Anticoagulant residues in rats and secondary non-target risk

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CONTENTS

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A B S T R A C T

Anticoagulant pesticides are widely used in New Zealand for vertebrate pest control. The occurrence of residues of the anticoagulant rodenticides brodifacoum, coumatetralyl, warfarin, pindone, and diphacinone in the livers of laboratory rats was measured after they had consumed bait products, under three different bait consumption scenarios for each anticoagulant: at death resulting from presentation of an approximate LD_{99} amount of anticoagulant bait over 4 days; after 1 day's feeding *ad libitum* on anticoagulant bait; and at death resulting from *ad libitum* feeding on a choice of anticoagulant bait and non-toxic pellets. Liver residue concentrations were used as the basis for a conservative assessment of the secondary poisoning risk to non-target predators and scavengers of rodents in New Zealand. Brodifacoum presented the highest overall theoretical risk of secondary poisoning to predators (especially mammals), and a high risk to small and medium scavengers (both birds and mammals). Of the first-generation anticoagulants, diphacinone is likely to present the overall lowest risk of acute secondary poisoning because of its relatively short persistence, a theoretical very low risk to birds, and low to medium risk to mammals. Warfarin has a longer persistence than diphacinone, but also a very low risk profile to birds, and medium risk to mammals. Coumatetralyl is the most persistent of the first-generation compounds, but also has a very low risk profile for birds and a medium risk to mammals. Although pindone has a short persistence similar to diphacinone, it has a high risk profile to birds and a medium risk to mammals. In general, mammals are at greater potential risk of acute secondary anticoagulant poisoning than birds. The efficacy and non-target impacts of diphacinone especially, but also coumatetralyl and warfarin, should be further evaluated as alternative vertebrate pesticides for field uses in New Zealand.

Keywords: anticoagulant, rodenticide, liver residues, non-target risk, predator, scavenger, New Zealand.

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1. Introduction

Many control strategies for rodents currently rely on the use of a range of anticoagulant rodenticide baits. The Department of Conservation (DOC) has indicated a need for more background data regarding the residual effects of anticoagulants. Brodifacoum is a highly effective toxicant for brushtail possums and rodents, but sustained use at mainland sites has led to residue contamination in a range of non-target species (Eason et al. 2002). Concerns for secondary poisoning effects of residues on wildlife (Eason et al. 2002), and for the potential for residues to have further-reaching effects, such as tertiary transmission of residues from feral pigs to humans (Clear 2003), have prompted investigation of alternative vertebrate pesticides and baiting strategies. Several conservancies have been trying alternatives such as warfarin, diphacinone, pindone, or coumatetralyl for rodent control, and now there is an urgent need for data on efficacy v. risk. Characterisation of the residue concentrations of each toxicant in rat carcasses at death was considered a key determinant of comparative risk of transfer of poison through the food chain. Because rodents in the field may be preyed on or scavenged at variable times after they have consumed variable amounts of bait, three different scenarios of bait intake and resulting residues in rodent liver were investigated, using five anticoagulants currently available in New Zealand. A comparative assessment of secondary poisoning risk to predatory and scavenging species was carried out on the basis of these residue results. This study was carried out between April 2000 and September 2002.

1.1 OBJECTIVES

The objectives of this study were to measure residues of brodifacoum, coumatetralyl, warfarin, pindone, and diphacinone in laboratory rat liver following bait uptake, and to use these hazard data in a comparative assessment of the potential risks of secondary anticoagulant poisoning to non-target predators and scavengers present in New Zealand.

1.2 ASSESSING SECONDARY NON-TARGET RISK

Non-target risk is a function of both exposure and hazard. Anticoagulant compounds used as vertebrate pesticides are highly toxic to mammals and birds, and baits represent a high hazard to both target and non-target species. Reports of anticoagulant residues in predatory birds (e.g. Newton et al. 2000) and other wildlife (e.g. Shore et al. 1999) appear to have increased over the last decade, heightening worldwide concern regarding non-target effects of rodenticide use. Recent data have shown that a range of non-target species in New Zealand (including game species and native birds) have been contaminated with the second-generation anticoagulant brodifacoum, either directly through consuming baits, or indirectly through secondary poisoning

(Gillies & Pierce 1999; Eason et al. 2002). While this increase may be due in part to more sensitive monitoring and analytical techniques, the presence of anticoagulant residues indicates that primary or secondary exposure of non-target wildlife occurs.

Anticoagulants are known to have a high affinity for liver tissue (Parmar et al. 1987), and second-generation anticoagulants in particular can persist for prolonged periods in live animals and carcasses. Soon after an anticoagulant is ingested, it will begin to be metabolised and excreted, although to different extents depending on the compound. However, animals may still have high concentrations in the liver, and to a lesser extent in other tissues, when they die. Because death from anticoagulant poisoning occurs some days after ingestion, target rodents can continue to eat baits after ingesting a lethal dose, increasing the concentration of anticoagulant in their body before they die. Secondary exposure of non-target species that prey on, or scavenge the carcasses of, poisoned rodents may result in sublethal or chronic poisoning, and represent an unrealised source of further environmental contamination.

Secondary poisoning hazard may be estimated by evaluating anticoagulant concentration in rodent carcasses following bait intake (Joermann 1998). The additional step of feeding contaminated rodents to predators provides an exposure component to an investigation of secondary poisoning risk. This approach has been used for a range of anticoagulant compounds and non-target species (e.g. Evans & Ward 1967; Townsend et al. 1981; Gray et al. 1992; O'Connor et al. 2003). Such studies often aim to present a worst-case scenario for secondary intake of selected anticoagulants by a model predator species, but their results are difficult to use for comparative risk assessment (Joermann 1998) as they cover a range of anticoagulant compounds and non-target species under different experimental conditions. Because of the high ethical cost of using some non-target species (e.g. birds of prey), these studies can also be limited by small sample sizes.

By measuring anticoagulant concentration in rodent carcasses following bait intake, and estimating toxicity and secondary exposure of non-target species, a relatively rapid and inexpensive comparative risk assessment can be established as a guide to future information requirements. We have evaluated, for the first time, the hazard presented by rodent carcasses in a comparative study of brodifacoum, coumatetralyl, warfarin, pindone, and diphacinone. A number of scenarios for residue burden in live or dead rodents following exposure to bait are possible, depending on the intake of bait over time, and whether the animal is preyed on or scavenged when it is sublethally poisoned, in the latent period before death or after it has died of toxicosis. We sought to quantify the liver residue profiles of the five anticoagulants in rats in three different laboratory-simulated scenarios:

- At death resulting from presentation of an amount of anticoagulant bait over 4 days containing an approximate LD_{99} dose* (no-choice trial)
- After 1 day's feeding *ad libitum* on anticoagulant bait (no-choice trial)
- At death resulting from *ad libitum* feeding on a choice of anticoagulant bait and non-toxic pellets (two-choice trial).

* A dose that kills 99% of the animals dosed; approximately a 2.5 LD_{50} dose

These laboratory trials were used as simulations of different bait uptake to estimate the corresponding mortality and liver residues that might be expected in field populations of rodents. Liver residues in rats after eating an approximate minimum effective dose (Trial 1) represented the lowest potential secondary hazard. Liver residues in rats euthanased after 1 day (approximately 24 h) of feeding *ad libitum* on bait (Trial 2) represented the secondary hazard in the period between rats eating a lethal dose and the onset of symptoms, which also might make them more susceptible to predation. Liver residues in Trial 3 represented a high hazard for scavengers of rodent carcasses, and were also considered the worst case for predators of moribund rodents. It was assumed that anticoagulant residues in liver have the same bioavailability as the active ingredients in bait and that highest concentrations of anticoagulant residue would be present in liver rather than other tissues (e.g. Parmar et al. 1987). The highest measured liver residue concentrations from each trial, rather than the average concentrations, were used as a worst case.

