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Anticoagulant effects and the persistence of brodifacoum in possums (*Trichosurus vulpecula*)

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Abstract Brodifacoum was administered to possums at a sub-lethal dose of 0.1 mg/kg to assess its persistence in blood, muscle, and liver. Only 1 of 68 possums died at this dose level. However, significant increases in one-stage prothrombin (OSP) and activated partial prothrombin times (APP) confirmed that the possum is susceptible to the anticoagulant effects of brodifacoum. Trace amounts of brodifacoum were detected in plasma for 35 days. Substantial concentrations of brodifacoum were retained in the liver for 8 months. Much lower concentrations were also retained in muscle tissue. The persistence of brodifacoum raises concerns about the possible transfer of this compound through the food chain to humans, dogs, or wildlife.

Keywords brodifacoum; possums; residues; toxicity

INTRODUCTION

Brodifacoum formulated into a cereal bait (Talon® containing 20 ppm brodifacoum) and distributed from bait stations is becoming increasingly used for killing possums (*Trichosurus vulpecula*). This toxicant (3-[3-(4'-bromobiphenyl-4-yl)-1,2,3,4-tetrahydro-1-napthyl]-4-hydroxycoumarin) is an anticoagulant, and therefore differs markedly in its mode of action to that of the acute toxins such as sodium monofluoroacetate (1080) or cyanide. Possums may take 3 or more weeks to die after eating a lethal dose (Eason et al. 1994a). Its delayed action obviates the need for pre-feeding with non-toxic bait. Although the manufacturers indicate that the LD_{50} is 0.17 mg/kg and that 60 g of bait will kill one possum, we suspect that the LD_{50} may be higher (Eason et al. 1994a). Field trials indicate that some possums will eat more than 1 kg of bait before dying, which can make the use of brodifacoum expensive (Henderson et al. 1994). Nevertheless, this toxicant can be cost-effective when used after 1080 and cyanide, or trapping, to "mop up" low-density possum numbers, as recommended by the manufacturers, ICI. Its advantages are lower toxicity to dogs than that of 1080 and the existence of an effective antidote.

A more significant disadvantage of brodifacoum is the persistence of residues in the liver. In contrast, 1080 is eliminated from the blood and tissues of sheep within a week (Eason et al. 1994b). Brodifacoum has been shown to persist in the liver of sheep for over 16 weeks (Laas et al. 1985). As a result, brodifacoum baits must be kept clear of livestock, and this is standard practice (Eason et al. 1994a). However, little consideration has been given to the potential dangers arising from brodifacoum residues in possum carcasses. Metabolism and excretion of foreign compounds such as toxins vary in different species, and the possum shows some idiosyncrasies in its responses (Eason & Jolly 1993; Jolly et al. 1994; Eason et al. 1994c). In earlier experiments with possums using one-stage prothrombin (OSP) and activated partial prothrombin (APP) times as indicators of susceptibility, we demonstrated that the possum was relatively tolerant to pindone, a first-generation anticoagulant (Eason & Jolly 1993). In this study we used these parameters to determine whether the lack of susceptibility of the possum to pindone extends to the second-generation anticoagulant brodifacoum. We set out to quantify the persistence of brodifacoum in possums subjected to a single sub-lethal dose. In this paper we report data that

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should be considered by wildlife managers and farmers when they evaluate the advantages versus disadvantages of using brodifacoum for possum control.

MATERIALS AND METHODS

Dosing and sampling procedures

A solution of brodifacoum (2.5%) was obtained from ICI, Richmond, New Zealand. Thirty-six possums weighing 2–4 kg were dosed by gastric cannula with brodifacoum at 0.1 mg/kg (2 ml/kg of 0.05 mg/ml). This would be equivalent to a possum eating 10–20 g of Talon cereal bait containing 20 ppm brodifacoum. We estimate that this dose is approximately one-quarter to one-half the LD_{50} of brodifacoum in possums. The animals were allowed free access to food and water before and after dosing.

A series of blood samples was taken from the jugular veins of three males and three female possums before dosing and at 4, 8, 24, and 48 h, and 7, 14, 21, 28, and 35 days after dosing. OSP and APP times were measured on platelet-poor plasma using a COBAS fibro analyser (Hoftman-La Roche Co., Switzerland). Rabbit brain thromboplastin with added calcium ions (Ortho Diagnostic Systems) was used for determining OSP times. Activated thrombotax-optimised reagent containing bovine-brain phospholipids and ellagic acid as contact activators was used for determining the APP times.

A further 32 possums were divided into groups of four animals (two male and two females). One group was killed before dosing and the other seven at 2, 7, 14, 35, 64, 126, or 256 days after dosing with brodifacoum (0.1 mg/kg). Blood samples were taken under anaesthesia and muscle tissue and liver samples were collected at post-mortem. The blood samples for brodifacoum residue analyses were centrifuged, and plasma and tissue were stored at –20°C for later analysis by high-performance liquid chromatography with fluorescence detection using published methods for determining brodifacoum in blood (Felice & Murphy 1989) and animal tissues (Hunter 1983).

