14 July 2016
EMA/CVMP/EWP/495905/2015
Committee for Medicinal Products for Veterinary Use (CVMP)

Overview of comments received on 'Guideline for the testing and evaluation of the efficacy of antiparasitic substances for the treatment and prevention of tick and flea infestation in dogs and cats' (EMEA/CVMP/EWP/005/2000-Rev.3)

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

Stakeholder no.	Name of organisation or individual
1	Beaphar BV
2	Swissmedic, Department Authorization Veterinary Medicines, TAM
3	Klifovet, Germany
4	FVE, FECAVA, UEVP
5	European Coalition to End Animal Experiments (ECEAE)
6	Association of Veterinary Consultants (AVC)
7	International Federation for Animal Health Europe (IFAH Europe)

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1. General comments – overview

Stakeholder No.	General comment (if any)	Outcome (if applicable)
2	For repeated infestations, collection of blood specimens would be of advantage e.g. for later pharmacological evaluations.	As a relationship between activity against external parasites and blood levels of the active substance does not exist for any ectoparasitic product (local versus systemic), this is not address in this general guidance (see also page 8).
3	We appreciate the revision of the existing guideline for the testing and evaluation of the efficacy of antiparasitic substances for the treatment and prevention of tick and flea infestation in dogs and cats. More detail has been provided on study design, conduct and evaluation in the current Draft and this is welcome. In addition, a new section on the specific requirements for generic ectoparasiticidal products for external topical use acting locally has also been included. This is a welcome addition to the existing guideline given the large number of generic compounds being developed in this therapeutic area. We would appreciate some further clarification as mentioned below. KLIFOVET AG is a contract development organisation highly engaged in the development of ectoparasitic products during the last decades.	Noted.
4	These guidelines are clear and have a number of commendable checks and balances that maximise confidence in the products meeting the criteria, while preserving animal welfare in laboratory conditions. For tick borne disease transmission, it should be emphasised that no product is able to completely prevent attachment and therefore prevent disease transmission. Unfortunately too many fatalities are still seen with the use of permethrin in cats through the use of topical antiparasitic products	Noted. Labelling of specific products is not within the scope of this guideline.

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	containing permethrin intended for dogs. These medicinal products can cause serious neurological disorders (trembling, convulsions, ataxia, agitation, coma) sometimes in conjunction with digestive symptoms such as hypersalivation, which can be fatal in cats. It is important to put very clearly label permethrin-possessing products as toxic for cats.	
5	*Need for a workshop prior to revision*. We think to release the guideline as it is would not result in any significant improvement in terms of the 3Rs. There are a number of areas within this guideline that would benefit from a workshop to determine if further improvements can be made in relation to the 3Rs. These include: • Need for individual versus group housing • Maximum length of studies • Need for untreated, infected controls as opposed to treated with existing product • Use of donor animals for infestation • Possibilities to reduction the number of studies (dose determination, etc) using combined studies or in vitro tests • Testing requirements for generics We request that serious consideration is made to the conduct of a workshop with manufacturers of these products. The workshop would determine, 1. The protocols manufacturers are already using and if there are any that are better in terms of the 3Rs than others (ie. Sharing best practice) and 2. To determine if further improvements could be made without affecting the rigor of the studies.	Proposal noted. Animal welfare issues raised discussed at an interested parties meeting held on 1st June 2016 in the margins of the EWP plenary meeting. It was agreed to modify the wording concerning the housing conditions to encourage applicants to ensure animal welfare of study animals in laboratory studies.

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5	*Individual housing* We appreciate that efforts have been made since the previous guideline (EMEA/CVMP/EWP/005/2000-Rev.2) to suggest that animals are singly housed only from infestation to counting. However, it could be made clearer in the guideline that 'counting' refers to the counting of the parasites shortly after infestation (i.e. to ensure that they have attached to the animals) rather than counting the number of live parasites after substance treatment, which would undoubtedly require much longer periods of single housing. We assume that the recommendation for single housing for 'up to 96 hours at the beginning of the trial' refers to the 'pre-allocation infestation' period. However, in the procedure table this appears to be a length of 7 days (Day-7 to Day 0) so this needs to be clarified. Nonetheless we remain concerned about the recommendation that animals must be singly housed for up to 96 hours at the beginning of the trial and then again for 48hrs during each subsequent period of infestation. Single housing is recognised to cause stress in social animals, especially dogs, which could confound experimental results. Housing social species on their own for prolonged periods is an example of a procedure that in itself can cause severe suffering under Annex VIII of the Directive 2010/63/EC We understand that the purpose of conducting a 'pre-allocation infestation' test is to determine how susceptible certain animals are to tick or flea infestations. Animals with a similar susceptibility can therefore be housed together and treated as a group throughout the study. However, it is not clear why the animals must again be separated for such long periods of time (48 hours for ticks and 24 hours) for each subsequent period of infestation. For animals in 'short-term' studies this will mean single housing every week for up to 4 weeks while	The topic of "individual housing" has been thoroughly discussed at the Working Party. It is acknowledged that single housing may cause stress in social animals which could confound experimental results. However, keeping dogs or cats in pairs or groups would also confound the results of infestation studies with ticks or fleas. It has to be noted that on the one hand social behaviour (e.g. mutual licking/grooming etc.) of animals kept in pairs or groups would have an influence on the status of infestation. On the other hand also the behaviour of the parasite (change of the host) could lead to changes regarding infestation status. In addition, for veterinary medicinal products to be applied topically, cross-contamination may occur when dogs or cats are kept in pairs or groups. In view of the statistical evaluation of such a study, an animal which is kept together with others cannot be considered as an independent 'being'/sample, meaning that the pair or the group of animals has to be seen as one statistical unit. Grouping of animals into one statistical unit would increase the overall number of study animals per group. Therefore, to take the number of study animals as low as possible it is considered justified to take a single animal as a statistical unit and accept short periods of single housing. The time period for attachment of an ectoparasite to a host – which in ticks can take several hours for finding a suitable attachment site - is not the only period to be considered. It

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	animals in 'long-term' studies will be isolated every 4 weeks over several months. We understand that animals will have to be separated while parasites are applied to their bodies and allowed to attach. However, it is not clear why a period of up to 48 hours is necessary. According to the Centers for Disease Control and Prevention, ticks can take between 10 minutes and 2 hours to attach to their host and start feeding (CDC, 2015). While fleas require even less time to attach, e.g. cat fleas will being to feed within just a few minutes and will become engorged (full of blood) after just one hour. (Cadierques et al 2000). We therefore ask that the duration of single housing for subsequent periods of infestation be reconsidered. The guideline states that it must be read in conjunction with the guideline on 'Demonstration of efficacy of ectoparasiticides' (Vol. 7AE17a, 1994). We would like to highlight the fact that this guideline does not mention a requirement for single housing and allows groups of animals to be used as long as control animals are separated from treatment groups. The World Association of for the Advancement of Veterinary Parasitology's (WAAVP) guideline for '*evaluating the efficacy of parasiticides for the treatment, prevention and control of flea and tick infestations on dogs and cats*' (2013) also does not require single housing. (Marchiondo et al., 2013). We request that this issue is discussed by the working group and advice from manufacturers and testing facilities sought.	has to be taken into account that the time periods for evaluation of efficacy of an ectoparasiticidal product, as outlined in the guideline, should be appropriate to allow the attachment of the required percentage of ectoparasites to the host and (to cover) the development of toxic signs in the ectoparasites to be able to conclude on efficacy. Since the susceptibility to acaricides/insecticides or repellents may be variable depending on parasite species and biological factors, e.g. age and size in ticks, a flexible approach was taken in the guideline. Therefore, a shortening of the mentioned flexible time periods (up to…) is not considered constructive. The guideline on 'Demonstration of efficacy of ectoparasiticides' (Vol. 7AE17a, 1994) is a general guidance document, which provides basic issues and no details. More detailed information for the conduct of efficacy studies is given in specific guidelines, at present available for dogs and cats, cattle and sheep. The general guideline does not address specific housing conditions, but states that "groups of animals should be maintained under such conditions to guarantee comparable parasite loads, but exclude interference between treatments and controls".
5	*Language on animal welfare and the 3Rs* Although the guideline refers readers to Directive 2010/63/EU and the 3Rs, the text could be elaborated on to clearly mention animal welfare, the definitions of the 3Rs and the obligations under the Directive.	In this guideline reference to Directive 2010/63/EU and the 3Rs is provided in section 3. Animal welfare aspects are further addressed in other parts of the guideline where considered relevant.

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	We suggest language of this nature: Wherever possible, a scientifically satisfactory method or testing strategy, not entailing the use of live animals, should be used instead of an animal study in accordance with Directive 2010/63. Where this is not possible, studies should be selected that (a) use the minimum number of animals; (b) involve animals with the lowest capacity of experiencing pain, suffering, distress or lasting harm; (c) cause the least pain, suffering, distress or lasting harm; and are most likely to provide satisfactory results. If animals are to be used, numbers of studies, animals, dose levels and routes of administration should be kept to a minimum to generate the required information. Provision of social housing, enrichment and humane endpoints wherever possible should be made. There could also be more specific references to how animal welfare standards will be maintained throughout the experiments, which require prolonged periods of testing (several months) and the possibility of distressing side effects as a result of repeated parasite infestations (e.g. bleeding, allergies, irritation etc.). For example, the WAAVP guideline clearly emphasises the importance of animal welfare throughout the guideline and highlights opportunities to reduce stress e.g. exercise and socialising routines as well as the use of environmental enrichment such as toys.	
5	*Update of related guideline* The purpose of updating this guideline was to address some new aspects for guidance not covered in the previous version, which were addressed in a Q&A document published in 2012. The main additions include information on the requirements for generics, clarification on how to calculate efficacy and provisions for testing tick species non-endemic within the EU. The aim of this guideline was to consolidate	The purpose of the guideline on 'Demonstration of efficacy of ectoparasiticides' (Vol. 7AE17a, 1994) is to provide the basic/general principles independent of animal species. As such it has its justification. It is not considered useful to repeat the general information in each specific guideline (dogs and cats, sheep, cattle). The specific guidelines provide details valid for the

