# Anticoagulant resistance in rodents

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Published by
Department of Conservation
Head Office, PO Box 10-420
Wellington, New Zealand

This report was commissioned by Science & Research Unit.

ISSN 1171-9834



Reference to material in this report should be cited thus:

Bailey, C. I.; Eason, C.T., 2000.
Anticoagulant resistance in rodents. *Conservation Advisory Science Notes No. 297,* Department of Conservation, Wellington.

Keywords: rodent control, anticoagulant resistance, warfarin, brodifacoum, resistance testing.

# Abstract

Current issues in rodenticide anticoagulant resistance testing are reviewed.

Research and management issues for New Zealand agencies include:

1. Assessing and monitoring the spread of resistance.

2. Standardising and improving resistance-testing techniques.

3. Identifying from the range of tests that have been developed for assessing rodenticide resistance which is the best to use.

4. Developing use patterns for anticoagulants, non-anticoagulant poisoning, and trapping to minimise the development of resistant populations of rodents.

Resistance to anticoagulants can develop in a population after 5-10 years sustained use of anticoagulant rodenticides. Monitoring the resistance status of rodent populations is a pivotal component of integrated risk management. No data exist on the baseline susceptibility of rodent populations in New Zealand to anticoagulants or their changing patterns of susceptibility in areas of sustained use. Monitoring systems for wild target populations and changes to poisoning methods will assist New Zealand rodent control groups in avoiding the resistance-induced control problems now seen overseas. We recommend the `blood clotting response test' (BCRT) as an integrated component of rodent control to maximise efficacy and safety of rodent population management.

# 1. Introduction

Anticoagulant rodenticide resistance is a worldwide phenomenon that occurs after sustained use of anticoagulant toxicants for rodent control. Landcare Research has briefly reviewed current developments in this field. This was facilitated by (i) attendance at the 2nd European Vertebrate Pest Management Conference and (ii) a QEII Technician's Scholarship to work in three leading centres in Europe involved in research and monitoring of anticoagulant resistance.

Resistance was first recognised following prolonged use of warfarin in the UK and has subsequently been detected throughout the world to a range of first- and some second-generation anticoagulants.

# 2. Background

The Department of Conservation (DOC) is committed to eradication or control of rodents to protect endangered native birds. Sustained control of rodents on the mainland is likely to be substantially dependent on toxicants, and anticoagulant poisons in particular, for the foreseeable future.

# 3. Objective

To briefly review current research and management knowledge relating to anticoagulant (poison) resistance in rodents, including the latest ideas with regard to resistance development, monitoring, and mitigation.

# 4. Methods

Material for this short review was gathered from published literature, a symposium on rodenticide resistance (2nd European Vertebrate Pest Management Conference), and through discussions with overseas anticoagulant researchers during a QEII Technicians Scholarship project in Europe (October 1999). The mode of action of anticoagulant poisons and hence the mechanisms of resistance are described. Methods for testing for resistance are discussed and strategies for monitoring resistance in populations outlined. Finally we present recommendations that are of particular relevance to sustained mainland island control.

# 5. Results

## 5.1 PREVIOUS ANTICOAGULANT RODENTICIDE RESEARCH IN NEW ZEALAND

Prior to 1999 the focus of rodent control research in New Zealand has been to (i) optimise baiting strategies for island eradication programmes and sustained rodent control on mainland islands; and (ii) to develop an understanding of the fate and non-target impact of rodenticides. The broad-scale field use of brodifacoum has raised concern regarding the toxicant's tendency to bioaccumulate in non-target species. This has led to some recent changes in the use of brodifacoum, in particular, within the framework of sustained rodent control on the mainland. Regardless of this, anticoagulant poisons are likely to remain a pivotal component of rodent control strategies. Hence,

anticoagulant resistance will potentially become an increasingly important issue.

