15 November 2010
EMA/CVMP/EWP/459883/2008
Committee for Medicinal Products for Veterinary Use (CVMP)

Guideline on veterinary medicinal products controlling *Varroa destructor* parasitosis in bees

Draft Agreed by Efficacy Working Party	September 2009
Adoption by CVMP for release for consultation	14 October 2009
End of consultation (deadline for comments)	30 April 2010
Agreed by Efficacy Working Party	October 2010
Adoption by CVMP	9 November 2010
Date for coming into effect	1 June 2011

This guideline replaces guideline Reference III/9283/90 "Veterinary Medicinal Products controlling *Varroa jacobsoni* and *Acarapis woodi* parasitosis in Bees"; last update September 1991, published in Vol. 7 (7AE16a) of "The Rules governing medicinal products in the European Union".

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Executive summary

This guideline outlines the conditions and criteria for the acceptability of data on efficacy and target animal safety for veterinary pharmaceutical products intended for varroosis control in honey bees and should be read in conjunction with current EU guidelines.

Varroa destructor control implies a number of measures, which can include treatment with veterinary medicinal products.

Availability of veterinary medicinal products is therefore considered as relevant.

This guideline aims to provide general guidance on issues that should be considered or addressed when designing and implementing studies to demonstrate efficacy and target animal safety. Study results should allow recommendations for use, to be made under various climatic conditions.

1. Introduction (background)

New veterinary medicinal products developed as antiparasitic treatments controlling *Varroa* mite infestation in bees should satisfy the usual requirements for authorisation. *Varroa* control implies a number of integrated pest management measures, which include routine beekeeping maintenance methods and the use of approved miticides. Veterinary medicinal products should therefore be regarded as an integrated part of *Varroa* control.

Considering that performance of veterinary medicinal products will partly depend on the climatic conditions under which the products are used, attention should be focussed on the collection of relevant information, e.g. data on temperature and rainfall should be recorded. Regarding differences in e.g. climate and bee keeping practices throughout the EU, it is recommended that applicants seek cooperation with regional / national experts, when considering the development of veterinary medicinal products for *Varroa* control.

2. Scope

The objective of this guideline is to provide applicants with general guidance on the demonstration of efficacy and target animal safety of products intended for the control of *Varroa destructor* parasitosis of the honey bee, in support of applications made for the authorisation of such products.

3. Legal basis

This guideline has to be read in conjunction with the introduction and general principles (4) and the Annex I to Directive 2001/82/EC, as amended.

It should also be read in conjunction with the guideline on Efficacy and target animal safety data requirements for veterinary medicinal products intended for minor uses or minor species (EMEA/CVMP/EWP/117899/2004).

Studies should follow the principles of VICH guideline GL9 on Good Clinical Practice, where relevant.

4. Assessment of efficacy and target animal safety

4.1. General aspects of studies

The following information will usually be required to demonstrate the efficacy and target animal safety of a proposed product:

- Data to characterise the mechanism of action and the known pharmacological effects of the active substance (including toxicological effects on bees and brood).
- Data to justify the recommended treatment dose, method, timing of administration and frequency.
- Data to justify the efficacy and safety of the product in the field.

The primary aim of *Varroa* mite control is a reduction in mite numbers and studies should investigate and document what can be achieved by treatment under different climatic conditions and various beekeeping practices.

Study results should lead to recommendations for use e.g. on dose, method of administration, treatment duration and frequency, time of treatment.

Dose finding and tolerance should be studied under controlled conditions / in an experimental setting.

Infested colonies are required for studying efficacy.

4.1.1. Study design

Study protocols should indicate the aim of the study and specify the relevant parameters. Variables should be recorded and monitored as appropriate throughout the study period; see under section 5.1 (“Details that should be included in the studies”).

The applicant is encouraged to standardise study protocols and study reports as far as possible, to facilitate the comparison of study results.

The implementation of small scale outdoor pilot studies on dose confirmation, efficacy and tolerance, in at least 10 colonies including control and a minimum of 5 test colonies, should be considered before large scale field studies are planned, as variables can be better controlled.

When carrying out pilot studies, colonies should preferably be comparable with respect to location, type of hive, level of infestation, size of hives, pre-treatment history, age of queen, presence of brood, and normal age distribution of worker bees.

As a general principle, if studies are carried out at different apiaries, habitats should be comparable (access to similar food supplies) and groups/colonies should be homogenous.

4.2. Statistical analysis

Statistical analyses should follow the principles of the CVMP guideline on Statistical Principles for Veterinary Clinical Trials (EMEA/CVMP/816/00).

Primary and potentially secondary endpoints, hypotheses, and statistical methods should be specified and justified in a protocol before beginning the experiments. Sample sizes, in terms of hives per area for climatologically different regions, should be large enough to provide sufficient statistical power. Whenever possible, results of the analyses should be accompanied by confidence intervals.

