Factors affecting 1080 pellet bait acceptance by house mice (*Mus musculus*)

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ABSTRACT

Avoidance of 1080 (sodium fluoroacetate) could be one of the main reasons why multi-species control operations sometimes do not produce high reductions in wild house mouse (*Mus musculus*) populations in New Zealand. This study investigated how the concentration of 1080 in pellet bait affects acceptance by mice; whether pre-feeding with non-toxic bait mitigates mouse avoidance of bait containing 1080; and whether a non-toxic bait containing a masking agent is acceptable to mice. Wild-caught mice demonstrated very low acceptance of, and subsequent low mortality (25%) from, baits containing 0.08% 1080 in a two-choice laboratory test. In a second test, mice ate comparatively more pellets containing 0.001% 1080, but there was no resulting mortality and the non-toxic alternative pellets were still significantly favoured. Pre-feeding for 3 days with non-toxic pellets did not improve the low acceptance of 0.15% 1080 pellet baits by mice. In two of the three two-choice tests, the intake of all food by mice was significantly reduced for 2 days following the introduction of 1080-treated food. This 'drop feed' effect was followed by an increase, mostly of non-toxic food, in daily intake over the next 3 days, to return to eating similar amounts to those measured before the introduction of 1080 (and to daily food intakes of control mice). Non-toxic bait was strongly preferred over two different types of non-toxic bait containing a masking agent. We suggest that avoidance of 1080 by mice is mediated by conditioned taste aversion. However, masking the taste of 1080 may not be effective if mice are micro-sampling and learning to associate sublethal poisoning effects with any distinctive taste. Improvement of bait efficacy may involve developing baits that delay the onset of symptoms of 1080 poisoning; or pre-feeding with baits containing a non-toxic substance with similar taste and/or odour to 1080.

Keywords: house mouse, *Mus musculus*, sodium fluoroacetate, 1080, bait, pre-feeding, acceptance, microencapulsants, New Zealand

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1. Introduction

House mice (*Mus musculus*) are widely distributed throughout New Zealand. They feed on a wide range of invertebrates and seeds, and possibly also on lizards and the eggs of small birds (Wilson et al. 2006). Mice are prolific breeders, and in some environmental conditions their population can irrupt, as reported every few years in various parts of New Zealand, particularly in the South Island (e.g. Ruscoe et al. 2004).

The negative impacts of mice on mainland New Zealand ecosystems are not as well characterised as those of other introduced pest mammals, such as ship rats (*Rattus rattus*) and brushtail possums (*Trichosurus vulpecula*). However, introduced mouse populations on other island ecosystems have been implicated in the decline or extinction of native species (Invasive Species Specialist Group 2006), and mice have recently been described as active predators of seabirds on Gough Island (Wanless et al. 2007). In New Zealand, mice may have a significant impact as predators of native invertebrates, although this requires further investigation (e.g. Jones & Toft 2006).

Current multi-species pest management approaches in mainland New Zealand include broad-scale aerial application of sodium fluoroacetate (1080) to control brushtail possums and ship rats, but this has not proven to be reliably effective against mice, particularly when rat numbers are suppressed (Gillies 2002; Sweetapple & Nugent 2005). The reasons for this are unclear, but avoidance of 1080 by mice is a potentially significant contributing factor in failures of 1080 operations to produce high mortality in wild mouse populations. Morriss et al. (2006) tested the effect of a range of variables on acceptance of cereal pellet baits by wild mice, and found that the presence of 0.15% 1080 was the only factor that significantly reduced acceptance.

We sought to further characterise 1080 avoidance by New Zealand house mice in a series of laboratory trials. We also tested mouse acceptance of a non-toxic bait containing a microencapsulant formulation (which could potentially mask odour or taste cues presented by 1080), as a step towards identifying 1080 bait formulations that could be more effective against house mice.

2. Objectives

The objectives of this study were to determine:

- Whether there is a detection threshold of 1080 concentration in bait that maximises bait acceptance and mortality in mice.
- Whether pre-feeding with non-toxic RS5 cereal baits affects subsequent acceptance of 0.15% 1080 RS5 cereal baits by mice (the formulation commonly used in aerial 1080 operations).
- The palatability to mice of two (non-toxic) bait formulations containing microencapsulant components.

3. Methods

3.1 ANIMAL HUSBANDRY

Wild adult house mice were caught by hand from oat stacks on farms around Lincoln, Canterbury, and housed at the Landcare Research animal facility, Lincoln. These mice were considered unlikely to have had previous exposure to 1080 baits, as 1080 is not applied close to buildings and pasture with livestock. The mice were individually housed in polycarbonate cages (40 × 20 × 15 cm high) that were lined with sawdust and shredded paper and had a wire lid. They had free access to water throughout the acclimatisation and trial periods, and were maintained during acclimatisation on commercial feed pellets for laboratory rodents (Weston Animal Nutrition, Rangiora), supplemented with fruit, sunflower seeds and cat biscuits. Mice were acclimatised to these conditions for a minimum of 21 days and were weighed regularly during acclimatisation to ensure that females were not pregnant and body weight was stable or increasing before they were used in trials.

Trials were conducted under the approval of the Landcare Research Animal Ethics Committee (Approval No. 06/06/01).

3.2 TRIAL 1: NON-TOXIC VERSUS 0.08% 1080 BAITS

A loose mixture of RS5 formulation was supplied by Animal Control Products (Waimate, date of manufacture 11 July 2006). Pellet baits (non-toxic and 0.08% w/w 1080) were then prepared from this by the Landcare Research toxicology laboratory.

To make the 0.08% w/w 1080 pellets, a stock solution of 1080 was made in distilled water to a maximum volume of 200 mL. A quantity of this solution was then slowly blended into 1.4 kg of non-toxic RS5 loose mix to produce the required concentration of 1080 in the mixture (0.08%). Pellets were formed by pressing the blended mixture into a cylindrical bait mould (20 mm diameter) under a pressure of 4500 p.s.i. using a hand-operated Blackhawk pump (model #65420) and press (model #65150). After being dried for 24 h at 30°C, each pellet weighed approximately 12 g. A six-pellet subsample of each 0.08% 1080 batch was assayed by the Landcare Research toxicology laboratory to confirm that the 1080 concentrations in the pellets were within an acceptable range (± 2%).