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	the two documents into one. However, the guideline also states that it must be read in conjunction with a completely separate and more general guideline on 'Demonstration of efficacy of ectoparasiticides' (Vol. 7AE17a, 1994). We suggest that it might also be useful to consolidate information from this guideline so that all of the important aspects of tick/flea testing can be found in one place. Group sizes are covered in one guideline and dose groups in the other. There are even inconsistencies between the two guidelines. For example, the draft guideline states that 95% of fleas must be killed to be deemed effective while the 1994 guideline requires a kill rate of 100%. It is unclear which value is the correct one.	respective target animal species. Therefore, with regard to efficacy thresholds for fleas in cats and dogs it is 95%.
5	*Opportunities to reduce the number of animal tests* The guideline recommends dose determination tests in animals, dose confirmation tests in animals and clinical field tests in animals. It also requires 'description of the mode of action' studies, which according to the 1994 guideline could include PK/PD studies in animals. In the interests of reducing unnecessary testing in animals, opportunities to combine some of these tests or waive them based on the results of previous tests, *in vitro* or existing data should be mentioned clearly throughout the guideline. *For example (1), PK/PD to avoid specific dose determination study:* Several studies have suggested that PK/PD studies can be used to determine appropriate dosage regimens for clinical trials, without the need for dose determination studies. (Toutain, 2002, McKellar, 2004). A study that compared a dose finding study in animals with a PK/PD study concluded that the PK/PD trial was able to predict the recommended dose regimen for the drug nimesulide in dogs, which was later confirmed in clinical trials. (Toutain, 2001).	As products can differ in the mechanism of action (systemic/local) the PK/PD should be described and the number of studies needed to support this depends on the type of product. Therefore guidance was not given concerning this specific point. Example 1: Information on dose determination studies are provided in the general guideline. The combination of PK/PD studies with dose determination studies is not addressed since most of the ectoparasiticides authorised so far are veterinary medicinal products which are to be applied topically. These products do not qualify for PK-endpoints like concentrations of the active substance in blood/serum. Therefore, PK/PD considerations are not appropriate for such kind of products, meaning that classical dose determination studies are not dispensable. In contrast, PK/PD considerations could be an option for systemically acting products. Once a revision of the general

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	These studies suggest that PK/PD studies can be combined with dose determination studies to reduce unnecessary testing. Furthermore, while dose finding studies traditionally require at least three doses to be tested in animals, the PK/PD approach only requires one single dose, which also leads to the use of fewer animals. *For example (2), in vitro studies to predict dose determination:* Whilst the possibility to use *in vitro* methods is briefly mentioned in the guideline, more detail on how and when this can be done is needed. While these tests might not be able to fully replace the use of animals in dose determination studies, they can provide useful information, which could potentially be used to lower the number of dose groups needed. For example, the 'adult immersion test' can be used in dose determination studies to ascertain what doses are effective at killing certain fleas/ticks. In these tests, 'engorged' adult parasites (collected right after feeding on the blood of naturally infected animals) are immersed in various concentrations of treatment solutions before being incubated. The parasites are then checked for their ability to reproduce, the hatch rate of their eggs and percentage of mortality. (Parveen et al., 2014). Another test called the 'larval packet test' can also be used to test the susceptibility of parasite larvae to certain doses of a test substance. In these tests, larvae are placed in between sheets of filter paper that have been soaked with the test substance before being incubated. The number of dead and living larvae is then counted after 24hrs. (Chagas et al., 2014). Another *in vitro* test involves the use of an artificial feeding system for parasites. In these tests, parasites are placed in plastic cages containing chambers separated by mesh (for egg collection). Blood	guideline is envisaged it would be meaningful to check this aspect and introduce respective information, if considered justified. Example 2: If at all, only well-established methods might be addressed in a guideline. With view to lowering the number of dose groups in dose determination studies in the target animal species it is assumed that there are no validated in vitro-tests which ensure scientifically robust results for that purpose. Example 3: Although allometric scaling may be used as a tool in early development stages to predict drug dose regimens, it cannot replace the studies currently required.

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	mixed with a test substance is then enclosed in an artificial membrane and placed into the cage so that the parasites can feed in a similar way to how they would normally. Hair can also be introduced to the system to make it more realistic. The whole unit is then incubated and reproduction, egg hatch rate and flea/tick survival are measured. (Williams et al., 2014). *For example (3), use of existing information to predict dose determination* 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 pharmacokinetic data often helps to save time 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).	
5	*Definition of negative and positive controls* The use of a negative control is mentioned in several places in the document. In this guideline, negative controls are animals that have not been given the treatment (i.e. antiparasitic substance) and are left with the infestation. In other scientific fora, such as toxicology, a negative control refers to a group of animals that are not given the infection and not treated while a positive control group refers to animals that are given the infection and not treated. To prevent any misunderstanding, it would therefore be beneficial to include the definitions of a positive and negative control in the guideline glossary.	Accepted.
5	*Extra testing for generics* According to the guideline, bioequivalence studies are not applicable	A classical bioequivalence (BE) study is only applicable for systemically acting products: It is assumed that, if an active

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	to generic locally acting ectoparasiticidal products for external topical use. It is not clear why these products have been left out of the scope of the bioequivalence guideline and we are concerned about the recommendation for additional animal tests. Is there a possibility to update this guideline to include locally acting products or perhaps produce a separate guideline to address this issue? It should also be made clear that the bioequivalence guideline is only not applicable for locally acting antiparasitic products but can be applied to systemic antiparastic substances. Section 7 of this draft guideline starts off by stating that efforts must be made to 'avoid unnecessary use of animals in experiments for generic antiparasitic products with local activity only'. However, this statement is contradicted by the rather weak recommendations that follow. The guideline mentions the possibility of confirming efficacy through the use of 'validated' in vitro methods but does not provide any references or make any suggestions of what types of methods would be considered. This is an example of another missed opportunity to encourage a reduction in animal tests.	substance of a test veterinary medicinal product reaches the systemic circulation with the same rate and extent as the active substance of a reference veterinary medicinal product, the local availability (concentration in tissue) of the active substance will be similar for the test and the reference products. The similarity of availability at the site(s) of action is the basis of concluding therapeutic equivalence of the products. Section 7 of the draft guideline for the testing and evaluation of the efficacy of antiparasitic substances for the treatment and prevention of tick and flea infestation in dogs and cats explicitly concerns generic ectoparasiticidal products for external topical use which are locally acting and normally poorly absorbed. Therefore, a classical BE study is not applicable. With view to the in vitro methods the following may be explained: For generic ectoparasiticides the required data base is already reduced in that only one controlled dose confirmation study per claimed parasite species has to be provided (instead of two under usual conditions). Depending on the number of claimed tick species it is possible to further reduce these requirements. If from a spectrum of claimed tick species the least susceptible tick species can be identified by a validated method, only controlled dose confirmation studies (i.e. at least 1) with this tick species will be required to get the claim for all. The option to determine the least susceptible tick species by in vitro methods is, however, only acceptable if the respective method is validated and the results correlate with in vivo

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		results. So far, no in vitro-method has been presented which has reliably proven these conditions.
6	The Association of Veterinary Consultants (AVC) appreciates the revision of the existing guideline for the testing and evaluation of the efficacy of antiparasitic substances for the treatment and prevention of tick and flea infestation in dogs and cats. More detail has been provided on study design, conduct and evaluation in the current Draft and this is welcome. In addition, a new section on the specific requirements for generic ectoparasiticidal products for external topical use acting locally has also been included. This is a welcome addition to the existing guideline given the large number of generic compounds being developed in this therapeutic area. However, one key item which always is unclear to applicants is the option to conduct combined studies (tick and fleas) and in particular combined field studies as this represents potential large savings to the development of novel compounds. Even though there is a reference to this option (combined studies) under the New section on Generics ectoparasiticidal, no mention is made for novel molecules. This should be clarified in the New Guideline and if acceptable for generic compounds should also be appropriate for novel compounds. Based on the experience conducting dose confirmation studies and the differences observed in speed of kill, we propose to use the exactly shown time periods to be written in SPCs rather than categorising such effect in immediate (<24 hours) or other (<48 hours). We believe that the consumer does not understand such terms and stating the timings as shown in the DC studies is the most appropriate way for a claim.	Following thorough reconsideration it has been decided that studies with mixed infestations are not considered justifiable in laboratory studies due to animal welfare reasons. The required infestation level will be too high. Consequently, this approach has been deleted from the generic section. When feasible, mixed infestations may be used in field studies.
7	IFAH-Europe welcomes the opportunity to comment on the revision of this guideline. The inclusion of guidance for systemic	Noted

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	ectoparasiticides is a positive step forward. We would like to see the inclusion of guidance for mixed infestations with multiple species of ticks and/or tick-flea infestations as this would reduce the number of animals required for studies and therefore support the 3Rs principles.	

2. Specific comments on text

Line no.	Stakeholder No.	Comment and rationale; proposed changes	Outcome
078	2	**Comments**: § 2 Scope The sentence gives the impression that IGRs are not efficacious against ticks, which is not correct according to literature, e.g. Microsc. Res Tech. 2013 Nov;76(11):1177-85. Action of the insect growth regulator fluazuron, the active ingredient of the acaricide Acatak®, in Rhipicephalus sanguineus nymphs (Latreille, 1806) (Acari: Ixodidae). Calligaris IB1, De Oliveira PR, Roma GC, Bechara GH, Camargo-Mathias MI **Proposed change**: …in combination with an ~~flea~~ adulticide. (delete the word “flea” in this sentence)	Accepted.
078	2	**Comments**: § 4. Data requirements Description of the mode of action - the meaning of it is not clear. The mode of action of a compound is usually elucidated within the framework of biochemical and molecular studies and is not the scope of this guideline.	Comment noted. The mode of action refers to the general guideline and is mentioned here as a reminder.
078-80	7	**Comment:** The text indicates that efficacy in the prevention of transmission of vector borne diseases is outside the scope of this guideline. However they are mentioned later in the guideline (e.g. lines 195, 196). We suggest that further mentions in the guidance are deleted.	Not accepted. The information that “transmission of infectious diseases by ticks cannot be excluded” is considered an important information. There is no contradiction to the wording in the scope.

