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

Draft

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This revision will replace the current version of the CVMP guideline on veterinary medicinal products controlling *Varroa destructor* parasitosis in bees (EMA/CVMP/EWP/459883/2008-Rev.1).

Comments should be provided using this template. The completed comments form should be sent to vet-guidelines@ema.europa.eu

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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 medicinal products intended for the control of varroosis in honey bees.

The guideline aims to provide general guidance on aspects to be considered or addressed when designing and implementing studies to demonstrate efficacy and target animal safety.

1. Introduction (background)

New veterinary medicinal products developed as antiparasitic treatments controlling *Varroa* mite infestation in bees should meet the standard requirements for authorisation. Veterinary medicinal products should be considered as an integrated component of *Varroa* control programmes. Such programmes employ pest management measures, including good beekeeping practices and the use of approved miticides.

Considering that performance of veterinary medicinal products may be influenced by the climatic conditions under which the products are used, attention should be focussed on the collection of relevant environmental information, e.g. data on temperature and rainfall should be recorded. Regarding differences in e.g. climate and beekeeping practices throughout the European Union, applicants are encouraged to cooperate 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 guidance on the demonstration of efficacy and target animal safety of veterinary medicinal products intended for the control of *Varroa destructor* parasitosis in the honey bees. The guideline is intended for applications for marketing authorisation of new veterinary medicinal products, as well as for variations to already authorised veterinary medicinal products.

3. Legal basis

This guideline should be read in conjunction with the data requirements set out in Regulation (EU) 2019/6 and, in particular, Annex II of that Regulation. Applicants should also refer to other relevant European and VICH guidelines as listed in the reference section of this guideline.

Considering the target animal species, veterinary medicinal products controlling *Varroa destructor* parasitosis in honey bees will be intended for a limited market (eligible or not for authorisation under Article 23 of Regulation (EU) 2019/6); in these cases, the corresponding guidelines highlighting the acceptable data gaps and regulatory flexibilities will also apply.

All animal experiments should be conducted taking into account section I.1.7 of Annex II of Regulation (EU) 2019/6 and the 3Rs principles (replacement, reduction and refinement), notwithstanding the place of conduct of the experiments. Alternatives to *in vivo* test methods should be employed whenever possible.

4. Pre-clinical studies

4.1. General aspects

The following information will generally 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 honey bees and brood);
- Data to justify the recommended treatment dose, method, timing of administration and frequency.

Study results should lead to recommendations for use e.g. regarding 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 honey bee colonies are required for the assessment of efficacy.

It is recommended that the tolerance of the product be initially investigated in caged honey bees under laboratory conditions.

The highest tolerated concentration/quantity can be used as an indication for concentrations/quantities that can be used in subsequent dose determination as well as dose confirmation studies and clinical trials.

The implementation of small-scale outdoor pilot studies on dose confirmation (see section 4.3), efficacy and tolerance, in at least 10 colonies including control and a minimum of 5 test colonies, should be considered before planning large scale clinical trials, as study variables can be more effectively controlled.

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

4.2. Dose determination studies

The aim of dose determination studies is to establish the recommended dose, dosing interval and duration of treatment of the product, taking into account the pharmaceutical form for which marketing authorisation is sought. It is preferred that dose determination studies are carried out under controlled laboratory conditions, e.g. using 10 bees per cage, 3 cages per concentration, 3 controls and one replicate, i.e. the studies should be performed twice. The experimental design should include an infested negative control group.

Dose determination studies should aim at identifying the minimum effective and maximum tolerated levels of the active substance reaching honey bees and *Varroa* mites. 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 in clinical trials.

4.3. Dose confirmation studies

The aim of dose confirmation studies is to confirm the efficacy of the selected dosage regimen under controlled clinical conditions. Dose confirmation studies can be conducted under small scale field conditions or combined with clinical trials (e.g. within a sequential design). These studies should be

conducted in natural honey bee colonies/hives under conditions similar to field conditions. A study should preferably include a negative control group (see section 6). When the use of a negative control is not possible, an appropriate positive control may be acceptable, provided the internal validity and sensitivity of the study are ensured (CVMP/EWP/81976/2010). Either way, the choice of control should be suitably justified.

Dose confirmation studies should use the final formulation of the veterinary medicinal product for which marketing authorisation is sought and at the recommended dosage.

5. Clinical trials

In order to confirm the efficacy and target animal safety of the proposed product under field conditions, appropriate clinical data should be presented.