Twenty-four mice (12 males, 12 females) were randomly selected from the acclimatised population and allocated to a control or treatment group ($n = 12$ for each group), to be presented with a standard two-choice test over 5 nights, commencing on 1 August 2006. The control mice were offered a choice of non-toxic RS5 and standard feed pellets (i.e. their accustomed diet), and mice in the treatment group were offered a choice of 0.08% 1080 RS5 cereal pellets and non-toxic RS5 cereal pellets. This choice between toxic and non-toxic RS5 pellets (rather than between standard feed pellets and toxic RS5 pellets) was

used to ensure that the presence of 1080 in food was the only variable presented to the treatment group.

A weighed amount (approximately 20 g) of each bait type was placed in separate feeding cups each choice night, and after approximately 24 h the remaining pellets and fragments were collected and reweighed to calculate the amount eaten over the period. The position of the treatments in the two feeding cups was alternated each 24-h period. The type and number of pellets removed from each feeding cup and the number of pellets that appeared to have been nibbled were recorded daily. Daily bait intake could not be measured with an accuracy of less than 0.1 g. The mean starting body weights of mice in the control (17.08 g) and treatment (16.25 g) groups were not significantly different (see section 4.1), so bait consumption estimates were not corrected for body weight.

For toxic and non-toxic RS5 pellets, three environmental controls were also randomly located in the room and weighed daily, to correct for any weight changes due to moisture loss or gain during this period.

After 5 days, surviving rodents were returned to their normal diet. All individuals were observed daily for signs of poisoning, which were recorded up to time of death. At 14 days, all surviving mice were euthanased.

To investigate whether mice might show avoidance of the newly presented bait choice (i.e. a potential neophobic response to any new food or object in a familiar setting), or avoid 1080-treated baits without sampling them, a sub-sample of mice (3 males, 5 females in the treatment group; and 1 male, 1 female in the control group) were videoed continuously for 3 h immediately after being first offered the choice of bait types. This sample comprised the maximum number of mouse cages that could be adequately monitored using the two available cameras, without significantly disturbing mice by moving their cages. The number and duration of visits to each bait type was recorded from the videotapes.

3.3 TRIAL 2: NON-TOXIC VERSUS 0.001% 1080 BAITS

Non-toxic and toxic pellet baits were prepared from the RS5 base using the same methodology as outlined above for Trial 1; however, the toxic baits were made to a concentration of 0.001% w/w 1080—the lowest concentration that could be accurately incorporated into the quantities of pellet matrix.

Another 24 mice (12 males, 12 females) were randomly allocated to either a control or treatment group (n = 12 for each group) to be presented with a standard two-choice test over 5 nights, commencing on 12 August 2006. Quantities and presentation of bait, conditions, procedures for measuring daily bait intake, environmental control baits, and the observation of mice for illness and mortality were conducted as for Trial 1. Again, the mean starting body weights of mice in the control (17.75 g) and treatment (17.08 g) groups were not significantly different (see section 4.2), so bait consumption estimates were not corrected for body weight.

3.4 TRIAL 3: EFFECT OF PRE-FEEDING ON 1080 BAIT ACCEPTANCE

Non-toxic RS5 pellets and 0.15% w/w 1080 RS5 pellets were obtained from Animal Control Products, Wanganui. This 1080 formulation is commonly used in aerial 1080 baiting operations targeting possums and rats, and ideally would also achieve high kills of mice. This trial investigated whether pre-feeding mice with the non-toxic version of the bait would increase their acceptance of toxic bait and resultant mortality. Twenty-four mice (12 males, 12 females) were randomly allocated to two treatment groups. Mice in the 'pre-fed' group were each given a weighed amount (approximately 20 g) of fresh, non-toxic RS5 pellets for three consecutive nights, while mice in the 'not pre-fed' group remained on their normal diet (standard feed pellets). The 3-day pre-feed phase commenced on 16 October 2006. Daily bait intakes of mice were estimated and were adjusted for changes in weight measured in three environmental control samples of each bait type (as described in sections 3.2 and 3.3).

Following the pre-feed phase, the two treatment groups were offered a two-choice test over 5 nights, using the same methods as described for Trials 1 and 2. Both groups were offered a choice of weighed amounts (approximately 20 g) of fresh 0.15% 1080 RS5 cereal pellets and standard laboratory feed pellets. The latter bait type was used as the 'choice' option (rather than non-toxic RS5 pellets as used in Trials 1 and 2) on the assumption that standard feed pellets represented 'normal' food for the mice in this trial, thus simulating the availability of an accustomed food alongside that of toxic baits that would be present in habitats of mice during an aerial 1080 baiting operation. Quantities and presentation of bait, the use of environmental controls to correct intake estimates, procedures for measuring daily bait intake, replacement with new bait each day, and observation of mice for mortality were conducted as for Trials 1 and 2. In these previous trials, mice had been found to urinate on baits (see section 4.1), potentially introducing an additional source of experimental error; therefore, for Trial 3 any remaining bait collected each morning was also dried for 24 h at 35°C before being weighed.

3.5 TRIAL 4: ACCEPTANCE OF NON-TOXIC MICROENCAPSULANT FORMULATIONS

For trial 4, two non-toxic bait formulations (Bait A and Bait B) were supplied by Connovation Ltd (Auckland) for testing. These contained unspecified components that could potentially be used to encapsulate 1080 to mask odour and/or taste cues presented to mice. Connovation Ltd also supplied a non-toxic 'challenge diet' (a loose mix comprising maize and oat mash) that met Environmental Protection Agency (USA) standards for palatability testing in rodents (e.g. Buckle & Kaukeinen 1988).

