VICH International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products

#224

GUIDANCE FOR INDUSTRY

BIOEQUIVALENCE: BLOOD LEVEL BIOEQUIVALENCE STUDY

VICH GL52

Draft Guidance

This guidance document is being distributed for comment purposes only.

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Additional copies of this draft guidance document may be requested from the Communications Staff (HFV-12), Center for Veterinary Medicine, Food and Drug Administration, 7519 Standish Place, Rockville, MD 20855, and may be viewed on the Internet at either http://www.fda.gov/AnimalVeterinary/default.htm or http://www.regulations.gov.

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VICH GL 52 (BIOEQUIVALENCE)
For consultation at Step 4

BIOEQUIVALENCE: BLOOD LEVEL BIOEQUIVALENCE STUDY

Recommended for Consultation
at Step 4 of the VICH Process
in November 2013
by the VICH Steering Committee

This draft guidance has been developed by the appropriate VICH Expert Working Group and is subject to consultation by the parties, in accordance with the VICH Process. At Step 7 of the Process the final draft is recommended for adoption to the regulatory bodies of the European Union, Japan and USA.

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BIOEQUIVALENCE: BLOOD LEVEL BIOEQUIVALENCE STUDY

This draft guidance, when finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

A. Objective:

This draft guidance is intended to harmonize the data recommendations associated with *in vivo* blood level bioequivalence (BE) for veterinary pharmaceutical products. To meet this objective, the draft guidance addresses the following topics:

- A harmonized definition of BE.
- Factors/variables that should be considered when developing scientifically sound blood level BE study designs.
- Information that should be included in a blood level BE study report.

The International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) strives to eliminate repetitious and unnecessary testing through harmonisation of regulatory recommendations for the registration of veterinary products, a goal that undoubtedly leads to a reduction in the number of animals used for product development and registration.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

B. Background:

Within the context of this draft guidance, BE is defined as the absence of a difference (within predefined acceptance criteria) in the bioavailability of the active pharmaceutical ingredient (API) or its metabolite(s) at the site of action when administered at the same molar dose under similar conditions in an appropriately designed study. When using blood drug concentrations as a surrogate for demonstrating product BE, there is an underlying assumption that two products

having an "equivalent" rate and extent of drug absorption, as measured in the blood, will be therapeutically indistinguishable and therefore interchangeable in a clinical setting.

The determination of product BE in animal species can present numerous statistical, logistical, and regulatory challenges. International differences in addressing these challenges and in the respective criteria for defining product BE can lead to barriers in data exchange and scientific confusion. Therefore, the development of a harmonized draft guidance will unify the global veterinary community understanding of the basic pharmacokinetics (PK), study design considerations, and statistical principles upon which BE determinations are based. By their nature, draft guidances address most, but not all possible eventualities. Alternative approaches can be used if they satisfy the requirements of applicable statutes and regulations.

C. Scope:

This draft guidance focuses on the study designs and principles specific to the determination of *in vivo* blood level BE for veterinary drug products. The following topics are outside the scope of this draft guidance:

- Biowaivers
- Biomass products
- Therapeutic proteins or peptides
- Medicated premixes
- Pharmacological endpoint studies
- Clinical endpoint studies
- *In vitro* dissolution tests
- Human food safety
- Products where the blood concentrations may not be indicative of drug levels at the site of action. Examples include topically active formulations, intramammary products, and intravenous administration of complex drug delivery systems that release the API directly at the site of action.
- The potential need for supportive studies, such as palatability or licking studies (e.g. transdermal products, medicated blocks).
- Animal species from which multiple blood sampling is difficult (e.g., fish, honeybee etc.).

BE is relevant not only for the comparison of generic (test) and reference products, but also in product development. For example, BE or relative bioavailability assessments can be used to bridge between different formulations, pharmaceutical forms, routes of administration, and comparison of formulations used in pivotal versus early clinical trials.

The glossary provides a definition of the various terms used in this draft guidance and provides some synonymous terms that can be applied in guidelines available in local jurisdictions.

An appendix is provided as additional clarification for the scientific and statistical concepts described in the draft guidance. Other relevant International Cooperation on Harmonisation of

Technical Requirements for Registration of Veterinary Medicinal Products (VICH) guidances should be consulted.

A sample exercise describing sample size estimation BE data statistical analysis, and a sequential analysis is provided in a separate, supporting document titled: "Supplemental Examples For Illustrating Statistical Concepts Described in the VICH In Vivo Bioequivalence Draft Guidance GL52."