The primary aim of *Varroa* mite control is a reduction in mite numbers, and clinical trials should investigate and document the efficacy of treatment under different climatic conditions and various beekeeping practices.

5.1. General aspects

Efficacy should preferably be studied across different regional/climatic conditions to enable 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, airflow, temperature 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 colony strength (Liebefeld method), should be monitored at regular intervals and documented, commencing prior to treatment. Infestation rates should be comparable across all test groups within the same study. The possible impact of strong but small (e.g. corresponding to one super in Langstroth hives) colonies on treatment outcomes should be considered. Weak colonies should not be included.

A sufficient number of hives per group is required in each of the apiary sites studied, representing relevant conditions of reproduction and honey production. The number of hives should be adequately justified. For each climatic condition, the number of study units should be large enough to allow a proper statistical evaluation of the results. The mite fall in treated and untreated control colonies should be compared to demonstrate efficacy of the product and to verify that the observed fall is not attributable to natural variation. The different habitats should be selected to account for weather influence and, where applicable, different conditions of nectar and pollen flow.

Clinical trials should use the final formulation of the veterinary medicinal product as intended for marketing.

5.2. 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.

Applicants are encouraged to standardise study protocols and study reports as far as possible, to facilitate the comparison of study results.

As a general principle, if the study is carried out at different apiaries, treatment and control groups/colonies should be comparable, e.g. with respect to their habitats (access to similar food resources).

5.3. Details that should be included in clinical trials

When reporting clinical trials, the following issues and recommendations should be taken into account.

5.3.1. Hives

Model and number of hives should be recorded.

Trays should be suitable for accurate mite counting and protected from ants. A mesh-fitted tray (diameter of 2.8-3 mm) is recommended.

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.

5.3.2. 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 clinical trial

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

5.3.3. Location

Apiaries involved should preferably be located at a sufficient distance from other apiaries to avoid disturbance and to reduce risk for re-infestation. The type and availability of food sources should be recorded. Depending on the mode of dispersion of the product, control and test apiaries should be located at a sufficient distance to prevent contamination of control groups by the tested product through drifting foragers, drones or robbers.

5.3.4. 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 into account the mode of action and the anticipated efficacy of the product. Treatment should preferably be performed at outdoor temperatures >5° C and in the absence of sealed brood, unless the product is intended to be effective under these conditions.

5.3.5. Observations and parameters

Both dead mites and dead bees should be counted at regular intervals before, during and after treatment. Mites should fall directly to the bottom of the hive. If the primary variable is mite mortality, dead mite counts should be carried out every 1-2 days during the observation period. Sublethal effects on mites can be recorded as a secondary endpoint, but only under controlled experimental conditions.

Bee mortality inside and adjacent to the hive should be recorded at regular intervals, preferably on a daily basis. 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 prior to administration of the treatment. Pre- and post-treatment counts should be performed 1-2 times per week. The observation period should continue after treatment. As observation frequency and duration 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.

5.3.6. 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.

6. Demonstration of efficacy

Evaluation of efficacy should be based on mite reduction as the primary endpoint. Mite reduction in treated colonies should be compared to that in control colonies. A follow-up treatment applied in both treated and control groups will reveal the residual number of mites.

The percentage of mite reduction 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 and with no resistance of the *Varroa* mites observed in the area of study/trial.

Before a clinical trial is initiated, applicants should ensure that mites within a representative number of hives in the participating apiaries are susceptible to the active substance of the intended follow-up treatment. The number of hives (from which mites are tested) per apiary should be based on statistical considerations, the origins of the hives, their treatment history, and colony strength.

It is preferable that applicants use bioassays (DNA-based assays for detection of mutations) to demonstrate the susceptibility of mite populations to the substance used in the follow-up treatment.

This follow-up treatment should take place shortly after treatment with the test product, in order to minimise reinfestation levels. The follow-up treatment should be administered according to the dose regimen for the treatment of varroosis.

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-treatment observation period should be kept as short as possible to reduce this risk. As progress in mite mortality depends on the acaricide used, it should be clearly stated to which timepoint and treatment the calculated values apply. It should also be stated whether results refer to a single or multiple administrations of the same veterinary medicinal product.

For those products which penetrate below brood cappings and kill mites in the cells, it is advisable to wait until the sealed cells are opened, i.e. for a minimum period of 14 days, as these mites will be released and fall to the bottom board only after adult bee emergence.