This trial investigated whether microencapsulant components affected the acceptance of bait by mice. Eighteen mice were randomly allocated to two treatment groups (7 females and 2 males each) for two-choice testing over 5 nights with either Bait A or Bait B versus the challenge diet. The trial began on 5 March 2007, and the same procedures as described previously were used for

weighing bait and estimating daily intakes. Palatability was defined as the amount of bait consumed by mice. This was expressed as a percentage of the combined weight of test (treated) and untreated pellets eaten, and then calculated as a mean value. A value of 50% indicated equal palatability of bait types.

3.6 DATA ANALYSES

Data from Trials 1 and 2 were analysed using Student's *t*-tests (mouse body weight data) and paired Student's *t*-tests (bait intake data) in the statistical package Genstat (Genstat Committee 2002). Data from Trial 3 were analysed using the Generalised Linear Mixed Models (GLMM) procedure in the statistical package Genstat (Genstat Committee 2002), where date and pre-feed treatment were included as fixed effects and mouse identification was included as a random effect. The Wald test was used to determine the significance of terms and interactions. Data from Trial 4 were analysed using the Linear Mixed Effect model procedure in GenStat (Genstat Committee 2002). Initial analysis showed that the distribution of the residuals showed heteroscedasticity (a fan shape to the residuals) and there was one extreme outlier, so a $\log_{10}$ transformation ($\log_{10}(x + 0.5)$) was applied to the data and the extreme outlier was removed from the analysis.

4. Results

4.1 TRIAL 1: NON-TOXIC VERSUS 0.08% 1080 BAITS

The laboratory-prepared 0.08% 1080 pellets assayed within acceptable ranges of the nominal concentrations. The non-toxic pellets had a 1080 concentration below the method limit of detection (< 2 ppm). Mice frequently removed the pellets from the feeding cups before partly consuming them, making it difficult to collect all very small fragments of uneaten bait from within the cage and separate these from small pieces of bedding material. Mice also frequently urinated on uneaten bait fragments (which were collected wet), which would have increased their weight.

At the start of the trial, there was no significant difference between groups in mouse body weights (*t*-test, $t = -0.779$, df = 22, $P = 0.444$), with control and treatment groups having mean weights of 17.08 g and 16.25 g, respectively.

Data from the first 2 days of the 5-day choice test for the control group were not recorded due to a technical oversight. However, over the last 3 days, mice in the control group ate significantly more non-toxic RS5 pellets than standard feed pellets (paired *t*-test, $t = 19.089$, df = 10, $P < 0.001$), with mean (± SEM) total intakes per mouse of 10.10 ± 0.36 g and 1.08 ± 0.37 g, respectively (Fig. 1A). In the treatment group, only three mice ate measurable amounts of the 1080 pellets, resulting in 25% ($n = 3$ of 12) mortality. One mouse was dead on the third morning and the other two were dead the following morning of the 5-day trial. (Note that non-toxic RS5 intakes by these three mice were included in the