Please note the examples provided in the supplemental material are intended solely for informational purposes.

Throughout this draft guidance, the terms blood, plasma and serum can be used interchangeably.

II. *IN VIVO* PROTOCOL DEVELOPMENT

All BE studies should be conducted in a manner that assures the reliability of the data generated. To be internationally acceptable, BE studies should be conducted in accordance with Good Laboratory Practice (GLP) or Good Clinical Practice (GCP) as detailed in the Organization for Economic Cooperation and Development "Series on Principles of Good Laboratory Practice" or the VICH, "Consensus Guideline" (VICH Topic GL9).

A. Product Selection:

Whereas the product selection for BE or relative bioavailability studies conducted during reference product development is not defined, the following conditions generally apply for product selection in BE studies supporting approval of generic veterinary drug products:

- BE studies should be performed on test and reference products that contain the same API.
- The test product should be representative of the final formulation of the product to be marketed.
- The reference product should be from a lot associated with a veterinary medicinal product that has been granted approval within the jurisdiction for which the generic product approval is being sought.
- The API content of the test and reference products should be assayed prior to conducting the BE study. To be internationally acceptable[1], it is recommended that the assay content of the batches from which test and reference products were obtained should differ by no more than ±5% from each other.
- The test product should originate from a batch of at least 1/10 of production scale, unless otherwise justified.

[1] Where this phrase is used, it indicates that within some jurisdictions, requirements may be less stringent. This difference may be a consideration if a study is to be submitted to support product marketing solely within a specific region.

- The characterization and specification of critical quality attributes of the API, such as dissolution, should be established from the test batch for which BE has been demonstrated.

The study report should include the reference product name, strength (including assayed content), dosage form, batch number, expiry date, and country of purchase. The test product name, strength (including assayed content), dosage form, composition, batch size, batch number, manufacturing date, and expiry date (where available) should be provided.

B. Dose Selection:

For blood level BE studies, it is recommended to dose animals according to the labeled dose as opposed to the assay content of the test and reference batches.

The blood level BE study should generally be conducted at the highest labeled (e.g., mg/kg) dose approved for the reference product. By using the highest approved dose, significant formulation differences are more easily detected in most cases. However, if it can be substantiated that the reference product exhibits linear PK across the entire dose range, then any approved dose can be used if a scientific justification is provided as to why the highest dose cannot be used. In exceptional cases where a batch of reference product with an assay content differing less than 5% from the test product cannot be found, the data could be dose normalized. In such cases, the procedure for dose normalization should be pre-specified and justified by inclusion of the results from the assay of the test and reference products in the protocol.

A BE study conducted at a higher than approved dose can be appropriate when a multiple of the highest approved dose is needed to achieve measurable blood levels. In general, the maximum dose would be limited to 3x the highest dose approved for the reference product. The reference product should have an adequate margin of safety at the higher than approved dose level and should exhibit linear PK (i.e., there are no saturable absorption or elimination processes). In this case, a scientific justification should accompany the choice of the dose.

For reference products with less than proportional increase in AUC with an increase in dose (nonlinear kinetics) across the therapeutic range, the following should be considered:

- When there is evidence indicating that the product absorption may be limited by saturable absorption processes, this can lead to two formulations appearing to be bioequivalent when administered at the highest labeled dose but fail to be bioequivalent when administered at lower approved doses. To avoid this situation, use of a dose that is less than the highest approved dose is preferable. In this case, a scientific justification should accompany the choice of the dose (showing that the dose is within the linear range).
- If there is nonlinearity over the therapeutic range due to low solubility, then BE should be established at both the highest labeled dose and at the lowest labeled dose (or a dose in the linear range), i.e. in this situation, two BE studies may be recommended.

In crossover studies, the same total dose should be administered to each animal in all study periods. The use of dose adjustments in those rare situations where large weight changes are anticipated (e.g., studies conducted in rapidly growing animals) should be considered on a case-by-case basis.

Where relevant, doses should be rounded up based on the available strength of the solid oral dosage form, or to the nearest upper division on the dosing equipment.

Solid oral dosage forms should not be manipulated in a way that could bias the study, e.g., by grinding or filing to achieve equal doses. Breaking tablets along score lines can be appropriate if the uniformity of the scored sections can be supported by pharmaceutical/manufacturing data (e.g., content uniformity of the halves). For reference products, in the absence of manufacturing or pharmaceutical data, the information included in the product labeling can be used as a guide for allowable tablet manipulation.