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 emergence of resistance.

Treatment efficacy can be calculated as follows:

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

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

For veterinary medicinal products which do not act through a direct varroacidal action, alternative criteria might be used for efficacy evaluation. These veterinary medicinal products could exert different effects on *Varroa* populations (e.g. RNA interference) and/or involve different types of target processes (e.g. they may interfere with reproduction). Therefore, specific endpoints, time points and possibly, criteria for efficacy evaluation, may be proposed and scientifically justified based on the involved mechanism of action. Finally, a statistically significant and clinically relevant mite reduction should always be demonstrated, as well as an appropriate maintenance of the bee colony strength over time. The demonstrated efficacy will also have to be balanced against the potential for resistance development induced by the treatment.

Statistical analysis

The results obtained for test and control groups should be statistically analysed, and the clinical relevance of the observed effects and the additional benefit in relation to possible adverse effects should be discussed.

Statistical analyses should follow the principles of the CVMP Guideline on statistical principles for clinical trials for veterinary medicinal products (pharmaceuticals) (EMA/CVMP/EWP/81976/2010).

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

7. Safety for the target animal (bee colonies)

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

7.1. Safety for worker bees

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 per week. In the second to fourth week following the end of treatment, dead bees should be collected at least twice per week. The numbers of dead bees in different test groups should be compared.

If applicable (e.g. when therapeutic use in autumn or winter is anticipated), 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 with positive or negative controls.

7.2. 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 evaluate the health of the queen through direct observations during the trials (e.g. presence of the queen), and use indirect methods to support queen tolerance over a longer time span, based on her ability for reproduction demonstrated by the colony strength, i.e. colony strength post-administration of the candidate product (i.e. short-term effects, refer to section 7.1) and colony development during the spring following treatment administration (i.e. longer-term effects, refer to section 7.3).

For products intended to be administered on multiple occasions during the year, colony development in spring should be evaluated after all possible treatments have been administered in the preceding year, as outlined in the product information.

As a rough estimate, the brood area of test colonies should be determined before and after administration 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.

Recommended method

Colonies with sealed and unsealed brood should be used. After applying recommended 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 due to feeding incompetence of worker bees and direct adverse effects on eggs and larvae following administration 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 lifespan of the worker bee at high production time (6-8 weeks).

7.3. 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 following several treatments, as well as the development of colonies at the time of first colony growth and honey production in spring.

This could be particularly important in case multiple administrations are proposed during the year and/or when used in presence of honey flow. Medium- and long-term data should be provided to justify safety versus a limited number of administrations over the year to evaluate these effects.

Especially when veterinary medicinal products are used repeatedly throughout the year, long term effects might occur after 1 year.

8. Specific requirements

8.1. Resistance pattern

The potential emergence of clinically relevant resistance for the claimed indication in the target animal species shall be addressed and clearly reflected in the product information. Where possible, information on the resistance mechanism(s), the molecular genetic basis of resistance, and the rate of transfer of resistance determinants shall be presented. Whenever relevant, information on co-resistance and cross-resistance shall be presented. Measures to limit resistance development shall be proposed by the applicant.

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 over several bee reproduction cycles could provide relevant information.

The treatment duration should cover several reproductive cycles of the parasite to investigate the development of resistance and the rate of such development. These data may be obtained under laboratory and/or field conditions.

Ectoparasitic resistance may vary between geographical locations. When known, the resistance profile of ectoparasites should be described; the location of studies and strains of investigated ectoparasites should take account of these resistance profiles to ensure that study findings are representative for the ectoparasites in the EU.

Suspected cases of lack of efficacy observed during pre-clinical studies or clinical trials should be appropriately discussed.

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

When resistance patterns are observed under study conditions, the treatment history of the colony, particularly the (reused and/or treated) frames and data about the wax reuse, could provide useful information.

8.2. Vapour products

The effectiveness of vapour acaricides is influenced by various factors.

For such products, airflow through the hive is important, as is the chemical behaviour of the compounds in relation to temperature. These factors can influence both the efficacy of the product and the safety of the colonies.

Therefore, for vapour products, data should be gathered under various temperature and airflow conditions, taking into account the relevance of these factors to the particular product and the technical feasibility. This would help characterise the conditions under which the product can be used safely and effectively.

If the product requires special equipment for administration, information on the equipment used in the clinical trials should be included in the product information. The product information should include guidance on appropriate use of the product.

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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