15 December 2023
EMA/CVMP/EWP/326568/2018
Committee for Veterinary Medicinal Products (CVMP)

Overview of comments received on Guideline on the conduct of pharmacokinetic studies in target animal species (EMEA/CVMP/EWP/133/1999-Rev.1)

Interested parties (organisations or individuals) that commented on the draft document as released for consultation.

Stakeholder no.	Name of organisation or individual
1	Zoetis
2	European Coalition to End Animal Experiments (ECEAE)
3	European Group for Generic Veterinary Products (EGGVP)
4	Elanco Animal Health

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1. General comments – overview

Stakeholder no.	General comment (if any)	Outcome (if applicable)
2	**Language on the 3Rs** Although Directive 2010/63/EU on the protection of animals used for scientific purposes is listed in the references section at the end of the guideline, we feel it would also be appropriate to clearly describe the obligations of the Directive and the 3Rs at the beginning of the document. This is in line with the EMA's ongoing commitment to support the implementation of the 3Rs principles: http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_001916.jsp&mid=WC0b01ac0580d52a5e. The following text has been accepted into the final versions of two other veterinary guidelines we commented on recently: '***In accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes and Directive 2010/63/EU on protection of animals used for scientific purposes), the 3R principles (replacement, reduction and refinement) should be applied***.' (EMA/CVMP/261180/2012 and EMA/CVMP/EWP/005/2000 – Rev 3). **Opportunities to reduce the number of animal pharmacokinetic tests** The opportunity should be taken with the revision of this guideline to also highlight how animal tests can be reduced as the science has progressed. Currently, we do not feel the draft 1). Highlights those opportunities that are presented clearly enough or 2). Includes all possible opportunities.	Thank you for your comments. The following text has been included in section 3-Legal basis: "*In accordance with Annex II of the aforementioned Regulation, all experiments on animals should be conducted taking into account the 3R principles (replacement, reduction and refinement) as laid down in Directive 2010/63/EU on protection of animals used for scientific purposes.*" The use of PK/PD modelling approaches, essentially to reduce the recourse to classical dose determination studies, is clearly welcomed by the guideline, although a proper dose optimisation process would rarely be completely "non-animal", except for situations where sufficient existing animal data are available and can be directly transposed to a candidate product. This is addressed in sections 6.1 and 5.4. The possibility to combine PK investigations with other tests in a same study, i.e. to collect PK data from dose determination, dose confirmation or target animal safety studies, is considered as appropriate depending on the situation and this is addressed in section 5.4. The possibility to collect PK data during clinical trials pertains to

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	Whilst the possibility of using in vitro methods to generate pharmacokinetic data is mentioned in the guideline for some of the pharmacokinetic factors (e.g. absorption and metabolism), more detail on how this can be done is needed. The guideline largely focuses on (and provides detailed methodology for) pharmacokinetic studies in animals without any description of the recent advances in in silico and in vitro-based prediction tools, which have gained significant ground since the implementation of the original guideline 18 years ago. In the interest of reducing unnecessary testing in animals (which is in line with the EMA's goal to help implement Directive 2010/63/EU and the 3Rs principles) we suggest that a more balanced approach be provided. Ideally, the guideline should be re-structured and re-worded in an appropriate way to ensure that in vitro and/or in in silico approaches are considered first, along with existing data, before new tests in animals are conducted. Also, any opportunities to waive these tests or combine them with other required toxicological tests e.g. repeated dose studies, should be described and prioritised. We suggest splitting section 5; 'methodology and conditions of study' into two parts – 1. In vitro/in silico test systems and 2. In vivo test systems, where specific information on both systems should be provided. Also, in section 4 'pharmacokinetic factors to be investigated', the relevant in vitro and/or in silico approaches that can be used for each pharmacokinetic factor (i.e. absorption, distribution, metabolism and excretion) should be mentioned at the beginning of	the special approach of population pharmacokinetics, and is addressed in section 6.2. It should be noted that the use of existing data on the active substance, where relevant, to reduce the need for PK studies with a new product, is welcomed and mentioned at several places in the guideline. According to the paper of Huang and Riviere, 2014, interspecies allometric scaling methods have been developed in veterinary medicine for the prediction of pharmacokinetic parameters or appropriate dosage. This is a potential tool for: (i) the preselection based on the interpretation of laboratory animal study results, of first in-species dose regimen(s). This may thus be part of the applicants' rationale to design target species studies from laboratory animal data; however, this is not really a means for replacement/reduction of animal tests. (ii) the practical use off-label of some products in wildlife or minor domestic species. In connection with that, it is noted that the possibility of pharmacokinetic data extrapolation to minor target species is addressed in the CVMP guidelines relating to limited market products. Furthermore, such approaches have limitations and many pitfalls may affect the performance of prediction, since the different animal species have their own unique pharmacokinetic processes characteristics. Notably, they display species-specific processes in drug disposition that