The study report should include the labeled dose administered to each animal in each period of the study.

C. Route of Administration Selection:

Unless otherwise justified when conducting an *in vivo* BE study:

- The same route and site of administration should be used for the test and reference products.
- Separate BE studies should be submitted for each route of administration approved for the reference product.

D. Study Design Considerations:

1. Crossover versus parallel study design:

A two-period, two-sequence, crossover study is commonly used in blood level BE trials because it eliminates a major source of study variability: between subject differences in the rates of drug absorption, drug clearance, and the volume of drug distribution. The study design is as follows:

Period	Sequence A	Sequence B
1	Test	Reference
2	Reference	Test

Note that to eliminate potential confounding by period effects, there should be two sequences included in the design of a two period crossover study.

Due to the potential risk of invalidating the crossover design, the treatment administered in Period 1 should not affect the PK associated with the treatment administered during Period 2. For this reason, the duration of the washout interval should ensure that the drug and its metabolites are essentially cleared from the body, and there are no residual physiological effects

that will alter how the drug administered in Period 2 is processed by the study subjects. Therefore, in addition to proof of absence of pre-dose concentrations, it is recommended that the duration of the washout interval should be at least 5 times the blood terminal elimination half-life of the API and its metabolite(s).

When dealing with endogenous substances, the presence of carry-over effects is very difficult to quantify. Therefore, caution should be exercised to ensure that the washout period is of an adequate duration. The length of the washout period should be addressed and justified *a priori* in the protocol. For endogenous substances the pre-dose (baseline) drug concentrations for Period 1 should be comparable to the pre-dose concentrations for Period 2.

A parallel study design may be preferable in the following situations:

- The parent compound and/or its metabolites induce physiological changes in the animal (e.g., liver microsomal enzyme induction, altered blood flow) that can alter the bioavailability of the product administered in Period 2.
- The parent compound/metabolites, or drug product (e.g., flip flop kinetics) has a terminal elimination half-life so long that a risk is created of residual drug present in the blood at the time of Period 2 dosing (i.e., a wash-out period is not practical).
- The duration of the washout for the two-period crossover study is so long as to result in significant physiological changes in the study subjects.
- The total blood volume of the species precludes the capture of blood concentration-time profiles for more than one period.

Alternative study designs can be considered. For example:

- Replicate study designs (See subsection II. D. 2)
- Sequential study designs (See subsection II. D. 3)
- To obtain approvals in multiple regions, a 3-treatment crossover or a multiple reference parallel study design can be considered when performing one study with two different reference products, depending on the products registered in the respective regions.

Alternative designs and corresponding proposed method of statistical analysis can be discussed with the regulatory authority prior to conducting the BE study. Pilot data or literature can be used in support of alternative study designs.

Regardless of how the study will be conducted, the design should be described *a priori* in the protocol.

2. Replicate study design:

A replicate study design is an investigation where at least one of the treatments is repeated.

If it is estimated that a traditional crossover design would not be feasible without the inclusion of a very high number of animals, replicate study designs can be considered using three (partial replication where for example, the reference is replicated in all subjects) or four (full replication, where each subject receives the test and reference products twice) periods within each group.

3. Sequential study design:

It is appropriate to use a sequential approach when attempting to demonstrate product BE. When employing a sequential study design, an initial group of subjects can be treated and their data analysed. If bioequivalence has not been demonstrated, an additional group can be recruited and the results from both groups combined in a final analysis.

If this approach is adopted, appropriate steps should be taken to preserve the overall Type I error of the experiment and the stopping criteria should be clearly defined prior to initiating the study. The analysis of the first stage data should be treated as an interim analysis and both analyses should be conducted at adjusted significance levels (with the confidence intervals corrected accordingly using an adjusted coverage probability that will exceed 90%). The plan to use a two-stage approach should be pre-specified in the protocol along with the number of animals to be included in each stage and the adjusted significance levels to be used for each of the analyses.

4. Single dose versus multiple dose study design:

In most situations, a single dose BE study is recommended for both immediate- and modified-release drug products because single dose studies are generally the more sensitive approach for assessing differences in the release of the API from the drug product into the systemic circulation.

For extended release formulations intended for repeated dosing, demonstration of BE should be based on multiple dose studies if there is accumulation between doses. In such cases, the C_{trough} could be an important parameter to consider, in addition to C_{max} and the AUC. It should be noted that C_{trough} can not be equal to C_{min} in the case of products with a lag time. If there is no or negligible accumulation, single dose BE data could also be sufficient for extended release formulations intended for repeated dosing.

