Animal Pharm
Agribusiness intelligence

Experts outline ramifications of revised European bioequivalence studies guideline

Cemile Jakupoglu of Cyton Germany and Maggie Fisher of the UK's Veterinary Research Management give Animal Pharm an overview of the major changes involved in the revised EU guideline on the conduct of bioequivalence studies for veterinary medicinal products, which came into play this year.

Bioequivalence (BE) studies are used in a variety of situations, most often when a sponsor wishes to manufacture a generic version of an already approved product for which the data protection period has expired.

Such studies often provide pivotal data within marketing authorization applications for generic veterinary medicinal products, as they allow bridging of the safety and efficacy data associated with the reference veterinary medicinal product.

The purpose of the European Medicine Agency's Committee for Medicinal Products for Veterinary Use (CVMP) bioequivalence guideline is to specify requirements for the design, conduct and evaluation of bioequivalence studies, for pharmaceutical forms with systemic action (it is limited to chemical entities, including endogenous substances).

The guideline also outlines how in vitro data may, in specific cases, be used to allow bridging of safety and efficacy data. Recommendations on when in vivo studies are mandatory and when in vitro data might be sufficient are given within the guideline. The intention behind the revision was not to increase the data requirements for marketing authorization applications but rather to clarify the standards applicable and improve consistency of approach.

Background to the revision

A need to update the CVMP 'Guideline on the conduct of bioequivalence studies for veterinary medicinal products' was identified with the

purpose to bring it in line with the VICH GL52 'Bioequivalence: blood level bioequivalence study', which came into effect in the European Union in August 2016. The revision of the CVMP guideline was intended to harmonize the data requirements associated with *in vivo* blood level BE for veterinary pharmaceutical products and provide internationally agreed guidance.

Additionally, the analytical methods used in BE studies must now, according to the revised guideline, comply with standard criteria of validation as given in the Committee for Medicinal Products for Human Use guideline on bioanalytical method validation (EMEA/CHMP/EWP/192217/2009-Rev.1). Standards are also therefore being harmonized with regard to human pharmaceuticals.

The updated Revision 3 came into effect on July 1, 2019.

In the updated Revision 3 of the guideline, sections two (scope), four (situations when BE may be applicable), six (study report), seven (waivers from BE study requirements for immediate release formulations) and eight (dissolution testing) remain unchanged.

Major changes

The main changes have been made in: section one (introduction), providing a new definition of BE; section three (legal basis), aiming to adapt to VICH GL52; and section five (design and conduct of BE studies).

Maggie Fisher

Ms Fisher is a veterinary graduate of the Royal Veterinary College, London, with a passion for parasitology and diagnostics. In 2008, she acquired Ridgeway Research – a thriving UK contract research organization with in-depth expertise in livestock, poultry, game birds and horse studies including parasitology.

In 2011, Ms Fisher was one of the two founding directors of Veterinary Research Management. She is an active member of the executive committee running the World Association for the Advancement of Veterinary Parasitology. She is also a diplomate of the European College of Veterinary Parasitology and a fellow of the Royal Society of Biology. She is certified in PRINCE2 project management.

The definition given in the guideline for bioavailability – "The fraction of an administered dose that reaches the systemic circulation as intact substance" – remains unchanged. Blood drug concentrations can be used as surrogates 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 definition of BE has been updated. In Revision 2, BE was defined as: "The similarity between two products that contain the same active substance(s) and shows similar rate and extent of absorption of the active substance(s). In most cases the rate and extent of absorption are expressed as Cmax and AUC. The aim is to show that two medicinal products are similar to such degree that their systemic effects, with respect to both efficacy and safety, will be essentially the same."

In Revision 3, the new definition of BE reflects the attention to metabolites and the site of action as following: "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."

In the updated guideline, the randomized, two-period, two-sequence, single-dose crossover study design is still recommended as a preferred first choice, where appropriate. However, alternative study designs are mentioned and in certain situations – a parallel study design might be more appropriate, for example in the following cases:

- When the parent compound and/or its metabolites induce physiological changes in the animal that can alter the bioavailability of the product administered in the second period of a crossover study;
- There is a risk of a prolonged half-life causing residual drug to be present in the second period;
- The washout period would be so long that the animals would have changed physiologically; or
- The total blood volume precludes collection for more than one period.

