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Authors

Morgan, David R.
Wright, G. R.
Ogilvie, Shaun C.
et al.

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ASSESSMENT OF THE ENVIRONMENTAL IMPACT OF BRODIFACOUM DURING RODENT ERADICATION OPERATIONS IN NEW ZEALAND

D. R. MORGAN, G. R. WRIGHT, and **S. C. OGILVIE**, Manaaki Whenua - Landcare Research, P.O. Box 69, Lincoln, New Zealand.

R. PIERCE, Department of Conservation, P.O. Box 842, Whangarei, New Zealand.

P. THOMSON, Department of Conservation, Private Bag 3072, Hamilton, New Zealand.

ABSTRACT: Although Talon® baits containing brodifacoum have been used successfully in eradicating rats from some of New Zealand's offshore islands, little is known about any environmental effects of this toxin. Invertebrates, blackbirds, soil, and water at intervals of two days to nine months were sampled to determine whether brodifacoum residues were present after aerial distribution of Talon® 20P cereal pellets on Red Mercury Island and after bait-station use of Talon® 50WB wax-coated cereal blocks on Coppermine Island. No brodifacoum residues were found in soil, water, or most (99%) invertebrate samples. Low concentrations (0.12 μg/g) were found in one sample of slugs collected two days after aerial sowing. Liver tissues from all birds (n=4) and rats (n=3) found dead, and from all six birds collected alive eight months after aerial baiting, also contained low-to-moderate concentrations of brodifacoum (0.004 to 11.0 μg/g). These preliminary results suggest that invertebrates are not likely to accumulate brodifacoum as a result of Talon® baiting. Laboratory studies showed that, although some invertebrates may eat Talon® baits, it appears that the brodifacoum is metabolized and/or excreted within a few days. The dead blackbirds found were, therefore, more likely to have been killed by primary rather than by secondary poisoning. Further monitoring for brodifacoum residues after Talon® operations should be undertaken to confirm that contamination of invertebrates, soil, and water is unlikely. Some bird species may be at risk from eating Talon® baits. Likely effects on population levels of such species should be assessed to help weigh the risk and benefits of Talon® use in rodent eradication.

KEY WORDS: animal damage control, rodenticides, field tests

INTRODUCTION

Three species of rats have been introduced to New Zealand. The Polynesian rat or kiore (*Rattus exulans*) was introduced by the Maori about 1,000 years ago. Ship rats (*Rattus rattus*) probably arrived with the early European colonists in the mid-nineteenth century, and Norway rats (*Rattus norvegicus*) arrived later. Rats have harmed the indigenous biota of New Zealand, reducing or eliminating populations of birds, reptiles and invertebrates (King 1990). Islands offer potential refuges for endangered wildlife, but many are inhabited by rats. Although Talon® cereal pellets (Talon® 20P) and wax-impregnated cereal blocks (Talon® 50WB) have been used successfully to eradicate rats from islands in New Zealand and overseas (Buckle and Fenn 1992), little is known about the environmental effects of control operations using these types of toxic baits in New Zealand. Brodifacoum, the active ingredient used in Talon® baits, acts by interfering with the normal synthesis of vitamin K-dependent clotting factors in the liver of vertebrates (Hadler and Shadbolt 1975). A review of the international literature (Eason and Spurr 1995) suggested that many of New Zealand's native vertebrates would be at risk if they ate Talon® baits directly or were exposed to brodifacoum via the food chain if the toxicant accumulates in invertebrate prey species. Invertebrates have been seen feeding on Talon® baits (Eason and Spurr 1995) and although no toxicological data were presented, Shirer (1992) considered it unlikely that invertebrates would be killed by brodifacoum as they have different blood clotting systems than vertebrates. However, if invertebrates became contaminated with brodifacoum, either directly through eating bait or indirectly through ingesting contaminated soil or water, they may pose a risk of secondary poisoning to vertebrates that prey upon invertebrates (Eason and Spurr 1995).

Assessment of the risks to non-target species presented by a vertebrate pesticide should be determined by considering the likelihood of non-target species being exposed to the compound, the persistence of the compound in different parts of the environment, and the susceptibility of non-target species to the compound (Brown et al. 1988). In assessing the possible environmental risks posed by Talon® usage, the authors, therefore, determined the potential for exposure of non-target species to brodifacoum by monitoring its fate in soil, water, invertebrates and vertebrates under field conditions after rodent control operations. They monitored over time to assess the persistence of the compound in these components of the environment. To assist in interpreting the field data, they also monitored the feeding response of three species of native insects in the laboratory when offered Talon® baits and conducted a study on the toxicity and persistence of brodifacoum in one of these species.