2. Methods

2.1 ANIMAL HUSBANDRY

All procedures involving the use of animals were carried out with the approval of the Landcare Research Animal Ethics Committee (AEC 01/07/03). Young adult (approximately 7 weeks old) female rats (*Rattus norvegicus* Wistar) were individually identified and housed singly in a controlled-temperature environment (18°C ± 2°C) at the animal facility, Landcare Research, Lincoln, using standard operating procedures (SOP 3.1). Rats were acclimatised for at least 14 days before the start of the trial, and throughout the trials had free access to water. Prior to and after the trials rats had free access to cereal feed pellets (Weston Animal Nutrition, Rangiora). Rats were weighed at the beginning of each trial when toxic baits were offered, and again at death, and daily during evident anticoagulant toxicosis in Trial 3 to determine changes in bodyweight.

2.2 FEEDING TRIALS AND ANALYSIS OF BAIT SAMPLES

Bait products currently registered and available in New Zealand for rodent control (Table 1) were offered to rats in three different trials. On each night of the three trials, a similar amount of toxic (baits) and non-toxic food (pellets) was weighed into containers (n = 3) and placed in the room housing the rats. These 'environmental controls' were reweighed on the following morning, and any change in weight averaged across the three samples, so that the amounts of bait consumed by the rats could be accurately adjusted for any changes in bait weight due to environmental conditions.

TABLE 1. ACTIVE INGREDIENT, CONCENTRATION OF ACTIVE INGREDIENTS AND SUPPLIERS OF THE FIVE ANTICOAGULANT BAIT TYPES OFFERED TO LABORATORY RATS IN FEEDING TRIALS.

ACTIVE INGREDIENT	PRODUCT–BAIT TYPE	NOMINAL CONC. (g/kg)	PRODUCER–SUPPLIER
Brodifacoum	PESTOFF® rodent bait 20R cereal pellets	0.02	Animal Control Products
Coumatetralyl	Racumin® paste	0.375	Bayer
Warfarin	PESTOFF® rodent bait cereal pellets	0.5	Animal Control Products
Pindone	PESTOFF® possum bait cereal pellets	0.5	Southern Pest Management
Diphacinone	Ditrac® All Weather Blox waxed cereal blocks	0.05	Bell Laboratories

As part of a quality assurance approach, in order to accurately estimate the amounts of anticoagulant ingested in bait by rats, samples of each bait type were analysed for anticoagulant concentrations at the toxicology laboratory at Landcare Research, Lincoln. The analysis of brodifacoum (TLM017) was based on the methods of Hunter (1983) and ICI (1983). A sample of bait was ground in a Retsch mill and a 5 g subsample was weighed into a centrifuge tube in duplicate. Anhydrous sodium sulphate was added and the mixture extracted three times with methanol. A small aliquot of the combined extracts was filtered and diluted in methanol for analysis by high-performance liquid chromatography (HPLC). Difenacoum was used as an internal standard for improved quantitation. A post-column pH switching technique, using 10% ammonia and 10% methanol (to reduce solvent gassing) as the post-column reagent, was used to fully exploit the natural fluorescence of the rodenticides.

The analysis for coumatetralyl (TLM068) was based on the methods of Hunter (1983) and Houghlum et al. (1989). The sample of paste bait was ground in a mortar and pestle with a silicaceous powdering agent, and duplicate samples were extracted with methanol. The extract was filtered, diluted as necessary, and injected into an HPLC as above. The analysis for warfarin (TLM029) was based on the methods of Hunter (1983), Steyn et al. (1986) and Houghlum et al. (1989). Warfarin cereal pellet bait was milled and duplicate samples were extracted with a solvent mixture of methanol/water/0.25% acetic acid, centrifuged, made up to volume and injected into an HPLC as above. The analysis for pindone (TLM014) was based on the method of Hunter (1984). Duplicate samples of homogenised bait were extracted on a shaking machine with a solvent mixture of acetonitrile/methanol/0.2% phosphoric acid, neutralised with triethanolamine buffer, filtered and injected into an HPLC, using paired-ion chromatography on an octadecylsilane (C18) column and a fixed-wavelength UV detector at 284 nm. The analysis for diphacinone (TLM072) was based on the method of Hunter (1984). Duplicate samples of homogenised bait were extracted on a shaking machine with a solvent mixture of acetonitrile/methanol/0.2% phosphoric acid, neutralised with triethanolamine buffer, filtered and injected into an HPLC, using paired-ion chromatography on a C8 column and a fixed-wavelength UV detector at 284 nm.

2.3 RATS OFFERED A LETHAL AMOUNT OF BAIT OVER 4 DAYS (TRIAL 1)

Seventy-five female rats (mean ± SE weight 259.63 ± 2.41 g) were randomly allocated into five treatment groups (brodifacoum, coumatetralyl, warfarin, pindone, or diphacinone) of 15 rats. Rats were offered an amount of the appropriate bait without alternative food, in order to deliver an estimated LD_{99} dose over 4 days. Published acute LD_{50} values for rats are sparse for some anticoagulant compounds, e.g. pindone, and variable for others, e.g. warfarin, diphacinone. Also, first-generation anticoagulants are generally most potent when eaten as small consecutive doses, whereas second generation compounds are usually lethal to rats in a single feed. As an approximation of a minimum effective dose, a cumulative 'target intake' over 4 days for each anticoagulant was set (Table 2). These target figures, as estimated LD_{99} doses, were set in an effort to account for data gaps, inconsistencies in published LD_{50} figures, and the different potency of each anticoagulant.

TABLE 2. ESTIMATED EFFECTIVE LETHAL DOSES OF FIVE DIFFERENT ANTICOAGULANTS FOR LABORATORY RATS TO BE DELIVERED IN BAIT CONSUMED OVER 4 DAYS IN TRIAL 1.

ANTICOAGULANT	ACUTE ORAL LD_{50} (mg/kg)	REFERENCE	TARGET INTAKE OVER 4 DAYS (mg/kg)
Brodifacoum	0.27	Godfrey 1985	0.54
Coumatetralyl	16.5	Hone & Mulligan 1982	32.0
Warfarin	3.3	Hone & Mulligan 1982	6.6
Pindone	100	Eason & Wickstrom 2001	200
Diphacinone	2.1	Ashton et al. 1987	4.3

Baits were weighed out and placed in the feeding troughs of rat cages in the morning (between 0800 and 1000 h) and then removed and weighed after approximately 24 h. Each morning the amount of bait replaced depended upon the acute LD_{50} value of the anticoagulant (Table 2), the amount consumed by each rat over the previous 24 hours, and the weight of the individual rat.

Within each treatment group, three rats randomly allocated as controls were offered an amount of non-toxic feed pellets approximately equal to the maximum amount of bait given each morning to rats receiving poison in that treatment group. Rats were returned to a normal diet when they had ingested the target intake of anticoagulant (Table 2), or after 4 days. After returning to normal diet, all rats were closely observed at least once a day for symptoms of anticoagulant poisoning, and were weighed weekly. Rats that had lost more than 25% of their bodyweight, or were deemed to be suffering unduly during toxicosis, were euthanased. Rats were euthanased by cervical dislocation whilst under carbon dioxide/oxygen anaesthesia (SOP 1.17). The livers were removed from each rat post-mortem and each sample was labelled (with animal number, toxicant dosed, and point in time sampled) and then frozen for residue analysis.

2.4 ONE DAY'S FEEDING *AD LIBITUM* ON BAIT (TRIAL 2)

Seventy-five female rats (mean ± SE weight 176.36 ± 1.99 g) were randomly allocated into five treatment groups (brodifacoum, coumatetralyl, warfarin, pindone, or diphacinone) of 15 rats. Rats were offered approximately 40 g of the appropriate bait, which, from the measurement of 2 days' feeding on non-toxic pellets, was expected to be in excess of the amount they would consume over 24 h. Within each treatment group, three rats randomly allocated as controls were offered approximately 40 g of non-toxic feed pellets. Rats were weighed just prior to offering baits. Baits were weighed out and offered to the rats in the morning (between 0800 and 1000 h) and then removed and weighed after approximately 24 h. Rats were returned to normal diet and euthanased as described above after approximately 24 h of normal diet. Liver samples for analysis were taken as described above. The dose of anticoagulant ingested by each rat was calculated according to the individual's bodyweight and the actual concentration of anticoagulant measured by analysis of each bait type.

