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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

**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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GUIDELINE FOR THE TESTING AND EVALUATION OF THE EFFICACY OF ANTIPARASITIC SUBSTANCES FOR THE TREATMENT AND PREVENTION OF TICK AND FLEA INFESTATIONS IN DOGS AND CATS

1. INTRODUCTION

1.1 General

This note for guidance is intended as an addition to the note for guidance on the demonstration of efficacy of ectoparasiticides dealing with general requirements for the assessment of efficacy of such products.

This note provides special guidance with respect to the testing and evaluation of efficacy of antiparasitic products that are intended for external use for the treatment and prevention of tick and flea infestations in dogs and cats. For products with insect growth regulating properties, some parts of this guideline may not be applicable.

This note should be read also together with Directive 92/18/EEC and the CVMP/VICH-Guideline on Good Clinical Practice (CVMP/VICH/595/98-FINAL).

1.2 Data requirements

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

- Description of the mode of action
- Determination of dose
- Dose confirmation trials, including persistent efficacy trials, where applicable
- Clinical field trials

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

1.3 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

Fleas:

Ctenocephalides canis

Ctenocephalides felis

2. 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 laboratory studies and field studies) for each species and stage.

As it is not appropriate to fix bags, capsules and the like to cats, results of laboratory studies in dogs to establish the efficacy in the treatment and prevention of tick infestations may be extrapolated to cats. However, a dose confirmation study in cats should be performed. In view of the difficulties of experimental infection studies in cats, pharmacokinetic parameters may be used instead. Claims for efficacy in cats should be supported by field studies.

2.1 Laboratory studies

2.1.1 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 checked in vitro before starting in vivo experiments, unless it can be demonstrated by bibliographic data.

Normally, at least 2 established tick strains per indication will be sufficient for laboratory testing. Where efficacy is claimed for strains of parasites resistant to e.g. organophosphates, defined resistant laboratory strains should be used for testing.

2.1.2 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.

2.1.3 Allocation

Animals should be maintained separated in individual accommodation during the trial to ensure that cross contamination does not occur. It is recommended to include at least 6 animals per treatment/control group.

2.1.4 Tick infestation

The infestation level should be approximately 50 unfed adult ticks (approximately sex ratio of 1:1, except for *Ixodes ricinus*) and of very similar age per test animal and infestation time point. Twenty five to fifty percent (e.g. 12-25 ticks) of these ticks should attach to the animal at each time point following infestation in the control group. This demonstrates that the tick population used is vigorous.

Whole body infestation

Ticks are applied at one or more points on the animal to allow them to distribute over the animal. For this procedure, the animals should be kept quiet for approximately 10 minutes if possible (e.g. by mild sedation) so that the ticks can attach firmly to the fur without being removed by the animal.

Site infestation

This can be used as an alternative method for testing an acaricidal effect of a product. Infestation takes place within a bag, capsule or other device. Such devices, attached to the body, ear, paw or tail, can also be utilised to examine the level of active substance at the extremities of the animal. The applicant should describe and justify the method used.

2.1.5 Criteria of efficacy

2.1.5.1 Repellent effect

A repellent effect means that no ticks will attach to the animal. Ticks already on the animal will leave the animal soon after treatment.

In general, no ticks should be detectable on the animal after 24 hours following administration of the product.

2.1.5.2 Acaricidal effect

In evaluating the acaricidal efficacy of a product, the feeding or engorgement of ticks should be taken into consideration in addition to the rate at which ticks are killed. It is recommended to assess the acaricidal effect on individual ticks according to the following parameters:

Definition of parameters			
Category	General findings	Attachment status	Effect
1	live	free	no
2	live	attached; unengorged	no (except single ticks)
3	live	attached; engorged	no (except single ticks)*
4	killed	free	yes
5	killed	attached; unengorged	yes
6	killed	attached; engorged	no (except single ticks)*

*Note: In the event of an occurrence of single ticks in the treatment group of categories 3 and 6, within 48 hours following infestation, a note corresponding in meaning to the lines proposed below should be included in the SPC and package insert:

There may be an attachment of single ticks. For this reason, a transmission of infectious disease by ticks cannot be completely excluded if conditions are unfavourable.

Indications such as „to prevent...“ or „for prophylactic use“ should be omitted if the effect is purely acaricidal, because as a rule, an attachment of the ticks is not prevented by the acaricidal substance. Thus, a preventive effect is not warranted. As a consequence, a note corresponding in meaning to that proposed below should be included in the SPC and package insert if an acaricidal effect has been claimed:

Ticks will detach from the host within 24 to 48 hours after infestation without having had a blood meal, as a rule. An attachment of single ticks after treatment cannot be excluded.

