ISBN 0-478-07710-6

37 ACVM 11/02

New Zealand Food Safety Authority
Post Office Box 2835
Wellington, New Zealand

ACVM REGISTRATION STANDARD AND GUIDELINE FOR EFFICACY OF VERTEBRATE PESTICIDES

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Endorsement:
Date:

CONTENTS

ACVM REGISTRATION STANDARD AND GUIDELINE FOR EFFICACY OF VERTEBRATE PESTICIDES

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 vertebrate pesticide, or to vary the conditions on a registered vertebrate pesticide. 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 vertebrate pesticides, 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 vertebrate pesticide or to vary the conditions on a registered vertebrate pesticide;
- all persons accredited under the Agricultural Compounds and Veterinary Medicines Act 1997 to undertake a risk assessment of applications made to register a vertebrate pesticide or to vary the conditions on a registered vertebrate pesticide.

The standard provides specifications for:

- general efficacy requirements;
- cage studies;
- pen studies; and
- field studies.

1.2 Definitions and abbreviations

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

Vertebrate pesticide
Any active ingredient formulated in a product to kill a mammalian pest (e.g. rat, ferret, rabbit, possum).

1.3 References

ACVM Research Standard
ACVM Registration Information Requirements for Vertebrate Toxic Agents including Vertebrate Pest Control Products (under construction)

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. Use patterns in field studies must be identical to those being proposed for registration.

2.1.4 Experimental data must be confirmed by data obtained under practical field conditions or in enclosed or laboratory conditions simulating 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.

2.2 Documentation

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

2.2.2 The applicant must state the overseas registration status of the pesticide. A reason must be given where the pesticide is not registered for use in the country of origin.

3 SPECIFIC REQUIREMENTS FOR EFFICACY OF VERTEBRATE PESTICIDES

Vertebrate pesticides are chemical substances used for killing mammalian pests (generally through ingestion). The most important features of a vertebrate pesticide that contribute to its performance are its toxicity and palatability in an appropriate delivery system.[1,3]

The sets of guidelines referred to in 'Further Reading' below include comprehensive series of test methods to be used in the evaluation of the toxicity and palatability of a vertebrate pesticide. Reference should be made to the published guidelines for the appropriate details. The test methods are set out in such a way that they form a series of stages in the development of a vertebrate pesticide from its preliminary screening as a potential useful chemical to its final evaluation in the field as the active ingredient in some suitable formulation. Tests that are designed to measure the toxicity and palatability of vertebrate pesticides to indicate what concentrations can be expected to give optimum results in the field are outlined. Efficacy studies should be conducted to determine the lowest possible dose to obtain consistently, statistically significant LD90-100 values.

The following are minimum study and reporting requirements (with guidelines) for evaluating the efficacy of vertebrate pesticides. They are additional to the general efficacy requirements above.

It is recognised that no two products are exactly alike; consequently, protocols may reflect design characteristics unique to a particular product and its mode of use. Regardless of a product's uniqueness, efficacy should be demonstrated through a combination of:

- cage studies and/or pen studies;
- field studies (actual use conditions).

Cage studies are more appropriate than pen studies for small animals (e.g. rodents) but pen studies will be appropriate for larger animals (e.g. possums or ferrets) during the initial stages of the assessment of a new active ingredient or formulation

3.1 General

3.1.1 Studies must be conducted under the supervision of appropriately qualified personnel, e.g. veterinarians or toxicologists, with support from trained animal technicians for cage and pen studies and wildlife management researchers for field studies.

3.1.2 For pen and cage studies, animals must be acclimatised. Acclimatisation is usually considered to be complete when the animals have established a pattern of regular daily consumption and body weight is stable or increasing.

3.1.3 The species of animal of interest (e.g. rat, possum) must be used. Extrapolation is possible if justified (e.g. laboratory rat to wild rat).

Study animals should be captive feral animals or appropriate surrogates (e.g. laboratory rats or wild rats).

Study animals should be healthy, active and sexually mature. Animals should fall into a specified weight category prior to the beginning of the study. Generally the weight variation in animals used in routine toxicity testing should not exceed ± 20% of their mean.[4]

3.1.4 All experimental feed or water must be clean and appropriate for the well-being of the animals (e.g. cereal pellets for rodents, meat for ferrets, cereals and vegetables for possums).

Water and feed should be provided *ad libitum*.

Feeders should be designed to minimise spillage. They should be maintained in such a way that feed intake can be measured.

The number of animals per feeder or water dispenser should be in keeping with international standards for toxicology studies, and will vary for each species.

