Environmental monitoring for brodifacoum residues after aerial application of baits for rodent eradication

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Abstract Aerial application of brodifacoum bait for eradication of invasive rodents from islands raises concerns about environmental contamination and adverse effects on non-target wildlife. We summarise results of environmental monitoring for brodifacoum residues after New Zealand eradications in a fenced reserve at Maungatautari and on the offshore islands Little Barrier, Rangitoto and Motutapu. Brodifacoum was not detected in extensive fresh water monitoring at Maungatautari, or in fresh water samples from Little Barrier Island. Residual concentrations were present in soil samples from underneath degrading bait pellets on Little Barrier, and decreased to near the limit of detection by *C*. 100 days after application. No brodifacoum was detected in marine shellfish sampled from Little Barrier, Rangitoto or Motutapu. A range of birds, including a kiwi from Little Barrier, were considered non-target mortalities. Residual brodifacoum concentrations detected in three of nine little blue penguins found dead on beaches outside the Rangitoto/Motutapu area after baiting were considered to represent sublethal exposure, with starvation considered the likely cause of death. This result highlights the crucial role of post-application environmental monitoring in rodent eradications, in addressing community concerns and filling information gaps regarding the movement, persistence and effects of brodifacoum in the environment.

Keywords: Laboratory testing, non-target species, residues, rodenticides, soil, water

INTRODUCTION

Brodifacoum is among the most toxic of the anticoagulants used against rats and mice (Erickson and Urban 2004), so they need to ingest a relatively small amount of bait for lethal exposure. The product has become a valuable tool for island conservation because of its delayed toxicity (Kaukeinen and Rampaud 1986), high rodenticidal efficacy, and bait formulations that are highly acceptable to rodents and can be applied aerially over large areas. To date, brodifacoum baiting has been used in an estimated 71% of campaigns to eradicate introduced rodents from islands (Howald *et al*. 2007). An important consideration has been assessing risk to non-target wildlife and the potential for environmental contamination. Increasingly, rodent eradication is being considered for islands that are inhabited or used by people or are close to highly populated mainland areas. Where the use of brodifacoum bait is proposed, particularly through aerial application, managers also need to address possible environmental contamination pathways that pose risks to humans, livestock and domestic animals.

Here we describe monitoring undertaken after three New Zealand eradications of rodents from islands involving aerial application of cereal pellet bait containing 20 ppm brodifacoum. We discuss the results in the context of environmental contamination and non-target risk. Under current New Zealand legislation, the aerial discharge of a contaminant such as brodifacoum to land and water (e.g., using helicopters for bait application) requires consent from a local government agency. While there are currently no prescriptive environmental monitoring regimes for residual brodifacoum, concerns addressed during the consent application process for each of the eradications focused attention on the fate of brodifacoum in water and soil as potential transfer pathways to human food and non-target wildlife. Where aerial application could result in bait entering the marine environment this has included monitoring of coastal marine fauna, especially shellfish commonly harvested for human food.

METHODS

Maungatautari water monitoring

The Maungatautari Ecological Island Trust (MEIT) aims to achieve complete pest mammal eradication in this mainland reserve in the central North Island, by pest-proof fencing and removal of pest mammals through aerial baiting and trapping within the fenced area (see www.maungatrust.org/index.asp). A pilot eradication programme in two fenced enclosures on the northern (*c*. 32 ha) and southern (*c*. 76 ha) sides of the mountain was undertaken in 2004. Each enclosure received two aerial applications of Pestoff Rodent Bait 20R at a rate of 15 kg/ha, applied in accordance with a Code of Practice (Anon. 2006). Streams flowing through both enclosures were used for human or livestock drinking supply by adjoining landowners. The resource consent specified that all water supplies drawn from the enclosures be disconnected before bait application, and to remain so until two water samples taken on consecutive days showed that any brodifacoum contamination was below the analytical method detection limit (MDL).