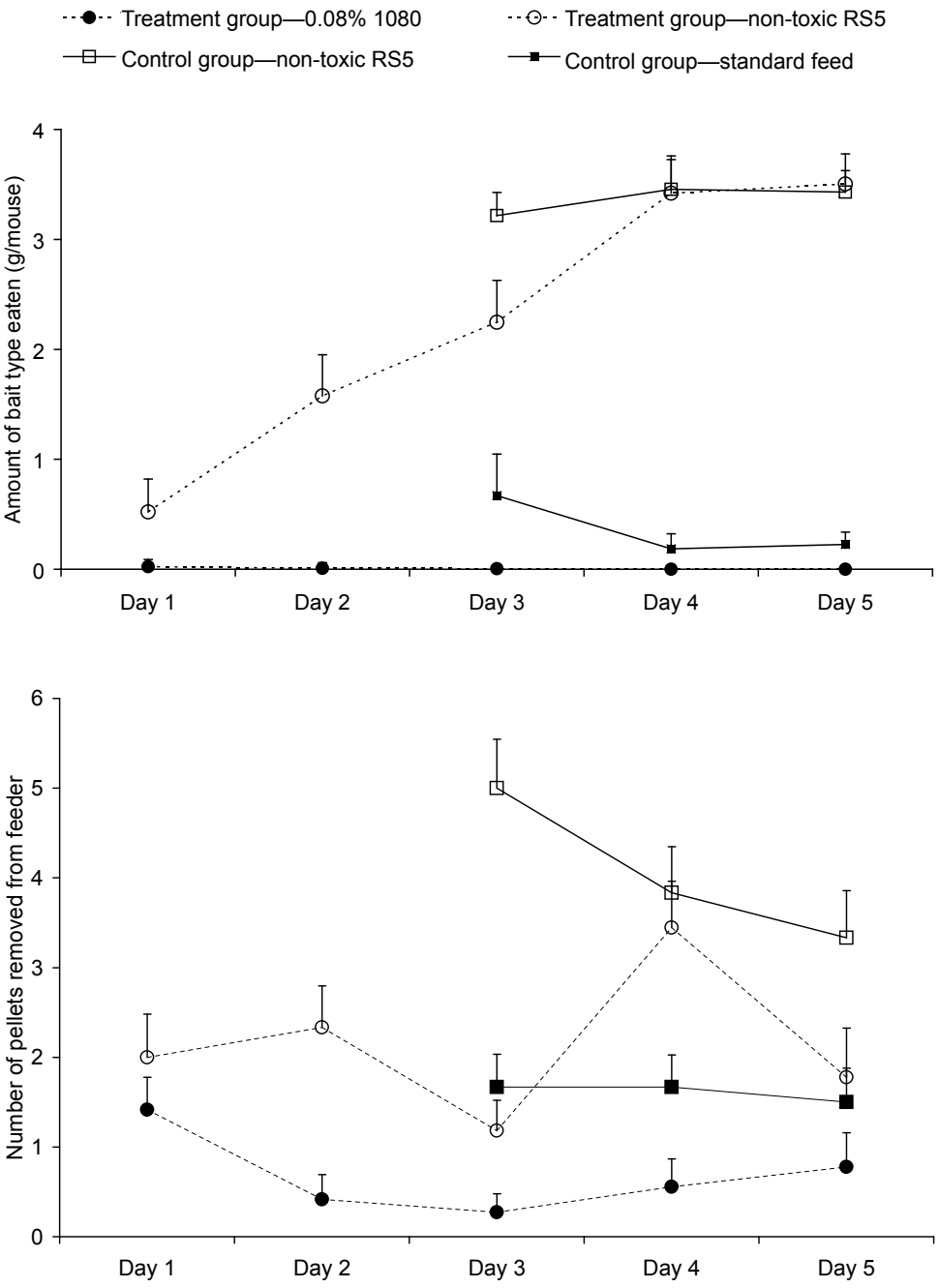


Figure 1. Daily intake of bait by house mice (*Mus musculus*) in the treatment (0.08% 1080 pellets v. non-toxic RS5 pellets) and control (non-toxic RS5 pellets v. standard feed pellets) groups over a 5-day two-choice test (Trial 1). A. Mean (± SEM) daily amounts eaten (g per mouse), and B. Mean number of pellets removed from feeders. Control group data for the first 2 days not available.

total mean estimates.) Figure 1A shows that mice in the treatment group ate little to no 0.08% bait over the 5 days, and gradually increased their daily intake of non-toxic RS5 pellets over this time from an initial relatively low intake. Over the 5 days, the mean (± SEM) total weights eaten were 9.35 ± 0.74 g non-toxic RS5 pellets per mouse, and only 0.04 ± 0.08 g 0.08% 1080 pellets per mouse, indicating a highly significant avoidance of baits containing 0.08% 1080 (paired *t*-test, $t = 5.378$, df = 10, $P < 0.001$). In the treatment group, there was also a steady increase in the mean quantity of non-toxic RS5 pellets eaten each day. Almost all of the 0.08% 1080 pellets that were eaten were taken on the first and second days. Fewer 1080-containing pellets than non-toxic RS5 pellets were also removed from the feeders, especially after the first day (Fig. 1B).

Table 1 summarises the video observations of mice in Trial 1. The angle of the video camera made it difficult to determine whether mice were feeding during

most visits, so a 'visit' was defined as the mouse putting its head into the feeding cup, presumably to either sniff, pick up or eat the bait type. Mice in the treatment group spent a significantly larger portion of the total observed time at the feeding cups containing 0.08% 1080 pellets than at those containing the non-toxic RS5 pellets (paired *t*-test, $t = -2.507$, df = 7, $P = 0.02$). The average duration of visits to the 1080 pellets was also significantly greater (paired *t*-test, $t = -3.156$, df = 7, $P = 0.08$). However, the number of visits to each bait type was similar. Control mice spent more time at and made more visits to the non-toxic RS5 pellets than to the standard feed pellets (their normal diet), although the sample size was too small to calculate whether this difference was significant.

TABLE 1. NUMBER AND DURATION OF VISITS MADE BY MICE (*Mus musculus*) TO FEEDING CUPS AND PROPORTION OF TIME SPENT AT BAIT DURING THE FIRST 3 h OF A TWO-CHOICE TEST IN TRIAL 1.

		TREATMENT ($n = 8$)		CONTROL ($n = 2$)*	
		RS5	1080 + RS5	RS5	FEED PELLETS
Number of visits	Mean ± SEM	9.88 ± 1.13	11.5 ± 2.09	25.5	10.5
	Range	4–13	5–25	31–20	11–10
Average duration of visit(s)	Mean ± SEM	4.75 ± 0.45	8.25 ± 1.13	24.0	11.0
	Range	3–6	4–13	22–25	7–15
Proportion of time spent at bait	Mean ± SEM	36 ± 5.53%	64 ± 5.53%	76%	24%
(% of total)	Range	13–54%	46–87%	77–75%	29–20%

* Sample size too small to calculate SEM.

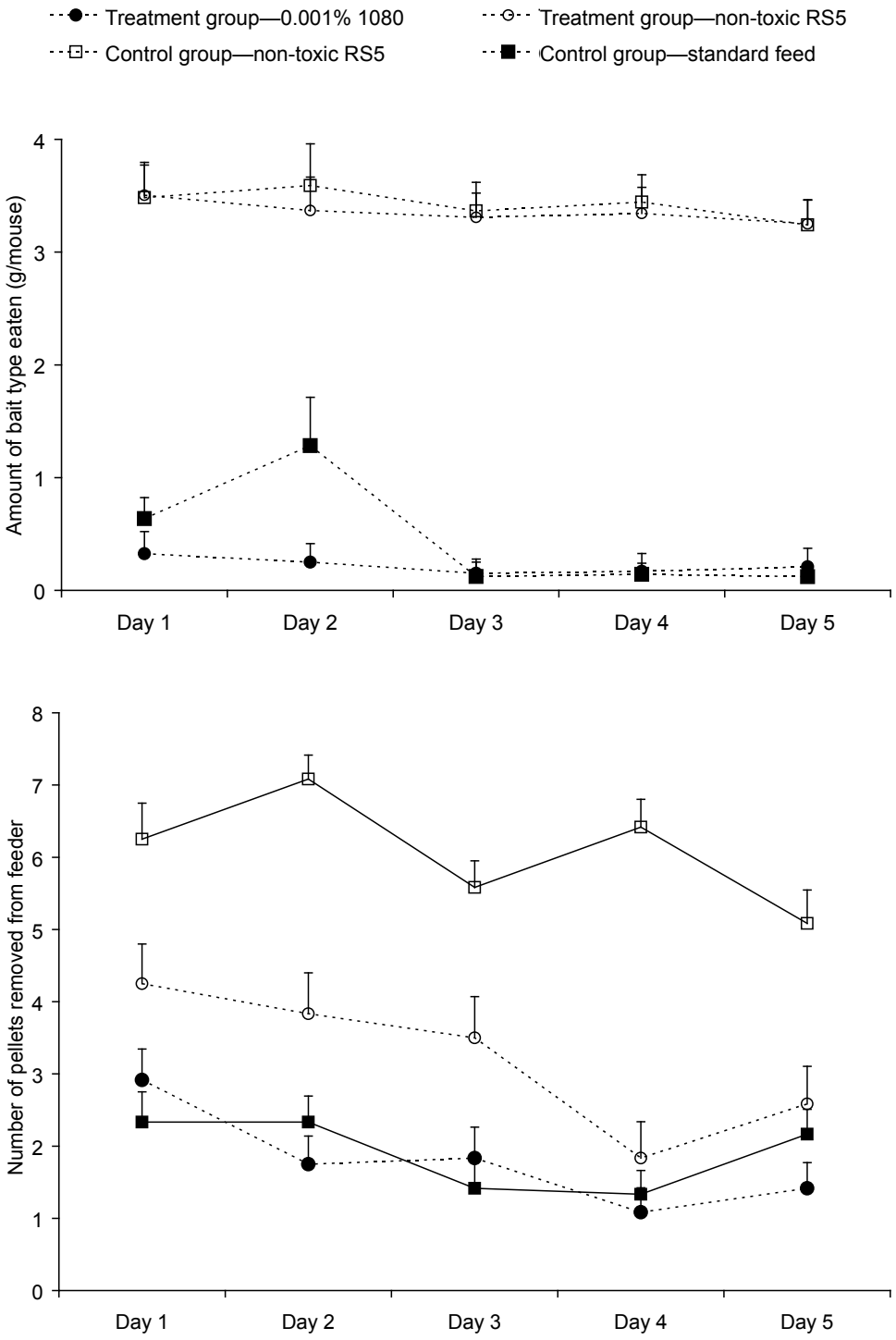
4.2 TRIAL 2: NON-TOXIC VERSUS 0.001% 1080 BAITS