METHODS

Field Monitoring

Environmental monitoring of brodifacoum was carried out after two Department of Conservation rodent control

operations—one using aerially sown bait, the other using baits in bait stations.

Talon® 20P pellets (ICI Crop Care Holdings, Richmond, New Zealand) of mean weight 2.5 g and containing green dye and 20 ppm brodifacoum were aerially sown at 15 kg/ha over the whole of Red Mercury Island (225 ha) on 1 October 1992. Live invertebrate samples (Table 1) were collected by hand at up to six widely distributed circular plots of 10 m radius (selected for relative abundance of invertebrates) 4 to 8 days before and 2 to 3, 9, 30, and 240 days after baits were sown. Samples were frozen shortly after collection using liquid nitrogen and returned to the laboratory for sorting and brodifacoum assay. Five dead blackbirds (*Turdus merula*) and three kiore (*Rattus exulans*) were collected during ground searches in the week after aerial sowing. Six live blackbirds were collected nine months after (four in mist nets, two by shooting). Soil and water samples were collected one month after aerial sowing. Samples (200 g) of topsoil were collected in plastic bags at nine widely distributed sites, and water samples (200 ml) were collected in glass bottles from small streams in different parts of the island. The sampling schedule is summarized in Figure 1.

Table 1. Numbers of samples of invertebrate types collected on Red Mercury and Coppermine Islands. Samples comprised from one to eight individuals depending on abundance at sampling sites. Each value indicates the number of samples pooled for an individual assay.

| Invertebrate Type (and Order, or Class where Order Unknown) | Red Mercury Island Sampling Schedule in Relation to Control Operation | | | | | Coppermine Island Sampling Schedule in Relation to Control Operation | | | | |
| | Days Before | Days after Sowing | | | | Days Before | Days after Start | | | |
	4-8	2-3	9	30	240	16-19	13	27-31	57	101
Slater (*Isopoda*)	11	6	5	3	0	4	2	3	6	2
Spider (*Araneae*)	7	3	4	6	4	4	3	6	4	4
Millipede (*Diplopoda*, order unknown)	11	2	2	2	4	12	5	8	5	7
Centipede (*Chilopoda*, order unknown)	4	1	1	2	0	2	3	4	5	6
Cockroach (*Blatoidea*, order unknown)	6	0	1	0	2	8	3	5	6	7
Ant (*Hymenoptera*)	0	0	1	1	0	0	0	0	0	0
Wasp (*Hymenoptera*)	0	0	0	0	0	1	0	0	1	0
Insect larvae (unidentified)	5	0	5	1	0	2	1	4	3	0
Ground weta (*Orthoptera*)	0	0	0	0	0	4	4	5	2	9
Cave weta (*Orthoptera*)	4	1	1	1	4	11	2	2	4	2
Slug (*Gastropoda*, order unknown)	0	2	1	2	1	0	0	0	0	0
Snail (*Gastropoda*, order unknown)	1	1	1	0	3	0	2	1	1	0
Worm (*Opisthopora*)	4	4	5	7	4	0	4	6	6	7
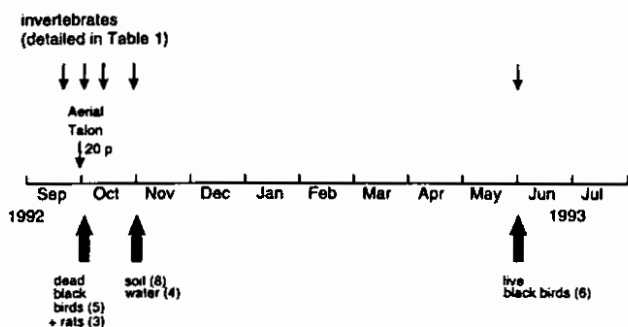
Figure 1. Schedule for collection of samples on Red Mercury Island.

