

**Avoiding Bait Shyness in Possums by  
Improved Bait Standards**

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## 1. Summary

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### 1.1 Project and Client

This study aimed to identify factors that cause possums to eat sublethal amounts of bait, with a view to recommending improved specifications for baits used during possum control. The work was done by Landcare Research and Lincoln University during 1997–1998 under contract R1457 to the Animal Health Board.

### 1.2 Objectives

To further develop bait specifications (for palatability, size, texture, toxicity, stability, prefeeding) aimed at avoiding sublethal 1080 poisoning and induction of bait shyness in possums, by:

- evaluating the toxicity of 1080 to possums in different bait types (cereal, paste, carrot, gel, apple, swede) and comparing results with published estimates of the acute toxicity of 1080 (administered in water);
- assessing the effect of 1080 concentration on the percentage of possums sublethally poisoned, and factors likely to affect the 1080 concentration in baits (variance in 1080 concentration during bait manufacture, bait storage, exposure to rainfall);
- assessing the effect of bait palatability on the percentage of possums sublethally poisoned, and factors likely to affect bait palatability (carrot variety, storage of carrot, soil residues on carrot, freezing of carrot, bait type, storage of manufactured baits, hardness of cereal baits, moisture content of cereal baits, lure concentrations, loss of cinnamon from baits, dyes);
- assessing the amount of bait eaten by possums and the effect of prefeeding on bait consumption;
- predicting the efficacy of baits used in bait stations by modelling the susceptibility of possums to 1080, concentration of 1080 in bait, possum body-weights, and amounts of bait eaten with and without prefeed;
- identifying bait factors that affect the efficacy of aerial control, from a database of aerial operations.

### 1.3 Methods

- Captive possums were used to assess the acute toxicity of 1080, bait palatability, and bait consumption. Wild possums' consumption of baits at bait stations was monitored using electronic scales and dataloggers.
- Predictions of bait efficacy at bait stations was modelled by randomly combining data on possums' susceptibility to 1080, variation of 1080 content in baits, possum bodyweights, and possums' consumption of baits.
- Bait factors that affect the success of aerial control operations were evaluated from a database of 209 aerial operations.

## 1.4 Results

### Acute toxicity

- The LD<sub>95</sub> of 1080 to captive possums was 3.6–4.7 mg/kg in different bait types.

### Toxin concentration

- Although many (>35%) possums survived baits containing 0.08% 1080 during cage studies and bait station control, few (<10%) survived baits containing 0.13% 1080. High 1080 concentrations (i.e., >0.15%) that are not adequately masked were aversive to possums, and caused 20–42% of possums to eat sublethal amounts of bait.
- Toxin concentrations were below specification because poorly calibrated equipment had not mixed appropriate amounts of poison throughout carrot bait, 1080 had biodegraded in stored baits, or rainfall had leached 1080 from baits.

### Bait palatability

- Without prefeeding, baits of low palatability (i.e., <30%) sublethally poisoned many (>35%) possums. Between 10% and 30% of possums survived baits of moderate palatability (i.e., 30–45%).
- Palatability was dependent on bait type, the variety of carrot used to make baits, the moisture content of cereal baits, cinnamon or orange concentration, bait hardness, and the duration of storage.
- Cinnamon was rapidly lost from bait, but tended to stabilise at about 25% of the nominal concentration after 2 days on carrot, and at 40% of the nominal concentration after 8 weeks in cereal baits.

### Amount of bait eaten

- The amounts of bait eaten by captive possums were extremely variable, but not affected by the weight of possums, or whether pens contained 1, 2, or 3 possums. The amounts eaten by wild possums were affected by site factors, season, bait palatability, aversion to 1080, and prefeeding.
- Prefeeding significantly increased amount of bait eaten from bait stations, reduced neophobia, and increased kills.

### Aerial control results

- Percentage kills during aerial control are significantly affected by bait palatability. Although concentrations of 1080 in bait had no measurable effect on the percentage kill, this result is assumed to be a consequence of aversions to 0.15% 1080 in existing baits. Prefeeding significantly increased kills with aerially broadcast carrot.