Samples from two streams in each enclosure were taken at zero hours (baseline) then at 1, 2, 3, 6, 9, 12, 24, 48 and 72 h after bait application, and thereafter at intervals of one week, two weeks and three months. Further samples were taken after ≥25 mm rainfall occurred in a 24-h period. Samples were taken from the point where each stream left the enclosure and at *C*. 800 m downstream. Samples taken up to 48 h after bait application were analysed within 24 h of receipt by the laboratory, to facilitate reconnection of water supplies once there were two consecutive below-MDL results.

Little Barrier Island water, soil, bait degradation and marine shellfish monitoring

Little Barrier Island is in the Hauraki Gulf 80 km north-east of Auckland (see www.doc.govt.nz/parks-and-recreation/places-to-visit/auckland/warkworth-area/little-barrier-island-hauturu-nature-reserve/). The Department of Conservation (DOC) aerially spread Pestoff Rodent Bait 20R at 11.7 and 6.2 kg/ha in June and July 2004, and the island was declared free of Pacific rats (*Rattus exulans*) in July 2006.

Carcass searches along the island's track network and grid-searches over *C*. 120 ha were undertaken during the week following each bait application. One kiwi carcass recovered was necropsied (IVABS, Massey University, NZ) with liver tissue analysed for residual brodifacoum (Table 1). Monitoring of bait degradation was used to

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Table 1 Testing laboratories, numbers analysed and detection limits for water, soil and animal tissue samples tested for residual brodifacoum following aerial bait application.

Island eradication	Sample type	No. tested	Testing laboratory	MDL (ppm)
Maungatautari	Water	217	LCR	0.00002
Little Barrier	Water	4	AQ	not specified
	Soil	8	AQ	not specified
	Shellfish	4*	AQ	0.001
	Kiwi liver	1	LCR	0.001
Rangitoto/Motutapu	Water	4	LCR	0.00002
	Shellfish	2*	LCR	0.001
	Penguin liver	9	LCR	0.001
	Dolphin liver	5	AQ	0.005
	Dolphin ingesta	5	AQ	0.005
	Dog vomit	1	AQ	0.005
	Pilchards	1*	LCR	0.001

* Each sample consisted of four or five individual shell/fish combined.
LCR = Landcare Research Toxicology Laboratory, Lincoln, New Zealand. AQ = Agriquality National Chemical Residue Laboratory, Upper Hutt, New Zealand. MDL = method detection limit.

determine timing of the release of three brown teal (*Anas chlorotis*) taken into captivity before the operation. At four sites representing grassland and forested habitats across the island, 20 bait pellets were placed under wire cages designed to exclude rodents and birds, and checked for condition scoring following the categories described by Craddock (2003a), over four months. Soil monitoring was undertaken after peg-marking the position of individual pellets so that soil samples could later be taken from the exact location. Soil (4-cm^3 plugs) collected at days 56 and 153 after the second bait application was stored frozen until analysis. Within 24 h after both bait applications, water samples were taken from one waterway, less than 1 m downstream from where bait pellets were visible in the water, and also from the island's bore water supply. At one and two weeks after the second bait application, samples (Table 1) of paua (*Haliotis iris*) and scallops (*Pecten novaezelandiae*) were taken from within 5 and 50 m of the shoreline, respectively.

Rangitoto and Motutapu islands residues in water, wildlife and marine shellfish

Rangitoto and Motutapu are connected islands in the inner Hauraki Gulf, approximately 8 km north-east of Auckland (see www.doc.govt.nz/parks-and-recreation/places-to-visit/auckland/auckland-area/rangitoto-island-scenic-reserve/ and www.doc.govt.nz/parks-and-recreation/places-to-visit/auckland/auckland-area/motutapu-island-recreation-reserve/). DOC undertook three aerial applications of Pestoff Rodent Bait 20R on 19-20 June, 9 July and 6 August 2009 with respective application rates of 22.1, 9.5 and 6.6 kg/ha. The initial high application rate was used to minimise the risk that uptake by rabbits would leave gaps in bait coverage intended for rodents (*Rattus rattus*, *R. norvegicus* and *Mus musculus*). Roof water-collection systems were disconnected before aerial application, and roofs and animal drinking troughs cleared of any bait afterwards. Four samples from drinking supplies on Motutapu were taken approximately 2 months after the last aerial application. Three weeks after the last application, 10 pipi (*Paphies australe*) from Motutapu and 10 mussels (*Mytilus edulis*) from Rangitoto were sampled for residue testing (Table 1).

The weeks following the baiting operation coincided with cases of domestic dogs (*Canis familiaris*) being poisoned on Auckland beaches. A vomit sample from one of five dogs that died was tested for residual brodifacoum (Table 1), although veterinary diagnoses and chemical testing later indicated that these cases were the result of dogs ingesting sea slugs (*Pleurobranchaea maculata*) containing tetrodotoxin (McNabb *et al*. 2009). The death of dogs soon after the Rangitoto/Motutapu brodifacoum applications increased public awareness of the aerial application of brodifacoum. National media and Internet coverage was given to assertions by various interest groups and individuals that marine wildlife, including little blue penguins (*Eudyptula minor*), dolphins (*Delphinus* sp.) and pilchards (*Sarditlops neopilchardus*), found dead on local beaches outside the eradication operational area had been poisoned as a result of the eradication operation. To address these concerns, brodifacoum testing was carried out on samples of liver from nine little blue penguins, samples of dolphins' stomach contents and samples of whole pilchards (Table 1). Necropsy data was also obtained to further diagnose whether brodifacoum poisoning was likely in these cases.

Residue analyses

Two accredited New Zealand laboratories analysed samples for brodifacoum, with method detection limit (MDL) values dependent on sample type (Table 1). The Landcare Research brodifacoum analyses used HPLC with fluorescence detection, with methods developed for different sample types based on those described by Hunter (1983), Booth *et al*. (1999), and Primus *et al*. (2001).

RESULTS

No brodifacoum was detected in 217 water samples from Maungatautari, in any of the four water samples tested from Little Barrier, or in the four drinking water samples from Motutapu. On Little Barrier Island, bait pellets in exclusion cages were nearly completely disintegrated by 100 days after bait application. Soil samples from a grassland site on Little Barrier had residues of 0.2 ppm (n=2 with the same concentration) on day 56 and 0.03 ppm on day 153. Soil samples from a forested site had residues of 0.9 and 0.5 ppm on day 56 and 0.07 ppm on day 153. Brodifacoum was not detected in any of the paua and scallop samples from Little Barrier, or in pipi or mussel samples from Motutapu and Rangitoto.

On Little Barrier Island, track searches recovered carcasses of a blackbird (*Turdus merula*) and a pukeko (*Porphyrio melanotus*). Grid searches recovered carcasses of two blackbirds, four pukeko, 14 morepork (*Ninox novaeseelandiae*), one harrier (*Circus approximans*), two North Island brown kiwi (*Apteryx mantelli*) and two kakariki (*Cyanoramphus spp.*). The carcasses were

too degraded for necropsy or liver sampling, except for one kiwi where necropsy gave a provisional diagnosis of bronchopneumonia with residual brodifacoum concentration in the liver of 0.26 ppm.

Following the Rangitoto /Motutapu eradication, no brodifacoum was detected in five dolphins or their stomach contents or in whole-body samples of pilchards collected from local beaches during July 2009. In some cases, degradation of penguin carcasses precluded necropsy. Of the seven penguins examined, there were no obvious signs of anticoagulant poisoning (such as haemorrhage) and in three of these necropsy indicated poor condition, i.e. no body fat, empty stomach. Of the total nine penguin livers tested, no brodifacoum was detected in six, but in three there were concentrations of 0.005, 0.007 and 0.17 ppm, respectively.

