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Article *in* Ecotoxicology and Environmental Safety · March 2015

DOI: 10.1016/j.ecoenv.2014.11.013

Ecotoxicology and Environmental Safety 113 (2015) 1–8

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Residue profiles of brodifacoum in coastal marine species following an island rodent eradication

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ARTICLE INFO

Article history:
Received 20 August 2014
Received in revised form
15 November 2014
Accepted 19 November 2014

Keywords:
Anticoagulant rodenticides
Invasive species management
Marine harvest
Monitoring
Secondary exposure

ABSTRACT

The second-generation anticoagulant rodenticide brodifacoum is an effective tool for the eradication of invasive rodents from islands and fenced sanctuaries, for biodiversity restoration. However, broadcast application of brodifacoum bait on islands may expose non-target wildlife in coastal marine environments to brodifacoum, with subsequent secondary exposure risk for humans if such marine wildlife is harvested for consumption. We report a case study of monitoring selected marine species following aerial application of brodifacoum bait in August 2011 to eradicate Norway rats (*Rattus norvegicus*) from Ulva Island, New Zealand. Residual concentrations of brodifacoum were detected in 3 of 10 species of coastal fish or shellfish sampled 43–176 d after bait application commenced. Residual brodifacoum concentrations were found in liver, but not muscle tissue, of 2 of 24 samples of blue cod (0.026 and 0.092 μg/g; *Parapercis colias*) captured live then euthanized for tissue sampling. Residual brodifacoum concentrations were also found in whole-body samples of 4 of 24 mussels (range=0.001–0.022 μg/g, $n=4$; *Mytilus edulis*) and 4 of 24 limpets (range=0.001–0.016 μg/g, $n=4$; *Cellana ornata*). Measured residue concentrations in all three species were assessed as unlikely to have eventually caused mortality of the sampled individuals. We also conducted a literature review and determined that in eleven previous accounts of residue examination of coastal marine species following aerial applications of brodifacoum bait, including our results from Ulva Island, the overall rate of residue detection was 5.6% for marine invertebrates (11 of 196 samples tested) and 3.1% for fish (2 of 65 samples tested). Furthermore, our results from Ulva Island are the first known detection of brodifacoum residue in fish liver following an aerial application of brodifacoum bait. Although our findings confirm the potential for coastal marine wildlife to be exposed to brodifacoum following island rodent eradications using aerial bait application, the risk of mortality to exposed individual fish or shellfish appears very low. There is also a very low risk of adverse effects on humans that consume fish or shellfish containing residual concentrations in the ranges reported here. Furthermore, any brodifacoum residues that occur in marine wildlife decline to below detectable concentrations over a period of weeks. Thus potential human exposure to brodifacoum through consumption of marine wildlife containing residual brodifacoum could be minimized by defining 'no take' periods for harvest following bait application and regular monitoring to confirm the absence of detectable residues in relevant marine wildlife.

1. Introduction

Anticoagulant rodenticides (ARs) inhibit the formation of blood coagulation factors in the liver, resulting in uncontrolled haemorrhaging and eventually death (Silverman 1980; Suttie 1985). The second-generation anticoagulant rodenticides in common use today are more persistent in animal tissue, particularly liver, than first-generation ARs (Hadler and Buckle 1992; Fisher et al., 2003). Second-generation ARs are currently used by many countries for rodent management in agricultural production and public health settings (Albert et al., 2010; Sage et al., 2010). Increasingly, such uses are attributed to residual AR concentrations in non-target wildlife, with a monitoring focus on secondary exposure of predatory and scavenging species (Tosh et al., 2012; Jacquot et al., 2013; Langford et al., 2013; Thompson et al., in press).

Specialised, large-scale broadcast bait applications of the second-generation AR brodifacoum for eradication of invasive rodents

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http://dx.doi.org/10.1016/j.ecoenv.2014.11.013
0147-6513/

from islands and fenced sanctuaries (Parkes et al., 2011) have been fundamental to significant successes in island biodiversity restoration (Dunlevy et al., 2011; Towns et al., 2013). Such applications also create the potential for unwanted exposure of non-target wildlife to brodifacoum through ingestion of bait (i.e. primary exposure) or ingestion of other animals containing residual concentrations of brodifacoum (i.e. secondary exposure). Brodifacoum has broad-spectrum toxicity to mammals and birds because of the common mode of action of ARs in reducing formation of blood coagulation factors in liver. Less information is available about the toxicity of brodifacoum to invertebrates but they are considered less susceptible to ARs (Pain et al., 2000; Brooke et al., 2011, 2013). Even if exposure of non-target wildlife to brodifacoum is insufficient to cause mortality, any exposure is undesirable because movement of residual brodifacoum through food webs may contaminate species that are eaten by humans, or other wildlife of high conservation status (e.g. Eason et al., 1999; Dowding et al., 2006).