## 1.5 Recommendations

That the revised specifications recommended in the Appendices be adopted as industry standards.

### Management

To meet the updated specifications developed through this research:

a) Manufacturers of commercial baits should be advised to consistently produce:

- cereal baits with a cinnamon concentration of 0.2% wt/wt;
- individual baits with 1080 concentrations within  $\pm 25\%$  of the nominal concentration;
- baits with a palatability exceeding 40% compared to recently manufactured RS5 baits. (Note: palatability trials should always compare baits of equivalent size);
- a label on all possum baits that clearly shows the date of manufacture and a recommended use-by date. (Note: baits are likely to be of substandard palatability after gel bait has been stored for 12 months, Pestoff® paste has been stored for 8 months, RS5 cereal bait (12% moisture) has been stored for 6 months, and when No.7 cereal bait (14% moisture) has been stored for 3 months);

- cereal baits with a moisture content that does not exceed 14% wt/wt;
- cereal baits of a hardness whereby a pointed 2-mm probe will penetrate baits when 2–7 kg pressure is applied to small (i.e., 1.5 g) and 5–12 kg pressure applied to large (i.e., 6 g) baits.

b) Control operation managers should be advised to:

- increase the concentration of cinnamon oil used on carrot baits to 0.3% wt/wt;
- use bait with 0.15% 1080 and routinely measure the toxin concentration of baits;
- use Royal Chantenay carrot in carrot operations, and ensure that they are harvested before they weigh 200 g or within 6 months of sowing;
- store unwashed carrot in a 'heap' for no more than 1 month and, if overnight frosts are likely, ensure that carrots are covered by tarpaulins;
- not use the Gibson carrot cutter for preparation of baits;
- adequately screen carrot baits;
- use prefeed before bait station control with 1080, cholecalciferol, and cyanide.

## Research

In order of priority, future research is needed to assess:

- the persistence of 0.20% and 0.40% cinnamon in cereal and carrot baits; the effectiveness of higher cinnamon concentrations at masking the standard 1080 concentration (i.e., 0.15% 1080) and baits such as carrot that on occasions are inadvertently manufactured with a higher concentration (e.g., 0.20% 1080); the effects of packaging on the persistence of cinnamon in baits; the amount of non-target interference (e.g., captive and wild birds) with baits (carrot and cereal) containing higher cinnamon concentrations;
- factors influencing the quality and efficacy of baits containing brodifacoum, cholecalciferol, and cyanide, and to then develop specifications for these products;
- the long-term cost/benefits of prefeeding before aerial and ground control;
- the effectiveness of carrot at sowing rates lower than those currently used (c. 11.5kg/ha);
- the non-target risks when aerial control with 1080 baits follows a previous application of prefeed;
- the accuracy and precision of 1080 concentrations in individual RS5, No.7, and carrot baits that are currently used during possum control;
- the effectiveness of prefeeding unfamiliar baits (e.g., carrot and apple) to mitigate bait shyness caused by sublethal amounts of cereal bait;
- dyes with greater persistence than Bayer V200 for use on carrots to ensure baits comply with colour specifications;
- the efficacy of elevated bait stations (i.e., out of reach of livestock), following the application of prefeed;
- the reasons for naive possums eating different per capita amounts of bait at different locations;
- the proportions of baits of different hardness that fragment on the spinner of a sowing bucket.

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## 2. Introduction

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This study aimed to identify factors that cause possums to eat sublethal amounts of bait, with a view to recommending improved specifications for baits used during possum control. The work was done by Landcare Research and Lincoln University during 1997–1998 under contract R1457 to the Animal Health Board.

