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Accidental Discharge of Brodifacoum Baits in a Tidal Marine Environment: A Case Study

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Brodifacoum is a second-generation anticoagulant used worldwide to control commensal rodents (Colvin et al. 1988; Shore et al. 1999). Brodifacoum has also been successfully used to eradicate rats (e.g. *Rattus exulans*, *R. norvegicus*) from offshore islands (e.g. Taylor and Thomas 1989; Empson and Miskelly 1999). Brodifacoum is highly toxic to mammals and birds (Godfrey 1985; Erickson and Urban 2004) and has a relatively long retention time in mammalian liver (Laas et al. 1985; Eason et al. 1996). Concerns exist regarding the secondary effects of brodifacoum residues on terrestrial non-target wildlife in New Zealand (Eason et al. 2002) and elsewhere (Stone et al. 2003).

As the result of a road transport accident on 23 May 2001, approximately 20 tonnes of brodifacoum bait (Pestoff® Rodent Bait) was discharged into the environment. This occurred on the east coast of the South Island of New Zealand, approximately 11 km southwest of Kaikoura (S 42.444° latitude, E 173.586° longitude). The area includes wave exposed rock reefs and headlands between steep gravel beaches and the is popular for whale-watching, fishing, diving and collection of marine invertebrates for human consumption, including koura (spiny rock lobster, *Jasus* spp.), kina (sea urchin, *Evechinus chloroticus*) and paua (abalone, *Haliotis iris*). The cereal pellet bait spilled was packaged in multiwalled paper bags with polyethylene liner, 25 kg per bag, 40 bags per pallet. The bait was formulated with a water-soluble green dye and 20 ppm brodifacoum as the active ingredient. Not all of the bait material entered the ocean, and it was estimated that the tidal environment was exposed to a maximum of 360 g of brodifacoum (from 18 tonnes of bait) as a point source, which was an unprecedented incident. Immediate monitoring was considered especially important given the use of the area for human food collection, and the relative lack of information regarding the toxicity and residual persistence of brodifacoum in marine species, including invertebrates. The local agency Environment Canterbury and later, Community and Public Health (Ministry of Health, New Zealand) enacted monitoring aimed at determining the impact of the spill on the proximal environment. Samples were taken immediately after the spill and then monthly for four months, then at three and six month intervals for the following 21 months.

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MATERIALS AND METHODS

Samples were analysed for brodifacoum residues by the Landcare Research toxicology laboratory, New Zealand. Water and sediment samples were collected by divers from locations at the spill site and up to 400 m north and south of the spill site. Water samples were collected on each of the two days following the spill, at 10 and 11 d, and at 1 and 1.5 mon afterwards. Water samples were collected in 1-L glass bottles and stored at 5°C until analysis. A C-18 solid- phase disk procedure was used to concentrate brodifacoum from 500mL of sample and eluted with aliquots of acetone. The solvent was evaporated and the residue reconstituted with 1.0 mL of methanol/acetic acid/water mobile phase and analysed by high performance liquid chromatography (HPLC). The method recovery was 100 ± 10% determined versus the internal (surrogate) standard (difenacoum) and the method detection limit (MDL) was 0.02 ppb. Sediment samples were collected at 2 d, 9 d and 14 d after the spill.

