than 5% (15% CV), there is a 10% risk of failing to meet the current *in vivo* bioequivalence criteria for a single target animal species (where approval is based on meeting for both *AUC* and *C_{MAX}*). This risk increases to 20% if we require that products meet current bioequivalence criteria in two target animal species. In fact, if two studies are required for products associated with a 20% CV (not a rare situation) and if treatment means differ by 5%, the sponsor incurs a 78% risk of failing to successfully demonstrate product bioequivalence. Based upon these simulation results, it is evident that this high risk of failure can be a strong disincentive for generic drug sponsors when considering the development of products approved for use in two or more target animal species. Therefore, participants were asked to explore potential alternatives for obtaining multiple species approvals.

Probability of Rejection: one species (RMSE df=22)

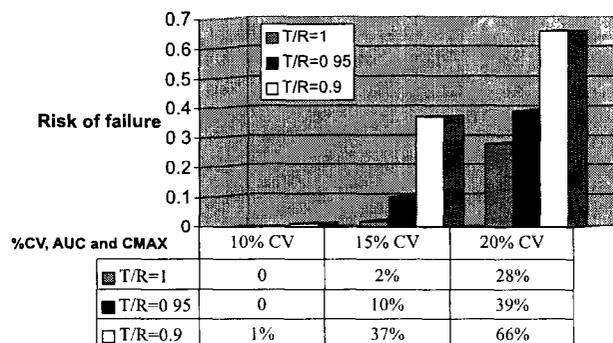


Fig. 12. Probability of study failure to meet confidence interval criteria for *AUC* and *C_{MAX}* based upon T/R ratios and parameter variability estimates.

Expressed views

Discussion of this issue lead to numerous additional questions. Despite an agreement that multiple species may present a disincentive for generic veterinary drug manufacturers, the use of interspecies extrapolation of product bioequivalence was met with concern. The question was also raised regarding the need for additional bioequivalence studies for different classes of animal. For example, would additional data be needed to support approvals in preruminant veal calves vs. mature ruminating animals? If additional studies are not needed in this case (going from a monogastric to ruminating state) how can we justify the need for other multiple study requirements? If multiple *in vivo* studies are required, could confidence criteria be loosened for one or both species? If wider limits are accepted, what degree of confidence do we still have that the products are truly interchangeable in each animal species? In this regard, it was noted that both fed and fasted *in vivo* bioequivalence studies are currently required for many human drugs. To accommodate the additional risk of failure, postprandial studies, when used as a supportive information, need only meet the requirement that test/reference ratio for *AUC* and C_{MAX} values fall within the limits of 80–125%.

It was noted that there are numerous literature examples of species-by-formulation interactions. A case in point is the impact of adding citric acid to the formulation of a drug that dissolves only in an acidic environment. When comparing the bioavailability of this drug when administered orally as a methocel suspension vs. a citric acid solution, a significantly larger difference in the oral bioavailability of the two formulations was observed in dogs as compared with rats. This species-specific difference in product relative bioavailability was largely attributable to species differences in gastric pH (Lin *et al.*, 1995). Other potential sources of species-by-formulation interactions include differences in the relationship between particle size vs. gastric emptying (Aoyagi *et al.*, 1992) and the impact of relationship between GI transit time vs. drug release rate on the bioavailability of sustained release formulations (Kabanda *et al.*, 1994).

In terms of study alternatives, the leading candidate was the application of BCS principles to support the development of *in vitro* dissolution criteria, where the BCS is used to characterize drug molecules in accordance with their intestinal permeability and solubility (Amidon *et al.*, 1995). This information has been combined with *in vitro* dissolution methods to provide for the granting of waivers for *in vivo* bioavailability study requirements for human drug products containing highly soluble and highly permeable compounds (CDER Guidance).⁶ Unfortun-

nately, minimal information has been obtained in veterinary species to support the use of this waiver mechanism for animal health products. The potential use of BCS principles in veterinary medicine has been extensively reviewed in two articles slated for publication in a special 2002 animal health issue *Advanced Drug Delivery Reviews* (Martinez *et al.*, manuscript in preparation).