5. Assessment of efficacy

The rate of mite mortality after treatment with the product under investigation should be determined, using a follow-up treatment in the treated colony itself (a so called "critical test"), with a chemically unrelated substance with >95% documented efficacy. This follow-up treatment should take place shortly after treatment with the test product, in order to keep the reinfestation level low.

To confirm the treatment effect of the product under investigation, controlled efficacy studies should be carried out by the inclusion of a placebo group. Placebo-treated colonies should be included to establish the effect of handling and the level of infestation. Follow-up treatment should be carried out in both groups at the same time.

The possibility of reinfestation of test groups through contact with neighbouring apiaries and hives of different groups should be carefully monitored and minimised as efficiently as possible. Depending on the timing of treatment, the post- observation period should be as short as possible to avoid this effect.

5.1. Details that should be included in the studies

In reporting study information the following issues and recommendations should be taken into account.

5.1.1. Hives

Type and number of hives should be recorded.

Trays should be appropriate for mite counting and protected from ants. Mites should fall directly to the bottom. A mesh-fitted tray (diameter of 2.8-3 mm) is preferred.

Temperature and relative humidity inside the hive(s) as well as exposure to solar radiation can be recorded, if considered relevant for the performance of the product.

5.1.2. Colony

The following items should be addressed and reported:

- Bee breed
- Colony strength evaluation (by the Liebefeld estimation method) in the early morning
- The presence of a queen before and after treatment
- Presence and amount of brood (by the Liebefeld estimation method)
- Brood development (if damage is expected)
- Flight activity of bees during the trial

Infestation level should be between 300 – 3000 mites per colony and infestation levels between hives included in the studies should be comparable. Weak colonies or colonies affected by diseases other than *Varroa* parasitosis should not be included.

5.1.3. Location

Apiaries involved should preferably have sufficient distance to other apiaries to avoid disturbance and to reduce risk for re-infestation. Type and availability of food sources should be recorded.

5.1.4. Treatment details

The following items should be addressed:

- Number of treatments
- Treatment period

- Treatment intervals, if more than one treatment is carried out.

The length of the study period should be justified, taking the mode of action and the anticipated efficacy of the product into account. Treatment should preferably be carried out at outdoor temperatures > 5° Celsius and in the absence of sealed brood, unless the product is intended to be effective this way.

5.1.5. Observations and parameters

Both dead mites and dead bees should be counted at regular intervals before, during and after treatment. The primary variable is mite mortality and during the observation period dead mite counts should be carried out every 1-2 days. Sublethal effects on mites can be recorded as a secondary endpoint, but only under experimental conditions. Bee mortality inside and adjacent to the hive should be recorded at regular intervals, preferably daily. The use of dead-bee traps is recommended. Studies should encompass both a pre-treatment and a post treatment period. Monitoring should begin 7-14 days before a treatment is carried out. Pre- and post treatment counts should be made 1-2 times per week. The observation period should be 7-14 days after treatment. As observation frequency and length of the observation period will depend on the mode of action of the substance/product, this should be taken into account and selected frequencies and intervals should be justified.

5.1.6. Reporting

Both positive and negative results should be reported, e.g. with respect to treatment effect, adverse effects on bees and/or brood, bee mortality, colony size and development, ease of product handling etc.

5.2. Dose titration studies

The aim of dose-titration studies is to establish the recommended dosage and dosing interval of the product, taking into account the pharmaceutical form for which marketing authorisation is sought. Implementation of dose-finding studies, carried out under controlled / laboratory conditions is preferred, e.g. using 10 bees per cage, 3 cages per concentration, 3 controls and one replicate, i.e. the studies should be carried out twice.

Dose-titration studies should aim at identifying the minimum effective and maximum tolerated levels of active substance reaching bees and parasites. As the treatment dose is usually close to the maximum tolerated dose, it is recommended to confirm efficacy and safety in a small scale study before implementing field studies.

5.3. Dose confirmation and field studies

Dose confirmation studies can be combined with clinical field studies, as purpose, design and implementation are similar. Dose confirmation and field studies should use the product in the pharmaceutical form for which marketing authorisation is sought. Dose confirmation studies should be carried out using the recommended dosage.

Efficacy should preferably be studied under different regional / climatic conditions, in order to allow extrapolation of results to regions / Member States with different climatic conditions if relevant.

All limiting factors for administration of the product (e.g. weather conditions or state of reproduction and honey flow) encountered in the studies should be reported and discussed. General conditions of the bee colony, such as the incidence of other diseases and the strength of the colony (Liebefeld method), should be monitored at regular intervals and documented, starting before treatment.