Monitoring undertaken after application of brodifacoum bait for island rodent eradication has detected residues in terrestrial birds (e.g. Masuda et al., 2014), insects (e.g. Ogilvie et al., 1997), and molluscs (e.g. Morgan et al., 1996). The terrestrial focus has probably reflected perceived risk. Compared with terrestrial organisms, marine species may be at low risk of being exposed (Empson and Miskelly, 1999), due to rapid disintegration of pellet bait in water and the low quantities which are expected to reach the coastal marine environment (Howald et al., 2010; Fisher et al., 2011). However, the imperative to achieve complete and consistent bait coverage of all rodent home ranges on an island, including those at the tide line, may conflict with the technical capacity to prevent some aerially-applied baits entering the ocean (Engeman et al., 2013). For example, following aerial application of brodifacoum bait on Palmyra Atoll, bait pellets were observed up to 7 m below the high tide line at 19.1% of the target density, despite the use of a deflector intended to limit pellet distribution to only above the high tide line (Pitt et al., 2012; Engeman et al., 2013). Even if bait can be completely prevented from entering the ocean, aquatic marine wildlife might be secondarily exposed to brodifacoum through consumption of other animals that ingested bait on land but then entered the ocean (e.g. crabs, carcasses of poisoned rodents).

Here we report the results of monitoring of coastal marine wildlife undertaken following an island rodent eradication. We determined the scope and extent of brodifacoum exposure following an aerial application to eradicate Norway rats from Ulva Island, New Zealand. We assessed whether exposure was likely to have been lethal or sub-lethal, and conducted a literature review to compare the methodology and results from our case study on Ulva Island to previous studies. We also determined the risk of secondary poisoning to humans who may consume coastal marine species.

2. Methods

2.1. Case study

In December 2010, invasive Norway rats (*Rattus norvegicus*) established a population on Ulva Island (267 ha), Stewart Island, New Zealand, which was previously predator free (Masuda and Jamieson, 2013). Cereal pellet baits containing 20 mg/kg brodifacoum (Pestoff 20R, Animal Control Products, New Zealand) were aerially applied on two occasions (18 August and 20 September 2011), at a total combined rate of 11.5 kg of bait per hectare across the entire island in an attempt to eradicate the rat population. For bait application around the coastline, a deflector was attached to the bait distribution hopper in an attempt to limit bait from directly entering the ocean.

At 43, 48, 77, 176, and 274 d following the first aerial bait application on Ulva Island, we tested for residual concentrations of brodifacoum in common coastal marine fish and shellfish, including species often harvested for human consumption: blue cod (*Parapercis colias*), mussels (*Mytilus edulis*), pāua/abalone (*Haliotis iris*) (Table 1). Initially, samples were collected at 43 d following the first application, although pipi/infaunal bivalve (*Paphies australis*) were collected at 48 d. Selection of the initial sampling timepoint was based on instances where previous monitoring of shellfish had detected residual brodifacoum following aerial bait application (Vestena and Walker, 2010, Table 3). Subsequent sampling was only conducted for species in which brodifacoum was previously detected, and was conducted at approximately double the number of days from the previous interval. Sampling of a species was stopped after residual concentrations of brodifacoum were no longer detectable. Divers collected sedentary species, and fish were collected by hook and line fishing. Sedentary species were killed by freezing and fish by cervical dislocation. Samples were taken in equal proportions from three randomly chosen locations on the west, southeast and north coasts of the island, at distances ranging from 1 m to approximately 50 m from the shoreline. Samples within each site were collected randomly, to account for possible variability between depth, water movements, and substrate. Samples were frozen within 5 h of collection, and transported to the Landcare Research toxicology laboratory (Lincoln, New Zealand) where they were stored at -20 °C before being defrosted for dissection of tissues just before testing. The testing of dead animals following humane killing is not regulated and therefore does not require Animal Ethics Committee approval. A collection permit was obtained from the New Zealand Ministry of Fisheries.

The entire liver and subsamples of muscle tissue (c. 10 g) were taken from each fish, with muscle dissected to simulate a small 'fillet' cut. Intact whole-of-body soft tissues were taken from each shellfish although testing of each individual animal collected was not possible due to budgetary constraints and because some individual shellfish, particularly limpets, were too small to provide a sufficient sample quantity that would allow replicated analysis if required. Tissues (whole fish livers, cuts of fish muscle or whole bodies of shellfish) from groups of individuals of the same species and sampling date were combined and homogenised to make a composite sample to prepare for analysis (Table 1). As a result, we report the estimated residue level per individual (i.e. residue level after dividing it by the number of animals per composite sample). All samples were analysed for brodifacoum concentration using high performance liquid chromatography with fluorescence detection and a post-column pH switching technique as described by (Primus et al., 2005). Difenacoum was used as an internal standard with an analytical detection limit of 0.001 μg/g, and uncertainty (95% CI) of $\pm 6\%$.