2.5 *AD LIBITUM* FEEDING ON A CHOICE OF BAIT AND NON-TOXIC PELLETS UNTIL DEATH (TRIAL 3)

Seventy-five female rats (mean ± SE weight 267.85 ± 2.51 g) were randomly allocated into five treatment groups (brodifacoum, coumatetralyl, warfarin, pindone, or diphacinone) of 15 rats, and three rats within each group were allocated as controls. The amount of non-toxic food consumed overnight was estimated by offering the rats approximately 40 g of non-toxic feed pellets on each of 2 nights, and weighing the remaining food the following morning. This enabled a known excess quantity of treatment bait (40 g) to be offered each night, alongside a 'maintenance diet' quantity (15 g, or approximately 5 g food per 100 g of bodyweight) of non-toxic feed. Baits were weighed out and offered to the rats in the morning (between 0800 and 1000 h), then removed and weighed after approximately 24 h, and replaced with fresh baits of the same amount, in alternate positions in the feeder each time. This choice was offered until the rats died.

Approval was given by the Animal Ethics Committee to use death by anticoagulant toxicosis as an endpoint in this trial; on the basis that wild rats being poisoned by field applications of these rodenticides were considered likely to undergo similar signs before death. The study, therefore, provided an opportunity to also collect behavioural (regular scan observations), time-to-death, and necropsy data that will be used in preparing comparative welfare assessments of the different anticoagulants in rats. It was also important to simulate a worst-case scenario for residue burdens in rats that could be available to non-target species.

Control rats were euthanased for sampling within 24 hours of all rats in a treatment group dying, with liver samples taken as previously described. Rats were weighed at the beginning and end of the trial. Bodyweights were not taken

daily as handling poisoned rats might have caused an increased likelihood of haemorrhage and influenced time to death. The dose of anticoagulant ingested by each rat was calculated according to individual weight and the actual concentration of anticoagulant measured by analysis of each bait type.

2.6 ANALYSIS OF TISSUE SAMPLES

All liver tissue was analysed for anticoagulant concentrations at the toxicology laboratory, Landcare Research, Lincoln. The method detection limit (MDL) and uncertainty for each analysis is summarised in Appendix 1. Analyses for brodifacoum, coumatetralyl, and warfarin were based on the methods of Hunter (1983). Liver samples were chopped and mixed with anhydrous sodium sulphate and the extraction solvent (chloroform/acetone). The mixture was homogenised with a tissue disperser, shaken and centrifuged. The supernatant was decanted and the extraction repeated twice more. The combined extracts were evaporated and taken up in hexane/chloroform/acetone for application to a gel permeation column for clean-up. The eluent from the column was again evaporated and taken up in mobile phase for HPLC determination, which employed post-column pH switching and fluorescence detection. Methods for pindone and diphacinone analyses were based on that of Hunter (1984). Tissue samples were chopped and mixed with anhydrous sodium sulphate and the extraction solvent (chloroform/acetone/formic acid). The mixture was homogenised with a tissue disperser, shaken and centrifuged. The supernatant was decanted and the extraction repeated twice more. The combined extracts were evaporated and taken up in hexane/chloroform/acetone for application to a gel permeation column for clean-up. The eluent from the column was again evaporated and taken up in mobile phase for HPLC determination, which employed ion-paired chromatography and UV detection at 284 nm.

Interlaboratory analyses of rat liver samples for anticoagulant concentrations were conducted by the Analytical Chemistry Project of the National Wildlife Research Center, US Department of Agriculture, Fort Collins, Colorado, USA (Appendix 2), as part of continuing quality assurance procedures conducted by Landcare Research under International Accreditation New Zealand (IANZ) accreditation.

2.7 CALCULATION AND COMPARISON OF POTENTIAL SECONDARY POISONING RISK

Theoretical estimates of risk to non-target species that scavenge rodent carcasses or prey on contaminated rodents can be made by considering the components of risk as 'hazard' (toxicity) and 'exposure' (likely access to, and uptake of, rodent tissue). The exposure component can be expressed as the proportion of usual daily food intake that non-target species would need to consume (as contaminated rodent tissue) in order to ingest an LD_{50} dose of anticoagulant. Nagy (2001) defined a series of exponential equations derived from allometric analyses of feeding rates v. body mass of a range of bird and

mammal species. These were used to predict feeding rates, in g of fresh matter intake (FMI) per day (Table 3). FMI was calculated in preference to dry matter intake (DMI) as rodent tissue is likely to have a reasonably high (e.g. > 60%) moisture content. Equations used were those most suited to diet classification, and were those that yielded an intermediate or lowest (i.e. conservative from a risk assessment perspective) estimate of daily FMI intake, which was calculated using appropriate bodyweights with an average error of 40% (Nagy 2001). Direct comparison of the 'hazard' component of secondary poisoning risk, as presented by the five different anticoagulants, needed to take into account the differences in acute toxicity to non-target species, and the bodyweight of non-target species. Approximate mean bodyweights of adults were used to define 'small', 'medium' and 'large' classifications of scavenger and predator species (Table 3).

TABLE 3. CLASSIFICATION BY APPROXIMATE ADULT BODYWEIGHT OF SMALL, MEDIUM, AND LARGE SCAVENGING AND PREDATORY BIRDS AND MAMMALS, WITH EXAMPLES OF SPECIES PRESENT IN NEW ZEALAND.

FMI = Fresh matter intake, calculated using predictive equations described by Nagy (2001).

	EXAMPLE	BODYWEIGHT (g)	FMI/DAY (g)
Birds—predators			
Small	Morepork	150	85.33[a]
Medium	New Zealand falcon	300	135.30[a]
Large	Australasian harrier	650	226.26[a]
Birds—scavengers			
Small	Starling	85	33.94[b]
Medium	Magpie	350	82.43[b]
Large	Black-backed gull, weka	700	127.31[b]
Mammals—predators			
Small	Stoat	500	91.18[c]
Medium	Ferret	1 000	164.12[c]
Large	Cat, small dog	3 000	416.65[c]
Mammals—scavengers			
Small	Rat	200	48.88[d]
Medium	Dog	8 000	596.13[d]
Large	Pig	40 000	1775.18[d]

[a] Equation number 64, carnivorous birds (Nagy 2001).

[b] Equation number 62, omnivorous birds (Nagy 2001).

[c] Equation number 26, carnivores (Nagy 2001).

[d] Equation number 34, omnivores (Nagy 2001).

In many cases, no acute lethal dose values for specific anticoagulants are published for non-target species and available figures can be variable between, and even within, species. Hence, low (conservative) lethal dose values available (Table 4) for representative predatory and scavenging birds and mammals were used to estimate non-target secondary poisoning risk for each of the anticoagulants.

TABLE 4. ACUTE ORAL LD_{50} VALUES FOR ANTICOAGULANTS IN MAMMAL AND BIRD SPECIES, USED IN ESTIMATES OF NON-TARGET RISK THROUGH SECONDARY EXPOSURE.

ANTI-COAGULANT	BIRD SPECIES	ORAL LD_{50} (mg/kg)	REFERENCE	MAMMAL SPECIES	ORAL LD_{50} (mg/kg)	REFERENCE
Brodifacoum	black-backed gull *Larus dominicanus*	0.75	Godfrey 1985	domestic pig *Sus scrofa*	0.1	Godfrey 1985
Coumatetralyl	chicken *Gallus gallus*	50	Worthing & Hance 1991	domestic pig *Sus scrofa*	1.0	Dobson 1973
Warfarin	mallard duck *Anas platyrhynchos*	620	Erickson & Urban 2002	domestic cat *Felis catus*	2.5	Erickson & Urban 2002
Pindone	wedge-tailed eagle *Aquila audax*	0.25	Twigg et al. 1999	domestic dog *Canis familiaris*	0.3	Twigg et al. 1999
Diphacinone	bobwhite quail *Colinus virginianus*	400	US EPA 1998	coyote *Canis latrans*	0.6	Savarie et al. 1979
				ferret *Mustela putorius*	0.6	Ogilvie et al. 1996

3. Results and discussion

3.1 LABORATORY ANALYSIS OF ACTIVE CONCENTRATION IN BAIT PRODUCTS

The active concentrations of anticoagulants measured in bait products were similar to those claimed by the manufacturers (Table 5). Baits for analysis were subsampled from the same large batch, and greater variability was present in the active concentration of the warfarin and pindone products than in the other three products. The measured active concentration was used in calculation of dose ingested by rats. No method detection limits (MDL) were specified for the analysis of bait materials, due to the relatively high working concentrations of anticoagulants in the samples.