## 5.2 MODE OF ACTION OF ANTICOAGULANTS

The anticoagulant action of rodenticides arises from inhibition of vitamin K metabolism in the liver (Bell & Caldwell 1973; Zimmerman & Matschiner 1974). Vitamin K is essential for the production of several blood-clotting proteins and, when greatly reduced in concentration, results in fatal haemorrhaging.

Vitamin K in its reduced form (vitamin K hydroquinone) is a co-factor for a carboxylase active in the production of proteins such as clotting factors II, VII, IX, and X. During this process, vitamin K is oxidised to vitamin KO and is then unavailable until recycled to vitamin K hydroquinone by the enzyme vitamin K epoxide reductase (VKOR). It is this enzyme that is inactivated by the action of anticoagulants, which have a similar structure to vitamin K and bind strongly to the enzyme, leaving it unavailable for the recycling of vitamin KO.

In animals resistant to anticoagulants, VKOR has become modified through point mutations of the DNA so that, while still remaining functional, it displays a reduced affinity for the toxicant or the toxicant is more easily replaced by the vitamin KO (Bell & Caldwell 1973; Zimmerman & Matschiner 1974). This modification is inheritable.

## 5.3 TOLERANCE V. RESISTANCE

Genetic variability of populations results in varying degrees of tolerance towards toxicants. Populations that have been subjected to sustained control using anticoagulants may exhibit an average $LD_{50}$ that is much higher than a newly treated population, but this is not resistance. In these cases, the population (after several generations) has had some of its less tolerant animals removed.

Resistance results from a random genetic event. It will become prevalent in populations that are strongly selected by application of the toxicant, killing most non-resistant individuals and allowing resistant animals a greater influence in the genetic pool of future generations. It is expected that, after such a selection commences, resistance will be observable in populations within approximately 10 generations (depending on the continuance of toxicant application) (A.D. MacNicoll, Central Science Laboratories, Sand Hutton,York, UK, pers. comm.).

The possibility of the development of resistant populations (in New Zealand mainland islands) underpins the importance of establishing a database containing:

- baseline data for future comparison,

- information on areas and level of exposure to anticoagulants,
- data on the susceptibility of previously untreated populations and those exposed by previous and/or current use of anticoagulant rodenticides.

Resistance to warfarin has been widely observed overseas. In these cases animals may freely feed on bait containing warfarin and show no harmful effects (MacNicoll 1993; Quy et al. 1995). There is, however, some debate over whether resistance to brodifacoum and other second-generation anticoagulants can develop. Regardless of this, a programme of selective resistance testing will allow DOC managers to pre-empt future difficulties.

## 5.4 TESTING FOR RESISTANCE

The techniques for monitoring resistance are evolving and currently include:

- feeding testing,
- blood clotting response testing (BCRT),
- hepatic vitamin K epoxide reductase (VKOR) assessment,
- specific genotypes that are markers of resistance.

For all the above tests the most useful comparative data will be obtained by trapping wild rodents and maintaining them under standardised dietary and other husbandry conditions in a laboratory environment prior to testing. There are advantages and disadvantages to all of these methods; these were extensively reviewed at the 2nd European Vertebrate Pest Management Conference, and are discussed below. The BCRT is the most widely used method of monitoring resistance, and considerable international effort has been expended in recent years to standardise this test method so that datasets and trends from around the world relative to the susceptibility of rodent populations to different anticoagulants can be compared. New Zealand has the opportunity, through the links established in this project, to become part of an international effort targeting the monitoring and mitigation of rodenticide resistance.

### Feeding testing (Drummond & Wilson 1968)

Captured wild animals are caged, allowed to acclimatise, and subjected to a 7-day or longer feeding regime where the food given contains 0.0005% toxicant (Gill & MacNicoll 1991). Surviving animals are considered resistant.The rationale behind this test is that animals surviving in the laboratory would have survived in a field situation. This type of trial is expensive, time-consuming, and ethically difficult. The standardised OECD testing protocols have a number of features that have caused some debate:

- Caged animals are limited to eating bait alone and thus may receive much higher doses than field situations.