Talon® 50WB baits (ICI Crop Care Holdings, Richmond, New Zealand) of mean weight 30 g and containing green dye and 50 ppm brodifacoum were placed in tunnel-type bait stations on Coppermine Island (80 ha) from October 1992 to May 1993. Live invertebrates were collected by searching vegetation from 0 to 1 m above ground within 12 m of six bait stations, 16 to 19 days before and 13, 27 to 31, 57, and 101 days after baiting started. Soil samples (200 g) were collected under five bait stations and at five sites equidistant between bait stations, one and nine months after bait stations were established. The sampling schedule is summarized in Figure 2.
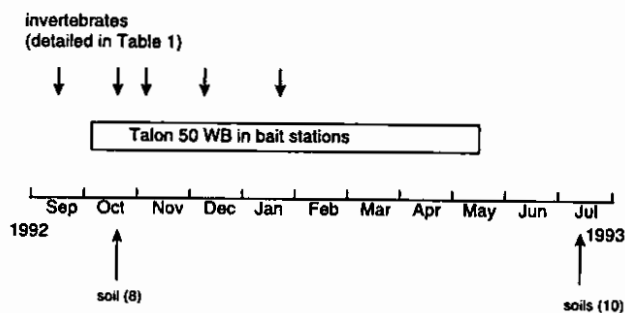

Figure 2. Schedule for collection of samples on Coppermine Island.

Invertebrate samples were sorted into orders (Table 1). To provide sufficient material for analysis, the invertebrate types (i.e., as orders) collected from all sites on each sampling occasion were pooled. Samples from the two islands were, however, sorted and pooled separately, and stored frozen until assay.

Pooled samples were weighed and chopped into small pieces with scissors before being assayed for brodifacoum content using High Performance Liquid Chromatography. The method used is described by Hunter (1983) and the detection limits are 0.0001 μg brodifacoum/ml in water, 0.004 $\mu g/g$ in vertebrate tissues (liver or gizzard), 0.02 $\mu g/g$ in soil, and 0.05 $\mu g/g$ in invertebrate tissues.

Laboratory Studies

Laboratory colonies were maintained of three native insect species. Large-headed weta (*Hemideina crassidens*), cave weta (*Gymnoplectron edwardsii*), and ground beetles (*Megadromus bullatus*) were kept in glass tanks (72 x 38 x 38 cm) with a floor-lining of soil, leaf-litter and sphagnum moss. Each tank housed up to six individuals of a single species. Small logs of approximately 30 cm length and 8 cm diameter with a hollow core of 20 cm were supplied in weta tanks for shelter, as recommended by Barret (1991). Fresh native plant material and apple was supplied every second day, along with processed pet meat supplied about every two weeks. Water was always freely available.

Each animal was uniquely marked with a small amount of white typing paint applied to the dorsal surface of the carapace. Feeding behavior of the animals towards Talon® baits (20P and 50WB) was recorded from continuous time-lapse video recordings of overnight (16 h) activity in each tank. Each group of animals in a tank was offered four 20P pellets or one 50WB bait on different nights. The baits were placed on a small petri dish on a piece of white paper to facilitate observation. The identity of individuals seen feeding on baits and the time spent feeding were recorded.

The acute toxicity of brodifacoum to large-headed weta (selected for relative ease of dosing) was determined by dosing weta with brodifacoum using a 10 μL syringe with a 28 gauge blunt needle (modified method of Sutherland et al. 1982). A constant dose of 1 $\mu L/g$ bodyweight was given to all weta, but different solutions were used to dose groups (three male and three female) at each of the following dose levels: 12.5, 25, 45 and 62.5 $\mu g/g$ bodyweight. The brodifacoum was administered with 10% dimethylsulfoxide (DMSO) in 60% monopropylene glycol (MPG). A control group of six male and six female weta was dosed with the DMSO and MPG mixture. Precise dosing proved to be difficult as some spillage of the mixture occurred and some weta regurgitated a small proportion of the mixture. Following administration, all animals were returned to their familiar glass tanks and closely monitored until no further deaths occurred.

The persistence of brodifacoum in live large-headed weta was determined by dosing 18 individuals nominally with 15 $\mu g/g$ brodifacoum in 10% DMSO and 60% MPG. This is equivalent to consumption of 6 g Talon® 20P pellets and such a large dose should be regarded as representing prolonged feeding by weta on Talon® pellets over a period of several days. At nine time points over a 14-day period, one male and one female weta were killed. Each entire animal was then macerated before analyzing for brodifacoum content.

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RESULTS
Field Monitoring

No residues of brodifacoum were found in soil, water, or most (99%) invertebrate samples from Red Mercury and Coppermine Islands. One sample of slugs, collected on Red Mercury Island two days after aerial sowing, contained 0.12 μg/g brodifacoum. Liver tissues from birds (n=4) and rats (n=3) found dead after aerial sowing contained low-to-moderate levels of brodifacoum (0.6 to 11.0 μg/g), and livers from all six birds collected alive contained low levels of brodifacoum (0.004 to 0.2 μg/g).

