Persistence of four anticoagulant rodenticides in the livers of laboratory rats

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P. Fisher, C. O'Connor, G. Wright, and C.T. Eason

Landcare Research, PO Box 69, Lincoln 8152, New Zealand
Email: fisherp@landcareresearch.co.nz

ABSTRACT

The persistence of sublethal (approximate LD_{15}) oral doses of brodifacoum, warfarin, pindone and diphacinone in the livers of laboratory rats was compared. At one end of the spectrum, retention of brodifacoum in liver was characterised by a relatively long half-life of 113.5 d, compared with half-lives of 26.2 d for warfarin, and 3 d and 2 d for diphacinone and pindone respectively. These results suggest that the indandione anticoagulants diphacinone and pindone present a shorter-term and, therefore, reduced risk of secondary poisoning to predators and scavengers than the coumarin anticoagulants warfarin and brodifacoum.

Keywords: anticoagulant, rodenticide, persistence, liver, half-life, residues

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1. Introduction

Brodifacoum is a potent, second-generation anticoagulant rodenticide developed in the mid-1970s. It is, however, highly persistent in mammalian and avian liver tissue (Eason et al. 2002). It has been successfully used in recent island rodent eradication programmes (see Empson & Miskelly 1999) and is currently used to control possums in areas of mainland New Zealand (Eason et al. 2000). Recent data have shown non-target animals (including game species and native birds) have been contaminated with brodifacoum, either directly through consuming baits, or indirectly through secondary poisoning (Eason et al. 2002). The need for sustained rodent control on the New Zealand mainland is likely to increase, and alternative rodenticides that are effective, but less persistent, are needed. Currently, no non-anticoagulant compounds are registered in New Zealand as rodenticides, and anticoagulants such as warfarin and pindone are not registered as rodenticides. In this study, Landcare Research investigated the persistence in rat liver of sublethal doses of four anticoagulants. This 2002 study is the first comparative study of hepatic pharmacokinetics of indandione versus coumarin anticoagulants in one species.

1.1 OBJECTIVES

The objectives of this study were to measure the persistence of brodifacoum, warfarin, pindone, and diphacinone in rat liver, following an approximate LD_{15} dose, and to compare the pharmacokinetics of the four anticoagulants.

2. Comparative pharmacokinetics

There is a need to identify suitable toxicants for field use that are effective, but less persistent than second-generation anticoagulants and, therefore, likely to be less hazardous to non-target species (Eason et al. 2002). The tendency for anticoagulants to persist in mammalian liver is influenced by the magnitude of the dose ingested and the relative affinity of the compound for receptors in the liver, which determine the hepatic elimination half-life and the proportion of the dose retained (Parmar et al. 1987). Accordingly, liver tissue is the focus for most investigations of anticoagulant persistence, although there are few data on the hepatic persistence of anticoagulant rodenticides in mammals, where either compounds or species have been compared. The hepatic half-life of brodifacoum has been reported as 130 d in rats (Parmar et al. 1987), > 252 d in possums (Eason et al. 1996) and > 250 d in sheep (Laas et al. 1985). Thijssen (1995) estimated a hepatic half-life of 7–10 d for warfarin in rats, while residues in the liver of pigs that survived a dose of warfarin had declined to near the analytical limit of detection by 30 d (O'Brien et al. 1987). The persistence of

pindone in mammalian liver has been described for dogs (Fitzek 1978) and sheep (Nelson & Hickling 1994). Cahill & Crowder (1979) found that mouse liver tissue showed high uptake of radio-labelled diphacinone. High concentrations of radioactivity were present in liver tissue 1.5 h after dosing with a slight increase in activity at 7.5 h, followed by an abrupt decrease and slower decline in activity to 96 h after dosing. Radioactivity, indicating the presence of diphacinone in liver tissue, was present at 192 h (8 days) after dosing, which was the last sampling period in the study. Yu et al. (1982) reported that rats and mice orally administered single, sublethal doses of radio-labelled diphacinone had highest concentrations in the liver 8 d and 4 d after dosing, respectively, although no further sample times were included. In contrast, Bullard et al. (1976) reported that cows dosed with diphacinone had detectable liver residues 90 d after dosing. None of these previous studies allow the accurate definition of persistence of any of the anticoagulants in tissues for comparative purposes.