The laboratory-prepared (nominally) 0.001% 1080 cereal pellets had a measured concentration of 0.00087% 1080. In the non-toxic RS5 cereal pellets, 1080 concentration was below the method limit of detection (< 2 ppm). As for Trial 1, there was likely error in intake estimates as a result of bait being removed from the feeders by mice and being urinated on.

At the start of the trial, there was no significant difference between groups in mouse body weights (*t*-test, $t = -0.668$, df = 22, $P = 0.510$), with control and treatment groups having mean weights of 17.75 g and 17.08 g, respectively.

Mice in the control group removed and ate significantly more non-toxic RS5 pellets than standard feed pellets (paired *t*-test, $t = 11.233$, df = 11, $P < 0.001$) over the 5 days, with mean totals (± SEM) of 17.12 ± 0.54 g/mouse and 2.31 ± 0.42 g/mouse, respectively. Mice in the treatment groups ate significantly more non-toxic RS5 pellets than 0.001% w/w 1080 pellets (paired *t*-test, $t = 15.045$, df = 11, $P < 0.001$) over the 5 test days, with mean totals (± SEM) of 16.77 ± 0.85 g/mouse and 1.10 ± 0.31 g/mouse, respectively. There was no mortality in the treatment group. The mean weight of 0.001% w/w 1080 pellets eaten was highest on day 1, as was the number of 0.001% w/w 1080 pellets removed from the feeding cups. The daily amounts of non-toxic RS5 eaten remained approximately consistent over the 5 days (Fig. 2A), although there was a gradual decline in the number of non-toxic RS5 pellets removed from feeding cups each day (Fig. 2B).

Figure 2. Daily intake of bait by house mice (*Mus musculus*) in the treatment (0.001% 1080 pellets v. non-toxic RS5 pellets) and control (non-toxic RS5 pellets v. standard feed pellets) groups over a 5-day two-choice test (Trial 2). A. Mean (± SEM) daily amounts eaten (g per mouse), and B. Mean number of pellets removed from feeders.



4.3 TRIAL 3: EFFECT OF PRE-FEEDING ON 1080 BAIT ACCEPTANCE

At the start of the trial, there was no significant difference between groups in mouse body weights (paired *t*-test, $t = -0.769$, df = 22, $P = 0.257$), with pre-fed and not pre-fed groups having mean weights of 18.08 g and 17.40 g, respectively.

On each of the 3 days in the 'pre-feed' phase, mice in the pre-fed treatment group consistently ate more of the non-toxic RS5 pellets than the standard feed pellets (Fig. 3A). However, during the pre-feed phase, both groups consumed similar total amounts of food per mouse per day ($t = 0.586$, df = 4, $P = 2.776$; Fig. 3A & B).

Laboratory assay showed that the 0.15% w/w 1080 pellets presented to the mice in the 5-day test phase were within an acceptable range of the nominal concentration. Three mice in each group died of poisoning, with mortalities occurring on days two, four and five for both groups. Group sizes were reduced accordingly when calculating mean daily intakes of food types on a g per mouse per day basis.

There was no effect of pre-feeding on the proportion of 1080 pellets removed from the feeders by each group (Wald test, $\chi^2 = 0.11$, df = 1, $P = 0.742$), and no significant difference between pre-fed and not pre-fed mice in the total amount eaten daily (Wald test, $\chi^2 = 0.42$, df = 1, $P = 0.52$). The mean (± SEM) amount of 0.15% 1080 pellets eaten daily was 0.53 ± 0.05 g/mouse for the pre-fed group (Fig. 3A) and 0.48 ± 0.05 g/mouse for the not pre-fed group (Fig. 3B). However, both groups showed a marked decrease in overall food intake on the day following presentation of the 0.15% 1080 RS5 pellets alongside the standard feed pellets. After this, the total daily amounts of the standard feed pellets eaten by each group then gradually increased over the 5 days, to return to approximately the same level as before exposure to 1080-treated pellets (Fig. 3A & B). When terms that were not significant were dropped from the model, 'day' was the only term remaining, and there was a highly significant difference in the total amount of food taken between days (Wald test, $\chi^2 = 69.29$, df = 4, $P < 0.001$).

