



Manaaki Whenua
Landcare Research

What concentrations of potential kea repellents in 1080 bait least affect possum and rat control efficacy?

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What concentrations of potential kea repellents in 1080 bait least affect possum and rat control efficacy?

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Summary

Project and client

- Manaaki Whenua – Landcare Research (MWLR) was contracted by OSPRI to identify the optimal concentrations in 1080 bait of known bird repellents that would reduce the poisoning risk to non-target birds, particularly kea, without an adverse effect on possum control efficacy.
- This report summarises the results from testing the repellent compounds, first on captive possums and rats in pen trials, and then on wild possums and rats in field trials.
- The trials were undertaken between April 2019 and May 2020, and write-up was completed in September 2020.

Objectives

- Determine the highest concentrations of previously identified potential kea repellents that could be incorporated into 1080 cereal baits without significantly reducing uptake by, and lethality to, captive possums and rats
- Determine, in a field test with ground-laid toxic 1080 bait, the effectiveness of two different candidate repellent-concentration combinations in reducing the abundance of wild possums and rats.

Methods

- Three candidate repellents were added to the RS5 cereal bait widely used in 1080 operations and tested for repellency to captive possums and rats. The repellents tested were:
 - 0.17% D-pulegone (DP)
 - 0.04% and 0.08% anthraquinone (AQ), in both 'old' and 'new' formulations
 - 2% and 4% tannic acid (TA).
- The repellents were either:
 - infused (I) throughout the bait matrix (all)
 - surface coated (SC) (DP and AQ) or
 - both infused and surface coated (I & SC) (DP only).
- Animals were offered a choice between a standard maintenance food (pellets) and either one of the repellent formulations or (as a control) non-repellent RS5.
- Bait consumption was monitored for 5 nights (with a 5-night gap between the 3rd and 4th nights to simulate the usual gap between pre-feeding and toxic baiting in an aerial 1080 operation, and to check for learned aversion after first exposure).
- In non-toxic trials, non-toxic bait was presented on all nights, whereas in the toxic trials bait containing 1080 was presented on the 4th and 5th nights. Bait acceptance (percentage of animal nights during which some of the bait was eaten), bait consumption (the weight of bait consumed per animal per night), palatability (the

relative consumption of bait and pellets), and, in toxic trials, lethality (the percentage of animals killed) were measured to compare repellent formulations.

- To determine whether adding repellents might reduce possum and/or rat kill, the control efficacy of the two most promising repellent formulations identified from pen trial outcomes and previous research, 4% TA and 0.08% AQ (in a new formulation [nf]), was compared to that for non-repellent RS5 bait in two groups of three 80 ha blocks in Southland.
- Each block was pre-fed with non-toxic baits and then subsequently poisoned with toxic (1080) bait. All baits were sown by hand. Control efficacy was assessed using tracking tunnels, chewcards, wax tags, and trail cameras. Post-poison monitoring was disrupted by the Covid-19 lockdown.

Results

- For possums, and across both toxic and non-toxic trials, the acceptance, consumption, and palatability metrics for 2% & 4% TA, 0.17% DP SC and 0.04% AQ-nf were close to or higher than those for non-repellent RS5. Modestly lower acceptance and lethality were recorded for 0.08% AQ-nf, and modestly lower palatability was recorded for 0.017% DP I & SC. Ultimately, the proportion of possums killed with any of the five repellent treatments for lethality did not individually differ from that for non-repellent RS5 ($p > 0.65$).
- Rats preferred pellets to bait, so all bait uptake metrics were lower than for possums. In the non-toxic trials all metrics for the two DP formulations tested were lower than for RS5. The same was true for all of the nine formulations tested in toxic trials, with evidence of reduced lethality ($p < 0.6$) for four of them (0.17% DP SC, and three 'old' AQ formulations). However, the lethality of 2% & 4 TA, and 0.04% & 0.08% AQ-nf matched that for RS5 ($p > 0.69$).
- In the field test, all three treatments resulted in major (>90%) reductions in possum abundance, with the number of possum visits to camera sites reduced to near zero immediately after the 1080 baiting, with no difference seen between treatments. For rats, trail camera monitoring showed a marked reduction (>90%) in activity in the 2 weeks immediately after 1080 baiting for all three bait types. However, rat activity then increased, with more rapid increases in the c. 80 ha blocks in which repellent bait was used (ANOVA, $p < 0.01$). The pattern was similar for mice.
- For all native birds combined, there were similar levels of activity over 6 weeks before and 6 weeks after 1080 baiting, whereas for all introduced birds combined (mainly thrushes and blackbirds) there was some reduction in activity, particularly where non-repellent bait was used.

Conclusions

- Possums clearly preferred RS5 bait to the plain cereal pellets, whereas rats preferred the pellets. That difference resulted in reduced consumption, palatability, and lethality for rats, suggesting the possibility of low control efficacy when alternative preferred food is abundantly available. In the non-toxic trials, bait acceptance and uptake increased on second exposure for all bait types for both possums and rats, so there was no indication that any of the repellents were acting as secondary repellents (i.e. none of them appeared to induce bait aversion as a result of illness after initial consumption).
- Overall, none of the repellent formulations tested had a major adverse effect on bait uptake by, and lethality to, captive possums. In contrast, the almost universally lower metrics (than for non-repellent RS5) for captive rats suggests that rats are much more readily repelled than possums. Despite this, rat lethality was not greatly reduced by the TA and AQ-nf repellents. The highest concentrations of each (4% TA and 0.08% AQ) were therefore chosen for field testing.
- Neither 4% TA nor 0.08% AQ had any detectable major effect on the efficacy of 1080 baiting in killing possums. In contrast, there was some loss of control efficacy for rats, but the effect appears to be modest, with no major difference between the two repellent formulations.
- The apparent smaller reduction in the activity of blackbirds and thrushes combined in the blocks where repellent 1080 bait was used suggests the candidate repellents did provide some protection to that group of bird species.
- Overall, neither repellent formulation poses a major threat to the level of possum control efficacy required for TB-related possum control. Although both repellent formulations may reduce efficacy against rats, most rats were killed. If rat numbers are low (as is typical between mast years), the use of either repellent formulation would still result in extremely low rat numbers. In mast years, however, rat numbers can be extremely high, which could unacceptably amplify the reduction in rat control efficacy.

Recommendations

- Testing the effectiveness of TA and AQ in deterring kea from eating non-toxic baits should be undertaken. Testing on captive kea is possible, but the number of birds available for that is low (DOC, pers. comm.), limiting the number of formulations that could be tested.
- An alternative option would be to use trail cameras to monitor non-toxic bait acceptance by wild kea. The feasibility of that approach is not known, but, if feasible, a much wider range of formulations could be tested. It would also avoid the risk that the responses of well-fed captive birds differ from those of wild birds.

1 Introduction

Manaaki Whenua – Landcare Research (MWLR) was contracted by OSPRI to identify the optimal concentrations of known bird repellents in 1080 bait that would reduce the poisoning risk to non-target birds, particularly kea, without an adverse effect on possum control efficacy. This report summarises the results from testing the repellent compounds, first on captive possums and rats in pen trials, and then on wild possums and rats in field trials. The trials were undertaken between April 2019 and May 2020 and write-up was completed in September 2020.

2 Background

OSPRI aims to eradicate bovine tuberculosis (TB) from possums (*Trichosurus vulpecula*) and other wildlife by 2055 (OSPRI 2020). In large areas of forest and mountainland in the South Island, aerial 1080 baiting is by far the most cost-effective tool for reducing the density of possums, the primary wildlife host of TB, to the low densities needed to break the TB cycle (Warburton & Livingstone 2015). However, an iconic native bird, the kea (*Nestor notabilis*), also occupies much of the area involved. While 1080 baiting can increase kea reproductive success by removing possums and other nest predators such as stoats (*Mustela erminea*) (Kemp et al. 2018), kea are sometimes also killed incidentally during such operations (Kemp et al. 2019).

As a result, the Department of Conservation (DOC) has imposed major constraints on where and when aerial 1080 baiting can be used (DOC 2020). Most importantly for OSPRI, DOC's current code of practice restricts aerial 1080 baiting of possums in areas where kea are present to mast years – years in which seedfall from beech trees and other species is exceptionally abundant, resulting in greatly increased rodent and predator numbers. In such years, kea breed more prolifically. If possums, rats, and other predators are reduced by 1080 baiting, a greater proportion of chicks survive, more than offsetting the risk of 1080-related kea mortality. Outside mast years, there is little kea breeding to offset that risk (Kemp et al. 2018).

The problem for OSPRI is that maintaining the low possum densities needed for TB eradication requires sustained control, which has traditionally been achieved by repeating aerial 1080 baiting two to three times at c. 4–5-year intervals (Warburton & Livingstone 2015). However, mast events occur at irregular intervals 2–6 years apart. Those events can be predicted, but only by about a year or so in advance (Kelly et al. 2013). It is therefore logistically difficult for OSPRI to schedule a long-term programme of sustained control with effort and expenditure spread evenly over time in which different groups of areas are poisoned each year on a 5-year cycle.

One way of overcoming this difficulty could be to reduce the risk of incidental kea mortality by making the 1080 baits less palatable to kea by adding a repellent, and there has been a substantial body of work undertaken to try to achieve this (Orr-Walker et al. 2012; Reardon 2014; Cowan et al. 2015; Van Klink & Crowell 2015; Cowan et al. 2016; Crowell, Martini et al.

2016; Crowell, Booth et al. 2016). While this research has identified a number of candidate repellents, questions remain about the maximum concentrations that are acceptable to rats and possums (Cowan et al. 2016; Crowell, Booth et al. 2016), with the result that there is still no operational solution available to OSPRI that deters kea from eating toxic bait without substantially reducing the effectiveness of 1080 baiting in reducing possum (and preferably also rat) densities.

The first aim of this project was therefore to identify, through testing on captive possums and rats, the maximum concentration (in cereal bait) at which several candidate kea repellents remained palatable and lethal to possums and rats. The second aim was to assess the likely effect of the two most promising repellent concentrations on the mortality of possums, rats, and other species in a small-scale field trial. The goal was to identify a repellent 1080 bait that could feasibly be deployed on a large scale in mountainland forests, which could then enable OSPRI to use aerial 1080 baiting at times best suited to TB eradication.

3 Objectives

- Determine the highest concentrations of previously identified potential kea repellents that could be incorporated into 1080 cereal baits without significantly reducing uptake by, and lethality to, captive possums and rats
- Determine, in a field test with ground-laid toxic 1080 bait, the effectiveness of two different candidate repellent-concentration combinations in reducing the abundance of wild possums and rats.

4 Methods

4.1 Candidate repellents and concentrations

We identified three candidate repellents (tannic acid, anthraquinone, and D-pulegone) through a literature search and through consultation with key researchers involved in previous kea repellent projects, most notably a former MWLR colleague Phil Cowan (Cowan et al. 2015) and Michelle Crowell, DOC (Crowell, Booth et al. 2016).