Laboratory Studies

Many individual insects were observed feeding on Talon® baits on overnight video-recordings (Table 2). Talon® 20P pellets were preferred by the three species. All ground beetles were seen feeding on these pellets, and individual cave weta were seen feeding for up to 64 min during a 16 h period. Talon® 50WB baits were less palatable and were not fed on at all by large-headed weta.

All weta survived the two highest doses of brodifacoum (Table 3). Three died at lower doses but

since three also died in the control group, it is probable that all deaths were due to the trauma of dosing rather than effects of the brodifacoum.

When administered at 15 μg/g, brodifacoum persisted in weta for a maximum of four days (Figure 3). There was no significant difference in the rate of elimination between males and females ($F_{1,14} < 0.001$, p=0.991) and so the data were pooled to produce a highly significant regression equation that accounted for a high proportion of the variation in the data ($r^2 = 0.946$, p < 0.001). The amount of brodifacoum recovered from weta during the first two hours after dosing was less than expected, the regression equation predicting a value of 12.8 μg/g brodifacoum at time zero. This is explained by the observed spillage during dosing and/or regurgitation after dosing, and possibly to incomplete recovery of the brodifacoum during laboratory analyses. The form of the curve suggests that most of the brodifacoum was eliminated within two days, and the rate of elimination decreased over time ($F_{1,14} = 40.3$, p < 0.001). This may have been due to some of the compound passing rapidly through the gut unchanged and some being metabolized.

Table 2. Feeding responses of three species of insect when presented with Talon® baits on separate occasions overnight.

		Talon® 20P		Talon® 50WB	
	Number of Individuals Observed	Number (and Percentage) Observed Feeding on Bait	Range of Individual Total Feeding Times (secs) During 16 h Overnight	Number (and Percentage) Observed Feeding on Bait	Range of Individual Total feeding Times (secs) During 16 h Overnight
Cave weta	17	10 (59)	12 - 3840	6 (35)	12 - 90
Large-headed weta	7	3 (43)	472 - 765	0 (0)	-
Ground beetle	15	15 (100)	1 - 151	7 (47)	1 - 24

Table 3. Proportions of large-headed weta dying after being dosed with brodifacoum.

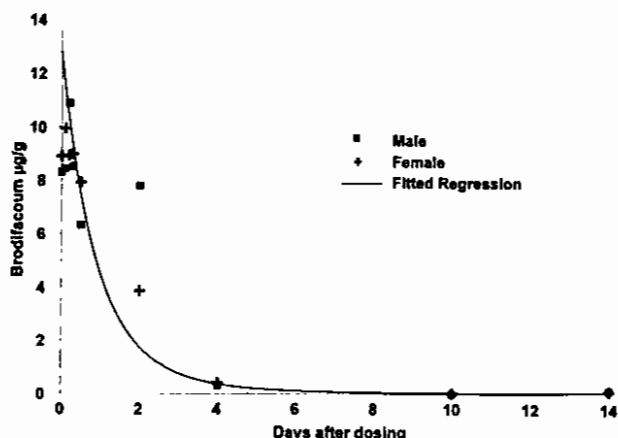|	(Control) 0 μg/g	12.5 μg/g	25 μg/g	45 μg/g	62.5 μg/g
Male	1/6	2/3	0/3	0/3	0/3
Female	2/6	0/3	1/3	0/3	0/3

Figure 3. Elimination of brodifacoum from male and female large-headed weta following a nominal dose of 15 $\mu g/g$.

DISCUSSION

Much of this study focused on the fate and persistence of brodifacoum in invertebrates as potential sources of secondary poisoning of birdlife. The findings suggest, however, that invertebrates are unlikely to accumulate brodifacoum as a result of bait station use of Talon® 50WB or aerial sowing of Talon® 20P. This could be because: 1) invertebrates do not find baits; 2) the baits are unpalatable to invertebrates; 3) natural food abundance or other environmental factors predisposed invertebrates against feeding on baits; 4) brodifacoum is readily metabolized and/or excreted by invertebrates; and/or 5) the brodifacoum assay is too insensitive or recovery of the brodifacoum incomplete.

Failure to find baits was improbable, especially after aerial sowing where baits were distributed on average at approximately 1 bait/1.3 m^2. The authors' observations of the feeding response of three insect species towards Talon® suggest that baits being unpalatable to invertebrates is also an unlikely reason for the lack of residues in invertebrates: all three species ate one or both types of Talon®, and 20P was clearly the bait type preferred. It also seems unlikely that such a wide range of invertebrates would have been predisposed not to feed on baits by environmental factors such as food abundance, particularly on Coppermine Island where Talon® 50WB baits were presented continuously over a seven month period.

