Toxicity and residues of brodifacoum in snails and earthworms

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ABSTRACT

A paucity of data regarding the effects of the vertebrate pesticide brodifacoum on soft-bodied invertebrates was addressed by laboratory evaluations of the toxicity and occurrence of residues of this anticoagulant in snails and earthworms. Brodifacoum was toxic to pasture earthworms at 500 and 1000 mg a.i./kg soil. These concentrations are higher than those likely to be found in the field following aerial application of Talon® baits; however, it is unknown what soil concentrations of brodifacoum may result from field applications in bait stations. We observed common garden snails feeding on cereal pellet baits containing brodifacoum, but no mortality was linked to exposure. Primary poisoning of native *Powelliphanta* snails by cereal brodifacoum bait is considered unlikely. The potential for secondary poisoning of these snails through consumption of invertebrates that have consumed brodifacoum should be further investigated.

Keywords: brodifacoum, toxicity, snails, earthworms, invertebrates, residues, non-target species

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1. Introduction

Brodifacoum is a potent second-generation anticoagulant vertebrate pesticide that has been successfully used in cereal baits (Talon®, PESTOFF®) in New Zealand for rodent eradications on islands, and is now used to control possums and rodents in bait station applications on the mainland. Brodifacoum is highly toxic to mammals and birds, and is persistent in mammalian tissues, particularly liver (Eason et al. 1996). A number of non-target mammal and bird species have been contaminated with brodifacoum, either directly through consuming baits, or indirectly through secondary poisoning (Eason et al. 2002). Invertebrates are also recognised as potential non-target species that may be subject to primary poisoning following application of brodifacoum baits. If they can carry residues of brodifacoum, they may also be potential sources of secondary exposure for insectivores and scavengers. The toxicity of brodifacoum and occurrence of brodifacoum residues in two invertebrate species, the common garden snail (*Cantareus aspersus*) and pasture earthworm (*Aporrectodea caliginosa*), was investigated by Landcare Research, Lincoln, for the Department of Conservation (DOC) in June 2002. This work aimed to provide baseline data on the susceptibility to brodifacoum of 'soft-bodied' invertebrates present in New Zealand, as a basis for risk assessment for secondary exposure.

2. Background

The importance of vitamin K-dependent carboxylation reactions in the mammalian blood-clotting cascade is well described, as is the toxic action of anticoagulant rodenticides, which cause fatal haemorrhage by inhibiting vitamin K metabolism in mammalian liver (e.g. Bell 1978; Thijssen 1995). Brodifacoum (a second-generation anticoagulant) is thought to lack insecticidal properties because invertebrates do not possess the same blood-clotting systems as vertebrates (Shirer 1992). However, there are few data on the toxicity of brodifacoum to invertebrates, and there are no published formal investigations of the absorption, distribution, metabolism and excretion of anticoagulants in invertebrates in the scientific literature. Vitamin K-dependent carboxylation reactions occur in mammalian tissues other than liver (Vermeer et al. 1992), and there is some evidence that these carboxylase enzyme systems are generally distributed in vertebrate and invertebrate systems (Walker et al. 2001). Therefore, physiology other than vertebrate haemostasis may also be affected by 'anticoagulants', as they bind to the enzyme vitamin K 2,3-epoxide reductase, thereby interrupting the cellular recycling of vitamin K.

Captive studies with large-headed tree wētā (*Hemideina crassidens*) and Ascension Island land crabs *(Gecarcinus lagostoma*) indicate that neither species appears highly susceptible to brodifacoum toxicity (summarised in Booth et al. 2001) in comparison with mammals. Following sublethal doses,

brodifacoum residues were not detectable after four days in wētā (Booth et al. 2001) and after one month in land crabs (Pain et al. 2000). Field residue and population monitoring studies have investigated the impact of aerial application of Talon® on a range of arthropod species found in New Zealand. These evaluations included arthropods on offshore islands following rodent eradication campaigns (Stewart Island in 1991, Coppermine Island in 1992 and 1997, Red Mercury Island in 1992, Chetwode Islands in 1993, and Lady Alice Island in 1994) and a mainland site (Pelorus Bridge in 1993) (Booth et al. 2001). Arthropods were observed feeding on cereal-based baits containing brodifacoum, and brodifacoum residues were detected in beetles (Coleoptera), cave wētā (*Gymnoplectron* sp.) and cockroaches (Blattoidea) (Ogilvie et al. 1997; Booth et al. 2001). Of insect species for which the toxicity of brodifacoum has been evaluated, few appear to be at risk from primary poisoning as a result of aerial bait application, and to date no deleterious effects on New Zealand arthropod populations have been detected at the family level (Booth et al. 2001). Similarly, non-target insects and millipedes in the Seychelles Islands consumed brodifacoum bait with no apparent adverse effects (Gerlach & Florens 2000).