Anthraquinone (AQ) (Chemical Abstract Services chemical identification number; CAS 84-65-1) is a specific isomer (9,10-anthraquinone) of an aromatic organic compound with the chemical formula $C_{14}H_8O_2$. It is a yellow, highly crystalline solid, poorly soluble in water, and is used in dye making. It has been used as a bird repellent since the 1940s (DeLiberto & Werner 2016). It does not appear to repel birds on initial contact or have an unpleasant taste, but causes post-ingestional illness (including vomiting), so anthraquinone repellency appears to be a learned behaviour (Avery et al. 1997). It is therefore classed as a secondary repellent (Day et al. 2012). At high concentrations (2.7%) it has been used to induce conditioned aversion in captive kea (Nichols et al. 2020). Captive kea have also been shown to be much

less likely to eat baits containing 0.1% AQ (plus 0.17% D-pulegone) than untreated baits (Orr-Walker et al. 2012).

D-pulegone (DP) (CAS No. 89-82-7) is a volatile peppermint compound ($C_{10}H_{16}O$) that has been used as a synthetic flavouring substance in food. It is repellent to a number of vertebrates, including mammals (Wager-Pagé & Mason 1996). It is classed as a primary repellent, and, in a New Zealand study, at a 2% concentration initially reduced wheat consumption by sparrows by almost 50% on the first day of exposure, but by lesser amounts at lower concentrations and on successive days (Day et al. 2012). Most trials undertaken since early 2000 have focused on using 0.17% DP (albeit mostly in combination with AQ), first on robins and tomtits (Day et al. 2003; Clapperton et al. 2014), then on captive kea (Orr-Walker et al. 2012). All reported reduced feeding of repellent baits by the target species, and that, for captive kea, the repellent effect could be maintained by DP alone.

Further research on captive possums and rats showed that palatability and consumption of 0.17% DP RS5 baits were similar for standard untreated RS5 baits, and therefore bait uptake by those species was unlikely to be affected in an operational control setting (Cowan et al. 2015). Based on these results, the effectiveness of DP in reducing kea mortality was operationally tested in the field (Van Klink & Crowell 2015), but 15% of 34 kea died in that trial. The apparent lack of any effective repellency may have reflected the 60% lower concentration (0.07%) in the 1080 bait when it was sown compared to the intended concentration of 0.17%, with the difference probably resulting from the well-known volatility of DP (Crowell, Booth et al. 2016). Subsequently, New Zealand's main 1080 bait manufacturer (Orillion) developed a more stable form of DP, so we tested that product.

Tannic acid (TA) (CAS 1401-55-4) is a specific form of tannin with a number of naturally occurring forms, sometimes represented as $C_{76}H_{52}O_{46}$. It may act as both a primary and a secondary repellent (Cowan et al. 2016). Tannins defend some plants against browsing by inhibiting herbivore digestion. Adding 20% TA to possum diet reduced consumption markedly, but initial responses began at 5%, suggesting that possums were unaffected by lesser concentrations (Burchfield et al. 2006). Although TA has not been tested on kea, it has been shown to reduce food consumption in a number of bird species, particularly at higher concentrations of up to 5% (Cowan et al. 2016). We therefore tested two concentrations (2% and 4%) below that level.

In addition to testing baits in which the repellents had been incorporated (i.e. infused) into the bait matrix during the manufacture process (as is standard practice), we also tested AQ baits that had been surface coated with the repellent, and DP baits that had been both surface coated and infused with the repellent (Table 1).

The pen trials were undertaken in MWLR's Animal Research facility at Lincoln under animal ethics approval (AEC Approval No. 18-12-03).

4.2 Captive pen trials

The pen trials consisted of a series of non-toxic and toxic 'two-choice' trials. These were structured to mimic pre-fed aerial 1080 poisoning operations in which non-toxic RS5 bait is

sown as a pre-feed, followed 5–10 days later by 1080-laden RS5 bait. The trials followed the protocols used by Cowan et al. (2015).

Captive possums ($n = 19–22$) and rats ($n = 6–12$) of known sex and body weight were offered a standard maintenance food (generic possum and rat feed pellets) and treatment food (Orillion's RS5 pellets with the designated repellent treatment) for 3 nights, returned to plain food for 5 nights, and then again offered a choice of standard and treatment food for 2 nights. For convenience, the standard maintenance food is hereafter referred to as 'pellets' and the RS5 treatment food as 'bait'. Possums and rats were each offered 70–100 g and 20 g, respectively, of pellets and bait nightly.

In the non-toxic trials, both the initial (nights 1–3) and second (nights 4 & 5, starting 5 days later) offerings of bait consisted of the various non-toxic RS5-repellent formulations. In the toxic trials, the initial baits offered were again non-toxic, but the second baits offered were toxic. Difficulties obtaining the required number of animals (mostly rats) resulted in some formulations being tested only in toxic trials. Details of the trials undertaken, and the numbers of possums and rats tested, are outlined in Table 1.

Baits were assayed to determine the concentrations of repellent compounds and 1080 (Appendix 1), but sometimes (as a result of manufacturing delays) this could not be done until during or after the trials were started. This testing identified a labelling error with non-toxic TA baits, with one batch labelled as 4% in fact containing c. 2%, and vice versa. However, the toxic TA baits in that trial were labelled correctly. Those treatments are therefore labelled 4%/2% TA and 2%/4% TA in Table 1 and in the results. In addition, these assays also detected a 10-fold error in the 1080 concentrations in the five bait formulations provided by Orillion in October 2019 (Appendix 1).

We also trialled a new AQ formulation thought likely to have greater bird repellency than the previously used formulation at equivalent concentrations (W. McCook, Orillion, pers. comm.).

In each of the trials, the weights of bait and pellets remaining after each of the 5 trial nights were recorded, both in the cages containing animals and in three empty cages with no animals. The data from the empty cages were used as environmental controls to correct for bait weight loss due to evaporation/absorption (i.e. the mean percentage change in weight of bait and pellets from the empty cages was used to adjust the weights of bait and pellets remaining in the cages containing animals). Negative adjusted weight changes were set to zero, as were weight changes of <0.1 g/night for rats and 0.3 g/night for possums, as these were considered likely to result from measurement error rather than actual consumption.

We used four metrics to compare treatments.

- *Acceptance*: the percentage of possum nights during which each animal ate at least some bait. This was assessed separately for the two multi-night periods during which bait was offered (i.e. exposure nights 1–3 and 4 & 5, respectively). It was simplistically assumed that possum nights were largely independent.
- *Consumption*: the mean weight of bait and pellets eaten per night.

- *Palatability*: the weight of bait eaten as a percentage of the total weight of food (bait+ pellets) eaten. A palatability score was calculated for each animal for each night on which that animal ate at least some food.
- *Lethality*: the percentage of animals that died during the first and second nights (and overall) was recorded in the toxic trials.

Table 1. Summary of treatments, trial dates, and numbers of animals used in the non-toxic and toxic trials for possums and rats. The repellent treatment acronyms are: TA = tannic acid, DP = D-pulegone, and AQ = anthraquinone (with -nf indicating a new formulation of anthraquinone). The 'I or SC' column indicates whether the repellent compounds were surface coated onto the baits (SC) or infused into the baits (I). In the toxic trials for possums, the mixed TA formulations (see text) are shown with the non-toxic concentration shown first and the toxic concentration second (e.g. 2%/4%).

Trial type	Species	Dates	Treatment	I or SC	Sample size (<i>n</i>)
Non-toxic	Possum	6/3–15/3/19	Non-repellent RS5	-	19
			TA 2%	I	20
			TA 4%	I	20
			DP 0.17%	SC+ I	20
			DP 0.17%	SC	20
Non-toxic	Rat	26/2–6/3/19	Non-repellent RS5		6
			DP 0.17%	SC+ I	12
			DP 0.17%	SC	12
Toxic	Possum	8/4–17/4/19	Non-repellent RS5	-	22
			TA 2%/4%	I	22
			TA 4%/2%	I	22
			DP 0.17%	SC	22
		14/10–24/10/19	AQ I 0.04%-nf	I	20
			AQ I 0.08%-nf	I	21/20
Toxic	Rat	2/4–11/4/19	Non-repellent RS5	-	12
			AQ S 0.04%	I	12
			AQ I 0.04%	I	12
			AQ S 0.08%	I	12
			AQ I 0.08%	I	12
		30/4–1/5/19	DP S 0.17%	SC	12
		14/10–24/10/19	Non-repellent RS5	-	12
			AQ I 0.04%-nf	I	12
			AQ I 0.08%-nf	I	12
			TA 2%	I	12
TA 4%	I		12		

4.3 Repellent effects on mortality of wild possums and rats

4.3.1 Design

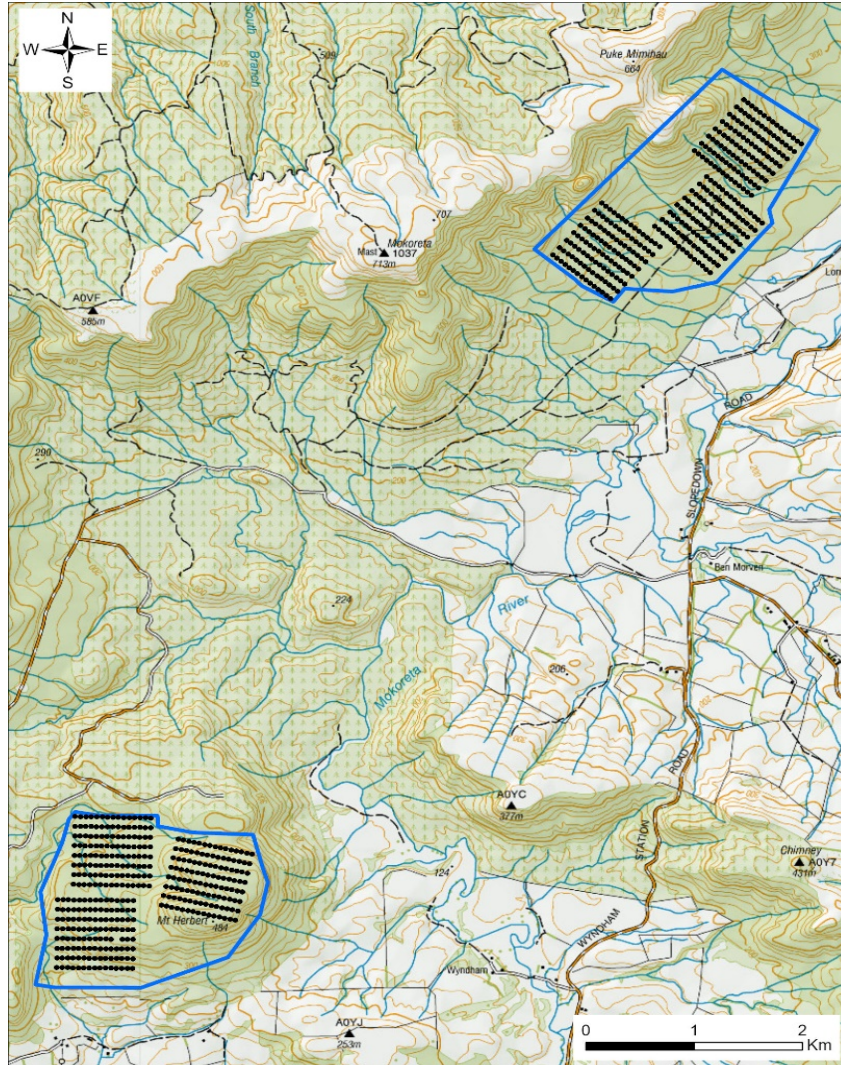
Two repellents (0.08% AQ and 4% TA) were chosen for field testing. The reasons for that choice are discussed in section 6.2.