Line no.	Stakeholder No.	Comment and rationale; proposed changes	Outcome
		Proposed change: Remove any further Vector borne disease related references.	
107	4	Where applicable, groups of treated and control animals should be established by random selection, and investigators should be blinded. **Comment**: "Where applicable" blinded, randomised trials are rarely not applicable unless they cannot practically be achieved **Proposed change**: Replace with "Where possible"	The sentence has been replaced by the recommendation to conduct clinical studies according to GCP.
109-124	4	**Comment**: Why is *Ixodes canisuga* omitted? It is arguably a more common and more clinically significant European ectoparasite than *Ctenocephalides canis*. **Proposed change**: Include *Ixodes canisuga*	Not accepted. With regard to tick species only the most relevant are listed. It is acknowledged that *Ixodes canisuga* can also be found on dogs and cats, however, addition of *I. canisuga* to the listed species is not considered necessary.
110-127	7	**Comment:** As mentioned in lines 550-552 the possibility of combining species should be referenced in this section. **Proposed change:** Add "Studies can be combined (multiple species in one study), e.g. infestation with both *Ct. felis* and one tick species in one study."	Not accepted. Following thorough reconsideration it has been decided that studies with mixed infestations are not considered justifiable in laboratory studies due to animal welfare reasons. The required infestation level will be too high. Consequently, this approach has been deleted from the generic section. When feasible, mixed infestations may be used in field studies.
117	7	**Comment:** The proposal to include the ability for claims on exotic tick species is welcome, in order to address not only global marketing strategies, but also the increased movement of animals and potential aspects such as overseas territories of EU countries.	Accepted.

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		However, the text does not take into account those animals living in these overseas territories. While we understand the rationale, and recognize that this is a minority of animals, in the interests of equality the text should be amended to reflect their existence. **Proposed change:** Applications may also concern non-autochthonous tick species which are of no epidemiological relevance for the EU continent area but might affect animals **living in EEC territories**, or travelling to or returning from areas where such ticks are endemic.	
118-123	7	**Comment:** The text states that deviations from the guidance may be acceptable if sufficiently justified in the context of non-autochthonous tick species. Well justified deviations should be acceptable in the full context of the guideline.	Not accepted to add further information. The wording as indicated in the Annex of Directive 2001/82/EC as amended, implies that well justified deviations may be acceptable. Therefore, it is not considered necessary to include such a general note in this guideline.
133	2	**Comments**: § 5 Extrapolation of study data from dogs to cats due to difficulties of experimental infestations (and in handling) on cats: such an argument should only be acceptable if study data extrapolations are scientifically doubtless. ***Proposed change:*** …if such study data extrapolations can be shown as scientifically correct.	Accepted.
143-144	7	**Comment:** 3 years is impossible in practice for a development program for selection of a field isolate for DC studies. As the time required for the regulatory	Accepted. The indicated frequency for enrichment of a laboratory strain

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		process (e.g. MRP) may be 2 years and the field trial can take about 6 months (in-life and reporting). This stipulation would therefore require the applicant to run the DC/DD in parallel to the field trial very close to the submission. GD on antimicrobials requires strains of 5 years old. What is the justification for the requirements for ectoparasiticides to be more stringent? It should also be noted that no such condition exists for the registration of biocides. Additionally whilst the possibility to use genetically enriched isolates is appreciated the frequency of enrichment (every 3 years) might not match with the biology of all ticks species. If *Ixodes* species are being introduced into a new colony, then it will take at least 3 years (2 life cycles) to have the necessary incorporation of the new ticks into the existing colony. In addition, after the new ticks have been introduced, a tick colony will need some time to stabilize and for data to be generated to ensure that there is no adverse impact of the introduction of the new ticks on the performance of the tick colony. Also it is a considerable risk to introduce new field isolates into laboratory colonies due to their potential individual vector borne disease pathogen load. Therefore this should be reduced to a minimum. As generation times of dog and cat ticks in nature are comparably long and population numbers on the host comparably low, development of genetic variation with respect to insensitivity or resistance is expected to be slow. Hence refreshment of laboratory colonies can without	with a field strain, i.e. about every 3 years, is deduced from the WAAVP guideline, second edition: Guidelines for evaluating the efficacy of parasiticides for the treatment, prevention and control of flea and tick infestations on dogs and cats (Marchiondo et al., 2013). However, as this time interval appears not to be appropriate with regard to ticks the proposed refreshment cycle in 6 years intervals may be acceptable.

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		any risk be set to wider intervals e.g. every 6 years. **Proposed change:** Replace: “which are genetically enriched with parasites from field isolates about every ~~3~~**6** years”	
146 and 326	2	**Comments**: § 5.1.1. and 6.1.1. (line 326) Ticks should be pathogen-free not only for animal welfare reasons. The presence of a pathogen will alter immunity and possibly metabolic parameters in the host animal, which in turn might have an impact on the efficacy of the compound tested. Should a tick population not be tested for the most important pathogens prior to the experiment? There is no mentioning of the origin of the flea populations used for efficacy testing in 6.1.1., to ensure strains to be used that are representative of the field situation.	Accepted. Ad 5.1.1.: It is agreed that testing of ticks for the most important vector pathogens prior to the experiment is reasonable. It is assumed that this is sufficiently covered by the last sentence of the second para, and it is also assumed that laboratory strains are well characterised. Ad 6.1.1.: A respective sentence is included.
149 and 329	2	**Comments**: § 5.1.2. and 6.1.2. (line 329) Ideally, the skin should not be extensively pigmented to make sure that the parasites are easily detected by the experimenter. **Proposed change:** For reasons of good parasite detection on the skin, the skin pigmentation should be considered in the selection process, too.	Accepted. A repetition of this information under 6.1.2. is, however, not considered necessary.
151	2	**Comments**: § 5.1.2. Selection of animals It should be ensured that there is no impact… **Proposed change:**	Not accepted, since this is a guideline and not a legal text.

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		We suggest to replace “should” by “must”	
151-152	7	**Comment:** “It should be ensured that there is no impact of a previous treatment with an ectoparasitic substance on the study outcome.” This requirement is not precise and is open to interpretation. **Proposed change:** “It should be ensured that ~~there is no impact of a previous treatment~~ included animals have not been treated with an ectoparasitic substance ~~on~~ within a timeframe that might impact the study outcome. Animals should be tested for their ability to carry adequate numbers of parasites prior to study start.”	Accepted.
157 160-163	5	“For example, during the time period(s) of infestation with ectoparasites, dogs and cats should be kept in individual accommodation, i.e. from the day of infestation until the day of ectoparasite counting (up to 96 hours at the beginning of the trial, and up to 48 hours after subsequent challenge infestations).” We assume that the recommendation for single housing for ‘up to 96 hours at the beginning of the trial’ refers to the ‘pre-allocation infestation’ period. change the above sentence to: ‘up to 96 hours at the beginning of the trial **for the pre-allocation infestation test..’**.	“Up to 96 hours” does not refer to the pre-allocation infestation. It refers to the first incidence where the test substance is used, i.e. day -2 with the tick infestation up to day + 2 (up to 48 hours after application of the test substance). To avoid misunderstanding an additional information has been included to the guideline text. For ticks the duration of pre-allocation infestation period may take about 48 hours.
157-158	7	**Comment:** We fully agree that only for the time periods of infestation with ectoparasites, dogs and cats should be kept in individual accommodation, i.e. from	Partly accepted. The wording in brackets is given as an example (e.g. up to

Line no.	Stakeholder No.	Comment and rationale; proposed changes	Outcome
		the day of infestation until the day of ectoparasite counting. However, the sentence given in parenthesis is redundant and may not apply to every product or study objective. **Proposed change:** ectoparasite counting ~~(up to 96 hours at the beginning of the trial, 157 and up to 48 hours after subsequent challenge infestations)~~. For	96…).
158-160	5	"For the other time periods, it may be considered to keep treated and control animals separately in respective groups with sufficient space according to species." This recommendation is rather weak and could be clarified as we have discussed in the general comment section above. If animals can be kept in groups then this should be Made very clear in the guideline. It would be unfortunate if some manufacturers remain ignorant of the possibility to group house animals in between infestation periods. **Proposed change:** "For the other time periods i.e. between infestations, ~~it may be considered to keep~~ treated and control animals should be kept separately in respective groups with sufficient space according to species."	Partly accepted. The comment is acknowledged, however, a guideline is not a legislative text rather it gives recommendations in which way a certain topic may be investigated/studied. In combination with the indication of the animal welfare Directive and the 3Rs principles in section 3 'Legal basis' the presented wording in section 5.1.3. and 6.1.3. is considered adequate. It is within the responsibility of the stakeholders to consider the Directive. Nevertheless following an interested parties meeting held on 1st June 2016 in the margins of the EWP plenary meeting, it was agreed to slightly modify the wording concerning the housing conditions to encourage applicants to ensure animal welfare of study animals in laboratory studies.
163 and 333	2	**Comments**: § 5.1.4. and 6.1.3. (line 333) For tick infestation, it has to be made sure that the sedative used for calming down the animals does not interfere in the experiment. In order to increase the	Partly accepted. With regard to the sedation concerning the tick infestation a respective sentence has been included. No further details on how to perform the infestation are considered necessary to be

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		efficiency of attachment one should probably apply the ticks at sites of the body that are not easily accessible for dogs to remove them, and where the experimenter finds them easily. Preferred sites for ticks are sites on the body with little hair and thin skin, such as head, ears, shoulder pit, and inner thigh.	included since applicants are expected to know the relevant sites on the body. A sedation is not considered necessary for flea infestation.
163	7	**Comment**: Rather than the proposed approximately 50 an indicative range of 25-50 (as for fleas: 50-100) should be defined. It is possible to have conclusive results with 25 ticks, which is a realistic number. Repeated infestations with 50 ticks can be very irritating to the skin. **Proposed change:** The infestation level should be ~~approximately~~ 25-50 unfed adult ticks	Not accepted. The comment is noted. However, a possible reduction from 'approximately 50' to '25-50 unfed adult ticks' concerning the infestation level is not considered acceptable for statistical reasons.
163-165	7	**Comment:** The proposed ratio for *Ixodes* spp. seems high, and would better be presented as a suggestion than a mandatory ratio. Alternative ratios should be considered acceptable (e.g. 60% females : 40% males) also in view of the difficulty in getting such high numbers of female *Ixodes* ticks for a study. **Proposed change:** females, except for Ixodes ~~ricinus~~ **spp.** with a sex ratio of **for example** approximately 10% males: 90% females).	Accepted.
165-167	7	**Comment:** The sentence "Twenty five to fifty percent (i.e. 12-25 ticks) of these ticks should attach to the animal at each time point following infestation in the control group." is very strict requirement if "to the animal" means each individual control animal. Also the range doesn't make a lot of sense.	Not accepted. The proposed approach to modify the expected infestation rate into 'an average of 25% attachment rate' would ignore a potential increased variability in the control group.