There is some indication that molluscs may be susceptible to brodifacoum poisoning. Gerlach & Florens (2000) reported 100% mortality of two Seychelles Islands snail species (*Achatina fulica* and *Pachnodus silhouettanus,* a common species used as a model for the threatened *P. fregatensis*) that had consumed brodifacoum bait. Lethal amounts appeared to vary according to the size of the snail. For the 15–20 mm *P. silhouettanus,* doses of 0.01–0.2 mg/snail caused death within 72 h, and 0.04 mg of brodifacoum resulted in the death of *A. fulica* in the same time. Gerlach & Florens (2000) also suggested that brodifacoum poisoning may have contributed to observations of significantly higher numbers of recently dead *Pachystyla bicolor*, lower numbers of live adult *P. bicolor*, and shells of the critically endangered *Erepta stylodon* at Mauritian field sites subject to rodent baiting. Existing New Zealand field monitoring data indicate that molluscs can retain brodifacoum. A sample of terrestrial slugs (*Gastropoda* spp.) collected two days after aerial application of brodifacoum baits on Red Mercury Island was found to contain brodifacoum (Morgan & Wright 1996).

New Zealand has a large number of endemic terrestrial snail species, many populations of which are threatened by habitat destruction and predation by introduced vertebrates and birds (Parrish et al. 1995, Brook 2002). In a number of small sites, brodifacoum has been used for many years to reduce rat populations and thereby lower the incidence of predation on large land snails (Meads et al. 1984). However, there are concerns that the poison may also be limiting snail population size (Kath Walker, DOC Nelson. pers. comm.). Numbers of *Powelliphanta* have declined in many sites, including areas where there has been use of brodifacoum baiting for vertebrate pest control (Meads et al. 1984). *Powelliphanta* are carnivorous, feeding primarily on live earthworms, and are unlikely to eat meat-based baits (Kath Walker, DOC Nelson, pers. comm.). However, no data are available regarding the toxic effects or persistence of brodifacoum in *Powelliphanta*, or in earthworms and other invertebrates on which they feed. Accordingly it is important to ensure that the benefits of reduced numbers of pest predators are balanced against

potential impacts of pest control on *Powelliphanta*, *Paryphanta*, and other rhytidids. Given the limited definition of experimental toxicology protocols for invertebrates, the need for family-specific toxicology data in New Zealand, the high conservation status of *Powelliphanta,* and the uncertain taxonomic status of earthworm species in New Zealand, the use of model invertebrate species for initial investigations was considered appropriate.

3. Objectives

- To assess the toxicity of brodifacoum to introduced earthworms and common snails as models for native species.
- To describe the occurrence of residues in introduced earthworms and snails following exposure to brodifacoum.
- To assess the toxicity and occurrence of residues of brodifacoum in *Powelliphanta* snails and native earthworms.

4. Methods

4.1 INTRODUCED EARTHWORM EXPERIMENTS

The pasture earthworm (*Aporrectodea caliginosa*) was chosen as a model species for native New Zealand earthworms, and laboratory colonies were established from adult earthworms collected in Canterbury, New Zealand. Colonies were maintained at the Landcare Research Animal Facility, Lincoln, in Templeton silt loam (3.8% organic matter) collected from the Selwyn District, Canterbury. The soil was air-dried for 24 h to kill any extant earthworms and other macroinvertebrates, then seived and rehydrated with distilled water to produce a moisture content of 25–30% by mass. Dry grass meal (from organic lawn clippings) was added at the rate of 14 g/kg of dry soil to provide food for the earthworms. The pH of the reconstituted soil was 6.5–7. Adult earthworms were maintained in this soil in 10 L plastic buckets. The soil was changed at 28 day intervals and cocoons were removed and maintained on wet filter paper until hatching. Juvenile earthworms were then placed in 10 L plastic buckets containing the previously described soil, and reared to maturity. All earthworms were maintained at 20°C during this rearing phase.