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## 3. Background

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Despite extensive research to identify causes for sublethal poisoning of possums, it is still commonplace for 15% or more of possums to survive an exposure to bait (Morgan 1994; Spurr 1993a; Henderson *et al.* 1997). Possums sublethally poisoned during control operations become "shy" towards bait (Hickling 1994; Morgan *et al.* 1996) by associating the illness caused by the toxin with the bait that was eaten. Possums that become shy may then refuse to eat bait for up to 5 years (O'Connor 1998). While recent research has focussed on ways of mitigating shyness in populations exposed to pesticides (e.g., Morgan *et al.* 1996; Ogilvie *et al.* in press) or developing cost-effective methods of controlling shy possums (e.g., Cook & Dean 1996; Henderson *et al.* 1997), the more fundamental question of how shyness can be avoided in the first place remains only partially resolved. A logical first step is to ensure that the quality of all baits used for control is of a standard whereby 95% or more of possums will ingest a lethal dose of toxin when first exposed to baits.

There are four key variables that determine whether individual possums consume a lethal or sublethal dose of toxic bait. These are:

- **the acute toxicity of the poison to the possum,**
- **the concentration of toxin in baits,**
- **bait palatability,**
- **the amount of bait eaten.**

The acute toxicity of 1080 has previously been estimated by administering aqueous solutions of 1080 to possums by oral gavage (Bell 1972; Rammell & Fleming 1978; McIlroy 1981, 1983). In the early years of possum control, the concentrations of 0.06–0.08% 1080 in bait was based on an LD<sub>95</sub> of 1.2 mg/kg (Rammell & Fleming 1978). However, the concentration was increased to 0.15% wt/wt by the New Zealand Forest Service following a review of the acute toxicity of 1080 by Peters & Frederic (1982 unpubl. report). In this study the toxicity of 1080 to possums was evaluated in bait using existing data for carrot and cereal baits compiled between 1990–1996, or results from recent dose-ranging experiments with apple, swede, paste, and carrot baits during 1997–1999. Our research aimed to define more accurately the acute toxicity of 1080 and the concentration of 1080 required in baits to ensure all possums ingest a lethal amount of poison.

Previous research has demonstrated that baits of low palatability cause many possums to survive control (Henderson & Morriss 1996). In this study, we examined in detail the relationship between bait palatability and factors that influence it, and the likelihood of possums eating sublethal amounts of bait as a consequence of low palatability.



Factors affecting the amounts of bait eaten by wild possums have not previously been assessed. In this study we estimated the amounts eaten from bait stations using electronic scales and dataloggers. These data were then used to determine factors affecting bait consumption (e.g., seasonal effects and prefeeding), and to model the percentage of possums killed by 1080 baits that were of high and low palatability.

After identifying factors that contribute to possums being sublethally poisoned during control operations, we then used information generated in the study to revise bait specifications to ensure that few, if any, possums are sublethally poisoned.

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## 4. Objectives

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To further develop bait specifications (for palatability, size, texture, toxicity, stability, prefeeding) aimed at avoiding sublethal 1080 poisoning and induction of bait shyness in possums by:

- evaluating the toxicity of 1080 to possums in different bait types (cereal, paste, carrot, gel, apple, swede) and comparing results with published estimates of the acute toxicity of 1080 (administered in water);
- assessing the effect of 1080 concentration on the percentage of possums sublethally poisoned, and factors likely to affect the 1080 concentration in baits (variance in 1080 concentration during bait manufacture, bait storage, exposure to rainfall);
- assessing the effect of bait palatability on the percentage of possums sublethally poisoned, and factors likely to affect bait palatability (carrot variety, storage of carrot, soil residues on carrot, freezing of carrot, bait type, storage of manufactured baits, hardness of cereal baits, moisture content of cereal baits, lure concentrations, loss of cinnamon from baits, dyes);
- assessing the amount of bait eaten by possums and the effect of prefeeding on bait consumption;
- predicting the efficacy of baits used in bait stations by modelling the susceptibility of possums to 1080, concentration of 1080 in bait, possum body-weights, and amounts of bait eaten with and without prefeed;
- identifying bait factors that affect the efficacy of aerial control from a database of aerial operations.