As with oral dosage forms, species-by-formulation interactions have been observed with parenteral products. Two published examples include differences in the relative bioavailability of an aqueous and oily formulation of ampicillin trihydrate when tested in swine, sheep and cattle (Martinez *et al.*, 2001), and two formulations of injectable ivermectin in cattle and swine (Lifschitz *et al.*, 1999). Currently, there does not exist a classification system analogous to the BCS for parenteral products. Moreover, while dissolution and release testing may be useful for evaluating manufacturing controls issues, it is unclear as to whether or not these tests would be predictive of *in vivo* bioavailability of injectable formulations. Extensive research is clearly needed in this area we can consider recommending the granting of waivers for parenteral dosage forms that are not aqueous solutions.

Exposure (partial AUC)

It is well recognized that C_{MAX} is not a pure measure of absorption rate, but rather reflects both rate and extent of absorption (Steinijans *et al.*, 1992; Bois *et al.*, 1994). For this reason, a variety of other metrics have been proposed, including partial areas (Midha *et al.*, 1994), center of gravity (Veng-Pedersen & Tillman, 1989), mean absorption time (Jackson & Chen, 1987), maximum entropy (Charter & Gull, 1987) and C_{MAX}/AUC (Endrenyi *et al.*, 1991). For the most part, none of these metrics have been viable alternatives in the assessment of product bioequivalence. As the objective of bioequivalence trials is to assure comparable product concentration–time profiles, the use of exposure concepts has recently been encouraged. For most oral dosage forms, CDER continues to use C_{MAX} as the metric for estimating similarity of product peak exposure. In this regard, CDER's recent guidance titled 'Bioavailability and Bioequivalence Studies for Orally Administered Drug Products – General Considerations' states the following:

Pharmacokinetic Measures of Systemic Exposure: Both direct (e.g. rate constant, rate profile) and indirect (e.g. C_{MAX} , T_{MAX} , mean absorption time, mean residence time, C_{MAX} normalized to *AUC*) pharmacokinetic measures are limited in their ability to assess rate of absorption. This guidance therefore recommends a change in focus from these direct or indirect measures of absorption rate to measures of systemic exposure. C_{MAX} and *AUC* can continue to be used as measures for product quality BA and BE, but more in terms of their capacity to assess exposure than their capacity to reflect rate and extent of absorption. Reliance on systemic exposure measures should reflect comparable rate and extent of absorption, which in turn should achieve the underlying statutory and regulatory objective of ensuring comparable therapeutic effects. Exposure measures are defined relative to early, peak,

⁶For additional information in this regard, please refer to the following two guidances:

- Chemistry: SUPAC IR: Immediate release solid oral dosage forms scale-up and post-approval changes.
- Biopharmaceutics: Waiver of *in vivo* bioavailability and bioequivalence studies for immediate release solid oral dosage forms based on a BCS.

Both documents can be obtained at <http://www.fda.gov/cvm/guidance/guidance.html>

and total portions of the plasma, serum, or blood concentration–time profile, as follows:

(a) *Early exposure:* For orally administered immediate-release drug products, BE may generally be demonstrated by measurements of peak and total exposure. An early exposure measure may be indicated on the basis of appropriate clinical efficacy/safety trials and/or pharmacokinetic/pharmacodynamic studies that call for better control of drug absorption into the systemic circulation (e.g. to ensure rapid onset of an analgesic effect or to avoid an excessive hypotensive action of an antihypertensive). In this setting, the guidance recommends use of partial AUC as an early exposure measure. The partial area should be truncated at the population median of T_{MAX} values for the reference formulation. At least two quantifiable samples should be collected before the expected peak time to allow adequate estimation of the partial area.