Aquatic biota sampled included marine invertebrates, (paua, koura, kina, limpets (*Cellana ornata*), mussels (*Mytilus edulis, Perna canaliculus*) and starfish (*Coscinaterias muricata*)), and fish, including herring (*Sprattus* sp.), butterfish (*Odax pullus*) and scorpion fish (*Scorpaena papillosus*). Of these, only paua, limpets, and mussels were collected over a 9-mon period. Limpets were initially collected as examples of molluscan grazers, in the absence of paua at the spill site. As paua recolonized the reef and were able to be sampled regularly, sampling of limpets was discontinued. Tissue samples from any dead animals observed in the area were also analysed. All biological specimens were collected in resealable plastic bags or containers and stored at –20°C. Depending on the species, liver, muscle and/or gut tissues were dissected from whole organisms and analysed using methods based on those of Hunter (1983). This method used a solvent mixture combined with a mechanical homogenizer to extract brodifacoum from the tissue sample followed by a gel permeation separation to clean up the sample extract. The solvent was evaporated and the residue reconstituted with 0.50 mL of mobile phase and analysed by HPLC. Brodifacoum concentrations were determined in all sample extracts by high performance liquid chromatography using a C-18 column with a reversed-phase mobile phase and detected by fluorescence. Homogenised fresh greenlip mussel tissue was used for internal quality control samples, analysed alongside samples in each batch. For the first three-quarters of the tissue samples analysed, the method recovery (corrected versus the difenacoum internal standard) was 75 ± 19% and the MDL was 0.020 ppm. The maximum acceptable concentration of brodifacoum in food for human consumption in New Zealand is 0.001 ppm (New Zealand Maximum Residue Limit (MRL) of the Agricultural Compounds Mandatory Food Standard 1999 as amended June 2001). At the time of the spill, the MLD for available analysis of brodifacoum in tissue was above the MRL, so the method was improved and revalidated to a MLD of 0.001 ppm with 75% recovery.

RESULTS AND DISCUSSION

Bait entering the water quickly began to soften and disintegrate, consistent with observations made by Empson and Miskelly (1999). An area of surrounding water, approximately 100 m wide by 300–700 m long, turned a cloudy green color due to the release of dye and particulates from the baits. The plume persisted for approximately 24 hr. The seawater contained visible quantities of suspended or congealed cereal grain material, which accumulated on the adjacent gravel beach. Congealed or partially disintegrated bait also accumulated in rock crevices and on sheltered areas of the seabed. Sampling of one area of the seabed illustrated that particles of bait had set into a layer >100 mm thick, with the consistency of thick porridge. This 'sediment' had a well-leached surface but deeper layers retained the colour and consistency of fresh-moistened baits. A sample of these layers taken 36 hr after the spill contained 7.6 ppm brodifacoum, compared with 19.3 ppm in a bait sample collected from the road. Measured concentrations of brodifacoum were not corrected for water retained by the sample, so dry weight concentrations could have been much higher. It took at least 1 wk for these congealed areas to be diluted and dissipated by wave action, so that kibbled grain material was no longer visible.

Measurable concentrations were detected in water at the immediate spill location within 36 hr of the spill, but between 36 hr and day 9 the concentrations were below MLD (<0.020 ppb). Brodifacoum has an estimated very low water solubility of <10 ppm at pH 7 (US EPA 1998). Given the non-polarity of brodifacoum molecules, and the ionic strength of seawater at 12–14°C (the average temperature of the ocean at the time of the spill) the solubility was probably in the low ppb range. A significant portion of the brodifacoum is likely to have remained as particulate matter adsorbed to bait particles or other organic material that accumulated on the seabed and among the rocks. One of seven sediment samples collected 1 d after the spill was positive for brodifacoum (0.060 ppm). Other sediment samples collected for 9 d after the spill were below the MLD (<0.020 ppm), as were two seaweed/kelp samples collected 64 and 91 d after the spill. A single starfish collected at day 16 had residues <0.020 ppm, as did the 13 crayfish and one crab sampled between 8 and 14 d after the spill at the point source. A butterfish sampled 9 d after the spill had residues of 0.040 ppm in liver and 0.020 ppm in gut, although muscle tissue was below MLD (0.020 ppm). Residues in other fish samples, a scorpion fish, two herrings, and an unknown species of fish collected between day 14 and 16, were all <0.020 ppm. Two seals (*Arctocephalus forsteri*), two black backed gulls (*Larus dominicanus*) and a cormorant (*Phalacorcorax* spp.) were found dead in the area following the spill. Necropsies found no sign of anticoagulant toxicity, and no tissue samples from carcasses contained detectable brodifacoum. The LC_{50} for bluegill sunfish (*Lepomis macrochirus*) exposed to brodifacoum in flow-through water for 96 hr was 0.025 ppm (US EPA 1998), indicating high toxicity. Marine fish species were observed eating non-toxic pellet baits dropped into the sea, and some mortality was reported in an aquarium trial where marine fish were exposed to relatively high concentrations of brodifacoum bait in water (Empson and Miskelly 1999).