Highly Variable Drug Products (HVDPs), now defined as those for which the intra-individual variability for a parameter for the reference product is larger than 30%, might need a replicate partial crossover study or crossover with four periods design. It is recommended scientific advice is sought before embarking on designs more complex than a simple crossover due to the nature of the animals or substances being evaluated.

The chosen study design should be described *a priori* in the protocol.

The dose to be tested should normally be at the highest labelled dose approved for the reference product. This shall allow the detection of significant formulation differences more easily. Lower or higher doses must be scientifically justified. Exceptions include where there are substances with non-linear pharmacokinetics, and where the highest level is undetectable in blood (so it may be necessary to go above the highest recommended dose rate).

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 where there is a risk of

differences in drug absorption, distribution, metabolism or elimination in period 1 vs 2 that could bias the within-subject comparison) will need to be considered on a case-by-case basis.

The sample size (number of subjects needed) should now be based on the pharmacokinetic parameter anticipated to have the greatest magnitude of variability and/or difference in treatment means (e.g. C_{max}). To maintain statistical power, replacement of study animals during an ongoing study might be allowed. However, the removal criteria should be provided in the protocol.

Minor changes

What might be referred to as a pharmacokinetic refinement, by replacing $C_{min, ss}$ by C_{trough} (the plasma concentration at steady state immediately before the administration of a next dose) throughout the guideline (the two parameters may not be equal in the case of products with a lag time between administration of the formulation and systemic appearance of the active substance), has been introduced for those substances where steady state is reached after repeated doses.

A definition is now given for acceptance criteria as: "The upper and lower limits (boundary) of the 90% confidence interval that is used to define product BE."

For orally administered products, it is recognized that prandial state of animals should be consistent with animal welfare (e.g. ruminants would not be fasted).

Regarding analytes to be measured, BE will generally be determined on the basis of the total (free plus protein-bound) concentrations of the active substance. Chiral methods may be necessary for detection of relevant enantiomers. In case of endogenous substances, baseline corrections may be required.

For blood sampling time considerations, for active substances with a long terminal half-life, the duration for which samples are collected should be scientifically justified, and this may result in the AUC being truncated.

For statistical analyses, natural log (ln) transformation is recommended. Other types of data transformation will be difficult to interpret. Analysis of variance (ANOVA) is mentioned explicitly also for studies with parallel design as the recommended statistical method.

In general, Revision 3 contains more specific instructions on what the final study report should contain, and which details should be defined a priori in the study protocol, although section six (study report) of the guideline has not changed.

Implications of the guideline

Considerations for BE study planning following introduction of third version of guideline

Stage				
Planning	**Protocol development**	***In vivo***	**Statistical analysis**	**Reporting**
Is scientific advice necessary e.g. due to the complexity of the design being considered due to animal or substance characteristics? Does the study include sufficient animals to allow for the study to remain valid even if animals have to be removed? Is it powered for the parameter with the largest variability? Are small pilot studies needed to establish e.g. the most variable parameter?		Address animal welfare needs e.g. by avoiding fasting ruminants. Ensure tolerance where the highest dose level is to be exceeded.	Normally natural log transformation should be used and one-way ANOVA for parallel study designs.	Should include details of the test and reference product including name, form, batch number, expiry, country of purchase (for the reference product).
	Ensure that the protocol contains necessary details including the design, randomization and analysis to be conducted *a priori*.			

Planning what should be included in the protocol and study design feature in the new guideline, and so it may be fair to assume that short shrift will be given to those who fail to undertake this aspect of the study sufficiently.

Knowing the likely impact on animal numbers required based on the parameter with the most variance, may require small pilot studies to establish the likely parameter, and its variance within the type of animals that will be used for the study. Including sufficient animals to ensure that some can be removed due to illness if necessary, without impacting the validity of the study, will be particularly important for some species such as broiler chickens.

Conclusion

The new guideline provides considerably more guidance on the details of crossover designs, when to choose parallel studies over crossover designs, and study animals – particularly considerations on numbers to be included and doses to be tested.

Broadly, this is likely to assist those designing studies and there remains the option to seek scientific advice in the event that the guidance fails to address a particular circumstance. As ever, care should be taken to ensure that sufficient planning is undertaken to address the specific requirements of a particular BE evaluation so that it has maximum opportunity to demonstrate BE sufficiently, and it addresses the key recommendations made in the guideline.