4.1.1 Experiment 1: Exposure to contaminated soil 1

Earthworms were exposed to 500 g of brodifacoum-spiked soil in 1 L glass jars. Brodifacoum pellet baits (Talon 20P, 20 mg/kg) were ground finely and mixed into the Templeton silt loam described above at 0, 1, 5, 10, 25, 50 and 100 g ground pellets/kg soil (dry weight) (0, 0.02, 0.1, 0.2, 0.5, 1, and 2 mg a.i./kg). Jars were maintained at 20°C, constant light and 25% moisture for the duration

of the experiment. There were three replicates of five earthworms per treatment and mortality was monitored after seven and 14 days' exposure. Mortality was assessed by emptying the soil onto a plastic tray, sorting earthworms from the soil and testing their reaction to a mechanical stimulus to the anterior part of the earthworm. At the termination of exposure, worms were depurated for 24 h, and frozen for later tissue analysis, using methods described below.

4.1.2 Experiment 2: Exposure to contaminated soil 2

Brodifacoum pellets were ground up and added to soil as described above at 250 and 500 g bait/kg soil, which was equivalent to 5 and 10 mg a.i./kg, and compared with controls spiked with 0, 250 and 500 g of ground non-toxic pellet bait. Soil treatments were then divided between two jars (500 g each) and maintained until any fungal growth had stopped. During this time, the soil was tipped out weekly, thoroughly mixed to aid this process, and placed back in the treatment jar. After fungal growth was no longer observed, 14 g/kg grassmeal was added to the soil. Five earthworms were weighed and randomly allocated to each jar and their weight was assessed weekly up to 28 days. Growth was expressed as the mean percent change in weight over the exposure period for each treatment (n = 4). Mortality was assessed as described above, and after each assessment earthworms were placed back into the test container with the contaminated soil for a further seven days. Jars were maintained at 20°C, constant light, and a moisture content of 25% throughout the experiment. At the termination of exposure, worms were depurated for 24 h, and frozen for later tissue analysis, using methods described below.

4.1.3 Experiment 3: Exposure to contaminated soil 3

Brodifacoum powder (Sulkem Co. Inc., 2%) was mixed with talcum powder to a concentration of 2.5% a.i.. This mixture was added to soil at 4, 20 and 40 g/kg (100, 500, and 1000 mg a.i./kg) and compared with a control containing 40 g of talcum powder and a control containing no talcum powder. There were four replicates (500 g soil in a 1 L jar) for each treatment and five earthworms per replicate. Earthworms were weighed prior to addition to the soil, and mortality and weight were assessed weekly up to 28 days. Jars were maintained at 20°C, constant light, and a moisture content of 25% throughout the experiment. At the termination of exposure, earthworms were weighed, depurated for 24 h and frozen for later tissue analysis, using methods described below.

4.2 INTRODUCED SNAIL EXPERIMENTS

As a model species for native New Zealand snails, common garden snails were maintained in groups in sealed 2-L plastic containers with small air holes in the lid, and fed rabbit food pellets (Western Milling, Rangiora) and fresh vegetable leaves. Leaves and three to five rabbit pellets were removed and replaced with fresh food twice weekly. Water was freely available in a shallow plastic container. Snails were obtained from an organic garden in Auckland and acclimatised to laboratory conditions for at least 7 days prior to experimentation.

4.2.1 Experiment 1: Exposure to contaminated soil

Soil was prepared as in 4.1.3 above at 0, 0.02, 0.1, 0.2, 0.5, 1, and 2 mg a.i./kg, and 250 g of prepared soil was placed into two replicate 1 L plastic containers for each treatment. Two snails were added to each container and exposed to the contaminated soil for 14 days, with water and food freely available. Mortality was assessed after 7 and 14 days, and at termination of exposure snails were frozen for analysis of brodifacoum in tissues.

4.2.2 Experiment 2: Exposure to contaminated soil and toxic baits

Soil was prepared as in 4.1.3 above at 0, 100, 500, and 1000 mg ai/kg, and 250 g of soil placed into six replicate 1 L plastic containers for each treatment. Three replicates, each comprising two snails of approximately 2 g each, were offered brodifacoum pellets (Talon 20P, 20 mg/kg) and three replicates were offered non-toxic pellets (RS5). A total of 5 g of bait per snail was offered and baits were removed and replaced with fresh ones every second day. Two snails of similar weight (within 2 g) were added to each container and mortality was monitored every second day. Soil was analysed for brodifacoum content at the commencement of exposure.