(b) *Peak exposure:* Peak exposure should be assessed by measuring the peak drug concentration (C_{MAX}) obtained directly from the data without interpolation.

With this in mind, participants were asked to consider if and when CVM should agree to use alternative metrics for evaluating product bioequivalence.

Expressed views

Participants noted that there exist conditions under which C_{MAX} may not be appropriate for ensuring profile comparability. These include:

- When it is important to rapidly obtain a minimal effective concentration, such as that which occurs with anesthetics and analgesics.
- When products, such as hormonal implants, exhibit complex input characteristics. An example of this is zeranol (Pusateri & Kenison, 1992), and a simulated example of the types of blood level profiles often seen with hormonal implants is provided in Fig. 13. In cases where profiles contain multiple maxima, metrics based upon a single peak concentration will fail to adequately describe the complex absorption characteristics.
- When products have peak concentrations that have little impact on efficacy or toxicity, such as the beta-lactam antibiotics (time-dependent killing dynamic).

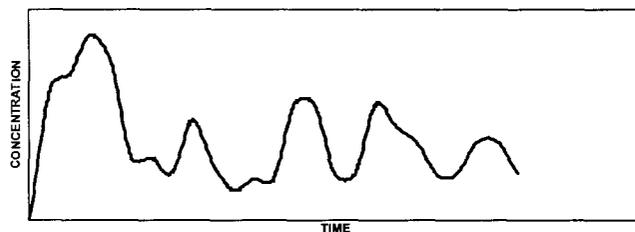


Fig. 13. Simulated example of the type of curves typically seen with many veterinary hormonal implants.

For these situations, alternative metrics (such as partial areas) may need to be considered. In this regard, working in collaboration with Drs Rescigno and Bartoszynski, Drs Jean Powers and Edward Herderick presented information summarizing their ongoing effort to develop an alternative method for evaluating the degree of parallelism of two concentration/time profiles. The resulting index provides an opportunity for differentiating the weights associated with specific portions of the profile, allowing for greater emphasis on one region over another. This proposed metric is an expansion of the method published by Bartoszynski *et al.* (2001) for comparing *in vitro* dissolution profiles. The authors are in the process of completing the performance characterization this new metric and have addressed additional thoughts on the potential application of this metric in a companion manuscript submitted to this journal.

Criteria for acute vs. chronic use products

In veterinary medicine, there are many situations when drug products are administered on an acute rather than a chronic basis. This is particularly true for food-producing animals. Conversely, companion animal medicine often mimics human dosing conditions where one animal may be exposed to several generic versions of a single pioneer product over the course of a lifetime. Therefore, the question asked was whether or not bioequivalence criteria should be different for acute vs. chronic use drugs?

Expressed views

The overall opinion was that bioequivalence criteria should be based upon the therapeutic window rather than product use characteristics. In this regard, greater regulatory emphasis was requested on the use of published literature for establishing approval criteria. With regard to use in food producing animals, many participants indicated that in farming situations, the patient being treated is the herd rather than the individual. Therefore, as herds may be repeatedly exposed to a product, switchability remains a critical issue for ensuring product bioequivalence.

Changing criteria

It was noted that over the lifetime of a pioneer product, both scientific information and technology can change. Accordingly, bioequivalence study requirements associated with these various generic applications may change over time. An example of this is pyrantel pamoate, where of four approved generic applications, two were granted a waiver of *in vivo* bioequivalence study requirements and two others were required to submit the results from *in vivo* bioequivalence studies. Similarly, for two Type A medicated feed products, one was approved on the basis of a clinical equivalence trial while the other was granted a waiver.

This experience raised the question of whether or not FDA should require that all generic sponsors be subjected to identical

bioequivalence study requirements or be allowed to modify study requirements in accordance with the most current information and technology?