TABLE 5. METHOD REFERENCE, UNCERTAINTY, CLAIMED AND MEASURED ACTIVE CONCENTRATIONS OF ANTICOAGULANT IN BAITS.

ANALYTE AND BAIT TYPE	ANALYSIS METHOD	UNCERTAINTY (± 95% CI)	ACTIVE CONCENTRATION OF ANTICOAGULANT (mg/g)		
			CLAIMED*	MEASURED: TRIAL 1	MEASURED: TRIALS 2 & 3
Brodifacoum PESTOFF® rodent bait 20R	TLM017	7%	20	19.7	18.3
Coumatetralyl Racumin® paste	TLM068	5%	375	410	358
Warfarin PESTOFF® rodent bait	TLM029	3%	500	443	580
Pindone PESTOFF® possum bait	TLM014	6%	500	690	810
Diphacinone Ditrac® All Weather Blox	TLM042	8%	50	56	60

* Claimed by the manufacturers

3.2 RATS OFFERED A LETHAL AMOUNT OF BAIT OVER 4 DAYS (TRIAL 1)

None of the control rats in any of the treatment groups died. Table 6 summarises the amount of bait eaten over 4 days, the corresponding dose of anticoagulant ingested, and the resultant mortality in each group. In the coumatetralyl group, the 10 rats that died consumed significantly more poison (mean 34.20 ± 0.54 mg/kg over 4 days) than the two rats that survived (Wilcoxon non-parametric test, P = 0.03), suggesting that the predicted effective dose of 32 mg/kg over 4 days was adequate to cause 100% mortality. In the warfarin group, 10 rats ate the predicted effective dose (6.6 mg/kg over 4 days) but only four of these died, so that eight rats survived a mean dose of 6.14 ± 0.69 mg/kg. Rats that died did not eat significantly more than rats that survived (*t*-test, Welsh correction, P = 0.12), suggesting that the predicted effective dose was slightly too low to produce 100% mortality. In the pindone group, no rats consumed the predicted effective dose (200 mg/kg over 4 days), although four rats died (mean 116.10 ± 6.28 mg/kg) and eight rats survived (mean 90.91 ± 7.15 mg/kg). There was some evidence that surviving rats ate a significantly lower dose (*t*-test, P = 0.05), indicating that the predicted effective dose was too high. In the diphacinone group, ten rats ate the predicted effective dose (4.3 mg/kg over 4 days), and six of these died (mean 4.36 ± 0.11 mg/kg). Of the six rats that survived (mean dose 2.84 ± 0.88 mg/kg), two did not appear to eat any bait at all over the 4 days. However, rats that died did not eat significantly more than rats that ate bait and survived (*t*-test, Welsh correction, P = 0.15), indicating that the predicted effective dose was slightly too low.

All rats that died in the treatment groups showed behaviour and post-mortem pathology indicative of anticoagulant poisoning, e.g. anaemic and ungroomed appearance, hunched posture, visible bleeding from nose, large internal haemorrhages. Anticoagulant residues measured in liver of rats from each treatment group—those that died of poisoning (samples taken within 8 h of death) and rats that survived and were euthanased 21 days after last ingestion of baits—are shown in Table 7. Except for the diphacinone group, liver residues were significantly greater in rats that died than in rats that survived

TABLE 6. SUMMARY OF THE AMOUNTS OF ANTICOAGULANT BAIT EATEN BY RATS OVER 4 DAYS IN TRIAL 1, RESULTING MORTALITY, AND TIME TO DEATH.

Mean doses and times to death are shown ± SEM.

TREATMENT	TARGET DOSE OVER 4 DAYS (mg/kg)	NO. REACHING TARGET BAIT INTAKE	MEAN DOSE OVER 4 DAYS (mg/kg)	MORTALITY	MEAN TIME TO DEATH (h)
Brodifacoum	0.54	11/12	0.64 ± 0.02*	12/12	102.0 ± 13.25
Coumatetralyl	32.0	10/12	33.93 ± 0.51	10/12	157.45 ± 11.64
Warfarin	6.6	10/12	6.57 ± 0.49	4/12	72.13 ± 10.90
Pindone	200	0/12	99.27 ± 6.16	4/12	96.00 ± 11.02
Diphacinone	4.3	10/12	3.60 ± 0.48	6/12	69.21 ± 4.43

* Brodifacoum rats were inadvertently offered more bait on the final day than intended, so had the opportunity to consume more than the target dose.

TABLE 7. MEAN (± SEM) ANTICOAGULANT RESIDUE CONCENTRATIONS IN LIVERS OF LABORATORY RATS THAT DIED AND SURVIVED IN TRIAL 1.

TREATMENT	RATS THAT DIED		RATS THAT SURVIVED	
	Liver (mg/g)	*n*	Liver (mg/g)	*n*
Brodifacoum	1.86 ± 0.07	12	-	-
Coumatetralyl	1.46 ± 0.29	10	0.07 ± 0.01	2
Warfarin	1.00 ± 0.08	4	0.41 ± 0.04	8
Pindone	1.81 ± 0.41	4	0.29 ± 0.16	8
Diphacinone	0.26 ± 0.06	6	0.18 ± 0.12	6

(coumatetralyl group $t_{10} = 4.77$, $P = 0.001$, warfarin group $t_{10} = 7.83$, $P < 0.001$, pindone group $t_{10} = 4.19$, $P = 0.002$).

3.3 ONE DAY'S FEEDING *AD LIBITUM* ON BAIT (TRIAL 2)

The average amounts of bait eaten in the five treatment groups offered *ad libitum* feeding over 1 day (24 h) are shown in Table 8, with rats in the coumatetralyl and warfarin groups eating considerably less bait than rats in the other three groups. Based on the LD_{50} values in Table 2, all 12 rats in both the coumatetralyl and warfarin groups consumed sufficient bait in 1 day to deliver an LD_{50} dose, and 11 rats in the brodifacoum group, nine rats in the diphacinone group, and no rats in the pindone group consumed in excess of an LD_{50} dose. The mean doses consumed by each treatment group, and corresponding mean liver residues, are shown in Table 8. In comparing the dose of anticoagulant consumed by a rat to the concentration of residues found in its liver, there was a very weak positive but non-significant correlation in the brodifacoum group ($r = 0.3142$, $P = 0.32$), no significant correlation in the coumatetralyl group ($r = 0.1982$, $P = 0.54$), a strong positive significant correlation in the warfarin group ($r = 0.7553$, $P = 0.005$) and a very strong positive significant correlation in the diphacinone group ($r = 0.9335$, $P < 0.001$). Overall, these results suggest that

TABLE 8. MEAN (± SEM) AMOUNTS OF BAIT AND CORRESPONDING DOSES OF ANTICOAGULANT INGESTED OVER 1 DAY BY RATS IN TRIAL 2, AND ANTICOAGULANT RESIDUES IN LIVER OF RATS SAMPLED THE DAY AFTER EATING BAITS.

The number of LD_{50} doses eaten was calculated using the values shown in Table 2.

TREATMENT	BAIT EATEN (g)	DOSE EATEN (mg/kg)	NO. OF LD_{50} DOSES EATEN	LIVER RESIDUE (mg/g)
Brodifacoum	12.73 ± 1.30	1.31 ± 0.13	4.85 ± 0.48	5.01 ± 0.82
Coumatetralyl	4.88 ± 0.25	29.26 ± 1.76	1.77 ± 0.11	9.92 ± 0.71
Warfarin	5.82 ± 0.51	33.63 ± 2.75	10.19 ± 0.83	6.13 ± 0.77
Pindone	13.39 ± 2.49	58.56 ± 3.34	0.59 ± 0.03	5.50 ± 0.37
Diphacinone	10.06 ± 0.46	4.22 ± 0.76	2.01 ± 0.36	3.90 ± 0.47

residue burdens in rodents are likely to increase with the amount of anticoagulant eaten, so that secondary hazard is likely to be greatest when rodents have unlimited access to bait over time.

