Review of house mouse (*Mus musculus*) susceptibility to anticoagulant poisons

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ABSTRACT

Appropriate baits, toxicants and management strategies have yet to be developed and tested for the control of field populations of house mice (*Mus musculus*) on the mainland of New Zealand. A review of toxicological information regarding the susceptibility of house mice to different anticoagulants was carried out, as a first step in assessing effective toxic loadings in bait for mouse control. In laboratory assessments of toxicity, house mice are generally less susceptible to anticoagulants than rats (*Rattus* spp.), and female mice seem to be less susceptible than male mice. Mice are most susceptible to second-generation anticoagulants, particularly brodifacoum, and more susceptible to first-generation anticoagulants in small consecutive oral intakes. However, the efficacy of toxic baits used for field control of mice needs to be balanced against their potential adverse effects on non-target species and the environment. On the basis of reviewed information, an efficacy and risk assessment study should be conducted for mice, comparing diphacinone with single-feed formulations of a second-generation anticoagulant.

Keywords: House mouse, *Mus musculus*, anticoagulant, efficacy, toxicity, New Zealand.

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1. Introduction

Anticoagulant poisons are used worldwide for the control of commensal rodents (rats and mice). In New Zealand especially, anticoagulants have also been used successfully for the eradication of field populations of rodents on offshore islands, and on the mainland for field rodent control. Baiting operations targeting 'rodents' have generally been prescribed for rat (*Rattus* spp.) control, and where island eradications of mice (*Mus musculus*) have been achieved incidentally to rat control, the reasons for success are not well understood. Appropriate baits, toxicants and management strategies have yet to be developed and tested for the control of field populations of house mice (*Mus musculus*) on the mainland of New Zealand.

Anticoagulants were considered the most appropriate toxicants to begin with because of their already widespread applications for control of commensal rodent populations and delayed onset of toxic action, which is thought to reduce the occurrence of bait-shyness and 'stop-feed' behaviour in target rodents. This is an important characteristic for a toxicant that could be re-applied to provide sustained control of mouse populations. This review of published anticoagulant lethal doses for mice is intended to begin the process of identifying appropriate toxicant(s) for mouse control in New Zealand, and associated information gaps. Toxicological information regarding the susceptibility of house mice to different anticoagulants was sought in the scientific and industry literature. Non-anticoagulant poisons, such as 1080, zinc phosphide and cholecalciferol have previously been used as rodenticides, but are not considered in this review. Zinc phosphide and 1080 toxicosis can cause learned aversion in survivors due to the rapid onset of symptoms (Kaukeinen et al. 2000), while cholecalciferol products for rodent control require relatively high concentrations of active ingredient, which may lead to bait aversion (Kaukeinen et al. 2000).

2. Background

Anticoagulants act by interfering with the normal synthesis of vitamin K-dependent blood-clotting factors in the liver. Clinical signs in mice and rats, as in other mammals undergoing anticoagulant toxicosis, usually reflect some degree of haemorrhage and commonly include anaemia and weakness. The main advantages of using anticoagulants as rodenticides are that death occurs several days after ingestion of bait (so that rodents eating anticoagulant baits do not develop learned aversion/bait shyness), and that Vitamin K_1 (phylloquinone) is an effective antidote. Anticoagulants can be classified according to chemical structure, as indandiones or coumarins, and also as first- or second-generation compounds, according to when they were developed (Fig. 1). The weaker potency of first-generation anticoagulants is related to a