Comparative pharmacokinetics is an important basis for formulating assessments of risk to non-target species (and minimising environmental effects). The hepatic persistence of second-generation anticoagulants, such as brodifacoum, in the liver of mammals is well characterised in comparison to first-generation anticoagulants, although estimates of plasma retention for the latter group are available in the literature. However, recommendations on preferred anticoagulants for field use in New Zealand are currently made on estimates of their persistence in the liver of a range of mammal species, based on relatively few published studies. This literature suggests low-single-dose-potency anticoagulants such as warfarin and pindone persist in the liver for 0.5–1 months, moderate-single-dose-potency anticoagulants such as diphacinone and coumatetralyl persist in the liver for approximately 6 months, and high-single-dose-potency anticoagulants such as bromadiolone, brodifacoum and flocoumafen persist in the liver for more than 12 months (Eason 1999).

3. Methods

3.1 ANIMAL HUSBANDRY

All procedures involving the use of animals were carried out under the approval of the Landcare Research Animal Ethics Committee (AEC 00/1/2). Young adult female rats (*Rattus norvegicus* Wistar) were individually identified and housed in pairs in a controlled-temperature environment (18°C ± 2°C) at the animal facility, Landcare Research, Lincoln. Rats were acclimatised for at least 14 d prior to starting the trial, and throughout the trial had free access to water and cereal feed pellets (Weston Animal Nutrition, Rangiora).

3.2 COMPARATIVE PERSISTENCE IN RAT LIVER

For diphacinone and warfarin in particular, published estimates of LD_{50} values for male and female *R. norvegicus* were numerous and extremely variable. There were fewer published estimates of LD_{50} values for brodifacoum for *R. norvegicus* and these were less variable, while comparatively few, but also variable, estimates were found for pindone. In the absence of detailed information regarding the shape and slope of dose-response (toxicity) curves for brodifacoum, warfarin, pindone and diphacinone, a sublethal (approximate LD_{15}) dose for each was estimated from an acute oral LD_{50} value for rats, assuming a directly proportional relationship. An LD_{50} value/range for each anticoagulant was chosen from the lowest reported values, thus assuming the highest toxicity for each compound. Where possible, these values were selected from the most recently published studies that included values derived using female rats and confidence interval calculations (Table 1). Thus, the doses administered reflected the relative toxicity of each anticoagulant for comparison of persistence but were also expected to be sublethal, unlikely to produce symptoms that would adversely affect rats throughout the trial, and result in measurable concentrations of residues in liver tissue. While rodents exposed to anticoagulant baits in field situations may consume much higher doses than those administered here, it was important to obtain a relative estimate of the persistence of residues that could be available to predators of healthy, active rats in the wild as a basis for risk assessment.

3.3 TRIAL 1: SCOPING PERSISTENCE

Rats were randomly allocated to treatment groups (Table 2) and weighed just prior to oral dosing by gastric intubation with a maximum dose volume per rat of 0.5 mL. At least one 'spare' rat per treatment was dosed to maintain sample size in case of mortality during the trial. For 5 d after dosing, all rats were closely observed at least once a day for symptoms of anticoagulant poisoning. Groups of six or seven rats were humanely euthanased at different set times for each different anticoagulant treatment group (Table 2). All rats were weighed just prior to euthanasia, and their livers removed post-mortem for analysis of the appropriate anticoagulant residue, as described below. Any rats that died during the trial were weighed, but not included in the liver samples. 'Spare' rats

TABLE 1. INFORMATION USED TO DERIVE SUBLETHAL (APPROXIMATE LD_{15}) DOSES OF FOUR ANTICOAGULANTS ORALLY ADMINISTERED TO LABORATORY RATS.

ANTICOAGULANT	ACUTE ORAL LD_{50} (mg/kg)	REFERENCE	ESTIMATED SUBLETHAL DOSE (mg/kg)
Brodifacoum	0.27	Godfrey (1985)	0.1
Warfarin	3.3	Hone & Mulligan (1982)	1.0
Pindone	75–100	Eason & Wickstrom (2001)	24.0
Diphacinone	1.93–2.7	Ashton et al. (1987)	0.8

TABLE 2. SUMMARY OF TREATMENT GROUPS, SUBLETHAL DOSES ADMINISTERED AND SAMPLE TIMES FOR TRIALS 1 AND 2.

TREATMENT	TRIAL	NO DOSED	DOSE (mg/kg)	SAMPLE TIMES AFTER DOSING
Brodifacoum	Trial 1	20	0.1	Week 1, 18, 24
Warfarin	Trial 1	25	1.0	Week 1, 3, 6, 12
	Trial 2	20	1.0	Week 1, 18, 24
Pindone	Trial 1	20	24	Week 1, 3, 6
	Trial 2	18	35	Day 2, Week 1, 2
	Trial 2 (replicate)	14	35	Week 1, 2
Diphacinone	Trial 1	32	0.8	Week 1, 6, 12, 18, 24
	Trial 2	18	1.5	Day 2, Week 1, 2
	Trial 2 (replicate)	13	1.5	Week 1, 2

that were dosed but did not die during the trial were sampled in the last set time group, which was different for each anticoagulant treatment.