DISCUSSION

Brodifacoum in water

The water monitoring implemented at Maungatautari (217 samples tested, no brodifacoum detected) appears the most comprehensive reported to date. Brodifacoum was also not detected in water samples from Little Barrier and Motutapu, consistent with previous small-scale monitoring on Red Mercury Island (Morgan and Wright 1996) and Lady Alice Island (Ogilvie *et al.* 1997). Interacting factors likely to have contributed to such results are brodifacoum's overall low water-solubility (0.24 mg/l at 20ºC and pH 7.4, British Crop Protection Council 2000), adsorption of brodifacoum to organic particles (World Health Organisation 1995), and dilution with water volume and flow rate. If aerially applied baits were to enter fresh water, only a limited amount of the brodifacoum in them would enter solution, being more likely to remain bound to bait or to other organic particles present in the water or sediment. Binding of brodifacoum would render it undetectable in water that could have been used for drinking supplies.

Bait degradation and brodifacoum in soil

Bait degradation on Little Barrier took a similar time to that described by Craddock (2003a) at Tawharanui (NZ) where 96.5% pellets had completely broken down by 120 days in open grassed area, although bait degradation was slightly slower in a forested site. Thus a universal degradation time for all situations cannot be defined, especially as rainfall (Bowen *et al.* 1995), among other climatic factors affecting degradation, can vary from island to island. In each instance, monitoring should ensure that uneaten baits have degraded sufficiently to no longer present a non-target hazard. Following aerial bait (Talon 20P) application on Red Mercury Island (Morgan and Wright 1996) and Lady Alice Island (Ogilvie *et al*. 1997), no brodifacoum was detected in topsoil sampled at one month and over days 2 to 34, respectively. Those soil samples are presumed not to have been specifically associated with degrading bait, noting that brodifacoum is relatively immobile in soil (Eason and Wickstrom 2001). Hence, any residual soil concentrations are most likely to be localised around uneaten, degrading bait, as indicated by the Little Barrier results. The relatively low brodifacoum concentrations (<1 ppm) in these samples may have been due to the presence of disintegrated bait particles in the sample, in addition to limited movement of brodifacoum from bait into the soil. A decrease in the concentrations (from maximum 0.9 ppm to minimum 0.03 ppm over *C*. 100 days) suggests degradation in soil over time. Degradation rates of brodifacoum in a sandy clay loam was estimated as 22.4 weeks (US EPA 1998), but probably varies with soil type at least. Thus soil invertebrates near degrading bait on Little Barrier may have been exposed to low brodifacoum concentrations for a limited period. While exposure of laboratory earthworms (*Apporectodea caliginosa*) to 500 ppm brodifacoum in soil resulted in 85% mortality after 28 day's exposure (Booth and Fisher 2003), this soil brodifacoum concentration was 25 times higher than that of bait. It is unknown whether soil concentrations in a much lower (*c.* 1 ppm) range, more representative of field results, would affect soil invertebrate survival or health, and for how long sublethal residual concentrations of brodifacoum persist in soil invertebrates.

Brodifacoum in marine shellfish

Following accidental spillage in 2001 of 18 tonnes of PestOff 20R into the ocean at Kaikoura, NZ, brodifacoum residues were detectable for some weeks in marine shellfish commonly harvested for human consumption (Primus *et al*. 2005), which raised awareness and concerns about potential human exposure. An important point of difference was that the spill comprised an extremely large quantity of bait entering the ocean at one point. In contrast, aerial application disperses individual pellets, resulting in much smaller quantities of brodifacoum entering the ocean around island shorelines. The results reported here suggest that contamination of marine shellfish is unlikely following aerial application of brodifacoum baits for rodent eradication. That there were no detectable results in marine shellfish following the Little Barrier and Rangitoto/ Motutapu eradications is consistent with previous small monitoring efforts following bait applications on New Zealand islands. Two oyster samples and three of four mussel samples from Motuihe Island in 1998 were <MDL, with one mussel sample reported as 0.02 ppm as a conservative interpretation by the analysing laboratory (Landcare Research) against the detection limit available at the time. Two mussel samples from aquaculture farms near Great Barrier Island (Hauraki Gulf) were also below detectable concentrations, following a 2008 rat eradication attempt.