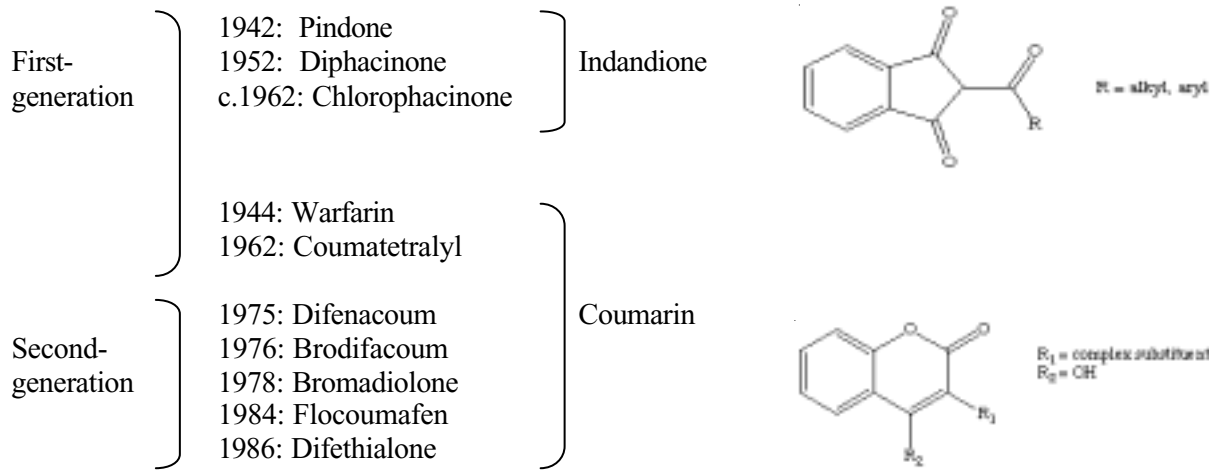

Figure 1. Summary of the dates of development as commercially available products for first- and second-generation anticoagulant rodenticides, and their grouping by chemical structure (inandione or coumarin).

generally lower binding affinity for sites in the liver when compared to second-generation compounds (Parmar et al. 1987). The main advantage of the second-generation rodenticides is their potency which makes them highly effective, even against warfarin-resistant rats. Primary and secondary poisoning of non-target species is a risk particularly with the second-generation anticoagulants because they are very toxic and persist in some body tissues. In comparison, the first-generation anticoagulants are more rapidly metabolised and excreted, and there is less risk to non-target species.

3. Objectives

The objective of this study was to review the susceptibility of the house mouse to anticoagulant poisons.

4. Methods

The scientific literature on the range of anticoagulant rodenticides was reviewed. Each anticoagulant compound was described in terms of its basic chemistry (Chemical Abstracts nomenclature, physical form, melting point and water solubility). A brief history, background of use as a rodenticide (especially against mice), and formulations were described. Generally, emphasis was given to searching for literature about the anticoagulants registered and used in New Zealand, although other anticoagulants used internationally were also considered.

A standard index of toxicity following ingestion of a pesticide is the lethal dose (LD_{50}). This is the amount of pesticide required to kill 50% of a population of animals. The oral LD_{50} value in bird and mammals is expressed as parts per million (ppm), equivalent to a milligram of pesticide per kilogram of animal bodyweight (mg/kg). Acute oral toxicity (LD_{50}) values for anticoagulants in rodents and especially for *M. musculus* were sought. If no published toxicity values for an anticoagulant compound were found for house mice, available single- or multiple-dose toxicity values for other rodent species, e.g. Norway (*Rattus norvegicus*) or black (*R. rattus*) rats, were noted. Despite recognised interspecific differences in acute toxicity, rats were considered the nearest indicative species for 'starting' susceptibility estimates if no values were available for mice. Based on available toxicity data for mice, the amounts of nominated bait formulation for each anticoagulant that mice would have to eat to consume twice an LD_{50} dose were then estimated.

5. Review

5.1 FIRST-GENERATION ANTICOAGULANTS

5.1.1 Pindone

2-(2,2-dimethyl-1-oxopropyl)-1H-indene-1,3(2H)-dione; C14H14O3; Molecular weight 230.3

Pindone is a yellow crystalline powder with a melting point of 108.5°C–110.5°C, with low solubility in water (18 mg/L at 25°C). The sodium salt (pival or pindone-sodium) is readily water soluble. Pindone was synthesised in 1942 and developed as a pesticide in the early 1940s. Pindone has been used worldwide to control rodents, but the introduction of the more potent first-generation indandiones, diphacinone and chlorophacinone (see below), and later the second-generation anticoagulants, contributed to a reduction in pindone use for rodent control. Tradenames worldwide include 'Pival' and 'Pivalyn' (the sodium salt). The susceptibility of mice to pindone is not known (Table 1), although it is likely that its toxicity to mice is enhanced when it is taken in small, consecutive doses, as for other first-generation anticoagulants. In New Zealand, ready-to-use baits for rabbit (*Oryctolagus cuniculus*) and possum (*Trichosurus vulpecula*) (but not rodent) control contain 0.025% to 0.05% pindone (w/w), and a soluble concentrate for addition to baits contains 3.4%w/v. Trade/product names registered in New Zealand include 'Pindone Rabbit Pellets' and 'Pindone Possum Pellets'. Use of pindone baits in New Zealand field applications were reported to produce 'mixed' and 'reliable' success in reducing rat tracking rates to < 5% in Northern Te Urewera (800 ha) and in Boundary Stream (800 ha) 'Mainland Islands' respectively (Gillies 2002). No reports of applications against field populations of mice were found.