Fewer sampling intervals were required to ensure an accurate estimate of the persistence of liver residues of brodifacoum due to the existing literature for this compound. Sampling intervals for the other three anticoagulants (Table 2) were initially selected on the basis of existing information regarding persistence and metabolism of these compounds in a range of species, which suggested that warfarin and pindone were likely to be less persistent than diphacinone. However, the results of this first trial indicated that the persistence of diphacinone, warfarin and pindone had not been accurately defined, so additional treatment groups were dosed with these anticoagulants, and sample intervals selected to provide data for appropriate time points that would improve the sample size and data for calculation of hepatic half-life.

3.4 TRIAL 2: REFINING ESTIMATES OF PERSISTENCE

In the second trial, young adult female rats (Wistar) were randomly allocated to warfarin (n = 20), pindone (n = 32) or diphacinone (n = 32) treatments (Table 2). Two spare rats were dosed in each treatment group as replacements for any mortality that might occur between dosing and sampling. Rats were weighed prior to their being dosed via oral gavage. For pindone and diphacinone, slightly higher sublethal doses (35 mg/kg and 1.5 mg/kg, respectively) were used in this trial because liver residues of both anticoagulants appeared to be eliminated well before 6 weeks in Trial 1. In this second trial, analyses of liver tissue were completed before the next sample interval, to ensure that the residue concentrations had not fallen to below the method limit of detection and that sampling was not proceeding unnecessarily. In the pindone and diphacinone groups this occurred at the 2 week sample, and the remaining dosed rats were considered unlikely to have detectable liver residues. To optimise the experimental use of these rats, they were maintained for 8 weeks to ensure

clearance of liver residues, before being weighed and orally gavaged with the same anticoagulant and dose as before. Rats in this 'replicate' within Trial 2 were sampled at weeks 1 and 2 after dosing (Table 2).

3.5 LABORATORY ANALYSIS OF LIVER RESIDUES

Stock solutions used to dose the rats were prepared at the IANZ-accredited Landcare Research toxicology laboratory. Monopropylene glycol (MPG) solutions of each of the anticoagulants were prepared as shown in Appendix 1. All liver tissue was analysed for anticoagulant concentrations at the toxicology laboratory, Landcare Research, Lincoln. Analyses for brodifacoum (TLM009: Determination of brodifacoum in liver tissue by HPLC) and warfarin (TLM057: Determination of warfarin in liver tissue by HPLC) 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.

For brodifacoum analyses, the HPLC was equipped with an Alltech 250 × 4.6 mm, 5 µm Econosil C18 column, fluorescence detector and post-column reagent pump. Aliquots of the sample were chromatographed on an Alltech Econosil, using acidified methanol and water as the mobile phase. The post-column reagent was ammonia/methanol/water (10: 10: 80). The flow rate of the ammonia solution was adjusted until the effluent had a pH of approximately 10.1. The HPLC was run at a flow-rate of 1.5 mL/min with helium sparging, using a gradient programme of: initial 65% A, 35% B; 5 min 84% A, 16% B; 10 min 95% A, 5% B; 15 min 95% A, 5% B; and 21 min 65% A, 35% B. The fluorescence detector was set at an excitation wavelength of 310 nm, with an emission wavelength of 390 nm, a gain of × 1000 and a filter setting of 1.5 for the Waters system. The retention time was 12–14 min. For the mobile phase, 2.50 mL of glacial acetic acid was added to HPLC methanol to 1 L and filtered through a 0.22 µm filter and 1.25 mL glacial acetic acid was added to deionised water to 500 mL and filtered through a 0.45 µm filter.

For the warfarin anayses, the HPLC was equipped with an Alltech 250 × 4.6 mm, 5 µm Econosil C18 column (acid), fluorescence detector and post-column reagent pump. Aliquots of the sample were chromatographed using acidified methanol and water as the mobile phase. The post-column reagent was ammonia/methanol/water (10: 10: 80). The flow rate of the ammonia solution was adjusted until the effluent had a pH of approximately 10.1. The HPLC was run at a flow-rate of 1.5 mL/min with helium sparging, using the gradient programme: initial 65% A, 35% B; 6 min 84% A, 16% B; 10 min 95% A, 5% B; and 15 min 65% A, 35% B. The fluorescence detector was set at an excitation

wavelength of 310 nm, with an emission wavelength of 390 nm, a gain of × 1000 and a filter setting of 1.5 for the Waters system. The retention time for warfarin and the internal standard (coumatetralyl) was 7.4 min and 8.8 min respectively.