Furthermore, a multiple dose study can also be appropriate when:

- There are saturable elimination processes.
- The assay sensitivity is inadequate to permit drug quantification that sufficiently characterizes the AUC after administration of a single dose (see section II. I. Blood Sampling Schedule).

Both single and multiple dose studies can be conducted using a crossover study or parallel design.

E. Subject and Species Selection:

The animals to be studied should be of the target species. For each jurisdiction within which registration is sought, the BE studies should be performed on each of the major target animal species included on the approved reference product label. Extrapolation of results from a major species in which BE has been established to minor species could be appropriate if valid scientific arguments are provided to support such extrapolation, taking into account species anatomy and physiology, and properties of the API and formulation.

The experimental animals should be free of any drug treatment for a minimum of two weeks prior to the *in vivo* phase of the study (a longer drug-free period can be important, depending on the half-life of the drug and/or its metabolites and physiological impact of the drug product used in prior investigations).

Studies should be conducted with healthy animals that are representative of the target population. Especially for parallel design studies, the animals/treatment groups should be homogeneous and comparable in all known and prognostic variables that can affect the PK of the API, e.g. age, body weight, gender, nutrition, physiological state, and level of production (if relevant).

Animals should be randomized and an equal number of animals should be assigned to each sequence (crossover design) or each treatment (parallel study design).

A complete description of the above information should be included in the study report.

F. Prandial State:

For all species prandial state and exact timing of feeding should be consistent with animal welfare (e.g., ruminants would not be fasted) and the PK of the API.

For canine and feline drug products administered via the oral route, studies should be conducted in fasted animals unless the approval for the reference product recommends administration in the fed state only, in which case the study should be conducted accordingly. Fasting should be a minimum of 8 hours prior to dosing and 4 hours after dosing.

For orally administered modified release formulations intended for non-ruminants, BE normally should be established under both fed and fasted conditions unless adequately justified.

The study protocol and study report should contain the rationale for conducting the BE study under fed or fasted conditions and should describe the diet and feeding regimen.

G. Exclusion of Data from Analysis:

There are numerous situations that can occur that could necessitate removal of all or a portion of an animal's data from the study. When this occurs, adequate justification for removal should be provided in the study report, and decisions to eliminate data should be made prior to analysis of blood samples to avoid bias.

There are situations that occur with sufficient frequency to require stipulation in the study protocol. For example, because there is the risk of losing all or part of the administered dose for oral formulations, the criteria for removal of subject data from analysis due to vomiting are expected to be specified *a priori* in the study protocol. Aspects to consider when defining such criteria are:

- What is an acceptable time between drug administration and a vomiting event (taking into account e.g. the expected time for the drug to exit the stomach, the prandial state of the animal)?
- What will be considered an allowable amount of material lost in the vomitus?

In addition, when re-dosing after vomiting is considered to be an option in the study, the criteria for re-dosing should be specified *a priori* in the study protocol. It is important that all available data be included in the statistical analysis. If for example, an animal is excluded from Period 2, the data gathered from that animal in Period 1 should not be excluded from the statistical evaluation.

H. Sample Size Determination:

Pilot studies are useful for estimating the appropriate sample size for the pivotal BE study.

Sample size calculations assume that the estimates used (e.g., treatment differences and variances) will be realized in the future study. Additionally, sample sizes are generally estimated as the "minimum number" needed to demonstrate BE if those estimates are realized. For that reason, the sponsor should calculate a sample size for multiple scenarios (larger variances and differences than expected) and consider if more animals should be included. A reference is provided that describes sample size calculations.

Sample size for a BE study should be based upon the number of subjects needed to achieve BE for the PK parameter anticipated to have the greatest magnitude of variability and/or difference in treatment means (e.g., C_{max}). Equations and examples are provided in the Appendix.

It should be noted that for a study to be internationally acceptable, a minimum of n=6 is necessary (i.e., the total number of study animals in a two-period, two-sequence crossover study, N, should be equal to or greater than 12).

When the risk of subject loss is a concern, the sponsor can elect to design the study to include additional animals. In this situation, if animals are removed as the study progresses (due to vomiting or dosing errors or death/injury), the additional animals placed on study can allow appropriate statistical power to be maintained.

Sample size selection should be justified *a priori* in the study protocol.