This investigation was carried out with the approval of the Landcare Research Animal Ethics Committee.

RESULTS

Before dosing, mean OSP and APP times were similar at 33.4 and 33.2 s, respectively. As no sex differences could be determined, the results for males and females were pooled. Seven days after dosing, the OSP and APP times had increased significantly, and APP times remained elevated for 21 days (Table 1). In one possum, OSP and APP times reached peaks of >180 and >300 s, respectively, 21 days after dosing. This animal died before the 28-day sample could be taken. Post-mortem examination revealed extensive haemorrhaging in the abdomen, thorax, and under the skin. No significant changes occurred in haematocrits of the survivors.

Brodifacoum residues were substantially eliminated from the blood of all the possums by 35 days (Fig. 1). The results from males and females were combined because there was no detectable

Table 1 Mean haematocrit, one-stage prothrombin (OSP) time, and activated partial prothrombin APP time in possums ($n = 6$) dosed with brodifacoum (0.1 mg/kg).

Time in days after dosing	Haematocrit (± SE)	OSP times (± SE)	APP times (± SE)
0	0.46 (0.01)	34.4 (3.0)	33.2 (3.3)
1	0.37 (0.01)	40.9 (3.2)	37.5 (1.8)
2	0.36 (0.01)	40.2 (4.0)	42.4*** (3.9)
7	0.39 (0.01)	57.0** (2.5)	86.2*** (4.6)
14	0.41 (0.02)	33.4 (2.8)	50.3** (6.3)
21	0.47 (0.05)	35.8 (1.8)	43.0** (4.2)
28	0.46 (0.01)	36.3 (3.3)	44.2 (7.7)
35	0.46 (0.03)	39.2 (3.2)	45.2 (5.5)

Values are means ± SE with significant changes indicated as ** $P < 0.01$; *** $P < 0.001$.

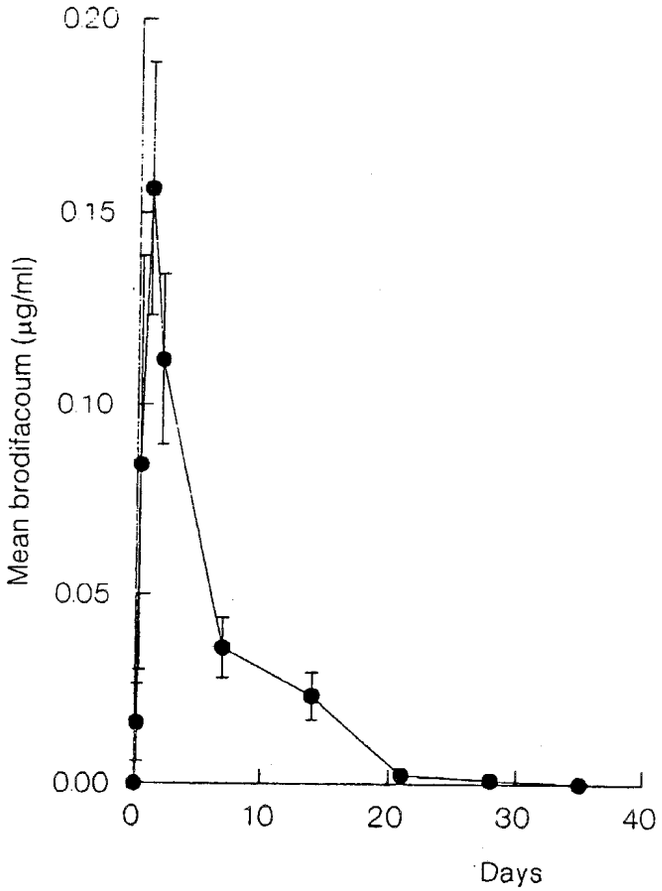

Fig. 1 Mean plasma brodifacoum concentrations in possums (n = 6) after oral administration of 0.1 mg brodifacoum/kg. Vertical bars represent SE.

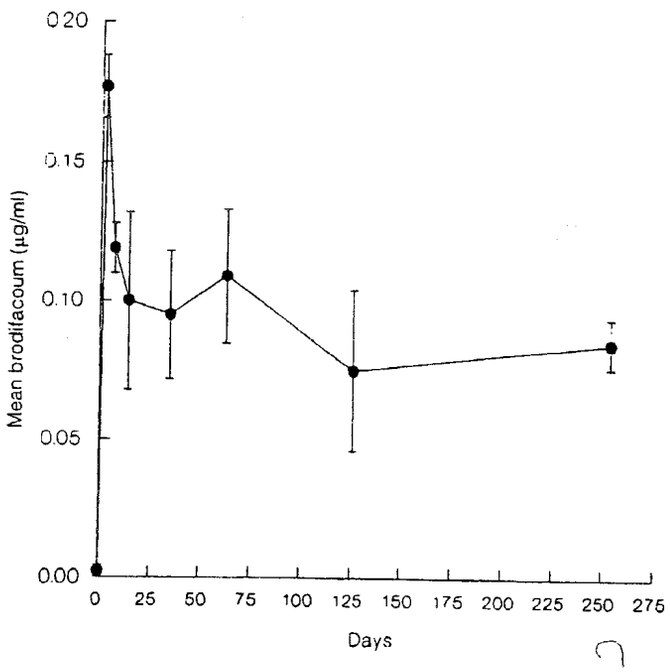

Fig. 2 Mean liver brodifacoum concentrations in possums (n = 4) after oral administration of 0.1 mg brodifacoum/kg. Vertical bars represent SE.

sex difference. The mean peak plasma concentration (C_{max}) was 0.167 µg/ml (SE ± 0.031) and the time taken to reach C_{max} (T_{max}) was 29.3 h (SE ± 6.4).

