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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

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This guideline replaces the 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).

Keywords	***Ectoparasitic veterinary medicinal product, tick, flea, dog, cat***

*The current revision consists of administrative changes made in order to align the guideline to the new definitions and terminology provided by Article 4 of Regulation (EU) 2019/6. The references to the legislation applicable and other scientific guidelines have also been updated. As no changes were made to the scientific content, no concept paper and no public consultation were deemed necessary.

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000 An agency of the European Union

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

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Executive summary

This guideline is intended as an addition to the guideline on the "Demonstration of efficacy of ectoparasiticides" dealing with general requirements for the assessment of efficacy of such products.

It provides specific guidance with respect to the testing and evaluation of efficacy of veterinary antiparasitic products for the treatment and prevention of tick and flea infestations in dogs and cats.

1. Introduction (background)

This guideline should be read in conjunction with the guideline "Demonstration of efficacy of ectoparasiticides", which provides guidance for general requirements for the assessment of efficacy of an ectoparasiticide preparation (Vol. 7AE17a, 1994). The aim of this guideline is to be more detailed for certain specific issues which are not addressed in the context of the general requirements.

2. Scope

This guideline provides specific guidance with respect to the testing and evaluation of efficacy of veterinary antiparasitic products that are intended for the treatment and prevention of tick and flea infestations in dogs and cats, and includes information for the testing of veterinary systemically and locally acting antiparasitic products and products containing substances with insect growth regulating properties (IGRs), either as mono-preparations or in combination with an adulticide. In addition, information for generic products is provided. It should be noted that this document does not give advice on how to design studies for the demonstration of efficacy in the prevention of transmission of vector-borne diseases.

3. Legal basis

This guideline should be read also together with Regulation (EU) 2019/6 and VICH GL9 Guideline on Good Clinical Practices (CVMP/VICH/595/98). In addition, the guideline on statistical principles for clinical trials for veterinary medicinal products (pharmaceuticals) (EMA/CVMP/EWP/81976/2010) should be considered unless otherwise stated. Furthermore, where applicable, the guideline on pharmaceutical fixed combination products (EMA/CVMP/83804/2005) and supplemental documents (Questions and answers on the CVMP guideline on pharmaceutical fixed combination products – EMA/CVMP/EWP/325284/2011) should be taken into account.

In accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes and Directive 2010/63/EU on protection of animals used for scientific purposes, the 3R principles (replacement, reduction and refinement) should be applied.

4. Data requirements

In principle, the demonstration of efficacy includes the following test phases:

- Description of the mode of action
- Determination of dose and dosing interval(s)
- Dose confirmation studies, including persistent efficacy testing, where applicable
- Clinical trials

Two types of studies should be performed: pre-clinical studies to establish immediate and persistent efficacy of a product, depending on the claim, and clinical trials to confirm the results of pre-clinical studies.

It is recommended to conduct pre-clinical studies according to Good Laboratory Practice (GLP). Good Clinical Practice (GCP) is also acceptable. Clinical trials shall be conducted in accordance with established principles of Good Clinical Practice (GCP), unless otherwise justified. In case GLP and/or GCP is not applied, traceability and integrity of data should be adequately guaranteed by other means.

4.1. Ectoparasite species

The choice of tick and flea species to be tested depends on their epidemiological status in the European Member State where the veterinary medicinal product is intended for marketing.

Most relevant tick and flea species in dogs and cats in Europe:

Ticks:

- *Dermacentor reticulatus*
- *Ixodes hexagonus*
- *Ixodes ricinus*
- *Rhipicephalus sanguineus*

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. In principle, the same standards/quality of data as outlined in this guideline would apply for documentation supporting such claims. However, depending on the tick species, deviations from the recommendations set out in this guideline and in other relevant EU guidance documents may be acceptable, if sufficiently justified. Reference to non-autochthonous species may only be made in the SPC and package leaflet if the efficacy has been reliably shown.

Fleas:

- *Ctenocephalides canis*
- *Ctenocephalides felis*

5. Study design for testing the efficacy of products for the treatment and prevention of tick infestation

Studies for each tick species and each stage of the life cycle against which efficacy is claimed should be provided. The applicant should justify the type of studies (*in vitro* and *in vivo* pre-clinical studies and clinical trials) for each parasite species and stage.

In view of the difficulties of experimental infestation studies in cats, results of pre-clinical studies in dogs to establish the efficacy in the treatment and prevention of tick infestations may be extrapolated to cats if scientifically justified. However, one dose confirmation study in cats for each claimed parasite species should be performed.

5.1. Pre-clinical studies

5.1.1. Tick species

For demonstration of the efficacy *in vivo* it will be sufficient to perform testing in adult ticks only since, in general, larvae and nymphs have a higher susceptibility. Nevertheless, the higher drug-sensitivity of larvae and nymphs of a claimed tick species should be confirmed through *in vitro* evaluation before starting *in vivo* experiments, unless it can be demonstrated by bibliographic data.

It is recommended to use different laboratory tick isolates, which are genetically enriched with parasites from field isolates about every 6 years, or tick species from recent field collections, which are multiplied in the laboratory for at least 2 generations. Such strains would be representative of the current field situation. For animal welfare reasons, the ticks used should be free of transmittable pathogens.

