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ACVM REGISTRATION STANDARD AND GUIDELINE FOR EFFICACY OF ZINC PROPHYLACTIC PRODUCTS FOR FACIAL ECZEMA

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1 INTRODUCTION

This document specifies the minimum study and reporting requirements, i.e. the standard, for efficacy studies submitted in support of an application to register a zinc prophylactic product for facial eczema, or to vary the conditions on a registered zinc prophylactic product for facial eczema. It also incorporates guidelines, which are intended to provide more detailed information and guidance to applicants to assist them in complying with the standard.

The requirements that form the standard are shown in this document in **bold font**, while the guidelines are in regular font.

Guidelines reflect principles commonly recognised by the scientific community as appropriate and necessary for collecting scientific data. It is recognised that there are acceptable methods, other than those described in these guidelines, that are capable of achieving the principles of this document.

The standard is compulsory in all cases where efficacy data are required to be provided for registration of zinc prophylactic product for facial eczema, unless a waiver has been granted by NZFSA.

Waivers may be granted to reduce the number of studies or type of data that an applicant must submit (e.g. by permitting cross-referencing to existing data held by NZFSA). *These waivers must be granted by NZFSA prior to the applicant submitting an application.* This standard will be reviewed periodically, and waivers incorporated if appropriate.

Applicants should note that they are responsible for providing all information required by the ACVM Group of NZFSA to make a decision on the application. Applications that do not contain the required information will not be assessed. If further advice is required, applicants are advised to contract the services of an appropriate consultant prior to submitting the application.

1.1 Scope

The standard must be followed by:

- all persons applying to register a zinc prophylactic product for facial eczema or to vary the conditions on a registered zinc prophylactic product for facial eczema;
- all persons accredited under the Agricultural Compounds and Veterinary Medicines Act 1997 to undertake a risk assessment of applications made to register a zinc prophylactic product for facial eczema or to vary the conditions on a registered zinc prophylactic product for facial eczema.

1.2 Definitions and abbreviations

FE
Facial eczema.

GD
Glutamate dehydrogenase.

GGT
Gamma-glutamyltransferase.

Target species
The species of animal for which the test substance is intended for final use.

1.3 References

ACVM Research Standard
ACVM Registration Information Requirements for Veterinary Medicines in New Zealand

2 GENERAL REQUIREMENTS FOR EFFICACY STUDIES

2.1 Clinical requirements

2.1.1 All studies must be conducted in accordance with the *ACVM Research Standard.*

2.1.2 The efficacy of the product and/or its active ingredients must be investigated in the target species.

2.1.3 Product formulation used in studies must be identical to that being proposed for registration.

2.1.4 Experimental data must be confirmed by data obtained under practical field conditions.

2.1.5 Sample sizes must be adequate to detect differences among treatment groups with a statistical power of at least 80%.

2.1.6 Adequate statistical methods must be used and justified. A 5% or lesser probability level ($P \leq 0.05$) should be used in deciding whether to accept or reject the null hypothesis.

2.1.7 Where a dose range is stated on the label, efficacy studies must be undertaken using the lowest dose rate.

2.2 Documentation

2.2.1 Reports must be presented in accordance with the *ACVM Research Standard.*

2.2.2 The applicant must state the overseas licensing status of the remedy. A reason must be given where the remedy is not licensed for use in the country of origin.

3 SPECIFIC REQUIREMENTS FOR EFFICACY OF ZINC PROPHYLACTIC PRODUCTS FOR FACIAL ECZEMA

The following are mandatory clinical study and reporting requirements (with guidelines) for evaluating zinc prophylactic products for facial eczema. They are additional to the general efficacy requirements above.

A number of the requirements listed below are also listed in the *ACVM Research Standard.* They are repeated here in order to expand on them for these particular products.

Experimental protocols should be designed to demonstrate that the zinc formulation and method of administration is effective as a preventative against facial eczema in the field yet does not debilitate or cause welfare problems in the animals or otherwise compromise the experiment.

3.1 General

3.1.1 Studies must be conducted using the class of animal for which the product is intended.

Healthy, vigorous animals should be used in the studies.

3.1.2 Study animals must be from commercial genotypes not selected for resistance to facial eczema (FE).

3.1.3 Study animals must not have been exposed to high *Pithomyces chartarum* spore count pasture within the previous six months.

3.1.4 Before the animals are randomised into groups, they must all be bled to ensure that pre-existing liver injury is not present. Because gamma-glutamyltransferase (GGT) activity in serum does not rise for about ten days after exposure to *Pithomyces chartarum* spores or sporidesmin, these samples must be examined for glutamate dehydrogenase (GD) as well as GGT.

3.1.5 If it is unavoidable that animals with minor FE liver injury must be used in studies, criteria on which they are selected and distributed into treatment groups must be reported. Full information on such animals must be submitted in the final study report.

If it is unavoidable that animals with minor FE liver injury must be used in studies, they should be distributed into experimental groups by random block design, based on GGT levels and body weights. It is better, however, that animals with high GGT levels be eliminated from the studies.