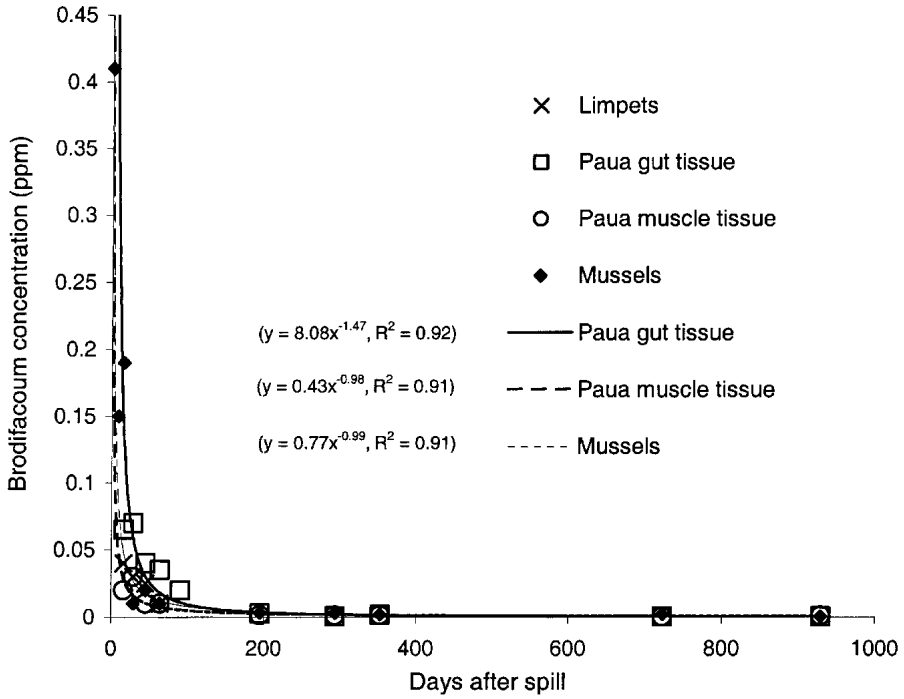

Figure 1. Average brodifacoum concentration in tidal marine invertebrates collected within 100 m of the spill location. Power equations with the correlation coefficient are included for mussel and paua tissues

Initial high environmental brodifacoum concentrations in the immediate locality were probably sufficient to cause mortality of some invertebrates and fish. No dead fish were found, however mortality would have been extremely difficult to measure in these mobile animals.

Brodifacoum concentrations in mussels peaked at 0.41 ppm 1 d after the spill, and averaged just above detectable concentrations by day 29. However, the average in five mussel samples collected at 353 d was still over (0.002 ppb) the MRL. Concentrations in mussels were initially higher than in other invertebrates, but decreased more rapidly over time. Mean concentrations in paua gut and muscle tissue were highest on day 29 and at day 191 there was a mean of 0.003 ppm brodifacoum for gut and of 0.0015 ppm for muscle tissue (Figure 2). At day 353, the next-to-last samples taken, there was a mean of 0.0017 ppm for paua gut and 0.0014 ppm for muscle. Detectable residues in limpet tissue persisted for approximately 80 d. The greatest exposure of marine invertebrates to brodifacoum occurred within 100 m of the spill location. Only minor exposure was observed in the 100–300 m range from the spill location, for one (a paua) of nine invertebrates sampled for up to 29 d, but none after this. Potential explanations for the pattern of residue concentrations found in the soft-bodied molluscs were 1) a relatively long half-life of brodifacoum in these animals (possibly in tissues with Vitamin K

dependent metabolism) and 2) re-exposure to brodifacoum through low concentrations remaining in sediment. While the persistence of brodifacoum residues (half-life) in mussels remains unknown, it was thought to be at least partially due to the continued exposure of mussels to brodifacoum through filter feeding.