Methods for pindone (TLM018: Determination of pindone in liver tissue by HPLC) and diphacinone (TLM048: Determination of diphacinone in liver tissue by HPLC) analyses were based on those of Hunter (1984), but used a smaller C8 HPLC column, and chloroform/acetone/formic acid for the tissue extraction. 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 evaporated again and taken up in mobile phase for HPLC determination, which employed ion-paired chromatography and ultraviolet-visible detection.

For pindone analyses, the HPLC was equipped with a Phenomenex Luna 5 µm C8, 250 × 4.6mm column (PIC column), and the UV detector set at 284 nm. Aliquots of the sample were chromatographed using 0.005 M of tetrabutyl ammonium phosphate in methanol, and Waters PIC Reagent A in water as the mobile phase. The HPLC was run at a flow-rate of 1.0 mL/min with helium sparging, using a gradient programme of: initial 50% A, 50% B; 0.5 min 50% A, 50% B; 4 min 90% A, 10% B; 6 min 90% A, 10% B; 7 min 50% A, 50% B; and 10 min 50% A, 50% B. An injection volume of 50 µL was used and the retention times for pindone and the internal standard (diphacinone) were 5.1 min and 5.8 min respectively. For the mobile phase, 1.7 g tetrabutyl ammonium dihydrogen phosphate was dissolved in HPLC-grade methanol and made up to 1 L, and filtered through a 0.22 µm solvent filter. One bottle of PIC Reagent A was added to a 1 L bottle and made up to volume with MQ water and filtered through a 0.45 µm filter.

For the diphacinone analyses, the HPLC was equipped with a Phenomenex Luna 5 µm C8, 250 × 4.6 mm column (PIC column), and the UV detector set at 284 nm. Aliquots of the sample were chromatographed using 0.005 M of tetrabutyl ammonium phosphate in methanol, and Waters PIC Reagent A in water as the mobile phase. The HPLC was run at a flow-rate of 1.0 mL/min with helium sparging, using the gradient programme: initial 50% A, 50% B; 0.5 min 50% A, 50% B; 4 min 90% A, 10% B; 6 min 90% A, 10% B; 7 min 50% A, 50% B; and 10 min 50% A, 50% B. An injection volume of 100 µL was used and the retention times for diphacinone and the internal standard (chlorophacinone) were 7.3 min and 7.7 min, respectively. For the mobile phase, 1.7 g of tetrabutyl ammonium dihydrogen phosphate was dissolved in HPLC grade methanol to 1 L, and filtered through a 0.22 µm solvent filter. One bottle of PIC Reagent A was made up to 1 L with MQ water and filtered through a 0.45 µm filter.

For all analyses, blank and spiked sample matrix materials were run with each sample-set to establish method recoveries. The least detectable level (LDL) and uncertainty for each analysis are summarised in Appendix 1 (Table A1.2).

3.6 STATISTICAL ANALYSIS

The equation:

$$log_e(residue) = a + bt$$

was used to model the liver residue concentration decline with time (t) for each anticoagulant. Large residues tend to have large variability, but the variabilty of $log_e(residue)$ was reasonably constant so in this form the equation could be fitted using ordinary least squares. For graphs, the equation was retransformed to:

$$residue = A \times B\text{^}t$$

which implies that A = exp(a) is the initial residue and B = exp(b) is the proportional decrease in residue in 1 week. The half-life was then estimated as:

$$log\ (0.5)/b.$$

4. Results and discussion

No rats died after dosing with brodifacoum (0.1 mg/kg), warfarin (1 mg/kg) or pindone (24 or 35 mg/kg). No symptoms of anticoagulant poisoning were observed in any rats in these groups prior to euthanasia. Post-mortem, a small mesenteric haemorrhage was observed in one rat 18 weeks after dosing with brodifacoum; and small abdominal haemorrhages in one rat at 1 week, and in another rat 6 weeks after dosing with warfarin. One rat died 10 days after being dosed with diphacinone (1.5 mg/kg), exhibiting symptoms of anticoagulant poisoning.

