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**COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE
(CVMP)**

**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 is to replace the current “Guideline on the testing and evaluation of the efficacy of antiparasitic substances for the treatment and prevention of tick and flea infestations in dogs and cats” (EMEA/CVMP/005/2000-Rev.1).

KEYWORDS	Guideline, veterinary medicinal product, efficacy testing, flea, tick, dog, cat
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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

EXECUTIVE SUMMARY

This revised note for guidance 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 special guidance with respect to the testing and evaluation of efficacy of veterinary antiparasitic products that are intended for external use for the treatment and prevention of tick and flea infestations in dogs and cats. Taking into account the developments during recent years, including feed back obtained from the users of the previous guideline, the revised document gives additional guidance for veterinary antiparasitic products for systemic use for the treatment and prevention of flea infestations in dogs, and information on the testing of antiparasitic products containing substances with insect growth regulating properties (IGRs).

1. SCOPE

This note provides special guidance with respect to the testing and evaluation of efficacy of veterinary antiparasitic products that are intended for external use for the treatment and prevention of tick and flea infestations, or systemic use for the treatment and prevention of flea infestations in dogs and cats. Information is also provided for the testing of veterinary antiparasitic products containing substances with insect growth regulating properties (IGRs), either as mono-preparations or in combination with a flea adulticide.

2. LEGAL BASIS

This note should be read also together with Directive 2001/82/EEC as amended and the CVMP/VICH-Guideline on Good Clinical Practice (CVMP/VICH/595/98-FINAL). In case of uncertainty in classifying a specific product either as veterinary medicinal product or as biocidal product it is recommended to consult a competent authority for the Veterinary Medicinal Products Directive 2001/82/EC as amended and a competent authority for the Biocidal Products Directive 98/8/EC. In addition, the Guidance Document of the Commission outlining criteria for borderline setting between biocidal products and veterinary medicinal products may be considered (Borderline between Directive 98/8/EC concerning the placing on the market of biocidal products, Directive 2001/83/EC concerning Proprietary Medicinal Products and Directive 2001/82/EC concerning Veterinary Medicinal Products, 2002).

3 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.

3.1 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 (dogs)

Fleas:

Ctenocephalides canis (dogs)
Ctenocephalides felis

4. 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 infestation studies in cats, studies on the distribution and concentration profile of the proposed products in the cat's skin and fur may be performed instead and used to conclude on the efficacy of the anticipated dose. Claims for efficacy in cats should be supported by field studies.

4.1 Laboratory studies

4.1.1 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, one established tick strain per tick species claimed will be sufficient for laboratory testing. The use of laboratory tick strains obtained from recent field collections and multiplied in the laboratory for at least 2 generations is encouraged. Such strains would be representative of the current field situation. It should be ensured, however, that the ticks used are free of transmittable pathogens. Where efficacy is claimed for strains of parasites resistant to e.g. organophosphates, defined resistant laboratory strains should be used for testing.

4.1.2 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.

4.1.3 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.

4.1.4 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. The applicant should describe and justify the infestation method.

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.

4.1.5 Criteria of efficacy

4.1.5.1 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.

4.1.5.2 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) *

Engorgement of ticks is indicated by the presence of blood in the digestive tract. Engorgement can be determined visually by squeezing technique or microscopically or by accurate weighing

* 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 be killed and fall off 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.

4.1.6 Efficacy testing

Products with repellent or acaricidal properties may demonstrate 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 dog and by visual assessment, as appropriate. Ticks should be removed from test animals after each counting.

4.1.6.1 Acaricides

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

Day -7:	Examination of tick strain for infestation rate and suitability of test animals. The number of ticks recovered from each dog should be rank ordered from highest to lowest tick counts and animals randomly allocated by blocks so that each treatment group has equal numbers of animals that are able to maintain high to low numbers of ticks.
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 4.1.5.2 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 on, pour on: 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.

4.1.6.2 Repellents

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 persistent 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.

4.1.7 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) and 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 more than 90 %.

4.1.8 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 according to VICH GL5 (photostability testing of new veterinary drug substances and medicinal products), and should be addressed in the Quality part of the dossier.

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

4.1.9 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 the maintenance of effectiveness of the product in these cases has not been tested.

4.2 Field studies

4.2.1 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 and should include a control group.

4.2.2 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.

4.2.3 Counting

Counts should be undertaken at weekly intervals and the tick species should be identified.

4.2.4 Treatment

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

4.2.5 Individual Animal Record

An example of an individual animal record for use in a clinical field study is attached to this guideline (Annex I).

5. 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).

5.1 Laboratory studies

5.1.1 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.

5.1.2 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 separately 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.

5.1.3 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. The applicant should describe and justify 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 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.

5.1.4 Testing for efficacy

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

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. It is recommended to count fleas by combing by trained personnel according to a standard procedure reliable.