2.1.6 Efficacy testing

Products with repellent or acaricidal properties may demonstrate short term (< 4 weeks) or long term (> 4 weeks) persistent effects. Products with short-term effects typically include shampoos, sprays and spot ons/pour ons. Products with long-term effects include typically collars. 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 palpating the dog and by visual assessment. Ticks should be removed from test animals after each counting.

2.1.6.1

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

Day -7:	Examination of tick strain for infestation rate and suitability of test animals.
Day -2:	Tick infestation
Day 0:	Application of test substance.
Immediate efficacy	Efficacy testing by palpating the dog and by visual assessment according to the definitions given under 2.1.5.2 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 ons, pour ons: Weekly infestation of ticks, efficacy testing up to 48 h following each challenge as described above.
Long-term persistent efficacy	Preparations with a claimed persistent efficacy for more than 4 weeks, e.g. collars: Tick infestation every 4 weeks over the period of effectiveness claimed, efficacy testing up to 48 h after each challenge as described above.
Last month of the period of effectiveness claimed:	For reasons of decreasing efficacy, infestation every 2 weeks.

2.1.6.2

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

Day -7:	Examination of tick strain for infestation rate and suitability of test animals.
Day 0:	Application of test substance.
Day 0 + 24 h *	Tick infestation.
Immediate efficacy	Efficacy testing by palpating the dog and by visual assessment up to 24 h after challenge
Long-term persistence efficacy	Tick infestation at 4-week intervals over the period of effectiveness claimed and efficacy testing up to 24 h after challenge.
Last month of the period of effectiveness claimed:	For reasons of decreasing efficacy, infestation every 2 weeks.

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

Note: Where effectiveness over several months is claimed, the ticks should be applied at 4-week intervals over the first three months because 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. Also, severe reactions at the site of application should be reduced to a minimum.

2.1.7 Evaluation of efficacy

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

$$\text{Efficacy (\%)} = 100 \times (m_C - m_T)/m_C$$

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

Treatment group (m_T): Mean number of live (categories 1-3) or killed, engorged ticks (category 6) on the host animals.

Arithmetic means are usually acceptable for this calculation. If geometric means are used, the transformation must be justified and the arithmetic means also recorded.

The efficacy of the proposed product should be > 90 %.

2.1.8 Testing for photostability

For products intended for external use, the final formulation intended for marketing should be tested for its photostability, e.g. by UV radiation under laboratory conditions.

2.1.9 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). 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 following warning should always be included in the SPC and package insert:

Avoid frequent swimming or shampooing the animal or remove collar beforehand because there may be a reduction of the effectiveness.

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

2.2 Field studies

2.2.1 General

Field studies should take place when the relevant tick species are abundant and should be performed in at least 2 different geographic regions. The results of the field study should largely confirm those of the laboratory study. Field studies should be performed for each animal species (dog/cat) claimed.

2.2.2 Selection of animals

The study should include animals confirmed to be infested with ticks by an appropriately qualified person who should record the initial level of infestation in the questionnaire. The tick species should be identified.

At least a total of 50 cases in each region should be available for efficacy evaluation. 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 infection (e.g. hounds) should be included if possible.

2.2.3 Counting

Counts should be undertaken at weekly intervals.

2.2.4 Treatment

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

2.2.5 Study protocol

An example study protocol is attached to this guideline.

3. STUDY DESIGN FOR TESTING THE EFFICACY OF PRODUCTS FOR THE TREATMENT AND PREVENTION OF FLEA INFESTATION

Both laboratory and field studies should be performed for each animal species claimed (dog/cat).

3.1 Laboratory studies

3.1.1 Flea species

Laboratory 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 laboratory studies) for each species and stage should be justified. If the laboratory studies have included the flea species commonly identified on the host species then specification of fleas is not usually required in the field studies. Where efficacy is claimed for strains of parasites resistant to e.g. organophosphates, defined resistant laboratory strains should be used for testing.

3.1.2 Allocation of test animals

The choice of experimental animals should be justified. It is desirable to include animals of a breed characterised by a fur of moderate hair length, so that the fleas are offered a chance of penetrating the hair and being maintained on the animal.

Animals should be maintained separated in individual accommodation during the trial to ensure that cross contamination does not occur. It is recommended to include at least 6 animals per treatment/control group.

3.1.3 Flea infestation

Studies to support claims for the treatment of adult fleas:

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. Fleas should be distributed over the entire host animal. Approximately 50 % of these fleas should be present on the control animals at each time point following infestation.

Studies to support claims for the prevention of flea infestations:

Depending on the specific nature of the claim, alternative study designs may be applicable, for example, using environments able to support flea infestations. The applicant should justify the choice of study design.

3.1.4 Testing for efficacy

Insecticidal products may demonstrate short term (< 4 weeks) or long term (> 4 weeks) persistent effects. Products with short-term effects typically include dusts, sprays and spot ons/pour ons. Products with long-term effects typically include collars.