Expressed views

Nearly all participants agreed that the FDA should be permitted to adjust its study requirements in accordance with changing science and technology. However, it was emphasized that CVM needs to effectively communicate these changes to the public. Participants recommended that if policy does change (e.g. such as the adoption of a BCS-based waiver policy), guidance to that effect should be issued. Alternatively, if new criteria were used on an individual basis, the rationale for using these alternative approval criteria should be clearly delineated in that product's Freedom of Information summary.

STATISTICS

Variability issues

The CVM Bioequivalence Guidance states that 'The sponsor and CVM should determine the acceptable bounds for confidence limits for the particular drug and formulation during the protocol development stage'. Unfortunately, for many compounds, the true magnitude of kinetic variability is unknown and difficult to accurately assess from pilot study data. Therefore, the following three questions were asked:

- What statistically valid methods are available for adding more subjects to the end of a study if adequate power is not achieved?
- How do variability comparisons fit into the evaluation of product bioequivalence (e.g. if one product exhibits greater variability than does the other)?
- Could acceptance criteria (wider confidence intervals) be scaled in accordance with the variability of the reference product (*post hoc* consideration) rather than maintained at some *a priori* bounds?

Expressed views

Concern was expressed about allowing *post hoc* changes in approval criteria. Discomfort was based upon the potential widening of confidence intervals based upon poor study design, the inability to control for interstudy variability, the potential for encouraging poorer study quality, and the lowering of consumer confidence in generic drug products. Participants agreed that product switchability must factor not only the similarity between *treatment means* but also the *magnitude of variability* associated with the test and reference products. The use of a confidence interval approach imposes a restriction on how different two products can be with regard to both mean and variability. By tightening these confidence intervals, products are required to be increasingly similar. This is particularly important when dealing with a narrow therapeutic window drug. However, by definition, if a drug has a narrow therapeutic window, the extent to which

drug concentrations can vary within a given individual is small. Accordingly, these products are generally found to exhibit low variability and therefore tend to readily meet traditional equivalence criteria.

Conversely, drugs with a wide therapeutic window may exhibit substantial within and between subject variability. Under these circumstances, it is possible that a reference product could fail to demonstrate bioequivalence even if tested against itself. For example, in an investigation where 59 subjects were administered a single drug product, the statistical analysis was repeated on the observed values but the data handled as if they were obtained from two separate treatment groups rather than from a single product (parallel design, 29 subjects per treatment group). Subjects were randomly designated as representing either Product A or Product B. Data randomization and bioequivalence testing was repeated three times. Table 2 provides a summary of one of these bioequivalence tests. Given the very large variability observed for the reference product, the reference product failed to demonstrate bioequivalence to itself in one out of three randomizations. These results raise the question of whether or not it is appropriate to impose the same confidence interval for a wide and narrow therapeutic index drugs. This point is graphically presented in Fig. 14.

Failure to meet *in vivo* bioequivalence criteria can be attributable to the magnitude of the differences between treatment means or to the variability associated with the blood level parameters. To further explore this point, we estimated the maximum allowable difference across a range of variability estimates (5–30% CV) and numbers of subjects included in the investigation. Given the differences in the estimation of degrees of freedom, this evaluation was conducted for both a two-period, two-sequence crossover and a parallel study design.⁷ It was assumed that the values for both treatments followed a normal distribution. The results of this estimation procedure are provided in Table 3. From these calculations, we see that if variability estimates are the same, there is little difference in allowable difference associated with a parallel vs. a crossover design.

Clearly, large differences between treatment means or a more variable test than reference formulation could seriously impair product switchability. However, if a high level of variability is observed with both products, or if the test product has substantially less variability than does the reference, average bioequivalence methods fail to adequately identify products that will produce the same therapeutic effects. For this reason, CDER has provided guidance on population and individual bioequivalence methods that allow the investigator to scale the

⁷It should be noted that a fundamental reason for selecting a crossover over a parallel study design is to minimize the standard error of the estimate of the difference between treatment means (within subject variability is generally smaller than the corresponding between subject variability). However, for the purpose of this exercise, no distinctions were made in this regard.

