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# Learned Bait-Shyness by Possums (*Trichosurus vulpecula*) towards Baits Containing Cyanide, 1080, Cholecalciferol, or Brodifacoum

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**Abstract:** Around NZ\$76 million per annum is presently spent on controlling and researching the introduced brushtail possum in New Zealand. The national control effort is likely to be further increased over the next decade in an attempt to eradicate bovine tuberculosis and to arrest the degradation of key conservation areas. There is a risk that repeated control operations using toxic baits could result in widespread development of learned "bait-shyness," behavior that develops when sublethally poisoned animals are able to link the experience of an illness with the memory of the bait eaten. Four studies are reviewed that aimed to determine the likelihood of possums becoming shy after sublethal doses imparted by different types of toxic bait, and to find solutions to the problem. Captive possums were presented with baits containing sublethal doses of the 4 vertebrate pesticides commonly used. Following a recovery period, possums were presented with lethal baits and their response compared with that of naive possums. Between 15% and 90% of possums previously dosed with sublethal doses of cyanide, 1080, or cholecalciferol developed bait-shyness, the proportion being dose and toxicant dependent. Although cholecalciferol induces toxicosis more slowly (i.e., >24 h) than cyanide (5 min) and 1080 (1 h), possums were nevertheless able to associate the effects of this toxicant with consumption of the bait in which it was delivered and avoid eating further baits. However, brodifacoum acts very slowly (1-4 weeks), and possums did not become shy towards baits containing this toxicant after first eating sublethal baits. Shyness induced by either 1080 or cyanide persisted for at least 12 and 24 months respectively, thus jeopardizing the effectiveness of "maintenance" control operations, which can be required every 12 months or less. The persistence of bait-shyness to cholecalciferol remains untested. Use of alternative baits (bait-switching) proved an effective solution for eliminating cyanide- and 1080-induced bait-shyness. Baits containing brodifacoum can also be used very effectively to "mop up" bait-shy populations, but care must be taken to minimize environmental contamination by this persistent compound.

**Key Words:** brushtail possum, *Trichosurus vulpecula*, possum control, cyanide, 1080, cholecalciferol, brodifacoum, bait-shyness, learned aversion

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## INTRODUCTION

The Australian brushtail possum (*Trichosurus vulpecula*) is New Zealand's foremost vertebrate pest because of its role as a reservoir of bovine tuberculosis (Tb) (Coleman and Caley 2000), its extensive damage to indigenous forests (Payton 2000), and its destruction of avifauna (Sadleir 2000). Additionally, this introduced nocturnal, arboreal marsupial causes significant damage to a variety of horticultural plantings, forestry and catchment plantings, and household gardens (Butcher 2000). The direct cost of production losses has been estimated at NZ\$40 million per annum (Hackwell 1999), but the potential loss of dairy, beef, and deer exports is far greater. The Animal Health Board (2001) estimated that losses of NZ\$1.29 billion have a 24% probability of occurring over a 12-year period. Spending on all possum control and research (for protection of both conservation and agricultural values) increased from NZ\$34 million in 1992/93 to \$76 million in 1998/99. A further doubling of annual possum control for Tb eradication is planned throughout the coming decade (Animal Health Board 2001).

Poisoning is currently the most widely used technique for the control of possums, supplemented by the use of traps and, to a lesser extent, shooting (Morgan and Hickling 2000). While a significant research effort is

presently under way to develop biological control methods to supplement conventional control (Cowan 2000), their introduction is still at least 10 years away, as many technical issues must be resolved and public acceptance sought. Hence, it is likely that possum control will depend on the use of poisons to a greater or lesser extent for many years yet. Major reliance is placed on the use of sodium monofluoroacetate (i.e., "1080"), supplemented by the use of cyanide (as the salt of either sodium or potassium), cholecalciferol (i.e., vitamin D<sub>3</sub>), and brodifacoum (an anticoagulant).