The following time schedule is recommended for an adulticidal compound:

Day -2:	Flea infestation, 2 days before treatment. Prior to day -2, the animals should be infested to assess ability of animals to maintain a flea population and the counts should be used to rank order the animals from highest to lowest flea counts and randomly allocated by blocks so that each treatment group has equal numbers of animals that are able to maintain high to low numbers of fleas.
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. 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. 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.

5.1.5 Evaluation of efficacy

For calculation of efficacy (%) towards adults, 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 at least 95% for adult fleas at each counting during the claimed efficacy period.

5.1.6 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 according to VICH GL5 (photostability testing of new veterinary drug substances and medicinal products), and should be addressed in the Quality part of the dossier.

5.1.7 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 or 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 the maintenance of effectiveness of the product in these cases has not been tested.

5.2 Field studies

5.2.1 General

Field studies should be performed when fleas are abundant, in at least two different geographic regions to confirm the efficacy and safety of the proposed product in the target species under practical use conditions. Specification of fleas is not usually required in field studies. Field studies should be performed for each animal species (dog/cat) claimed.

5.2.2 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 biocides (e.g. Insect Growth Regulators) should be avoided during the study.

5.2.3 Counting

Actual flea counts e.g. through combing should be performed every two weeks. 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 field studies will usually be lower compared to those of controlled laboratory studies due to the reinfestation pressure from the environment.

5.2.4 Treatment

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

5.2.5 Individual animal record

An example of an individual animal record for use in a clinical field study is attached to this guideline (Annex I).

5.3 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 treatment and prevention of flea infestations. Although it is acknowledged that some few IGRs could also affect ticks, IGRs are not considered suitable in the treatment and prevention of tick infestations, because the tick species common in Europe (*Dermacentor reticulatus*, *Ixodes ricinus*, *I. hexagonus*, *Rhipicephalus sanguineus*) are three-host ticks. Laboratory and field studies demonstrating the IGR properties should be provided. The applicant should justify the type of study (ovicidal/larvicidal activity).

5.3.1 Specific laboratory studies recommendations for IGRs

5.3.1.1 *In vitro* studies

Substances with insect growth regulating properties (IGRs) prevent the females from laying viable eggs and/or the larvae from turning into adults.

5.3.1.1.1 *In vitro* studies to demonstrate ovicidal activity

The effect of an insect growth regulator *via* contact on flea metamorphosis (sterilisation of eggs/ inhibition of egg hatching and the formation of cocoons) should be demonstrated and the LC₅₀ and LC₉₀ calculated, using justified recognized methods.

Demonstration of ovicidal activity (Egg hatch test)

Since young 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 (LC₅₀ and LC₉₀) should be calculated statistically according to an appropriate linear regression method.

5.3.1.1.2 *In vitro* studies to demonstrate larvicidal activity

To determine the larvicidal LC₅₀ and LC₉₀ 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 LC₅₀ and LC₉₀. 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* formula¹ as stated below. The dose-effect relationship should be calculated statistically by using an appropriate linear regression method.

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

M_{corr} = corrected mortality at each concentration tested

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

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

5.3.1.2 *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. Percent efficacy at each time point can be evaluated according to the formula given below.

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. 200/animal) and/ or extend the study period in order to generate adequate numbers of eggs for the calculation of the ovicidal activity. Reinfestations 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.

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:

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

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

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

¹ J.R. Busvine: Toxicological Statistics – A Critical Review of the Techniques for Testing Insecticides. 2nd Edition (1971), page 263-288

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 at least 95% for adult fleas and at least 90 % for the inhibition of the emergence to adults (IGR).

5.3.2 Specific Field 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.

If prevention of flea multiplication by inhibiting egg development is claimed only (e.g. an IGR mono product), the study should be performed on animals harbouring apparently no or low numbers of fleas (0 – 3 fleas/animals) at the commencement of the trial period. During the study 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 study due to animal welfare reasons.

Note: Recommendation should be given in the SPC and product literature of IGR-mono products: “Concurrent use of an adulticidal may be necessary at the beginning of treatment if severe flea infestation is present.”

If treatment and prevention of flea infestations (e.g. product combining an IGR and an adulticidal) is claimed, animals enrolled in the study should harbour a natural flea burden of at least 5 to 10 fleas per 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 5.2)

References

Directive 2001/82/EC of the European Parliament and of the Council as amended.
Official Journal L 311, 28/11/2001 P. 0001/0066

Directive 98/8/EC concerning the placing of biocidal products on the market

CVMP/VICH Guideline on Good Clinical Practice (CVMP/VICH/595/98/Final)

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

Guidance Document of the Commission, Borderline between Directive 98/8/EC concerning the placing on the market of biocidal products, Directive 2001/83/EC concerning Proprietary Medicinal Products and Directive 2001/82/EC concerning Veterinary Medicinal Products (2002).

Marchiondo et al. (2007):

WAAVP guidelines for evaluating the efficacy of parasiticides for the treatment, prevention and control of flea and tick infestation on dogs and cats (Veterinary Parasitology, 145, 322-344.)

ANNEX I

Example of an individual animal record for use in a clinical field study testing product efficacy in dogs and cats infested with 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: 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 (for acaricides only)		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