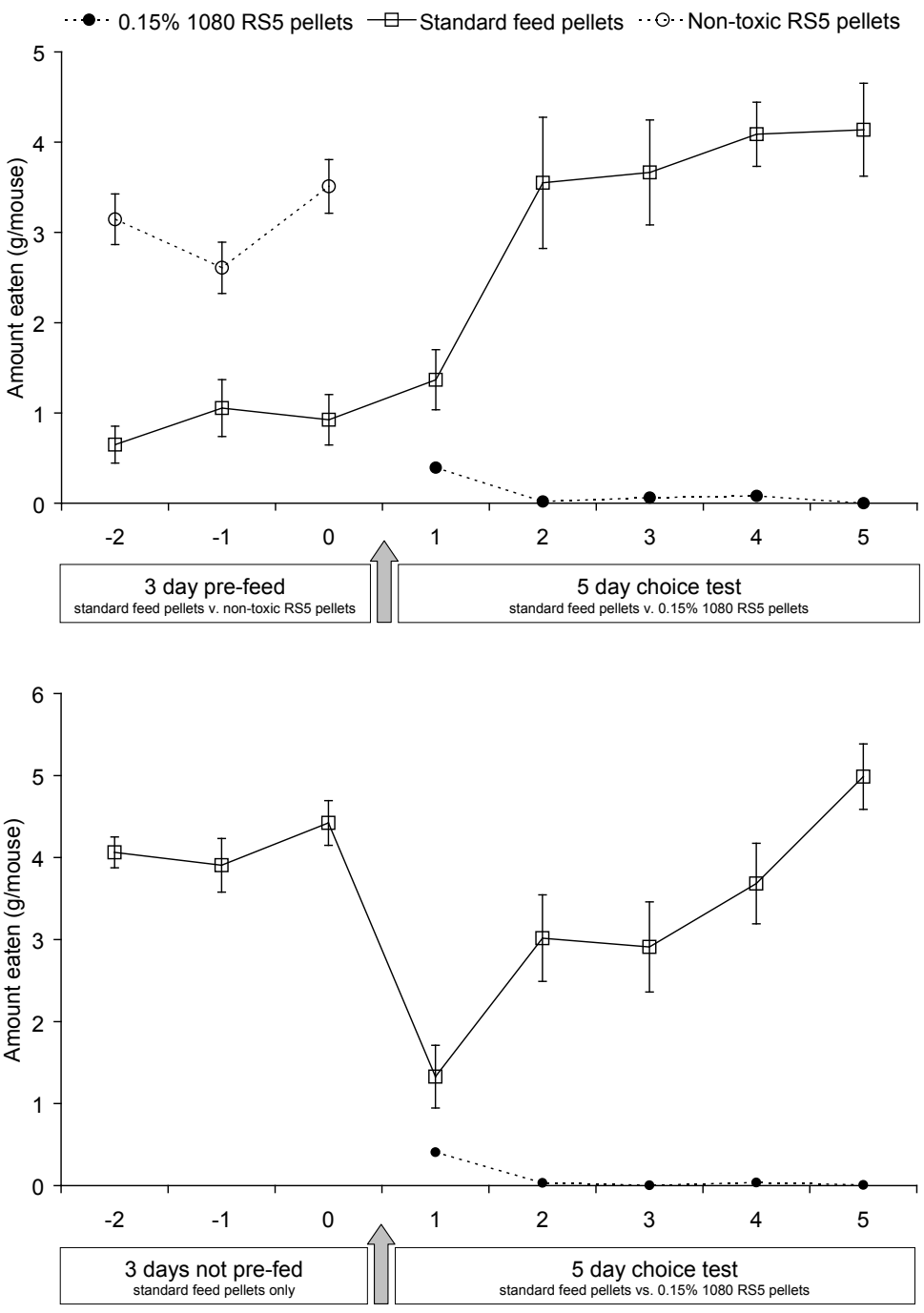


Figure 3. Mean (± SEM) daily intakes (g/mouse) of baits by mice (*Mus musculus*) over a 5-day two-choice test period (Trial 3). A. Non-toxic RS5 and standard feed pellets offered to 12 mice during a 3-day 'pre-feed' phase, and subsequent daily intakes of 0.15% 1080 RS5 pellets and standard feed pellets over a 5-day two-choice test period. B. standard feed pellets by 12 mice during a 3-day, no-choice (not pre-fed) phase, and subsequent daily intakes of 0.15% 1080 RS5 pellets and standard feed pellets. Note that the SEMs are too small to be shown on the 1080 pellet data.

4.4 TRIAL 4: ACCEPTANCE OF NON-TOXIC MICROENCAPSULANT FORMULATIONS

The non-toxic challenge diet was strongly preferred by mice over both non-toxic microencapsulant bait types (Fig. 4). The palatability of Bait A was estimated as 0.027% and Bait B as 0.053%. There was no statistically significant difference between the amounts of microencapsulant bait eaten by the two groups ($\chi^2 = 1.20$, df = 4, $P = 0.274$).

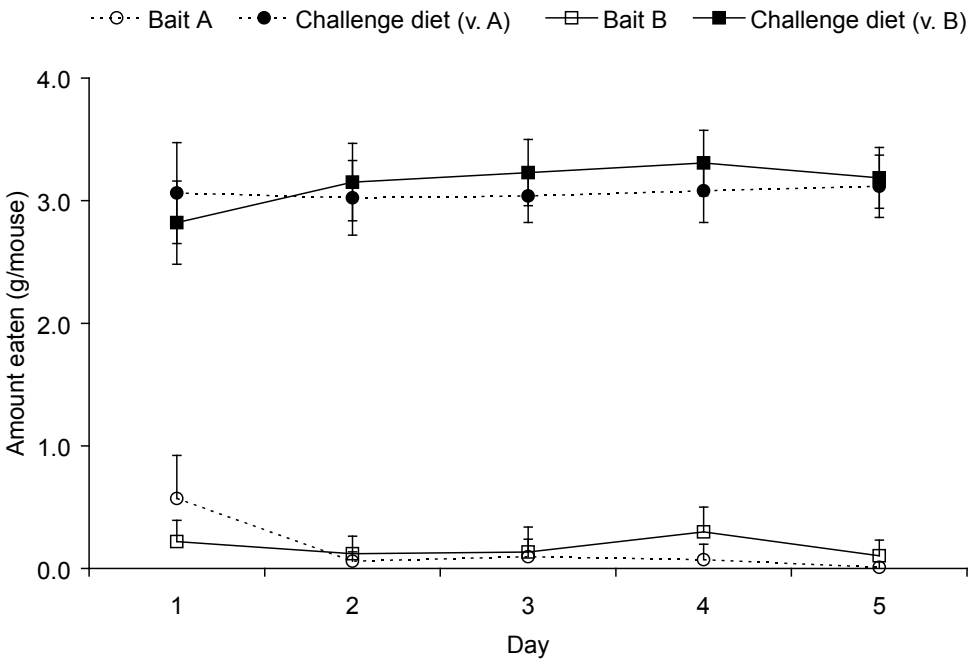


Figure 4. Mean (± SEM) amounts (g/mouse) of microencapsulant test baits and challenge diet eaten daily by groups of mice ($n = 9$ each) during two separate 5-day choice tests (Trial 4).

5. Discussion

Overall, the non-toxic RS5 formulation was well accepted by mice, with control groups of Trials 1 and 2, and the pre-fed group of Trial 3 showing a clear preference for the non-toxic RS5 pellets over the standard laboratory pellets. Our results suggest that the addition of 1080 to the RS5 formulation substantially reduces its acceptability to mice. The very low acceptance by mice of bait containing 0.08% 1080 in Trial 1 and of bait containing 0.001% 1080 in Trial 2 supports the finding of O'Connor et al. (2005) that mice can detect and avoid 1080 in cereal pellet bait.

The concentration of 1080 in food probably influences acceptance, as mice in Trial 2 removed and ate comparatively more 0.001% 1080 pellets than mice presented with 0.08% 1080 pellets in Trial 1. However, this difference was not sufficient to produce a corresponding increase in mortality in Trial 2, as considerably larger intakes of food containing 0.001% 1080 than were eaten here would be required for mice to ingest a lethal dose. Oral LD_{50} values reported for 1080 in mice are variable: 4 mg/kg (Fairchild 1977); 8.3 mg/kg (McIlroy 1982); 17 mg/kg (Fairchild 1977); and ranging between 2.6 and 12.8 mg/kg depending on the ambient temperature (Oliver & King 1983). Emlen & Strecker (1951) concluded that laboratory tests of 1080 toxicity in mice were likely to be indicative of its lethality in field conditions. Assuming a body weight of 20 g, and using the highest ('least susceptible') LD_{50} value of 17 mg/kg, our test mice would each have to have eaten approximately 0.425 g of 0.08% 1080 bait (Trial 1), or 34.0 g of 0.001% 1080 bait (Trial 2) for 50% of the population to be killed.

Mice are considered intermittent feeders, taking small but frequent meals. Witmer & Jojola (2006) estimated food intake at 10% to 20% of body weight per day; and Clapperton (2006) estimated that mice need to eat approximately 17% of their body weight per day to maintain mass. In other feeding trials, mice have been reported visiting a food tray approximately 200 times per night, taking about 20 mg of food per visit (Lund 1988). Despite limitations to the accuracy with which bait intake could be measured in Trials 1 and 2, the three mice that died in Trial 1 were each estimated to have eaten a total of < 0.1 g to 0.2 g of 0.08% 1080 pellet bait within the first 3 days of the two-choice test. The nine surviving mice were each estimated to have eaten a total of < 0.1 g to 0.1 g over the 5 days. So although quite small quantities of the 0.08% bait (e.g. 0.425 g, as estimated above) were expected to produce mortality, most mice in Trial 1 did not even eat sufficient for this. The mice that died may have had higher susceptibility than surviving conspecifics to 1080 and/or a reduced ability to detect it before consuming a lethal amount. The feeding habits of mice (large numbers of small meals) also suggest that the current strategy for aerial possum and rat control, i.e. relatively low sowing rates (2–5 kg/ha) of relatively large baits (6–12 g), may also contribute to inconsistent mouse control by limiting opportunities for all mice to find sufficient toxic bait soon enough to cause mortality.

Other species will also avoid normally acceptable food following the addition of 1080. Morgan (1982) suggested that some possums may have an aversion to the smell or taste of 1080, and others will avoid 1080 baits after surviving a sublethal exposure. Similarly, Sinclair & Bird (1984) attributed the refusal

of hungry dunnarts (*Sminthopsis crassicaudata*) to eat 1080-treated meat to an olfactory aversion, a taste aversion, or a bait-shyness response following previous sublethal exposure. McFadden & Towns (1991) reported that kiore (*Rattus exulans*) avoided eating kibbled maize baits containing 0.08% 1080, after readily eating dyed, non-toxic prefeed baits during an eradication attempt on Rurima Rocks and Korapuki Island. It was unlikely that this kiore population had experienced previous exposure to 1080, suggesting that they were able to detect and avoid 1080 without previous experience. Since the mice in our trials were captured from habitats close to areas of human habitation, their responses were considered unlikely to be a result of previous exposure of the population to 1080.

The mechanism(s) through which mice detect 1080 and reject it as a toxic component of otherwise demonstrably palatable food (e.g. non-toxic RS5 pellets) remains unclear, but taste and learned aversion seem more likely explanations than odour cues. From a human perspective, 1080 is stated to have no odour (Rammell & Fleming 1978) or a slight acetate smell (Pattison 1959). While the videotaped behaviours in Trial 1 were not taken from a suitably large sample size to make formal experimental comparison, mice did not appear to show less initial investigation of the 0.08% 1080 pellets or reduce their investigation of them over the first 3 h of the two-choice test, as might have been expected if the 1080 presented an unattractive odour. The mice made similar numbers of visits to 0.08% w/w 1080 pellets as to non-toxic food and spent more time, on average, 'visiting' the 1080 pellets.