There is a lack of information regarding potential differences in exposure pathways between sediment and water-column-feeding shellfish species and the persistence of residual brodifacoum in shellfish. On this basis, residues may still be found in marine shellfish following aerial bait application, but the evidence so far suggests that the risk of secondary brodifacoum exposure to humans harvesting and eating shellfish is relatively low. Where this is a concern for proposed eradications, stipulating a no-harvest period linked to post-application monitoring is a prudent approach to confirming that there is no potential secondary human exposure as a result of consuming shellfish.

Brodifacoum in non-target wildlife

Brodifacoum is highly toxic to mammals and birds (Erickson and Urban 2004). Consequently, rodent bait presents a primary poisoning hazard to non-target mammals and birds. If exposure to the baits is not lethal, residual brodifacoum can persist for months in the livers of mammals (Eason *et al*. 2002; Fisher *et al*. 2003; Spurr *et al*. 2005) and birds (Fisher 2009), but is eliminated within days from blood and other tissues (e.g., Fisher 2009). Liver residues and stomach contents containing partially digested brodifacoum bait present the highest secondary hazard for mammalian and avian species that prey on rodents or scavenge carcasses (e.g., Howald *et al*. 1999; Shore *et al*. 1999). Some terrestrial invertebrates will feed on cereal-based bait and then contain residual concentrations of brodifacoum (e.g., Booth *et al*. 2001; Craddock 2003b; Bowie and Ross 2006). Secondary mortality of insectivorous New Zealand dotterels (*Charadrius obscurus aquilonius*) may have been through this environmental pathway (Dowding *et al*. 1999). Unpublished evidence of suspected secondary brodifacoum poisoning of two tuatara (*Sphenodon punctatus*) held in a zoo was the basis

for implementing several mitigation measures to prevent brodifacoum exposure of tuatara held in outdoor enclosures on Little Barrier.

The 27 bird carcasses found on Little Barrier were of species previously reported as non-target mortalities in other New Zealand eradications using brodifacoum (e.g., Towns and Broome 2003), and in the absence of residue testing or necropsy data, the conservative assumption is they represent non-target mortality. Of 10 radio-tagged little spotted kiwi (*Apteryx owenii*), one was confirmed to have died of brodifacoum poisoning following rodent eradication on Kapiti Island, with haemorrhage found at necropsy, and with liver residues of 1.2 ppm (Robertson and Colbourne 2001). Wild kiwi have occasionally been recorded eating softened or degraded cereal bait, but their main prey are soil invertebrates such as earthworms, cicada nymphs and grass grubs (Robertson *et al*.1999), so primary and secondary exposure to brodifacoum was possible for the two brown kiwi found dead on Little Barrier Island. Better understanding of invertebrates as a residue vector is required to identify the most likely pathways of environmental exposure by kiwi to brodifacoum, and also to direct improved non-target risk mitigation measures for insectivores. Most morepork carcasses were found in areas where historical densities of Pacific rats had been highest, so presenting a possible increased risk of secondary poisoning. Since the bait application in 2004, morepork have remained abundant on Little Barrier and kiwi surveys show that the non-target mortality following the eradication did not have a population-level effect (Wade 2009). However, while this outcome supports an overall, long-term ecological benefit of rodent eradication to these populations, some community groups consider that any non-target bird mortality (especially iconic native species) is unacceptable.