3.4 *AD LIBITUM* FEEDING ON A CHOICE OF BAIT AND NON-TOXIC PELLETS UNTIL DEATH (TRIAL 3)

During prefeeding, rats in all groups combined ate a mean of 15.96 ± 0.49 g of non-toxic feed pellets on the first night, and 16.39 ± 0.38 g on the second night. Thus, the 15 g of non-toxic pellets offered alongside toxic bait approximated the expected overnight food intake, so that consumption of toxic baits by rats was a free choice. Food intake by 12 rats in each of five treatment groups and resultant survival are shown in Figs 1–5. Although this was not a trial of the acceptance of baits by rats, these results suggest that brodifacoum, coumatetralyl and diphacinone baits were palatable to rats, as relatively small amounts of non-toxic feed pellets were consumed in these three treatment groups (Figs 1, 2 and 5). Rats in the warfarin group ate relatively more non-toxic feed on the first day, but an increasing amount of bait over the following 8 days (Fig. 3). In the pindone group, rats consistently ate more non-toxic food over 13 days, suggesting that pindone pellets were not as palatable to rats as non-toxic feed (Fig. 4).

Mortality was first observed after Day 4 of feeding on bait in the diphacinone group, Day 6 in the brodifacoum, coumatetralyl and pindone groups, and Day 7

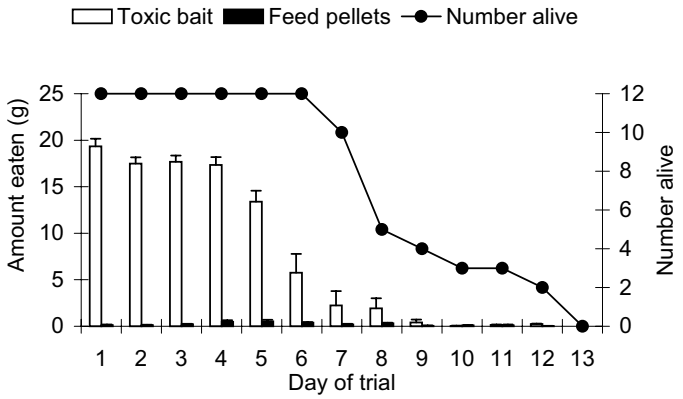

Figure 1. Mean intake (SEM as bars) of PESTOFF 20R (nominal concentration 20 ppm brodifacoum) baits and feed pellets (non-toxic) eaten, and mortality in rats in Trial 3.

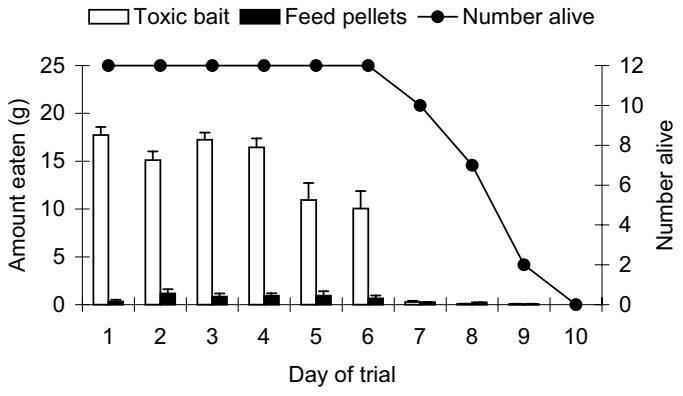

Figure 2. Mean intake (SEM as bars) of Racumin (nominal concentration 375 ppm coumatetralyl) baits and feed pellets (non-toxic) eaten by rats in Trial 3.

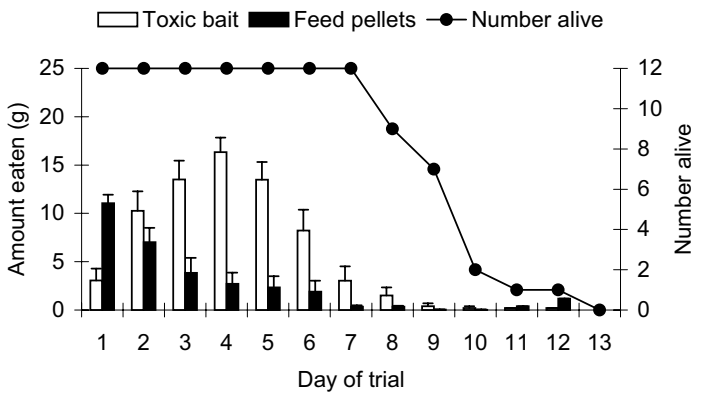

Figure 3. Mean intake (SEM as bars) of PESTOFF pellet (nominal concentration 500 ppm warfarin) baits and feed pellets (non-toxic) eaten and mortality in rats in Trial 3.

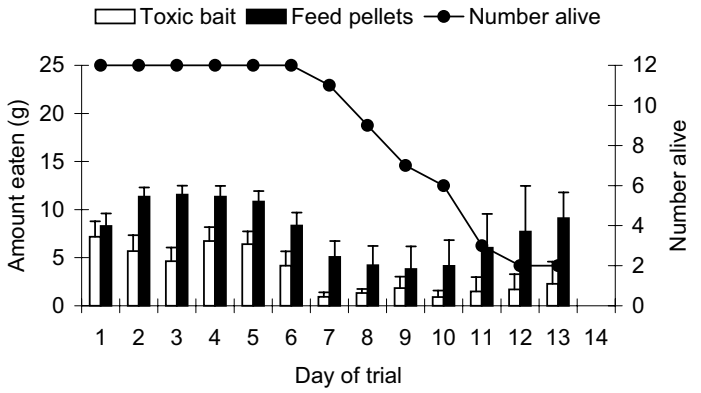

Figure 4. Mean intake (SEM as bars) of PESTOFF possum pellet (nominal concentration 500 ppm pindone) baits and feed pellets (non-toxic) eaten and mortality in rats in Trial 3.

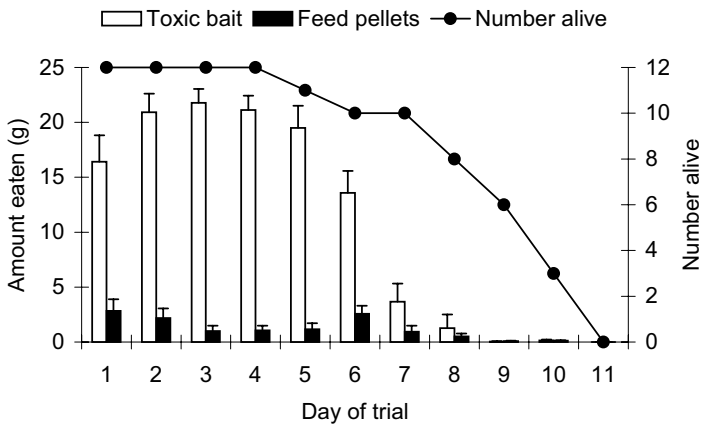

Figure 5. Mean intake (SEM as bars) of Ditrac bait blocks (nominal concentration 50 ppm diphacinone) baits and feed pellets (non-toxic) eaten and mortality in rats in Trial 3.

in the warfarin group. All rats (n = 12) were dead by Day 10 in the coumatetralyl group (mean time to death 7.25 ± 0.34 days), by Day 11 in the diphacinone group (mean time to death 8.24 ± 0.38 days) and by Day 13 in the brodifacoum group (mean time to death 8.02 ± 0.56 days) and warfarin group (mean time to death 7.68 ± 0.58 days). Ten of the 12 rats in the pindone group were dead by Day 14 (mean time to death 8.39 ± 0.54 days). There was no significant difference in the times to death between the treatment groups ($F_{4,53}$ = 0.88, P = 0.48). The two rats in the pindone group that were not dead by Day 14 were not showing obvious symptoms of anticoagulant poisoning and were increasing their consumption of non-toxic feed in relation to the pindone pellets. They were euthanased on Day 18 with the control rats in this group.