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		Proposed change: Add clarification that an average (arithmetic mean) of 25% attachment rate is expected in the control group animals at each time point.	
After line 167 (section 5.1.4)	7	**Comment:** Include the possibility for mixed infestation with multiple species of ticks – this would reduce the number of animals required for studies and therefore would support the 3Rs. **Proposed change:** add: "When feasible, infestation with multiple species of ticks is acceptable as this will reduce the number of animals required for studies."	Not accepted. For a new product dose finding and dose determination studies should produce meaningful results. It should be considered that an infestation rate of approximately 50 unfed ticks per species is considered adequate to be able to finally calculate the efficacy. In case of infestation with 2 tick species a reduction to 25 ticks per species would have an impact on the validity of the results (e.g. difficulties to evaluate efficacy for the individual tick species). An infestation with 50 ticks per species (i.e. 100 ticks when 2 tick species are included) might be too stressful for the individual animal. For animal welfare reasons it has therefore been decided that studies with mixed infestations are not considered justifiable in laboratory studies. Consequently, this approach has been deleted from the generic section. When feasible, mixed infestations may be used in field studies.
171-176	7	**Comment:** We would propose removing the details of infestation method given in the text; all methods of infestation should be acceptable as long as they result in the required level of infestation in the control animals. For example alternatives are available to mild sedation to keep the animals calm such as crates.	Partly accepted. It is considered useful to give some information with regard to the infestation method. Therefore, the currently existing text will be kept. However, a sentence which allows alternative methods for infestation has been included.
181	3	**Comment:** We would propose removing the details of infestation method given in the text; all methods of infestation should be acceptable as long as they result	Partly accepted. See previous comment.

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		in the required level of infestation in the control animals. For example alternatives are available to mild sedation to keep the animals calm such as crates.	
181	6	We fully support the proposed change for the evaluation of systemically acting products. However, in case that prevention or repellency is claimed, the assessment of the engorgement status of the ticks on the host may be of additional value. We therefore propose to consider to keep the previous evaluation categories for ticks for measuring the repellency or prevention.	Not accepted. It should be noted that repellents should be studied within 24 hours following application of the test substance. Under normal conditions no engorgement of ticks will happen in this time period, rather ticks will be in the process of attachment. Thus, for repellents it is not reasonable to mention engorgement. Concerning products with acaricidal effect it is considered important to have comparable criteria for efficacy assessment for topically applied products with local action and systemically acting products. As the engorgement status is no suitable parameter with regard to systemically acting products, it has no longer been taken into account.
Table between 181 and 182	7	**Comment:** For systemic acting products live free ticks must be regarded as “not attached yet”; therefore ticks in this category cannot contribute to efficacy assessment. In line with the WAAVP Guidelines we propose to add a statement with regard to systemic acaricides. **Proposed change:** **In general** ~~I~~**i**t is recommended to assess the acaricidal effect according to the following parameters: about the effect of the product with or without attachment. If scientifically justified, the assessment of efficacy might be adapted for systemically acting	Accepted.

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		products.	
185-187	7	**Comment:** We do not really understand the term "immediate acaricidal efficacy" being seen in contrast to "persistent acaricidal efficacy" in these lines. (see also line 199: "a claim for *immediate* and/or persistent tick killing activity"…) **Proposed change:** Please rephrase these lines to clarify the intent	Not accepted. The terms "immediate acaricidal efficacy" and "persistent acaricidal efficacy" is deducible from the time schedule for testing acaricides. No change of the wording.
192	5	"In addition, for this type of products a general note should be included in the SPC and package leaflet […]" Typo: the 's' at the end of the word 'products' should be deleted.	Accepted.
192 -193	3	**Comment**: We propose that the proposed term makes reference to the period of time proven in studies, which was shown in the dose confirmation studies and not use a generic term. **Proposed change:** Use the following term: *ticks would be killed and fall off the host within x to y hours after infestation without having had a blood meal: as shown in dose confirmation studies*	Accepted. The option to refer to the period of time proven in studies has been added.
192-193	6	**Comment**: We propose that the proposed term makes reference to the period of time proven, which was shown in the dose confirmation studies and not use a generic term. **Propose change**: use the following term: *ticks would*	Accepted, see above.

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		be killed and fall off the host within x to y hours after infestation without having had a blood meal *X and y: as shown in dose confirmation studies*	
195 Section 5.1.5.1	4	It should be reflected in the product literature that a transmission of infectious diseases by ticks cannot be excluded. **Comment**: "If applicable" If it is correctly being acknowledged that individual ticks may attach post treatment then this infers that disease transmission will be possible **Proposed change** (if any): remove "If applicable"	Accepted.
195-196	7	**Comment:** Please refer to comment to line 78-80. These lines are speculative and provide no guidance for the applicant. In addition, this topic will be the topic of the upcoming vector borne disease GL. **Proposed change:** Delete sentence as this is out of scope of this guideline.	Not accepted. This sentence is of course valid for this guideline.
199	7	**Comment:** The use of the word "only" indicates an inferiority of systemic acting products compared to others. **Proposed change:** substance(s), ~~only~~ a claim for immediate	Accepted.
202	7	**Comment:** We are not sure if the term "feeding" is best in this respect. There is a very complex interaction of the tick and the host for the first hours that expose the tick already to some host fluids while the true feeding doesn't start yet. This contact may be	Not accepted. The term feeding activity is already accepted in centralised marketing authorisation procedures, i.e. "Ticks (and fleas) must attach to the host and commence feeding in order to be

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		sufficient to expose ticks to enough active ingredient to kill. It might be beneficial to agree upfront on a valid terminology in order to avoid discussions in the future (as happened for the term "repellency"). **Proposed change:** must attach to the host ~~and commence feeding~~ in order to be exposed to the active substance.	exposed to the active substance". Also in the WAAVP guideline this expression is used (Marchiondo et al., 2013).
203	7	***Comment:*** The general statement for all oral products is not acceptable. If demonstrated through appropriate studies that specific diseases are not transmitted this statement should not be required. Alternately refer to the upcoming vector-borne disease guideline. ***Proposed change****: "*Furthermore, under such conditions the transmission of tick-borne diseases cannot be *automatically* excluded. Consequently, *if not demonstrated through appropriate studies that specific diseases are not transmitted*, reference should be made in the SPC and package leaflet that *a transmission of infectious diseases by ticks cannot be excluded since ticks have to attach to the host to reach an acaricidal effect"*.	Accepted. The sentence under debate is considered justified. However, with view to possible demonstration of prevention of transmission of certain vector borne disease pathogens the guideline text has been modified corresponding to the proposal.
205-206	7	**Comments:** Label language should be restricted to facts based on studies and not necessarily listing all potential risks because of the presence of the ticks; to draw a comparison with antibiotic, the label does not require "due to the necessary presence of bacteria to the animal other effects like hyperthermia, infections, tachypea, wounds, anaphylactic reactions may occur".	Partly accepted. The respective note as presented in the guideline should be given if appropriate and is, therefore, considered justified. To avoid any misunderstanding the sentence may read: As far as based on study results, a further note may address that…*may occur*, as appropriate.

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222 229 (229-235)	7	**Comment:** The phrase “ideally no ticks …” implies a repellence rate of 100% which is not in line with the efficacy rate of 95% in line 280. Please also see our comment to line 280 and harmonise on 90%.	Not accepted. Ideally a repellence rate of 100 % would be best. However, this value is not realistic to be constantly reached in biological systems, therefore the efficacy threshold for repellents has been set at the minimum of 95%.
224	7	**Comment:** Repellency should not be based on “… the presence/absence of live ticks (attached, free) …” ignores fast acting acaricides. If many dead attached ticks would be present, we would not be looking at a repellent. **Proposed change:** Please clarify that if dead attached ticks are present, repellency cannot be claimed.	Accepted.
235	2	**Comments**: § 5.1.6. It should be mentioned how long these animals will be exposed to the ticks before the counting is done.	Respective information is given in the time schedule under 5.1.6.1.
238	5	“For the assessment of efficacy under laboratory conditions the inclusion of untreated animals (negative control group) is considered necessary. In both the 1994 guideline and the current draft guideline, the use of an untreated control group in clinical field trials can be waived based on animal welfare concerns. It is not clear why this would not also apply to laboratory trials.	The assessment of efficacy under laboratory conditions is based on efficacy thresholds of 90 % / 95% parasite count reduction. Thus, the inclusion of untreated infested animals is necessary to conclude on the vigour of the ticks for infestation to be able to reliably conclude on efficacy. In field trials the focus lies on confirming efficacy and target animal safety of the product under different environmental field conditions. In the cited guidelines no respective information is included that in clinical field trials there is a waiver for untreated control groups.
241	2	**Comments**: §.5.1.6.1. Table for acaricides, immediate efficacy	Not accepted.

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		The term “immediate efficacy” may not be defined with enough clarity. People may expect that ticks will not attach at all (repellent efficacy) or fall off the host within minutes if the product has a claim for immediate efficacy. Would it make sense to declare “immediate efficacy” as the period of up to 2 hours after treatment with the product, and declare efficacy between 2 and 48 hours as e.g. “rapid”? **Proposed change:** add another line to the table to distinguish immediate from rapid efficacy and redefine the periods for the measured efficacy.	The term immediate efficacy is generally accepted for studying insecticidal / acaricidal efficacy and is also used in the WAAVP guideline (Marchiondo et al., 2013) and in scientific literature. It is not considered useful to introduce an additional term. In addition it should be taken into account that a differentiation between ‘immediate’ and ‘rapid’ is difficult to explain and also an exact translation into other languages could be a problem. The proposed change is not supported to avoid confusion.
Table between 241 and 242	7	**Comment:** We think that the examination of tick strain for infestation for infestation rate and suitability of test animals by a pre-allocation infestation does not have to be on Day -7 as long as it is prior Day -2. **Proposed change:** Please harmonize with the respective flea section (line 357 Table) and replace ‘Day -7’ with ‘Prior to Day -2’.	Accepted.
241-242	7	**Comments:** As highlighted by the discussions during the previous revision of the guideline, a systemic product may take longer to act than a contact product. The design should take this into account by allowing counts at timepoints later than 48h (*e.g.* 72h). It is known that ticks spend several hours (4-6 hours for some ticks) searching for a feeding location, during this period they may bite and detach several times before starting to feed. Feeding starts slowly: secretion	Partly accepted. The change of the term “application” by “administration” is accepted. Concerning the second point no addition to the text is considered necessary. Count time points later than 48 hours are adequately covered by the existing wording.