5.1.2. Selection of animals

The choice of experimental animals should be justified by the applicant. It is desirable to have animals of a breed characterised by a fur of moderate hair length, so that the ticks are offered a chance of penetrating through the hair and being retained on the animals. For reasons of good parasite detection on the skin, the skin pigmentation should be considered in the selection process, too. It should be ensured that included animals have not been treated with an ectoparasitic substance within a timeframe that might impact the study outcome. Animals should be tested for their ability to carry adequate numbers of parasites prior to the start of the study.

5.1.3. Housing and allocation

The housing conditions should be selected in careful consideration of animal welfare aspects implying that prolonged isolation should be avoided as far as possible. 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 (e.g. up to 96 hours at the beginning of the trial from day -2 to day 2, and up to 48 hours after subsequent challenge infestations). In order to reduce stress, enrichment of the environment should be considered.

However, for the other time periods, housing of animals in groups should be considered (treated and control animals separately) with sufficient space according to animal species. All in all it should be ensured that the housing conditions do not adversely affect the integrity of the study.

It is recommended to include at least 6 animals per treatment/control group.

5.1.4. Tick infestation

The infestation level should be approximately 50 unfed adult ticks (approximately sex ratio of 50% male: 50% females, except for *Ixodes* spp. with a sex ratio of for example approximately 10% males: 90% females) and of very similar age per test animal and infestation time point. Twenty five to fifty percent (i.e. 12-25 ticks) of these ticks should attach to each animal at each time point following infestation in the control group. The number of live attached and dead attached ticks should be provided per animal in order to evaluate the adequacy of infestation at the individual animal level in the control group. This demonstrates that the tick population used is vigorous. It should be considered that the specific grooming behaviour of cats may have an impact on the efficacy assessment.

Ticks are applied at one or more sites of the animal's body to allow them to distribute over the animal. For this procedure, the animals should be kept calm for at least 30 minutes, if possible (e.g. by mild sedation) so that the ticks can attach firmly to the fur without being removed by the animal. It has to be ensured that the sedative used for calming down the animals does not interfere with the experiment/objectives of the study. Especially with regard to topically applied products for studying repellency it should be observed that the induced infestation should not be performed near the application site of the test product. The applicant should describe the infestation method. Alternative ways of infestation may also be acceptable if justified.

5.1.5. Criteria of efficacy

5.1.5.1. Acaricidal effect

For systemically or locally acting products with acaricidal properties efficacy evaluation is based on the differentiation between live and dead ticks.

It is recommended to assess the acaricidal effect according to the following parameters:

General findings	**Attachment status**	**Acaricidal effect**
live	free*	no
live	Attached	no
dead	free	yes
dead	attached	yes

* For systemically acting acaricides, live free ticks should be recorded but counts may be excluded from the efficacy calculation.

The number of ticks per category (free live, free dead, live attached and dead attached ticks) for each animal should be recorded giving information about the effect of the product with or without attachment.

While the demonstration of an immediate acaricidal efficacy is the precondition for the indication *treatment of* existing *tick infestations* the following should be considered with regard to persistent acaricidal efficacy:

Locally acting products

For locally acting products with a pure acaricidal effect where persistent acaricidal efficacy against new infestations is shown, the indication *prevention of tick re-infestation through an acaricidal effect* further specified by the period of time proven (see table under 5.1.6.1.) is acceptable.

In addition, for this type of product a general note should be included in the SPC (section 3.4) and package leaflet that *ticks would be killed and fall off the host within 24 to 48 hours after infestation without having had a blood meal*, *as shown in dose confirmation studies*, but that *an attachment of single ticks after treatment cannot be excluded.* Product specific time periods can be included if the speed of kill is sufficiently demonstrated (see 5.1.6.1.). It may also be reflected in the product literature that *a transmission of infectious diseases by ticks cannot be excluded*, unless demonstrated through appropriate studies on the prevention of transmission of infectious diseases by ticks.

Systemically acting products

For systemically acting products where acaricidal efficacy depends on the attachment of ticks to the host and the ingestion of a toxic dose of the active substance(s), a claim for *immediate and/or persistent tick killing activity* further specified by the period of time proven is justified. In addition, information should be included in the product literature (SPC section 3.2 - "indications") that the ticks must attach to the host and commence feeding in order to be exposed to the active substance. Furthermore, under such conditions the transmission of tick-borne diseases cannot be excluded. Consequently, unless demonstrated through appropriate studies on the prevention of transmission of specific vector-borne diseases, reference should be made in the product literature that *a transmission of infectious diseases by ticks cannot be excluded since ticks have to attach to the host to achieve the acaricidal effect*. As far as based on study results, a further note may address that *due to the necessary attachment of the ticks to the host other effects like skin irritation, skin damage, wounds, allergic or toxic reactions may occur*, as appropriate.

5.1.5.2. Repellent effect

In addition to acaricidal activity, some substances might also exhibit repellent properties. In general a repellent effect means that ectoparasites will avoid the contact with the treated animal. In crawling arthropods like ticks, however, various reactions may be caused by substances with repellent effects depending on the nature of the product.

A strict repellent effect *("sensu stricto")* is characterized by an irritant effect on the tick (e.g. "hot foot" effect) which causes the tick to move away from the treated animals, falling off soon after the contact with the host's coat, usually within 6 – 8 hours. In the subsequent time period up to 24 hours following the initial infestation, other effects like inhibition of attachment of new infesting ticks or disruption of attachment of ticks which are in the attachment process (detachment) may occur, which can be subsumed under the definition of repellency in the wider sense (*"sensu lato")*. For such products the indication *prevention of tick infestation through a repellent effect* further specified by the period of time proven (see table in 5.1.6.2) is considered acceptable.