During Trial 3, there was no mortality in control rats, which appeared healthy and gained a mean 5.02% of starting weight. In general, rats offered toxic food in all treatment groups in Trial 3 lost weight as a consequence of anticoagulant toxicosis. The measured, rather than claimed, concentration of active ingredients in each bait type (Table 4) and the starting bodyweights of treatment rats were used to calculate the average total doses of anticoagulant (mg/kg) ingested in groups during Trial 3. As rats lost weight during the trial, this probably underestimated the total dose eaten (shown in Table 9 with the mean concentrations of liver residues in rats in each group).

In all treatment groups, rats ate in excess of an LD_{50} dose. In the brodifacoum group, all rats consumed approximately five times an LD_{50} dose on the first day, and a total dose of approximately 25 times the LD_{50}. In the coumatetralyl group, most rats consumed an LD_{50} dose on the first day, and a total dose of approximately 10 times the LD_{50}. In the warfarin group, six rats consumed an LD_{50} dose on the first day, and the total dose was approximately 43 times the LD_{50} value. In the pindone group, four rats did not ingest an LD_{50} dose during the trial. In the diphacinone group, all rats had consumed more than an LD_{50} dose by Day 2 and the total dose was approximately 12 times the LD_{50}. These results indicate that there is potential for field populations of rodents with constant access to bait to consume an amount in excess of an effective lethal dose, with implications for environmental contamination in the form of excreted and retained anticoagulant residues.

3.5 SECONDARY POISONING HAZARD AND RISK TO NON-TARGET SPECIES

The three laboratory trials were used as simulations of different bait uptake by rodents, to estimate the corresponding mortality and liver residues that might be expected in field populations of rodents. Mean liver residues in rats that died of anticoagulant poisoning (Trial 1 and Trial 3) were used in estimates of risks to scavengers because it was considered unlikely that predatory species would feed on carcasses. Mean residues in rats that were euthanased following consumption of anticoagulant bait (Trial 1 survivors, Trial 2 and Trial 3 survivors) were used to estimate risks to predators that would take live rodents in field conditions. Overall, mean anticoagulant residue concentrations increased from Trial 1 to Trial 3 (Table 9). Liver residues in rats that died after eating an approximate minimum effective dose (Trial 1) represented the lowest potential secondary hazard to predators. Liver residues in rats that survived after being offered an approximate effective dose but did not voluntarily consume sufficient bait (Trial 1) were in general much lower than residues in those that died in Trial 1, and these latter were lower than liver residues in rats euthanased after 1 day of feeding *ad libitum* on bait (Trial 2). Trial 1 represented the secondary hazard in the period between rats eating a lethal dose and the onset of symptoms, which might also make them more susceptible to predation. Liver residues in Trial 3 represented the highest hazard for scavengers of rodent carcasses, and was also considered the worst case for predators of moribund rodents. It was assumed that anticoagulant residues in liver have the same bioavailability as the active ingredients in bait and that

TABLE 9. MEAN TOTAL AMOUNTS (± SEM) OF BAITS EATEN BY TREATMENT GROUPS DURING TRIAL 3, MEAN DOSES OF ANTICOAGULANT RESULTING FROM THESE INTAKES, THE NUMBER OF LD_{50} VALUE DOSES EATEN, AND MEAN (± SEM) RESIDUES OF ANTICOAGULANT IN LIVER.

TREATMENT	BAIT EATEN BEFORE DEATH, TOTAL (g)	DOSE EATEN BEFORE DEATH, TOTAL (mg/kg)	NO. OF LD_{50} DOSES EATEN	LIVER RESIDUE (mg/g)
Brodifacoum	93.67 ± 3.27	6.55 ± 0.19	24.26 ± 0.72	10.7 ± 1.1
Coumatetralyl	87.80 ± 4.56	117.41 ± 5.62	7.12 ± 0.34	15.8 ± 2.1
Warfarin	69.27 ± 8.44	144.83 ± 16.02	43.69 ± 4.85	10.4 ± 1.0
Pindone*	40.41 ± 5.51	119.65 ± 16.67	1.20 ± 0.17	8.6 ± 1.0
Diphacinone	113.39 ± 7.40	25.53 ± 1.54	12.16 ± 0.73	4.7 ± 0.8

* Excludes data from two rats in the pindone group that did not die. They ingested doses of 73.90 and 148.37 mg/kg and had liver residues of 6.6 and 11.0 mg/g respectively.

highest concentrations of anticoagulant residue would be present in liver rather than in other tissues (e.g. Parmar et al. 1987). The highest measured liver residue concentrations from each trial, rather than the average concentrations, were used as a worst case. For birds and mammals, LD_{50} values (Table 4) and estimates of FMI requirements (Table 3) were used with the maximum rat liver residues (Table 10) to estimate the risk of acute secondary toxicity for each anticoagulant (Figs 6–10). The 'highest risk' category was where the amount of contaminated liver required to deliver an LD_{50} dose of anticoagulant was < 10% of the estimated g FMI/day, 'medium risk' was where this amount of liver was 10–50% of the estimated intake (g) of FMI/day, and 'low risk' was where the amount was 50–100% of the intake (g) of FMI/day.

The *y*-axes of Figs 6–10 indicate the percentage of daily FMI required for ingestion of a lethal dose of contaminated tissue. Intakes over 200% were considered improbable and hence to represent extremely low risk. In New Zealand, there is high concern regarding the potential for acute or sublethal secondary poisoning of native bird species such as weka, morepork, Australasian harrier, and New Zealand falcon. Mammalian species most of concern tend to be medium-to-large feral animals, such as deer, goats, and pigs, that are sometimes taken as game meat for human consumption. This assessment should be used as a theoretical basis for selecting appropriate

TABLE 10. SUMMARY OF MEAN AND RANGE OF LIVER RESIDUES MEASURED IN THE FIVE TREATMENT GROUPS IN TRIALS 1, 2, AND 3.

TREATMENT	MEAN (RANGE) LIVER RESIDUES (µg/g)			
	Trial 1 (survived)	Trial 1 (died)	Trial 2	Trial 3
Brodifacoum	–	1.86 (1.5–2.2)	5.01 (1.6–11.0)	10.7 (6.7–17.0)
Coumatetralyl	0.07 (0.06–0.08)	1.46 (0.46–3.4)	9.92 (5.7–14.0)	15.8 (5.1–33.0)
Warfarin	0.41 (0.25–0.56)	1.00 (0.82–1.2)	6.13 (2.0–10.0)	10.4 (6.6–15.0)
Pindone	0.85 (<0.2–1.40)	1.81 (0.94–2.7)	3.90 (3.1–7.2)	8.6 (6.6–16.0)†
Diphacinone	0.45 (<0.1–0.8)	0.30 (<0.1–0.4)	5.50 (<0.1–5.6)	4.7 (<0.1–9.0)

† Excludes data from two rats that survived

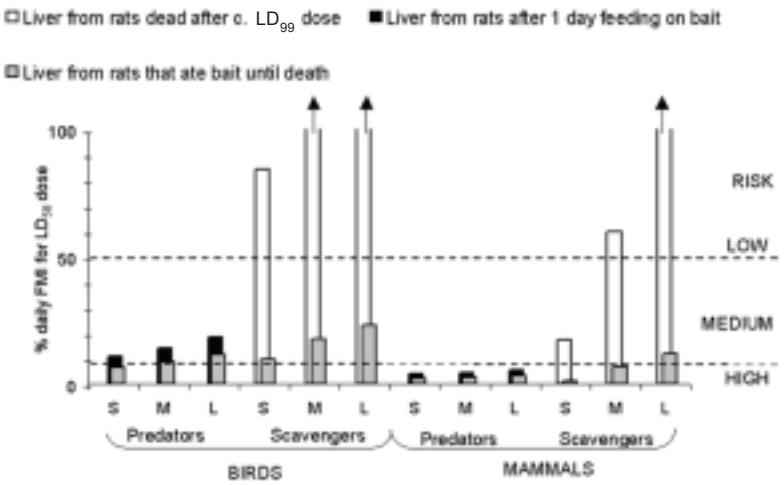

Figure 6. Theoretical risk of acute secondary brodifacoum toxicity to avian and mammalian predators and scavengers through consumption of contaminated rat liver, calculated using FMI requirements for small (S), medium (M) and large (L) predators and scavengers (Table 3), acute oral toxicity of brodifacoum to birds and mammals (Table 4) and maximum mean values for brodifacoum residues in rat liver (Table 10). Arrows indicate values higher than 100% daily FMI intake for an LD_{50} dose.