5.1.2 Diphacinone

2-(diphenylacetyl)-1H-indene-1,3(2H)-dione; C23H16O3; Molecular weight 340.4

TABLE 1. SINGLE (mg/kg) AND MULTIPLE-DOSE (mg/kg/DAY) ORAL TOXICITY (LD_{50}) VALUES FOR FIRST-GENERATION ANTICOAGULANTS FOR HOUSE MICE AND OTHER RODENT SPECIES. DETAILS OF STRAIN ARE PROVIDED, WHERE KNOWN. UNLESS OTHERWISE INDICATED, LD_{50} VALUES PERTAIN TO BOTH SEXES.

COMPOUND	SPECIES	ORAL LD_{50} (mg/kg)	REFERENCE
Pindone	House mouse	NA[1]	-
	Southern bush rat (*R. fuscipes*)	2–8 for 5 days	Twigg et al. 1999
	Norway rat	50	Dubock & Kaukeinen 1978
	Norway rat	75–100	Eason & Wickstrom 2001
	Norway rat (lab. strain)	280 (233–336)[2]	Gaines 1960
Diphacinone	House mouse (lab. strain male)	0.42 for 5 days	Ashton et al. 1987
	House mouse (lab. strain female)	2.83 for 5 days	Ashton et al. 1987
	House mouse (lab. strain both sexes)	1.41 for 5 days	Ashton et al. 1987
		28.3 (25–32)[2]	Kusano 1974
	House mouse (lab. strain female)	30 (26–34.7)[2]	Kusano 1974
	House mouse (lab. strain male)	340	Correll et al. 1952
	House mouse		
Chlorophacinone	House mouse	NA[1]	-
	Deer mouse (*Peromyscus maniculatus*)	0.49	PMEP 2001
		6.26	Tomlin 2000
	Norway rat (assume lab. strain)	20.5	PMEP 2001
	Norway rat (assume lab. strain)	2.1	PMEP 2001
	Norway rat (assume lab. strain)		
Warfarin	House mouse (lab. strain)	374	Hagan & Radomski 1953
	Norway rat (lab. strain)	1 for 5 days	Tomlin 2000
	Norway rat (lab. strain)	3.0 (2.3–3.8)[2]	Gaines 1960
	Norway rat (lab. Sherman strain)	14.5–186	Cited in Erickson & Urban 2002
	Norway rat (lab. strain male)	323	Hagan & Radomski 1953
	Norway rat (lab. strain female)	58	Hagan & Radomski 1953
	Norway rat (lab. strain male)	100	Cited in Erickson & Urban 2002
	Norway rat (lab. strain female)	8.7	Cited in Erickson & Urban 2002
Coumatetralyl	House mouse	>1000	Tomlin 2000
	House mouse	2000-4000	Popischil & Schnorbach 1994
	House mouse	3.5 for 18 days	Popischil & Schnorbach 1994
	Norway rat (lab.strain)	16.5	Dubock & Kaukeinen 1978
	Rat (assume *R. norvegicus* lab. strain)	0.3 for 5 days	Popischil & Schnorbach 1994

[1] NA indicates no published toxicity value sourced.
[2] Where available, 95% lower and upper confidence intervals of values are shown in brackets.

Diphacinone is a yellow crystalline powder melting at 145–147°C and is practically insoluble in water (0.3 mg/L, assume at 20°C). The rodenticidal activity of diphacinone was first described in 1952. In terms of available data, the toxicity of diphacinone to mice is the best characterised of the first-generation anticoagulants and it appears to have a similar oral toxicity to that of warfarin (Table 1). Diphacinone is produced and primarily used in the USA, formulated as weather-resistant baits (pellets or meal) to control mice, rats, prairie dogs, ground squirrels, voles, and other rodents (Tomlin 2000). In New Zealand, registered bait products for rodents contain 0.005% diphacinone (w/w), and a paste bait for ferret control contains 0.03% (w/w). Formulations include grain and ready-to-use block bait for the control of commensal rodents. Trade/product names of diphacinone baits registered, or being used under permit, for rodent

control in New Zealand are 'Pest-Gone Rodent Bait' and 'Ditrac'. No reports of field applications of diphacinone against rodents in New Zealand were found; however, diphacinone is currently undergoing registration in the United States of America, for aerial applications in Hawaii for broad-scale rat control (e.g. Lindsey & Mosher 1994; Nelson et al. 2002).