3.1.5 The number of animals placed within each pen/cage must be reported.

3.1.6 Numbers, replicate numbers per experimental group, and the number of times the experiment is repeated will depend upon differences expected, and the results obtained.

3.1.7 Health check vaccination programmes, if warranted, should be implemented to protect the animals against prevalent infectious disease(s), prior to experimental use in order to protect the animals and the handlers.

3.1.8 Environments for cage studies should be as uniform as possible for all experimental groups in a study. Stringent control of environmental conditions and proper animal care techniques are essential for meaningful results. The behaviour of an animal, for example, can be adversely affected by unstable or unfavourable environmental conditions.

3.1.9 Sufficient blood clinical chemistry and histopathology in recently dead animals should be available to determine mode of action of the product in the target species. Pre-existing literature data may be used if the product is well established.

3.1.10 The method of sexing animals should be identified. Sufficient animals of each sex should be included to demonstrate whether there is a sex difference or not. If there is a sex difference, the focus of the toxicity testing and product development should be on the least susceptible sex of the animal.

3.1.11 The protocol should include a method that provides for:

- accountability for the animals;
- verifying the randomisation of animals to pens or cages;
- controlling animal/pen mix-ups;
- detecting migration or misplacement of animals;
- humane endpoints. (It is usual in such investigations to acknowledge the need for humane sacrifice of animals that are moribund or *in extremis*.[4])

3.1.12 The available pen area or cage size for study animals and other husbandry considerations should reflect minimum international standards for the welfare of experimental animals.

3.1.13 Animals should be observed with adequate frequency to appropriately manage the study, monitor the welfare of animals and collect dead animals for necropsy prior to decomposition.

A detailed descriptive account of the animal before, during, and after exposure to the test substance may reveal behavioural, physiological and pathological changes that can be used to assess the degree of suffering and pain. The timing of observations will be critical and should be performed at a similar frequency prior to, and throughout the course of the study. The observer should be familiar with the normal appearance, performance, and behaviour of the animal prior to the start of the study.

3.1.14 Any deaths of non-target animals during field experiments should be reported. The effects on non-target animals thought to be at risk should be considered. This may require laboratory studies and these animal populations may need to be monitored during field trials. The effects of secondary poisoning of non-target animals should also be considered for those animals that may not be detected during field monitoring.

3.2 Cage studies

3.2.1 Experimental design

3.2.1.1 Experimental groups must comprise:

- **non-medicated controls; and**
- **poisoned animals at different dose levels.**

3.2.1.2 Treatments should define LD50 and/or LD90.

If there is little or no information about the toxicity of a vertebrate pesticide, lethal dose levels should be established prior to mixing the toxicant into a bait. Once an LD50 or LD90 is obtained, 'no-choice feeding tests' should be conducted. The target species should be offered toxic bait without an alternative food source. All other food, including food scattered on the cage floor, must be removed during no-choice tests though water must be provided *ad libitum*. Candidate vertebrate pesticides that have performed well

in toxicity and no-choice tests should have their effectiveness more clearly determined by assessing the palatability of the toxic bait alongside an equivalent non-toxic formulation.

N.B. LD90 is more important than LD50 when defining the amount of pesticide that should be added to a bait.

3.2.1.3 The duration of the experiment must be sufficient to determine if there is a welfare concern involving any animal, including those surviving.

For example, for anticoagulant vertebrate toxicants a 28-day observation period is recommended. For all others, 14 days will allow for the evaluation of any untoward effects in survivors or delayed mortality.

3.2.1.4 Experimental subjects must be challenged with sufficient quantities of pesticide to cause death as swiftly and humanely as possible.

3.2.1.5 Cage studies should be conducted using both genders unless specific evidence is provided that demonstrates that a gender-toxicant interaction does not exist. Male and female animals should be caged separately.

3.2.1.6 Dose determination studies should be conducted.

3.2.1.7 Animals should be weighed at the beginning of the study.

3.2.1.8 Food consumption should be measured concurrently with body weight. If body weights are measured once per week, food consumption should still be measured daily.

3.2.1.9 A sanitation and biosecurity programme should be adopted to prevent the inadvertent introduction of pathogens.

3.2.1.10 Behavioural observations should be undertaken on a daily basis.

3.2.2 Pesticide or bait

3.2.2.1 The product must be freshly made or known to be in a stable condition.

Information on the shelf life and field life of the bait should be provided.

3.2.2.2 The concentration of each test substance must be verified in the concentrate used (active ingredient) and the final bait formulation.