2.2. Literature review

To compare our results with other monitoring of marine wildlife following the aerial application of brodifacoum bait to eradicate rodents, we reviewed the literature using the search terms 'brodifacoum' and 'marine' in Web of Knowledge (http://apps.webofknowledge.com), as well as 'brodifacoum in marine species' and 'brodifacoum in marine environment' in Google Scholar, April 2014. We also searched the New Zealand Department of Conservation database for additional records of monitoring in the marine environment following aerial brodifacoum applications, as well as citations in relevant articles and reports for any additional studies. We excluded monitoring results from

Palmyra Atoll, where an exceptionally high brodifacoum bait sowing density of 156.2 kg/ha was applied (Engeman et al., 2013). The remaining monitoring regimes included in our review were conducted following the application of less than 39 kg/ha of brodifacoum bait.

3. Results

In blue cod, brodifacoum was detected in liver but not muscle tissues in two of six samples collected at 43 d after the first aerial application of bait on Ulva Island (Table 1). However, brodifacoum was below detectable concentrations in the liver and muscle of four additional blue cod samples collected at this timepoint (Table 1). No brodifacoum was detected in samples collected at 77 d ($n=4$) and 176 d ($n=4$) nor in any of the other fish species sampled (Table 1).

Of the five species of invertebrates sampled, we detected brodifacoum residue in mussels and limpets (Table 1). A third of samples ($n=9$) of mussels collected at 43 d, and a sixth of samples ($n=6$) collected at 176 d contained detectable concentrations of brodifacoum (range 0.003–0.022 μg/g; Fig. 1). A quarter of samples ($n=8$) collected at 43 d and a third of samples ($n=6$) collected at 77 d contained detectable concentrations of brodifacoum (range 0.001–0.016 μg/g; Fig. 1). The highest concentrations detected in samples of mussels or limpets occurred in those collected 43 d after the application (Fig. 1).

Our literature review identified that brodifacoum residues in marine species have been monitored following 10 aerial applications between 1997 and 2011 (Table 2). Nine of these studies were in New Zealand, with the remaining in the United States. Brodifacoum residue has been detected in 11 of 196 marine invertebrate samples (5.6%) across all case studies, including our results from Ulva Island (Tables 1 and 3). Residues have been detected in pipi ($n=2$), mussels ($n=5$) and limpets ($n=4$), and concentrations ranged from 0.001 to 0.022 μg/g (mean=0.008 μg/g ± 0.003 SE, $n=11$). Brodifacoum residue was also detected in 2 of 65 (3.1%) fish samples across all case studies.

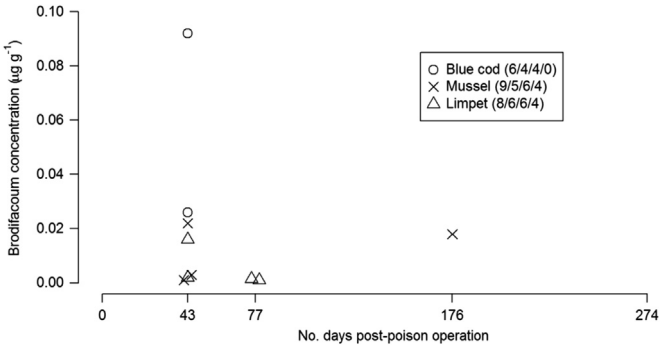

Fig. 1. Brodifacoum concentrations detected in blue cod liver, mussel and limpet samples collected 43, 77 and 176 d following an aerial application of brodifacoum baits on Ulva Island in 2011. Samples were also collected 274 d following the application, but residue was not detected. Only positive results (i.e. > MDL) are indicated in the figure, and the MDL for all samples was 0.001 μg/g and the uncertainty was 95%CL ± 6%. The total number of laboratory samples tested from each of the four sampling periods is indicated in parentheses. Some data points are shifted to prevent overlap.

4. Discussion

We detected brodifacoum residue concentrations in some samples of 3 of 10 marine species collected (blue cod, mussel, limpet) from Ulva Island. Based on our literature review, these results were not entirely unexpected. Across ten published case studies, including our results from Ulva Island, brodifacoum was detected in 5.6% (11 of 196) of marine invertebrate samples and 3.1% (2 of 65) marine vertebrate samples. Despite the detection of brodifacoum residue in multiple samples, the concentrations detected likely represented sub-lethal exposure because all of the animals tested were alive and apparently healthy when collected. As nearly all samples comprised homogenised tissue from multiple individuals (Tables 1 and 3), the exact number or identities of individual animals with residues in each sample could not be determined. As a result, a positive detection indicated that at least one individual in the sample had been exposed to brodifcacoum. Nevertheless, the presence of brodifacoum in these species allows for the possibility that brodifacoum could move through the food web, from invertebrates and fish, to predators, then to decomposers, although this determination is beyond the scope of our current study.