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		of cement (a few hours), secretion of enzymes (24h…..) and then ingestion increases after 48h. This makes a 72h assessment more relevant in this specific context. Frequencies and number of infestations should not be imposed (e.g. : "*every 4 weeks then every two weeks*…"), applicants should be allowed to define numbers and frequencies of assessments according to their objectives. **Proposed changes:** Changes suggested for the table: Day 0: Change "application" by "administration" (to include systemic products) Immediate efficacy: add the systemic product at the end of the sentence. : "Efficacy testing in situ according to the parameters given under 5.1.5.1. at day 0 up to 48h or longer if appropriate (e.g. collars or systemic product)."	
242 -256	1	**Comments**: 5.1.6.1. Laboratory studies - Speed of kill. According to the draft guideline any time point up to 48 hour after administration, after re-infestation and during the whole period of the claimed persistent effect can be used to determine the speed of kill. If the speed of kill should be investigated (next to the efficacy after 48 h) a time interval to undertake tick counts should be specified.	Within the period of 48 hours after administration etc, it is up to the applicant to define time points for evaluating the speed of kill since this depends on the product's performance. It is considered self-evident that the same pattern of chosen time points should be taken after each re-infestation. Note, that the efficacy threshold is at least 90% also for speed of kill at all time points until the end of the persistence period claimed. For better understanding the sentence "Within the 48-hour period always the same pattern of selected time points should be used throughout the study" is introduced.

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242-256	7	**Comment:** The guidance should permit the differentiation of the curative speed of kill (existing infestation) and persistent speed of kill (new infestations) where this can be justified.	Not accepted. Such an approach would be confusing. If the speed of kill is studied it should result in the same time period throughout the duration of the study. Different 'speed of kill'-figures for existing or new infestation cannot be supported.
243-244	7	**Comment:** Speed of Kill – Change "*The speed of kill is the time point when at least 90% of ticks have been killed*" to "*The speed of kill is the time point when at least 90% acaricidal efficacy is achieved*" or clearly define 'kill' based on the counts in both control and treated groups (Similar comment for section 6.1.4) **Proposed change:** "The speed of kill is the time point when at least 90% ~~of ticks have been killed~~ <u>acaricidal efficacy is achieved</u>"	Partly accepted. Wording has been specified by adding "based on counts in both control and treated groups".
247-248	1	**Comments**: It is unclear if a different speed of kill can be claimed for immediate efficacy (onset), if results are variable over the period of claimed persistent effect.	It should be noted that the threshold for determining the speed of kill in ticks is always 90% as defined. At the start of the trial and during the period of claimed persistent efficacy always the same pattern of time points within the 48 hour period should be taken for studying the speed of kill. For a product only one figure for indicating the speed of kill is considered acceptable. If the speed of kill-time points are variable throughout the duration of the study (covering the period of claimed persistent effect) then the range covering the shortest and the longest speed of kill period, until at least 90 % of ticks have been killed, will be taken.
247 -248	1	**Comments**: It is unclear if ticks need to be removed during every tick	In principle it might be acceptable to count ticks through palpation and visual inspection without removing them at every tick count if this is based on a standardized procedure.

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		count (TC). To ensure compliance with the 3 R's (Replacement, Reduction and Refinement), it should be determined if it is acceptable to count ticks through palpation and visual inspection of the animal's skin and coat at TCs before 48 h post infestation or treatment (determine if ticks are alive or dead by visual inspection) and to remove the ticks at TCs at 48 h post infections or treatment. *E.g. for a study with TCs at 12 h, 24 h and 48 h post infestation or treatment, three control and three treatment groups would be required if removal is required all times and one control and one treatment group would be required if removal is only required at 48 h.*	It is the responsibility of the applicant to adequately demonstrate the speed of kill. No change to the guideline text.
249 259	7	**Comment:** Applicants should have the ability to also assess the status of live/moribund fleas/ticks removed from dogs/cats at each protocol specific timepoint in specifically designed SOK studies in order to more fully assess the immediate but short term exposure to a toxic dose of the drug being studied. This has been termed immediate efficacy and resultant or induced mortality in the published literature (F. Beugnet et al.: Parasite 2014, 21, 42). With short exposure times to pulicides or acaricides, the true phenotypic endpoint of death may not be adequately assessed unless removed live/moribund fleas/ticks (from both treated and control animals) are maintained under appropriate (humidity/temperature) *in vitro* conditions for up to 24 after their removal from the animal so that the true	Not accepted. The aspect of induced mortality (or delayed killing effect) has intentionally not been included in the guideline because of methodological difficulties (incl. lack of standardisation). Therefore, in previous marketing authorisation procedures this approach was not accepted by CVMP.

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		effects of the ingested toxic doses of a systemically acting drug have had time to be more accurately measured. These additional *in vitro* assessments and measurements of the systemically acting drugs to demonstrate induced flea/tick mortality after they have been removed off of dogs/cats, without any additional drug exposure, will allow a more complete and holistic assessment of resultant or induced mortality after a very short exposure to the drug being evaluated. **Proposed change:** "At each assessment time selected all live and killed parasites should be counted. If desired, collected live ticks/fleas may be kept under controlled conditions and re-assessed (live/dead) after 24 hours. The speed of kill ~~should~~ could be based on either the immediate killing effect at the time of counting on the animals or on the subsequent counts after incubation."	
250	7	**Comment:** the term "immediate killing effect" is an unnecessary repetition and causes confusion because the 'immediate effect' is already defined as efficacy on an existing infestation following treatment, please rephrase. **Proposed change:** should be based on the ~~immediate~~ killing effect at the time of counting on the animals	Accepted.
251 368	7	**Comment:** "Only animals treated with the minimum recommended dose are considered acceptable." It remains unclear how the definition of the minimum recommended dose should be understood. Particularly,	For a reliable evaluation of the speed of kill a study with animals treated with the minimum recommended dose of a veterinary medicinal product is the preferred approach. To take into account that this approach could be difficult for some

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		what does it mean for unit dose medicinal products (tablets, collars…), suitable for treatments of ranges of weights, which delivered dose cannot be adjusted to the exact weight of animal to be treated? In addition, this definition should not be stricter for some pharmaceutical forms on the sole reason that they allow adjustment to the exact weight of the animal compared to the others (solutions…). **Proposed change:** Please define what a "minimum recommended dose" is in practical conditions, taking into account that it has to be applicable in an adequate way for the different pharmaceutical forms (spot-on solutions, collars, tablets, shampoos).	pharmaceutical forms the wording in the guideline text has been changed as follows: "Animals should be selected in such a way that dosing as close as possible to the minimum recommended dose is possible."
253-254	7	**Comment:** Why should the time of speed of kill be moved to section 5.1 of the SPC? We are unable to see a benefit in this move.	The speed of kill is a pharmacological property of the product. It does not fit under 4.2, 4.4 or other sections in the clinical particulars of the SPC.
254-271	7	**Comment:** The term onset can be difficult to translate clearly to other languages we would suggest an alternative. **Proposed Change:** Use 'start' instead of 'onset'	Accepted.
254-255	7	**Comment:** When a product 'starts killing significant numbers of ticks' this can be relevant information for dermatologists specifically in relation to flea allergy dermatitis (FAD). A statistically significant reduction in the number of live ticks showing certainly when an incremental raise in efficacy during subsequent time	Not accepted. It was decided not to include the "onset of kill activity" as a parameter in the guideline because a statistically significant reduction in the number of live ticks below 90 % in the treated versus control group is not considered clinically relevant.

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		points can be interesting to define onset of kill. (Similar comment for section 6.1.4 **Proposed change:** "The initial onset of killing activity after ~~application~~ administration of the product ~~meaning a kill activity below the threshold of 90% is considered not to be clinically relevant, and such information should not be included in the SPC and product literature~~ may be stated as the time point when the product begins to kill a statistically significant number of ticks in the treated versus the control group".	Note beside: It is assumed that the relationship between ticks and FAD is erroneously mentioned in the stakeholder's comment.
258-265	7	**Comments:** Frequencies and number of infestations should not be imposed e.g.: "every 4 weeks then every two weeks…" applicants should be allowed to define numbers and frequencies of assessments according to the study objectives.	It should be noted that the frequencies and numbers of infestations as given in the tables are recommended and not imposed. No change of the wording necessary.
261-262	5	"Where effectiveness over several months is claimed, the ticks should be applied at 4-week intervals over the first three months […]" Are you able to recommend a limit to how long these studies can be carried out? Repeated periods of sedation, single housing and tick/flea bites will cause increasing distress to the animals.	Partly accepted. The duration of the persistent efficacy – as claimed by the applicant – has to be shown. Therefore, a limitation of the duration of such studies cannot be specified in the guideline, but some wording has been amended.
264-265	7	**Comment:** "Also, severe reactions at the site of application should be reduced to a minimum." This consideration is implied from an animal welfare point of view and therefore should not be stated in the guideline. Additionally, the meaning of "the site of	Accepted.