I. Blood Sampling Schedule:

The sampling schedule should include frequent sampling around T_{max} to provide a reliable estimate of C_{max}. For routes of administration other than intravenous injection, the sampling schedule should avoid situations where the first sampling time corresponds with $C_{max.}$ The duration of blood sampling should provide a reliable estimate of the extent of exposure which is achieved if $AUC_{0\text{-}LOQ}$ is at least 80% of $AUC_{0\text{-}\infty}$. At least 3 samples are recommended during the terminal log-linear phase in order to reliably estimate k_e and obtain an accurate estimation of $AUC_{0\text{-}\infty.}$

For an API with a long terminal elimination half-life, BE can be based on AUC values that are less than 80% of total systemic exposure (in addition to C_{max}) as long as the absorption phase has been completed during the applied sample collection period.

In multiple dose studies, the pre-dose sample should be taken immediately before dosing and the last sample is recommended to be taken as close to the end of the dosing interval as possible to ensure an accurate determination of AUC_{τ}. Sampling should also be performed to show that steady state conditions are reached (i.e. trough concentrations should be sampled sequentially until C_{trough} is stable).

For endogenous compounds, the predose sampling schedule should be consistent with the method of baseline correction (see section II. J. Blood Level BE Parameters).

The planned and actual timing of blood sample collections for each individual should be included in the study report.

J. Blood Level BE Parameters:

The following parameters should be collected. Some of these parameters will not be used for the statistical BE parameters (see section II. D. Study Design Considerations).

In single dose studies, C_{max}, T_{max}, $AUC_{0\text{-}LOQ}$, and $AUC_{0\text{-}\infty}$ should be determined.

In multiple dose studies, the AUC_{τ}, steady state C_{max} values ($C_{max\ ss}$), steady state C_{min} values ($C_{min\ ss}$), and steady state T_{max} values ($T_{max\ ss}$) should be determined.

If the API is an endogenous compound, the calculation of BE parameters should include a correction for baseline concentrations. The method for baseline correction should be specified and justified *a priori* in the study protocol. The recommended method of baseline correction is subtraction of the mean endogenous concentrations obtained from the pre-dose concentrations estimated at the same time on three consecutive days. If diurnal variations in the concentrations of the endogenous compound are anticipated, profiles characterizing this variation can be appropriate.

Additional parameters that may be relevant to report include k_e, terminal elimination half-life and T_{lag}.

Non-compartmental methods should be used for the determination of PK parameters in BE studies.

The study report should state the method used to derive the PK parameters from the raw data.

K. Defining the Analyte:

In principle, BE evaluations should be based upon measured concentrations of the parent compound because the C_{max} of a parent compound is usually more sensitive to differences between product absorption rates as compared to the C_{max} of a metabolite. In general, product BE will be determined on the basis of total (free plus protein bound) concentrations of the API.

1. Pro-drugs

BE demonstration should be based upon the parent compound unless the parent compound is a pro-drug and that pro-drug is associated with negligible blood concentrations. In cases where there are negligible systemic concentrations of the pro-drug, the active metabolite (the compound formed upon absorption of the pro-drug) should be measured. Sponsors should provide scientific rationale for the compound to be quantified in the study report.

2. Enantiomers

Under most situations, use of an achiral assay will suffice for the assessment of product bioequivalence. However, the use of an enantiomer-specific analytical method is recommended when all of the following conditions are met:

- The enantiomers exhibit different PK.
- The AUC of the enantiomers is modified by a difference in their respective rates of absorption.
- The enantiomers have different pharmacodynamics characteristics.

If all three conditions are met, chiral (stereospecific) analytical methods are recommended. In addition, chiral methods may be important when the test or reference products include the use of a stereospecific (chiral) excipient that can selectively alter the absorption of one or both enantiomers. It may also be important when a drug is a single enantiomer that undergoes *in vivo* chiral conversion.

L. Bioanalytical Method Validation:

The bioanalytical phase of the BE study should be based upon an appropriately validated bioanalytical method. The following aspects of bioanalytical method validation and performance should be summarized in the study report:

- Concentration range and linearity
- Matrix effects
- Limit of detection (LOD)
- Limit of quantitation (LOQ)
- Specificity
- Accuracy
- Precision
- Stability of analyte and internal standard

The following data from quality control (QC) samples obtained during in-phase analytical runs containing incurred samples should be provided:

- Precision
- Accuracy

III. STATISTICAL ANALYSIS:

The statistical BE evaluation is best generated by the use of 90% confidence intervals (i.e., the two-sided confidence interval approach). The two-sided confidence interval for the ratio of the treatment parameter means can be characterized as follows: "If an investigator repeatedly calculates these intervals from many independent and random samples, 90% of these intervals would correctly bracket the true population ratio".