In all treatment groups, rats had anticoagulant residues in liver tissue at the first sample after dosing, with the mean concentrations of residues in liver declining over subsequent samples (Table 3). After dosing with 0.1 mg/kg brodifacoum, mean concentration of residues in liver tissue declined from 1.27 µg/g at 1 week, to 0.49 µg/g at 24 weeks. After dosing with 1.0 mg/kg warfarin, mean concentration of residues in liver tissue declined from 1.26 µg/g at 1 week, to below the analytical limit of detection at 24 weeks after dosing (combined results of Trial 1 and Trial 2). After dosing with 24 mg/kg pindone in Trial 1, mean concentration of residues in liver tissue declined from 0.61 µg/g at 1 week, to below the anaytical limit of detection at 3 and 6 weeks after dosing. After dosing with 35 mg/kg pindone in Trial 2, mean concentration of residues in liver tissue declined from 1.75 µg/g at 2 days to 0.28 µg/g at 1 week, and after the replicate dosing with 35 mg/kg pindone in Trial 2, mean residues declined from 4.38 µg/g at 2 days to 0.24 µg/g at 1 week. After dosing with 0.8 mg/kg diphacinone in Trial 1, mean concentrations of residues in liver tissue declined from 0.08 µg/g at 1 week after dosing to below the analytical limit of detection at 6 to 24 weeks. After dosing with 1.5 mg/kg diphacinone in Trial 2, mean concentration of residues in liver tissue declined from 0.54 µg/g at 2 days to 0.08 µg/g at 1 week; and after the replicate dosing with 1.5 mg/kg diphacinone in Trial 2, mean residues declined from 0.91 µg/g at 2 days to 0.20 µg/g at 1 week.

TABLE 3. MEAN (± SEM) OF LIVER RESIDUE CONCENTRATIONS IN RATS AT SELECTED TIMES AFTER A SUBLETHAL DOSE OF ANTICOAGULANT (MDL = METHOD DETECTION LIMIT).

SAMPLING TIME AFTER DOSING	DOSE (mg/kg)	NO OF RATS	MEAN LIVER RESIDUE CONCENTRATION (µg/g)
Brodifacoum			
1 week[a]	0.1	6	1.27 ± 0.09
18 weeks[a]	0.1	6	0.59 ± 0.05
24 weeks[a]	0.1	8	0.49 ± 0.04
Warfarin			
1 week[a]	1.0	6	1.26 ± 0.07
3 weeks[a]	1.0	6	0.72 ± 0.05
6 weeks[a]	1.0	7	0.40 ± 0.04
12 weeks[a]	1.0	6	0.29 ± 0.07
12 weeks[b]	1.0	6	0.09 ± 0.01
18 weeks[b]	1.0	6	0.03 ± 0.00
24 weeks[b]	1.0	8	< MDL
Pindone			
2 days[b]	35.0	6	1.75 ± 0.22
2 days[c]	35.0	7	4.38 ± 0.94
1 week[b]	35.0	6	0.28[d]
1 week[c]	35.0	7	0.24 ± 0.02
1 week[a]	24.0	6	0.61 ± 0.08
2 week[b]	35.0	6	< MDL
3 weeks[a]	24.0	7	< MDL
6 weeks[a]	24.0	7	< MDL
Diphacinone			
2 days[b]	1.5	6	0.54 ± 0.09
2 days[c]	1.5	6	0.91 ± 0.08
1 week[a]	0.8	7	0.08 ± 0.01
1 week[b]	1.5	6	0.12[d]
1 week[c]	1.5	7[e]	0.20 ± 0.10
2 weeks[b]	1.5	6	< MDL
6 weeks[a]	0.8	6	< MDL
12 weeks[a]	0.8	6	< MDL
18 weeks[a]	0.8	6	< MDL
24 weeks[a]	0.8	6	< MDL

[a] Trial 1.

[b] Trial 2.

[c] Replicate group in Trial 2.

[d] Only one liver sample with detectable residues and remainder of samples < MDL.

[e] One rat died 10 days after dosing with 1.5 mg/kg diphacinone in Trial 2.

While the 8-week period between the first dose in Trial 2 and the replicate dose for the pindone and diphacinone treatments was expected to allow clearance of detectable residues from the liver, it is unknown whether the differences in the magnitude of the mean day 2 residues, in the pindone treatment particularly, were influenced by the previous dose. There may have also been an effect on residue concentrations of the larger body size of the rats when dosed in the replicate. However, the combined day 2 results provide an indication of the range of residues that could be expected in rat liver soon after ingestion of a

sublethal dose of pindone or diphacinone, and are not inconsistent with the overall trend of declining residues over time. Liver microsomes contain a selective, saturable binding site for anticoagulants, and the extent of persistence depends upon the chemical structure of the anticoagulant and the subsequent affinity for the binding site (Thijssen 1995). In this study, liver residues in the first samples (2 days) after dosing with pindone and diphacinone were variable in comparison to the first samples (1 week) after dosing with warfarin and brodifacoum, and this may be due to differences in the chemical structures of anticoagulant compounds, which are linked to their persistence in liver tissue (Thijssen 1995).