5.1.3 Chlorophacinone

2-[(4-chlorophenyl)phenylacetyl]-1H-indene-1,3(2H)-dione; C23H15ClO3; Molecular weight 374.8

Chlorophacinone forms pale yellow crystals, with a melting point of 140°C, and which are slightly soluble in water (100 mg/L at 20°C). Chlorophacinone was developed in the early 1960s as a single-dose rodenticide. No toxicity values for chlorophacinone in mice were found. International trade names include 'Redentin', 'Rozol', 'Ramucide' and 'Ramik', with formulations for commensal rodent control containing 0.005% to 0.025% chlorophacinone (w/w). Chlorophacinone is not registered as a vertebrate pesticide in New Zealand.

5.1.4 Warfarin

4-hydroxy-3-(3-oxo-1-phenylbutyl)-2H-1-benzopyran-2-one; C19H16O4; Molecular weight 308.3

Warfarin forms colourless crystals with a melting point of 161–162°C. It has very low solubility in water (17 mg/L at 20°C), although the sodium salt is fully soluble in water (400 g/L). The anticoagulant properties of warfarin were first reported in 1944, with the (*S*)-isomer having seven-fold greater rodenticidal activity than the (*R*)-isomer (Tomlin 2000). It was the first anticoagulant to be developed and widely used for the control of commensal rodents. Only one (acute) oral toxicity value of 374 mg/kg was found for mice and the bulk of data pertain to toxicity in rat species. It is assumed that, as in rats, the toxicity of warfarin to mice is enhanced when it is taken in small consecutive doses, as for other first-generation anticoagulants. International trade/product names include 'Sakarat', 'd-Con' and 'Rodex', and include concentrate formulations for mixing with bait material. Loadings of warfarin in baits vary from 0.1% to 1% (w/w) for use in rodent holes and runs. Warfarin is not currently listed by the Agricultural Compounds and Veterinary Medicines (ACVM) group of the New Zealand Ministry of Agriculture and Forestry (MAF) as a registered, commercially available vertebrate pesticide in New Zealand. However, trial field applications in New Zealand of warfarin in cereal pellet baits (0.05% w/w) against rat and mouse populations had had mixed or short-term success at suppressing mouse numbers (Gillies 2002).

5.1.5 Coumatetralyl

4-hydroxy-3-(1,2,3,4-tetrahydro-1-napthalenyl)-2H-1-benzopyran-2-one; C19H16O3; Molecular weight 292.3

Coumatetralyl forms colourless to yellowish crystals, with a melting point of 172–176°C and a low-to-good solubility in water, depending on pH (4 mg/L at pH 4.2, 20 mg/L at pH 5 and 425 mg/L at pH 7; all at 20°C). Coumatetralyl was developed and introduced as a rodenticide in the 1960s by Bayer AG. The

available single-dose (acute) toxicity figures for coumatetralyl in mice appear relatively high (indicating lower toxicity), in comparison to the reported toxicity of coumatetralyl to rats and to the other first generation anticoagulants to mice. Like other first-generation anticoagulants, coumatetralyl has increased toxicity to mice in successive intakes (Table 2). In New Zealand, registered bait products contain 0.0375% to 0.74% coumatetralyl (w/w). Formulations include tracking powder, and ready-to-use block and paste bait for the control of commensal rodents. Trade/product names of coumatetralyl baits registered in New Zealand are 'Racumin' and 'No Rats and Mice'. In a comparative pen trial of different anticoagulant baits, wild house mice ate approximately one-third less of 'Racumin' than of two other anticoagulant bait formulations ('Pestoff' and 'Talon 50WB'), indicating that increased palatability of this product would be required for consistent consumption of a lethal dose by mice (O'Connor & Booth 2001).

TABLE 2. SINGLE-DOSE (mg/kg) ORAL TOXICITY (LD_{50}) VALUES FOR SECOND-GENERATION ANTICOAGULANTS FOR HOUSE MICE AND OTHER RODENT SPECIES. DETAILS OF STRAIN ARE PROVIDED, WHERE KNOWN.UNLESS OTHERWISE INDICATED, LD_{50} VALUES PERTAIN TO BOTH SEXES.