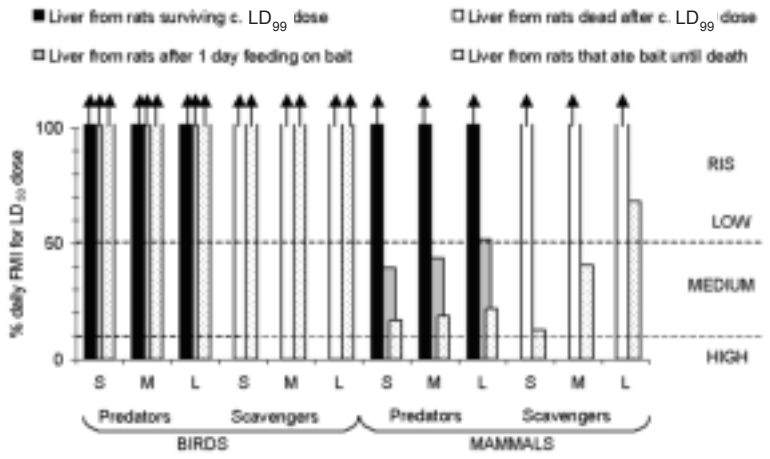

Figure 7. Theoretical risk of acute secondary coumatetralyl toxicity to avian and mammalian predators and scavengers, through consumption of contaminated rat liver, calculated using FMI requirements for small (S), medium (M) and large (L) predators and scavengers (Table 3), acute oral toxicity of coumatetralyl to birds and mammals (Table 4) and maximum mean values for coumatetralyl residues in rat liver (Table 10). Arrows indicate values higher than 100% daily FMI intake for an LD_{50} dose.

anticoagulant uses with minimised residue risks in field situations. In general, smaller birds and mammals were more at potential risk of acute secondary poisoning than larger birds and mammals, and mammals faced greater potential risks of secondary poisoning than birds.

Brodifacoum presented the highest overall theoretical risk of secondary poisoning to predators (especially mammals), and a high risk to small and medium scavengers (both birds and mammals). At best, scavengers of brodifacoum-poisoned rodents had a medium theoretical risk. Coumatetralyl presented a low risk of acute secondary poisoning to birds, and a medium-to-low risk to mammals depending on the level of coumatetralyl contamination in liver. This assessment is consistent with results reported in a secondary

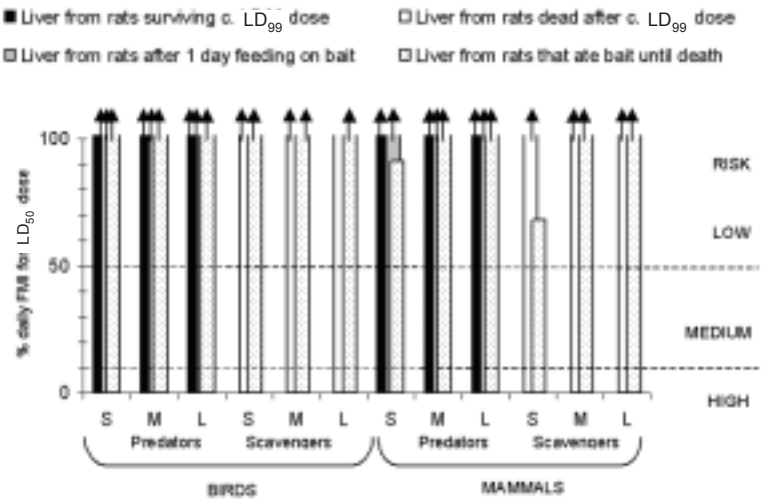

Figure 8. Theoretical risk of acute secondary warfarin toxicity to avian and mammalian predators and scavengers, through consumption of contaminated rat liver, calculated using FMI requirements for small (S), medium (M) and large (L) predators and scavengers (Table 3), acute oral toxicity of warfarin to birds and mammals (Table 4) and maximum mean values for warfarin residues in rat liver (Table 10). Arrows indicate values higher than 100% daily FMI intake for an LD_{50} dose.

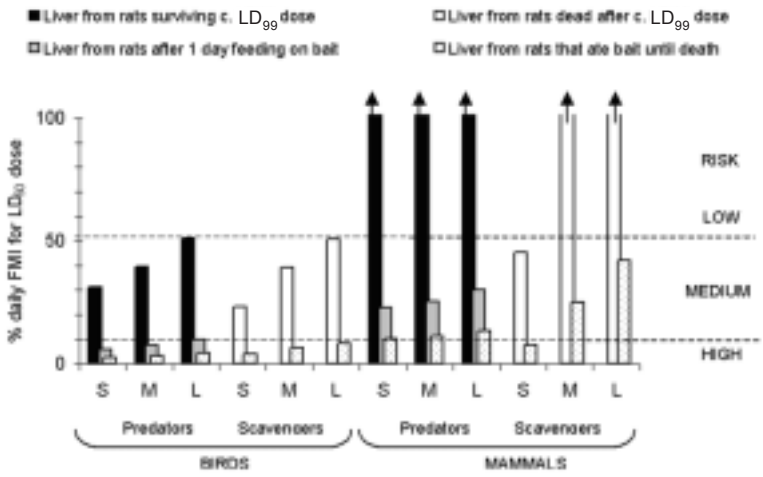

Figure 9. Theoretical risk of acute secondary pindone toxicity to avian and mammalian predators and scavengers, through consumption of contaminated rat liver, calculated using FMI requirements for small (S), medium (M) and large (L) predators and scavengers (Table 3), acute oral toxicity of pindone to birds and mammals (Table 4) and maximum mean values for pindone residues in rat liver (Table 10). Arrows indicate values higher than 100% daily FMI intake for an LD_{50} dose.

poisoning study of weka and ferrets, where these potential non-target animals were fed coumatetralyl-poisoned rats (O'Connor et al. 2003). Warfarin presented, in general, a low risk to mammalian predators and scavengers, and a very low risk to birds. Pindone presented a high-to-medium risk to bird predators and scavengers, and a high-to-low risk to mammals depending on the level of pindone contamination in liver. Diphacinone presented a very low risk to birds, and a medium-to-low risk to mammals depending on the level of diphacinone contamination in rat liver.

Erickson & Urban (2002) made a comparative assessment of secondary poisoning risk for nine anticoagulant and non-anticoagulant rodenticides used

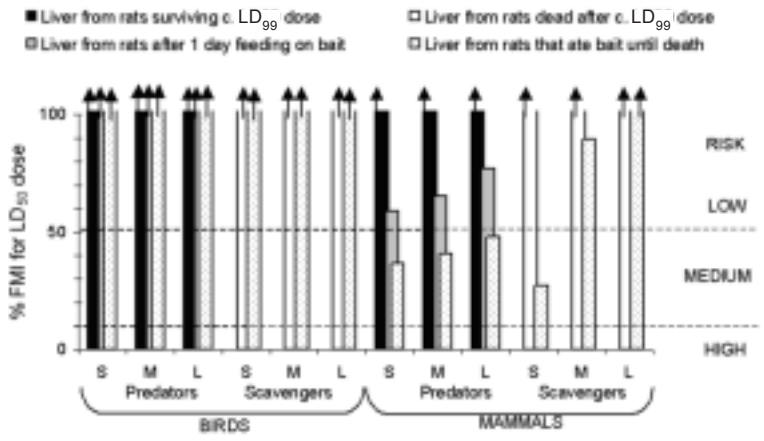

Figure 10. Theoretical risk of acute secondary diphacinone toxicity to avian and mammalian predators and scavengers, through consumption of contaminated rat liver, calculated using FMI requirements for small (S), medium (M) and large (L) predators and scavengers (Table 3), acute oral toxicity of diphacinone to birds and mammals (Table 4) and maximum mean values for diphacinone residues in rat liver (Table 10). Arrows indicate values higher than 100% daily FMI intake for an LD_{50} dose.

in the USA, including brodifacoum, diphacinone and warfarin. Their risk ranking of these three anticoagulants was similar to the results reported here: of the nine rodenticides considered in their study, brodifacoum posed the greatest overall potential risk to birds and non-target mammals, diphacinone posed greater potential risk to mammals than birds, and warfarin presented a very low risk to birds and mammals. However, Erickson & Urban (2002) utilised literature reviews and modelling rather than comparative laboratory estimates of residues in rodents, and did not include pindone and coumatetralyl.