The field trial involved a small-scale (<500 ha in total) hand-laid pre-fed 1080 baiting operation intended to simulate an aerial 1080 operation. It was undertaken in the Slopedown area, Southland (Figure 1a), in mixed broadleaf/hardwood forest, with low to moderate densities of both possums and rats. The trial began in November 2019, with 1080 baiting in late summer 2020. Post-1080 monitoring was disrupted and extended by the Covid-19 Alert Level 4 lockdown in March so could not be completed until late May 2020.

The field design consisted of two replicates of three treatments, located as two groups of c. 80 ha blocks, with each replicate comprising a non-repellent standard RS5 cereal bait block, a 4% TA repellent block, and a 0.08% AQ repellent block (Figure 1a). In each block, non-toxic 6 g pre-feed baits of the respective types were first sown (along eight 800 m-long transects at c.100 m spacing) at the rate of 1 kg/ha. Matching toxic 12 g baits were later sown at 2 kg/ha along the same transects 1–5 weeks later.

Pre-feeding was undertaken in mid-January 2020. Bait was broadcast by hand (i.e. baits were thrown laterally from the transects as far as possible). Sowing of the toxic 1080 bait commenced in late January, but only one block (a non-repellent treatment block) had been completed before poor weather caused an interruption that resulted in the remaining five blocks not being poisoned until late February.

(a)



(b)

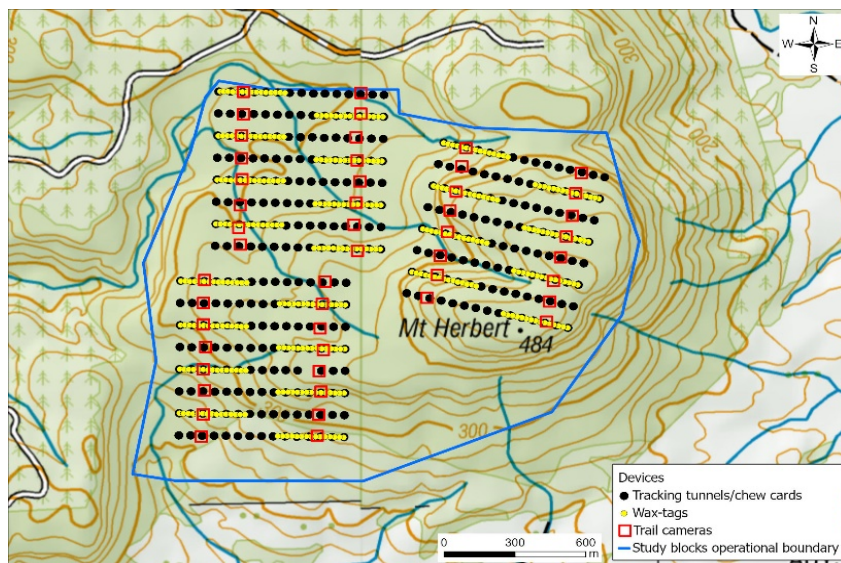


Figure 1. Maps of the study area in Sloedown, Southland, showing (a) the two groups of three study blocks and (b) the layout of chewcard and wax-tag monitoring transects and camera locations for one group of study blocks.

4.3.2 Assessing 1080 baiting outcomes

The relative abundances of possums and rats (and, incidentally, a range of non-target species) present at the Slopdown site was assessed before and after the 1080 baiting. In an effort to maximise our chances of detecting any difference between treatments, and recognising the potential limitations of the various monitoring tools available, we used four different monitoring indices:

- tracking tunnel indices (TTIs) (King & Edgar 1977) for detecting rats, mice and stoats
- chewcard indices (CCIs) (Sweetapple & Nugent 2011) and wax-tag indices (WTIs) (Thomas et al. 2003) for detecting possums, rats, and mice
- trail cameras (Glen et al. 2013; Dilks et al. 2020) for detecting both target and non-target species (such as birds and deer).

The tracking and bite-mark devices were deployed along 750 m of the eight baiting transects within each block, with tracking tunnels ($n = 120$) and chewcards ($n = 120$) spaced at 50 m intervals, and wax tags ($n = 120$) spaced at 20 m intervals (Figure 1b). The wax tags were deployed on two lines located at alternating ends of the transects, with the pattern reversed between pre-and post-control monitoring to minimise previous exposure to wax tags during the post-baiting period.

We originally planned to monitor chewcard and wax-tag bite-mark interference over both short (7-night) and long (c. 28–35 night) intervals before and after poisoning. However, the Covid-19 lockdown in late March 2020 prevented the planned 7-night check and extended the post-1080 assessment period to c. 55 nights. The pre-1080 data were therefore combined into a single c. 42-night index. It is presumed that this index will be biased low compared to the 55-night index, but not by nearly as much as the usual shorter-interval indices (Nugent et al. 2019).

Trail cameras (a mixture of Bushnell Aggressor and Bushnell Core DS cameras) ($n = 16$ per block) were deployed at the centre of each wax-tag line. Cameras were deployed at least 10 weeks before toxic baiting. Memory cards and batteries were changed when other devices were checked, and cameras were eventually recovered in mid- to late May, c.10 weeks after the main 1080 baiting. Images were scanned and the species, number of animals, and date and time of visits were recorded. A total of 81,501 animal images were recorded, of which 33% were of possums, 27% of rats, and 20% of mice. A further 22 animal species were also recorded. A visit was defined as a series of images separated by more than 5 minutes from any other such series of images of indistinguishable animals.

As the 1080 baits were deployed on different dates, images were classified according to the number of days before or after the 1080 deployment date, and then into fortnightly periods before or after. Cameras that were not functional for most of the c. 6-month period we monitored were excluded from these analyses.

We also attempted to compare between bait types the behavioural responses of possums and rats to baits when they first encounter them. For this, a single, non-toxic 12 g bait (using the designated repellent treatment for the particular block) was nailed to a tree (or tree root)

at ground level at a distance of c. 0.5–1.5 m from each camera. These baits were deployed a few days ahead of when pre-feed was sown.

The effects of the various treatments on the relative abundances of species were assessed by comparing pre- and post-poison indices. For interference indices (TTIs, CCIs and WTIs), the line was used as the sampling unit, while for the photographic indices, each camera was used as an independent sampling unit. Some of the per-line interference indices (CCIs and WTIs) were highly saturated (i.e. all or most devices on the line detected animals), so the per-line indices were Poisson-transformed in an effort to increase the linearity of the assumed relationship between the index and animal density (Caughley 1977).

Some per-line or per-camera index values increased from very low or zero levels before 1080 baiting to much higher levels after control, resulting in positive (and sometimes infinitely high) estimates of the percentage reduction, so simple percentage-reduction comparisons could sometimes be biased high by just one or two extreme data points. A relative-change-in-activity index (RCAI; Nugent et al. 2011) was therefore calculated for each line or camera, as follows (using CCIs as an example):

$$\text{RCAI} = (\text{CCI}_{\text{post}} - \text{CCI}_{\text{pre}}) / (\text{CCI}_{\text{pre}} + \text{CCI}_{\text{post}}).$$

This metric is constrained to vary between -1.0 (*no* activity recorded after 1080 baiting) and $+1.0$ (*all* activity recorded after 1080 baiting), with a value of 0 indicating an equal number of bite marks, and therefore no apparent change in activity, recorded in the designated pre- and post-1080 intervals.

For the CCI and WTI data, the post-control indices were adjusted downward by simple linear scaling (i.e. 42/55) to account for the mean difference in the number of days that devices were deployed. The RCAI therefore nominally represents a comparison of activity over c. 7 weeks before and 7 weeks after poisoning. For trail cameras, we compared relative abundances over the 6 weeks immediately before poisoning and either 2 or 6 weeks immediately after (6v2-week and 6v6-week indices). The 6v6-week indices had greater precision as a result of increased sample size (numbers of images), but had greater potential to be affected by post-poisoning changes in abundance due to immigration or recruitment. For the 6v2-week RCAI, the count of post-poisoning images was multiplied by 3 so that pre- and post-poisoning counts were equivalent (i.e. so that an RCAI of 0.0 still indicated no apparent change in relative abundance).

5 Results

5.1 Repellency to captive possums and rats

5.1.1 Effect of repellents on non-toxic bait uptake

Possums

Non-toxic bait acceptance by possums was very high (>95%) for all five bait types and both periods, and was as high or higher for all repellent formulations than for non-repellent RS5 (Figure 2). Acceptance was also as high or higher on nights 4 and 5 than on nights 1 to 3 for all repellent bait types, so there was no evidence that 'pre-feeding' with repellent baits on nights 1 to 3 reduced subsequent bait acceptance by possums.

Mean consumption per night of bait by possums was higher than for pellets for all bait types (Figure 2). Similar consumption of non-repellent RS5 bait was recorded during the first and second exposure periods, whereas higher consumption was recorded during the second exposure for all four repellents. Consumption of repellent baits was mostly close to or higher than that for non-repellent RS5, except for the SC & I 0.17% DP formulation during the first exposure period. Mean palatability (excluding nights on which no baits or pellets were eaten) was likewise similar for all bait types in both exposure periods, except again for the SC & I 0.17% DP formulation. There was therefore no evidence that the 2% TA, 4% TA, and SC 0.17% DP formulations substantially reduced the willingness of possums to eat baits.

Rats

Full 5-night non-toxic trials were completed for only two formulations of DP (Figure 2). Rats were less inclined to feed than possums, with no food of either kind (pellets or bait) being consumed on 25–56% of rat nights during first exposure to any of the treatments (including non-repellent RS5). That reduced to just 12–17% during second exposure, indicating a greater willingness to feed with increased exposures. Bait acceptance was far lower (all formulations <42%) than for possums (all >92%), but with no major difference between treatments.

Mean consumption per night of bait by rats was lower than for pellets for all three treatments (Figure 2). There was a clear familiarisation effect, as mean consumption of both pellets and baits increased between exposure periods for all three treatments. The increased consumption was higher for pellets than for bait, resulting in slightly lower mean palatabilities being recorded during the second exposure period. As for possums, the lowest mean consumption was recorded for the SC & I 0.17% DP formulations, particularly during the first non-toxic period, and mean palatability was also lowest for that formulation.

5.1.2 Effect of repellents on toxic bait acceptance, consumption, and lethality

Possums

Acceptance of non-repellent RS5 and of most of the repellent formulations by possums was very high (>95%) during the non-toxic pre-feeding stage (Figure 3). The exception was 0.08% AQ 'old' formulation (84% acceptance). However mean consumption and palatabilities of baits were much the same for all treatments, and consumption was clearly higher than for pellets.

Acceptance of toxic bait was mostly slightly lower, and again lowest (74%) for the 0.08% SC AQ formulation. Consumption of toxic bait was about half that of non-toxic bait, and consumption of pellets reduced even more markedly, presumably because possums tended to eat the preferred food (bait) first, and then ceased feeding as toxicosis developed.