The confidence interval approach should be applied to the individual parameters of interest, typically AUC and C_{max}. The sponsor should use the natural logarithmic transformation (Ln-transformation) of the parameters prior to statistical analysis.

A. The Statistical Model:

The precise model to be used for the Analysis of Variance (ANOVA) should take into account sources of variation that can be reasonably assumed to have an effect on the response variable.

For a two-period, two-sequence, two-treatment crossover study, model terms usually include (but are not limited to) sequence, animal within sequence, period and treatment. Fixed effects, rather than random effects, should be used for testing of period and treatment effects. When using a parallel study design, the treatments are generally compared using a one-way ANOVA (i.e., treatment is the sole effect being tested by the statistical model). Accordingly, the residual error

(random effect) is the appropriate error for statistically comparing the test and reference products.

Other statistical methods may be appropriate, depending upon study design.

The statistical model and randomization process should be defined *a priori* in the study protocol.

B. Ln-Transformation:

Ln transformation should be used for BE evaluation because it generally improves our ability to meet the assumptions of the ANOVA. Reasons for this include:

- PK models are multiplicative rather than additive
- Ln transformation stabilizes the variances
- BE comparisons are generally expressed as ratios rather than differences

Other types of data transformation will be difficult to interpret.

C. Dose Normalization:

Dose normalization is not appropriate, except as described in section II. B. Dose Selection.

D. Confidence Interval Acceptance Criteria:

To be internationally acceptable:

- The acceptance criteria for AUC and C_{max} should be 0.80 to 1.25, and
- In cases where multiple dose studies have been employed for extended release formulations and there is drug accumulation, these criteria will also be applied to C_{trough} values.

In cases where a sponsor intends to use an alternative study design to allow for an adjustment to the acceptance criteria based upon the variability of the reference product, the regional authorities could be consulted about appropriate statistical methods and study designs.

E. Statistical Report:

At a minimum, the study report should include the individual subject concentration versus time data for each study period (indicating period and treatment associated with each blood level profile), subject allocation to sequence, individual parameter estimates, methods used for parameter estimation, summary statistics, and the statistical output (e.g., ANOVA). This would enable regulatory authorities to perform PK and statistical analyses if necessary.

IV. GLOSSARY

- **Acceptance criteria (syn: confidence bounds):** The upper and lower limits (boundary) of the 90% confidence interval that is used to define product BE.

- **Active pharmaceutical ingredient (API) (syn: active substance)**: A substance used in a finished pharmaceutical product, intended to furnish pharmacological activity or to otherwise have direct effects in the diagnosis, cure, mitigation, treatment or prevention of disease or to have direct effect in restoring, correcting or modifying physiological functions of the body.

  Note: Due to international differences in the interpretations of what is considered to be the "same API" when considering, for example, different salts and esters, no agreed upon definition is provided. Sponsors should consult with the local regulatory authority for that jurisdiction's interpretation of what could be considered the "same API".

- **Area under the curve (AUC)**: Area under the plasma drug concentration versus time curve, which serves as a measure of drug exposure. It includes several different types of AUC estimates:

  - **$AUC_{0\text{-}LOQ}$**: AUC to the last blood sampling time associated with quantifiable drug concentrations. The last quantifiable concentration (the limit of quantification, LOQ) is determined by the sensitivity of the analytical method. The last quantifiable drug concentration may occur prior to the last blood sampling time.

  - **$AUC_{0\text{-}\infty}$** $AUC_{0\text{-}LOQ}$ with the addition of the extrapolated area from the last quantifiable drug concentration to time infinity. The terminal area from the last quantifiable drug concentration to time infinity is estimated as C_{last}/λ_e, where C_{last} is the last quantifiable drug concentration and λ_e is the terminal slope of the Ln concentration-time profile.

  - **AUC_{tau} (AUC_{τ})**: AUC over one steady state dosing interval. Mathematically, the quantity equals $AUC_{0\text{-}\infty}$ of the first dose if there is linear (non-saturable) PK.

- **Assay content**: The amount of the analyte in a sample.

- **Bioavailability**: The rate and extent to which the API or active metabolites enters the systemic circulation.

- **Bioequivalence**: the absence of a difference (within predefined acceptance criteria) in the bioavailability of the API or its metabolite(s) at the site of action when administered at the same molar dose under similar conditions in an appropriately designed study.