- Caged animals are limited in their ability to move about. Activity is likely to enhance the likelihood of lethal haemorrhaging.

**Blood clotting response testing (BCRT) (Kerins et al. 1993)**

Captured wild animals are caged, allowed to acclimatise, subjected to a single discriminating dose of toxicant, a blood sample is collected, and clotting time assayed. A further sample is taken at 24 hours and again assayed for clotting time. Animals that have markedly reduced clotting activity are considered susceptible, while those showing shorter clotting times are considered resistant.This testing method is much swifter in obtaining results and causes little suffering in the test animals.

**Hepatic vitamin K epoxide reductase (VKOR) assessment (MacNicoll 1985; Thijssen et al. 1986)**

In all observed cases of resistance, modified biochemistry of the VKOR enzyme is observed. This produces either reduced affinity for the toxicant or allows easier replacement of the toxicant by vitamin KO. Hepatic VKOR assessment is carried out in vitro by monitoring the activity of VKOR in the presence and absence of the toxicant. Susceptible samples show minimal VKOR activity when anticoagulant is present, while enzyme activity in resistant samples remains above 20% of original levels.

**Genotypic testing**

Attempts have been made to identify the genomic changes that give resistance to anticoagulants. Even once established, this form of testing may never be useful other than to identify which form of a known resistance is present. This test would be specific for each resistance-causing mutation identified, but would not detect new mutations that might result in resistance.

Overseas laboratories are presently using the first three methods in identifying resistance in rodents. Landcare Research has developed the capability to undertake all three tests, and has developed considerable experience with BCRT during 1999/2000.

## 5.5 DEALING WITH RESISTANT POPULATIONS

As few data are currently available on the resistance status of rodents in New Zealand, any observed failure of poisoning may be due to resistance or may be the result of operational difficulties or population tolerance.

After locating resistant populations and determining the spectrum of resistance, strategies to deal with a resistant population must be developed. A commonly held and heavily debated point of view is that stopping the use of anticoagulants will result in the disappearance of all resistance because such animals are less fit to survive. This is based on studies of Norway rats *(Rattus norvegicus),* which showed that resistant animals had reduced fitness/sur-

vival. Recent work with new strains of resistant animals shows no reduction in fitness, thus throwing doubt on this hypothesis.

There is considerable debate as to how anticoagulant rodenticides should be best deployed to avoid the production of resistant populations. Some hold the view that weaker anticoagulants are more likely to permit the faster development of resistant populations due to a greater number of survivors to any dosing regime. They then argue that potent anticoagulants (such as brodifacoum) should be used exclusively. However, such a strategy can be expected to lead to an increasing incidence of non-target species poisoning or contamination (Eason et al. 1996).

The most obvious strategy to avoid and mitigate resistance is to integrate anticoagulant and non-anticoagulant rodenticides. This of course reintroduces the problems related to the latter that made anticoagulant use so popular (i.e. bait shyness). Similarly, use of brodifacoum in a pulse fashion (a cycle using warfarin followed by short-term use of brodifacoum) could function in a similar fashion while reducing environmental exposure to brodifacoum. There is scope for applied research on optimum baiting strategies for rodent control and rodenticide use in New Zealand.

# 6. Conclusions

We can expect that, using current poisoning methods, resistance to anticoagulants in New Zealand rodent populations will develop over time.

Opportunities exist to limit the rate of onset and severity of anticoagulant rodenticide resistance in New Zealand. Selective monitoring of populations using BCRT is necessary to establish the current situation and provide wildlife managers with information that they can integrate into their management strategies. The capability of monitoring rat BCRT response to anticoagulants has been developed as part of DOC Research Investigation No. 3111 (Non-invasive determination of anticoagulant effects). The first stage of this study, which includes method validation before and after repeated exposure to sub-lethal doses of brodifacoum, has been successfully completed in laboratory rats using the BCRT methodology.