Higher mean nightly consumption and palatabilities were recorded in the toxic phase for all of the repellent baits than for RS5 bait, and lethality was high (>91%) for all bait types other than 0.08% SC AQ (85%).

The proportion of possums killed with any of the five repellent treatments did not differ from that for non-repellent RS5 (Fishers Exact Test [FET], $p > 0.65$ for all pairwise comparisons). Overall, even the highest concentrations of TA and AQ were eaten by, and subsequently killed, a high proportion of possums.

Rats

As in the non-toxic trials, acceptance of all bait types during the pre-feeding stage was lower than that for possums, and mean consumption of pellets was higher than mean consumption of bait in all but one of the 11 trials (Figure 4). Acceptance of the TA bait and five of the six AQ formulations was similar (56–75%) to that for RS5 (69%), but lower for the 0.08% AQ new-formulation and the 0.17% DP SC baits (30-42%). Contradictorily, the mean palatability of the latter matched that of RS5 bait, whereas the palatability for all other repellent baits was lower (Figure 5).

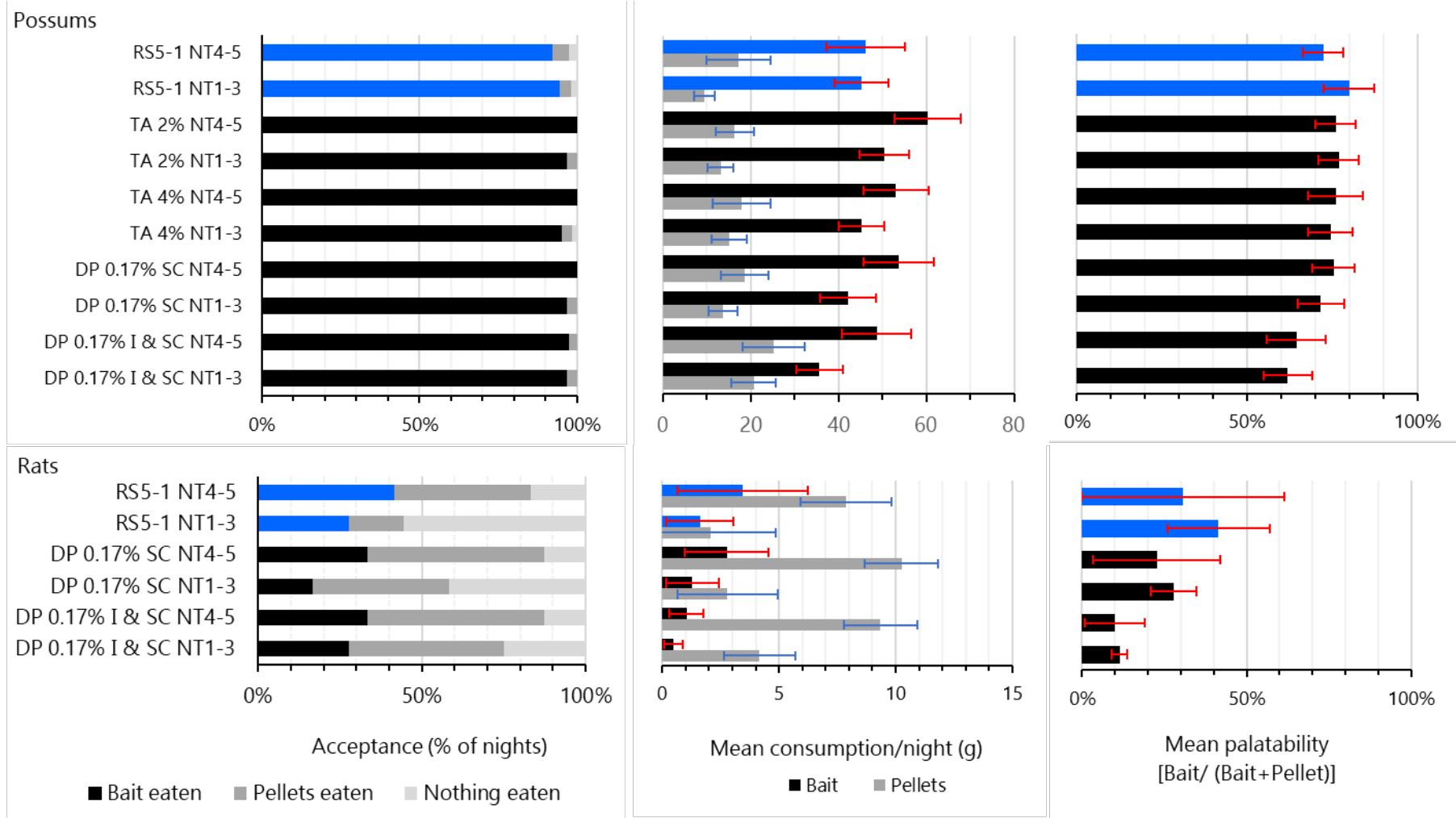


Figure 2. Acceptance, mean consumption (\pm 95% CL) and mean palatability (\pm 95% CL) of non-toxic cereal baits of different bait types and cereal feed pellets by possums (upper row) and rats (lower row), showing: the percentage of nights on which nothing was eaten, pellets but not bait were eaten, and bait was eaten (left panel); mean consumption of baits and pellets separately (central panel), and the mean palatability (per night) of baits for nights on which at least some food was consumed (right panel). Data are shown separately for the first 3 nights (NT1–3) on which bait was presented, and for the 4th and 5th nights (NT 4-5 [which were 5 and 6 days after night 3, respectively]). Data for non-repellent RS5 bait are shown in blue.

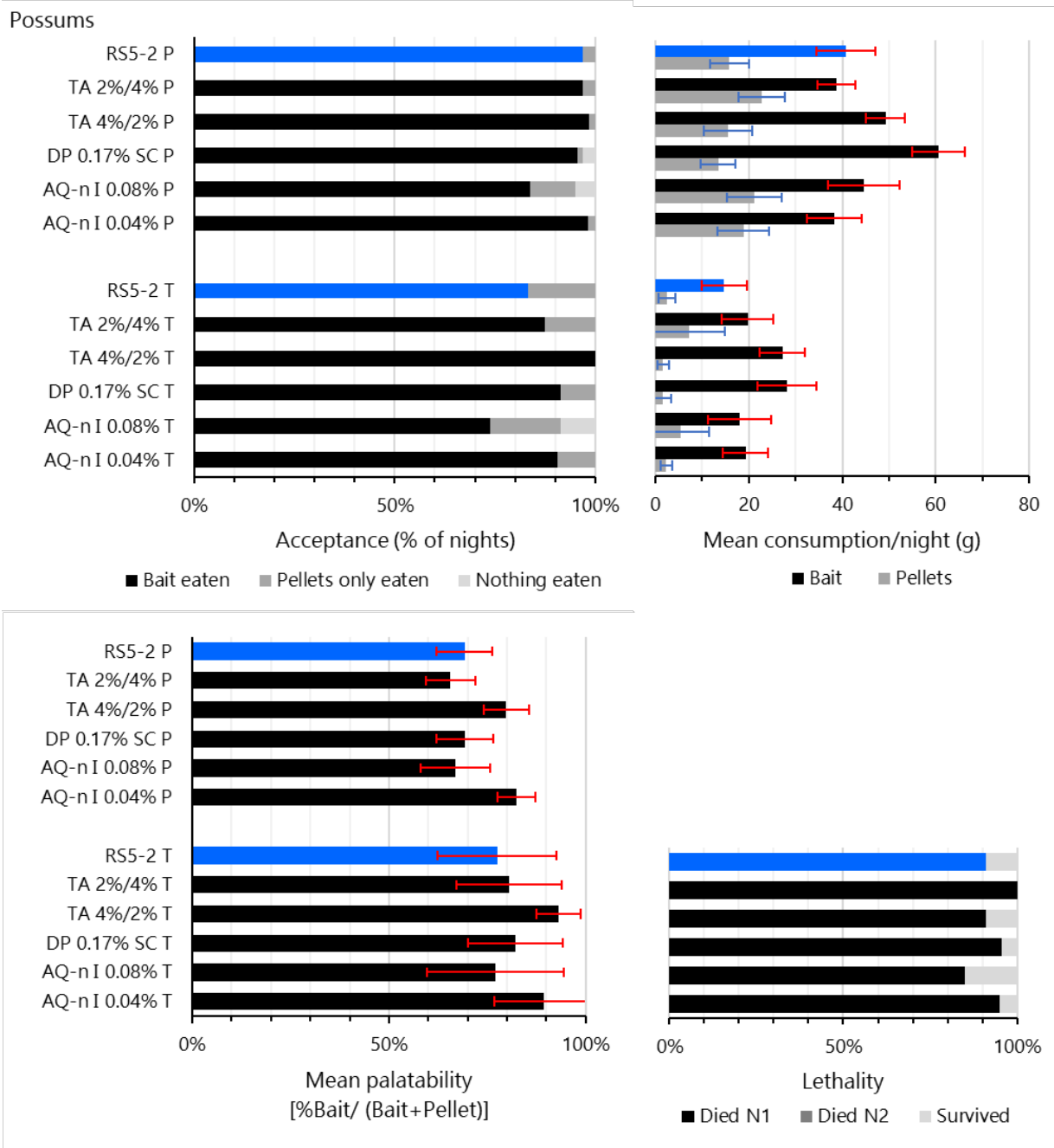


Figure 3. Acceptance, mean consumption (\pm 95% CL) and mean palatability (\pm 95% CL) and lethality of toxic cereal baits of different bait types and cereal feed pellets for captive possums, showing: the percentage of nights on which neither bait nor pellets were eaten, pellet but not bait were eaten, and bait was eaten (top left); mean consumption of baits and pellets separately (top right); mean palatability (per night) of baits for nights on which at least some food was consumed (bottom left); and lethality by night (bottom right; N1 = night 1, N2 = night 2). Data are shown separately for the initial exposure to non-toxic 'pre-feed' bait (P) and the subsequent exposure to toxic bait (T). The non-repellent treatment is shown in blue.