Blue cod collected from Ulva Island was the only instance in which residues were detected in fish across all case studies (Tables 1 and 3), possibly due to the relatively sedentary nature of this species (Mace and Johnston, 1983). Residue was only detected in two of five blue cod liver samples, but not in any of the five muscle samples taken from the same individuals. Previous monitoring for brodifacoum in marine fish has tested whole bodies or muscle, rather than liver tissue as we report here. For example, a mean of 0.337 ± 0.067 SE μg/g ($n=24$) of residue was measured in composite samples of fish carcasses following a very-high-density aerial application of brodifacoum bait on Palmyra Atoll (Pitt et al., 2012). However, it is likely that residue levels detected in whole-body samples of fish are not directly comparable with those in liver alone; in mammals, brodifacoum accumulates and persists primarily in liver and to a much lesser extent in muscle and other tissues (Huckle et al., 1988). Our results suggest this may also be the case in fish, and thus using whole-body or muscle samples to monitor brodifacoum exposure in fish appears to be less sensitive than using liver tissue alone. The retention of brodifacoum in liver but not muscle tissue may have contributed to non-detection of residues in muscle or whole-body samples of fish collected during previous monitoring efforts (Table 3). The relative roles of liver and muscle tissue in retaining brodifacoum residues in fish also have implications for secondary exposure risks to human consumers of fish, as discussed further below.

The maximum brodifacoum concentrations we measured in composite samples of mussels (0.022 μg/g) and limpets (0.016 μg/g) were among the highest reported in similar monitoring of marine invertebrates following island rodent eradications (Table 3). Prior to our study, brodifacoum was detected in mussels (0.02 μg/g) and pipi (0.0019 and 0.0012 μg/g) following island rodent eradications (Table 3). Higher concentrations of brodifacoum in marine invertebrates (e.g. up to 0.41 μg/g in mussels) were measured in an exceptional situation of accidental spillage of a large quantity of bait into a small, localised area of coastal marine environment (Primus et al., 2005). The relatively higher brodifacoum concentrations measured in mussels, limpets and pāua sampled following this bait spill were assumed to represent sub-lethal exposure as the marine molluscs were alive and apparently healthy when collected for testing (Primus et al., 2005). On the basis of a small number of captive and field-based studies, invertebrates are in general considered less susceptible to brodifacoum toxicity than mammals and birds (Booth et al., 2001). However, to date, most relevant investigations have concerned terrestrial arthropods (e.g. Pain et al., 2000; Bowie and Ross, 2006) or molluscs. There is limited and conflicting information on the susceptibility of terrestrial molluscs to brodifacoum toxicity; in some species of land snails mortality can result after feeding on brodifacoum bait (Gerlach and Florens, 2000) whereas other snail

Table 1
Laboratory results of marine organisms sampled from Ulva Island and tested as composite tissue samples for brodifacoum residue following aerial application of brodifacoum baits in 2011 to eradicate *Rattus norvegicus*. The method detection limit (MDL) for all samples was 0.001 μg/g and the uncertainty 95%CL ± 6%. Positive results (i.e. where brodifacoum was detectable) are indicated in bold. 'Days' is the number of days after the first aerial bait application (18 August 2011) at which the samples were collected.