COMPOUND	SPECIES	ORAL LD_{50} (mg/kg)	REFERENCE
Brodifacoum	House mouse (assume lab. strain)	0.4	Godfrey 1985
	House mouse (wild NZ)	0.52	O'Connor & Booth 2001
	Norway rat	0.27	Godfrey 1985
	Norway rat (lab. strain male)	0.41 (0.35–0.50) [1]	US EPA 1998
	Norway rat (lab. strain female)	0.56 (0.47–0.66) [1]	US EPA 1998
	Black rat	0.77	Mathur & Prakash 1981
	Ship rat (wild NZ)	0.46	O'Connor & Booth 2001
	Kiore (R. exulans, wild NZ)	0.32	O'Connor & Booth 2001
Bromadiolone	House mouse (lab. strain)	1.75 (0.2–3.3) [1]	Erickson & Urban 2002
	House mouse (T.O. strain male)	0.86	Erickson & Urban 2002
	House mouse (T.O. strain female)	1.13	Erickson & Urban 2002
	House mouse (T.O. strain both sexes)	0.99	Erickson & Urban 2002
	Norway rat (Wistar strain both sexes)	0.65	Meehan 1978
	Norway rat (lab. strain)	1.13	Poche 1986
Flocoumafen	House mouse	0.8	Tomlin 2000
	Norway rat (assume lab. strain)	0.25	Huckle et al. 1989
	Norway rat (assume lab. strain)	0.46	WHO 1995
	Rice rat (*R. argiventer*, male)	0.25	Lam 1990
	Rice rat (*R. argiventer*, female)	0.37	Lam 1990
Difenacoum	House mouse (lab. strain male)	0.8	Bull 1976
	Norway rat (wild male)	2.5	Bull 1976
	Norway rat (wild male)	3.5	Bull 1976
	Norway rat (lab. strain)	1.8	Dubock & Kaukeinen 1978
	Roof rat (*R. rattus minadensis*, male)	7.0	Bull 1976
	Roof rat (*R. rattus minadensis*, female)	2.5	Bull 1976
	Rice rat (*R. argiventer*)	0.7	Bull 1976
Difethialone	House mouse (assume lab. strain)	1.29	Tomlin 2000
	Rat (assume lab. strain *R. norvegicus*)	0.56	Tomlin 2000

[1] Where available, 95% lower and upper confidence intervals of values are shown in brackets.

5.2 ANTICOAGULANT RESISTANCE IN RODENTS

The well-documented occurrence of warfarin resistance in rat populations in the UK and continental Europe prompted the development of the more potent second-generation anticoagulants. Accordingly, the issue of anticoagulant resistance should be borne in mind in New Zealand, where alternative anticoagulants to brodifacoum are currently being considered. Resistance in this context is defined as where rodents have a heritable trait that allows individuals to survive a dose that would otherwise kill most (99% is the technical definition) susceptible individuals. Resistance results from naturally occurring variation but will become prevalent in populations that are strongly selected by ongoing application of the poison, which kills susceptible individuals but allows those with the resistance gene(s) to pass it on to their offspring. In Norway rats, resistance to first-generation anticoagulants, such as warfarin, is the result of inheritance of a single autosomal gene on chromosome 1 (MacNicoll et al. 2003), and the presence of this gene also confers varying resistance to other first-generation anticoagulants, e.g. diphacinone and coumatetralyl (A. MacNicoll, Central Science Laboratories, UK, pers. comm. 2003). A recent example of the development of a warfarin-resistant strain of mice occurred on Lord Howe Island (offshore from New South Wales, Australia), where rodent control between 1986 and 1989 used warfarin at 0.025% w/w in grain baits, and since 1989 at 0.08% w/w (Eason 1996). Currently, this 0.08% bait is registered with an approved label specifically for use on Lord Howe Island as the product 'Rentokil Baits for Rats and Mice' (Rentokil 2003). Following field studies of the consumption of warfarin grain bait by mice on Lord Howe Island, Billing & Harden (2000) suggested that the mice had reduced susceptibility to warfarin. Feeding tests established that house mice on Lord Howe Island were resistant to warfarin (Billing 2000).

While the specific mechanism of anticoagulant resistance in mice has not been as well studied as that in rats (Sutcliffe et al. 1987), the presence of genetically mediated anticoagulant resistance in mouse populations has the same practical implications for the use of first-generation anticoagulants for control of these populations, i.e. first-generation compounds will have reduced efficacy against resistant rodents and their use is expected to create further selection for resistance in a population. While there is evidence of physiological resistance of some strains of rats to second-generation anticoagulants (e.g. Thijssen 1995), compounds such as brodifacoum currently still provide effective control of resistant rodent populations in conventional bait applications.