Infestation rates should be comparable in all test groups included in the same study. The possible impact of strong as well as small (e.g. corresponding to one super in Langstroht hives) colonies on treatment result should be taken into consideration. Weak colonies should not be included.

A sufficient number of hives per group in each of the apiary sites studied, representing relevant conditions of reproduction and honey production, is recommended. The number of hives should be justified. For each climatic condition, the number of study units should be large enough to allow a proper statistical evaluation of the results. The different habitats should be chosen to account for weather influence and, where applicable, different conditions of nectar and pollen flow.

5.4. Evaluation of efficacy

Evaluation of efficacy should be based on mite mortality rate as the primary variable. Mortality rate in treated colonies should be compared to that in control colonies. A follow-up treatment will reveal the residual number of mites.

As progress in mite mortality depends on the acaricide used, it should be stated clearly to which moment and treatment calculated values apply. It should also be stated if results refer to a single treatment or a number of treatments.

The influence on the treatment effect, due to differences in study conditions, should be reported. The level of control after treatment should preferably be 95% or higher for synthetic substances and 90% or higher for other non-synthetic substances. This level of efficacy will help reduce the risk for induction of resistance.

Treatment efficacy can be calculated as follows:

$$\%\ \text{Mite Reduction (**)} = \frac{\text{No. of mites in test group killed by treatment}}{\text{No. of mites in test group killed by treatment + No. of mites killed in test group after follow-up treatment}} \times 100$$

Data from colonies with abnormally high bee mortality should not be included in the efficacy evaluation.

5.5. Resistance pattern

The possibility of resistance emerging after several treatments should be taken into account. If observed, a dose-lethality relationship of the product or active substance(s) after regular use of the product for several reproduction periods of the bees could provide relevant information, but is difficult to carry out.

The application should cover several reproductive cycles of the parasite to show the development of resistance and the rate of such development. These studies can be performed under laboratory and/or field conditions.

The product information should include guidance on appropriate use to minimise the risk of resistance development.

6. Safety for the target animal (Bee colonies)

The data submitted should characterise the safety of the product after its application at the highest therapeutic level. In these studies, the long-term effects must be determined and possible effects on reproduction as well as honey production should be observed and measured.

6.1. Safety for worker bees

It is recommended that the tolerance of the product is first tested in caged bees in the laboratory.

The highest tolerated concentration/quantity, can be used as an indication for concentrations/quantities that can be used in subsequent dose-titration as well as dose-confirmation/field studies.

Dead bees should be collected one week before, at the time of and for four weeks after the end of treatment. During treatment dead bees should be collected either daily or at least three times a week. In the second to fourth week following the end of treatment dead bees should be collected at least twice a week. The numbers of dead bees in different test groups should be compared.

If applicable (envisaged therapeutic use in autumn or winter), the morbidity, mortality and colony number, as well as the development of colonies, should be carefully observed at the time of the first flight in spring and thereafter and compared to positive or negative controls.

6.2. Safety for bee reproduction (brood, queen, drones)

Results of studies to demonstrate that treatment does not lead to intolerable effects on the health and reproductive capacity of queens and drones should be submitted. These studies should cover the lifetime of queens (from egg stage to normal time of replacement) and drones. As a rough estimate, the brood area of test colonies should be determined before and after application of the product and compared to the negative control group. In cases where the product is intended for use in colonies with brood, the demonstration of safety for all stages of brood should be carried out.

6.2.1. Recommended method

Colonies with sealed and unsealed brood should be used. After applying therapeutic doses of the test product, frames with eggs and larvae should be left to develop in the hive for certain periods of the larval stage and the development and behaviour of bees included in the test should be compared. Feeding behaviour of the brood in the hive should be monitored by measuring the amount of food found with the larvae and taking the age of the larvae into account.

By comparing both parameters – development of brood and feeding behaviour of bees, including the ratio between brood and number of worker bees – it should be possible to differentiate between effects of feeding incompetence of worker bees and direct adverse effects on eggs and larvae after application of the product. Control groups should be used.

Safety should be demonstrated for all stages of development (egg stage, larvae of several stages and pupae), and should cover the normal life span of the worker bee at high production time (6-8 weeks).

6.3. Long-term observations on colony strength

Long-term observations can establish the influence of any treatment on winter survival and colony strength and should cover at least one winter period after several treatments and the development of colonies at the time of first colony growth and honey production in spring.

Definitions

Brood:	Eggs, embryo's larval and pupal stages of bees. In man made brood frames, brood is inside (hexagonal) cells.
Capped brood:	Brood cells that have been sealed or capped
Liebefeld method:	A method developed by the Swiss Agroscope-Liebefeld-Posieux Research Station ALP to estimate the strength of a bee colony, by counting the number of bees on a dm^2 of occupied honeycomb surface at three-week intervals.

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