Species	Tissue tested	Days	No. individuals represented in composite sample	Positive samples of total tested	Brodifacoum concentration detected (μg/g)
Fishes					
Banded wrasse (*Notolabrus fucicola*)	Muscle	43	5	0/1	< MDL
Girdled wrasse (*Notolabrus cinctus*)	Muscle	43	1	0/1	< MDL
Spotty (*Notolabrus celidotus*)	Muscle	43	4 (n=2), 6 (n=2)	0/4	< MDL
Trumpeter (*Latris lineata*)	Muscle	43	5	0/2	< MDL
Blue cod (*Parapercis colias*)	**Liver**	**43**	**5**	**2/6**	**0.026; 0.092**
Blue cod (*Parapercis colias*)	Muscle	43	5	0/2[a]	< MDL
Blue cod (*Parapercis colias*)	Liver	77	5	0/4	< MDL
Blue cod (*Parapercis colias*)	Muscle	77	5	0/4	< MDL
Blue cod (*Parapercis colias*)	Liver	176	5	0/4	< MDL
Blue cod (*Parapercis colias*)	Muscle	176	5	0/4	< MDL
Invertebrates					
Kina (*Evechinus chloroticus*)	Whole body	43	5	0/3	< MDL
Limpet (*Cellana ornata*)		**43**	**5 or 10**	**2/8**	**0.002** (composite n=5); **0.016** (composite n=10)
Mussel (*Mytilus edulis*)		**43**	**7 or 10**	**3/9**	**0.001** (composite n=10) **0.003** (composite n=10) **0.022** (composite n=7)
Pāua (*Haliotis iris*)		43	5	0/3	< MDL
Pipi (*Paphies australis*)		48	10	0/2	< MDL
Mussel (*Mytilus edulis*)		77	10	0/5	< MDL
Limpet (*Cellana ornata*)		**77**	**5 or 10**	**2/6**	**0.001** (composite n=5); **0.0015** (composite n=10)
Mussel (*Mytilus edulis*)		**176**	**5 or 10**	**1/6**	**0.018** (composite n=5)
Limpet (*Cellana ornata*)		176	5 or 10	0/6	< MDL
Limpet (*Cellana ornata*)		274	10	0/4	< MDL
Mussel (*Mytilus edulis*)		274	10	0/4	< MDL

[a] The two muscle tissue samples tested corresponded to the two positive composite liver samples from the same sample interval of 43 d, i.e. livers and muscle from the same fish were tested separately.

species appear to be at no risk of mortality after exposure to bait (Booth et al., 2003; Brooke et al., 2011). The vitamin-K-dependent systems best described as being affected by anticoagulant toxicity are the carboxylation reactions that produce blood coagulation factors in mammalian liver, however, vitamin-K-dependent metabolic processes also occur in other tissues (Vermeer et al., 1992). Carboxylase enzyme systems are widely distributed in invertebrate metabolic systems (Walker et al., 2001), so brodifacoum toxicity in invertebrates may be mediated through modes of action other than anticoagulation, which are not yet described. There appear to be no published assessments of the toxicity of brodifacoum to marine invertebrates.

In some previous monitoring efforts where residual brodifacoum was detected in marine invertebrates, samples were deliberately collected from within known close proximity to bait pellets. For example, one of four mussel samples collected from a pool into which baits were intentionally dropped on Motuihe Island contained detectable concentrations of brodifacoum (Fisher et al., 2011), as did two of five pipi samples collected within 20 cm of bait pellets placed in coastal waters of Urupukapuka Island (Vestena and Walker, 2010). In contrast, the locations from which marine animals were sampled from around Ulva Island were chosen without reference to known locations of bait. Overall we found a greater frequency of detectable brodifacoum in samples of marine vertebrates and invertebrates, compared with previous monitoring efforts around other islands and sanctuaries (Tables 1 and 3), which may have been in part due to a relatively more intensive sampling regime. Compared with other monitoring

Table 2
Monitoring of marine wildlife following aerial applications of brodifacoum (< 39 kg/ha). Data compiled from a literature review (see Methods).

Site	Country	Year of application	Total sowing density (kg/ha)	Type of brodifacoum bait applied	Residue detected in fish/invertebrates
Motuihe I	New Zealand	1997	11.5	Talon 7–20	N/Y
Anacapa I	USA	2001	15	nd	N/N
Hauturu (Little Barrier) I	New Zealand	2004	17.9	Pestoff 20R	N/N
Kaikoura I	New Zealand	2008	nd	nd	N/N
Rangitoto/Motutapu I	New Zealand	2009	38.2	Pestoff 20R	N/N
Urupukapuka I	New Zealand	2009	23.3	Pestoff 20R	N/Y
Moturua I	New Zealand	2009	23.3	Pestoff 20R	N/N
Shakespear Sanctuary	New Zealand	2011	31.5	Pestoff 20R	N/N
Taranga (Hen) I	New Zealand	2011	36	nd	N/N
Ulva I	New Zealand	2011	11.5	Pestoff 20R	Y/Y

'nd' indicates data unavailable.

Table 3
Laboratory results of marine fishes and invertebrates tested for brodifacoum residue following the aerial application of < 39 kg/ha of brodifacoum to eradicate invasive rodents. Results from Ulva Island, New Zealand, are excluded (see Table 1). Data compiled from a literature review (see Methods). Positive results (i.e. > MDL) are indicated in bold.