Long-term prevention of ecological damage and spread of bovine Tb often requires repeated control operations (i.e., "maintenance control"). Typically, for example, control is repeated annually in forest-farm edge habitat where transmission of Tb from possums to cattle occurs. Where poison baits are used repeatedly, there is the possibility that possums may develop bait-shyness. This problem is well documented for a range of pest rodent species exposed to baits containing acute (fast-acting) toxicants (Prakash 1988). Animals that ingest only a sublethal dose of poison learn to associate the illness induced by the toxicants with the memory of the bait eaten, and subsequently avoid further bait consumption. We could not assume, however, that possums have the same tendency to develop bait-shyness

as rodents, since data on the comparative learning ability of possums and eutherian mammals are conflicting. For example, possums performed as well as carnivores, skunks, and 3 species of monkey in learning to respond to varying position and light as cues for a reward (Kirkby and Williams 1979), but were less successful than cats and rats in learning to negotiate mazes based on visual and tactile cues (Pollard and Lysons 1967). Furthermore, the Virginia opossum (*Didelphis virginiana*) performed poorly in a reward-based test of ability to learn to discriminate between the presence or absence of an odor (Tilley et al. 1966). Nevertheless, a strong suspicion that possums can learn to avoid baits is provided by empirical observations made by Hickling (1994) on the decline in efficacy of 1080 baits in annual control operations at Mapara Forest in the central North Island. Over a period of 3 years (1991-93), population reductions of 75%, 30%, and 0% were recorded, suggesting that either there had been selection for an intrinsic physiological resistance, or that possums had become bait-shy. These possibilities pose a major threat to the sustainable control of possum populations using toxic baits. Research was therefore undertaken to assist managers in preventing the development of widespread loss of efficacy. Our research has focused initially on the possible development of bait-shyness as the more likely explanation for declining efficacy.

This paper summarizes 4 similar experiments conducted to determine if bait-shyness is induced in possums by sublethal doses of the 4 main possum toxicants described above. We also assessed the persistence of bait-shyness to 2 toxicants (cyanide and 1080), and examined ways of overcoming shyness based on the use of alternative bait types and toxicants (i.e., "bait-switching"). The main components of this research have been published in detail elsewhere (Morgan et al. 1996, 2001; Morgan and Ross 2001; Morgan and Milne 2002). In this paper we summarize and synthesize this work, focussing in particular on the differences between the 4 toxicants, and the opportunities those differences provide for overcoming the problem.

## METHODS

### Possum Capture and Husbandry

Possums were captured in the Waipara Gorge district of North Canterbury (latitude 43° 15'S, longitude 172° 80') using metal box traps baited with apple. After transfer to the Landcare Research animal facility, they were housed in individual cages (530 × 400 × 1000 mm) that contained nest boxes (230 × 200 × 350 mm), and were maintained on a diet of fruit, vegetables, and supplementary feed pellets. Drinking water was continually available. The animals were acclimatized to captivity for at least 4 weeks, as recommended by Day and O'Connor (2000). Ambient temperature was maintained within the range 18-22° C, and lighting on a 12:12 h light:dark cycle, with complete refreshment of air

every 4 minutes. Possums were routinely weighed at least weekly to assess general health, enabling estimation of doses (i.e., mg/kg body weight) of toxicant ingested. The studies were conducted with the approval of the Landcare Research Animal Ethics Committee.