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	the sub-section in order to encourage their implementation and routine use before resorting to animal tests. **Non-animal approaches in pharmacokinetic-pharmacodynamic (PK/PD) modelling** While we appreciate the inclusion of a new 'special approaches' section (section 6) that encourages the use of PK/PD modelling (which can be used to replace standard dose determination studies, and thereby reduce the use of animals) we are disappointed that no examples of non-animal PK/PD approaches have been included. According to a recent review of PK/PD modelling in veterinary medicine, '*advancement in modern technology allows us to use more computer-based techniques for the investigation of complex PK-PD relationships. Progressively, simulation-based techniques are mostly used in therapeutic areas and made available for a quantitative description of the time course of drug effects, which have great ability for achieving a more optimal drug therapy that can also reorganise the development of drug and help in critical decisions. The decisions include designing and planning of the most favourable dosing regimen in clinical trials*.' (Ahmad et al., 2016). The PK/PD section of the draft guideline currently only focuses on PK/PD tests in animals and does not take into account any of the recent technological advances in in vitro/in silico modelling systems. As with the previous sections we suggest that some examples of non-animal PK/PD approaches be included at the beginning of this section and considered before PK/PD studies in animals. Could the working group talk to model developers and companies to provide some examples, even if they are only described in general terms?	might result in tremendous differences in pharmacokinetic parameters. Therefore, not all active substances are amenable to allometric scaling and not all species can be bridged. Also, not all parameters are suitable for such approach or correspond to validated scaling methods. In addition, the authors point out that allometric scaling methods may generate wide prediction intervals, i.e. be poorly precise, when used in a prospective manner. For these reasons, it is not considered appropriate to recommend the use of allometric scaling methods to replace animal testing in the context of the present guideline, i.e. in order to predict the PK properties of a substance in a specific commercial formulation and in the target animal (and outside of the context of limited market products). As a general remark, it should be reminded that the generation of preliminary PK data for a new active substance in laboratory animals is not in the scope of the present guideline, which rather addresses the investigation of the pharmacokinetic properties of a final or nearly-final formulation in the target animal species, with a view to ensuring optimal safety and efficacy in clinical conditions. Overall, it is considered that there are not enough validated and well-established *in vitro* or *in silico* models that could replace pharmacokinetic investigations in animals, to completely restructure the guideline and recommend them as the new standard approach, i.e. as the mainstay in pharmacokinetic studies. A radical change in requirements

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	The option to combine PK/PD studies with other toxicological tests should also be included. **Use of existing information to predict pharmacokinetics** Opportunities to use existing pharmacokinetic data in target animals and on existing compounds should also be included in the guideline as a way to further encourage 3Rs implementation. For example, a technique known as 'allometric scaling', was used to predict dose regimens and pharmacokinetic profiles of 85 veterinary drugs based on a database of existing information. The study concluded that '*the analysis of available published pharmacodynamic data often helps to save time and to estimate the first in-species dose regimen and important pharmacokinetic parameters for human and animal species during drug development and extra-label use in veterinary medicine'*. (Huang et al, 2014).	is currently not sufficiently supported. Also, it is out of the scope of this guideline revision to review all possible alternative methods and assess their validity and possibility of use in the specific context of VMP clinical safety and efficacy demonstration. Rather, the use of such methods is recommended on a case-by-case basis, depending on their applicability in specific situations and on the availability of existing relevant *in vivo* data. A general paragraph emphasizing that the CVMP is open to alternative methods, while remaining prudent as to their ability to effectively and accurately support the determination of an appropriate dosing regimen, is included in section 5-Methodology and conditions of study.
3	Many thanks to CVMP/EWP for the updated guideline which is an important tool to support effective and safe dosage regimens. The provisions in the proposed guideline are welcomed and largely supported by EGGVP. However, EGGVP would suggest addressing also in this guideline: • guidance/scope for the use of radiolabel material for the conduct of PK studies • guidance on approach for the conduct of PK studies in ruminants and/or non-ruminants species. This should include advise on extrapolation of data from laboratory animals - rodents - to the ruminants (since most of PK	Thank you for your comments. The use of radiolabelled substances is expected to be rare in the context of pharmacokinetic testing of a final or near-final formulation in the target animal. It would rather pertain to early pharmacokinetic or pharmaco-toxicological studies in laboratory animals. For this type of studies, other guidance documents are relevant, for example, the guidelines on metabolism and residue kinetics studies in the context of MRL establishment and consumer safety (VICH GLs 46, 47, 48, 49). Indeed, the pre-selection of doses may be less straightforward in ruminants, and in such situation more pilot studies in the target species may be required. It is