In support of a repellency claim, ideally no ticks (see 5.1.7) should be on a treated animal after 24 hours following administration of the product. Thus, evaluation of a repellent effect should be based on the presence of live and dead ticks (attached, free) on the animal.

If an earlier repellent effect e.g. "hot foot" effect is claimed, suitable time points for demonstrating repellency may be chosen accordingly within the 24 hour period of time after administration of the product.

If single ticks in the treatment group would attach within a period up to 24 hours following infestation, reference to this should be made in the SPC and package leaflet as appropriate.

5.1.6. Efficacy testing

Products with acaricidal and repellent properties may demonstrate respective immediate effects and/or short term (up to 4 weeks) or long term (more than 4 weeks) persistent effects. Efficacy should be established at intervals throughout the period of effect claimed. The applicant should justify the methods used for the assessment of efficacy. It is recommended that tick counts are made by comb counting or by palpating the animal and by visual assessment, as appropriate. Ticks should be removed from test animals upon completion of the counting procedure. For the assessment of efficacy under laboratory conditions the inclusion of untreated animals (negative control group) is considered necessary.

5.1.6.1. Acaricides

For **acaricides** the following time schedule is recommended:

Prior to Day -2:	Examination of tick strain for infestation rate and suitability of test animals by a pre-allocation infestation: The animals should be infested to assess the ability to maintain a tick population. The tick counts should be used to rank order the animals from highest to lowest tick counts and randomly allocate them to blocks so that each group has equal numbers of animals that are able to maintain high to low numbers of ticks. Animals with a very low number of ticks should be excluded.
Day -2:	Tick infestation
Day 0:	Administration of test substance.
Immediate efficacy	Efficacy testing in situ according to the parameters given under 5.1 at day 0 up to 48 h or longer if appropriate (e.g. collars).
Short-term persistent efficacy	Preparations with a claimed persistent efficacy for up to 4 weeks, e.g. shampoo, spray, spot on, tablets: Weekly infestation of ticks, efficacy testing *in situ* up to 48 h following each challenge as described above.
Long-term persistent efficacy Last month of the period of effectiveness claimed:	Preparations with a claimed persistent efficacy for more than 4 weeks, e.g. collars, tablets: Tick infestation every 4 weeks over the period of effectiveness claimed, efficacy testing *in situ* up to 48 h after each challenge as described above. * For reasons of decreasing efficacy, infestation every 2 weeks may be considered.

** Where effectiveness over several months is claimed it should be taken into account that a too frequent application of ticks may induce an individually varying immunity to ticks in the test animal. In turn, this may adversely affect the infestation rate.*

Speed of kill

Speed of kill-studies are only required if a claim is made for speed of kill of less than 48 hours. The speed of kill is the time point when at least 90% of ticks have been killed based on counts in both control and treated groups (see 5.1.7). It should be studied within 48-hours after the first administration of the product and after each re-infestation. The speed of kill should be studied for the whole period of claimed persistent effect, i.e. the last assessment should be performed after the last challenge. Within the 48-hour period the same pattern of selected time points should always be used throughout the study.

At each assessment time point selected all live parasites should be counted. The speed of kill should be based on the killing effect at the time of counting on the animals. Delayed mortality should not be considered.

Animals should be selected in such a way that dosing as close as possible to the minimum recommended dose is possible. All assessments should be performed in comparison with an untreated control group.

Veterinary medicinal products may only be characterised with one time period for the parameter 'speed of kill'. If the resulting speed of kill-time points are variable throughout the duration of the study (covering the period of claimed persistent effect), the speed of kill should be indicated by the range covering the shortest and longest time period until at least 90 % of the ticks have been killed.

Respective information addressing the point of time of the speed of kill should be given in section 4.2 of the SPC (pharmacodynamics). The start of kill activity after application 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 product literature.

5.1.6.2. Repellents

For **repellents** the following time schedule is recommended:

Prior to Day -2:	Examination of tick strain for infestation rate and suitability of test animals (see 5.1.6.1).
Day 0:	Application of test substance.
Day 0 + 24 h *	Tick infestation.
Immediate efficacy	Efficacy testing *in situ* up to 24 hours after challenge according to the parameters given under 5.1.5.2. Depending on the nature of the product early time points within the given 24 hour-period may be selected.
Short-term persistent efficacy	Preparations with a claimed persistent efficacy for up to 4 weeks, e.g. shampoo, spray, spot on: Weekly infestation of ticks, efficacy testing *in situ* up to 24 h following each challenge as described above.
Long-term persistent efficacy	Tick infestation at 4-week intervals over the period of effectiveness claimed and efficacy testing *in situ* up to 24 h after challenge (see also above). **
Last month of the period of effectiveness claimed:	For reasons of decreasing efficacy, infestation every 2 weeks may be considered.

** The period of time required for distribution of the active substance may vary depending on the product formulation and may be longer.*

*** Where effectiveness over several months is claimed it should be taken into account that a too frequent application of ticks may induce an individually varying immunity to ticks in the test animal. In turn, this may adversely affect the infestation rate*.