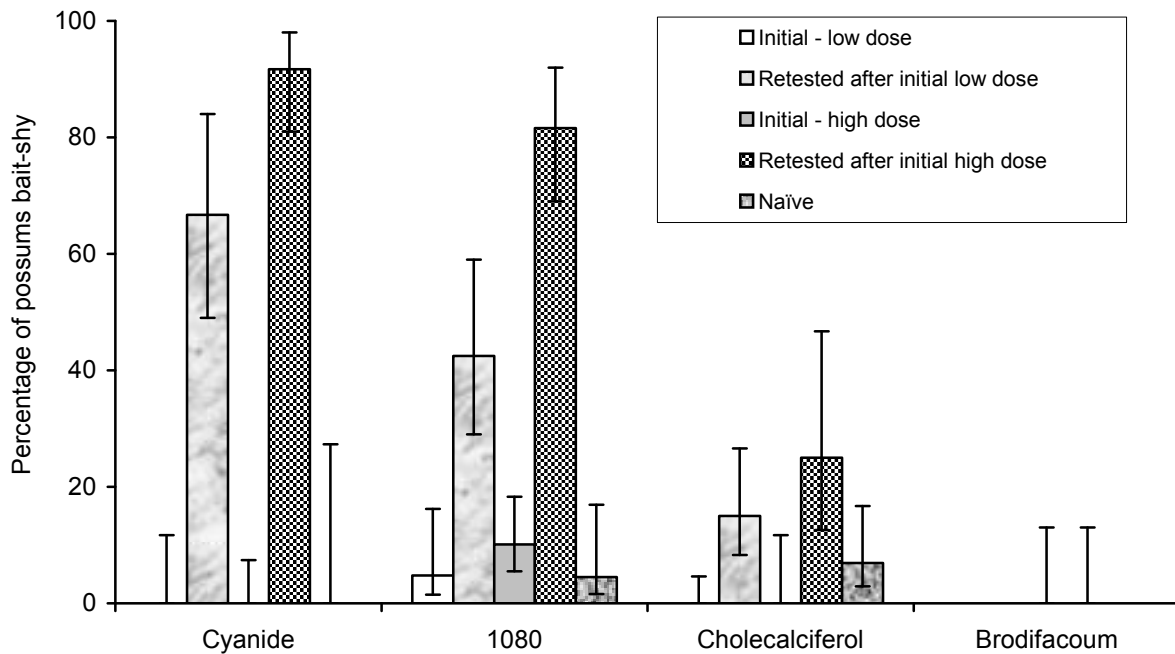
### Testing for Induction of Bait-Shyness

Possums were randomly allocated to treatment groups with approximately equal sex ratios. Dose rates and presentation matrices are summarized in Table 1. Brodifacoum, 1080, and cholecalciferol were presented in 'low' and 'high' sublethal doses in cereal pellet baits (Animal Control Products, Waimate) flavored with cinnamon as a toxicant mask (Morgan 1990). Cyanide was incorporated in a petrolatum-based paste (Animal Control Products, Wanganui) at a nominal concentration of 55% wt:wt, and presented with orange-flavored, sweetened flour as an attractant as is standard field-practice. Samples of all baits were assayed for toxicant concentrations so that known doses of bait could be presented to possums. The low and high doses given corresponded approximately to LD<sub>10</sub> and LD<sub>40</sub> values based on a published dose-response curve for 1080 (Henderson et al. 1999) and linear extrapolation from published LD<sub>50</sub> data for cholecalciferol (Jolly et al. 1995), cyanide (Bell 1972), and brodifacoum (Eason et al. 1994). Different doses of cholecalciferol were given to males and females because the sexes differ in their susceptibility to this toxicant (Jolly et al. 1995). A single high sublethal dose of brodifacoum bait was presented, as no shyness was expected for this slow-acting toxicant. The choice of doses aimed to represent approximately the range of sublethal doses that most possums surviving control operations may have ingested.

Baits containing cyanide, 1080, or cholecalciferol were presented for 16 h overnight, while the slower-acting brodifacoum baits were made available for 4 days. Possums that did not eat the portions of 1080 bait (Figure 1) containing these doses (i.e., innately bait-shy) were removed from the experiment at this stage to maintain the focus of the study on learned bait-shyness. Surviving possums' appetite was monitored to determine their return to full health, and they were then presented with a lethal quantity of the same bait/toxicant offered initially. This was after 7 days for cyanide and 1080, 21-30 days for cholecalciferol, and 34 days for brodifacoum. Four groups of naïve possums (i.e., individuals not previously been exposed to sublethal baits) were also presented with baits containing the different toxicants. Bait-shyness in survivors was defined as consumption of less than 1 g of bait by surviving possums, as some possums use taste as well as olfactory and visual cues to recognize baits (Morgan et al. 1996). The relationship between the percentages of possums showing shyness and toxicant, dose (i.e., low or high sublethal dose), and experience (i.e., initial versus subsequent exposure), was investigated using logistic regression in S-Plus 6 (S-Plus 6 2001).