	Reference mean	CV reference mean (%)	Diff (%)	Mean Lower CI (%)	Mean Upper CI (%)
AUC_{0-4}	7.24	31.18	11.15	79	98
C_{MAX}	0.14	25.38	9.25	83	99

Table 2. Failure of reference product to demonstrate product bioequivalence to itself

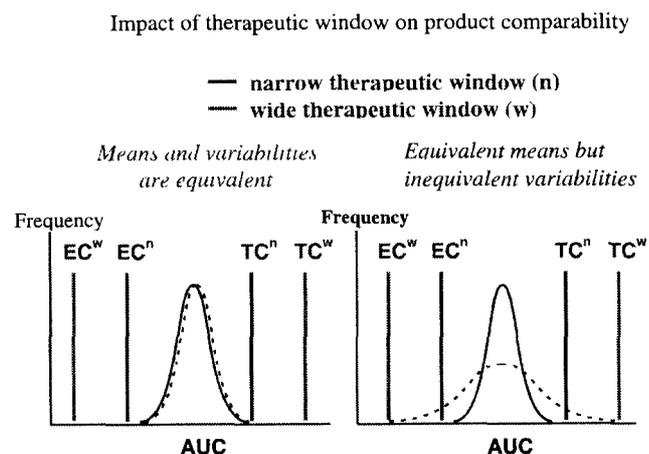


Fig. 14. For narrow therapeutic window drugs, the within-subject variability tends to be small because systemic concentrations must be maintained within very tight limits. The effective concentration (EC) and the toxic concentration (TC) provide the boundaries defining this window of allowable variability. In contrast, for wide therapeutic window drugs, the range between EC and TC is much broader. Therefore, these compounds often exhibit much greater pharmacokinetic variability. While this large variability will not compromise the clinical safety or effectiveness of wide therapeutic compounds, it can lead to difficulty in meeting traditional bioequivalence criteria when a generic product is tested against the corresponding innovator formulation.

bioequivalence criteria on the basis of the variability associated with the reference product.⁸

The CDER guidance defines three methods for estimating product bioequivalence:

Average bioequivalence: This method represents the traditional confidence interval approach for establishing product bioequivalence, and is recommended by CDER for the majority of bioequivalence studies. Here, the population difference between two treatment means are expected to fall within some predetermined criteria. As we are dealing with a small subset of the true population, we can only 'estimate' the true ratio of the treatment means. Accordingly, this approach requires the calculation of a 90% confidence interval about the ratio of product averages. When product bioequivalence is declared, we are 90% confident that the true ratio of treatment means is contained within the limits of 0.80–1.25.

⁸CDER Guidance to Industry: Statistical Approaches to Establishing Bioequivalence, January, 2001.

Table 3. Maximum allowable difference between treatments as a function of variability and the number of observations per treatment (n). These values represent the maximum differences (expressed as the percentage difference between test and reference formulations) that will allow for the confidence limits to be contained within the bounds of 0.80–1.20 (estimates based on untransformed data). In a crossover study, 'n' represents the total number of subjects included in the trial. For a parallel study, the total number of subjects = $2n$

n	CV (%)	Allowable difference	
		Crossover	Parallel
12	10	12.4	12.9
20		14.5	14.7
24		15.0	15.2
30		15.6	15.7
36		16.0	16.1
12	15	8.6	9.3
20		11.8	12.0
24		12.6	12.7
30		13.4	13.5
36		14.0	14.1
12	20	4.8	5.8
20		9.0	9.3
24		10.1	10.3
30		11.2	11.4
36		12.0	12.1
12	25	1.0	2.2
20		6.3	6.7
24		7.6	7.9
30		9.0	9.2
36		10.0	10.2
12	30	NA	NA
20		3.5	4.0
24		5.1	5.5
30		6.8	7.1
36		8.0	8.2

The use of the two one-sided test procedure is to ensure that the test product is not significantly less bioavailable than the reference product (20% allowable difference, $\alpha = 0.05$ level) and that the reference product is not significantly less bioavailable than the test product (20% allowable difference, $\alpha = 0.05$ level). If we sum the alpha values associated with the two one-sided test procedures, we obtain an overall alpha value of 0.10. It is for this reason that we state that this test provides for the 90% confidence limits. The variance used for estimating this interval is based upon the average residual (unexplained) error associated with the statistical model. Any difference in the variability of the test or reference product is not considered.