5.1.7. Evaluation of efficacy

For calculation of acaricidal or repellent efficacy (%), the following formula (according to Abbott's formula)[1] is recommended:

Efficacy (%) = 100 x (m_C – m_T)/m_C

For acaricidal efficacy:

Control group (mc): Mean number of live ticks (attached, free) on the host animals

Treatment group* (m_T): Mean number of live ticks (attached, free) on the host animals

**For the systemically acting product, live free ticks may not be considered for efficacy evaluation*

For repellent efficacy:

Control group (mc): Mean number of live ticks (attached, free) on the host animals

Treatment group (m_T): Mean number of live and dead ticks (attached, free) on the host animals

In case of controlled studies (i.e. pre-clinical studies for dose determination and dose confirmation) calculation of efficacy should be based on the arithmetic mean – irrespective of whether the count data are skewed or not – since efficacy estimates based on geometric means tend to be larger, might potentially mask treatment failures, and carry the risk of misinterpretation of results. Efficacy calculation based on geometric mean may also be reported. Geometric mean calculations will, however, not be decisive for efficacy assessment in this type of study.

The acaricidal efficacy of the proposed product should be at least 90% at each counting during the claimed efficacy period. The same efficacy threshold is valid for studying the speed of kill. Regarding repellency the efficacy of the proposed product should be at least 95% at each counting. In any case the difference in counts between treated and untreated animals must be statistically significant at a level of 5 %.

5.1.8. Testing for water stability

For products intended for external use, the water stability of the formulation intended for marketing should be demonstrated, especially for products with a claimed duration of efficacy for 2 or more weeks. The impact of exposure to water e.g. through shampooing, swimming, rainwater on the acaricidal/repellent effect should be evaluated at regular intervals (e.g. once a week). The conditions and duration of exposure to water should be justified. Alternatively, data on the concentration time course of the active substance in the fur after single/repeated washing after treatment can be provided. If the water stability of the product intended for marketing could not be demonstrated, or data are not available, the warning should always be included in the SPC and package leaflet to avoid

[1] W.S.Abbott (1987)
Abbott's Formula - A Method of Computing the Effectiveness of an Insecticide
Journal of The American Mosquito Control Association, Vol.3, 2, 302-303

frequent swimming or shampooing the animal, or to remove an antiparasitic collar beforehand because the maintenance of effectiveness of the product in these cases has not been tested.

5.2. Clinical trials

5.2.1. General

Clinical trials should take place when the relevant tick species are abundant and should be performed in at least two different geographic and climatic regions. Animals with mono- or mixed infestations with ticks and fleas are eligible for inclusion. Depending on the trial design a positive or negative control group should be included. Clinical trials should be performed for each target animal species (dog/cat) claimed.

5.2.2. Selection of animals

The trial should include animals confirmed to be infested with ticks by an appropriately qualified person who should record the initial level of infestation. The tick species should be identified. The tick species included in the list of indications should be represented among the included animals, whenever possible.

The number of animals (sample size) required in the trial should be statistically justified in advance and based on the hypothesis tested (e.g. superiority design versus non-inferiority) and consider both, efficacy and the safety aspects of the trial. The animals should belong to a variety of breeds of different hair length and to different husbandries. Furthermore, animals exposed to a high risk of infestation (e.g. hunting dogs) should be included, if possible. It should be ensured that included animals or other animals within the same household have not been treated with an ectoparasitic substance within a timeframe that might impact on the trial 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. With regard to efficacy only one predefined animal as representative unit per household should be included in the analysis, however, with regard to safety aspects all treated animals should be considered.

The owners of the animals should be advised not to treat the home environment during the duration of the trial (especially with regard to *R. sanguineus*). When a non-inferiority evaluation is planned it should be ensured that the infestation rate is large enough in the test and the positive control group to obtain sufficient assay sensitivity.

5.2.3. Counting

Counts should be undertaken at weekly intervals and the tick species should be identified. For products with a claimed persistent efficacy of more than 4 weeks the intervals for tick counting may be extended (corresponding to those in controlled pre-clinical studies, i.e. intervals of 4 weeks and in the last month a 2-week interval). For topically used products which are locally acting the distal parts of the body like paws and tail but also the inner thighs should be carefully considered.

5.2.4. Treatment

The final formulation intended for marketing should be used at the recommended dose and route. Any deviation should be justified by the applicant.

6. Study design for testing the efficacy of products for the treatment and prevention of flea infestation

Both pre-clinical studies and clinical trials should be performed for each target animal species claimed (dog/cat).

6.1. Pre-clinical studies

6.1.1. Flea species

Pre-clinical studies for each flea species and each stage of the life-cycle against which efficacy is claimed should be provided. The type of studies (*in vitro* and *in vivo* pre-clinical studies) for each species and stage should be justified. The flea strains used for pre-clinical studies should be representative of the current field situation. If the pre-clinical studies have included the flea species commonly identified on the host species, then specification of fleas is not usually required in the clinical trials.

6.1.2. Housing and allocation of test animals

In principle the same conditions apply as given for the pre-clinical studies with ticks under 5.1.2 and 5.1.3. Regarding the necessary periods for individual accommodation, the respective time period from the day of infestation with fleas until the day of counting should be considered.

6.1.3. Flea infestation

Studies to support claims for the treatment of flea infestations

It is recommended to infest the test animals with 50-100 unfed adult fleas of very similar age for each infestation. Each animal should be infested with the same number of fleas. The applicant should describe the infestation method. Fleas should be distributed over the entire host animal at the time of treatment. Approximately 50% of these fleas should be present on each control animal at each time point following infestation.

Studies to support claims for the prevention of flea re-infestations

Depending on the specific nature of the claim, alternative study designs may be applicable, for example, using environments able to support flea infestations (e.g. simulated home environmental studies (SHE)). The applicant should justify the choice of study design.

6.1.4. Testing for efficacy

Insecticidal products may demonstrate immediate insecticidal effects and/or short term (up to 4 weeks) or long term (more than 4 weeks) persistent effects.

Demonstration of an immediate insecticidal efficacy is the precondition for the indication *treatment of* existing *flea infestations*. For locally acting products where persistent insecticidal efficacy against re-infestations with fleas is shown, the indication *prevention of re-infestation with fleas through insecticidal effect* further specified by the proven period of persistent efficacy is acceptable. In consequence, efficacy should be established at intervals throughout the claimed time. With regard to systemically acting products, in principal the same applies as indicated for ticks, i.e. a claim for immediate and/or persistent flea killing activity is justified.

The applicant should justify the methods used for assessment of efficacy and the time from treatment to assessment of efficacy. It is recommended to count fleas by combing by trained personnel according to a reliable standard procedure. For the assessment of efficacy under laboratory conditions the inclusion of untreated animals (negative control group) is considered necessary.

The following time schedule is recommended for an adulticidal compound:

Prior to day -1	The animals should be infested to assess the ability of animals to maintain a flea population. The flea counts should be used to rank order the animals from highest to lowest flea counts and randomly allocate them to blocks so that each treatment group has equal numbers of animals that are able to maintain high to low numbers of fleas.
Day -1*:*	Flea infestation.
Day 0:	Administration of test substance.
Immediate efficacy	Efficacy testing with a recognised method, e.g. counting by combing, at day 0 up to 24 h following treatment or longer, if appropriate (e.g. collars).
Short-term persistent efficacy	Preparations with a claimed persistent efficacy for up to 4 weeks. Weekly infestation, efficacy testing up to 24 h following each challenge.
Long-term persistent efficacy Last month of period of effectiveness claimed:	Preparations with a claimed persistent efficacy for more than 4 weeks. Flea infestation every 4 weeks over the period of effectiveness claimed, efficacy testing up to 24 h after each challenge. For reasons of decreasing efficacy, it may be considered to infest the animals every 2 weeks.

Speed of kill

Speed of kill-studies are only required if a claim is made for speed of kill of less than 24 hours. The speed of kill is the time point when at least 95% of fleas have been killed based on counts in both control and treated groups. It should be studied within 24 hours after the first administration of the product and after each re-infestation. The speed of kill should be studied for the whole period of claimed persistent effect, i.e. the last assessment should be performed after the last challenge. Within the 24-hour period the same pattern of selected time points should always be used throughout the study.

At each assessment time selected all live parasites including moribund fleas should be counted. The speed of kill should be based on the killing effect at the time of counting on the animals. Delayed mortality should not be considered.

Animals should be selected in such a way that dosing as close as possible to the minimum recommended dose is possible. All assessments should be performed in comparison with an untreated control group.

Veterinary medicinal product may only be characterised with one figure for the parameter 'speed of kill'. If the resulting speed of kill-time points are variable throughout the duration of the study (covering the period of claimed persistent effect), the speed of kill should be indicated by the range covering the shortest and longest time period until 95 % of the fleas have been killed.

Respective information addressing the point of time of the speed of kill should be given in section 4.2 of the SPC (pharmacodynamics). The start 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 product literature.

6.1.5. Evaluation of efficacy

For calculation of efficacy (%) towards adults, the following formula (according to Abbott's formula) is used:

Efficacy (%) = 100 x (m_C – m_T)/m_C

Control group (m_C): Mean number of live fleas on the host animals.

Treatment group (m_T): Mean number of live fleas on the host animals.

In case of controlled studies (i.e. pre-clinical studies for dose determination and dose confirmation) calculation of efficacy should be based on the arithmetic mean – irrespective of whether the count data are skewed or not – since efficacy estimates based on geometric means tend to be larger, might potentially mask treatment failures, and carry the risk of misinterpretation of the results. Efficacy calculation based on geometric mean may also be reported. Geometric mean calculations will, however, not be decisive for efficacy assessment in this type of study.

The efficacy of the proposed product should be at least 95% for adult fleas at each counting during the claimed efficacy period. This efficacy threshold is also valid for studying the speed of kill. In any case the difference in counts between treated and untreated animals must be statistically significant at a level of 5 %.

6.1.6. Testing for water stability

Please, see under section 5.1.8.

6.2. Clinical trials

6.2.1. General

Clinical trials should be performed when fleas are abundant, in at least two different geographic and climatic regions, to confirm the efficacy and safety of the proposed product in the target species under practical use conditions. Specification of flea species is not usually required in clinical trials. Animals with mono- or mixed infestations with fleas and ticks are eligible for inclusion. Depending on the trial design a positive or negative control group should be included. Clinical trials should be performed for each animal species (dog/cat) claimed. Field data is needed to support claims related to flea allergy dermatitis (FAD).

6.2.2. Selection of animals

The trial should include households, where one or more target animals is confirmed to be infested with fleas by an appropriately qualified person who should record the initial level of infestation. The number of animals (sample size) required in the trial should be statistically justified in advance and based on the hypothesis tested (e.g. superiority design versus non-inferiority) and consider both, efficacy and the safety aspects of the trial. The host animals should belong to a variety of breeds of different hair length and to different husbandries. Furthermore, animals exposed to a high risk of infestation should be included if possible. It is recommended that animals should harbour a number of at least 5 – 10

fleas at the time point of inclusion. It should be ensured that included animals or other animals within the same household have not been treated with an ectoparasitic substance within a time frame that might impact on the trial outcome. If possible, treatment of all animals of the target species in a household with the same product is the preferred option to avoid any bias. With regard to efficacy only one predefined animal as representative unit per household should be included in the analysis, however, with regard to safety aspects all treated animals should be considered. Treatment of the home environment with biocides (e.g. Insect Growth Regulators) should be avoided during the trial. When a non-inferiority evaluation is planned it should be ensured that the infestation rate is large enough in the test and the positive control group to obtain sufficient assay sensitivity.

6.2.3. Counting

Actual flea counts e.g. through combing should usually be performed every two weeks. For products with a claimed persistent efficacy of more than 4 weeks the intervals for flea counting may be extended (corresponding to those in controlled pre-clinical studies, i.e. intervals of 4 weeks and in the last month a 2-week interval). Alternative appropriate intervals for counts may be proposed by the applicant depending on the specific product characteristics, particularly its recommended duration of efficacy. However, for products with a short term residual activity more frequent counts should be performed.

The method of flea counting used should be justified. It should be considered that the level of efficacy in clinical trials will usually be lower compared to those of controlled pre-clinical studies due to the re-infestation pressure from the environment.

6.2.4. Treatment

The final formulation intended for marketing should be used at the recommended dose and route. Any deviation should be justified.

6.3. Specific recommendations for efficacy testing of veterinary medicinal products containing insect-growth regulators (IGRs) against fleas

The use of IGRs in cats or dogs is limited to the prevention of flea reproduction by inhibiting the development of eggs and/or juvenile stages. It is often combined with an adulticide for treating existing flea infestations. Although it is acknowledged that some IGRs could also affect ticks, IGRs are not considered suitable in the prevention of tick reproduction, because the tick species common in Europe are three-host ticks (*Dermacentor reticulatus, Ixodes ricinus, Ixodes hexagonus, Rhipicephalus sanguineus*). Pre-clinical studies and clinical trials demonstrating the IGR properties should be provided. The applicant should justify the type of study (ovicidal/larvicidal activity).

6.3.1. Specific pre-clinical study recommendations for IGRs

6.3.1.1. In vitro studies

Substances with IGR properties prevent the females from laying viable eggs and/or the larvae from turning into adults.

6.3.1.1.1. *In vitro* studies to demonstrate ovicidal activity

The effect of an insect growth regulator on flea metamorphosis (sterilisation of eggs/ inhibition of egg hatching and the formation of cocoons) should be demonstrated and the LC_{50} and LC_{90} calculated, using justified recognized methods.

Demonstration of ovicidal activity (Egg hatch test)

Since young flea eggs are much more sensitive to treatment than older ones, only newly laid flea eggs of a well-established flea strain freshly collected from donor animals should be used *in vitro*.

In order to calculate adequately the dose-effect relationship, it is recommended to use at least 10 viable eggs for each test concentration, and to run at least 5 to 10 replicates/test concentration in order to ensure that a sufficient number of eggs are exposed to each concentration.

Normally, when incubated under optimal conditions, e.g. at 25°C temperature and 75 +/- 10% humidity, flea eggs will hatch about three days after being laid. Thus, eggs should be observed at least for 96 h after treatment in order to ensure that all surviving eggs have sufficient time to hatch.

Any test replicate where egg hatching of the control is less than 30% should be excluded from the calculation and should be repeated. The results of all replicates should be pooled allowing adequate calculation of the mean efficacy at each concentration. Mortality can then be calculated according to *Busvine* formula as stated below. The dose effect relationship (LC_{50} and LC_{90}) should be calculated statistically according to an appropriate linear regression method.

6.3.1.1.2. *In vitro* studies to demonstrate larvicidal activity

To determine the larvicidal LC_{50} and LC_{90} of an insect growth regulator *in vitro* (e.g. juvenile hormone antagonist) preferably 2nd or early 3rd instar larvae of a well-established flea strain should be used because of convenience in handling. In order to adequately calculate dose effect relationship of an IGR, it is recommended to use at least 20-50 viable larvae at each test concentration.

2nd instar larvae are normally reared under conditions of 25°C temperature and approx. 70-80% humidity. Under these conditions, adult emergence is normally completed maximally at day 21 after the pupation started. The larval rearing media used *in vitro* should preferably be screened twice weekly for pupation and emergence of adults.

It is recommended to run at least 2-4 replicates/test concentration in particular at lower concentrations in order to ensure adequate calculation of LC_{50} and LC_{90}. In principle, each replicate should run with its own untreated control. Any test where emergence to adult of the control is less than 80% should be withdrawn from the calculation.

At the end of the study the total inhibition of cocoon formation as well as the inhibition of emergence to adults should be recorded for each concentration tested.