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	preliminary studies are conducted in rodents in laboratory conditions). It would also be appreciated if the guideline could provide an example study design to facilitate the planning of the study.	difficult to give specific guidance on that issue, however, this is reflected in a general manner in the amended introduction to section 5: "[…] *preliminary studies, usually single-dose, investigating the overall pharmacokinetic behaviour of different dose levels and/or formulations. The need for these studies will depend on the availability of existing data (e.g. from literature or laboratory animal studies) relevant for the intended active substance, pharmaceutical form, and target species."*

2. Specific comments on text

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
45	4	Comment: Consider amending the sentence: "… for the purpose of supporting the clinical part of the dossier for a veterinary pharmaceutical product" to reflect that pharmacokinetic studies support not only the clinical part but also the pre-clinical and safety part of the dossier Proposed change: "… for the purpose of supporting the **efficacy (pre-clinical and clinical) and safety** part of the dossier for a veterinary pharmaceutical product"	Accepted. The text has been amended in that sense; please note that it has been reworded more extensively.
99	4	Comment: Consider amending the sentence "… the rate of absorption of the active substance should be quantified" to be consistent with line 93 and the definition of bioavailability. Proposed change: "… the rate **and extent** of absorption of the active substance should be quantified"	Accepted.
103	4	Comment:	Accepted.

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		Suggest to add blood as a suitable matrix in addition to plasma, in alignment with line 95. Proposed change: "a precise pharmacokinetic analysis of the entire **plasma/blood** concentration profile should be made"	
122-125	2	'*As an alternative to in vivo studies, the use of suitably validated in vitro models to demonstrate non-absorption of the active substance(s) may be considered. Models must be relevant to the species for which the product is intended. Usually, such models will have been described in recognised peer-reviewed literature and will have been shown to be repeatable across different laboratories'*. Comment: We think it would be beneficial to provide some solid examples of in vitro models that can be used to replace animals in studies of absorption. The consideration of these methods should be encouraged before the conduct of new animal tests. Suggested change: *'As an alternative to in vivo studies, the use of suitably validated in vitro models to demonstrate non-absorption of the active substance(s)* ***should*** ~~***may***~~ *be* ***first*** *considered. Models must be relevant to the species for which the product is intended. Usually, such models will have been described in recognised*	Partly accepted. The specific text referred to has been amended to "*should be considered*". After further revision the concerned paragraph reads: "*As an alternative to in vivo studies, the use of in vitro/in silico models to demonstrate non-absorption of the active substance(s) should be considered. Models must however be suitably validated and relevant to the species for which the product is intended. In vitro testing should preferably involve the final product formulation, unless it can be justified that the excipients and physico-chemical properties of the product will not have a significant impact on (non-)absorption.*" As regards the provision of examples, this has not been done since it is considered that no test method completely replacing *in vivo* studies in the context of pharmacokinetic testing of a (near-)final formulation in the target species can currently be recommended as a general standard or as well-established for a given purpose. Please see also our answer to your general comment above.

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		peer-reviewed literature and will have been shown to be repeatable across different laboratories'. ***(Include examples).***	
124	4	Comment: Suggest to delete "recognised" as this is arbitrary and not defined. Proposed change: ".. such models will have been described in ~~**recognised**~~ peer-reviewed literature"	Accepted. The word "*recognised*" was deleted and the paragraph was further amended, notably to include the word "*validated*".
137-138	4	Comment: The volume of distribution, as defined in the text, can only be measured following intravenous administration. Only apparent volume of distribution can be measured following extravascular route. We suggest to clarify this in the text. Proposed change: The volume of distribution (Vd) is a measure of the extent of distribution, determined by the ratio of the amount of drug in the body (i.e. dose) to the plasma drug concentration, and should be reported **following intravenous administration.**	Accepted.
146-147	4	Comment: The requirement to identify metabolites and determine pathways seems too absolute, suggesting that all metabolites and all pathways would have to	Partly accepted. The text has been reworded as:

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		be investigated We suggest that it should only apply to the major metabolites and major pathways. Proposed change: This should comprise not only identification of the **major** metabolites themselves, but also the determination of the **major** pathways	*"(…) the identification of metabolites present in potentially clinically significant amounts, and also the determination of the major pathways involved (…)".*
146-149	2	'Unless otherwise justified, the formation of metabolites should be investigated. This should comprise not only identification of the metabolites themselves, but also the determination of the pathways involved in the metabolism of the active substance, in other to establish potential drug interactions. In vitro methods (e.g. hepatic microsome assays) may be considered as an option to generate such data'. Comment: This is slightly better as the mention of in vitro methods is at the beginning of the section and one example is provided. However, the recommendation to use these methods as a first step before resorting to animal tests should be made stronger and more examples could be provided. Proposed change: 'Unless otherwise justified, the formation of metabolites should be investigated. This should comprise not only identification of the metabolites	Partly accepted. The specific wording proposed has been implemented, with the addition of "*if adequately validated*" (see next comment). As regards the provision of further examples, this has not been done since it is considered that no test method completely replacing *in vivo* studies in the context of pharmacokinetic testing of a (near-)final formulation in the target species can currently be recommended as a general standard or as well-established for a given purpose. Please see also our answer to your general comment above.

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		themselves, but also the determination of the pathways involved in the metabolism of the active substance, in other to establish potential drug interactions. In vitro methods (e.g. hepatic microsome assays) **should** ~~may~~ be **first** considered as an option to generate such data.' ***(Include more examples).***	
147-149	1	Comment: The tools to conduct this type of analysis are not currently available in all species. Specifically, metabolic pathways in many livestock species have not been extensively studied and species specific probe substrates are not available for CYP-P450 phenotyping in these species.	Accepted. The text "*if adequately validated*" has been added.
160-161	1	Comment: This wording is copied from the old version but it is unclear what is meant by validated in terms of study design, this term is generally used for bioanalytical assays and biomarkers.	Accepted. The concerned sentence has been deleted.
160	4	Comment: Not all pharmacokinetic studies require validated methods (e.g. early phase studies does not require such rigor). Suggest to amend the sentence to clarify that this is required for pivotal studies. Proposed change:	Partly accepted. The problematic sentence has been deleted (see also previous comment).

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		All **pivotal** pharmacokinetic studies should be performed according to validated and internationally accepted	
196-197	4	Comment: Pharmacokinetic interaction can not only lead to a lack of activity, but also to decreased or increased activity. Suggest to amend the sentence. Proposed change: In combining substances into a fixed combination product, unintended pharmacokinetic interactions might occur, leading to a ~~**lack of**~~ **modified** activity and/or adverse effects.	Partly accepted. The wording "*(…) a lack of activity and/or adverse effects (…)*" has been replaced by "*altered exposure*".
205	4	Comment: Can you provide a definition of "equivalence testing" and make it clear that bioequivalence is not a requirement, since pharmacokinetic, together with efficacy and safety data should be considered.	Accepted. The words "*equivalence testing*" have been deleted and it is only referred to bioequivalence in accordance with the current CVMP bioequivalence guideline. It is now expressed that PK data from a study that is not designed to show bioequivalence and which therefore, only indicate an absence of difference (superiority analysis with no rejection of the null hypothesis), are acceptable if associated to evidence of an absence of clinically significant interaction. Please see amendments in the text.
206	1	Comment:	Not accepted.