It was also noted that the reason for setting the confidence limits to -20 and $+25\%$ is to set a lower limit for $T/R = 0.80$

and a lower limit for R/T = 0.80. If R/T (= 0.80 or 4/5) is then expressed as T/R (= 5/4), the upper limit confidence bound translates to a value of 1.25. Thus, our bioequivalence limits, expressed as T/R, is 0.80–1.25 (or –20%, +25%).⁹

Population and individual bioequivalence:

Population and individual bioequivalence approaches are based on the comparison of an expected squared distance between the test and reference formulation to the expected squared difference between two administrations of the reference formulation. An acceptable test formulation is one where the distance between the test and reference products is not substantially greater than that between repeated measures of the reference product. Both the individual and population approaches allow for scaling the acceptance criteria by either the reference variability or by some alternative constant total variance (σ_{T0}^2 and σ_{W0}^2 for the specified constant total and within variances, respectively).

The population method scales in accordance with the total reference variance (within plus between subject variance). This approach is intended to address the issue of PRESCRIBABILITY (the assurance that a given dose of a drug, when administered for the first time to a patient, will provide a profile of safety and effectiveness expected for that patient population). In contrast, the individual bioequivalence approach factors only the within-subject variances and is intended to address issues of SWITCHABILITY (the assurance that switching formulations in a patient already titrated to a specific dose of a drug, will be invisible to that patient). With both methods, the confidence limit criteria can be widened if the reference product is associated with large variability. Moreover, for each of these approaches, any difference in the variance of the test and reference products must be considered, and if the test product is more variable than the reference, can result in failure to demonstrate product bioequivalence. In other words, for any given difference between the means, if the test product exhibits greater variability than the reference, the likelihood for failure increases. Lastly, individual bioequivalence also adds a term for subject-by-formulation interaction into the width of the confidence interval (see below), thereby ensuring that there does not exist a particular subpopulation for which the test and reference products are not interchangeable.

Population bioequivalence:

$$\frac{(\mu_T - \mu_R) + (\sigma_{TT}^2 - \sigma_{TR}^2)}{\sigma_{TR}^2} \leq \theta_p$$

Individual bioequivalence:

$$\frac{(\mu_T - \mu_R) + \sigma_D^2 + (\sigma_{WT}^2 - \sigma_{WR}^2)}{\sigma_{WR}^2} \leq \theta_p$$

where μ_T is the population average response of the log-transformed measure for the T formulation, μ_R the population average response of the log-transformed measure for the R

formulation, σ_{TT}^2 the total variance (i.e. sum of the within- and between-subject variances) of the T formulation, σ_{TR}^2 the total variance (i.e. sum of the within- and between-subject variances) of the R formulation, σ_D the subject-by-formulation interaction variance component, σ_{T0}^2 some specified constant total variance, σ_{WR}^2 the within subject variance of the R formulation, σ_{WT}^2 the within subject variance of the T formulation and θ_p the bioequivalence limit.

Participants requested that CVM consider issuing a similar guidance to industry in this regard.