Efficacy should be established at intervals throughout the claimed time.

The applicant should justify the methods used for assessment of efficacy and the time from treatment to assessment of efficacy. Where possible flea counts should be made by combing. However it is important to demonstrate that reproducible results can be achieved by combing.

The following time schedule is recommended:

Day -2:	Flea infestation, 2 days before treatment.
Day 0:	Application of test substance.
Immediate efficacy	Efficacy testing with a recognised method, e.g. counting by combing, at day 0 up to 48 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, e.g. shampoo, spray. Weekly infestation, efficacy testing up to 48 h following each challenge.
Long-term persistent efficacy	Preparations with a claimed persistent efficacy for more than 4 weeks, e.g. collars. Flea infestation every 4 weeks over the period of effectiveness claimed, efficacy testing up to 48 h after each challenge.
Last month of period of effectiveness claimed:	For reasons of decreasing efficacy, infestation every 2 weeks.

Other effects such as repellent, insect growth regulatory or insect development inhibitory effects are not considered in this guideline but will be dealt with at a later stage. If claimed these effects must be demonstrated according to a recognised scientific standard.

3.1.5 Evaluation of efficacy

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

$$\text{Efficacy (\%)} = 100 \times (m_C - m_T)/m_C$$

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.

Arithmetic means are usually acceptable for this calculation. If geometric means are used, the transformation must be justified and the arithmetic means also recorded.

The efficacy of the proposed product should be approximately 100 %.

3.1.6 Testing for photostability

For products intended for external use, the final formulation intended for marketing should be tested for its photostability, e.g. by UV radiation under laboratory conditions.

3.1.7 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 weeks or more. The impact of exposure to water e.g. through shampooing, swimming, rainwater on the insecticidal effect should be evaluated at regular intervals (e.g. once a week). Alternatively, data on the concentration time course of the active substance in the fur after single/repeated washing can be provided.

If water stability of the final product could not be demonstrated, or data are not available, the following warning should always be included in the SPC and package insert:

Avoid frequent swimming or shampooing the animal or remove collar beforehand because there may be a reduction of the effectiveness.

3.2 Field studies

3.2.1 General

Field studies should be performed when fleas are abundant, in at least two different geographic regions. Specification of fleas is not usually required in field studies. The results of the field study should confirm those of the laboratory study. Field studies should be performed for each animal species (dog/cat) claimed.

3.2.2 Selection of animals

The study should include animals confirmed to be infested with fleas by an appropriately qualified person who should record the initial level of infestation in the questionnaire. At least a total of 50 cases in each region should be available for efficacy evaluation. 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. Treatment of the home environment with pesticides (e.g. Insect Growth Regulators) should be avoided during the study.

3.2.3 Counting

Actual flea counts e.g. through combing should be performed every two weeks. However, for products with a short term residual activity more frequent counts should be performed. The method of flea counting used should be justified.

3.2.4 Treatment

The final formulation intended for marketing should be used at the recommended dose and route. Any deviation should be justified. The study should include a control group.

3.2.5 Study protocol

An example study protocol is attached to this guideline.

Clinical Testing of Efficacy in Dogs and Cats Infested by Ticks and Fleas

I. Initial veterinary examination

Veterinarian (address):

Animal owner (address):

1. Animal data:

Name:

Breed:

Age:

Sex:

Hair length: short:

moderate:

long:

Weight: under 4 kg:

under 10 kg:

under 25 kg:

above 25 kg:

2. Beginning of treatment (date):

3. Counting prior to initiation of treatment

Ticks	Fleas
Number of ticks	Number of fleas

4. Were ticks removed prior to treatment?

yes

no

5. Previous treatment (name of preparation, date of last treatment):

6. Clinical observations: Pruritus:

Loss of hair:

Allergy (to fleas):

II. Data to be given by animal owner/ appropriately qualified person

1. Husbandry:

Home:

Urban area:

Forest and pastures:

2. Occurrence of ticks and/or fleas:

Date	Detection of ticks		48 hours later		Flea infestation
	Number of ticks		Number of ticks		Number of fleas
	none	attached to skin	none	attached to skin	

3. Were preparations for flea control used in the home environment? yes no

If yes, which ones ?

Frequency of use:

4. Presence of other dogs/cats: yes no

5. Does the animal swim in open waters (lake)? daily weekly monthly

6. Does care of the animal include shampooing? daily weekly monthly

7. Side-effects observed:

8. Handling of product: easy slightly difficult difficult

9. Remarks:

III. Final veterinary examination

1. Treatment period:

2. Evaluation of efficacy (absence of ticks/fleas):

3. Remarks:

Date and Signature