3.2.2.3 Experimental subjects must be challenged with sufficient quantities of each pesticide for which a claim is made to ensure that the vertebrate pesticide kills the pest species, and to establish lethal dose levels and/or amounts of bait to kill an individual.

3.2.3 Reporting

3.2.3.1 The method of exposure of animals to the vertebrate pesticide must be described.

3.2.3.2 The following parameters must be reported:

- **feed and water intake;**
- **pathology reports;**
- **time to onset, duration and intensity of sickness behaviour prior to death;**
- **percentage mortality;**
- **time to unconsciousness;**
- **time to death and diagnosis of cause of death.**

3.2.3.3 Data that should be provided for registration purposes include:

Body weight:	recorded regularly throughout the study. (Daily body weights will not necessarily provide useful data and the regular disturbance and stress of the animals is likely to have other adverse effects, thus influencing toxicology and time to death.)
Food and water intake:	recorded on a daily basis.
Aspects of appearance:	e.g. condition and appearance of coat, eyes, nose, faeces and the posture of the animal.
Changes in vocalisation	
Changes in undisturbed behaviour	e.g. change in the sleep/wake cycle; feeding and drinking; characteristics such as gait, co-ordination, changes in movement or convulsion.
Changes in provoked behaviour	animals' response during routine husbandry to approach, capture and handling.
Physiological signs:	e.g. cardiac rate, respiratory rate, body temperature, sweating and degree of piloerection.
Postmortem examination:	e.g. inflammation, haemorrhage, enlarged or atrophied organs, congestion or obstruction in alimentary, respiratory or excretory tracts.

3.3 Pen studies

The use of groups of animals instead of single animals has advantages and disadvantages. Information about single animals is lost but, on the other hand, the influence of social interactions on the take of bait can be determined. However, to establish clear-cut efficacy results, social interactions are less important than bait consumption data for individual animals, which cannot be readily obtained with group housing.

3.3.1 Experimental design

3.3.1.1 Experimental groups must comprise at least:

- **non-medicated control;**
- **poisoned animals at different dose level.**

3.3.1.2 The duration of the study must be sufficient to determine if there is a welfare concern in any animal, including those surviving.

For example, for anticoagulant vertebrate toxicants a 28-day observation period is recommended. For all others, 14 days will allow for the evaluation of any untoward effects in survivors or delayed mortality.

3.3.1.3 Experimental subjects must be challenged with sufficient quantities of pesticide or bait for which a claim is made to ensure that the vertebrate pesticide kills the pest species.

3.3.1.4 Equal proportions of male and female animals should be reared together, or in separate pens depending on the behaviour of the target species.

3.3.1.5 Individual body weights should be recorded on all animals before treatment, and at death where applicable.

3.3.1.6 Weight gain or loss should be calculated.

3.3.1.7 Food consumption should be measured concurrently with body weight. If body weights are measured once per week, food consumption should still be measured daily.

3.3.2 Reporting

3.3.2.1 Method of exposure of animals to the pesticides and their means of identification must be described.

3.3.2.2 The following parameters must be reported:

- **pathology reports;**
- **time to onset, duration, and intensity of sickness behaviour prior to death;**
- **percentage mortality and species involved;**
- **time to death and diagnosis of cause of death.**

3.3.2.3 Data that should be provided for registration purposes include:

Body weight:	recorded regularly throughout the study. (Daily body weights will not necessarily provide useful data and the regular disturbance and stress of the animals is likely to have other adverse effects, thus influencing toxicology and time to death.)
Food and water intake:	recorded on a daily basis.
Aspects of appearance:	e.g. condition and appearance of coat, eyes, nose, faeces and the posture of the animal.
Changes in vocalisation	
Changes in undisturbed behaviour	e.g. change in the sleep/wake cycle; feeding and drinking; characteristics such as gait, co-ordination, changes in movement or convulsion.
Changes in provoked behaviour	animals' response during routine husbandry to approach, capture, and handling.
Physiological signs:	e.g. cardiac rate, respiratory rate, body temperature, sweating and degree of piloerection.
Postmortem examination:	e.g. inflammation, haemorrhage, enlarged or atrophied organs, congestion or obstruction in alimentary, respiratory or excretory tracts.

3.4 Field studies

3.4.1 Experimental design

3.4.1.1 A single study with adequate replication of treatments will suffice.

This may include the stratification of sites into forest and open country, for example, to give a more comprehensive result. Two (of the same) treatment and two control sites (at least) are preferable to analyse results.

3.4.1.2 A control group (area) must be included. Significant mortality in the control area would negate the study, even if 100% mortality has been achieved in the test group.