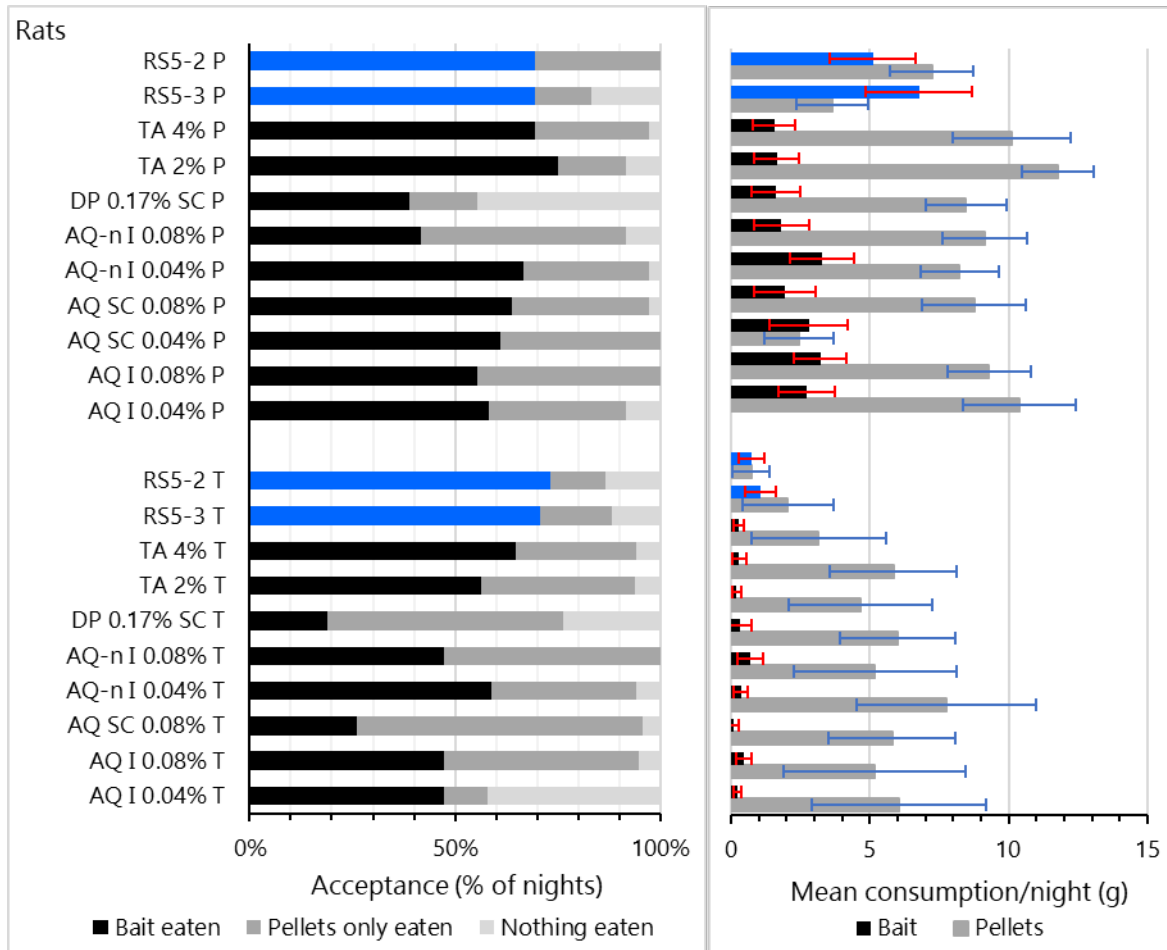


Figure 4. Acceptance and mean consumption (\pm 95% CL) of toxic cereal baits of different bait types and cereal feed pellets for captive rats. The data and layout are as for possums in Figure 3. The non-repellent treatments are shown in blue.

In the toxic phase, highest acceptance was recorded in the two RS5 trials (71 and 73%) and the lowest 0.08% AQ SC (26%) and the 0.17% DP SC baits (19%) (Figure 4). Consumption of bait was greatly reduced, but the reductions in consumption of pellets were less marked, except for in the RS5 treatments. Consequently, the highest mean palatabilities were recorded in the two RS5 treatments (Figure 5).

Pooled across two trials, 79% of 24 rats were killed with non-repellent RS5 bait. Lethality was similar to that for both concentrations of TA and both concentrations of the new formulation of AQ (FET, $p > 0.69$ for all four pairwise comparisons against RS5). In contrast, lethality was lower for three of the other four AQ formulations, and also for the DP formulation (FET, $p < 0.06$ for all four pairwise comparisons against RS5), with the 0.04% AQ SC formulation intermediate (Figure 5). The differences in lethality could plausibly have reflected the higher 1080 concentrations for all four 'high-lethality' repellent formulations (Appendix 1), but the lethality of non-repellent RS5 in the two trials appeared to be unaffected by 1080 concentration (83% and 75% of 12 rats killed with 0.13% and 1.4% 1080, respectively).

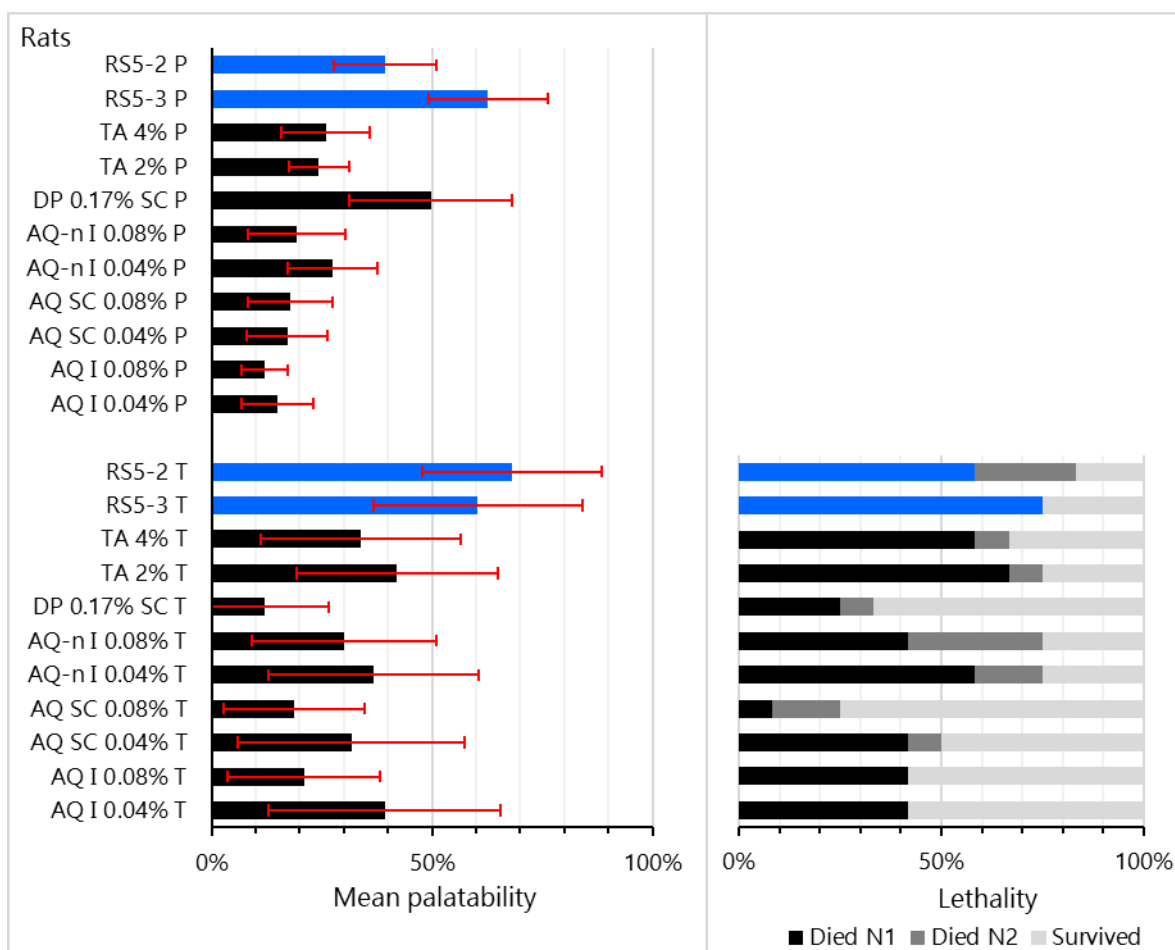


Figure 5. Palatability and lethality of non-toxic and toxic cereal baits of different bait types and non-toxic cereal feed pellets for captive rats, showing: the mean palatability (per night) of baits for nights on which at least some food was consumed (left), and lethality by night (bottom right; N1 = night 1, N2 = night 2). The palatability data are shown separately for the initial exposure to non-toxic 'pre-feed' bait (P) and the subsequent exposure to toxic bait (T). The non-repellent treatments are shown in blue.

5.1.3 Choosing candidate repellent formulations for field testing

After consultation with OSPRI (R. Curtis), DOC (N. Gorman) and Phil Cowan (MWLR Research Associate) the formulations chosen to test in the toxic field trial were the highest concentrations of AQ (0.08%) and TA (4%) (see section 6.2).

5.2 Repellent effects on control efficacy against wild possums and rats

5.2.1 Reduction in possum abundance

All three treatments resulted in major reductions in possum CCIs and WTIs that were greater than the reductions for rats and mice (Figure 6). There was no indication from CCIs and WTIs of any major loss of possum control efficacy with the repellent baits relative to the non-repellent RS5 bait.

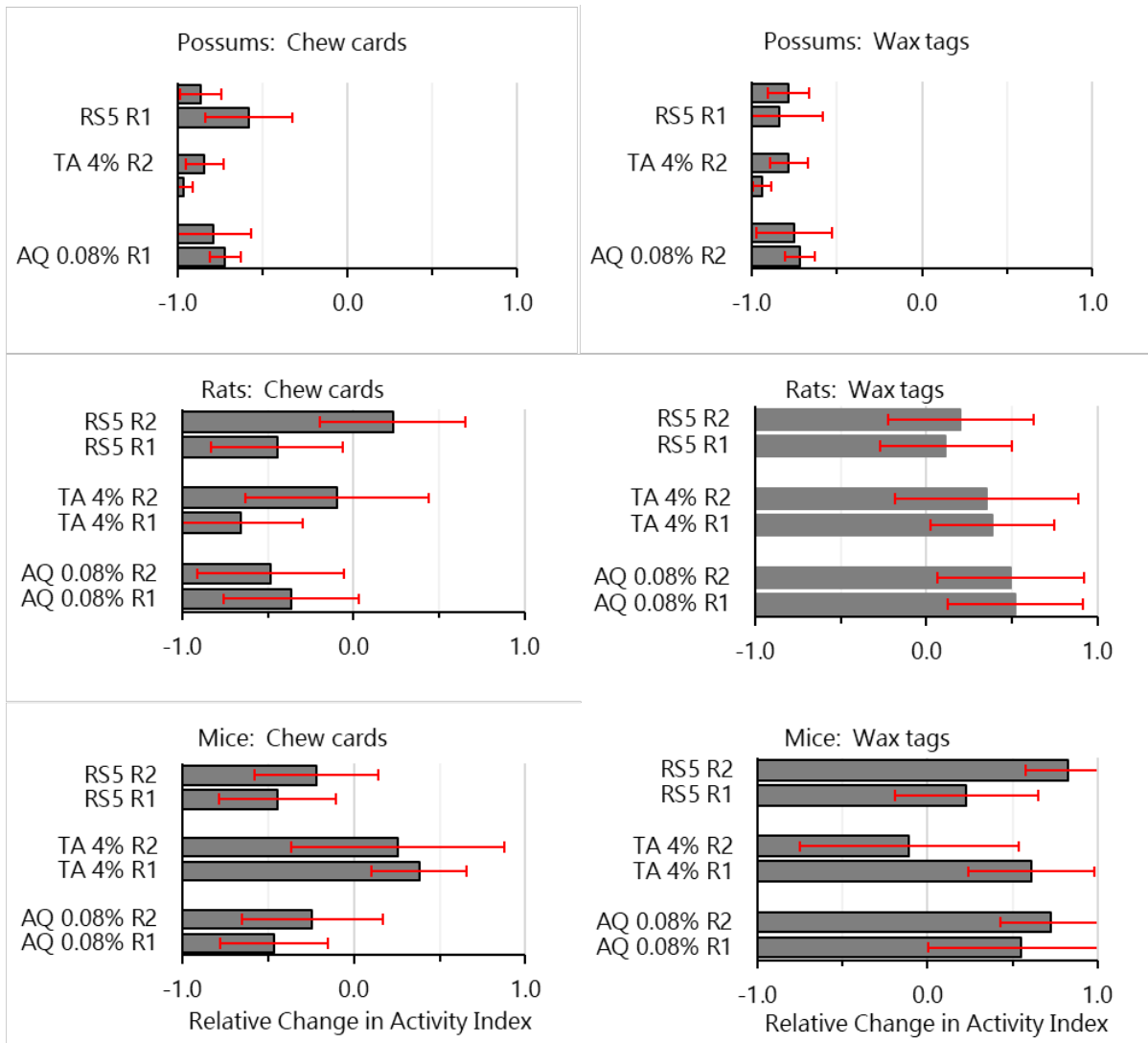


Figure 6. Post-control chewcard and wax-tag indices (CCI & WTI, respectively) expressed as a relative change in activity index (RCAI) of the combined total of the Poisson-transformed averages of number of bite marks recorded both before and after 1080 baiting. RCAIs of -1 indicate all activity was recorded before 1080 baiting (i.e. very high control efficacy), while an RCAI of +1 indicates all bites were recorded after 1080 baiting, and an RCAI of 0 indicates no apparent change in activity. The post-control indices were scaled down to account for the longer assessment period (see Methods).