The use of an individual bioequivalence approach requires that the trial employ an extended crossover design. Such designs may not be feasible in studies where subjects are growing animals, when using species subject to stress responses, in animals with limited blood supply, or for products with long half-lives (because of either prolonged elimination or absorption processes). In these cases, alternative study designs may be necessary. Alternatively, as mentioned in the CDER guidance, the population bioequivalence approach can be applied to a parallel study. However, sponsors must be aware that this approach can decrease the probability of demonstrating product bioequivalence if the reference product is not a highly variable drug or if the test product exhibits greater variability than the reference. Moreover, for reasons previously mentioned, use of this method should be limited to questions of prescribability and not switchability.

Finally, concerning the use of sequential analysis, this method has been very useful when applied to large clinical field trials where it may be highly cost-effective to conduct interim data analysis (Durrleman & Simon, 1990). When conducting a sequential analysis, the study is conducted on n consecutive patients per group for a maximum of K analysis. However, when constructing confidence intervals on each interim analysis, it is necessary to conserve the overall probability of a type I error. Therefore, the sum total of α_K must equal 0.05 per tail (i.e. $\alpha = 0.10$). While algorithms for adjusting α vary, statisticians are consistent in asserting that it is critical to establish an *a priori* specification of the rate at which the error level α is spent (Pocock, 1977, 1982; Geller & Pocock, 1987). Accordingly, because of the smaller value of α , the corresponding t -value will increase, causing each interim analysis to have greater difficulty in obtaining limits contained within the conventional bioequivalence bounds of 80–125%. For this reason, its utility within the bioequivalence arena is limited.

Subject-by-formulation interactions

The potential for subject-by-formulation interactions, while infrequent, is clearly a recognized concern in the evaluation of human generic drug products. An example of this was the pH-specific excipient effects associated with certain diazepam formulations, resulting in product inequivalence when administered to individuals with elevated gastric pH (Ogata *et al.*, 1982). Therefore, participants were asked to consider whether or not similar concerns needed to be addressed with veterinary

⁹Refer to the current version of the Orange Book in s.1.3 for an explanation of the statistics.

pharmaceuticals. If so, should study populations include representatives of different breeds, genders and other potential phenotypic subtypes?

Expressed views

It is well recognized that there exists breed (Opdycke & Menzer, 1984; Danielson & Taylor, 1993; Court *et al.*, 1999) and sex-related differences (Cristofol *et al.*, 1998) in drug pharmacokinetics among veterinary species. However, to date, there is little if any evidence to support concerns regarding breed-by-formulation or gender-by-formulation interactions with veterinary pharmaceuticals. Moreover, it is unlikely that subject-by-formulation interactions will be detected unless this interaction is extremely large or unless there is a very large number of subjects included in the investigation. The latter would render such bioequivalence trials economically prohibitive. Therefore, unless there is strong evidence to the contrary, the general consensus was that companies should be allowed to base their bioequivalence study design on an assumption of population homogeneity.

CONCLUDING COMMENTS

Based upon this mini-workshop, certain revisions of the existing CVM bioequivalence guidance were recommended. These include:

- The need to allow sponsors to use the highest recommended dose rather than the highest tablet strength when conducting *in vivo* bioequivalence trials.
- The clarification of conditions under which one may apply dose normalization and corresponding specifications for conducting normalization procedures.
- Guidance on the use of alternative statistical procedures.
- Alternatives to C_{MAX} for situations with complex absorption kinetics or where C_{MAX} has little therapeutic or toxic implication.
- Identification of methods that can be used for assessing product bioequivalence under conditions necessitating the use of destructive sampling techniques.
- Guidance on the use of the BCS to support waiver requests and multiple species approvals.
- Guidance on study designs that can be used for assessing the bioequivalence of nonsystemically absorbed products.
- Guidance on the use of clinical endpoint trials for confirming *in vivo* bioequivalence.

In certain cases, the solution to issues was unambiguous. For example, participants agreed that it is preferable to conduct blood level bioequivalence studies rather than clinical endpoint bioequivalence trials whenever possible. When using comparative blood level profiles, the total concentration of the parent compound is adequate for ensuring product comparability in the vast majority of cases. In most situations, participants encouraged the incorporation of new technologies into drug regulation, even if previous approvals have been held to more rigorous standards. However, changes in policy need to be

clearly delineated in either a regulatory guidance or in FOI summaries.