4.3 ANALYSIS OF WORM AND SNAIL TISSUE FOR BRODIFACOUM RESIDUES

Earthworms were depurated prior to analysis. Shells were removed from snails, which were then dissected into body (including gut) and foot (muscle) tissue for separate analysis. All worm and snail tissues were analysed for brodifacoum concentrations at the IANZ-accredited Toxicology Laboratory, Landcare Research, Lincoln, using Toxicology Laboratory Method TLM012 Determination of brodifacoum in insect tissue and soil by HPLC, based on the methods of Hunter (1983). Tissue was finely chopped and weighed. Anhydrous sodium was added followed by a chloroform/acetone/acetic acid extraction solvent. The mixture was homogenised and separated by centrifuging. After repeat extraction, the combined extract was evaporated to dryness on a savant evaporator and then taken up in mobile phase for HPLC analysis. The least detectable limit (LDL) of this analysis was 0.06 mg/g.

4.4 NATIVE SNAIL AND EARTHWORM EXPERIMENTS

Once the experiments with introduced worms and snails were completed, the results were analysed in order to determine appropriate methods and levels of experimental exposure of native worms and snails to brodifacoum. The identification, sourcing and husbandry of native species were investigated as they were expected to be different from those required for housing and maintaining the introduced species.

5. Results

5.1 INTRODUCED EARTHWORM EXPERIMENTS

In experiment 1, no mortality was observed for any treatment. Traces of brodifacoum were found in earthworms exposed to the four highest concentrations (Table 1), indicating that earthworms do consume bait fragments that are mixed in soil, and retain brodifacoum within body tissues. Under these experimental conditions, use of ground cereal (RS5) baits produced excessive fungal growth in the soil in both the treated and control groups. The presence of the fungus may have interfered with earthworm health (as evidenced by their appearance). To circumvent confounding effects of fungal growth on earthworm health, in experiment 2 the ground bait mixtures were left in the soil until the fungal growth had subsided before adding the earthworms.

TABLE 1. MEAN BRODIFACOUM RESIDUES IN EARTHWORMS (*Apporectodea caligniosa*) EXPOSED FOR 2 WEEKS TO SOIL CONTAINING GROUND TALON 20P BAIT. LEAST DETECTABLE LEVEL (LDL) WAS 0.06 mg/g.

GROUND BAIT IN SOIL (g/kg dry wt)	BRODIFACOUM IN SOIL (mg/kg dry wt)	BRODIFACOUM RESIDUES IN EARTHWORM (µg/g wet wt)	
		Replicate 1	Replicate 2
0	0	<LDL	<LDL
1	0.02	<LDL	<LDL
5	0.1	<LDL	<LDL
10	0.2	<LDL	0.17
25	0.5	<LDL	0.07
50	1	0.90	0.07
100	2	0.20	0.12

In experiment 2, all earthworms exposed to soil containing finely ground bait (both toxic and non-toxic) (equivalent to 0, 5 and 10 mg a.i./kg) died within 7 days, but all earthworms in the control containing no ground bait survived. Soil residues were not analysed in experiment 2, and residue analysis was not carried out on worms that died in this experiment. All mortality occurred in the first week of treatment, and no bodyweight data were taken from worms found dead. In spite of the absence of fungal growth at the time of addition of the earthworms, it is possible that fungal toxins may have remained in the soil after the growth of fungus appeared to have stopped, especially as none of the earthworms in the control (no bait) treatment died.

In experiment 3, brodifacoum mixed with talcum powder and added to the soil was toxic to worms at 500 mg/kg (85% mortality) and 1000 mg/kg (100% mortality). A one-way ANOVA showed that growth was also significantly affected by exposure to all treatments, including the talcum powder control ($P < 0.05$ in all cases). Earthworms exposed to talcum powder for 28 days

sustained a slight weight loss (mean 10 ± 8.78% (SE)) compared with controls (no talcum powder), which showed a 29.2 ± 4.65% (SE) increase in weight. Earthworms exposed to 100 mg brodifacoum/kg soil experienced no real change in weight (0.75 ± 2.19% (SE)). Surviving earthworms exposed to 500 mg brodifacoum/kg soil showed 71.5 ± 15.18% (SE) loss of body mass. Soil residues were not analysed in experiment 3, and residue analysis was not carried out on earthworms that died in this experiment.

