

VETERINARY MEDICINAL PRODUCTS CONTROLLING VARROA JACOBSONI AND ACARAPIS WOODI PARASITOSIS IN BEES

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Additional Notes	The objective of this document is to provide specific guidance in respect of the documentation of the efficacy of veterinary medicinal products intended to control Varroa jacobsoni and Acarapis woodi parasitosis of the bee. It should be read in conjunction with Directive 81/852/EEC as amended, and the note for guidance on <i>Good Clinical Practice for the Conduct of Clinical Trials on Veterinary Medicinal Products in the European Union</i> .

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VETERINARY MEDICINAL PRODUCTS CONTROLLING VARROA JACOBSONI AND ACARAPIS WOODI PARASITOSIS IN BEES

1. INTRODUCTION

New veterinary medicinal products developed as antiparasitic drugs controlling varroa and acarapis mite in bees must satisfy all the usual requirements for authorisation. These include the demonstration of safety for the consumer of honey, for the environment and for the user. This note for guidance deals with certain specific requirements with respect to efficacy and target animal safety, depending on the nature of the parasite and the host species.

This note serves as a supplement to the note for guidance: *Good Clinical Practice for the Conduct of Clinical Trials on Veterinary Medicinal Products in the European Union* and Directive 81/852/EEC. Its purpose is to provide specific guidance with respect to the demonstration of efficacy of products intended to control *Varroa jacobsoni* and *Acarapis woodi* parasitosis of the honey bee.

2. ASSESSMENT OF EFFICACY

2.1 General dose-effect studies (laboratory studies)

If applicable, the assessment of efficacy should include an *in vitro* study. Using the envisaged route of administration and type of formulation, the effect of active compound(s) on parasites should be demonstrated as a function of dose or concentration in relation to the influence of other physical factors such as temperature, humidity, etc..

This should include a dose-titration study for the parasite (ED 50 -100). The results of these studies should be used to define a minimum effective dose of active compound(s).

In the case of products to control *Acarapis woodi*, a sufficient number of bees from highly infested colonies should be used to define the dose-lethality relation of parasitising mites.

The results of these tests may be used as the basis for studies of the development of resistance. Where applicable, it is recommended also to test sub-groups of parasites showing different degrees of resistance to other anti-parasitic chemicals.

2.2 Dose titration studies

Experimental conditions:

The studies should establish the recommended dosage of the product as well as the preference for the pharmaceutical form for which marketing authorisation is sought. The number of bee colonies to be used in these studies depends on the variation of resulting data. The study should demonstrate minimum and maximum levels of active compound reaching bees and parasites under different conditions.

Where the effect of products to control varroa is expected to be achieved partly or fully by way of penetrating wax and reaching parasites in sealed and unsealed brood, adequate studies

demonstrating the amount of compound reaching the appropriate target and the percentage of killed varroa within brood cells should be submitted.

In products to control acarapis or varroa, a rough estimation of the amount of active compound reaching the brood should be possible, even where the effect is not claimed to be achieved in this way.

2.3 Dose confirmation studies (field trials)

Experimental conditions:

Dose confirmation studies should use the new product in the pharmaceutical form for which marketing authorisation is sought.

These studies should be carried out using the recommended dosage. The *in vivo* dose-effect relationship for the product concerning efficacy and safety for the target animal (individual bees and colony) should be demonstrated.

Efficacy should be demonstrated under different conditions of temperature, humidity, brood and feeding conditions in accordance with normal conditions of beekeeping in the Member States in which marketing authorisation is sought. All limiting factors for administration of the product (weather conditions or state of reproduction and honey flow) encountered in the studies should be documented and discussed.

General conditions of the bee colony, such as the incidence of other diseases and the strength of the colony, should be documented before and at the end of treatment.

When the product is claimed to be effective and safe in the treatment of natural and artificial swarms, adequate studies are to be submitted.

A minimum of 10 hives per group in each of the apiary sites studied, representing relevant conditions of reproduction and honey production, is recommended. The different habitats should be chosen to account for weather influence and, where applicable, different conditions of nectar and pollen flow.

Breeds of bees to be tested: When the social behaviour of bees plays a role in the spreading of the compound and in efficacy, several breeds of bees are to be used in field trials and in critical trials in accordance with the differences in social feeding habits of breeds of bees commonly used in the Member States.

Building of random test groups: Randomly chosen treatment and control groups are to be formed.

The possibility of reinfection of test groups through contact with neighbouring apiaries and hives of different groups should be carefully monitored and minimised as efficiently as possible.

The inclusion of positive control groups treated with approved products against varroa or acarapis mites is recommended.

Strong as well as weak colonies are to be taken into consideration.

There should always be a sufficient number of highly infested colonies. The infestation rates should be comparable in all test groups included in the same test.

The test groups should be comparable in all other aspects relevant to efficacy testing.

2.4 Mode of evaluation of efficacy data

a) Products intended for use against varroa:

Appropriate methods of determination of parasites killed on each day of testing should be used. Differentiation should be made between viable and non viable mites falling to the bottom of the hive. Appropriate procedures should be used to determine the number of surviving mites.