To extend risk assessment to field situations, the influence of factors such as bait concentration and application, the availability of residues in carcasses or live animals, and the food intake (i.e. what proportion of diet consists of poisoned rodents) need to be measured in field studies. In field applications of anticoagulants, rodents may die in places inaccessible to scavengers e.g. in burrows (Brown & Singleton 1998), and removal or degradation of carcasses by other means such as insects and bacteria may mean reduced availability of carcasses to scavengers. Predators and scavengers are likely to consume other tissues, as well as the liver, of contaminated rats. Average liver weight in adult laboratory rats has been estimated at 9.62 g, and probably comprises about 3.2% of the total bodyweight (Landcare Research, unpubl. data). Other tissues are likely to contain lower concentrations of anticoagulant residues than liver (e.g. Eason et al. 1996; Fisher et al. 2003b) and comprise larger proportions of food intake by predators and scavengers. By using the liver concentrations of anticoagulants measured in rats to estimate intakes required for secondary poisoning, a conservative case for risk is presented, i.e. where a predator or scavenger selectively feeds on rat livers. It should be noted that these estimates of secondary poisoning risk are based on LD_{50} estimates for non-target species, which allow some comparison of the risk of mortality across species and from residues of different anticoagulants. However, these estimates cannot account for risks of sublethal secondary poisoning. In reality, a risk of mortality in approximate 50% of a non-target population may also be unacceptable, so it is

important to follow up these estimates of non-target risk with field studies of non-target populations during the use of anticoagulants for pest animal control.

Secondary poisoning risk in field situations is also influenced by the retention time of the different anticoagulants in tissue. Retention of brodifacoum in liver is characterised by a relatively long half-life of 113.5 days, compared with a half-life of 26.2 days for warfarin, and 3 days and 2.1 days for diphacinone and pindone respectively (Fisher et al. 2003a). Hepatic half-life of coumatetralyl is less than 70 days (Eason et al. 2003.). Longer hepatic half-life is anticipated to increase secondary poisoning risk because residues are available in tissue for longer, so within the first-generation anticoagulants coumatetralyl will present a more persistent secondary poisoning hazard than warfarin, diphacinone and pindone, respectively. The first-generation anticoagulants can be given a lowest-to-highest risk ranking by combining the hazard data from this study and persistence data: diphacinone is likely to present the overall lowest risk of acute secondary poisoning because of its relatively short persistence, a theoretical very low risk to birds, and low-to-medium risk to mammals; warfarin has a longer persistence, but also a very low risk profile to birds, and medium risk to mammals; coumatetralyl has the longest persistence of the group, but also a very low risk profile for birds and a medium risk to mammals; and pindone, while having a short persistence similar to diphacinone, has a high risk profile to birds and a medium risk to mammals. Given the contribution of persistence of residues to estimates of secondary non-target risk, future consideration should be given to strategies of timed pulse baiting with anticoagulants in order to minimise the potential for cumulation of sublethal residues. The selection of a vertebrate pesticide to minimise secondary poisoning risk needs to be balanced by its efficacy as a control tool. There is sufficient evidence to suggest that brodifacoum, while highly effective against rodents and possums, carries a high risk of secondary poisoning. Within the first-generation anticoagulants, bait products containing coumatetralyl, warfarin and diphacinone have been used for effective control of commensal rodents. However, there are few efficacy data regarding the use of these anticoagulants against field populations of pests in New Zealand.

4. Conclusions and recommendations

Brodifacoum presents high potential risk of acute secondary poisoning in comparison to first-generation anticoagulants, and of the latter, diphacinone presents the lowest potential risk of acute secondary poisoning, followed by warfarin, coumatetralyl, and pindone, respectively. Anticoagulant residues in rats that have ingested sublethal doses are lower than those likely to be present in carcasses of poisoned rats. However, rats can potentially consume many times a lethal dose of anticoagulant in bait before death, so that the secondary

poisoning hazard increases with the amount of bait eaten. In general, mammalian predators and scavengers have greater risk of acute secondary poisoning through consuming anticoagulant-contaminated rodents than do avian predators and scavengers, and smaller birds and mammals are at greater risk than larger birds and mammals.

The efficacy and non-target impacts of diphacinone especially, but also coumatetralyl and warfarin, as alternative vertebrate pesticides for field use in New Zealand should be further evaluated. As an adjunct to efficacy testing, non-target risk of first-generation anticoagulants should also be evaluated in field studies to validate theoretical estimates of secondary poisoning risk and laboratory trials to investigate adverse effects of sublethal or chronic secondary exposure on birds.

5. Acknowledgements

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Appendix 1

ANALYSIS DATA FOR ANTICOAGULANT CONCENTRATIONS IN TISSUES

METHOD REFERENCES, LIMITS OF DETECTION (MDL), AND UNCERTAINTY FOR ANALYSIS OF ANTICOAGULANT CONCENTRATIONS IN TISSUE CARRIED OUT AT THE LANDCARE RESEARCH TOXICOLOGY LABORATORY

ANALYTE	ANALYSIS METHOD	UNCERTAINTY (± 95% CI)	MDL (mg/g)
Brodifacoum*	TLM009	20%	0.01
Coumatetralyl	TLM041	9%	0.02
Warfarin	TLM057	6%	0.1
Pindone	TLM018	41%	0.2
Diphacinone*	TLM048	38%	0.1

* IANZ-accredited assay

Appendix 2

INTERLABORATORY ANALYSIS OF LIVER SAMPLES

Results of analyses of rat liver samples for anticoagulant concentrations conducted by the Analytical Chemistry Project of the National Wildlife Research Center (NWRC), United States Department of Agriculture, Fort Collins, Colorado, USA and by Landcare Research (LCR) under International Accreditation New Zealand (IANZ) accreditation. Coumatetralyl analyses were completed at LincLab, Lincoln University, New Zealand, rather than NWRC, USA. Results are listed in corresponding order to sample in each column. Methods limits of detection (MDL) for each analysis are shown in Appendix 1. NT = not tested.

ANALYTE	LABORATORY	LIVER SAMPLE RESIDUES (mg/g)					
		1	2	3	4	5	6
Brodifacoum	LCR	0.05	0.99	2.3	4.5	6.0	6.7
	NWRC	<MDL	1.4	7.9	8.2	10	9.5
Coumatetralyl	LCR	0.63	0.67	0.72	1.1	0.77	0.60
	LincLab	0.82	0.80	0.70	1.1	0.50	0.79
Warfarin	LCR	<MDL	2.4	7.0	7.1	10	15
	NWRC	<MDL	1.8	7.9	8.4	9.3	14
Pindone	LCR	NT	0.38	4.2	3.7	6.6	6.6
	NWRC	<MDL	0.33	1.0	2.6	6.8	3.5
Diphacinone	LCR	<MDL	0.26,0.48	3.8	3.1	5.6	5.5
	NWRC	<MDL	0.34	3.5	3.3	5.1	6.6

An initial analysis of the components of variation examined the contribution of different sources of variation (between laboratories, between samples and error). This type of analysis was not ideal as there was a large between-sample variation and no replicates were taken. However, it indicated that the greater part of the variation in the interlaboratory results came from differences between samples, with variance components for laboratories all being negative. This means that the laboratories varied less than the samples. Paired *t*-tests between concentrations measured in the same samples by the different laboratories revealed no significant differences between laboratories for all analyses (coumatetralyl $t_4 = -0.511$, $P = 0.631$; warfarin $t_4 = 0.043$, $P = 0.968$; pindone $t_4 = 1.9927$, $P = 0.117$; diphacinone $t_4 = -0.34$, $P = 0.752$), except the brodifacoum analyses ($t_4 = -3.87$, $P = 0.018$). One possible explanation for the difference in the brodifacoum analyses is variation in the concentration of residue in different parts of the liver, and further interlaboratory comparisons are recommended to identify the source of the variation responsible.