5.3 SECOND-GENERATION ANTICOAGULANTS

5.3.1 Brodifacoum

3-[3-(4'-bromo-[1,1'-biphenyl]-4-yl)-1,2,3,4-tetrahydro-1-naphthylenyl]-=4-hydroxy-2H-1-ben-zopyran-2-one; C31H23BrO3; Molecular weight 523.4

Brodifacoum is an off-white to fawn-coloured powder with melting point of 228–232°C. It is practically insoluble in water, although solubility increases

slightly with pH (3.8×10^{-3} mg/L at pH 5.2, 0.24 mg/L at pH 7.4, 10 mg/L at pH 9.3; all at 20°C). Brodifacoum is a synthetic compound, which was first developed in 1976. It is a very potent (highly toxic) anticoagulant, active against rats and mice including strains resistant to warfarin and other anticoagulants. Brodifacoum is marketed worldwide for the control of commensal rodents. In New Zealand, ready-to-use baits contain either 0.005% or 0.002% brodifacoum (w/w). Various cereal pellet and wax block formulations are registered for use against commensal rodents, brushtail possums and field populations of rodents on offshore islands. Mice appear to have a similar high susceptibility to brodifacoum as various rat species (Table 2). LD_{50} estimates for mice range from 0.4 to 0.52 mg/kg and do not appear to vary greatly from LD_{50} estimates for rats, which range from 0.27 to 0.77 mg/kg (Table 2).

Trade/product names for brodifacoum baits registered in New Zealand include 'Talon' and 'Pestoff'. Of four anticoagulant baits tested against captive wild-caught house mice, 'Pestoff' rodent bait was the most palatable, and was highly effective (100% mortality). 'Talon 20P' appeared unsuitable for mice control, with poor palatability resulting in a low mortality rate (O'Connor & Booth 2001). House mice were eradicated from Enderby Island, New Zealand (710 ha) using two aerial applications of brodifacoum in Wanganui No. 7 pellet baits (Torr 2002). Ship rats and mice have been eradicated from three islands in the Indian Ocean by applying brodifacoum baits in a combination of techniques (Bell 2002; Merton et al. 2002). Ship rats were eradicated in an aerial operation on Saint Paul Island, French Southern Territories, using 'Pestoff 20R' baits, but mice survived and recovered to higher densities than before control (Micol & Jouventin 2002).

5.3.2 Bromadiolone

3-[3-(4'-bromo[1,1'-biphenyl]-4-yl)-3-hydroxy-1-phenylpropyl]-=4-hydroxy-2H-1-benzopyran-2-one; C30H23BrO4; Molecular weight 527.4

Bromadiolone is a yellowish powder with a melting point of 200–210°C, with low solubility in water (19 mg/L at 20°C). Its properties as a rodenticide were first reported in 1976. It is also a potent anticoagulant, active against rats and mice including strains resistant to warfarin. Amongst the second-generation anticoagulants, the toxicity of bromadiolone to mice is relatively well characterised (Table 2) and is similar to that for rat species. In New Zealand, ready-to-use baits contain 0.005% or 0.01% (w/w) bromadiolone and are formulated as wax blocks for commensal rodent control. Trade/product names registered in New Zealand include 'Bromtrol', 'Rid Rat Super Wax Baits' and 'Contrac All Weather Blocks'. In New South Wales (Australia) authorised control officers prepare bait for mouse control by mixing one litre of bromadiolone ('Bromakil') per 19 kg of wheat. Most mice need to eat about 0.5 g of bait or 13 treated wheat grains for a lethal dose. Mice die about 4–10 days after eating the bait (Eason 1996). No reports of field application of bromadiolone against rodent populations in New Zealand were found.

5.3.3 Flocoumafen

4-hydroxy-3-[1,2,3,4-tetrahydro-3-[4-[[4-(trifluoromethyl)phenyl]=methoxy]phenyl]-1-napthalenyl]-2H-1-benzopyran-2-one; C33H25F3O4; Molecular weight 542.6

Flocoumafen forms an off-white solid, of *cis*- and *trans*- isomers with melting points of 181–191°C and 163°C –166°C, respectively. It has very low solubility in water (1.1 mg/L, assume at 20°C). Its action as a rodenticide was reported in 1984, and it has been used against a range of commensal and agricultural rodent pests, including house mice. As with other second-generation anticoagulants, it is effective against warfarin-resistant rodent populations. Only one toxicity value was found for flocoumafen in mice, which was the same as that for difenacoum (Table 2). In New Zealand, ready-to-use baits contain 0.005% w/w flocoumafen in 'Storm' block baits. House mice were eradicated from Mana Island, New Zealand (217 ha) partly through the use of aerial application of flocoumafen baits (Storm®) on steep cliff areas (Hook & Todd 1992).