It is, therefore, possible that some invertebrates did feed on Talon® baits, but the brodifacoum was eliminated quickly through metabolism and/or excretion. This was supported by the results of the laboratory study, where elimination of a large (nominal) dose of brodifacoum (15 $\mu g/g$) in four days was recorded. This rate of elimination is much more rapid than the four months reported in vertebrate tissues (e.g., Lass et al. 1985; Eason et al. 1996). It also contrasts with a previous field finding at

Puketi forest of 1080 residues in invertebrates after aerial poisoning (Eason et al. 1993) where 1080 was found in two species of weta and a cockroach species for up to three weeks. The result requires further verification in weta and other invertebrates allowed free access to baits, but if elimination of brodifacoum by weta is representative of invertebrates, there is little likelihood that brodifacoum would accumulate in invertebrates following prolonged feeding on Talon® baits. Furthermore, the finding that most weta dosed with brodifacoum survived concentrations up to 62.5 $\mu g/g$ (a dose well in excess of the LD_{50} for all vertebrates listed by Eason and Spurr 1995) supports Shirer's (1992) view that invertebrates are unlikely to be killed by brodifacoum, but additional studies are required with other species to confirm that Talon® baiting has an insignificant impact on invertebrate species.

The sample pooling procedure adopted was designed to increase the chances of detecting brodifacoum contamination, and although contamination of individual animals may be diluted by pooling with uncontaminated animals, it is believed the procedure is more likely to detect residues than the same amount of laboratory analysis applied to spot-sampling of individual animals. The limit of detection equates to consumption of 0.002 g of bait by a large invertebrate, such as a weta, weighing 1 g, or a pooled 1 g sample of a smaller invertebrate, such as millipedes. Even if it is assumed that all invertebrate tissues contain brodifacoum at a concentration of 0.045 $\mu g/g$, which is just below the lower limit of detection (i.e., 0.05 $\mu g/g$), the risks of secondary poisoning are very low. For example, a southern black-backed gull [the most susceptible avian species for which data are given in Eason and Spurr (1995)] weighing 1 kg would have to consume 16.6 kg of such contaminated invertebrates to receive an LD_{50} dose of the toxin. Therefore, even if the laboratory assay for brodifacoum in invertebrate tissues was too insensitive, it is nevertheless extremely unlikely that undetected levels of brodifacoum presented a hazard to insectivorous birds. It is possible that a small proportion of brodifacoum was not fully recovered in laboratory analyses of invertebrates, but the low initial concentrations recorded in laboratory-dosed weta were believed to be due to observed spillage and regurgitation rather than poor recovery.

As so few invertebrates were found contaminated, the brodifacoum residues found in dead blackbirds and those collected alive on Red Mercury Island probably resulted from direct consumption of bait rather than from feeding on contaminated invertebrates. [This suggests that the green dye that is incorporated in pest baits in New Zealand to deter feeding by birds (Caithness and Williams 1971) may not be a completely effective measure.] However, primary and secondary poisoning of blackbirds, and perhaps other bird species, did not result in measurable reductions in bird populations on Red Mercury Island (Robertson et al. 1993). Similarly, although a few individual birds were found dead after aerial poisoning of rabbits and kiore with Talon® 20P on Stanley Island, Towns et al. (1993) found no evidence of a detrimental effect on the population of any species, including the ground-feeding Saddleback (*Philesturnus*

carunculatus) and Red-crowned Parakeet (*Cyanoramphus novaezealandiae*) and the predatory Morepork (*Ninox novaezealandiae*). The possibility of non-target kills occurring after aerial operations may be outweighed by the benefits of habitat improvement gained by rapid removal of rodents. Nevertheless, such a management decision should be supported by information on the likely response of populations of non-target species to Talon® 20P baits.

Since no dead birds with brodifacoum residues were found on Coppermine Island, it appears that the risk of poisoning birds may be avoided by placing bait in bait stations. However, this may be impractical on larger islands with inaccessible terrain.

As no residues were detectable in the soil or water samples, significant soil and water contamination appear unlikely as a result of Talon® baiting, either from aerial or bait-station applications. Nevertheless, further monitoring for brodifacoum in invertebrates, soil, and water under normal operational use should be undertaken at other sites to determine the general applicability of these findings. Additional studies are also required to determine which species of wild birds (particularly in prospective wildlife refuges) are most likely to feed on Talon® baits, and the potential impact on bird populations.

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