- **Biomass**: Crude products of fermentation, where the fermentation derived product is not extracted or purified; rather the resulting fermentation mixture, including the API and fermentation broth, is dried and used as is in the manufacture of medicated feeds or feed additives.

- **Biowaiver**: A waiver of the requirement to demonstrate *in vivo* BE between a test and reference drug product.

- **Blood:** Within this draft guidance, the terms blood, plasma, and serum are used interchangeably.

- **C_{max}**: The maximum (or peak) concentration of API or its metabolite(s) in blood.

- **C_{min}**: The minimum concentration of the API or its metabolites in blood at steady state. When there is no measurable lag time between administrations, C_{min} equals C_{trough}.

- **C_{trough}**: The concentration of API or its metabolite(s) in blood at steady state immediately prior to the administration of a next dose.

- **Dosage form (syn: pharmaceutical form):** The physical form of a dose of a medication such as tablet, capsule, paste, solution, suspension, etc.

  Note: Due to international differences, what is considered to be the "same dosage form" in some jurisdictions may be considered as different dosage forms in other jurisdictions. Drug sponsors should consult with the local regulatory authority for that jurisdiction's interpretation of what could be considered the "same dosage form".

- **Drug product (syn: medicinal product)**: A finished dosage form that contains the API usually in association with one or more excipients.

- **Elimination rate constant (k_e):** The first-order rate constant describing drug elimination from the body. Although the amount of drug eliminated in a first-order process changes proportionally with concentration, the fraction of a drug eliminated remains constant. The elimination rate constant is, then, a fraction of a drug that is removed from the body per unit of time.

- **Excipient (inactive ingredient):** A substance other than the API that has been appropriately evaluated for safety and is included in a drug product to either aid in its manufacturing; protect, support or enhance stability, bioavailability, or target animal acceptability; assist in product identification; or enhance any other attribute of the overall safety and effectiveness of the drug product during storage or use.

- **Extended release formulation:** A dosage form that is deliberately modified to protract the release rate of the API compared to that observed for an immediate release dosage form. This term is synonymous with prolonged or sustained release dosage forms.

- **Finished dosage form:** A dosage form of the API which is intended to be dispensed or administered to the animal and requires no further manufacturing or processing other than packaging and labelling.

- **Good Clinical Practice (GCP):** An international ethical and scientific quality standard for designing, conducting, monitoring, recording, auditing, analyzing, and reporting clinical studies designed to evaluate the effectiveness of drug products.

- **Good Laboratory Practices (GLP)**: Quality standards for conducting non-clinical laboratory studies and field trials. Regional standards/regulations are specified by each regulatory jurisdiction.

- **Highest labeled dose**: The highest approved dose of the reference product as indicated on the label (usually defined as strength per unit body weight, e.g., mg/kg). If there is an approved dose range, the highest labeled dose would be the highest dose in that range.

- **Linear pharmacokinetics:** When the concentration of the API or its metabolite(s) in the blood increases proportionally with the increasing dose, and the rate of elimination is proportional to the concentration, the drug is said to exhibit linear PK. The clearance and volume of distribution of these drugs are dose-independent.

- **Modified release formulation:** Drug products where the rate and/or place of release of the API(s) is different from that of an immediate release dosage form administered by the same route. This deliberate modification is achieved by a special formulation design and/or manufacturing method.

- **Medicated Premix**: A veterinary medicinal product which has been granted marketing authorization and is intended for oral administration following its incorporation into animal feedstuffs. The medicated premix frequently consists of the API, a carrier, and a diluent.

- **Nonlinear pharmacokinetics**: As opposed to linear PK, the concentration of the API or metabolites in the blood does not increase proportionally with the increasing dose. The clearance and volume of distribution of these may vary depending on the administered dose. Nonlinearity may be associated with any component of the absorption, distribution, and/or elimination processes.

- **Pharmacokinetics (PK)**: The study of the absorption, distribution, metabolism, and excretion of an API and/or its metabolite(s).

- **Reference product**: The drug product to which the *in vivo* BE and, in some instances, the *in vitro* equivalence of the test drug product is compared.

- **Replicate study design**: an investigation where at least one of the treatments is repeated.

- **Relative bioavailability**: The bioavailability of a drug product when compared with another formulation of the same drug administered by an extravascular route.

- **Steady state (ss):** The condition where the API input rate is in dynamic equilibrium with its output (elimination) rate.

- **Strength**: The amount of API in a drug product expressed in specific unit of measurement (e.g., 10 mg/mL, 25 mg/tablet).