3.4.1.3 A validated method of monitoring wild animal populations before and after use of the vertebrate pesticide containing bait must be used.

An appropriate species-specific monitoring technique to determine efficacy by monitoring number before and after use of bait should be used (e.g. determining the mortality of radio-collared wild animals is the most accurate technique for efficacy assessment).

3.4.1.4 Where a dose range is to be stated on the product label, field efficacy studies must include studies using the lower dose rate. The reason for having lower and higher doses in the range must be justified and included on the label.

Field efficacy studies should include replicates at different rates of application of the product.

3.4.1.5 Replicated field studies should be conducted to confirm the efficacy of a new toxic bait type and baiting strategy as able to kill at least 80% and preferably 90% of the population present.

3.4.1.6 Treatment groups should use:
- experimental block where animals are controlled;
- a matched block where no control occurs.

3.4.1.7 Field efficacy studies should include studies at different rates of application of the product.

3.4.2 Reporting

The following parameters must be reported:
- **details of the formulation used;**
- **mortality and species involved;**
- **environmental conditions during the study, temperature, rainfall, date.**

It has been established that there can be significant seasonal variation in the baiting success for some species (e.g. ferrets); hence the season, topography, temperature, rainfall, and habitat must be reported.

4 FURTHER READING

1. EPA (1982). *Pesticide Assessment Guidelines, Subdivision G: Product Performance*. United States Environmental Protection Agency, Washington, USA. Documents can be ordered from the EPA (http://www.epa.gov/epahome/publication).

2. EPA (1984). *Pesticide Assessment Guidelines, Subdivision F: Hazard Evaluation: Human and Domestic Animals*. United States Environmental Protection Agency, Washington, USA. Documents can be ordered from the EPA (http://www.epa.gov/epahome/publication).

3. EPPO (1982). *Guidelines for the Biological Evaluation of Rodenticides. Laboratory Tests for Evaluation of the Toxicity and Acceptability of Rodenticides and Rodenticide Preparations.* European and Mediterranean Plant Protection Organization, Set 10, No.113.32 p. Documents can be ordered from the EPPO (http://www.eppo.org/html/books.html#guidelin).

4. OECD (1987). Organization of Economic Cooperation and Development (Paris). Guidelines for testing of chemicals. Section 4 (Health Effects). (3rd addendum to the 1981 edition). Documents can be ordered from the OECD (http://www.oecd.org/ehs/test/health.htm).

APPENDIX A

CONTENT OF AN EFFICACY DATA PACKAGE SUMMARY

1 Identity

1.1 Applicant

1.2 Trade name of product

1.2 Active ingredient(s) and concentration

1.3 Chemistry

Provide references to the section(s) in the chemistry data package that describe the properties of relevance to the assessment of efficacy.

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2 Proposed use pattern

2.1 Use situation

2.2 Administration method

2.3 Dosage

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3 Studies

Provide a concise summary of all efficacy studies provided and their conclusions, including the level of efficacy proven and the statistical methods used.

APPENDIX B

CONTENT OF A RISK ASSESSOR'S REPORT FOR EFFICACY DATA

CONCLUSIONS

1 Identity

1.1 Applicant

1.2 Trade name of product

1.3 Active ingredient(s) and concentration

1.4 Chemistry

Comment on the key properties of the active ingredient(s) that impact on the efficacy of the trade name product.

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2 Proposed use pattern

2.1 Use situation

2.2 Administration method

2.3 Dosage

Comment on the appropriateness of the dosages proposed in terms of efficacy of the product.

2.4 Changes to practice

Include comments on any specific aspects of proposed use that are novel or at variance with similar compounds or common practices.

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3 Supporting data

3.1 Provide a concise statement on the quantity, quality, validity and completeness of the supporting data. Identify the level of efficacy proven.

3.2 Comment on the suitability of the method(s) of statistical analysis used.

3.3 If both New Zealand and overseas study data are submitted, indicate the level of consistency between them.

3.4 Advise whether the data are sufficient to assess efficacy of the trade name product.

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4 Conformance

State whether the supporting data conform to the *ACVM Registration Standard and Guideline for Efficacy of Vertebrate Pesticides*, the *ACVM Research Standard*, guidelines and information waivers. Where information waivers have been granted, comment on their impact.

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5 Risk statements

Provide a statement on the risk of the proposed use resulting in animal welfare thresholds being exceeded as a result of inefficacy.

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6 Further work or information

Identify any work that may reduce the level of uncertainty to an acceptable level, assist in the explanation or extrapolation of the data or provide a more complete database.

Assessor's name:
Signature:
Date:
