Environmental impact of brodifacoum use – monitoring residues in wildlife

Envirolink 1029-HBRC146

Landcare Research
Manaaki Whenua

Environmental impact of brodifacoum use – monitoring residues in wildlife

Lynn H. Booth, Penny Fisher, Matt Campion, Les Brown

Landcare Research

Prepared for:

Hawke's Bay Regional Council

159 Dalton Street
Napier 4110
New Zealand

July 2012

Landcare Research, Gerald Street, PO Box 40, Lincoln 7640, New Zealand, Ph +64 3 321 9999, Fax +64 3 321 9998, www.landcareresearch.co.nz

Reviewed by:	*Approved for release by:*
Grant Morriss Researcher Landcare Research	Phil Cowan Science Team Leader Wildlife Ecology & Management

Landcare Research Contract Report:	LC 1056

Disclaimer

This report has been prepared by Landcare Research for Hawke's Bay Regional Council. If used by other parties, no warranty or representation is given as to its accuracy and no liability is accepted for loss or damage arising directly or indirectly from reliance on the information in it.

Contents

Summary

Project and Client

- Hawke's Bay Regional Council contracted Landcare Research (Envirolink 146-HBRC131) and Habitat Biodiversity and Pest Management Ltd in 2012 to survey representative wildlife for residues of the vertebrate toxic agent brodifacoum.

Objective

- To measure residual brodifacoum concentrations in samples of indicator wildlife (hedgehogs, introduced birds, invertebrates) from four sites in Hawke's Bay with differing histories of brodifacoum application in bait stations for possum control.

Methods

- Four farms of different size were selected as monitoring sites. Sites were at least30 km apart, which was considered sufficient to exclude movement of wildlife between the sites during the sampling period.Three were part of the Possum Control Area (PCA) programme coordinated by the regional council and one was an organic farm that does not use any toxins for pest control (i.e. a control site). The three sites with brodifacoum use had been part of the PCA programme for different durations (3, 7 and 11 years).
- A minimum of six samples of each wildlife indicator (hedgehogs, introduced birds, and invertebrates) were collected from each site. Samples were taken from near the middle of each site to minimise the possibility of sampling an animal that had recently entered the site or had significant foraging range outside the site.
- Vertebrate specimens were killed to obtain liver tissue for brodifacoum residue testing. For invertebrate samples the whole bodies of small invertebrates were combined to make a composite sample for testing. We used HPLC with fluorescence detection to determine the concentration of brodifacoum in tissue samples. The method detection limit (MDL) was 0.001 µg/g for liver and 0.01 µg/g for invertebrate tissues.

Results

- Fifty-six vertebrate samples were collected from the four sites (6, 9, 6, and 6 hedgehogs and 7, 11, 5, and 6 birds from Sites 1, 2, 3 and 4 respectively).
- Of the 27 hedgehogs collected, 17 contained detectable brodifacoum residues, ranging from 0.001 to 0.14 µg/g with a mean of 0.022 µg/g. Site 1 (the unpoisoned control) contained the highest number of positives (5/6). Site 4 (11 years in the PCA) had the lowest number of hedgehogs with residues (2/6). Site 3 (3 years in the PCA) had 4 positives out of 6 samples and Site 2 (7 years in the PCA) had 6/9 samples positive.
- The birds collected at Sites 1 and 4 were mainly blackbirds and thrushes, at Site 2 were mainly magpies, and Site 3 had a range of bird species. Of the 29 birds collected, 12 had detectable brodifacoum residues, ranging from 0.0013 to 0.053 µg/g, with a mean of 0.010 µg/g. Site 2 had the largest number of positive samples (8/11). Site 4 had only one bird with residues.

- The invertebrate samples (n = 11) collected included slugs, snails, grasshoppers, cicadas and beetles. No residues were found in any of the invertebrate samples.
- Brodifacoum residue concentrations were not significantly different between sites (Kruskal–Wallis $\chi^2_3 = 6.82$, $p = 0.08$), but there were marginally significant differences between sites in the proportion of samples that contained brodifacoum ($\chi^2_3 = 7.89$, $p = 0.05$). A slightly higher proportion of birds and hedgehogs sampled from Sites 1 and 2 contained residues than did those from Sites 3 and 4. This marginally significant difference was not related to the duration of use of brodifacoum in the immediate environment (i.e. in bait stations).
- The control site, on which there had been no pesticide use at all, had the highest proportion of animals with brodifacoum residues.

Conclusions

- The approximately 50% incidence of brodifacoum-positive hedgehogs and birds across all sites, and the slightly higher proportion of vertebrates with residues in Sites 1 and 2 (with no or least brodifacoum use), indicates that brodifacoum exposure of some vertebrate wildlife is ubiquitous in Hawke's Bay.
- The lack of significant differences between the sites monitored suggests that brodifacoum has a wide capacity for secondary exposure through trophic pathways, with potential sources from both field bait station applications and its use for rodent control in and around farm and urban buildings.
- The frequency and concentration of residual brodifacoum in the wildlife sampled were not related to the length of time brodifacoum had been used at a particular site. The ongoing use of brodifacoum in bait stations as part of the PCA programme in Hawke's Bay does not therefore appear to be resulting in localised, relatively high levels of environmental contamination as indicated by the wildlife sampled.

Recommendations

- A survey of at-slaughter tissue (liver and muscle) from livestock from PCA farming areas would be useful to confirm that brodifacoum exposure is not as widespread in livestock as in the indicator wildlife sampled in this study.
- Quantifying the amounts of bait that are typically removed from different types of bait stations to the wider environment by rodent or possum behaviour could help to refine best practice in the PCA programme, by identifying 'least spillage' station types and / or modifications to minimise bait loss.
- Survey information about household or on-farm uses of brodifacoum (and other second-generation anticoagulant rodenticides) in Hawke's Bay would help to determine the relative contribution of the PCA programme to the overall widespread but low-level environmental brodifacoum residues measured in this study.

1 Introduction

Hawke's Bay Regional Council contracted Landcare Research (Envirolink 146-HBRC131) and Habitat Biodiversity and Pest Management Ltd in 2012 to survey representative wildlife for residues of the vertebrate toxic agent (VTA) brodifacoum. Four sites with different histories of brodifacoum use were monitored using selected wildlife (hedgehogs, introduced birds and invertebrates) as indicator species.

Research in New Zealand and internationally has raised issues around the residual environmental persistence and non-target effects of the anticoagulant VTA brodifacoum. However, no longitudinal studies or long-term monitoring have been conducted to address these concerns. This study was undertaken to measure the occurence of residual brodifacoum in selected environmental samples from the Hawke's Bay area where brodifacoum use is widespread and, in some places, has been used repeatedly for possum management.

2 Background

Pest animals have a significant impact on flora and fauna in New Zealand, and in many cases are subject to control using VTAs. However, VTAs can have negative environmental and ecosystem impacts. In New Zealand brodifacoum is used on a large scale for pest management. Sound advice on the toxin's non-target impacts will allow pest managers to better assess if the net benefits of using brodifacoum outweigh the potential environmental impacts. The results will have a potentially wide application for pest managers that already have similar large-scale pestcontrol programmes or are in the process of developing them.

In 2002 the Hawke's Bay Regional Council (HBRC) initiated the first Possum Control Area (PCA) in the Hawke's Bay Region. This was the start of a long-term plan to have all Hawke's Bay farmland under either Animal Health Board (AHB) driven possum control or under a council PCA. The PCA programme has now grown to 30 pest control areas covering 440 000ha. The initial pest control in a PCA is carried out by an approved contractor required to reduce the residual trap catch (RTC) to less than 3%. After this initial knockdown it is then the farmers' responsibility to maintain the RTC below 5%. The primary method used by most farmers is to set up a network of bait stations that are pulse-baited at intermittent intervals (generally 12–18 months). Most landholders use contractors to carry out this work.

New Zealand field research in the 1990s demonstrated mortality of some predatory and scavenging non-target wildlife through secondary brodifacoum exposure and the occurrence of residual brodifacoum in a range of other non-target wildlife (e.g. Eason & Spurr 1995). This led to the Department of Conservation implementing major restrictions on brodifacoum application on Conservation land, but brodifacoum remains the main VTA used in bait stations in many other pest management contexts. HBRC requires confidence that the toxins being used to control possums in the PCA are not incurring unacceptable environmental or ecosystem costs through contamination of animals. In 2010 the HBRC commisioned a literature review to summarise current knowledge about the environmental effects of the use of brodifacoum, particularly residual persistence and potential effects on non-target wildlife and livestock. This review (Fisher 2010) concluded that there has been little ongoing monitoring or investigation of the longer-term implications of the continued field use of brodifacoum for possum and rodent control on the environment in New Zealand.

The HBRC PCA creates an opportunity to characterise the environmental occurrence of residual brodifacoum at selected sites with known use patterns. Monitoring would provide a basis for clarifying the likely extent of environmental contamination by brodifacoum (using representative wildlife species as indicators) and to assess the potential environmental effects of the long-term use of brodifacoum. Furthermore it would provide a reference point for future monitoring of the same sites to determine whether changes in wildlife residue profiles could be linked to patterns in brodifacoum use.

The results are expected to indicate whether there is some level of environmental contamination associated with the use of brodifacoum in bait stations, and whether there is any association between the extent of contamination and duration of brodifacoum use. If environmental residues are detected at frequencies and concentrations of concern, this could suggest a future need to revise optimal possum control strategies where bait stations are used in order to reduce reliance on brodifacoum. This could include cycling of bait types containing alternative, less persistent VTAs or modifications to bait station design so as to minimise bait spillage and non-target access, and ensure delivery of lethal amounts of bait to target species.

3 Objective

- To measure residual brodifacoum concentrations in samples of indicator wildlife (hedgehogs, introduced birds, invertebrates) from four sites in Hawke's Bay with differing histories of brodifacoum application in bait stations for possum control.

4 Methods

4.1 Study sites

The PCA programme in Hawke's Bay has been running since 2002, so we can monitor the potential occurrence and variation in brodifacoum residue profiles at sites with different durations of brodifacoum exposure (intervention). Three sites were selected with existing bait station networks that had been serviced by a contractor who could provide information on when baiting had occurred. The three sites had had intervention in place for 2, 4 and 11 years respectively, and results were contrasted with a fourth site with no known history of brodifacoum use.

Four farms of varying size from different parts of the Hawke's Bay Region were selected as sampling sites. Three of the sites are part of the Possum Control Area (PCA) programme, while one is an organic farm that does not use any form of toxins for pest control. The PCA sites had all joined the PCA programme at different times and therefore had different histories of brodifacoum application prior to this study. All PCA sites were baited with 300 g of brodifacoum bait per bait station per year. The baits used were a cereal pellet formulation (Pestoff Brodifacoum Possum bait, Animal Control Products) containing 0.02 g/kg brodifacoum. Farms were at least 30 km apart, which was considered sufficient to exclude movement of wildlife between the sites during the sampling period.

Site 1 was the control site for the trial and has no previous documented applications of brodifacoum bait. Being a long-term organic farm means that this site had not used any form of toxin for pest control prior to the trial. This 1035-ha farm is in the Te Onepu South/Raukawa area.

Site 2 was located inland near the southern Hawke's Bay town of Takapau. This land holding had two parcels in different PCAs, Pukenui and Rakautathi. The trial was conducted in the eastern block, which is part of the Pukenui PCA. This 447-ha farm entered the PCA in 2006 and had 81 bait stations. Approximately 170 kg of brodifacoum bait had been used prior to the trial, with the most recent servicing of bait stations (before samples were taken) in May 2011 .

Site 3 was located on the coastline of northern Hawke's Bay. This large block (3509 ha) has been part of the Ridgemount PCA since 2010 and had 106 bait stations . A total of 95.4 kg of brodifacoum bait had been used prior to the trial, with the most recent servicing of bait stations in January 2012.

Site 4 was located near the coastline in southern Hawke's Bay. This land holding had two parcels, both of which are in the Baker 2 PCA. The site entered the PCA in 2002 and had 46 bait stations. A total of 151.8 kg of brodifacoum bait had been used on the block prior to the trial, with the most recent servicing of bait stations in July 2011.

4.2 Wildlife sampling

To determine the extent of residual brodifacoum in wildlife, a minimum of six samples of each non-target species (hedgehogs, birds, and invertebrates) were collected from each site. Hedgehogs were considered as appropriate mammal species as they are omnivorous (i.e. potentially exposed to brodifacoum through primary or secondary pathways) and easily trapped. Blackbirds and magpies were selected as suitable bird species representing potential primary (bait) and secondary (invertebrates, scavenged carcasses) exposure pathways. Hedgehogs were captured by hand while spotlighting (80%) or trapped (20%) using Victor rat traps baited with raspberry jam or cherries, covered DoC 200 traps baited with hen eggs and fish, or Victor No. 1 leg hold traps set at ground level lured with a flour and icing sugar blaze and baited with fish. Birds were captured by spotlighting and shooting using a .22 or high-powered air rifle or trapped using Victor rat traps or Victor No. 1 leg-hold traps. Various insects known to feed on cereal pellet baits (e.g. weta, beetles, and gastropods) were collected at night from rotten logs and litter from within 20 m of bait stations.

Samples were taken from near the middle of each of the sites to minimise the possibility of sampling an animal that had recently entered the area or had significant foraging range outside the site. The geographic coordinates of the capture sites were recorded and a central point between these sites selected. A circular buffer zone was then marked out around the central point to determine the amount of brodifacoum that had been used in proximity to the capture sites. The size of the buffer for each study site was selected to include as many bait stations and capture sites as possible. Distances of 1–3 km were selected as it is unlikely that many hedgehogs or invertebrates would venture outside that area during the sampling period. Samples were taken from Site 1 in December 2011; of 18 captures, 11 were within the 1-km buffer zone. Samples were taken from Site 2 in February 2012; of 22 captures, 18 were in the 1-km buffer zone, which contained 45 bait stations with total bait use of 94.5 kg. Samples

were taken from Site 3 in April 2012; of 13 captures, 10 were within the 3-km buffer zone, which contained 60 bait stations with total bait use of 54 kg. Samples were taken from Site 4 in May 2012, with all 13 captures within the 1-km buffer zone, which contained 29 bait stations with total bait use of 95.7 kg.

Whole carcasses and invertebrates were stored frozen and transported to the laboratory, where samples (2 g) of liver and muscle were taken for analysis (Appendix 1). Liver and invertebrate samples were analysed for brodifacoum by the Landcare Research toxicology laboratory (Lincoln), using an IANZ/ LAS accredited method (TLM070) for the determination of brodifacoum in animal tissues to food standards. In brief, this method used HPLC with fluorescence detection to determine the concentration of brodifacoum in tissue samples. A post-column pH switching technique was used to exploit the natural fluorescence of brodifacoum, using difenacoum as an internal standard. The method detection limit (MDL) was 0.001 µg/g, with method uncertainty (95% CI) of ±6%. For invertebrate samples the whole body was tested, and at least 1 g of sample was required, which generally meant that smaller invertebrates sampled from each site needed to be combined to make up one testable sample. The method detection limit was 0.01 µg/g, with method uncertainty (95% CI) being greater at ±27%.

4.3 Statistical analysis

Kruskal–Wallis chi-squared tests on residue data were carried out using the R statistical computing environment (version 2.15.0) (R Development Core Team 2012).

5 Results and discussion

5.1 Invertebrate samples

Eleven pooled invertebrate samples were collected from the four sites, and included slugs, snails, grasshoppers, cicadas and beetles. No residues were found in any of the invertebrate samples (Table 1). This is in contrast to a field study at Tawharanui where terrestrial invertebrates (weta, cockroaches, beetles and other 'miscellaneous' species) were monitored for residues before, during and after application of brodifacoum in bait stations (Craddock 2003). At that site, 'trace' concentrations of brodifacoum were apparently present prior to baiting, and levels up to 7.47 µg/g were found in invertebrates after bait was placed in bait stations. Residue concentrations were dependent on the amount of toxic bait available in stations. Invertebrates carrying brodifacoum dispersed up to 10 m from the bait stations and residue concentrations in them decreased significantly the further away from the bait stations they were sampled. In the study reported here, invertebrates were sampled from within 20 m of bait stations.

Captive studies (summarised in Booth et al. 2001) indicate low toxicity of brodifacoum to invertebrates. Craddock (2003) found that captive locusts fed readily on cereal-based brodifacoum baits with no significant increase in mortality. In captive weta, weight loss and mortality was not significantly higher in weta exposed to brodifacoum bait over 60 days (Bowie & Ross 2006). On this basis, consumption of brodifacoum bait by invertebrates is not expected to result in high mortality. The potential role of invertebrates as environmental

vectors of residual brodifacoum is then dependent upon the persistence of brodifacoum in their tissues. Limited data are available regarding anticoagulants in invertebrates but generally indicate lower persistence than in mammals. Following sublethal doses, brodifacoum residues were not detectable after 4 days in captive weta (Booth et al. 2001) and captive locusts appeared to excrete brodifacoum rapidly, indicating that long-term bioaccumulation was unlikely (Craddock 2003).

The overall lack of detectable residues in the invertebrate samples in this study indicates that sustained use of brodifacoum in bait stations for the PCA programme is not resulting in widespread or consistent transport of residues by invertebrates. There were different intervals between collection of samples and the most recent application of baits in stations at the three PCA sites. For Site 2 this was 9 months, in Site 3 was 4 months (bait stations serviced January 2012, samples collected April 2012) and in Site 4 was 10 months (bait stations setviced July 2011, samples collected May 2012). The presence of bait in stations at the time of sampling at each site was not formally measured, but it was observed by personnel collecting wildlife samples that approximately 20% of the bait stations at the three sites still contained baits from the last service. This approximation of bait availability in stations over time is considered accurate for the Hawke's Bay region in general, where in vegetated areas of rat habitat the majority of baits in newly filled stations would be present for two to three months with remaining baits being removed more slowly over the period before the next service. (*pers. comm*., L. Simmons, Habitat Biodiversity and Pest Management Ltd.). The possibility therefore remains that invertebrates in close proximity to bait stations may carry residues while baits are available, but such residues are unlikely to have a prolonged persistence once baits are removed.

5.2 Vertebrate samples

Vertebrate samples (*n* = 56) were collected from the four sites: 27 of these were hedgehogs and 29 were birds (magpies, blackbirds and song thrushes). Brodifacoum residues were found in 29 samples (52%). Despite no baiting at Site 1 it yielded residues in 8/13 samples (62%), whereas at the baited sites, Site 2 had residues in 14/20 samples (70%), site 3 in 4/11 samples (36%), and Site 4 in 3/12 samples (25%) (Table 1).

Table 1 Summary of brodifacoum residues found in 'indicator' wildlife in Hawke's Bay sampling sites

Site	Time in PCA	Species (number tested)	Number of samples with residues	Range (mean) of residues in positives (µg/g)
1	None (Control)	Hedgehogs (*n* = 6)	5	0.002–0.14 (0.030)
		Birds (*n* = 7)	3	0.006–0.022 (0.016)
		Invertebrates (*n* = 3)	0	NA
2	7 years	Hedgehogs (*n* = 9)	6	0.0012–0.11 (0.021)
		Birds (*n* = 11)	8	0.0013–0.053 (0.009)
		Invertebrates (*n* = 1)	0	NA
3	3 years	Hedgehogs (*n* = 6)	4	0.001–0.014 (0.006)
		Birds (*n* = 5)	0	NA
		Invertebrates (*n* = 5)	0	NA
4	11 years	Hedgehogs (*n* = 6)	2	0.0027–0.072 (0.037)
		Birds (*n* = 6)	1	0.0016
		Invertebrates (*n* = 2)	0	NA

Hedgehogs

Seventeen of the 27 hedgehogs sampled (63%) contained residues. Site 1 (control) contained the highest number of positives (5/6) and also the animal with the highest concentration of brodifacoum in liver (0.14 µg/g). There was no relationship between the number of animals containing residues and the length of time pest control with brodifacoum has been carried out, although excluding the control site, the average residue concentration in hedgehogs was higher in sites with the longest duration of brodifacoum use.

Residues found in hedgehogs ranged from 0.001 to 0.14 µg/g, with a mean of 0.022 µg/g. This is approximately 10-fold lower than levels reported by Spurr et al (2005) in hedgehogs sampled from the St Arnaud area during a period of brodifacoum use in bait stations. In that study residues (mean 0.20 mg/kg) were found in 47.6 % of hedgehogs (21 animals tested). Anticoagulant residues have also been reported in livers of hedgehogs from the United Kingdom (Dowding et al. 2009). Overall, 57.5% of the UK hedgehogs tested had residues of at least one second-generation anticoagulant, although brodifacoum was the least common (in 5% of hedgehogs) with a mean concentration of 0.05 µg/g. It is noteworthy that UK uses of brodifacoum and other second-generation anticoagulants are highly restricted to commensal rodent control in and around buildings, with no field applications as occurs in New Zealand. This suggests that at least some of the residue burden measured here in hedgehogs from sites in Hawke's Bay could originate from household, industry or farm building uses of brodifacoum for rodent control.

Hedgehogs are omnivores and feed upon worms, insects, slugs, spiders, eggs and carrion and are also opportunistic predators of small rodents, frogs and young birds. Brodifacoum exposure in hedgehogs may thus be through bait ingestion (primary exposure) or through ingestion of invertebrates, other small prey or carcasses containing residual brodifacoum (secondary exposure). The extent to which primary or secondary exposure contributed to the residue profile in hedgehogs is not known. Spillage of bait from stations by possums or rodents would increase the opportunity for hedgehogs to encounter and consume bait, thus it would be of use to quantify the proportion of bait applied in stations that can be spilled in normal field conditions.

The nil detection of brodifacoum in invertebrate samples collected from the same areas suggests that, at the time of sampling, secondary exposure of hedgehogs via invertebrates was not a significant pathway; however, the persistence of brodifacoum residues in invertebrates is less than in mammals, thus the presence of residues in invertebrates is likely to coincide with the availability of bait in their immediate foraging environment. Similarly, the presence of possum or rodent carcasses poisoned by brodifacoum will coincide with the availability of bait in stations, so that these periods probably represent peak exposure potential for hedgehogs.

Birds

Twenty-nine birds were collected from the four sites. The most common birds collected were magpies, blackbirds, and song thrushes (Table 2). All three species are omnivorous. Magpies generally feed on the ground and eat invertebrates, but will take carrion and small live prey such as skinks, frogs or mice – and grain, tubers, figs and walnuts have also been noted in their diet. Song thrushes and blackbirds both eat a wide range of invertebrates, especially

earthworms and snails, as well as soft fruits and berries. Blackbirds also eat seeds, and occasionally hunt small vertebrates, such as frogs, tadpoles and lizards.

Brodifacoum residues were found in 12 of the 29 samples (i.e. 41%), and the pattern of residues differed from that for hedgehogs. This may have possibly been related to the bird species collected at each site. Site 2 had the largest number of positive samples, with 8/11 positives, all but one of which was from magpies. Site 4 had only one bird with residues –a thrush – and the only sample of this species returned a positive result. Of the other positive bird samples 3/4 were blackbirds, with the fourth from an unidentified bird (Figure 1).

Residues found in birds ranged from 0.0013 to 0.053 µg/g, with a mean of 0.010 µg/g. The bird sample containing the highest brodifacoum residues was a magpie, but the mean concentration of residues found in blackbirds appeared to be higher than the mean for magpies.

Table 2 Bird species found at each site

Site	Bird species (number with residues)	Range of residues
1	3 blackbirds (2)	<MDL-0.022
	2 thrush	<MDL
	2 unidentified birds (1)	0.006
2	10 magpies (7)	<MDL-0.0531
	1 blackbird (1)	0.0025
3	2 magpies	<MDL
	1 myna	<MDL
	2 unidentified birds	<MDL
4	3 thrush (1)	0.0016
	3 unidentified birds	<MDL

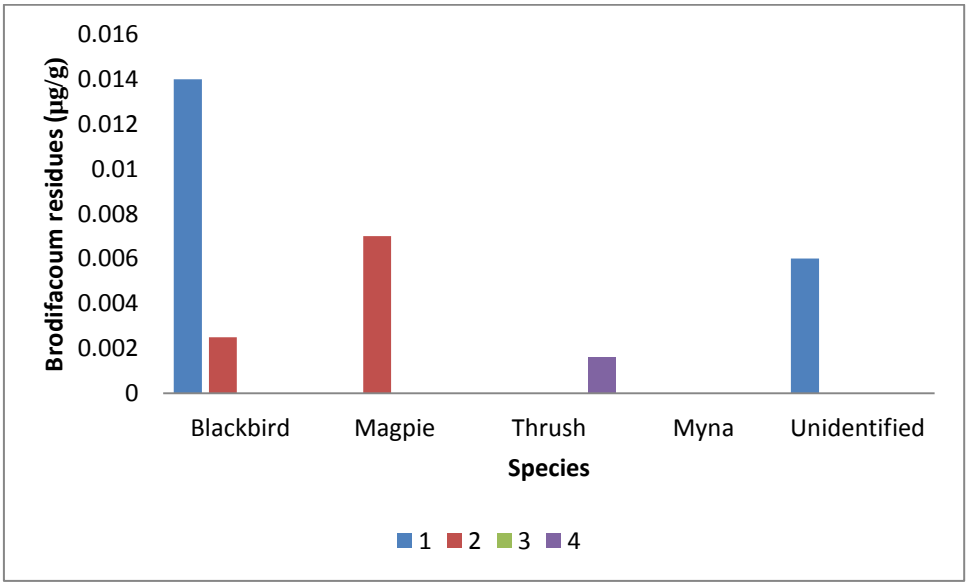

Figure 1 Mean brodifacoum residues in different bird species at each site.

5.3 Relationship between animal residues and site

Brodifacoum residue levels were not significantly different between sites (Kruskal–Wallis $\chi^2_3 = 6.82$, $p = 0.08$, but there was a marginally significant difference between sites in the proportion of samples that contained brodifacoum ($\chi^2_3 = 7.89$, $p = 0.05$). Samples from Sites 1 and 2 had a slightly higher proportion of animals containing residues than those from Sites 3 and 4. Site 1 is a long-term organic farm (control site), and thus had no known history of pesticide use. Site 2 has been in the PCA since 2006, and Site 4, which has been in the PCA the longest, had the lowest incidence of residues in the samples. The control site is surrounded by farms that have bait stations, and hedgehogs in New Zealand have a summer home range in the order of 30–90 ha, depending on food availability (Thomsen et al. 2000). Hedgehogs sampled from the control site may thus have originated from, or previously foraged in, neighbouring farms where brodifacoum was being used.

While the sampling sites were selected on the basis of their known history of brodifacoum use under the Hawke's Bay PCA programme, the extent of brodifacoum use for commensal rodent control on and near the sampling sites was not known. Rodent bait formulations containing brodifacoum are available over the counter to the public for use in houses or on the farm in and around buildings. Such applications are an additional input of brodifacoum into the environment. Spurr et al. (2005) showed that use of 'household' anticoagulant rodenticides was likely to have resulted in the occurrence of residues in wildlife trapped up to 8 km away. If such uses of brodifacoum are extensive in the Hawke's Bay Region, this may be contributing to the fairly consistent pattern of brodifacoum residues found in hedgehogs and introduced birds sampled from all four sites in this study. A survey of household and on-farm anticoagulant use, or of sales figures in the Hawke's Bay area, would help to determine the quantities of brodifacoum being used for commensal rodent control.

The use of brodifacoum as part of the PCA programme in Hawke's Bay is the most likely cause of the consistent, approximately 50% frequency of low-level residual brodifacoum found in the indicator wildlife from all four sites monitored in this study. Uncertainty remains about the contributions of primary exposure (i.e. bait spillage from stations) and secondary exposure (i.e. scavenging on the carcasses of poisoned animals) to the residue profiles described. This could be clarified by measuring the extent of bait spillage from stations used by possums and rodents, especially with respect to the potential for non-target animals, including grazing livestock, to be exposed to brodifacoum through bait spillage. Currently the Ministry for Primary Industries provides recommendations for the procurement of wild game animals (e.g. feral pigs, deer) to minimise the risk of brodifacoum residues in game meat for human consumption and export, but no similar recommendations are made for farmed livestock. A survey of at-slaughter tissue (liver and muscle) from livestock from PCA farming areas would be useful to confirm that brodifacoum exposure is not as widespread in livestock as in the indicator wildlife sampled in this study.

5.4 Environmental implications of brodifacoum residues

The long-term implications of sublethal brodifacoum exposure for survival or reproductive fitness of affected individuals are not clear. Given the persistent, slowly eliminated nature of

brodifacoum in liver; if non-target animals are repeatedly receiving nominally sublethal exposures, potential exists for liver residues to accumulate and eventually exceed an (undefined) threshold for toxic effects. The relationship between liver concentrations of brodifacoum and mortality is unclear. Use of 'threshold' liver concentration as a determinant of acute toxicity in mammals or birds has been suggested, with estimates of 0.7 µg/g (Kaukeinen et al. 2000) and 0.5 µg/g (Dowding et al. 1999). However, Littin et al. (2002) measured concentrations as low as 0.33 µg/g in livers of lethally poisoned possums, whereas sublethally exposed chickens had liver residues of 0.45–1.00 µg/g (Fisher 2009). Relatively high liver concentrations (> 1 µg/g) appear more strongly associated with lethal exposure, but there is overlap between the lowest 'lethal' and highest 'sublethal' concentrations reported. On this basis it seems more valid to relate increasing probability of lethal exposure with increasing liver concentration – as done by Myllymäki et al. (1999), who estimated that survival probability in voles started to decrease at 0.20 µg/g in liver. The residue concentrations measured in hedgehogs and birds in this study were generally well below this level – although the highest concentration of 0.14 µg/g in a hedgehog from Site 1 was approaching it.

Other coumarin anticoagulant compounds such as warfarin have been shown to affect bone mass (e.g. Price 1988) and have teratogenic effects (e.g Astedt 1995). The literature on reproductive or teratogenic effects of brodifacoum is limited almost entirely to mammalian studies, which overall suggest maternal toxicity (haemorrhage) resulting in abortion, rather than direct effects on the fetus. Brodifacoum given by oral gavage to female rats at daily doses of 0.001, 0.01 or 0.02 mg/kg during days 6–15 of pregnancy produced no apparent effects on fetuses, but daily doses above 0.05 mg/kg caused an anticoagulant effect in the dams and a high incidence of abortion (Hodge et al. 1980 cited by World Health Organisation 1995). Female rabbits dosed daily with 0.005 mg/kg brodifacoum over days 6–18 of pregnancy showed a high incidence of haemorrhage, and resultant mortality. In surviving dams that showed signs of haemorrhage there were no effects on the developing fetuses (Hodge et al. 1980 cited by World Health Organisation 1995). Twigg and Kay (1995) cite unpublished data where brodifacoum caused a 50% increase in aborted or still-born lambs when administered to pregnant ewes 7 weeks after mating, and a 22% increase in lamb mortality when pregnant ewes were administered brodifacoum one week before giving birth.

Whether the residues measured in hedgehogs and birds in this study represent single or multiple exposures to brodifacoum is not known, so it is difficult to determine the potential for multiple, consecutive exposures to brodifacoum in non-target wildlife or livestock that could cause the maternal toxicity effects described in laboratory studies. Because exposure of livestock is most likely to occur through bait spilled or carried from stations by possums and rodents, more information about the extent of this occurrence in operational conditions would be of value in improving risk assessment around unwanted effects of brodifacoum use on meat quality or livestock health.

6 Conclusions

The overall approximately 50% incidence of brodifacoum-positive hedgehogs and birds across all sites, and the slightly higher proportion of vertebrates with residues in Sites 1 and 2 (with no or least brodifacoum use), indicates that brodifacoum exposure of some vertebrate wildlife is ubiquitous in Hawke's Bay.

The lack of significant differences between the sites monitored suggests that brodifacoum has a wide capacity for secondary exposure through trophic pathways, with potential sources from both field bait station applications and its use for rodent control in and around farm and urban buildings.

The frequency and concentration of residual brodifacoum in the wildlife sampled were not related to the length of time brodifacoum had been used on a particular site. The ongoing use of brodifacoum in bait stations as part of the PCA programme in Hawke's Bay does not therefore appear to be resulting in localised, relatively high levels of environmental contamination as indicated by the wildlife sampled.

7 Recommendations

- A survey of at-slaughter tissue (liver and muscle) from livestock from PCA farming areas would be useful to confirm that brodifacoum exposure is not as widespread in livestock as in the indicator wildlife sampled in this study.
- Quantifying the amounts of bait that are typically removed from different types of bait stations to the wider environment by rodent or possum behaviour could help to refine best practice in the PCA programme, by identifying 'least spillage' station types and/or modifications to minimise bait loss.
- Survey information about household or on-farm uses of brodifacoum (and other second-generation anticoagulant rodenticides) in Hawke's Bay would help to determine the relative contribution of the PCA programme to the overall widespread but low-level environmental brodifacoum residues measured in this study.

8 Acknowledgements

This report was produced for the Hawke's Bay Regional Council under a small advice grant in the Envirolink (Ministry of Business, Innovation and Employment) scheme. We acknowledge the collaborative input of Colin Pirie, Leighton Simmons and Brian Ronke (Habitat BPM Ltd) into selection of monitoring sites, field collection of samples and compilation of site intervention data. Phil Cowan and Grant Morriss propvided review comments on earlier drafts and Christine Bezar editing services.

9 References

Astedt B 1995. Antenatal drugs affecting vitamin K status of the fetus and the newborn. Seminars in Thrombosis and Hemostasis 21: 364–370.

Booth LH, Eason CT, Spurr EB 2001. Literature review of the acute toxicity and persistence of brodifacoum to invertebrates and studies of residue risks to wildlife and people. Science for Conservation 177. Wellington, Department of Conservation.

Bowie M, Ross J 2006. Identification of weta (Orthoptera: Anostomatidae and Rhaphidophoridae) foraging on brodifacoum cereal bait and the risk of secondary poisoning for bird species on Quail Island, New Zealand. New Zealand Journal of Ecology 30: 219–228.

Craddock P 2003. Aspects of the ecology of forest invertebrates and the use of brodifacoum. Unpublished PhD thesis, University of Auckland, Auckland, New Zealand.

Dowding CV, Shore RF, Worgan A, Baker PJ, Harris S 2009. Accumulation of anticoagulant rodenticides in a non-target insectivore, the European hedgehog (*Erinaceus europaeus*).Environmental Pollution 158: 160–165.

Dowding JE, Murphy EC, Veitch CR 1999. Brodifacoum residues in target and non-target species following an aerial poisoning operation on Motuihe Island, Hauraki Gulf, New Zealand. New Zealand Journal of Ecology 23: 207–214.

Eason CT, Spurr EB. 1995. Review of the toxicity and impacts of brodifacoum on non-target wildlife in New Zealand. New Zealand Journal of Zoology 22: 371–379

Fisher P 2009. Residual concentrations and persistence of the anticoagulant rodenticides brodifacoum and diphacinone in fauna. Unpublished PhD thesis, Lincoln University, Lincoln, New Zealand.

Fisher P 2010. Environmental fate and persistence of brodifacoum in wildlife. Landcare Research Contract Report to Hawkes Bay Regional Council (Envirolink 884-HBRC131).

Kaukeinen DE, Spragins CW, Hobson JF 2000. Risk–benefit considerations in evaluating commensal anticoagulant impacts to wildlife. In: Salmon TP, Crabb AC eds Proceedings of the 19th Vertebrate Pest Conference. USA, University of California, Davis. Pp. 245–266.

Littin KE, O'Connor CE, Gregory NG, Mellor DJ, Eason CT 2002. Behaviour, coagulopathy and pathology of brushtail possums (*Trichosurus vulpecula*) poisoned with brodifacoum. Wildlife Research 29: 259–267.

Myllymäki A, Pihlava J, Tuuri H 1999. Predicting the exposure and risk to predators and scavengers associated with using single-dose second-generation anticoagulants against field rodents. In: Cowan DP, Feare CJ eds Advances in Vertebrate Pest Management Fürth, Filander. Pp. 387–404.

Price PA 1988. Role of vitamin K-dependent proteins in bone metabolism. Annual Review of Nutrition 8: 565–583.

R Development Core Team (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Spurr EB, Maitland MJ, Taylor GE, Wright GRG, Radford CD, Brown LE 2005. Residues of brodifacoum and other anticoagulant pesticides in target and non-target species, Nelson Lakes National Park, New Zealand. New Zealand Journal of Zoology 32: 237–249.

Thomsen T, Bowie M, Hickling G 2000. The potential for eradication of hedgehogs (*Erinaceus europaeus*) from Quail Island, Banks Peninsula. Lincoln University Wildlife Management Report 20.

Twigg LE, Kay BJ 1995. The effect of sub-lethal doses of bromadiolone on the breeding performance of house mice (*Mus domesticus*). Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology 110: 77–82.

World Health Organisation 1995. Anticoagulant rodenticides. Environmental Health Criteria 175. Geneva, World Health Organisation.

Appendix 1 – Toxicology reports

Landcare Research Toxicology laboratory reports for brodifacoum testing in hedgehog and bird liver, and composite invertebrates samples from sites in Hawke's Bay.

Manaaki Whenua
Landcare Research

Gerald Street
P.O.Box 40
Lincoln, 7640
Ph: +64 3 321 9999
Fax: +64 3 321 9998

Toxicology Laboratory Analysis Report

Report No: T4798

CLIENT: Penny Fisher, Landcare Research, Gerald Street, P.O. Box 40 Lincoln 7640
CLIENT REFERENCE No.: 796 209 0146 **Telephone No:** 03 321 9999
SAMPLES: Thirteen liver samples
REQUIREMENT: Examine for brodifacoum
RECEIVED: 16 February 2012 225

Sample/s were received for analysis. The details were entered into the laboratory sample system and the sample/s given a reference number. The sample details and results are as follows:

No. samples: 13

LabNo.	Description	Brodifacoum, µg/g
16558	Liver tissue, Site 1, Te Onepu South, Control, Hedgehog #1, 1/12/11	0.002
16559	Liver tissue, Site 1, Te Onepu South, Control, Hedgehog #2, 1/12/11	0.14
16560	Liver tissue, Site 1, Te Onepu South, Control, Hedgehog #3, 1/12/11	<MDL
16561	Liver tissue, Site 1, Te Onepu South, Control, Hedgehog #4, 2/12/11	0.002
16562	Liver tissue, Site 1, Te Onepu South, Control, Hedgehog #5, 2/12/11	0.003
16563	Liver tissue, Site 1, Te Onepu South, Control, Hedgehog #6, 3/12/11	0.003
16564	Liver tissue, Site 1, Te Onepu South, Control, Bird #1, 1/12/11	0.006
16565	Liver tissue, Site 1, Te Onepu South, Control, Bird #2, 2/12/11	0.022
16566	Liver tissue, Site 1, Te Onepu South, Control, Bird #3, 2/12/11	0.020
16567	Liver tissue, Site 1, Te Onepu South, Control, Bird #4, 1/12/11	<MDL
16568	Liver tissue, Site 1, Te Onepu South, Control, Bird #5, 3/12/11	<MDL
16569	Liver tissue, Site 1, Te Onepu South, Control, Bird #6, 3/12/11	<MDL
16570	Liver tissue, Site 1, Te Onepu South, Control, Bird #7, 3/12/11	<MDL

Manaaki Whenua
Landcare Research

Gerald Street
P.O.Box 40
Lincoln, 7640
Ph: +64 3 321 9999
Fax: +64 3 321 9998

Toxicology Laboratory Analysis Report

Report No: T4823

CLIENT: Penny Fisher, Landcare Research, Gerald Street, P.O. Box 40 Lincoln 7640
CLIENT REFERENCE No.: 796 209 0146 **Telephone No:** 03 321 9999
SAMPLES: Twenty one tissue samples
REQUIREMENT: Examine for brodifacoum
RECEIVED: 9 March 2012 225

Sample/s were received for analysis. The details were entered into the laboratory sample system and the sample/s given a reference number. The sample details and results are as follows:

No. samples: 21

LabNo.	Description	Brodifacoum, µg/g
16658	Liver tissue, Site 2, Paget Road, Hedgehog #1	<MDL
16659	Liver tissue, Site 2, Paget Road, Hedgehog #2	0.002
16660	Liver tissue, Site 2, Paget Road, Hedgehog #3	0.0078
16661	Liver tissue, Site 2, Paget Road, Hedgehog #4	0.11
16662	Liver tissue, Site 2, Paget Road, Hedgehog #5	0.0012
16663	Liver tissue, Site 2, Paget Road, Hedgehog #6	<MDL
16664	Liver tissue, Site 2, Paget Road, Hedgehog #7	<MDL
16665	Liver tissue, Site 2, Paget Road, Hedgehog #8	0.0013
16666	Liver tissue, Site 2, Paget Road, Hedgehog #9	0.0016
16667	Invertebrate, Site 2, Paget Road, Invertebrates #2	<MDL
16668	Liver tissue, Site 2, Paget Road, Magpie #1	<MDL
16669	Liver tissue, Site 2, Paget Road, Magpie #2	<MDL
16670	Liver tissue, Site 2, Paget Road, Magpie #3	0.053
16671	Liver tissue, Site 2, Paget Road, Magpie #4	0.0019
16672	Liver tissue, Site 2, Paget Road, Magpie #5	0.0018
16673	Liver tissue, Site 2, Paget Road, Magpie #6	0.0013
16674	Liver tissue, Site 2, Paget Road, Magpie #7	0.0031
16675	Liver tissue, Site 2, Paget Road, Magpie #8	0.0019
16676	Liver tissue, Site 2, Paget Road, Magpie #9	0.0061
16677	Liver tissue, Site 2, Paget Road, Magpie #10	<MDL
16678	Liver tissue, Site 2, Paget Road, Blackbird #1	0.0025

Manaaki Whenua
Landcare Research

Gerald Street
P.O.Box 40
Lincoln, 7640
Ph: +64 3 321 9999
Fax: +64 3 321 9998

Toxicology Laboratory Analysis Report

Report No: T4839

CLIENT: Penny Fisher, Landcare Research, Gerald Street, P.O. Box 40 Lincoln 7640
CLIENT REFERENCE No.: 796 209 0146 **Telephone No:** 03 321 9999
SAMPLES: Twelve tissue samples
REQUIREMENT: Examine for brodifacoum
RECEIVED: 2 May 2012 225

Sample/s were received for analysis. The details were entered into the laboratory sample system and the sample/s given a reference number. The sample details and results are as follows:

No. samples: 12

LabNo.	Description	Brodifacoum, µg/g
16755	Liver tissue, Site 3, Moeangiangi Station, Hedgehog #1	0.001
16756	Liver tissue, Site 3, Moeangiangi Station, Hedgehog #2	<MDL
16757	Liver tissue, Site 3, Moeangiangi Station, Hedgehog #3	0.014
16758	Liver tissue, Site 3, Moeangiangi Station, Hedgehog #4	0.001
16759	Liver tissue, Site 3, Moeangiangi Station, Hedgehog #5	<MDL
16760	Liver tissue, Site 3, Moeangiangi Station, Hedgehog #6	0.008
16761	Liver tissue, Site 3, Moeangiangi Station, Bird #1	<MDL
16767a	Invertebrate, Site 3, Moeangiangi Station, Invertebrates - snails	<MDL
16767b	Invertebrate, Site 3, Moeangiangi Station, Invertebrates - worms	<MDL
16767c	Invertebrate, Site 3, Moeangiangi Station, Invertebrates - big white worm	<MDL
16767d	Invertebrate, Site 3, Moeangiangi Station, Invertebrates - big green slugs	<MDL
16767e	Invertebrate, Site 3, Moeangiangi Station, Invertebrates - assorted	<MDL

Manaaki Whenua
Landcare Research

Gerald Street
P.O.Box 40
Lincoln, 7640
Ph: +64 3 321 9999
Fax: +64 3 321 9998

Toxicology Laboratory Analysis Report

Report No: T4868

CLIENT: Penny Fisher, Landcare Research, Gerald Street, P.O. Box 40 Lincoln 7640
CLIENT REFERENCE No.: 796 209 0146 **Telephone No:** 03 321 9999
SAMPLES: Sixteen tissue samlples
REQUIREMENT: Examine for brodifacoum
RECEIVED: 30 May 2012 225

Sample/s were received for analysis. The details were entered into the laboratory sample system and the sample/s given a reference number. The sample details and results are as follows:

No. samples: 16

LabNo.	Description	Brodifacoum, µg/g
16828	Liver tissue, Hedgehog 1, Site 4, 15/5/12	<MDL
16829	Liver tissue, Hedgehog 2, Site 4, 15/5/12	0.003
16830	Liver tissue, Hedgehog 3, Site 4, 15/5/12	0.072
16831	Liver tissue, Hedgehog 4, Site 4, 15/5/12	<MDL
16832	Liver tissue, Hedgehog 5, Site 4, 15/5/12	<MDL
16833	Liver tissue, Hedgehog 6, Site 4, 15/5/12	<MDL
16834	Liver tissue, Bird 1, Site 4, 15/5/12	0.002
16835	Liver tissue, Bird 2, Site 4, 15/5/12	<MDL
16836	Liver tissue, Bird 3, Site 4, 15/5/12	<MDL
16837	Liver tissue, Bird 4, Site 4, 15/5/12	<MDL
16838	Liver tissue, Bird 5, Site 4, 15/5/12	<MDL
16839	Liver tissue, Bird 6, Site 4, 15/5/12	<MDL
16841	Liver tissue, Bird 3, Site 2, 15/4/12	<MDL
16842	Liver tissue, Bird 4, Site 2, 15/4/12	<MDL
16843	Liver tissue, Bird 5, Site 2, 15/4/12	<MDL
16844	Liver tissue, Bird 6, Site 2, 15/4/12	<MDL

Manaaki Whenua
Landcare Research

Gerald Street
P.O.Box 40
Lincoln, 7640
Ph: +64 3 321 9999
Fax: +64 3 321 9998

Toxicology Laboratory Analysis Report

Report No: T4879

CLIENT: Penny Fisher, Landcare Research, Gerald Street, P.O. Box 40 Lincoln 7640
CLIENT REFERENCE No.: 796 209 0146 **Telephone No:** 03 321 9999
SAMPLES: Five invertebrate samples
REQUIREMENT: Examine for brodifacoum
RECEIVED: 13 February 2012 225

Sample/s were received for analysis. The details were entered into the laboratory sample system and the sample/s given a reference number. The sample details and results are as follows:

No. samples: 5

LabNo.	Description	Brodifacoum, µg/g
16571	Invertebrate, Site 1, Control, Sample 1	<MDL
16572	Invertebrate, Site 1, Control, Sample 2	<MDL
16573	Invertebrate, Site 1, Control, Sample 3	<MDL
16840a	Invertebrate, Site 4, Slugs	<MDL
16840b	Invertebrate, Site 4, Snails	<MDL

The results have been adjusted for method recovery. All results are reported to two significant figures.

The determination was carried out using TLM070, the determination of brodifacoum in animal and invertebrate tissue by HPLC. The method detection limit (MDL) is 0.001µg/g and the uncertainty (95% c.l.) is ± 6%

TESTED BY: leb **WORKBOOK REF:** 76/11
TEST PERIOD: 21-26/6/12

AUTHORISED BY:

L.H.Booth, L.E. Brown

Date: 26/06/2012

547

All tests reported herein have been performed in accordance with the laboratory's scope of accreditation

These results relate only to the samples as received and tested. This report may be reproduced in full only. The samples relating to this report will be disposed of after two months from the report date unless requested otherwise by the client. Where appropriate, the above results will be included in th National Vertebrate Pesticide Residue Database.

Report No: T4879 Page 1 of 1