- **Test product**: The drug product used for BE comparison to the reference product.

- $\mathbf{T_{lag}}$: The duration of time between drug administration and the appearance of the API in the systemic circulation.

- $\mathbf{T_{max}}$: Time to the C_{max}.

- **Transdermal product**: A dosage form designed to be applied to intact skin for the purpose of delivering the API for absorption through the skin and into the systemic circulation.

V. APPENDIX

An example of the sample size needed to attain a power of 80% at $\alpha = 0.05$ for a single variable in the case of the multiplicative model is provided in Table 1 below. Since the BE assessment is based upon the two one-sided tests procedure, the sample size calculation is based upon $\alpha = 0.05$ per tail, which translates into as a 90% confidence interval ($2\alpha = 0.10$). The number of subjects provided in the table (N), is the total number of subjects required in a two-period crossover design (where N = 2n and n = the number of subjects per sequence) for a given ratio of the test/reference product.

Table 1: An example of sample size estimates based upon a given ratio of test and reference treatment means and within subject variability where the confidence bounds (acceptance criteria) are 0.80 to 1.25.

	Ratio Test/Reference Products							
%CV	**0.85**	**0.9**	**0.95**	**1**	**1.05**	**1.1**	**1.15**	**1.2**
12.5	56	16	10	8	10	14	30	118
15	78	22	12	10	12	20	42	170
17.5	106	30	16	14	16	26	58	230
20	138	38	20	16	18	32	74	300
22.5	172	48	24	20	24	40	92	378
25	212	58	28	24	28	50	114	466
27.5	256	70	34	28	34	60	138	>500
30	306	82	40	34	40	70	162	>500
35	414	112	54	44	52	96	220	>500
40	>500	146	70	58	68	124	288	>500
50	>500	226	108	88	104	192	446	>500

If, for example, a multiplicative model is used, where the within-subject %CV is 20 and the ratio of the test/reference products = 0.95, the equation results in an estimate of 20 subjects (10 in Group 1, 10 in Group 2). In this example, if the %CV reflected the between-subject error in a parallel design study, the estimate would be 10 subjects per treatment (N = 20).

<u>Sample Size Estimation</u>

Ln-Transformed Data (based upon Hauscke et al., 1992)**.**

In a crossover study, the number of subjects needed to achieve a 1-β power at the α nominal level is termed N, and N = 2n, where n is the number of subjects required per sequence. For the multiplicative model, the number of subjects can be estimated as follows:

If $\theta = 1$, then: $n \geq [t(\alpha, 2n\text{-}2) + t(\beta/2, 2n\text{-}2)]^2 \; [CV/\ln 1.25]^2$
If $1 < \theta < 1.25$, then: $n \geq [t(\alpha, 2n\text{-}2) + t(\beta, 2n\text{-}2)]^2 \; [CV/(\ln 1.25 - \ln \theta)]^2$
If $0.8 < \theta < 1$, then: $n \geq [t(\alpha, 2n\text{-}2) + t(\beta, 2n\text{-}2)]^2 \; [CV/(\ln 0.8 - \ln \theta)]^2$

Where:
n = the number of subjects per sequence
t(α, 2n-2) = t-value associated with the estimated confidence interval
α = Type 1 error = 0.05 for a one tailed test or 0.10 for a two-tailed test. For example, for a two tailed test (α = 0.05 per tail) and with 10 degrees of freedom, the corresponding value from a T distribution table = 1.812
2n-2 = the error degrees of freedom used to estimate the confidence interval
β = Type II error (usually 0.20). For example, with 10 degrees of freedom, the corresponding value from a T distribution table = 0.879. Similarly, β/2 (used when θ= 1) = 1.372.
μ_T = the expected population mean for the test product (log-transformed value)
μ_R = the expected population mean for the reference product (log-transformed value)
$\theta = (\mu_T - \mu_R)$
CV = coefficient of variation. This is calculated as the square root of the variance (i.e., the standard error) divided by the mean of all of the study observations

This same equation can be applied to situations when a parallel rather than a crossover design study is used. However, when this equation is applied to a parallel study design, n = the number of subjects per treatment. Therefore, N =total number of subjects = n (test) + n (reference).

Note: This is an iterative equation. Because of the potential for greater differences and variances to occur when the pivotal study is performed, it may be prudent to repeat sample size estimates using both greater variability and both higher and lower estimates of ratios between treatment means. Based upon this additional information, the number of animals can be selected that will provide the best chance for success using the resources available.

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