Species	Sample or non-target[a]	Tissue tested	Days[b]	Proportion of positive lab samples (residue level detected; μg/g)	No. individuals per lab sample	MDL	Site	Reference
Fishes								
Blue cod (*Parapercis colias*)	Sample	Flesh	33	0/1	nd	0.001	Shakespear Sanctuary	Maitland (2012)
Moki (*Latridopsis ciliaris*)	Sample	Flesh	33	0/1	nd	0.001	Shakespear Sanctuary	Maitland (2012)
Parore (*Girella tricuspidata*)	Sample	Flesh	33	0/1	nd	0.001	Shakespear Sanctuary	Maitland (2012)
Pilchard (*Sarditlops neopilchardus*)	Non-target	Comp	12–42	0/1	4 or 5	0.001	Rangitoto/Motutapu Is	Fisher et al. (2011)
Snapper (*Chrysophrys auratus*)	Sample	Flesh	33	0/1	nd	0.001	Shakespear Sanctuary	Maitland (2012)
Spotty (*Notolabrus celidotus*)	Sample	Flesh	33	0/1	nd	0.001	Shakespear Sanctuary	Maitland (2012)
Tidepool sculpin (*Oligocottus maculosus*)	Sample	nd	15, 30 and 90	0/26	nd	0.001–0.07	Anacapa I.	Howald et al. (2005, 2010)
Triple fin (*Forsterygion varium*)	Sample	Flesh	33	0/1	nd	0.001	Shakespear Sanctuary	Maitland (2012)
Invertebrates								
Cockle (*Austrovenus stutchburyi*)	Sample	Flesh	41	0/1	nd	0.001	Shakespear Sanctuary	Maitland (2012)
Crab (unknown species)	Sample	Comp	41	0/1	nd	0.001	Shakespear Sanctuary	Maitland (2012)
Crab (*Pachygrapsus crassipes*)	Sample	Comp	15 and 30	0/42	nd	0.001–0.07	Anacapa I.	Howald et al. (2005, 2010)
Crayfish (*Jasus edwardsii*)	Sample	nd	1	0/2	3	0.0005	Taranga (Hen) I.	Broome et al. (2012)
Crayfish (*Jasus edwardsii*)	Sample	nd	9	0/2	3	0.0005	Taranga (Hen) I.	Broome et al. (2012)
Crayfish (*Jasus edwardsii*)	Sample	Flesh	33	0/1	nd	0.001	Shakespear Sanctuary	Maitland (2012)
Cushion star (*Asterina* sp.)	Sample	Comp	33	0/1	nd	0.001	Shakespear Sanctuary	Maitland (2012)
Cushion star (*Asterina* sp.)	Sample	Comp	41	0/1	nd	0.001	Shakespear Sanctuary	Maitland (2012)
Hermit crab (*Coenobita* sp.)	Sample	Comp	15, 30 and/or 90	0/6	nd	0.001–0.07	Anacapa I.	Howald et al. (2010)
Kina (*Evechinus chloroticus*)	Sample	Comp	1	0/2	3	0.0005	Taranga (Hen) I.	Broome et al. (2012)
Kina (*Evechinus chloroticus*)	Sample	Comp	9	0/2	3	0.0005	Taranga (Hen) I.	Broome et al. (2012)
Kina (*Evechinus chloroticus*)	Sample	Flesh	33	0/1	nd	0.001	Shakespear Sanctuary	Maitland (2012)
Kina (*Evechinus chloroticus*)	Sample	Flesh	41	0/1	nd	0.001	Shakespear Sanctuary	Maitland (2012)
Limpet (*Lottia gigantea*)	Sample	Comp	15, 30 and/or 90	0/1	nd	0.001–0.07	Anacapa I.	Howald et al. (2010)
Mussel (unknown species)	**Sample**	**nd**	**nd**	**1/4[c](0.02)**	**nd**	**0.01**	**Motuihe I.**	**Fisher et al. (2011), Broome et al. (2012)**
Mussel (unknown species)	Sample	nd	nd	0/2	nd	0.001	Kaikoura I.	Broome et al. (2012)
Mussel (*Mytilus edulis*)	Sample	Comp	21	0/1	4–5	0.001	Rangitoto I.	Fisher et al. (2011)
Mussel (*Mytilus californianus*)	Sample	Comp	15 and 30	0/22	nd	0.001–0.07	Anacapa I.	Howald et al. (2005, 2010)
Mussel (*Perna canaliculus*)	nd	Comp	1	0/1[d]	3	0.001	Moturua I.	Vestena and Walker (2010)
Mussel (*Perna canaliculus*)	nd	Comp	2	0/1[d]	3	0.001	Moturua I.	Vestena and Walker (2010)
Mussel (*Perna canaliculus*)	nd	Comp	21	0/1[d]	3	0.001	Moturua I.	Vestena and Walker (2010)
Mussel (*Perna canaliculus*)	nd	Comp	22	0/1[d]	3	0.001	Moturua I.	Vestena and Walker (2010)
Mussel (*Perna canaliculus*)	nd	Comp	35	0/1[d]	3	0.001	Moturua I.	Vestena and Walker (2010)
Mussel (*Perna canaliculus*)	nd	Comp	48	0/1[d]	3	0.001	Moturua I.	Vestena and Walker (2010)
Mussel (*Perna canaliculus*)	nd	Comp	76	0/1[d]	3	0.001	Moturua I.	Vestena and Walker (2010)
Mussel (nd)	Sample	Flesh	41	0/1	nd	0.001	Shakespear Sanctuary	Maitland (2012)
Pacific Oyster (*Crassostrea gigas*)	Sample	Comp	Nd	0/2	nd	0.01	Motuihe I.	Fisher et al. (2011)
Pacific Oyster (*Crassostrea gigas*)	Sample	Flesh	41	0/1	nd	0.001	Shakespear Sanctuary	Maitland (2012)
Pacific Oyster (*Crassostrea gigas*)	Sample	Flesh	147	0/6	nd	0.001	Shakespear Sanctuary	Maitland (2012)
Pāua (*Haliotis iris*)	Sample	Comp	7–14	0/2	4–5	0.001	Hauturu (Little Barrier) I.	Fisher et al. (2011)
Pipi (*Paphies australis*)	Sample	Comp	21	0/1	4–5	0.001	Rangitoto/Motutapu Is.	Fisher et al. (2011)
Pipi (*Paphies australis*)	nd	Comp	1	0/1[d]	3	0.001	Urupukapuka I.	Vestena and Walker (2010)
Pipi (*Paphies australis*)	nd	Comp	2	0/1[d]	3	0.001	Urupukapuka I.	Vestena and Walker (2010)
Pipi (*Paphies australis*)	nd	Comp	7	0/1[d]	3	0.001	Urupukapuka I.	Vestena and Walker (2010)
Pipi (*Paphies australis*)	nd	Comp	21	0/1[d]	3	0.001	Urupukapuka I.	Vestena and Walker (2010)
Pipi (*Paphies australis*)	**nd**	**Comp**	**22**	**1/1[d](0.0019)**	**3**	**0.001**	**Urupukapuka I.**	**Vestena and Walker (2010)**
Pipi (*Paphies australis*)	nd	Comp	28	0/1[d]	3	0.001	Urupukapuka I.	Vestena and Walker (2010)