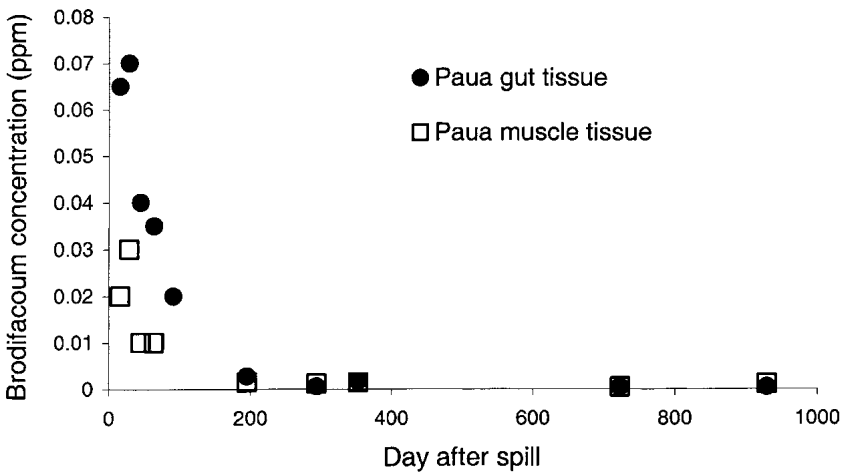

Figure 2. Concentration of brodifacoum in paua gut versus muscle tissue.

Initial high exposure of mussels to bait fragments suspended in the water would have decreased as the bait particles were dispersed from the immediate spill site over the first week. After this, mussels may have had low-level exposure to brodifacoum from sediments that were being constantly dispersed in the tidal marine environment. Fragments of bait settling in rock crevices may have presented a more prolonged exposure risk to grazing invertebrates such as paua and limpets. The decline rate of residues was used to guide decisions on the duration of a ban on collection of shellfish for human consumption in the immediate area. In general, paua 'foot' (muscle) tissue is preferred for human consumption, and the soft body of mussels are eaten whole. Figure 1 shows equations derived from the residue data, from which the time to clearance of brodifacoum to acceptable levels was extrapolated. Clearance of brodifacoum from paua and mussels to MRL concentrations, as specified under the current New Zealand food standards (0.001 ppm) has taken approximately 471 d for paua and is estimated to take approximately 796 d for mussels. From samples collected in December 2003, two mussel samples and one paua muscle sample were below 0.001 ppm, while one sample of paua muscle tissue showed a mean concentration of 0.0016 ppm. Public health warnings issued by Community and Public Health against shellfish collection at the site were lifted by the New Zealand Food Safety Authority in May 2004.

The retention of brodifacoum by invertebrates is not well described. Following sublethal exposures, brodifacoum residues were not detectable after 4 d in the terrestrial large-headed tree wēta (*Hemideina crassidens*) (Booth et al. 2001), nor

after 1 mon in Ascension Island land crabs (*Gecarcinus lagostoma*) (Pain et al. 2000). Terrestrial slugs (*Gastropoda* spp.) collected 2 d after aerial application of brodifacoum baits on Red Mercury Island, New Zealand, were found to contain brodifacoum residues (Morgan et al. 1996). Brodifacoum residues were found in gut (3.9 ppm) and foot tissue (1.2 ppm) of common garden snails (*Helix aspersa*) 14 d after they were exposed to soil containing 2 mg brodifacoum/kg (Booth et al. 2003). Brodifacoum is highly toxic to aquatic organisms on the basis of an EC_{50} of 0.98 ppm after 48 h of exposure in static water for the freshwater invertebrate *Daphnia magna* (US EPA 1998), although there are no data regarding the acute toxicity of brodifacoum to marine invertebrates. As summarised by Booth et al. (2001), neither terrestrial weta nor land crabs appeared highly susceptible to brodifacoum. Brodifacoum in soil was toxic to earthworms (*Apporectodea caliginosa*) at the relatively high concentration of 500 ppm, and common garden snails were observed to feed on cereal pellet brodifacoum baits over 2 wk without mortality (Booth et al. 2003). However, Gerlach and Florens (2000) reported mortality in Seychelles Islands snails that consumed brodifacoum baits. On the basis of this limited information, soft-bodied aquatic invertebrates may be more susceptible than insects or crustaceans to brodifacoum. Observations after the spill suggested that paua were initially absent from the intertidal areas of the reef at the point source, and appeared to repopulate slowly over the following year.