The number of possum visitors recorded at camera sites dropped to near zero immediately after the 1080 baiting, and then remained low for 2 months before increasing slightly (Figure 7). RCAIs comparing possum activity between the 6 weeks before and the 2 weeks after 1080 baiting (6v2-week RCAIs) were all close to -1 (Figure 8a), with no difference between repellent treatments (Table 2). Although 6v6-week RCAIs were slightly higher (Figure 8b), they did not differ between treatments or sites (Table 2).

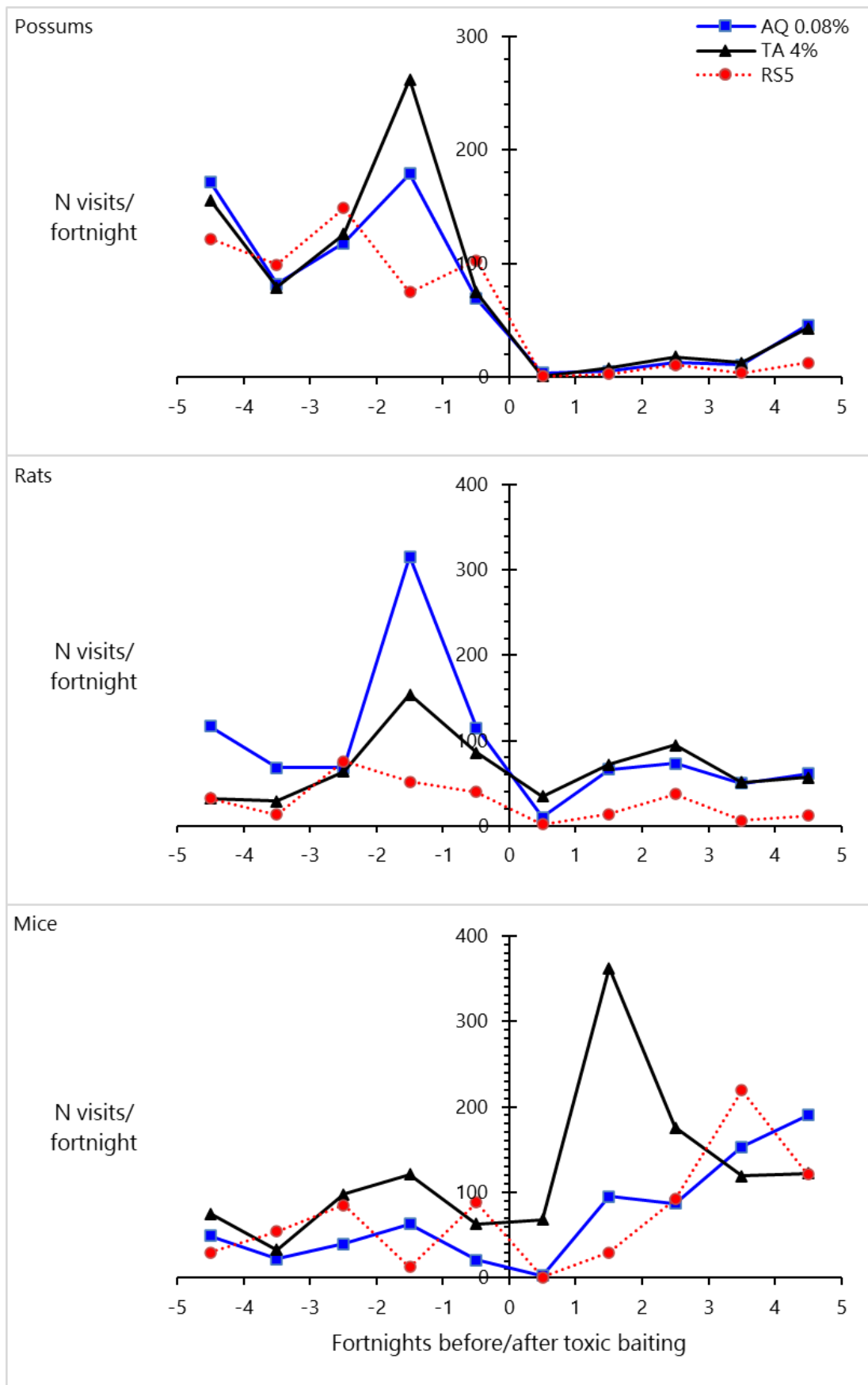


Figure 7. Trends in the numbers of possum, rat, and mouse visits recorded at camera sites during fortnight periods before and after 1080 with two repellent bait types, anthraquinone (AQ) and tannic acid (TA), and non-repellent bait (RS5).

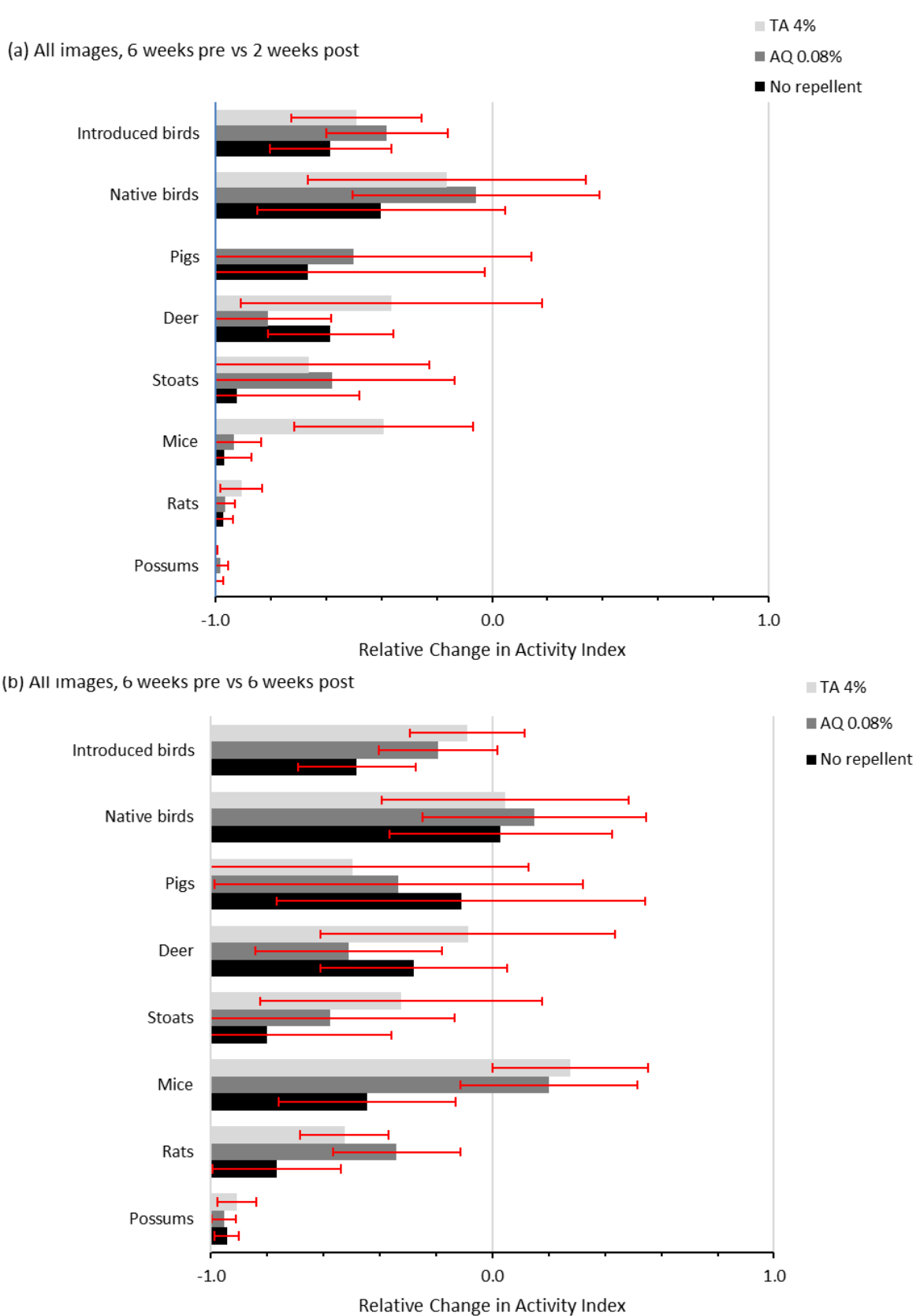


Figure 8. Mean (per-camera) relative change index (\pm 95% CL), by repellent treatment, for species groups, with the relative change in activity indices derived from comparison of the number of images recorded over (a) 6 weeks before and 2 weeks after 1080 baiting, and (b) 6 weeks before and 6 weeks after (see Methods).

Table 2. Statistical outcomes of a two-factor ANOVA comparing the mean (per-camera) relative change index by repellent treatment at two sites at Slopdown, Southland, showing the overall across-site mean indices for each treatment, and the F statistics and associated probability that the means differed by chance between treatments, sites, and treatments × site. The change indices were derived by comparing the number of images recorded over (a) 6 weeks before and 2 weeks after 1080 baiting, and (b) 6 weeks before and 6 weeks after (see Methods).