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		Difference testing should be the correct term	The term "difference testing" is considered as vague and not statistically correct. Please see also previous comment and amendments in the text.
210-211	4	Comment: This is a general statement that doesn't provide real guidance. Can the sentence be more specific? Does that mean that if the drug isn't absorbed, then the study isn't needed? Proposed change: For certain topical or local treatments, such data may not be required **e.g when when there is no systemic absorption;** in these cases, the omission of data should be justified.	Partly accepted. The sentence was completely deleted. The fact that the treatment is locally acting, even if absorption is very low, does not exclude any concern in regard of interactions. Moreover, the scope of this guideline is already restricted to "a locally-acting substance with potentially unintended systemic effects" as per section 2.
216-218	4	Comment: "However, kinetic data obtained from target animal safety studies or dose determination studies may also be accepted as a means to determine dose proportionality." Is identifying dose proportionality in a target animal safety (TAS) study too late? Less than proportional exposure in a TAS study has been evaluated critically (i.e. non-acceptable) by global regulatory agencies. However, altering the dosing scheme within the TAS study to achieve dose proportionality has also been evaluated critically by other global regulatory	Accepted. Indeed, evaluating dose proportionality at the stage of TAS studies will not generally be appropriate. The sentence was replaced by the following one: *"These data may be collected in dose determination or PK/PD studies, where sufficient existing PK data are available to support the selection of the tested dose levels."*

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		agencies. Is there an opportunity for alignment globally with what is required regarding dose proportionality and how this data impacts both safety and effectiveness?	
231-235	4	Comment: "the duration of such studies should exceed the time required to reach steady-state, thereby clearly demonstrating the time at which steady-state is attained" Can this be more specific and clarify time required beyond steady-state? Is this requirement for daily product only? What would be the requirement for drugs having a very long half-life or administered monthly?	Partly accepted. The sentence has been simplified and now reads:*" In the case of products intended for long-term continuous use, the duration of such studies should exceed the time required to reach steady-state."* It has been further clarified that: "*The rule of thumb that steady-state conditions are reached after approximately 4-5 half-lives (i.e. around 95% of the plateau) should be considered when planning the duration of a repeated-dose study."*
234-235	1	Comment: How is steady-state defined? Is 90% of plateau acceptable?	Accepted. The following sentence was included: "*The rule of thumb that steady-state conditions are reached after approximately 4-5 half-lives (i.e. around 95% of the plateau) should be considered when planning the duration of a repeated-dose study."*
290-291	1	Comment: In some cases destructive sampling is used so composite PK profiles would need to be used	Not accepted. This is true but such situation is expected to be rare in the context of final product testing in the target animal; therefore, including specific guidance is not considered necessary.

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			However, the word "*generally*" was included in the concerned sentence.
296-299	2	'Selection of dose level and dosing interval by means of a PK/PD modelling approach may be considered, though the duration of treatment would have to be demonstrated by other means. Such data may replace standard dose determination studies provided that the selected dose level and dosing interval are supported by dose confirmation studies'. Comment: We appreciate the option to replace standard dose determination studies with PK/PD studies. However, it would be useful to mention that this comes with the added benefit of saving animals. Proposed change: 'Selection of dose level and dosing interval by means of a PK/PD modelling approach may be considered, though the duration of treatment would have to be demonstrated by other means. Such data may replace standard dose determination studies, **which could lead to a reduction in animal tests,** provided that the selected dose level and dosing interval are supported by dose confirmation studies.	Accepted; note however that the concerned paragraph has been extensively reworked.
326	4	Comment:	Accepted. It is understood that the term "*endpoint*" should refer to a variable directly measured, while "*parameter*" is rather a

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		Is " PD parameter" the correct word (PD parameters are e.g. Emax, EC50 and gamma)? "Enpoint" seems more appropriate in the context of this sentence. Proposed change: The selected PD ~~**parameter**~~ **endpoint** should be relevant, sensitive and reproducible.	value (or its estimation) characterising the distribution of such variable. Nevertheless, the distinction between the two is not always straightforward, e.g. in PK the C_{max} could be viewed as a parameter or as an endpoint, although strictly speaking the endpoint would be plasma concentration. Please note also that other amendments were made in the concerned paragraph.
389	3	Comment: see proposed change Proposed change: Include the definitions of PK and PD	Accepted. In addition, a definition of PK/PD modelling is provided.
390	4	Comment: Propose to amend accumulation definition. Proposed change: Accumulation: The increase in drug concentration that occurs with each additional dose, **until steady state has been reached.**	Accepted. In addition, "*concentration*" was changed to "*exposure*". Please note that the notion of *accumulation ratio* has been included as well.
393	1	Comment: A term such as $AUC_{t(last)}$ would be better to distinguish from partial AUC to time t	Partially accepted. The definition was amended to explain that, while in general "AUC_t" may denote any partial AUC to a given time t, in the context of this guideline, this is employed as "$AUC_{t(last)}$", i.e. this refers to the AUC to the last sampling time associated with quantifiable drug concentrations.