3.1.6 Animals must be weighed, using calibrated scales, at the beginning and end of the study, and at monthly intervals during the study (at least).

Animals should be weighed fortnightly. The weighing procedure should be standardised across all groups. Animals should be weighed as soon as possible after being removed from feed to eliminate differences due to variable gut-fill.

3.1.7 Regular clinical appraisals, including evidence of photosensitisation, must be carried out during the study and reported.

Clinical appraisals should be carried out at least twice weekly. Other parameters that may be reported include general demeanour, milk yield (where applicable) and body condition. There may be further parameters specifically related to the circumstances of the study and the species studies.

3.1.8 Pre-sporidesmin (or pre-challenge) and regular blood samples must be taken for gamma-glutamyltransferase activity during the study.

Bleeding for subsequent GGT analysis should be done at regular intervals but preferably weekly for four weeks after a major spore rise in order to detect peak levels of GGT.

3.1.9 Administration of the zinc product must be started at least 24 hours before the facial eczema challenge.

While good protection may be gained by beginning zinc administration 24 hours before the facial eczema challenge, 48 hours is considered to be a better choice in most cases.

3.1.10 If severe photosensitisation causes death or warrants euthanasia of any animals from experimental plots in which sporidesmin has not been dosed as a challenge, or where high spore counts of *Pithomyces chartarum* have not been demonstrated on the pastures being grazed, samples of liver and any other organs showing changes at postmortem examination must be taken for routine histopathology from recently dead animals.

A suitably qualified person must carry out the examination of animals. The histopathological examination must be carried out by a person with recognised expertise or qualifications in veterinary pathology. The purpose of these examinations is to ensure that the photosensitisation observed in field studies is indeed facial eczema.

If neither of these occasions arise then at least one animal with clinical signs or, failing that, one with a high GGT, must be sacrificed to ensure that the condition is caused by sporidesmin.

3.1.11 Any animals dying or needing to be sacrificed during the study must be subjected to a postmortem examination to determine the cause of death or debilitation.

Animals should be observed with adequate frequency to appropriately manage the study, observe clinical signs and to collect dead animals for necropsy prior to decomposition.

3.1.12 The conditions of the study being reported must include the type of pasture grazed.

3.1.13 Study animals must not have access to other supplementary forms of zinc, e.g. boluses, treated water.

3.1.14 The age, sex, entirety or neutered condition of animals, breed and species of all animals in studies must be reported.

3.1.15 The method that provides for preserving the identification of animals must be reported.

Eartagging is a suitable method for preserving the identification of animals in these studies.

3.1.16 The welfare of the study animals is the prerogative of the Animal Ethics Committee authorising the study. However, it is strongly recommended that:

- provision of shade be made for all animals;
- either veterinary assistance be called for animals with signs of photosensitisation, or euthanasia be considered for animals with severe clinical signs of photosensitisation. In the latter case, a predetermined liver score should be assigned to the animals if liver scoring is done;
- regular weighing and clinical appraisal are assessed to determine the extent to which animals are affected by facial eczema, e.g. inappetance.

3.1.17 The number of animals per experimental group, and the number of times the study is repeated will depend upon differences expected, and the nature of the experimental challenge.

3.1.18 Experimental design, group size and analysis of data recorded should be done in consultation with a biometrician with appropriate experience in the conduct of animal studies and some knowledge of the variability of responses to facial eczema challenges.

3.1.19 Environments should be as representative as possible of the expected conditions under which the product will be used, for all groups in a study.

3.1.20 The stocking density for study animals should reflect commercial practices.

3.1.21 The stockmanship of animals should be equivalent to that of commercial practices.

3.1.22 Water and feed should be provided *ad libitum*.

3.1.23 Animals should be stressed as little as reasonably possible as this could affect their susceptibility to facial eczema.

3.1.24 Dose determination studies should be conducted using the same salts of zinc as used in the product and should be in the same form as that to be used in the product.

3.1.25 Demonstration of a dose-response with the product or its active ingredient should be a necessary feature of development work, giving a good indication of efficacy.

3.1.26 A sanitation and biosecurity programme should be adopted to avoid the inadvertent occurrence of disease other than that intended in the challenge.

3.2 The facial eczema challenge

3.2.1 The facial eczema challenge comprises a toxicological challenge to the zinc protection by any of the following methods:

- dosing with sporidesmin;
- dosing with a crude extract of Pithomyces *chartarum* spores;
- a field challenge in which the test animals and controls are exposed by grazing on paddocks with high spore counts of *Pithomyces chartarum*. Both test and control groups of animals must be grazed together on the same pastures.

The former two methods may be used in the development of the product. The final efficacy studies with the product should be carried out as a field challenge.

3.2.2 In all studies, the facial eczema challenge must be sufficiently intense to demonstrate good protection.

As a guide, this challenge should be sufficient to raise the average GGT serum levels of the control animals to a maximum of at least 200 IU/l for sheep, 400 IU/l for cattle, 300 IU/l for goats, or 300IU/l for deer.

3.2.3 The dose of sporidesmin administered either as the pure sporidesmin or as extracts of *Pithomyces chartarum* spores, or the *Pithomyces chartarum* spore counts of pastures grazed during the challenge must be reported.