	Overall RCAI mean			ANOVA results						
	Treatment			Treatment (df = 2)		Site (df = 1)		Interaction (df = 2)		
	No repellent	0.08% AQ	4% TA	F	P	F	P	F	p	Error df
(a) 6 weeks vs 2 weeks										
Possums	-1.00	-0.98	-1.00	2.68	0.075	0	0.999	0	0.999	75
Rats	-0.97	-0.97	-0.91	1.57	0.215	2.7	0.105	0.9	0.411	76
Mice	-0.97	-0.93	-0.39	8.58	<0.001	0.04	0.842	0.35	0.706	51
Stoats	-0.92	-0.58	-0.66	1.57	0.215	2.7	0.104	0.9	0.411	76
Deer	-0.58	-0.81	-0.36	1.88	0.167	98.5	<0.001	1.95	0.157	36
Pigs	-0.67	-0.50	-1.00	–	–	–	–	–	–	–
Native birds	-0.40	-0.06	-0.16	0.51	0.605	0.37	0.547	0.17	0.844	34
Introduced birds	-0.58	-0.38	-0.49	0.72	0.490	0.03	0.863	0.38	0.685	69
(b) 6 weeks vs 6 weeks										
Possums	-0.94	-0.95	-0.91	0.92	0.403	0.46	0.500	2.53	0.086	79
Rats	-0.77	-0.34	-0.53	6.61	0.002	0.6	0.441	12.38	<0.001	83
Mice	-0.44	0.20	0.28	6.63	<0.001	0	0.999	5.64	0.006	66
Stoats	-0.80	-0.58	-0.32	1.23	0.307	0.02	0.889	2.78	0.078	30
Deer	-0.28	-0.51	-0.09	1.15	0.326	1.65	0.206	2.29	0.114	43
Pigs	-0.11	-0.33	-0.50	0.76	0.481	0.19	0.668	1.19	0.326	19
Native birds	0.03	0.15	0.05	0.11	0.896	3.93	0.054	1.6	0.214	41
Introduced birds	-0.48	-0.19	-0.09	3.44	0.037	0.06	0.807	1.08	0.344	74

5.2.2 Changes in rat and mouse abundance indices

Rats

One-night rat-tracking tunnel indices (TTIs) were recorded 12 weeks before and 2–3 weeks after the 1080 baiting for all blocks except one of the non-repellent blocks, where the post-1080 check was delayed by poor weather for 5 weeks. The pre-1080 indices were low to moderate (range 5.8–19.2%). After control, the TTI had increased by a third in the non-repellent block in which monitoring was delayed until 2 months after the 1080 baiting, but in all the other blocks TTIs were a quarter to two-thirds lower (Table 3).

Table 3. Pre- and post-1080 1-night tracking tunnel indices (TTI%) for rats, and the percentage reduction, by treatment

Replicate- block	Treatment	Rat TTI% pre-1080	Rat TTI% post-1080	% reduction
1-1	No repellent	15.0	21.7	(+30.9)
1-2	0.08% AQ	5.8	4.2	27.6
1-3	4% TA	15.8	6.7	57.6
2-1	No repellent	10.8	4.2	61.1
2-2	0.08% AQ	15.8	5.8	63.3
2-3	4% TA	19.2	12.4	35.4

The RCAIs for rat CCIs measured over 7 weeks before and 8 weeks after the 1080 baiting were much higher than for possums, but were below zero (indicating some reduction in rat bite-mark activity) in three blocks (one of each of the bait types; Figure 6). In contrast, the RCAIs based on wax-tag bite-mark WTIs were all close to, or exceeded, zero. The reasons for the difference between CCIs and WTIs are not known.

However, trail camera monitoring showed a marked (albeit temporary) reduction in rat activity in the 2 weeks immediately after 1080 baiting, with the 6v2-week RCAIs indicating reductions of >90% for all three bait types (Figure 8a). In the 6v6-week comparison, however, the RCAIs for the repellent baits were markedly higher than for the non-repellent bait, but there was no difference between the two repellent types (Figure 8b, Table 2).

Mice

Chewcard indices were lower after 1080 baiting for non-repellent RS5 and 0.08% AQ, but not for 4%TA. As for rats, wax-tag indices indicated no change or increased activity for all treatments.

As for rats, trail camera monitoring showed some marked but temporary reductions in mouse activity in the 2 weeks immediately after 1080 baiting, with the 6v2-week RCAIs indicating reductions of >90% for non-repellent RS5 and 0.08% AQ, but not for 4% TA (Figure 8a). In the 6v6-week comparison, however, the RCAIs for the repellent baits were

markedly higher than for the non-repellent baits, but there was no difference between the two repellent types (Figure 8b, Table 2).

5.2.3 Behavioural responses of wild possums and rats to non-toxic baits

There was no discernible difference in the behaviour of possums and rats when they encountered non-toxic bait in the field (Figure 9). In fact, a higher percentage of possum encounters with repellent bait resulted in some, or all, of the bait being consumed.

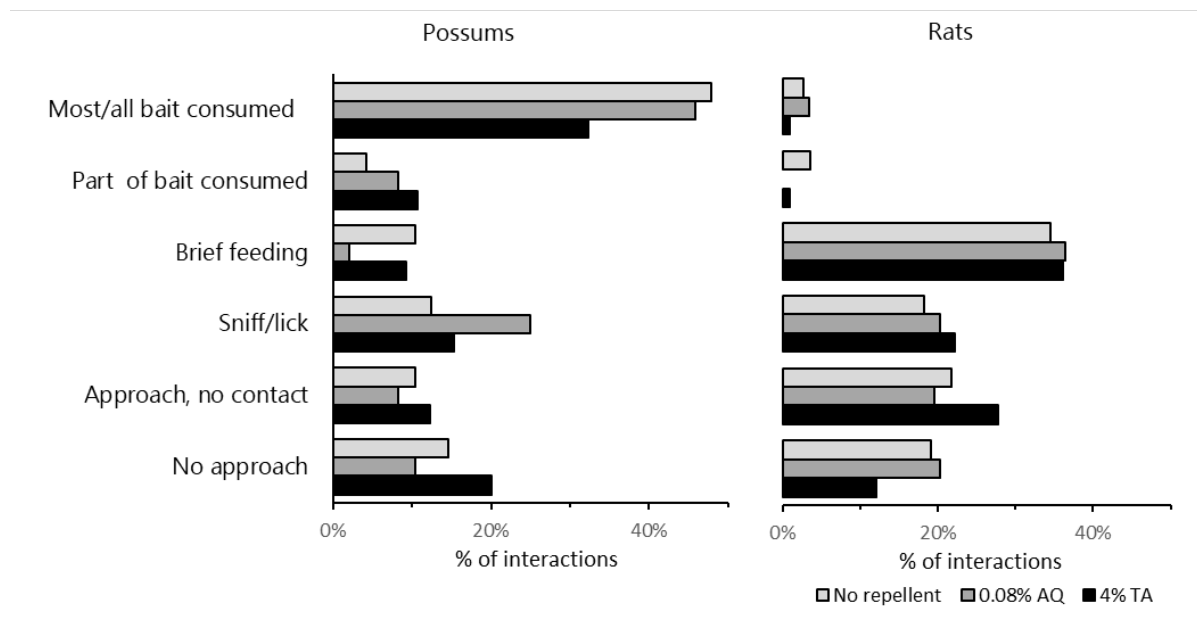


Figure 9. Responses of wild possums and rats to non-toxic bait formulations during the pre-feeding phase of a 1080 baiting operation at Slopdown, Southland, showing the percentage of interactions recorded by trail cameras for each of six behavioural responses (y axis). An interaction was recorded for each visit by an animal at a time when a bait was present within the camera’s field of view.

5.2.4 Changes in abundance indices of non-target species

The numbers of camera images for stoats, pigs, and deer were small, and sometimes zero for whole blocks. There was weak evidence of a reduction in stoat activity, particularly in the no-repellent blocks (Figure 8), but the difference was not statistically supported (Table 2). The results for deer and pigs also indicate some short-term reductions in activity, but no difference between bait types.

For all native birds combined, the mean 6v2-week RCAIs indicated possible reductions, but all the 95% confidence intervals overlapped zero (Figure 8a). The 6v6-week RCAIs indicated similar levels of activity before and after 1080 baiting (Figure 8b).

For all introduced birds combined (mainly thrushes and blackbirds), the 6v2-week RCAIs indicated some reduction in activity, with none of the 95% confidence intervals overlapping zero (Figure 8a). The 6v6-week RCAIs were higher, but still below zero for at least the no-

repellent blocks (Figure 8b). The difference between bait types was significant (Table 2), indicating more introduced-bird activity in the blocks where repellent was used.

6 Conclusions

6.1 Responses of captive possums and rats

Possoms clearly preferred RS5 bait to the plain cereal pellets, whereas rats preferred the pellets. That difference had substantial flow-on effects on reduced consumption, palatability scores, and lethality for rats. The result suggests the possibility that RS5 bait might be a sub-optimal bait for aerial baiting to control rats. It is also consistent with the poorer-than-expected outcomes of DOC's aerial 1080 baiting operations targeting rats in 2019, when it is suspected that a familiar and high-quality alternative food (beech seed) was abundant. In the non-toxic trials, acceptance, consumption, and palatability increased with familiarity (i.e. in the second exposure) for all bait types for both possums and rats, highlighting and affirming the well-known benefits of pre-feeding, particularly for rats (Coleman et al. 2007; Nugent et al. 2011).

In non-toxic bait, D-pulegone bait was as acceptable to possums as RS5 bait, but observed consumption and palatability of the infused-and-surface coated formulation was lower, particularly on first exposure. Despite that, lethality to possums of a surface-coated toxic DP formulation matched that for RS5. For rats, the toxic trial scores for acceptance, consumption, palatability, and lethality for that formulation were all lower than for RS5. We conclude that our DP formulations probably have a higher potential to deter rats than the other repellent formulations we tested. Adding to that, there are likely to be practical difficulties in operational-scale manufacturing of a surface-coated DP bait, particularly because the volatility of DP can result in reduction in DP concentration depending on storage time (Crowell, Booth et al. 2016).

Addition of tannic acid to RS5 bait had no major detrimental effect on bait acceptance, consumption, or lethality for possums or rats, but may have reduced palatability to rats. For possums, in the 13 comparisons of metrics shown in Figures 2–4, the scores for RS5 were matched or exceeded in 12 comparisons for 2% TA, and 11 comparisons for 4%TA. That suggests that neither concentration of TA had any negative effect, and further suggests the possibility that TA may have even improved palatability and lethality for possums slightly. These results support previous suggestions that possums readily cope with TA because it is found in many of the plants eaten by possums in their native Australia (Cowan et al. 2016).

Our anthraquinone formulations appeared to adversely affect rat responses. In the toxic trials, the maximum score for any of the six AQ formulations was always below the average RS5 score for all of the seven metrics compared in Figures 3 & 4. Of the six formulations, the two new-formulation AQ baits appeared to be most effective, with acceptance and palatability scores slightly higher for the 0.04% concentration. For possums, all the AQ metrics were similar to those for RS5, with little difference between AQ concentrations.

6.2 Choice of candidate repellent formulations for field testing

None of the repellent formulations had a major impact on lethality to possums. The formulations chosen for field testing were therefore those with little apparent impact on lethality (i.e. TA and new-formulation AQ; Figure 5). The logic behind our choice was that OSPRI's primary need is for a bait that can be used to kill possums between mast years, as ordinary bait not repellent to kea can still be used in mast years (DOC 2020). As rat numbers are low between mast years, and because rat control is not a primary objective for TB management, achieving a high rat kill would be seen as an additional ancillary benefit rather than an essential requirement; i.e. achieving a good possum kill but a poor rat kill with an effective kea repellent bait would not detract from TB eradication objectives nor adversely affect kea conservation objectives.