Table 3 (*continued*)

Species	Sample or non-target[a]	Tissue tested	Days[b]	Proportion of positive lab samples (residue level detected; μg/g)	No. individuals per lab sample	MDL	Site	Reference
Pipi (*Paphies australis*)	**nd**	**Comp**	**35**	**1/1[d](0.0012)**	**3**	**0.001**	**Urupukapuka I.**	**Vestena and Walker (2010)**
Pipi (*Paphies australis*)	nd	Comp	48	0/1[d]	3	0.001	Urupukapuka I.	Vestena and Walker (2010)
Pipi (*Paphies australis*)	nd	Comp	76	0/1[d]	3	0.001	Urupukapuka I.	Vestena and Walker (2010)
Scallop (*Pecten novaezelandiae*)	Sample	Comp	7–14	0/2	4–5	0.001	Hauturu (Little Barrier) I.	Fisher et al. (2011)
Sea cucumber (nd)	Sample	Comp	33	0/1	nd	0.001	Shakespear Sanctuary	Maitland (2012)
Seastar (*Coscinasterias* sp)	Sample	Comp	33	0/1	nd	0.001	Shakespear Sanctuary	Maitland (2012)
Sea urchin (*Strongylocentrotus franciscanus*)	Sample	nd	15, 30 and/or 90	0/10	nd	0.001–0.07	Anacapa I.	Howald et al. (2010)
Shrimp (nd)	Sample	Comp	41	0/1	nd	0.001	Shakespear Sanctuary	Maitland (2012)
Cat's eye (*Turbo* sp.)	Sample	Flesh	33	0/1	nd	0.001	Shakespear Sanctuary	Maitland (2012)
Cat's eye (*Turbo* sp.)	Sample	Flesh	41	0/1	nd	0.001	Shakespear Sanctuary	Maitland (2012)
Whelk (nd)	Sample	Flesh	33	0/1	nd	0.001	Shakespear Sanctuary	Maitland (2012)

'nd' indicates that data were not provided in the publication; 'comp'=composite sample.

[a] Specimen tested was alive (i.e. sample) or dead (i.e. non-target) at the time of collection.

[b] Number of days following the poison application during which the samples were collected. This value was calculated from the first poison application in cases where multiple applications were conducted as part of an individual eradication attempt.

[c] Samples were collected from pool where bait was deliberately dropped.

[d] Samples were deliberately collected from within 20 cm of poison bait.