3.2.4 Sporidesmin is an exceptionally toxic substance, subject to deterioration and is dangerous to the operator. Scientists experienced in the use of sporidesmin should be consulted, or preferably subcontracted, for the dosing procedure.

3.2.5 Administration of sporidesmin/crude sporidesmin is recommended outside of the FE season (January to April inclusive) in the FE susceptible areas or all year in areas in which FE does not occur. Field challenge is recommended in FE susceptible times and regions. Use of sporidesmin/crude sporidesmin in the FE season is not recommended unless careful spore counts have shown no evidence of spore rises.

3.2.6 Ideally the sporidesmin challenge should be administered early in the recommended time period for the product's use, some few days after the commencement of the zinc prophylaxis regime, and also towards the end of the zinc administration regime.

3.3 Field studies

3.3.1 At least two treatment groups must be used:

- **a non-zinc dosed group;**
- **a zinc product dosed or administered group.**

In field studies it is advisable to have larger groups of animals as there is greater variation between animals in their intake of spores and, hence, the challenge to each animal. It is recommended that the minimum group size should be 16 animals.

3.3.2 Animals must be selected to represent the section of the animal population for which the product is intended.

3.3.3 Paddocks must be observed to ensure that other factors (eg ragwort or other toxins or factors likely to compromise the study) are not present.

Pastures may be topped before the study starts or in the early stages of the study in an effort to encourage higher spore counts. If this is done it is not advisable to run the commercial animals together with the study animals. Likewise, grazing pressure can be adjusted to maximise the likely intake of *Pithomyces chartarum* spores.

3.3.4 All animals in the study must be grazed together on the same pastures for the entire study.

All groups of the study should be run together in the same paddocks preferably either as one single study group or, if more convenient, with the commercial animals from which the groups were formed. The animals external to the study may or may not be treated with zinc although, if their number is great compared with the study numbers, it should be realised that there is a small possibility that zinc contamination may lessen the differences between the study groups.

Occasional *Pithomyces* spore counts should be performed on the pastures being grazed to ensure that the animals are receiving an adequate and correct challenge.

3.3.5 Studies should be designed and commenced before pastures reach dangerous spore count levels.

3.3.6 Results may also be expressed as the effect of the zinc product on liver injury as judged by a veterinary pathologist's scoring of FE liver injury in livers passing through an appropriate meat processing plant. Such scoring must be done without any knowledge of the group origin for each liver. A scoring system for FE liver injury is given in the appendix.

3.4 Results

3.4.1 The effectiveness of the prophylactic regime is judged by:

- **the use of serum GGT analyses; and**
- **clinical observation of photosensitisation.**

3.4.2 The following parameters must be reported as evidence of protection:

- **weight changes;**
- **serum GGT levels;**
- **serum GD levels where taken;**
- **clinical recordings of facial eczema.**

Raw data as well as statistical analyses must be reported.

3.4.3 Reductions in:

- **the mean levels serum GGT; or**
- **poor performance (eg liveweight); or**
- **numbers of clinical cases of FE**

must show statistical significance at least at the 5% level.

3.4.4 Acceptable levels of protection against facial eczema for the final product are:

- **75% reduction in the numbers of animals showing photosensitisation;**
- **75% reduction in the mean peak serum GGT levels**

except where extenuating circumstances (eg an exceptionally heavy challenge) allow for variance to these figures.

APPENDIX

FACIAL ECZEMA LIVER INJURY SCORING SYSTEM

(after Smith *et al* [1977] The protective effect of zinc sulphate in experimental sporidesmin poisoning of sheep. *New Zealand Veterinary Journal 25*: 124-127)

Both sides of the liver are examined visually and by palpation and flexing of the liver to determine the firmness due to fibrosis. Minor injury is usually confined to the ventral lobe of the liver. The gall bladder is removed and holding the liver in the left hand and starting with the tip of the ventral lobe sections cut across the liver with a sharp skinning knife. As this is done the extent and magnitude of changes such as intralobular, interlobular and peribiliary fibrosis is observed, the degree of icterus and the amount of distortion of the liver noted. Changes due to other causes such as congenital changes, larval tracts, parasitic cysts etc are ignored.

The livers MUST be identified by number only with the operator having no knowledge of the group of origin of the liver.

Grades

1. No visible signs of toxicological injury.

2. Minor thickening of bile ducts confined to the ventral lobe. Mass of liver affected less than 20%.

3. Mild thickening of bile ducts, minor fibrosis, liver mass affected 20-40% still confined to ventral lobe.

4. Moderate thickening of bile ducts, moderate fibrosis, majority of ventral lobe affected and signs of fibrosis in dorsal lobe under area or gall bladder. 40-60% 0f liver mass affected.

5. Major changes throughout liver, gross thickening of bile ducts, both major lobes affected, fibrosis throughout most of parenchyma.

6. Gross thickening of all bile ducts, severe interlobular fibrosis of both lobes and caudate lobe also affected. Discolouration of parenchyma with bile pigments.