5.3.4 Difenacoum

3-[3-(1,1'-biphenyl)-4-yl-1,2,3,4-tetrahydro-1-napthalenyl]-4-hydroxy-=2H-1-benzopyran-2-one; C31H24O3; Molecular weight 444.5

Difenacoum forms colourless crystals, with a melting point of 215–217°C, and is practically insoluble in water, although solubility increases with pH (31×10^{-3} mg/L at pH 5.2, 2.5 mg/L at pH 7.3, 84 mg/L at pH 9.3; all at 20°C). Difenacoum was first described as effective against rats and mice in 1975, and as being effective against warfarin-resistant rodents. While the bulk of toxicity data for difenacoum pertains to rat species, it appears to have the same acute toxicity to mice as flocoumafen (Table 2). International product names include 'Neosorexa' and 'Ratak'. Difenacoum is not registered as a vertebrate pesticide in New Zealand.

5.3.5 Difethialone

3-[3-(4'bromo[1,1'-biphenyl]-4-y-l)-1,2,3,4-tetrahydro-1-napthalenyl-=4-hydroxy-2H-1-benzothiopyran-2-one; C31H23BrO2S; Molecular weight 539.5

Difethialone is a white, slightly yellowish powder, with a melting point of 233–236°C. It is slightly soluble in water (0.39 mg/L at 25°C). It was first developed as a rodenticide in 1986, and introduced for use in 1989 against commensal rats and mice (including warfarin-resistant strains) in France. It was introduced to the United States market in 1994. In New Zealand, registered baits contain 0.0025% (w/w) difethialone and are formulated as grain and ready-to-use block baits for commensal rodent control. Trade/product names registered in New Zealand include 'Baraki Rodenticide Pellets'. No reports of field application of difethialone against rodent populations in New Zealand were found.

5.4 RELATIVE SUSCEPTIBILITY OF MICE TO DIFFERENT ANTICOAGULANTS

While there are differences in the oral toxicity of different anticoagulants to house mice, relative potency is better illustrated by comparing the amount of rodenticide baits, using nominal concentrations in current formulations, that must be eaten by mice to equal an LD_{50} dose. The values shown in Table 3 were calculated using the LD_{50} figure for mice (where available), and the mean bodyweight range for wild populations of house mice in New Zealand, which was 16.7–24.9 g (Murphy & Pickard 1990). However, in using these values as an index of susceptibility of mice, consideration must be given to the differences in the quality of toxicity data available. In some cases no values for house mice were available; or they were obtained from older studies where it is difficult to compare methodologies; or values obtained for the same anticoagulant from different studies varied considerably. There can also be inter-sex and inter-strain differences in toxicity of the same anticoagulant to rodents (e.g. Ashton et al. 1987). On the basis of the toxicity figures in Tables 2 and 3, female mice appear to be less susceptible than males to anticoagulants. Future toxicity or efficacy assessments of anticoagulants against mice should consider sex-related differences.

In general, the susceptibility of mice to anticoagulants appears to be slightly higher than that of rat species. Where no values were available for mice, no extrapolations were made using toxicity values from other rodent species, as that was considered inappropriate due to the variation present within species susceptibility to different anticoagulants. Finally, most toxicity values were

TABLE 3. ESTIMATED AMOUNTS OF BAIT THAT NEED TO BE EATEN BY MICE TO INGEST AN LD_{50} DOSE. ESTIMATES BASED ON MOUSE BODYWEIGHT OF 16.7–24.9 g, KNOWN ORAL LD_{50} VALUES FOR *MUS MUSCULUS* (TABLE 1), AND CONCENTRATIONS OF ANTICOAGULANT IN BAIT FORMULATIONS. WHERE THE TOXICITY FOR AN ANTICOAGULANT WAS UNKNOWN, NO CALCULATIONS WERE POSSIBLE (–).