Exponential decay curves for each anticoagulant were graphed and liver elimination half-lives calculated (Figs 1–4). Liver elimination half-life ($t_{1/2}$) values are shown as an index of the persistence of residual concentrations of each anticoagulant in rat liver. For both pindone and diphacinone, detectable liver residues were present at only two points, day 2 and week 1, after dosing. Since the curve will fit any two points perfectly this provides no check at all that the assumed shape of the curve is correct. To give a more robust estimate, week 2 values set at half of the LDL were included in the analysis. These values were a little higher than the concentrations predicted for week 2 by the two-point curve, so the effect was to give an estimate of half-life that was likely to overestimate persistence in liver and provide a conservative basis for risk assessment.

The brodifacoum liver-elimination half-life was estimated at 113.5 d (Fig. 1), which is consistent with previously published reports of hepatic half-lives of brodifacoum, of > 80 d (Bachmann & Sullivan 1983) and 130 d (Parmar et al. 1987). The latter authors found the elimination of brodifacoum from the liver was biphasic, consisting of a more rapid initial phase up to 8 d after dosing, and a slower terminal phase. While coumatetralyl was not evaluated in this study, Parmar et al. (1987) estimated a liver-elimination half-life of coumatetralyl in rats of 55 d. On this basis, the hepatic elimination rate of coumatetralyl in rats appears to be between that of warfarin and brodifacoum, as in this study we estimated a liver-elimination half-life of warfarin in rats of 26.2 d (Fig. 2). The plasma elimination half-life of pindone in dogs was approximately 100 h after a

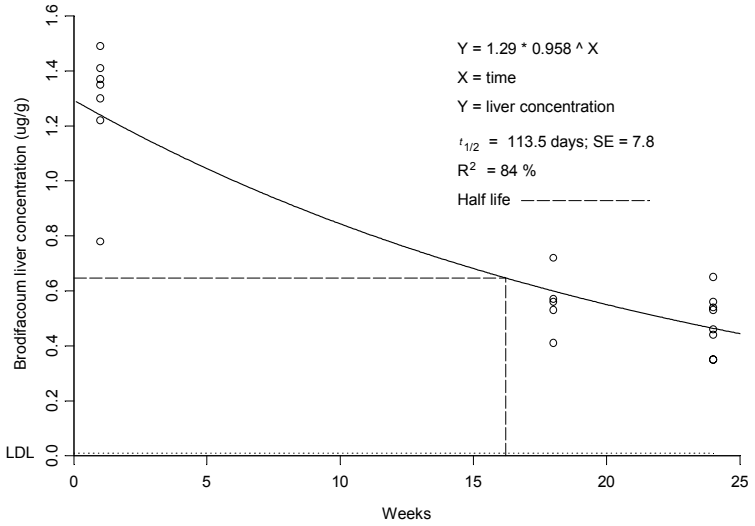

Figure 1. Concentrations of brodifacoum residues in liver of rats following an approximate LD_{15} (0.1 mg/kg) gavage dose ($t½$ = hepatic elimination half-life; SE = standard error of $t½$; R^2 = proportion of variability explained by the curve, based on untransformed data. Least detectable level (LDL) was 0.01 mg/g).

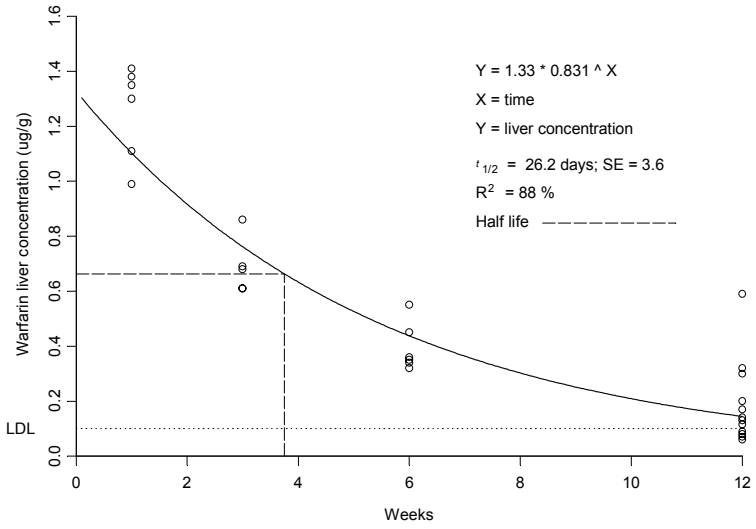

Figure 2. Concentrations of warfarin residues in liver of rats following an approximate LD_{15} (1.0 mg/kg) gavage dose ($t½$ = hepatic elimination half-life; SE = standard error of $t½$; R^2 = proportion of variability explained by the curve, based on untransformed data. Least detectable level (LDL) was 0.1 mg/g).

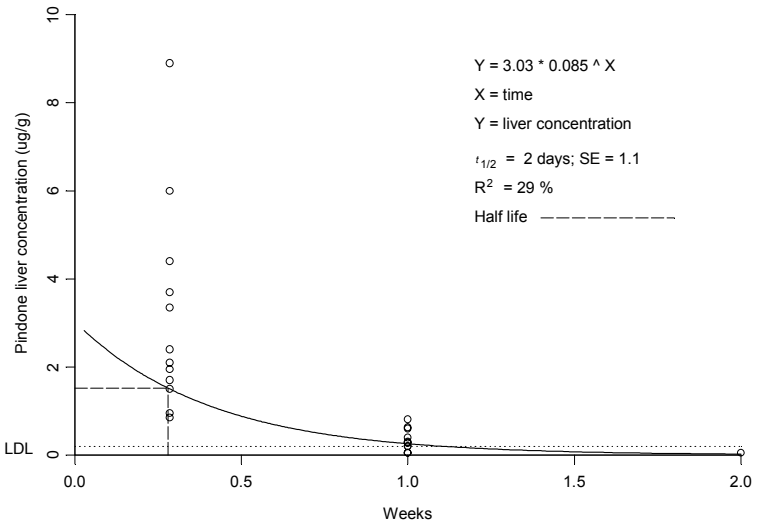

Figure 3. Concentrations of pindone residues in liver of rats following an approximate LD_{15} (35 mg/kg) gavage dose ($t½$ = hepatic elimination half-life; SE = standard error of $t½$; R^2 = proportion of variability explained by the curve, based on untransformed data. Least detectable level (LDL) was 0.2 mg/g).

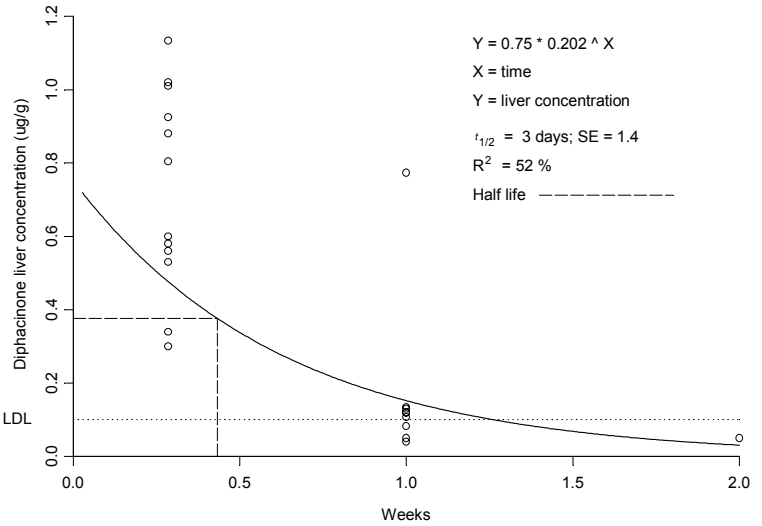

Figure 4. Concentrations of diphacinone residues in liver of rats following an approximate LD_{15} (1.5 mg/kg) gavage dose ($t½$ = hepatic elimination half-life; SE = standard error of $t½$; R^2 = proportion of variability explained by the curve, based on untransformed data. Least detectable level (LDL) was 0.1 mg/g).

3 mg/kg dose (Fitzek 1978), and sheep dosed with 10 mg/kg pindone had detectable liver residues at 8 d after dosing and no detectable liver residues 16 d after dosing (Nelson & Hickling 1994). We estimated the liver-elimination half-life of pindone in rats as 2 d (Fig. 3).