In other cases, resolution to problems was not clear-cut. For example, while data were presented whereby clinical bioequivalence trials could be used in lieu of *in vivo* blood level bioequivalence trials, the costs associated with such clinical endpoint trials are likely to be prohibitive, particularly if conducted in accordance with CDER recommendations. Moreover, the appropriate clinical endpoint is not always obvious.

With regard to the use of traditional bioequivalence parameters, while AUC and C_{MAX} remain the metrics of choice in most situations, there may be conditions under which alternative metrics are needed. For these situations, additional research is needed to develop and support alternative metrics that can withstand the rigors of the regulatory environment. Similarly, more research is needed to ascertain appropriate methods for determining product bioequivalence in species where only one or two blood samples can be obtained per animal.

With regard to multiple species approvals, there is currently no adequate alternative to the need for demonstrating product bioequivalence in each major target animal species. However, the adoption of *in vivo/in vitro* correlations as mechanisms for product regulation remains a promising venue for future development within veterinary medicine.

Finally, the evaluation of product bioequivalence for highly variable drugs and drug products remains a significant problem. In some cases, replicate study designs and population bioequivalence methods (as defined in the CDER guidance) can be employed. However, the use of extended crossover designs can be more expensive than traditional two-period crossover designs. Moreover, in some situations, only parallel study designs can be employed. In these cases, more investigations into alternative statistical methods are needed. However, regardless of what type of statistical procedure is employed, method of analysis and corresponding equivalence criteria need to be clearly defined within the protocol. *Post hoc* adjustments in equivalence criteria (other than scaling procedures as described above) are strongly discouraged.

At this time, no additional bioequivalence workshops were recommended. Rather, it was suggested that issues be addressed systematically by the FDA. Whenever possible, collaboration between CDER and CVM should be encouraged to maximize efficient use of resources.

Ultimately, the participants agreed that it is critical to encourage the use of scientific principles and 'state of the art' technology in an attempt to minimize drug development costs. This must be a cooperative effort by everyone associated with or affected by the use of animal drugs. Through interactions such as this mini-workshop sponsored by the AAVPT, it is our hope that we can enhance communication between experts from academia, regulated industry, government and practitioners. Working together, our goal is to encourage better use of these principles and technologies in an effort to ensure the availability of effective animal drugs, food additives, feed ingredients, medicated feeds, and animal devices that are safe to animals, humans, and the environment.

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APPENDIX

Guidances information related to this workshop

1. CDER GUIDANCES: <http://www.fda.gov/cder/guidance/index.htm>

BIOPHARMACEUTICS:

- Bioavailability and Bioequivalence Studies for Orally Administered Drug Products – General Considerations (<http://www.fda.gov/cder/guidance/3615fnl.pdf>)
- Statistical Approaches to Establishing Bioequivalence (<http://www.fda.gov/cder/guidance/3616fnl.pdf>)
- Waiver of *In vivo* Bioavailability and Bioequivalence Studies for Immediate release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System (<http://www.fda.gov/cder/guidance/3618fnl.pdf>).

CHEMISTRY: SUPAC IR: Immediate Release Solid Oral Dosage Forms Scale-up and Post-Approval Changes (<http://www.fda.gov/cder/guidance/cmc5.pdf>)

2. CVM GUIDANCE:

<http://www.fda.gov/cvm/guidance/guidance.html>

Guidance for Industry: Bioequivalence guidance (Guidance #35) (<http://www.fda.gov/cvm/guidance/bioeqapril1996.html>)

3. EMEA (European Agency for the Evaluation of Medicinal Products): Guidelines for the Conduct of Bioequivalence Studies for Veterinary Medicinal Products. <http://www.emea.eu.int/pdfs/vet/ewp/001600en.pdf>