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		application" seems to correspond to "the site of infestation" and therefore is confusing. **Proposed change:** Propose to delete this sentence	
272-277	7	**Comment:** The justification for using exclusively arithmetic means seems to be weak or personal opinion but lacks any science based factors. Expressing that geometric means are not relevant even if the count data are skewed seems to be quite odd. **Proposed change:** In cases in which the distribution is skewed the use of geometric mean should be allowed.	Not accepted. For calculating percentage efficacy in any case arithmetic means should be used: The main point is that an efficacy of x% usually will be interpreted as "x% of the parasites are killed by the treatment". This exactly corresponds to the efficacy based on arithmetic means (irrespective of the distribution of data, i.e. whether they are skewed or not) while geometric mean efficacies could not be interpreted in such an easy way.
278 and 386	2	**Comments**: § 5.1.7 and 6.1.5. (line 386) Why is acaricidal efficacy 90% and repellent efficacy 95%, and not higher? If a small subset of parasites is not affected by the compound, selection for resistance could become an issue as soon as the tested compound is marketed and widely used.	The efficacy threshold for acaricidal products (at least 90%) has not been changed. The threshold for repellent efficacy has been set at 'at least 95 %', since a constant level of efficacy of 100 %, which would be the ideal case, is an unrealistic approach in a biological system.
279	7	**Comment:** The increase in the level of requirement in EU, to a level that is so high, is inconsistent with the other effect on ticks (so how will a user understand the rationale behind the regulatory requirement and so understand the label?) and hardly achievable. It should be noted that for biocides such requirement does not exist nor in any other region. So it pushes the requirements in Europe on ectoparasites to unfair and	Not accepted. In the currently valid guideline it says in section 4.1.5.1. 'Repellent effect' that "in general no ticks should be detectable on the animal after 24 hours following administration of the product". This is corresponding to an efficacy threshold of 100% for repellents. Thus, there is no increase in the requirements in the revised guideline. The efficacy threshold for acaricidal products has not been changed. The 95 %

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		unrealistic levels and therefore places European manufacturers for export at a disadvantage, (Free sale certificate necessary for export markets). In addition it is unclear where the level of 95% of repellents derives from. In addition this level differs from the level expressed in line 222. We would suggest the level is set to 90% **Proposed change:** The acaricidal efficacy of the proposed product should be at least 9~~5~~**0**% at each counting during the claimed efficacy period. The same efficacy threshold is valid for studying the speed of kill and repellency.	efficacy threshold for repellent products is based on the consideration that 100 % efficacy cannot be constantly reached in a biological system.
281-282 (section 5.1.7) (see also lines 387 and 517	7	**Comment:** A recommended method for statistical hypothesis testing should be included (Similar comment for sections 6.1.5 and 6.3.1.2).	Not accepted. Methods for statistical hypothesis testing are adequately addressed in the statistical guideline.
274-275 and (section 5.1.7 and 6.1.5)	7	**Comment:** Please substantiate or justify the statement "since efficacy estimates based on geometric means tend to be **biased** upwards". While it is recognised that GM typically lead to higher estimates of efficacy, they are not necessarily biased estimates. See also our comment to lines 272-277. **Proposed change:** *"since efficacy estimates based on geometric means tend to be ~~biased upwards~~ larger and potentially mask treatment failures"*	Accepted.
286-287	5	"The impact of exposure to water e.g. through shampooing, swimming, rainwater or the	Concerning the testing for water stability the same criteria for efficacy testing are to be used as in section 5.1.6 (or 6.1.4

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		acaricidal/repellent effect should be evaluated at regular intervals (e.g. once a week)." How will this be assessed? These methods appear to vary quite widely and may have a positive or negative or neutral impact on animal welfare as well as the rigour of the test and the claims made as a result. Can this be elaborated on?	with regard to fleas).The applicant has to justify the conditions and durations of exposure. No details can be given in the guideline. Specific conditions of the chosen test will be presented in the product literature as background information for the user.
294 and 392	7	**Comment:** Guidance should be provided for the evaluation of efficacy in field studies. Either Abbott's formula (in the exceptional case of negative control) or the % reduction: % red = 100 x (Count at T_0 – Count at T_i)/Count at T_0 (when positive control is used) is recommended. For ethical reasons we consider the use of a negative control in cats and dogs to be unlikely, however we do not wish to exclude the possibility for exceptional circumstances.	Not accepted. As the study design is in the responsibility of the applicant no guidance for calculating efficacy will be provided in the guideline. In this respect it has also to be considered that generally for ticks field data is difficult to interpret since infestation with ticks under field conditions is variable over the months.
296-297	6	**Comment**: Field studies should be conducted in two geographic regions. We believe the concept of geographic regions is ambiguous. Assuming that the objective of having two geographic regions is to include different species of ticks perhaps the concept of "two climatic regions" should be used as this will guarantee different tick species in dry versus humid regions. **Proposed change**: Field studies should be conducted	Accepted.

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		in two geographic or climatic regions.	
296, 311 and 399	2	**Comments**: § 5.2.1. and 6.2.2. (line 399), Field studies, and tick counting (line 311) of 5.2.3. Field studies for ticks should take into account the biological differences and the peak occurrence of the different tick species. In Switzerland, *Ixodes* is typically prevalent from March to June and from September to November, and the development from larva to nymph to adult takes place outdoors, and can take 2-3 years. Infestations with *Dermacentor* can occur from February until December, depending on the climatic conditions. *Rhipicephalus sanguineus* on the other hand completes its life cycle typically within 3 months, and larval, nymph and adult development can take place indoors. Therefore, for *R. sanguineus* treatments, additional measures are often required to get rid of the infection in the real life situation, such as acaricidal treatment of the environment where the dog/cat is actually housed. Therefore field efficacy testing has to take into account that reinfection with *R. sanguineus* can take place very easily. Maybe the owners of the animals should be advised not to treat the home environment during the duration of the trial? For counting of ticks (5.2.3.), larval stages and nymphs should be counted, too. Usually, a compound should act on all three stages. If possible, blood samples should be obtained from animals that are involved in a field study (e.g. for later	Partly accepted. It is agreed to include a note that the owners of the animals should be advised not to treat the home environment during the duration of the trial. Based on *in vitro* experiments it should have been confirmed that larvae and nymphs have a higher susceptibility than adults (see section 5.1.1.). Thus it is assumed that efficacy testing using adult ticks is generally sufficient. To perform blood sampling in a field study for certain later evaluations should be up to the applicant's own decision. It is not considered necessary to include a respective note into the

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		pharmacological evaluations).	guideline.
298	7	**Comment:** The control group should be positive. **Proposed change:** species (dog/cat) claimed and should include a positive control group	Partly accepted. It is up to the applicant to choose an appropriate study design. The study design is decisive for the selection of the control group. Respective general information is indicated in section 5.2.1. Information on the kind of control group is also given in section 5.2.2. indicating that a non-inferiority evaluation includes a positive control group
301-302	7	**Comment:** The sentence that "*The tick species included in the list of indication should be adequately represented among the included animals*" may imply that this is a requisite for inclusion of the tick species in the list of indication (4.2). The potential impact is that a claim for efficacy is not granted because, while the efficacy was tested in the laboratory studies, the tick species was not found in the field. Given the number of animals enrolled in a field study, it is theoretically possible that certain (rare) tick species are not encountered at all in a study even if known to be endemic in the EU, or the ticks are found but at very low levels: in the latter case it has to be determined what is the threshold of "adequately represented" as currently in the draft guideline text. The field study is not meant to be an epidemiological survey or confirmation of efficacy vs each species, as this is the objective of laboratory studies. **Proposed change:** Delete the sentence: "~~The tick species included in the list of indication should be~~	Partially accepted. It is agreed that efficacy is predominantly demonstrated in laboratory studies. However, apart from the safety aspect, efficacy should also be shown under field conditions. In this context it is of course expected that the claimed tick species are covered as best as possible. The term 'adequately' does not imply that with regard to each claimed tick species a statistical evaluation is required. However, to avoid a false impression 'adequately' has been deleted and the respective sentence has been modified by adding "… whenever possible".

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		~~adequately represented among the included animals~~"	
303-304	6	**Comment**: Comment: A minimum of 50 animals per treatment group and region is required (i.e. minimum of 200 animals per study). This number does not take into account the study design (superiority or non-inferiority) and appears arbitrary and without scientific justification. In a superiority study versus placebo the likely number will be lower and in the non-inferiority (vs Positive control) the numbers may be higher. The number of animals required should be based on the tested hypothesis and other variables (statistical power, non- inferiority vs superiority, expected efficacy in each treatment group, significance level, etc.). **Proposed change**: The number of animals (sample size) required in the study should be statistically justified upfront and based on the hypothesis tested (e.g. superiority design versus non-inferiority) and consider both, efficacy and the safety aspects of the study.	Accepted.
303-308	7	**Comment:** The required numbers are not possible in field situations (client owned animals not a laboratory test). Number of animals by group and region seems unrealistic, the way it is defined means that at least 200 animals will be used and in 2 regions. **Proposed change:** Replace the paragraph as follows: When a non-inferiority evaluation is planned it	In principal accepted. See above.

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		should be ensured that the enrolled animals are living in infested area large enough in the test and the positive control group to obtain sufficient assay sensitivity. Adequate number of animals to respond to the objective will be used and with representativity in all regions.	
305	7	**Comment:** There is a typo in this line **Proposed change:** "….exposed to high risk of infe~~ction~~estation…"	Accepted.
306-307	7	**Comment:** "It should be ensured that there is no impact of a previous treatment with an ectoparasitic substance on the study outcome." This requirement is not precise and is open to interpretation. **Proposed change:** "It should be ensured that ~~there is no impact of a previous treatment~~ included animals have not been treated with an ectoparasitic substance ~~on~~ within a timeframe that might impact the study outcome.	Accepted.
310 -313	1	**Comments**: 5.2.3 Field studies - Counting It is not feasible for owners of animals treated with a long-term efficacious product to attend weekly veterinary examinations. Instead of a time interval, a minimum number of veterinary examinations evenly spread over the treatment period (or tick season if efficacy duration exceeds tick season) should be defined.	Accepted. A respective proposal for products with long-term efficacy has been included.
311, 399	2	See above	Partly accepted.

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and 296			See above.
311	7	**Comment:** Weekly intervals of tick counts may not be feasible for long-acting products under field conditions with privately owned animals (e.g. owners will not comply with weekly visits). We should keep the identical rules as in the controlled laboratory efficacy studies (table in line 258) **Proposed change:** cross reference to counting intervals in table of line 258.	See above. A respective proposal has been included.
315 and 419	2	**Comments**: § 5.2.4. and 6.2.4. (Treatment) For flea field studies (line 419), the inclusion of a positive control group is recommended. Why not for field studies for ticks?	For ticks an information with regard to control groups has been introduced in section 5.2.1. Analogously for fleas the same information has been included in section 6.2.1. In consequence the mentioning of a positive control group for fleas in section 6.2.4. has been deleted.
326	2	See above	See above (representativeness of the laboratory strains for current the field situation).
After 327 (section)	7	**Comment:** Include in this section or somewhere in the guideline the possibility that mixed flea and tick infestations can be performed – this would reduce the number of animals required for studies and therefore would support the 3Rs. **Proposed change:** Add: "<u>When feasible, infestation with multiple species of tick and fleas is possible as this will reduce the number of animals required for studies.</u>"	Partly accepted. An infestation with multiple species of ticks and fleas is only considered acceptable for field studies since usually the infestation level is assumed to be lower compared to the required infestation level for each ectoparasite species in laboratory studies. Therefore, with regard to field studies the option to use animals with mixed infestations has been introduced.

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5.2.4	7	**Comment:** The inclusion of a control group is not stated here which is different to the respective section in the flea part 6.2.4. Why is there a difference in both sections? **Proposed change:** Please harmonize with section 6.2.4.	Accepted. Sections have been harmonised.
329 and 149	2	See above	See above (housing and allocation). No need for repetition here.
333 and 163	2	See above	Partly accepted. Please, see above (sedation of animals)
337 (section 6.1.3)		**Comment:** Please specify in the control group. **Proposed change:** Replace: "*Approximately 50% of these fleas should be present on the control animals at each timepoint following infestation*" with "Approximately 50% of these fleas should be present on the ~~control~~ animals at each timepoint in the control group (arithmetic mean) ~~following infestation".~~	Not accepted. Please, see argumentation above (ticks).
338	7	**Comments:** It should be acceptable to occasionally have intermediate time-points with less than 50% of presence in control animals (not applicable to the immediate efficacy time-point and last time-point). **Proposed change**: remove "at each time point"	Not accepted. The wording in the guideline allows for some flexibility. It is said "approximately 50%...".
343	2	**Comments**: § 6.1.4. The inclusion of a negative control group (without treatment) is regarded as necessary for studies with fleas and ticks; there is no mentioning of a positive control group (treated with a product of known efficacy). This would provide important information on	Not accepted. In laboratory studies a positive control group is not considered appropriate because this would not allow to assess whether the ectoparasites are vigorous or not. However, the vigour of ticks or fleas is important to reliably conclude on efficacy.

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		if the tested formulation is more efficacious compared to the one already on the market. **Proposed change:** Inclusion of a positive control group to the study should be considered.	
351	7	**Comment:** The use of the word “only” indicates an inferiority of systemic acting products compared to others (see also comment to 199). **Proposed change:** ticks, i.e. ~~only~~ a claim for immediate and/or persistent flea killing activity is justified.	Accepted.
Table between 357-358	7	**Comment:** The recommended counting intervals of 24 hours forbid the co-infestation of ticks and fleas which is in contrast to the 3 Rs principles that are mentioned in lines 96-98. Furthermore, the assessment of speed of kill (described from line 358 onwards) already covers the 24 hours counting interval and is sufficient to ensure that flea efficacy at 24 hours is demonstrated for a product. **Proposed change:** Please harmonize with the respective tick-section (Table between lines 241 and 242) to allow flea assessments up to 48 hours after treatment and 48 hours after re-infestation.	Not accepted. The stakeholder’s comment on the concept paper (Jan 2013) to this guideline was followed, i.e.: “Some clarification or review of the efficacy assessments for early time points might be useful. - Fleas: There is no scientific rationale to infest fleas 48 hours prior to treatment. Fleas start feeding rapidly and a Day -1 infestation is sufficient. In cats, due to their grooming behaviour, Day -2 infestation and Day 2 efficacy assessment (4 days in between) may already lead to very low counts in the controls. In addition, flea counts at 24 hours would be preferable, particularly in the context of avoiding flea bites and FAD prevention effect.” This recommendation was considered reasonable and,

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			therefore, accepted by the EWP. Apart from this it has to be considered that a co-infestation of ticks and fleas cannot be supported for laboratory studies because the required infestation level for each parasite species would not be compatible with animal welfare aspects. Therefore, the counting interval will not be changed. Besides this, it should be noted that speed of kill-studies are not mandatory.
358	7	**Comment:** The header is bold here whereas in the respective section in the tick part it is underlined. **Proposed change:** Please harmonise format.	Accepted.
363	7	**Comment:** The last part of the sentence is unclear. What is meant with “the range of time points should be mentioned”? **Proposed change:** Please re-phrase so that the intent is clearer.	Products should only be characterised with one figure for the parameter ‘speed of kill’ which is valid for the whole duration of the study. As the time periods evaluated for the speed of kill may be variable throughout the study period the range covering the period between the shortest and the longest time period until 90 %/95 % of ticks/fleas have been killed would be acceptable to be mentioned. Sentence rephrased accordingly.
365	7	**Comment:** Efficacy assessment is based on live fleas. **Proposed change:** At each assessment time selected all live ~~and killed~~ parasites should be counted.”	Accepted with modification.
366	7	**Comment:** The term “immediate killing effect” is an unnecessary repetition and causes confusion because the ‘immediate effect’ is already defined as efficacy on an existing infestation following treatment, please rephrase.	Accepted.

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		Proposed change: The speed of kill should be based on the ~~immediate~~ killing effect at the time of counting on the animals	
366-367	7	**Comment:** The delayed mortality is not mentioned in the tick part of the GL; this sentence should be deleted. **Proposed change:** "~~Delayed mortality should not be considered~~"	Not accepted. Originally the delayed mortality was considered more relevant for fleas, therefore, it was not mentioned in the tick section. With regard to fleas the experience with studies on delayed mortality have shown unreliable results (highly variable viability of untreated control fleas under in vitro conditions). That is the reason why delayed mortality is not considered a reliable parameter. To be complete a respective information with regard to 'delayed mortality' has now been included in the tick part as well.
370-373	7	**Comment:** Why should the time of speed of kill be moved to section 5.1 of SPC? We are unable to see an improvement with regards to efficacy assessment **Proposed change:** Remove this sentence.	Not accepted. The speed of kill characterises a pharmacological property of the product and does not fit in the clinical section of the SPC.
373	7	**Comment:** At the end of the sentence it should read "SPC and product literature" (consistency to line 256). **Proposed Change:** The onset of kill activity after application of the product, meaning a kill activity below the threshold of 95% is considered not to be clinically relevant and such information should not be included in the SPC and product literature.	The term product literature includes the SPC. Therefore, no change necessary at this place. Line 272 was corrected accordingly.
380-385	7	**Comment:** The justification for using exclusively arithmetic means seems to lack scientific justification. Expressing that geometric means are not relevant even if the count data are skewed seems to be odd. **Proposed change:** In cases in which the distribution	Not accepted. See above.

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		is skewed the use of geometric mean must be allowed	
386 and 278	2	See above	With regard to fleas the efficacy threshold of at least 95% has not been changed compared to the currently valid guideline. This threshold corresponds to 'approximately 100%' according to the general guideline from 1994. However, a constant level of efficacy of 100 %, which would be the ideal case, is an unrealistic approach in a biological system. No need for a change. See also page 37/63.
394-395	6	**Comment**: same comment as for comment for lines 296-297 (see above) **Proposed change**: same proposal as for lines 296-297 (see above)	Accepted (to add climatic regions).
394-398	7	**Comment:** The inclusion of a control group is missing here and would not allow testing of efficacy in ticks and fleas in one field study (3 R principles). **Proposed change** Please add a control group to harmonize with the respective tick section.	Accepted.
397	7	**Comment:** Almost all existing products and generics have a statement "aid in control", so that the revised guidance would create an unfair negative balance to innovation from now. A regulatory threshold of 95% is as a matter of fact a straightforward guarantee to act against FAD. **Proposed change:** Field data is needed to support claims related; when not provided, a statement on the section of SPC 5.1 is accepted.	Not accepted. It is necessary to include FAD cases to corroborate the performance of a new product under field conditions.
399, 311	2	See above	Partly accepted.

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and 296			See above (*home environment, larvae and nymphs, blood sampling*)
400	3	**Comment**: The experimental unit should be the household in flea field studies to avoid bias. **Proposed change:** "The study should include *households*, *where at least one target species* animal is confirmed to be infested with fleas by an appropriately qualified person who should record the initial level of infestation.	Accepted.
400	6	**Comment**: The experimental unit should be the household in flea field studies to avoid bias. **Proposed change**: "The study should include *households*, *where at least one target species* animal is confirmed to be infested with fleas by an appropriately qualified person who should record the initial level of infestation.	Accepted.
401-402	6	**Comment**: same comment as for comment for lines 303-304 (see above) **Proposed change**: same proposal as for lines 303-304 (see above)	Accepted. (reg. sample size)
404	3	Comment: it should be considered that not only previous treatments may interfere, but also treatments to other animals in a household. If animals are treated with different local treatments, there may be cross-over of pharmaceutical actives from one animal to	In principle accepted. The sentence reads now "It should be ensured that included animals or other animals within the same household have not been treated with an ectoparasitic substance within a

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		another but also influence of such active on the efficacy and safety. **Proposed change:** It should be ensured that there is no impact of previous treatment or treatment of other animals with the same or other ectoparasitic substance on the study outcome. If necessary, treatment of all animals of the target species in a household with the same product is the preferred option to avoid any bias.	timeframe that might impact on the study outcome."
404	6	**Comment**: it should be considered that not only previous treatments may interfere, but also treatments to other animals in a household. If animals are treated with different local treatments, there may be cross-over of pharmaceutical actives from one animal to another but also influence of such active on the efficacy and safety. **Proposed change**: It should be ensured that there is no impact of previous treatment or treatment of other animals with the same or other ectoparasitic substance on the study outcome. If necessary, treatment of all animals of the target species in a household with the same product is the preferred option to avoid any bias.	Accepted.
408	3	Comment: In flea field studies, the infestation with fleas is not limited to one animal, but to all animals in a household. Therefore, the household (one animal as the representative unit) represents the experimental unit. To assure animal welfare, all other animals in	Accepted.