# 7. Recommendations

Targeted resistance-testing of New Zealand rodents to anticoagulant poisons should be introduced. Primary sites for monitoring should include areas where anticoagulant poisons have been used for an extended period of time, and areas where there is known to have been very little or no use of anticoagulants.

There are valuable opportunities to establish baseline susceptibility in naive populations and trends in areas where there has been anticoagulant use for several years. Testing using the BCRT will provide an indication of trends far more clearly than field efficacy data, which can be confounded by environmental (e.g. weather) and operational (e.g. poor bait quality) effects. Early warning of impending resistance can be used to trigger control practice aimed at avoiding or mitigating resistance.

Changes to poisoning strategies should be implemented (Quy et al. 1995), including alternating between anticoagulant and other types of poison.Total exclusion of potent anticoagulants such as brodifacoum from such poisoning protocols may be counter-productive, since there may be a requirement for the use of brodifacoum in populations that are becoming resistant to other anticoagulants and shy to baits containing non-anticoagulant. Population modelling based on actual field data of bait uptake, frequency of use, and efficacy should be used to predict optimum strategies for susceptible and resistant populations.

# 8. Acknowledgements

This research was funded by the Department of Conservation. Dr C.T. Eason's attendance at the 2nd European Vertebrate Pest Management Conference was funded by Landcare Research. C. I. Bailey's research at CSL York, UK, was funded by the QEII Technician's Scholarship programme.

# 9. References

Bell, R.G.; Caldwell, P.T. 1973: Mechanism of warfarin resistance. Warfarin and metabolism of vitamin K1. *Biochemistry 12:* 1759-1762.

Drummond, D.C.; Wilson, E.J. 1968: Laboratory investigations of resistance to warfarin of *Rattus norvegicus* Berk in Montgomeryshire and Shropshire. *Annals of Applied Biology 61:* 303-312.

Eason, C.T.; Wright, G.R.; Batcheler, D. 1996: Anticoagulant effects and the persistence of brodifacoum in possums *(Trichosurus vulpecula). New Zealand Journal of Agricultural Research 39*: 397-400.

Gill J E.; MacNicoll, A.D. 1991: Determination of the susceptibility of wild populations of Norway rat *(Rattus norvegicus)* to the anticoagulant rodenticide brodifacoum. *Zeitschrift fur Angewandte Zoologie 78*: 101-117.

Kerins, G.M.; Langton, S.D.; MacNicoll A.D. 1993: The development of a blood clotting response test for discriminating between difenacoum resistant and susceptible Norway rats *(Rattus norvegicus* Berk.). *Comparative Biochemistry and Physiology 104C*: 29-36.

MacNicoll, A.D. 1985:A comparison of warfarin resistance and liver microsomal vitamin K epoxide reductase activity in rats. *Biochimica et Biophysica Acta 840*: 13-20.

MacNicoll, A.D. 1993:Anticoagulant rodenticides : tolerance and resistance. *Phytoparasitica 21:* 185-188.

Quy, R.J.; Cowan, D.P; Prescott, C.V ; Gill, J.E.; Kerins, G.M.; Dunsford, G.; Jones, A.; MacNicoll, A.D. 1995: Control of a population of Norway rats resistant to anticoagulant rodenticides. *Pesticide Science 45*: 247-256.

Thijssen, H.H.W; Janssen, C.A.T.; Drittij-Reijnders, M.J. 1986: The effect of S-warfarin administration on vitamin K 2,3-epoxide reductase activity in liver, kidney, and testis of the rat. *Biochemical Pharmacology 35:* 3277-3282.

Zimmerman, A; Matschiner, J.T. 1974: Biochemical basis of hereditary resistance to warfarin in the rat. *Pharmacology 23:* 1033-1040.