**Table 1. The sublethal doses of four toxicant-bait combinations presented to possums, and the resultant mortality.**

Toxicant	Bait Type	Actual (and Nominal) Concentration of Toxicant in Bait (% wt:wt)	Sublethal Dose Given (mg/kg)	No. Possums Dosed	Mortality (%)
1080	Cereal pellet with cinnamon	0.13 (0.15)	Low = 0.4	42	0
			High = 1.0	89	36
Cholecalciferol	Cereal pellet with cinnamon	0.84 (0.8)	Low:		
			females = 2.5	30	0
			males = 4.3	34	12
			High:		
females = 6.7	23	35			
males = 8.5	16	44			
Cyanide	Paste covered with sweetened flour	53.9 (55.0)	Low = 2.2	24	0
			High = 5.9	36	0
Brodifacoum	Cereal pellet with cinnamon	0.0025 (0.002)	High = 0.3	22	41



**Figure 1. The percentage of possums that were bait-shy on initial presentation of either low (approx. LD<sub>15</sub>) or high (LD<sub>40</sub>) sublethal doses, on re-testing after these doses, and in control groups of naive animals offered free access to baits, on first exposure. Brodifacoum baits were not used at a low initial dose or among naive possums for assessing shyness as no shyness was expected or found, even at the high sublethal dose tested. Vertical bars indicate binomial confidence limits.**

The persistence of bait-shyness towards 1080 baits was further tested in low-dose-group possums at 90 days, and in combined low- and high-dose groups at 365 and 730 days. The persistence of bait-shyness towards cyanide baits was retested in combined low-dose and high-dose groups at 90 and 300 days. These tests resulted in some deaths on each occasion and a consequent decline in sample size.

### Overcoming Bait-shyness by Bait-Switching

Different combinations of bait type, toxicant, and flavor (Table 2) were presented to bait-shy possums to determine if shyness could be overcome by changing the cues by which possums recognized baits. Since a full assessment of the importance of each of these bait factors (and their interactions) would have been prohibitively costly, a few practical options (i.e., bait formulations that were already registered for use) were tested for overcoming bait-shyness. Lethal quantities of alternative baits were therefore presented between 35 and 100 days after initial baits were eaten, and shyness assessed as described above. The percentages of groups shy to initial baits were confirmed before bait-switching by either: (1) testing a separate group (1080), (2) testing all possums selected for bait-switching (cholecalciferol), or (3) testing a subsample of those selected for bait-switching (cyanide). Due to the restricted design of these experiments, statistical analysis of the data was not possible. Instead, comment is made on which options worked.

## RESULTS

### Testing for Induction of Bait-Shyness

The low and high sublethal doses of 1080 and cholecalciferol given resulted in sufficiently similar mortalities (low: 0-12%; high: 35-44%) (Table 1) to

permit comparison of the bait-shyness induced. Comparison with cyanide-induced shyness must be made with caution since no possums were killed by the estimated LD<sub>15</sub> and LD<sub>40</sub> doses of cyanide, indicating that Bell's (1972) LD<sub>50</sub> overestimated lethality.

Bait-shyness was caused by sublethal doses of all 3 fast-acting toxicants, cyanide, 1080, and cholecalciferol; but no bait-shyness was found after possums ate sublethal doses of the slow-acting brodifacoum (Figure 1). Averaging the percentages of survivors that were bait-shy after low- and high-initial doses gave 79% bait-shy after cyanide, 62% after 1080, 20% after cholecalciferol, and 0% for brodifacoum. Detailed statistical analysis was performed on all 3 factors (toxicant, experience, and dose) for all toxicants except brodifacoum, as it did not induce bait-shyness in any possums. The percentage of possums becoming bait-shy was strongly influenced by dose ( $\chi^2 = 16.9$ ,  $df=1$ ,  $P<0.001$ ) independently of toxicant ( $\chi^2 = 2.42$ ,  $df=2$ ,  $P=0.30$ ) and experience ( $\chi^2 = 0.40$ ,  $df=1$ ,  $P=0.53$ ): higher initial doses resulted in greater shyness. The type of toxicant used also affected the proportion becoming bait shy, but this was dependent on experience ( $\chi^2 = 2.81$ ,  $df=2$ ,  $P=0.001$ ) due to the different responses to 1080. While 8% of naive 1080 animals were innately shy, as has also been shown in other studies for 1080 baits (Morgan 1990), no naive animals were bait-shy for the other toxicants. Shyness in experienced animals was much lower for brodifacoum (0%) and cholecalciferol (18%) than 1080 (62%) and cyanide (82%) baits. There was no evidence for a 3-factor interaction between the 3 factors ( $\chi^2 = 0$ ,  $df=2$ ,  $P=1$ ).

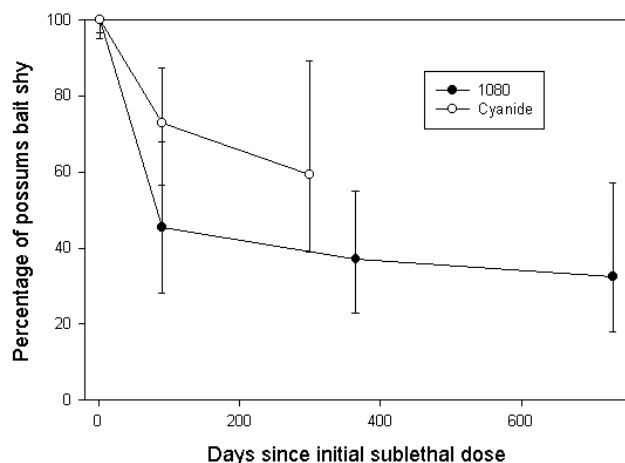
### Persistence of Bait-shyness

Bait-shyness towards cyanide and 1080 baits declined significantly during the first 90 days (as indicated by non-overlapping confidence intervals,

**Table 2. The effectiveness of bait-switching in overcoming bait shyness as indicated by the percentage of possums shy towards alternative baits. All baits were presented for 1 night except brodifacoum pellets, which were presented for 5 consecutive nights.**

Initial Bait				Alternative Bait					
Toxicant	Bait Type	Flavor	Mean Initial Dose (mg/kg)	Days Since Initial Dose	No. Tested	Toxicant	Bait Type	Flavor	No. Shy (and %)
1080	Pellet	Cinnamon	0.73	90	14	1080	Pellet	Orange	11(79)
			0.77		7	Brodifacoum	Pellet	Cinnamon	3 (43)
			0.74		13	1080	Carrot	Orange	0 (0)
Cholecalciferol	Pellet	Cinnamon	5.48	35-45	20	Cholecalciferol	Gel	Orange	9 (45)
			5.85		20	1080	Gel	Orange	7 (35)
Cyanide	Paste and flour	Orange	8.80	100	22	1080	Pellet	Cinnamon	2 (9)
			9.97		21	1080	Paste	Apple	0 (0)
			8.85		20	Cholecalciferol	Gel	Orange	0 (0)

Figure 2.). Thereafter, however, most possums that were bait-shy at day 90 remained bait-shy for the duration of each study (i.e., 1 year for cyanide, and 2 years for 1080) suggesting that they probably would have remained bait-shy even longer. The decline of shyness appears more rapid for 1080 than cyanide (Figure 2), but the data are too limited to confirm this statistically, and bait-shyness at day 90 was probably underestimated for 1080 because only possums from the low-dose group were re-tested at this time.