Persistence in the liver differed markedly from persistence in the blood. High mean concentrations were found in the liver 254 days after dosing in all animals. Lower concentrations of brodifacoum were also found in muscle tissue for 254 days (Table 2; Fig. 2). All 32 animals in this part of the experiment survived up to the time they were killed for sample collection.

Table 2 Mean brodifacoum concentrations (± SE) in possum meat, liver, and plasma after oral administration of 0.1 mg/kg to each group (n = 4).

Time in days after dosing	Concentration (µg/g or µg/ml)		
	Liver	Meat	Plasma
2	0.177 (0.011)	0.016 (0.001)	0.118 (0.021)
7	0.119 (0.009)	0.011 (0.007)	0.034 (0.009)
14	0.100 (0.032)	0.011 (0.000)	0.004 (0.003)
35	0.095 (0.023)	0.010 (0.002)	0.002 (0.002)
63	0.109 (0.024)	0.008 (0.001)	0.000 (0.00)
126	0.075 (0.029)	0.009 (0.002)	0.000 (0.00)
254	0.085 (0.009)	0.007 (0.002)	0.000 (0.00)

DISCUSSION

Our study confirmed that possums are susceptible to the anticoagulant effects of brodifacoum by showing significant increases in OSP and APP times after a single dose of brodifacoum at 0.1 mg/kg. However, there was no significant change in the mean haematocrit, indicating that haemorrhaging, if it occurred at this dose, was minor in most possums. In contrast, the first-generation anticoagulant pindone did not alter OSP and APP times at a dose of 25 mg/kg. A dose of 100 mg/kg of pindone was needed before there was a significant increase in APP times, but even at this high dose OSP times were unchanged (Eason & Jolly 1993), indicating that possums are not particularly susceptible to pindone.

The reasons for the normalisation of clotting parameters (OSP and APP times) in possums when brodifacoum is still present in the liver are difficult to elucidate at this stage. However, rats receiving similar sub-lethal doses of brodifacoum showed similar responses; OSP times were elevated more

quickly, and returned to normal more quickly (within 4–5 days) than in possums, but brodifacoum residues were again retained in the liver (Bachmann & Sullivan 1983). This implies that possums will have to eat considerably more than 60 g of Talon bait to receive a lethal dose.

The persistence of brodifacoum in possums highlights the potential secondary and tertiary risks associated with brodifacoum when used for possum control. For example, pigs will scavenge possum carcasses and it is apparent from this study that possums dying up to 1 year after being exposed to brodifacoum may contain residues that could be transferred to pigs. As feral pigs are hunted as a food source in New Zealand, there is a risk of tertiary poisoning of humans. Pigs and possums should not be hunted for human consumption from areas where brodifacoum has been used. A sensible precaution would be to recommend that the livers from all game are discarded since highest residue concentrations occur in liver versus muscle tissue. Our results indicating the persistence of brodifacoum in muscle tissue were surprising since an earlier study in sheep had reported brodifacoum present in the muscle for 2 weeks (Laas et al. 1985). However, in the study by Laas et al. (1985), the limit of detection for the assay method was not reported. To confirm our residue results in muscle samples we sent samples to a second laboratory and good agreement between our original results and those of the second laboratory was obtained. Even if residues in most animals never reached levels capable of causing serious harm to meat eaters (and this is difficult to judge because of the lack of toxicity data on brodifacoum in humans), the presence of brodifacoum in any meat products would have a negative effect on exports. Mean concentrations of brodifacoum in possum liver 25 days after oral administration of 0.1 mg/kg brodifacoum was approximately 0.08 μg/g. A 15 kg dog would need to eat over 500 kg of possum liver containing this amount of brodifacoum to receive a lethal dose given the oral acute toxicity (LD_{50}) for brodifacoum in dogs of 3.5 mg/kg (Eason & Spurr 1995). In our study, possums were given a small dose of brodifacoum equivalent to 10–20 g of Talon cereal bait. Although the lethal dose of Talon is thought to be between 60 and 200 g of bait, field trials have shown that possums can eat in excess of 1 kg of Talon over a period of 1–3 weeks before death (Henderson et al. 1994). Hence, considerably higher concentrations than those recorded in this study could be anticipated in dead and surviving possums in areas where Talon is used for possum control. The presence of brodifacoum in possum carcasses is also likely to pose a hazard to predators such as ferrets, feral cats, and harrier hawks, as well as farm dogs.

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