The presence of residual brodifacoum in livers of three of nine penguins cannot be confirmed as sourced from the Rangitoto/Motutapu bait applications. Brodifacoum bait stations are commonly used for commensal rodent control in New Zealand, and also for field use against brushtail possums and rodents (see Hoare and Hare 2006). Exposure of the penguins to brodifacoum before the Rangitoto/Motutapu aerial operation cannot be ruled out because brodifacoum was almost certainly being used in the Hauraki Gulf area, potentially around buildings or on boats in coastal areas near terrestrial penguin habitat, before June 2009. The presence of brodifacoum in the penguins also cannot be confirmed as a direct cause or contributor to their mortality, as brodifacoum can be retained in liver at sublethal concentrations, as reported in a range of live-sampled, apparently healthy mammals and birds (see Fisher 2009). Relatively high liver concentrations (< 1 ppm) are more strongly associated with lethal exposure, but there is overlap between the lowest lethal and highest sublethal concentrations reported. For example, Littin *et al.* (2002) measured concentrations as low as 0.33 ppm in livers of lethally poisoned possums, but sublethally exposed chickens (*Gallus gallus*) had liver residues of 0.45-1.00 ppm (Fisher 2009). Rather than estimating a threshold liver concentration definitive of lethal brodifacoum exposure (e.g., Kaukeinen *et al*. 2000), it is more valid to attribute increasing certainty of lethal exposure with increasing liver concentration. For example, Myllymäki *et al.* (1999) estimated that survival probability in voles (*Microtus* sp.) started decreasing at 0.20 ppm in liver. Necropsy observations of fresh carcasses may assist in determining the cause of death (e.g., Hosea 2000; Stone and Okoniewski 2003), and in some cases can be supported by information on the circumstances of carcass recovery and expert knowledge of common causes of mortality in the species concerned.

The 0.26 ppm liver concentration in the kiwi from Little Barrier Island was in the 'overlap' concentration range with low certainty, but possible lethal exposure. While necropsy did not indicate haemorrhage, the recovery of the carcass in the operational area soon after bait application and previous confirmation of kiwi mortality in similar circumstances (Robertson and Colbourne 2001) support a conservative diagnosis of brodifacoum poisoning. In all of nine penguin carcasses found on beaches outside the operational area in the month following the Rangitoto/Motutapu operation, necropsy indicated starvation with no evidence of haemorrhage considered typical of anticoagulant poisoning. In some years, many little blue penguin carcasses are washed ashore in New Zealand, probably as the result of food shortage or biotoxins (e.g., Heather and Robertson 1996). For the six penguins in which no brodifacoum was detected, starvation was the most likely cause of death. In two of the three penguins with detectable liver residues, starvation was also most likely because the very low brodifacoum concentrations of 0.005 and 0.007 ppm were most representative of sublethal exposure. The penguin with 0.17 ppm liver concentration was within the 'overlap' range with low-certainty, but possibly lethal exposure. Because the carcass was found outside the operational area and with no haemorrhage seen at necropsy, the known seasonal starvation in local penguin populations was considered the more likely cause of death than brodifacoum poisoning. However, it is unknown whether brodifacoum exposure in this penguin was a contributing factor to mortality.

Importance of monitoring

While environmental sampling and subsequent analysis adds labour and operating cost to eradication programmes, monitoring data from completed eradications have undoubted value in supporting future risk assessments. When budgeting to cover mandated monitoring, generally as stipulated by the conditions of a regulatory approval, eradication planners should retain the flexibility to obtain additional environmental samples that can be stored pending analysis; it is better to have samples that don't need testing than to need to test and not have samples. Even if the potential for brodifacoum contamination is considered low, directly addressing concerns through analysis for residues may have greater 'public relations' value than the dollar cost of a laboratory test, especially if confirmation or assurance is provided by nil-detected results from a locally relevant environment. Where brodifacoum is detected in environmental samples, this contributes to future risk assessments and mitigation approaches. The detection of residual brodifacoum in little blue penguins shows the role of monitoring in identifying new information. In this case, it has raised questions about the pathways and extent of exposure in penguins and the significance of sublethal residual concentrations for longer-term survival fitness. The Rangitoto/Motutapu bait application also attracted media attention and public concern that contributed to increased publicising of both factual and inaccurate information about brodifacoum and its effects. For managers planning eradications on inhabited islands, failure to clearly address the information gaps identified by community concerns around the aerial application of brodifacoum will mean that clear justification of eradication benefits will become increasingly difficult.

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