**Figure 2. The percentage of possums confirmed as bait-shy after different time intervals following consumption of sublethal doses of bait. Vertical bars indicate binomial confidence limits.**

### Overcoming Bait-shyness by “Bait-Switching”

For each toxicant, the mean initial doses consumed by possums used in bait-switching tests were similar, rendering the effectiveness of alternative baits comparable (Table 2). Although cyanide induced the highest proportion of bait shyness initially, it was most readily overcome by bait-switching using 3 types of bait that differed from the initial bait in all 3 characteristics (i.e., toxicant, bait type, and flavor). Despite the lower level of bait-shyness induced by cholecalciferol, bait-switching was only moderately effective, even when all 3 characteristics were changed. Bait-shyness induced by 1080 was effectively overcome by a change of bait type (to carrot) and flavor (to orange), but changing the flavor or toxicant alone was not very effective, suggesting that possums’ bait-shyness is based primarily on recognition of the bait base.

### CONCLUSIONS

These studies suggest that possums’ learning ability, with regard to poison bait avoidance, is at least as well developed as that of rats. Like rats (Prakash 1988), some possums surviving sublethal doses of acute toxicants developed a learned aversion to the bait eaten, and the proportion of possums developing such behavior was

influenced by the initial dose ingested, the toxicant used, and the latency of the toxicant.

Of the acute toxicants tested, cholecalciferol appeared the least likely to induce bait-shyness due to its much slower mode of action than cyanide and 1080. Prescott et al. (1992) showed that laboratory rats developed a weak learned aversion to cholecalciferol baits (i.e., toxic baits were consumed less but not avoided) reflecting both the relatively low doses given (an approximate LD<sub>10</sub>) and the long latency of 13 h for this toxicant in rats (Andrews and Braveman 1975). By comparison, after eating baits with LD<sub>15</sub> doses, possums not only ate significantly less toxic baits than nontoxic baits, but about 15% of possums became bait-shy, despite the longer latency of about 24 h for cholecalciferol in possums (Morgan and Milne 2002).

Bait-shyness was also extremely persistent, exceeding that previously recorded in rats. The most persistent bait-shyness reported (summarized in Prakash 1988) in rodents was 374 days for laboratory rats and 8 months for deer mice. Some possums in our cage trials remained bait-shy for at least 2 years after eating sublethal 1080 baits. Additionally, O’Connor and Matthews (1999) have shown that 18% of wild possums exposed to a 1080 control operation in the field 3 years previously were still bait-shy when tested under controlled, captive conditions. Allowing for a doubling of the residual population in the area over the 3 years (and hence approximate halving of the proportion of shy survivors), this finding is consistent with our data, which showed that about 33% of the survivors were still bait-shy after 2 years, a level expected by extrapolation to be little different at 3 years.

Shyness towards cyanide may have been even greater if correct LD<sub>15</sub> and LD<sub>40</sub> initial doses of cyanide had been used. In turn, this may have resulted in less effective bait-switching, and persistence of shyness in a greater percentage of possums. Nevertheless, bait-shyness in cyanide survivors appeared similarly persistent to that for 1080, and cyanide-induced food aversions have been shown in another study (O’Connor and Matthews 1997) to persist for at least 2 years. While a smaller percentage of possums developed shyness towards the slower-acting cholecalciferol baits, it is likely that shyness in these animals would be equally long-lived, but this remains to be tested.

### Management Implications

Current possum control strategies often require annual maintenance control to keep possum density below a threshold for Tb persistence or acceptable levels of conservation damage. Available control techniques should therefore be used with some understanding of their consequences so that “integrated” strategies can be developed for long-term use. Bait-shyness is clearly a potential threat to long-term sustainability of control: modeling predicts that the efficacy of annual control will be reduced by >30% where bait-shyness develops in 80%

of survivors and persists for at least a year (Hickling 1995).

The simplest way of avoiding the development of learned bait-shyness is to minimize the likelihood of possums finding and eating sublethal baits. This can be achieved by the highest standards of bait preparation and presentation. Nevertheless, the possibility remains that some possums will encounter and eat only sublethal amounts of bait.