Contamination of the marine environment by the spill of a large quantity of brodifacoum baits at one point was localised to an approximately 100-m^2 area. Residues in water and sediment declined to below detectable concentrations within 3 d and 9 d respectively. Residues in shellfish, including edible mussels and paua, took up to 31 mon to decline to concentrations below the MLD and therefore to acceptable levels for human consumption. This persistence of brodifacoum was thought to be due to a combination of a prolonged half-life in these invertebrates and re-exposure of the invertebrates to brodifacoum in the highly wave exposed and dynamic tidal marine environment.

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REFERENCES

Booth LH, Fisher P, Hepplethwaite V, Eason CT (2003) Toxicity and residues of brodifacoum in snails and earthworms. DOC Science Internal Series 143, Dept of Conservation, Wellington, New Zealand, p 14.

Booth LH, Eason CT, Spurr EB (2001) Literature review of the acute toxicity and persistence of brodifacoum to invertebrates and studies of residue risks to wildlife and people. Science for Conservation 177, Dept of Conservation, Wellington, New Zealand, p 23.

Colvin BA, Hegdal PL, Jackson WB (1988) Review of non-target hazards associated with rodenticide use in the USA. Bulletin OEPP/EPPO Bull 18:301–308.

Eason CT, Wright GR, Meikle L, Elder P (1996) The persistence and secondary poisoning risks of sodium monofluoroacetate (1080), brodifacoum, and cholecalciferol in possums. In: Timm RM, Crabb AC (Eds), Proceedings of the 17th Vertebrate Pest Conf, March 5-7 1996, Rohnert Park, California., University of California, Davis. pp 54–58.

Eason CT, Murphy EC, Wright GRG, Spurr EB (2002) Assessment of risks of brodifacoum to non-target birds and mammals in New Zealand. Ecotoxicology 11:35–48.

Empson RA, Miskelly CM (1999) The risks, costs and benefits of using brodifacoum to eradicate rats from Kapiti Island, New Zealand. New Zealand J Ecol 23:241–254.

Erickson W, Urban D (2004) Potential risks of nine rodenticides to birds and nontarget mammals: a comparative approach. Office of Pesticides Programs Environmental Effects and Fate Division, United States Environmental Protection Agency. Washington DC 20460, USA. p 230.

Gerlach J, Florens V (2000) Considering mollsucs in rodent eradication projects. Tentacle – The Newsletter of the IUCN/SSC Mollusc Specialist Group 9:7–8.

Godfrey MER (1985) Non-target and secondary poisoning hazards of 'second generation' anticoagulants. Acta Zool Fenn 173:209–212.

Hunter K (1983) Determination of coumarin anticoagulant rodenticide residues in animal tissue by high-performance liquid chromatography: I. Fluorescence detection using post-column techniques. J Chromatog 270:267–276.

Laas FY, Forss DA, Godfrey MER (1985) Retention of brodifacoum in sheep and excretion in faeces. New Zealand J Agric Res 28:357–359.

Morgan DR, Wright GR, Ogilvie SC, Pierce R, Thomson P (1996) Assessment of the environmental impact of brodifacoum during rodent eradication operations in New Zealand. In: Timm RM, Crabb AC (eds) Proc 17th Vertebrate Pest Conf:213–218.

Pain DJ, Brooke M, Finnie J, Jackson A (2000) Effects of brodifacoum on the land crab on Ascension Island. J Wildl Manage 64:380–387.

Shore RF, Birks JDS, Freestone P (1999) Exposure of non-target vertebrates to second-generation rodenticides in Britain, with special reference to the polecat *Mustela putorius*. New Zealand J Ecol 23:199–206.

Stone WB, Okoniewski JC, Stedelin JR (2003) Anticoagulant rodenticides and raptors: Recent findings from New York, 1998–2001. Bull Environ Contamin Toxicol 70:34–40.

Taylor RH, Thomas BW (1989) Eradication of Norway rats (*Rattus norvegicus*) from Hawera Island, Fiordland, using brodifacoum. New Zealand J Ecol 12:23–32.

United States Environmental Protection Agency (US EPA) (1998) Reregistration eligibility decision (RED) rodenticide cluster. Prevention, Pesticides and Toxic Substances (7508W). EPA738-R-98-007.