While it could not be discerned from the videotapes whether the mice were eating pellets from either feeder (as opposed to merely sniffing or investigating them), our observations suggest that the mice were showing neophilic responses to new food types. This could have been as part of a cautious 'micro-sampling' feeding strategy. In Trial 2, mice ate the greatest daily amount of 0.001% 1080 pellets on the first night of the two-choice trial, and also removed the greatest number of 0.001% pellets from the feeder on the first night (Fig. 2B). This could indicate initial sampling of the 0.001% w/w 1080 baits before the overall decline in the daily amounts removed and consumed, suggesting an association of taste with sublethal effects may have been a factor in determining avoidance after initial exposure. Lund (1988) suggested that taste is a more important associative cue than odour for development of aversion in rats.

There was a noteworthy effect of time (day) on the total food intake by mice in the presence of 0.08% 1080 pellets (Trial 1) or 0.15% 1080 pellets (Trial 3), but not 0.001% 1080 pellets (Trial 2). The apparent 'drop feed' effect in Trials 1 and 3 may have been due to mice initially sampling both non-toxic and 1080 treatments but recognising the presence of a toxic food type through experiencing sublethal effects soon after 'micro-sampling' sufficient of the 1080 treatment. If it took several bouts of micro-sampling and experience of sublethal effects for mice to discern which of the two choices was the toxic food type, e.g. through an association with taste, this could explain the 'drop feed' effect as a cautionary, adaptive response to the new presence of a toxicant in their environment. In the case of 1080, an acute poison with very high oral toxicity to most mammals, the difference between a lethal and sublethal intake of 0.08% or 0.15% bait for a mouse is likely to be in the order of milligrams.

Given the relatively high energy requirements of mice, a significant decrease in their intake of all food for up to 2 days would be expected to represent a growing metabolic cost. Therefore, it would be important for them to be able to discern toxic from non-toxic food within 3 days or so. In Trials 1 and 3, mice showed a gradual increase in total intake, almost entirely of non-toxic pellets, over the days following the initial 'drop feed', until total mean daily intakes were similar to those before exposure to 1080, and to those measured for control mice. This is consistent with the observation by Lund (1988) that 'poison shyness' in mice is more readily developed than bait shyness, so that mice become suspicious of any bait after sublethal poisoning, but this reaction then disappears in 1–2 days. In another study, Nachman & Hartley (1975) reported that laboratory rats injected intraperitoneally with a sublethal dose of 1080 after drinking a novel solution of sucrose developed strong sucrose aversions. Howard et al. (1977) conditioned deer mice (*Peromyscus maniculatus*) to avoid eating oat kernels treated with sublethal doses of 1080; when the conditioned deer mice were retested for aversion after 1, 2, 4 and 8 months without access to oats, none ate all four oat kernels offered each day (although 9% ate fatal amounts).

Compared with rats, mice are considered opportunistic and neophilic feeders (Lund 1988; Witmer & Jojola 2006). Pre-feeding is designed to overcome neophobic responses to an unfamiliar food type in a familiar environment, and to optimise the subsequent efficacy of using that food to deliver poison to a pest population. Pre-feeding can increase kill rates for possums (e.g. Coleman & Fraser 2006) and is generally recommended where acute toxicants are used for commensal rodent control. However, our results showed that pre-feeding mice with non-toxic RS5 cereal pellet baits was not followed by an increase in acceptance of the same bait type containing 0.15% 1080, suggesting that previous exposure to the bait type is unlikely to be an important factor determining acceptance of 1080 baits by mice. On the basis of the results reported here, we believe a micro-sampling strategy, taste and learned poison shyness are responsible for poor 1080 bait acceptance by mice.

While the components of the non-toxic microencapsulant bait types A and B were unknown (as the formulation of the bait was unknown), the finding that these were unpalatable to mice in Trial 4 highlights the importance of ensuring that new bait matrices used for delivery of poison have a suitably high acceptance to the target pest species before extensive development of toxic formulations is undertaken. If learned taste avoidance is one basis of poor efficacy of 1080 baits against mice, then microencapsulant formulations may need to delay onset of the signs of poisoning (to weaken the association of illness with bait ingestion), as well as mask taste or odour cues. If the rapid onset of the sublethal effects of 1080 is the driving cue for a 'stop feed' survival response in mice, delaying these effects should be the focus of improved delivery formulations. Simply masking the taste of 1080 or improving the palatability of the bait matrix may not be effective if mice can use a micro-sampling strategy to quickly associate sublethal effects of 1080 with any distinctive taste of a bait type and avoid the bait thereafter.

6. Conclusions

In the presence of alternative, acceptable non-toxic food, mice appear to have feeding strategies that allow them to detect and avoid 0.08% or 0.15% 1080 in pellet baits, which may partially account for low field efficacy of 1080 baiting against wild mouse populations in the context of multi-pest species management in New Zealand. If 'micro-sampling' of bait by mice is part of the mechanism by which they avoid 1080-treated food, this problem may be addressed by increasing the concentration of 1080 in baits, or by presenting an effective lethal dose of 1080 in one 'micro-sample' portion of a bait (rather than 1080 being uniformly distributed throughout the bait matrix). These approaches could be tested using smaller-sized baits that may be more readily handled and, potentially, more quickly consumed by mice.

Our results suggest that mice readily accept the non-toxic RS5 bait formulation, but inclusion of 1080 in this bait matrix significantly reduces acceptance, probably due to an association of sublethal effects with a taste imparted by 1080. Hence, modification of taste or odor cues presented by 1080 baits are less likely to improve efficacy against mice than a presentation that can delay the onset of the effects of 1080 poisoning in this species.

Pre-feeding with non-toxic bait did not improve the efficacy of the bait against mice, presumably due to some taste or odour cue imparted by the inclusion of 0.15% 1080 that was then associated by mice with sublethal effects of toxic bait ingestion. Pre-feeding mice with bait containing a non-toxic substance that has similar taste or odour to 1080 may reduce the likelihood of mice developing a learned aversion before ingesting a lethal amount of toxic bait, thereby increasing bait efficacy.

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