The effectiveness of bait-switching in overcoming shyness differed between toxicants. For 1080 bait-shyness, neither a change in the toxicant nor flavor alone was sufficient, but changing the bait base and flavor was effective, as also found by O'Connor and Matthews (1999). However, changing bait characteristics appears to be less effective in overcoming cholecalciferol-induced shyness. This may reflect the considerably longer latency of cholecalciferol, the possum's consequent inability to remember which food induced toxicosis, and a resultant generalized wariness to new foods. Against cyanide-induced bait shyness, the clear effectiveness of bait-switching contrasted with the results of field trials in which no additional kill was achieved using either 1080 pellets (2 trials) or cholecalciferol pellets (2 trials) 5-25 days after populations were reduced by 60-84% using cyanide paste (Henderson et al. 1997). While these conflicting findings warrant resolution, it must be noted that cyanide paste has been used extensively for retrieval of possum fur for 40 years with no other reliable reports of possum control being compromised as a result. The trend towards use of an encapsulated form of cyanide, "Feratox<sup>®</sup>," may reduce the likelihood of cyanide-induced bait-shyness as the small, odorless capsule is embedded in a paste bait and the cyanide is released during mastication, preventing rejection.

Alternative approaches to mitigating bait-shyness include the use of prefeeding, anticoagulant poisons (i.e., a particular type of bait-switching), and traps and other killing devices. Prefeeding is done primarily to increase the effectiveness of control through familiarizing pests with a bait-type before introducing the toxicant. Like black rats (Bhardwaj and Prakash 1982), possums are far less likely to develop bait-shyness if they have been pre-fed (Moss et al. 1998, Ross et al. 2000). The animals learn to identify the bait as "safe" and are then less likely to associate it with toxicosis if sublethal baits are subsequently ingested. The benefits of, perhaps, a 10% improvement in efficacy and avoidance of bait-shyness must therefore be carefully considered against the additional cost of prefeeding, which may exceed that of applying only toxic bait, depending on how many "prefeeds" are applied.

In the studies reported here, switching to the anticoagulant brodifacoum in cereal pellets for 5 days was only partially effective in overcoming shyness to 1080 cereal pellet baits. However, we have also found that more prolonged presentation of brodifacoum, starting a fortnight after possums became 1080 bait-shy, resulted

in a peaking of nightly bait intake after 14 days and a consequent mortality of 73-75% (Ross et al. 1997, Morgan and Ross 2001). In field trials, delaying the bait-switching for 2.5 months after initial control resulted in even greater efficacy (88-94% mortality), (Henderson et al. 1997). In effect, a bait-type containing an anticoagulant acts as its own prefeed. The possum cautiously eats the bait, falsely learning that the bait is "safe," and nightly intake increases. Although outward symptoms of toxicosis are first seen at about 13-15 days (Littin et al. 2000), the possum continues to consume the bait, often right up to the point of death (Morgan and Ross 2001) indicating that learned food-safety, like learned bait-shyness, is a very rigid behavior once formed. Brodifacoum baits have become regarded as a less desirable control option because they are relatively expensive, and because the toxicant is highly persistent in animal tissues, posing risks of secondary poisoning. However, restricted use of brodifacoum baits in "mopping up" populations that have already been heavily reduced by other control options is considered to be a desirable and responsible use.

Using these findings, strategies for sustained control of possums can be designed to minimize the development of bait-shyness. For example, following initial "knockdown" with aerially-delivered 1080 carrot baits, maintenance control could then be based on the use of Feratox<sup>®</sup> cyanide baits after one year, "long-life" cholecalciferol baits (Morgan 2000) permanently placed for the next 2 years, and brodifacoum cereal pellets in the fourth year. Humane kill-traps (Warburton et al. 2001) in the fifth and subsequent years could then be used efficiently (since they can remain in place for prolonged periods without being checked) to indicate population recovery and the need for further, cost-effective toxicant-based control. There is a need to evaluate such strategies in terms of not only cost-effectiveness, but also the long-term environmental impacts and benefits.

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