We estimated liver-elimination half-life of diphacinone in rats as 3 d (Fig. 4), which is consistent with the findings of two previous studies of tissue distribution and metabolism of diphacinone, using radio-labelled compound in rodents (Cahill & Crowder 1978; Yu et al. 1982). In contrast to findings in rodents, Bullard et al. (1976) reported that cows dosed with 1 mg/kg diphacinone by intraruminal injection had almost constant liver residues of up to 0.15 mg/g from 30 d to 90 d after dosing. However, rats fed for 14 d on the livers of the cattle dosed with diphacinone in that study showed no signs of toxicity during the test period or for 14 d afterwards. Liver samples taken from the rats at the end of that period contained no detectable diphacinone residues (Bullard et al. 1976), which is suggestive of a relatively rapid clearance of sublethal doses of diphacinone from the livers of rats.

While it is common practice to use indices, such as acute LD_{50} values, to compare the toxicity of vertebrate pesticides to different species, variability in the susceptibility of different rodent species (and between the sexes of one species) to anticoagulant compounds has been noted (Ashton et al. 1987). Variation in the elimination kinetics of anticoagulants in different pest rodent species, e.g. *Rattus rattus* and *R. norvegicus*, in field conditions should also be considered in future, as this may contribute to different risks of residues and secondary poisoning, according to the species of rodent being targeted.

5.0 Conclusions and recommendations

Our study suggests that warfarin appears to be slightly more persistent in rat liver than previously reported; Thijssen (1995) reported a half-life of 7–10 d for warfarin in rats, whereas our study measured a hepatic half-life of 26.2 d. Although no previous estimates of the hepatic half-life of pindone or diphacinone were found, in this study both pindone and diphacinone were less persistent than previously reported retention times in mammalian livers. Whilst second-generation anticoagulants are generally recognised as being more persistent than first-generation compounds, this trial has refined estimates of the persistence of warfarin, pindone and diphacinone with respect to brodifacoum, allowing comparison of these anticoagulants in future assessments of secondary poisoning risk. Because vertebrate pesticides that are rapidly metabolised or excreted by the target pest species may carry a reduced risk of secondary poisoning of non-target species, in contrast to a more persistent or bioaccumulative compound (e.g. Hosea 2000), diphacinone

appears the most promising potential alternative anticoagulant for mainland rodent control in New Zealand since it appears to have relatively low persistence but also medium potency to rodents.

Based on the results of this study, we recommend that further research be undertaken to assess the secondary hazard of different anticoagulants, including diphacinone, to New Zealand species of predators and scavengers, and also to specifically assess the efficacy of diphacinone formulations in field control of pest rodent species in New Zealand.

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Appendix 1

TABLE A1.1. PREPARATION OF STOCK SOLUTIONS BY THE LANDCARE RESEARCH TOXICOLOGY LABORATORY, LINCOLN.

ANTICOAGULANT AND DOSE (mg/kg)	MADE FROM % ACTIVE	STOCK SOLUTION (mg/mL)	STOCK SOLUTION CERTIFICATE NO	CERTIFICATE DATE	DOSE DATE
Brodifacoum					
(0.1)	95.4	0.05	P00/08	5 Apr 00	11 Apr 00
Warfarin					
(1.0, trial 1)	98	0.5	P00/18	14 Aug 00	15 Aug 00
(1.0, trial 2)	98	0.5	P01/64	26 Nov 01	27 Nov 01
Pindone					
(24, trial 1)	98	12.0	P00/17	14 Aug 00	15 Aug 00
(35, trial 2)	87	17.5	P01/62	26 Nov 01	27 Nov 01
(35, trial 2)	87	17.5	P02/03	29 Jan 02	5 Feb 02
Diphacinone					
(0.8, trial 1)	99	0.4	P00/10	11 Apr 00	19 May 00
(1.5, trial 2)	99	0.75	P01/63	26 Nov 01	27 Nov 01
(1.5, trial 2)	99	0.75	P02/04	29 Jan 02	5 Feb 02

TABLE A1.2 ANALYTICAL LIMITS OF DETECTION AND METHOD UNCERTAINTY, LANDCARE RESEARCH TOXICOLOGY LABORATORY, LINCOLN.

ANTICOAGULANT (TOXICOLOGY LABORATORY ANALYSIS REFERENCE NUMBER)	LEAST DETECTABLE LEVEL(µg/g)	METHOD UNCERTAINTY
Brodifacoum (TLM009)*	0.01	20%
Warfarin (TLM057)	0.1	6%
Pindone (TLM018)	0.2	41%
Diphacinone (TLM009)*	0.1	38%

* IANZ-accredited assay.