Mortality can be calculated according to *Busvine* formula[2] as stated below. The dose-effect relationship should be calculated statistically by using an appropriate linear regression method.

$$m_{corr} = \frac{m_o - m_c}{100 - m_c} \times 100$$

m_{corr} = corrected mortality at each concentration tested (in percent)

[2] J.R. Busvine: Toxicological Statistics – A Critical Review of the Techniques for Testing Insecticides. 2nd Edition (1971), page 263-288

m_o = mean observed mortality in the treated groups (in percent)

m_c = mean observed mortality in the control groups (in percent)

6.3.1.2. In vivo studies

Insect growth regulators will interrupt the life cycle of the flea by acting mainly on immature stages of the parasite. Efficacy can be mediated both indirectly by acting on egg development via a blood meal or contact with female fleas or directly via contact with flea eggs in the animal's fur.

Prior to the experimental infestation each animal should initially be treated (day 0) with the test product. Normally on day 1 of the experiment each animal should then be infested with at least 50–100 unfed adult fleas. Then, depending on the claim, i.e. the persistent efficacy of the proposed product or the frequency of treatment, each animal should be re-infested weekly. It is recommended to collect flea eggs at least twice a week or even more frequently. Approximately 50 collected eggs per control animal and time point are required to allow an adequate comparison of the percentage inhibition of adult emergence. Percent efficacy at each time point can be evaluated according to the formula given below provided that eggs are collected from the treated group.

In case of a combination product containing both an IGR and an adulticidal, the demonstration of the IGR efficacy may be markedly impeded by the rapid killing effect of the adulticidal compound. In such a case it may be necessary to increase the number of fleas for infestations in the controlled study according to the WAAVP guidelines (e.g. up to 200/animal) and/or to extend the study period in order to generate adequate numbers of eggs for the calculation of the ovicidal activity. Re-infestations should preferably be carried out at the end of the claimed persistent period, where it can be anticipated that the residual activity declines, resulting in a sufficient number of surviving egg laying fleas. Alternatively, a controlled study under simulated home environmental condition may also be appropriate to compare both the effect of the adulticidal product alone and the adulticidal compound in fixed combination with the insect growth regulator.

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 groups. Furthermore, a stabilized quantified infestation status of the animals should be ensured before starting the experiment, i.e. prior to treatment initiation, the weekly mean of 2 consecutive flea counts should not differ by more than 10% within the pens.

If appropriate, it may also be considered to use an approach with a specific challenge model for studying efficacy of a combination product with an adulticide and an IGR in which actively reproducing fleas are transferred from untreated animals (donor animals) to animals treated with the test product and to untreated control animals. Such a model may allow assessing separately adulticidal and ovicidal activity and also inhibition of hatching.

Criteria of the efficacy of a fixed combination product should then be based on both the statistical analysis of the weekly mean number of fleas in each study group tested and the mean number of emerged adults from all harvested eggs during the study period.

The percentage inhibition of the emergence can be calculated using the following formula:

Efficacy (%): $100 \times (a_c - a_t) / a_c$

Control group (a_C): mean number of emerged adult fleas/ mean number of collected eggs in the control group.

Treatment group (a_T): mean number of emerged adult fleas/ mean number of collected eggs in the treatment group.

Please, see point 6.1.5 for efficacy calculation of controlled studies.

The efficacy of the proposed product should be at least 95% for adult fleas and at least 90% for the inhibition of the emergence to adults (IGR). The difference in counts between treated and untreated animals must be statistically significant at a level of 5%.

6.3.2. Specific clinical trial recommendations for Insect Growth Regulator (IGR)

The conditions for the efficacy evaluation of an IGR under field conditions should strictly follow the claimed indications on the label to gain experience on the efficacy and safety of the product.

If prevention of flea reproduction by inhibiting egg and/or larval development is claimed only (e.g. an IGR mono product), the trial should be performed on animals harbouring apparently no or low numbers of fleas (0–3 fleas/animal) at the commencement of the trial period. During the trial any concomitant treatment of the animals with other ectoparasiticides (e.g. adulticides) or treatment of the home environment with a biocidal product should be avoided, since this may interfere with the test product, unless otherwise justified. Inclusion of a negative control group is recommended. If necessary, untreated control animals can be withdrawn from the trial due to animal welfare reasons.

A recommendation should be given in the SPC and product literature of IGR-mono products that *concurrent use of an adulticidal may be necessary at the beginning of treatment if severe flea infestation is present*.

If treatment of flea infestation and prevention of flea reproduction (e.g. product combining an IGR and an adulticidal) is claimed, animals enrolled in the trial should harbour a natural flea burden of at least 5-10 fleas/animal on average. Appropriate control should be included, e.g. an approved adulticidal product alone or a fixed combination product of an adulticidal and an insect growth regulator.

The frequency of flea count should follow the recommendations for adulticidal products (section 6.2).

7. Requirements for generic ectoparasiticidal products for external topical use which are locally acting

The principle is that generic ectoparasiticides with local activity only should be therapeutically equivalent to a reference product which is based on a full application for marketing authorisation. However, the guideline "Conduct of bioequivalence studies for veterinary medicinal products (EMA/CVMP/016/2000)" is not applicable for locally acting products such as ectoparasiticides for external topical use. In consequence, Article 19 of Regulation (EU) 2019/6 applies, i.e. data demonstrating efficacy and tolerance should be provided. In such cases equivalence between test and reference product has to be demonstrated by appropriate clinical trials.