5.2 INTRODUCED SNAIL EXPERIMENTS

In experiments 1 and 2, no mortality was observed in any of the snails exposed to brodifacoum in soil or offered brodifacoum pellet baits. Snails were observed on baits and brodifacoum residues were found in both the body (3.9 µg/g) and foot tissue (1.2 µg/g) of snails exposed to soil containing ground bait at 2 mg a.i./kg soil.

6. Discussion

The potential toxicity of brodifacoum to native invertebrates was investigated using two surrogate species, the pasture earthworm and the garden snail. Earthworms were found to be adversely affected by soil amended with brodifacoum at 500 and 1000 mg a.i./kg. These concentrations are equivalent to the distribution of brodifacoum from 25 or 50 kg Talon® cereal pellets (containing 20 mg a.i./kg bait) into 1 kg of soil. In the field, this scenario is unlikely to occur during the aerial application of brodifacoum baits, because of the application rates used (generally 3–15 kg bait/ha), and because of the low potential for brodifacoum, which is not water-soluble, to leach through soil. Higher 'application' rates of brodifacoum baits to soil could potentially occur in the field where spillage occurs from bait stations that are regularly refilled, or if rodents remove the pellets from bait stations and cache them at specific locations, although it is unknown what concentrations of brodifacoum would occur in soil in these instances. Earthworm populations and concentrations of brodifacoum in soil and earthworms found beneath bait stations could be monitored to investigate whether primary poisoning of earthworms might occur and to what extent earthworms are likely to be contaminated with brodifacoum residues. Contaminated earthworms are a potential source of secondary exposure of non-target birds and invertebrates to brodifacoum, and theoretical assessments of acute toxicity risk can be made. For instance, the acute oral LD_{50} of brodifacoum to pūkeko (*Porphyrio porphyrio*) has been reported as 1.0 mg/kg (Godfrey 1985). Using the highest residues reported here in earthworms (0.90 µg/g), and assuming a bodyweight of 850 g for a pūkeko, the bird would have to consume approximately 940 g of contaminated earthworms to ingest a lethal dose. While this scenario makes a number of assumptions, the risks of sublethal secondary exposure to brodifacoum and accumulation of brodifacoum in higher animals through repeated sublethal exposure warrant further investigation.

The presence of brodifacoum residues in snails exposed to brodifacoum indicates that common snails will eat brodifacoum pellet bait, and can retain residues of brodifacoum. Therefore, as for earthworms, contaminated snails may be a potential source of secondary poisoning for non-target birds. Using the above calculation, and the highest residues reported here in snails (3.9 mg/g), an 850 g pūkeko would have to consume approximately 220 g of contaminated snails to ingest a lethal dose. Given the very slow elimination rates for brodifacoum from the liver of exposed animals, it is therefore theoretically possible that a pūkeko could accumulate brodifacoum to a level that could cause toxicity, and this should be further investigated.

The identification and acquisition of sufficient native earthworms requires further resources, as these species are not abundant in natural populations, are not well-described taxonomically, and have no previously established protocols for captive husbandry and breeding for laboratory trials. Future evaluations in native earthworm species might include subsamples of mixed earthworm species collected from the field, and mixed earthworm communities including native species that could be used to determine the toxicity of brodifacoum. All of these points need to be addressed before a meaningful assessment of the toxicity and residues of brodifacoum could be made for native earthworms. The above results with the common pasture earthworm suggest that aerial application of brodifacoum is unlikely to result in soil concentrations that will pose a significant risk of primary poisoning to native earthworm species. The risk of exposure to earthworms that inhabit lower horizons of soil (rather than topsoil) may be further reduced, as brodifacoum has been shown to be relatively immobile in soil (Booth et al. 1999). However, secondary poisoning of *Powelliphanta* by consumption of surface-dwelling earthworms (or other invertebrates) cannot be ruled out.

Given the above result where common snails do not appear to be affected by exposure to very high concentrations of brodifacoum in soil, and the importance of earthworms as a food source for *Powelliphanta*, primary exposure to brodifacoum via soil or cereal bait is considered much less likely than secondary exposure by consuming contaminated earthworms. Accordingly, it remains important to evaluate both the toxicity and residual characteristics of brodifacoum in native earthworms and snails as a basis for estimating risks to native invertebrates from the use of this vertebrate pesticide.

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