(Table 3), the sampling regime off the coast of Ulva Island involved a greater number of species, larger samples sizes for each interval, and longer sampling intervals (Tables 1 and 3), all of which were expected to increase the likelihood of detecting residual brodifacoum if it was present.

Differences in detectable brodifacoum in samples of marine vertebrates and invertebrates between sampling regimes may also be due to differences in bait application rates. For example, the extremely high bait application rate used on Palmyra Atoll (Engeman et al., 2013) may have increased the likelihood of marine invertebrates being exposed to brodifacoum, as residue was detected in 22 of 35 samples (62.9%) of two crab species (*Uca tetragonon* and *Coenobita perlatus*) (Pitt et al., 2012). Although the overall aerial application rate of brodifacoum bait on Ulva Island (11.5 kg/ha) was among the lowest rate than previous baiting operations for which marine monitoring was undertaken (range=11.5–38 kg/ha; Table 3), there were some areas of the coastline (up to ~100 m inland) where a higher density of 23 kg/ha of bait pellets was applied because Norway rats are more likely to be found along the coastline (Harper, 2006). Furthermore, all islets off of Ulva Island were sowed with 24 kg/ha of bait pellets.

In contrast, bait pellets were sowed uniformly throughout Shakespear Sanctuary (Maitland, 2012). We were unable to obtain corresponding information for the remaining islands and sanctuaries examined in our literature review. It may be that the use of higher brodifacoum bait application rates along a coastline is one factor in the occurrence of residues in coastal marine environments, and this would be useful to consider in planning future monitoring programmes.

The presence of low concentrations of brodifacoum in blue cod liver, mussels and limpets from around Ulva Island presents a possible, though unlikely poisoning hazard to people who harvest these species, despite the lack of residues detected in any fish muscle samples which is the tissue most commonly eaten in blue cod. On the basis of a 'no observed effects level' (NOEL) value for daily brodifacoum exposure of 0.001 mg/kg, derived from laboratory animal studies (Anonymous, 2010), and the highest residual concentration we measured in our monitoring (in blue cod liver at 0.092 μg/g), we estimate that a person could consume at least 10 g of cod liver for every kilogram of their bodyweight daily (e.g. a 60-kg adult could eat 600 g of cod liver per day) without affecting blood coagulation capacity. Such a dietary scenario is considered very unlikely because relatively little fish liver is consumed by humans, and thus even at the highest residue concentrations measured here it is even less likely that an individual person could consume sufficient seafood over a short time to produce a harmful effect on human coagulation. Also, the majority of samples in our monitoring did not have detectable residues and each sample was composed of multiple individuals, thus the potential for secondary exposure of people harvesting would have been further reduced.

Although our results suggest that exposure in marine species was sub-lethal and unlikely to become a secondary hazard to humans, if conservation managers would like to achieve zero brodifacoum exposure to marine species during rodent eradications, then additional research is needed. Assuming that the deflector successfully prevented brodifacoum bait from directly entering the marine environment, brodifacoum detected in coastal marine species likely originated from bait applied on land. For example, bait pellets could have degraded, particles of brodifacoum bound to organic matter (e.g. soil and sediment) (World Health Organization, 1995; Housenger and Melendez, 2012), and travelled via surface runoff into the ocean, becoming suspended in the pelagic water column or settled on benthic substrates where blue cod, mussels and limpets could directly absorb brodifacoum particles in sea water, or indirectly consume contaminated prey such as crustaceans (for blue cod) and algae (for limpets) (U. S.

Environmental Protection Agency. 1998; Eason and Wickstrom, 2001; Jiang and Carbines, 2002; Fisher et al., 2011). Future research could focus on this pathway, such as a reduction in the amount of bait applied on land.

Our results, as well as those from a previous report (Primus et al., 2005), indicate that the concentration of any residues in marine wildlife will decrease over time and will eventually decline below detectable concentrations. It would be useful in the future to better define the rate of such a decline by extending the duration of residue monitoring, or conducting laboratory experiments. While a theoretical assessment indicates that there is a very low risk to people, understandably the mere potential for secondary exposure through seafood could be of concern to affected communities. The conservative approach to minimising this potential would be to define 'no take' periods for harvest of shellfish and fish livers following bait applications for island eradications, and to support these with monitoring of appropriate marine species to confirm residues are not detectable.

Acknowledgements

We thank staff from the New Zealand Department of Conservation Stewart Island Field Centre, especially Paul Jacques, for assisting with collection of the samples. Thanks also to Lynn Booth, Landcare Research Toxicology Laboratory for assistance in organising and interpreting residue data and to Phil Cowan for improvements to the draft manuscript. This study was funded by the New Zealand Department of Conservation, and Landcare Research, New Zealand, as a Crown Institute.

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