COMPOUND	CONCENTRATION IN BAIT (PPM)	LD_{50} (mg/kg)	ACUTE LD_{50} BAIT DOSE (g)	LD_{50} BAIT DOSE/ BODYWEIGHT [1]
First-generation:				
Pindone	250 or 500	Unknown	–	–
Diphacinone	50	30	10.02–14.94	60
Diphacinone	50	1.41 mg/kg/day[2]	0.40–0.87 mg[2]	60
Chlorophacinone	50	Unknown	–	–
Warfarin	250	374	24.98–37.25	149.6
Coumatetralyl	375	> 1000	44.53–66.40	266.7
Coumatetralyl	500	> 1000	33.40–49.80	200
Second-generation:				
Brodifacoum	20	0.52	0.43–0.65	2.6
Brodifacoum	50	0.52	0.17–0.26	1.04
Bromadiolone	50	1.75	0.58–0.87	3.5
Flocoumafen	50	0.8	0.27–0.40	1.6
Difenacoum	50	0.8	0.27–0.40	1.6
Difethialone	25	1.29	0.86–1.28	5.2

[1] Weight of LD_{50} dose of bait as a percentage of mouse bodyweight.

[2] Based on the multiple-dose oral LD_{50} of 1.41 mg/kg /day, a mouse would need to eat 0.3–0.8 mg of 50 ppm diphacinone bait per day for 5 days.

derived using laboratory strains of mice, which may have different susceptibilities to field populations of mice in New Zealand. However, as a starting point, these values were the most appropriate to use to make an estimate of the likely susceptibility of wild mice to different anticoagulants.

Ideally, for effective control, ingestion of 'one good mouthful' of a toxic bait by a target rodent should contain a lethal dose. Limited efficacy of the first-generation compounds against house mice of has often resulted from the sporadic feeding behaviour of mice and their lower susceptibility to these compounds (Kaukeinen et al. 2000). It would be useful to quantify the amounts of bait likely to be consumed by wild New Zealand house mice in single and consecutive feeds, in order to best design a toxic bait for mice. A palatable bait matrix for house mice, which can demonstrate high acceptance in field conditions, will be an important component in the establishment of an effective control tool for field populations of house mice in New Zealand. In addition, the development of effective baiting strategies for mice will need to account for the presence of field populations of rats.

On the basis of 'single-feed' toxicity, the second-generation anticoagulants are likely to be more effective control agents for mice (Table 3). The idea of increasing the toxic loading of baits to target mice, i.e. 'fortified' formulations, should be explored with an awareness of the concomitant non-target and environmental contamination risks. Brodifacoum is the most toxic of the second-generation compounds to mice; followed by flocoumafen and difenacoum, which appear to have similar acute toxicity; and then bromadiolone and difethialone, which also have similar acute toxicity. However, efficacy needs to be balanced against potential adverse effects in terms of hazard to non-target species and persistence of residues in the environment. While data gaps in the multiple-dose oral toxicity of first-generation anticoagulants do not allow a full comparison of likely efficacy of first- versus second-generation anticoagulants, the former are expected to pose reduced risks in terms of persistence and secondary poisoning. Diphacinone is the only first-generation anticoagulant with published chronic toxicity values for mice. The increased potency of diphacinone over consecutive intakes (as opposed to a single feed) should also be considered in designing a baiting strategy that is effective against field populations of mice in New Zealand.

6. Conclusions

There is currently very little information regarding effective baiting strategies for control of field populations of house mice in New Zealand. In laboratory assessments of toxicity, mice are generally less susceptible to anticoagulants than rats, but like rats, are more susceptible to first-generation anticoagulants in multiple, consecutive oral intakes than in single, larger intakes. From available toxicity data, mice appear to be more susceptible to second-generation than first-generation anticoagulants, and particularly susceptible to brodifacoum. On

this basis, second-generation compounds are likely to be effective toxicants for use against mice. Of the first-generation compounds, diphacinone appears to be the most toxic to mice (and has the most complete toxicity data for mice) suggesting that it could also be used effectively against them.

7. Recommendations

Based on this review, the author recommends that:

- The amounts of acceptable bait likely to be ingested over time by wild house mice in New Zealand should be quantified, to provide a baseline parameter for determining appropriate toxic loadings of potential toxicants for testing against mice.
- An efficacy study should be conducted using New Zealand wild house mice, comparing diphacinone with single-feed formulations of a second-generation anticoagulant. Initial pen-based assessments of efficacy of bait concentrations should be based on previously-used field concentrations and toxicity data, and extended to field trials to confirm efficacy of the most promising formulations against mice.
- Concurrent with pen and field assessments of efficacy, evaluations of persistence and secondary poisoning risks (i.e. tissue residue profiles and field monitoring)—at least of diphacinone and brodifacoum in wild house mice—should be carried out.

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