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		such household should receive appropriate treatment and should be observed for field safety. See also WAAVP guideline on this topic. **Proposed change:** add the above mentioned sentence to this paragraph.	
408	6	**Comment**: In flea field studies, the infestation with fleas is not limited to one animal, but to all animals in a household. In this case, the household (one animal as the representative unit) represents the experimental unit. To assure animal welfare, all other animals in such household should receive appropriate treatment and should be observed for field safety. See also WAAVP guideline on this topic. **Proposed change**: add the above mentioned sentence to this paragraph.	Accepted.
410	7	**Comment:** For counting intervals see the comment for tick studies, line 311	Accepted.
419 and 315	2	See above	For ticks and fleas an information with regard to control groups has been introduced in section 5.2.1. resp. 6.2.1. In consequence the mentioning of a positive control group for fleas in section 6.2.4. has been deleted. Thus, the respective sections for ticks and fleas are in accordance now.
421	2	**Comments**: § 6.3. IGRs against fleas Here the authors of the guideline state that IGRs could	Comment noted. No change to the guideline text.

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		also affect ticks, but are not considered suitable. However, larval and nymph stages must also consume blood, and if exposed to IGRs that hamper their development, this could lead to a reduction in the overall tick burden in the environment (see also Microsc. Res Tech. 2013 Nov; 76 (11): 1177-85.). One could imagine that at least for *Rhipicephalus* with a rather short time span to complete its life cycle and the ability to do this indoors, this could be relevant. However, this is rather speculative and not clear whether this has been shown in real life or not.	
421	3	**Comment**: it would be valuable to give more precise indication of an appropriate product used in the control group. **Proposed change:** It is recommended to include a positive control group that has the same claim as the intended claim for the IVP, is registered in the EU, is applied via the same route of administration and has a similar mode of action, wherever possible.	The sentence with regard to a positive control group has been deleted in section 6.2.4.. A respective information has now been included in section 6.2.1. More details with regard to a positive control are given in the definitions section.
421	6	**Comment**: it would be valuable to give more precise indication of an appropriate product used in the control group. **Proposed change**: It is recommended to include a positive control group that has the same claim as the intended claim for the IVP, is registered in the EU, is applied via the same route of administration and has a	See above.

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		similar mode of action, if possible.	
429	7	**Comment:** Should be singular **Proposed Change** 6.3.1. Specific laboratory stud~~ies~~**y** recommendations for IGRs	Accepted.
434-436	7	**Comment:** It might be very well the case that activity is not related to contact but via other ways. **Proposed change:** Please add: "<u>If scientifically justified, other methods might be applied".</u>	In principle accepted. The wording "via contact" has been deleted to allow for other ways of acting. Thus, the proposed sentence is not considered necessary.
477-478	7	**Comment:** It makes sense to also assess immediate efficacy for IGR. **Proposed change:** Please add possibility of infestation on Day -2 and egg collection on Day 0 after treatment and before the next infestation on Day 1.	Not accepted. Other approaches than those given in the guideline may be described and justified by the applicant. However, no addition of further details to the current wording is considered necessary.
495	5	"However, as many factors can influence the development of fleas under such conditions, an infested untreated group should be included in each study for control, kept under the same environmental conditions as the treatment group." How long are the untreated animals left with the infestation for? Some of the efficacy studies can last for months. Can the group size be reduced? Is it really necessary to always have this control? A workshop could identify if this is possible.	A negative control group is necessary to assess the vigour of the fleas. This is important for efficacy evaluation. Furthermore, to account for possible variability within the control group a certain minimum number of control animals is necessary to come to valid results (from a statistical point of view). The negative control group (infested, not treated) is left with the infestation in correspondence to the group treated with the test product. For IGR alone: weekly re-infestation. For combinations of adulticide and IGR: Re-infestations should be carried out at the end of the persistent efficacy of the

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			adulticide.
501-503	5	"[…] actively reproducing fleas are transferred from untreated animals (donor animals) to animals treated with the test product and to untreated control animals." Can recommendations be made as to the number and source of donor animals such to reduce the number of animals used overall?	The number of donor animals will depend 1) on the number of unfed fleas with which the donor animals will be infested, 2) on the number of healthy appearing fleas (motility, viability check), 3) on the group size of treated and untreated study animals and 4) on the intended number of fleas to be transferred to each study animal. The procedure of producing actively reproducing fleas and their transfer to study animals will be performed in defined intervals until the end of the claimed persistent efficacy of a product. These are reasons why no exact number of donor animals can be recommended.
527	7	**Comment:** It will be very difficult to find dog/cat owners willing to participate in a field study that includes a negative control group. **Proposed change:** Please change the sentence to: "Inclusion of a ~~negative~~ positive control group is recommended."	Not accepted. It is acknowledged that it might be difficult to get acceptance for a negative control group in a field study. However, a negative control group is considered important for the validity of the study in order to see any effect in case of a mono-preparation (*effect rather late, no efficacy against adult fleas*). Therefore, the guideline text will not be changed.
550-552	6	**Comment**: This section on requirements for generic ectoparasitical products describes the option of conducting combined studies (fleas and ticks) in the same study. It is not clear whether this paragraph refers to laboratory (artificial infestation) studies or field studies. If the description refers to field studies,	Accepted. For generic products, dose confirmation studies should be performed under laboratory conditions. Respective information is included now. Conducting combined studies under laboratory conditions has been reconsidered. Such studies are not recommended,

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		combined field studies (fleas and ticks) should be acceptable not only for generics but also for novel compounds. This should be clarified in the present guideline as it is not clear if field studies evaluating both ticks and fleas simultaneously are acceptable. **Proposed change**: Clarification required	because the resulting infestation level would not be compatible with animal welfare aspects. Under laboratory conditions the required infestation level per parasite species is necessary to get meaningful results and can, therefore, not be reduced.
565	3	**Comment**: we consider that in case an applicant is able to provide two dose confirmation studies showing a longer persistent efficacy than the reference product, that an additional field study does not provide further value in regard to the persistent efficacy claim. We therefore propose to grant such claim based on two additional studies, irrespective of being a 2 dose confirmation studies or one DC and one field study. However, in case an additional claim shall be made for Flea Allergic Dermatitis, we consider that a field study should be mandatory. **Proposed change:** adapt text accordingly	Partly accepted. The comment with regard to FAD is considered acceptable and a respective wording has been introduced into the guideline. A longer persistent efficacy than the reference product is a new claim which has to be proven according to requirements for products with a full application. Text remains unchanged in this respect.
565	6	**Comment**: we consider that in case an applicant is able to provide two dose confirmation studies showing a longer persistent efficacy than the reference product, that an additional field study does not provide further value in regard to the persistent efficacy. We therefore propose to grant such claim based on two studies,	Partly accepted. See above.

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		without confirmation of an additional field study. This follows the RRR recommendations. **Proposed change**: delete the words “and adequate field study”	
584	3	Comment: we think that in case that any of the mentioned criteria are different, that a field study is also needed. **Proposed change:** [dose confirmation and field] studies will be required.	Partially accepted. The sentence has been modified: “In case there is a difference in the qualitative or quantitative composition of the excipients which may affect absorption, the rate and extent of distribution and persistence of the active substance, further studies, e.g. dose confirmation and/or field studies, may be necessary.”
584	6	**Comment**: we think that in case that any of the mentioned criteria are different, then a field study is also needed. Proposed change: [dose confirmation and field] studies will be required.	See above.
	5	**References:** Cadiergues at al. (2000). First bloodmeal of Ctenocephalides felis felis (Siphonaptera: Pulicidae) on cats: time to initiation and duration of feeding. Journal of Medical Entomology, 37(4): 634-636. Centers for Disease Control and Prevention. (2015). Life cycle of hard ticks that spread disease. Last	

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		updated on 1 June 2015: http://www.cdc.gov/ticks/life_cycle_and_hosts.html Chagas et al. (2014). In vitro and in vivo acaricide action of juvenoid analogs produced from the chemical modification of Cymbopogan spp. ad Corymia citriodora essential oil on the cattle tick Rhipicephalus (Boophilus) microplus. Veterinary Parasitology, 205(1-2): 227-284. Marchiondo et al. (2013). World Association for the Advancement of Veterinary Parasitology (WAAVP) second edition: Guidelines for evaluating the efficacy of parasiticides for the treatment, prevention and control of flea and tick infestations on dogs and cats. Veterinary Parasitology, 194: 84-97. McKellar 2004. Pharmacokinetic/pharmacodynamics integration in drug development and dosage-regimen optimization for veterinary medicine. AAPS PharmSci, 4 (4): Article 38. Parvenn et al. (2014). In vitro evaluation of ethanolic extracts of Ageratum conyzoides and Artemesia absinthium against Cattle Tick, Rhipicephalus microplus. Scientific World Journal, doi:	

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		10.1155/2014/858973. Toutin et al, 2001. A pharmacokinetic/pharmacodynamics approach vs. a dose titration for the determination of a dosage regimen: the case of nimesulide, a Cox-2 selective nonsteroidal anti-inflammatory drug in the dog. Therap. 24, 43-55. Toutain, 2002. Pharmacokinetic/pharmacodynamics integration in drug development and dosage-regimen optimization for veterinary medicine. (2002). AAPS PharmSci, 4 (4): Article 38. Walker et al. (2012). A less stressful alternative to oral gavage for pharmacological and toxicological studies in mice. Toxicology and Applied Pharmacology, 260: 65-69. Williams et al. (2014). Fluralaner, a novel isoxazoline, prevents flea (Ctenocephalides felis) reproduction in vitro and in a simulated home environment. Parasites & Vectors, 7: 275.	
588	7	**Comment:** This sentence also needs to refer to repellency	Accepted.

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		Proposed Change: Persistent efficacy: Refers to active substances with repellent/acaricidal/insecticidal activity for an extended period of time after treatment.	
597	7	**Comment:** The sentence In fleas, the effect is usually very rapid, and no specific studies are required to prove the repellent effect. is confusing. There is no section in the document that addresses flea repellency and we are not aware that any product currently on the market claims flea repellency. We would strongly suggest deleting this sentence for consistency reasons with current products on the market. **Proposed Change:** ~~In fleas, the effect is usually very rapid, and no specific studies are required to prove the repellent effect.~~	Accepted.
603	7	**Comment:** The term “immediate killing effect” is an unnecessary repetition and causes confusion because the ‘immediate effect’ is already defined as efficacy on an existing infestation following treatment regarded as overdone, please rephrase. **Proposed change:** respectively, based on the ~~immediate~~ killing effect	Accepted.