Recommended methods for demonstrating quantitatively the reduction rate of parasites in efficacy tests:

After the end of the treatment, either the bee colonies used in the test at the same time in all test and control groups should be killed and washed in petrol to determine the number of parasites remaining on the bees after treatment, or a therapeutic product of known high efficacy (> 95%) used to determine the number of remaining mites. The necessary efficacy of the second product must be proven in the apiary at the beginning of the study.

Results should be expressed by the total number of bees in the colony and by the number of bees infested by the parasite.

The reduction rate (%) of parasites is determined as follows:

$$\% \text{ Reduction} = \frac{\text{killed parasites}}{\text{killed} + \text{remaining parasites}} \times 100$$

If an effect on the reproductive capacity of mites is claimed, the potential of the remaining mites to reinfest colonies should be evaluated by appropriate methods. For this purpose another set of tests should be performed in which the number of parasites is determined ca 4 weeks after administration of the product to brood-free colonies, which are allowed to breed after the end of treatment.

If applicable, the infestation of brood cells with viable parasites and their larval stages should be compared in test and control groups.

b) Products intended for the control of *Acarapis woodi*:

Appropriate procedures should be used to determine the number of surviving mites.

To demonstrate quantitatively the reduction rate of parasites, 60 to 100 individuals of each colony used in the test should be killed at the same time before and after treatment and the number of living parasites remaining on the bees determined. The evaluation of vitality of the parasites should be based on microscopic observation of movements of the mites in the dissected tracheas of the bees. The preparation methods should be carefully adjusted so as not to destroy the mites. The results obtained after treatment should be differentiated by the number of bees in the colony and parasite infestation rate before treatment.

If the product is intended to be used in colonies with brood, specific information should be gained by determining the infestation rate among young bees.

In the case of products intended for administration in late summer or autumn, the study of efficacy should cover the following winter and spring. At least 60 bees in each test colony should be killed in the spring, and infestation rates determined.

2.5 Statistical analysis of efficacy to determine significance

Appropriate statistical methods should be used and justified to compare the reduction rate in treated stocks with the reduction rate in control groups.

Statistical analysis is based on the reduction rate of parasites (varroa) and the reduction rate of living parasites in the observed sample (acarapis), in individual test hives and the measured variance of results in the test groups.

In the case of products claimed to be efficacious against *Acarapis woodi*, variations in the number of parasites in negative control groups should be measured carefully before and after the end of therapy and used to correct efficacy figures for treated groups.

If claimed, the reduction of parasites in brood cells should be taken into account (varroa).

2.6 Resistance pattern

The resistance pattern emerging after several treatments should be demonstrated.

For this purpose the dose-lethality relationship of the product or active ingredient(s) after regular therapeutic use of the product for several reproduction periods should be determined.

To save time, it is recommended to use small, artificially formed bee colonies (nuclear colonies) and apply the lowest quantity of product per treatment which under normal therapeutic conditions would reach the relevant parts of the hive. The application should cover several reproductive cycles of the parasite to show the development of resistance and the rate of such development.

3. 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 of application of the product must be looked for and possible effects on reproduction as well as honey production should be observed and measured.

3.1 Safety for worker bees

The tolerance of the product should be tested first in caged bees in the laboratory.

A therapeutic index should be established by comparing the highest amount of the compound reaching the bees under field conditions with the dose-lethality relationship found in the laboratory tests.

In the field tests these theoretical results should be differentiated by factors influencing the safety of the product under normal conditions of use.

Dead bees should be collected one week before, at the time of and for four weeks after the end of treatment. At the time of treatment dead bees should be collected either daily or at least three times a week. In the second to fourth week following treatment dead bees should be collected 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.

3.2 Safety for bee production (brood, queen, drones)

Results of special tests should be submitted to demonstrate that treatment does not lead to intolerable effects of the health and reproductive capacity of queens and drones. The study should cover the lifetime of queens (from egg stage to normal time of replacement) and drones.

The safety for bee brood should be determined in each case in which the product is not solely used in artificial swarms or individually treated animals. In such cases a reliable method should be used to quantify a possible reduction of viability.

As a rough estimate, the brood area of test colonies should be determined before and after application of the product and compared to negative or positive controls.

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 with particular accuracy.

Recommended method:

Colonies with sealed and unsealed brood should be used. After applying therapeutic doses of the product in question, frames with eggs and larvae should be left to develop in the hive or in an incubator for certain periods of the larval stage and the development and behaviour of animals 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.

By comparing both parameters – development of brood and feeding behaviour of bees – it should be possible to differentiate between effects of feeding incompetence of worker bees and direct adverse effects to eggs and larvae after application of the product. Control groups with and without formulation base treatment should be used.

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

3.3 Long-term studies

Long-term studies to establish the influence of the maximum number of treatments possible per year and the effects of residues in wax and hive on fertility and reproduction should be submitted. Such studies 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.