For rats, surface-coated DP appeared to be least effective, so it was excluded. For AQ and TA, the observed outcomes were broadly consistent with the intuitively logical assumption that higher concentrations of repellents are generally more repellent than lower ones, a generality that is well supported by many previous tests of bird repellents (Day et al. 2012; Cowan et al. 2016; DeLiberto & Werner 2016). As noted in section 5.1, we therefore decided (in collaboration with OSPRI and our advisors) to proceed with field testing the effect of 4% TA and 0.08% AQ on possum and rat control efficacy.

6.3 Repellent effects on control efficacy against wild possums and rats

Neither 4% TA nor 0.08% AQ had any detectable effect on the efficacy of 1080 baiting in killing possums. There is some suggestion in the trend data obtained from trail cameras (Figure 7) of a greater increase in activity in repellent-baited blocks after 6 weeks, but the chewcard and wax-tag RCAIs suggest activity was similar or lower in those blocks (Figure 6).

All bait types reduced rat activity markedly, but only for a few weeks. We presume that the subsequent large increases were a result of immigration from unpoisoned areas nearby and/or rapid recruitment. It is plausible that some of the apparent 'immigration' effect may have been from areas between transects, as the swaths of hand-laid bait will have been only 5–10 m wide, resulting in >90 m-wide unbaited swaths between monitored transects. A circular home range centred midway between baited areas with a radius of 45 m would cover 0.63 ha, much larger than some of the smallest home range sizes reported for rats elsewhere (e.g. a mean female home range of 0.12 ha (Harper & Rutherford 2016)). It is therefore likely that many rats and mice had weekly or monthly home ranges that fell entirely within the unbaited strips, and so did not encounter toxic bait.

The large reductions in rat activity in the first 2 weeks after 1080 baiting suggest that all bait types killed a large majority of rats in the baited areas. However, the lesser reductions for the 6-week period after baiting in repellent areas indicate some loss of control efficacy, but the effect appears to be modest, with no marked difference between the two repellent formulations. The immediate reductions in mouse activity were also high with the non-repellent RS5 and 0.08% AQ bait types, but much lower for 4% TA. This difference between repellents was no longer apparent by 6 weeks after baiting, which suggests that both repellents reduced efficacy against mice (unless the change in apparent efficacy between

assessment periods was an immigration effect). Although not statistically supported, the stoat data appear consistent with the rodent data, with a suggestion of larger reductions in the no-repellent areas than in either of the repellent areas. The 6v6-week pattern for stoats closely matched the 6v2-week pattern for mice, with the smallest reductions recorded for 4% TA. Collectively, the rat, mouse and stoat data suggest that 4% TA could be more generally repellent than 0.08% AQ

The repellents did not appear to greatly change non-target impacts on deer and pigs. None of the bait types appeared to greatly affect native bird activity, with slightly more birds in total recorded in the 6 weeks after baiting than in the 6 weeks before. However, the repellents did appear to result in lesser reductions in recorded activity of introduced birds, with the smallest 6v6-week effect (RCAI reduction) again being for 4% TA.

We conclude that neither of the 4% TA nor 0.08% AQ formulations poses any threat to the level of possum control efficacy required for TB-related possum control. Both repellent formulations may reduce efficacy against rats and be even less effective against mice (and possibly also stoats). However, it appears that most rats were killed. Given that rat numbers in between-mast years are typically already low, the use of either repellent formulations would still result in extremely low rat numbers.

7 Recommendations

- Testing the effectiveness of TA and AQ in deterring kea from eating non-toxic baits should be undertaken.
- One option is conducting testing on captive kea, but the number of birds available for this is low, possibly limiting the number of combinations of repellent product and concentration that can be tested to just two, or possibly three.
- An alternative option would be to use trail cameras to monitor non-toxic bait acceptance by wild kea. The feasibility of that approach is not known, but if feasible, this would increase the number of formulations that could be tested and also avoid the risk that the responses of well-fed captive birds differ from those of wild birds.
- Concurrently with the non-toxic testing above, OSPRI should consider initiating planning for an operational-scale field test of the survival of radio-collared kea in winter 2021, with TA as the 'default' repellent (i.e.; unless AQ performs better in the non-toxic trial above). The trial block should be remote from human influence and in an area where no mast is predicted. Pre-operational monitoring should be undertaken to confirm rat densities are low.

8 Acknowledgements

We would like to thank the many people who have contributed to this project: Phil Cowan (MWLR), Michelle Crowell and Nic Gorman (DOC), for advice on choice of repellents and project design; MWLR animal facility staff (Jane Arrow, Mike Werner, Leigh Elmers); MWLR toxicology laboratory staff (Lynn Booth, Amelia Gibbs); Michelle Ewans (Contract Wild animal Control Ltd) for help with permitting and consents; Ollie Halleux (Rayonier Forestry) for

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Appendix 1. Measured concentrations of (a) the repellent compounds (in alphabetical order) and (b) the sodium fluoroacetate (1080) in the baits used in the pen and field trials, as determined by laboratory assays of the RS5 and RS5+repellent baits.

The trial type (i.e. pen or field) and non-toxic (NT) or toxic (T), the times at which assay were undertaken relative to when they were used, whether or not the bait contained 1080 (toxic or non-toxic), and the measured concentrations of repellent or 1080 are shown for each of the nominal formulations. Repellent codes are: AQ = anthraquinone [-nf = new formulation], TA = tannic acid, DP = D-pulegone. The repellents were either infused through the bait matrix (I) or applied as a surface coating (SC). All repellent formulations were applied to Orillion RS5 bait.

Nominal formulation	Trial	Assay timing	Analysis date	Bait type	Measured concentration (% wt)	Report no.
(a) repellent concentrations						
AQ 0.04% SC	Pen	On receipt from Orillion	21/2/2019	Non-toxic	0.051	T7010
	Pen	Immediately prior to trial start	8/4/2019	Non-toxic	-	-
		Immediately prior to trial start	17/4/2019	Toxic	0.050	T7168
AQ 0.04% I	Pen	On receipt from Orillion	21/2/2019	Non-toxic	0.057	T7010
	Pen	Immediately prior to trial start	8/4/2019	Non-toxic	-	-
		Immediately prior to trial start	17/4/2019	Toxic	0.039	T7168
AQ-nf 0.04% I	Pen	On receipt from Orillion	-	Non-toxic	-	-
		Immediately prior to trial start	10/10/2020	Non-toxic	0.074	T7328
		Immediately prior to trial start	25/10/2020	Toxic	0.047	T7239
AQ-nf 0.08% SC	Pen NT	On receipt from Orillion	21/2/2019	Non-toxic	0.075	T7010
	Pen T	Immediately prior to trial start	-	Non-toxic	-	-
		Immediately prior to trial start	10/4/2019	Toxic	0.058	T7168
AQ 0.08% I	Pen NT	On receipt from Orillion	21/2/2019	Non-toxic	0.114	T7010
		Immediately prior to trial start	-	Non-toxic	-	-
		Immediately prior to trial start	10/4/2019	Toxic	0.076	T7168
AQ-nf 0.08% I	Pen T	On receipt from Orillion	-	Non-toxic	-	-
		Immediately prior to trial start	10/10/2020	Non-toxic	0.096	T7238

Nominal formulation	Trial	Assay timing	Analysis date	Bait type	Measured concentration (% wt)	Report no.
		Immediately prior to trial start	25/10/2020	Toxic	0.095	T7239
	Field	On receipt from Orillion	20/1/2020	Non-toxic	0.077	T7384
				Toxic	0.079	T7384
		Immediately prior to trial start	23/2/2020	Non-toxic	0.087	T7423
				Toxic	-	-
DP 0.17% SC	Pen NT	On receipt from Orillion	21/2/2019	Non-toxic	0.052	T7004
		Immediately prior to trial start	22/2/2019	Non-toxic	0.054	T7018
		2 weeks post-trial start	14/3/2019	Non-toxic	0.051	T7058
	Pen T	Immediately prior to trial start	8/4/2019	Non-toxic	0.043	T7059
		Immediately prior to trial start	17/4/2019	Toxic	0.032	T7232
		3 weeks post-trial start	9/5/2019	Non-toxic	0.031	T7232
DP 0.17% SC & I	Pen NT	On receipt from Orillion	21/2/2019	Non-toxic	0.13	T7004
		Immediately prior to trial start	22/2/2019	Non-toxic	0.092	T7018
		2 weeks post-trial start	14/3/2019	Non-toxic	0.079	T7058
TA 2% I	Pen NT	On receipt from Orillion	21/2/2019	Non-toxic	3.77	T7233
		Immediately prior to trial start	6/3/2019	Non-toxic	-	
	Pen T	Immediately prior to trial start	8/4/2019	Non-toxic	3.72	T7233
		Immediately prior to trial start	17/4/2019	Toxic	2.04	T7233
	Pen T	Immediately prior to trial start	10/10/2019	Non-toxic	2.10	T7326
		Immediately prior to trial start	25/10/2019	Toxic	1.70	T7327
TA 4% I	Pen NT	On receipt from Orillion	21/2/2019	Non-toxic	2.09	T7233
		Immediately prior to trial start	6/3/2019	Non-toxic	-	
	Pen T	Immediately prior to trial start	8/4/2019	Non-toxic	2.00	T7233
		Immediately prior to trial start	17/4/2019	Toxic	4.10	T7233
	Pen T	Immediately prior to trial start	10/10/2019	Non-toxic	3.70	T7326
		Immediately prior to trial start	25/10/2019	Toxic	4.40	T7327

Nominal formulation	Trial	Assay timing	Analysis date	Bait type	Measured concentration (% wt)	Report no.
	Field	On receipt from Orillion	20/1/2020	Non-toxic	3.50	T7383
				Toxic	3.00	T7383
		Immediately prior to trial start	23/2/2020	Non-toxic	3.20	T7497
				Toxic	3.70	T7420
(b) 1080 concentrations						
0.15% 1080 non repellent RS5	Pen T	Immediately prior to trial start	17/4/2019	Toxic	0.13	T7103
0.15% 1080 DP 0.017% SC			17/4/2019		0.14	T7103
0.15% 1080 TA 2% I			17/4/2019		0.13	T7103
0.15% 1080 TA 4% I			17/4/2019		0.14	T7103
0.15% 1080 non repellent RS5	Pen T	Immediately prior to trial start	14/10/2019	Toxic	1.41	T7318
0.15% 1080 AQ-nf 0.04% I			14/10/2019		1.38	T7318
0.15% 1080 AQ-nf 0.08% I			14/10/2019		1.34	T7318
0.15% 1080 TA 2% I			14/10/2019		0.92	T7318
0.15% 1080 TA 4% I			14/10/2019		1.27	T7318
0.15% 1080 non repellent RS5	Field T	On receipt from Orillion	20/1/2020	Toxic	0.133	T7381
0.15% 1080 AQ-nf 0.08% I					0.121	T7381
0.15% 1080 TA 4% I					0.116	T7381
0.15% 1080 non repellent RS5	Field T	Immediately prior to trial start	23/2/2020	Toxic	0.15	T7422
0.15% 1080 AQ-nf 0.08% I					0.13	T7422
0.15% 1080 TA 4% I					0.11	T7422