To allow a reduced number of clinical trials and to avoid unnecessary use of animals in experiments for generic antiparasitic products with local activity only, at least the following data package should be provided:

The efficacy of a proposed generic product should be confirmed under laboratory conditions in at least one controlled dose confirmation study (GCP) for each parasite species proposed for the generic product, on the target animal.

The origin of tick and flea population used should be representative of the current European field situation. With respect to fleas, *Ct. felis* is known to be the most prevalent species on dogs and cats, and the flea species that can be routinely reared in laboratories.

The dose confirmation studies should be in accordance with the provisions of this guideline. Insecticidal/acaricidal efficacy of at least 95% (fleas) and at least 90% (ticks), respectively, to be based on arithmetic means, should be achieved for the entire treatment period claimed by the applicant. Regarding repellent efficacy in ticks a threshold of at least 95 % should be realised.

Comparison to a reference product is not relevant as evaluation of efficacy is based on the threshold values specified in the guideline. The applicant needs to demonstrate the efficacy of the product in every target species for which a claim is made, e.g. in both dogs and cats. The evaluation of the persistent efficacy will be based on the proven duration of efficacy in the dose confirmation studies and cannot be longer than that of the reference product unless there is respective proof from both a second dose confirmation study for each parasite species and adequate clinical trials. Otherwise, clinical trials are not considered necessary.

No extra study for flea allergy dermatitis (FAD) would be requested provided suitable persistence of efficacy against fleas was confirmed. However, a clinical trial is requested in case that an additional claim for FAD is made for the generic product which is not covered by the reference product.

The option to confirm efficacy of a generic topically applied ectoparasiticide by using at least one controlled pre-clinical study with the least susceptible tick species determined *in vitro* can be accepted only if both, a validated *in vitro* method for ticks, and a clear correlation between *in vivo* and *in vitro* results are available.

In general, local tolerance data should be provided according to the requirements of the "Guideline on target animal safety for veterinary pharmaceutical products (EMEA/CVMP/VICH/393388/2006)". Systemic tolerance should be investigated additionally if the composition of the generic product is different from the reference product, in particular if the absorption of the active substance(s) in a generic product is expected to be higher than that of the reference product (e.g. because of a different composition or concentration), unless otherwise justified.

Efficacy or tolerance studies are not considered necessary in the case that the composition (i.e. quality and quantity of the active substance(s) and excipient(s)) and the physico-chemical properties of the generic product and the reference product are identical and the generic is to be administered at the same dose and route of administration as the reference product. If 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 studies and/or clinical trials, may be necessary.

Definitions

Clinical trial
Means a study which aims to examine under field conditions the safety or efficacy of a veterinary medicinal product under normal conditions of animal husbandry or as part of normal veterinary practice for the purpose of obtaining a marketing authorisation or a change thereof.

Insect growth regulator (IGR)
Active substance that interrupts or inhibits the development of different stages (eggs, larvae, pupae) in the life cycle of an insect.

Persistent efficacy
Refers to active substances with repellent/acaricidal/insecticidal activity for an extended period of time after treatment.

Pre-clinical study
Means a study not covered by the definition of clinical trial which aims to investigate the safety or efficacy of a veterinary medicinal product for the purpose of obtaining a marketing authorisation or a change thereof.

Prevention
Refers to the prevention of re-infestation for topically applied products with local action in ticks and fleas, reached by a persistent acaricidal/insecticidal efficacy (short-term/long-term persistent efficacy). A prevention of (re)-infestation can also be achieved by a repellent effect. In addition, a preventive effect can be stated for IGRs with long term inhibitory activity against eggs and/or juvenile stages of fleas.

Repellent effect
A product with a repellent effect will cause the parasite to avoid contact with a treated animal completely and/or to leave a host.

Speed of kill
The time after treatment when there is a percentage of mortality of the given threshold of at least 90% or at least 95% for ticks and fleas, respectively, based on the killing effect determined at the time of ectoparasite counting on the animal.

Treatment
Refers to immediate acaricidal and insecticidal efficacy of a product against existing infestations.

Negative control
Animals treated with placebo, or left untreated.

Positive control
Animals treated with an appropriate product other than the test product (a product authorised in at least one EU member state in the target animal species for the same indication(s), applied via the same route of administration and with a similar mode of action, whenever possible…

References

CVMP Guideline on statistical principles for clinical trials for veterinary medicinal products (pharmaceuticals) (EMA/CVMP/EWP/81976/2010).

CVMP Guideline on pharmaceutical fixed combination products (EMA/CVMP/83804/2005).

Regulation (EU) 2019/6 of the European Parliament and of the Council of 11 December 2018 on veterinary medicinal products and repealing Directive 2001/82/EC.

Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Official Journal of the European Union L 276, 20/10/2010, p. 33-79.

Guideline on the demonstration of efficacy of ectoparasiticides; Vol. 7AE17a, 1994.

CVMP Guideline on the conduct of bioequivalence studies for veterinary medicinal products (EMA/CVMP/016/2000)

Marchiondo *et al.* (2013): WAAVP guideline for evaluating the efficacy of parasiticides for the treatment, prevention and control of flea and tick infestations on dogs and cats, 2nd edition (Veterinary Parasitology, 194, 84-97).

Questions and answers on the CVMP guideline on pharmaceutical fixed combination products (EMA/CVMP/EWP/325284/2011), 8 May 2014.

VICH GL9: Guideline on Good Clinical Practices (CVMP/VICH/595/98).