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## 5. Methods

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### 5.1 Acute toxicity

Wild possums were trapped, individually caged, and acclimatised to captivity at the Landcare Research animal facility on a diet of pelleted cereals, fruit, vegetables, and herbaceous plants (Duckworth & Meikle 1995). Within 4–6 weeks of captivity those which had acclimatised were gaining weight and were thus considered suitable for inclusion in trials. The concentration of 1080 in apple, swede, gel, paste, carrot, and cereal baits was measured by gas liquid chromatography (Bowen *et al.* 1995, Landcare Research Toxicology Laboratory Method TLM023). During trials possums were either fed *ad libitum* on toxic baits and supplementary foods or given restricted amounts of bait that would administer a small dose of toxin to each animal. The weight of bait eaten by each possum was measured next day and combined with the assayed toxin concentrations to determine the amount of 1080 ingested (in mg/kg). All possums were observed periodically and their fate recorded during the week following administration of 1080.

The gender, colour phenotype, and bodyweight of possums (n=1435) were recorded before animals were given baits. This information together with the dose (in mg/kg) and fate of each animal was used to assess the susceptibility of possums to 1080 in different bait types. Lethal dose estimates were calculated by regressing the proportion of animals killed against dose (in mg/kg) and then estimating by probit analysis (Finney 1971) the dose giving an LD<sub>50</sub> (i.e., 50% of animals dying), LD<sub>90</sub>, or LD<sub>95</sub>. The statistical package POLO (Russell *et al.* 1977) was used to calculate lethal dose intercepts, slopes of probit regression curves, and lethal dose estimates. Where the acute toxicity of 1080 may have been affected by bait type, gender, diet, or phenotype, pairwise comparisons of intercepts and slopes of probit regression curves were made using POLO.

### 5.2 Toxin concentrations as a cause of sublethal poisoning

The effect of 1080 concentration on the percentage of captive possums sublethally poisoned was assessed on five groups of individually caged possums (n=53–229 per group) feeding on baits containing five different concentrations (0.08–0.10, 0.12–0.14, 0.14–0.16, 0.16–0.18, 0.18–0.20% wt/wt) of 1080. The proportions in different groups that survived after eating either 1080 (n=494 total) was compared using chi-square tests.

The effects of two concentrations of 1080 on the percentages of wild possums surviving bait station control was assessed in replicated field trials undertaken in either plantations of exotic trees or remnant patches of indigenous forest of 10–50 ha. Before each field trial the relative abundance of possums at each site was estimated using Victor No.1 leg-hold traps that allow possums to be caught and released with minimal injury (Warburton 1992). At each field site, 100 traps were set for 2 nights at intervals of 20–30 m on marked sites along lines that were spaced so that there were no more than seven traps per hectare.

Cereal baits were presented in each trial in bait stations distributed at approximately 1 per hectare when baits were not prefed (Henderson & Morriss 1996) or 1 per 2 hectares when prefeed was applied before toxic baits (Thomas *et al.* 1996). They were located mainly around the edge of each forest block at

spacings of approximately 100–150 m, with some situated within the forest to ensure that bait was readily accessible to all possums. At those sites where prefeed was used, 2 kg of non-toxic RS5 was applied weekly during a 2-week period before the application of toxic baits. Three-hundred grams of bait containing 1080 (0.08% or 0.15% wt/wt) was placed in each station, and bait consumption monitored for 1 week (prefeed bait stations) or for 5 weeks if bait stations were not prefeed (by which time negligible amounts of bait are eaten and all possums are likely to have been exposed to bait; Henderson *et al.* 1994).

On completion of each field trial, the sites used for pre-poison estimates of possum abundance were retrapped and the percentage of the population killed was estimated from the percentage decline in the proportion of traps catching possums. The percentages of possums surviving bait station control with baits containing different toxin concentrations were compared by analysis of variance.

### **Stability of 1080 in baits**

(a) *During storage of manufactured baits:* Four batches of commercial 1080 bait (Pestoff® paste, No.7 bait (both Animal Control Products, Wanganui), RS5 bait (Animal Control Products, Waimate), and Kiwicare gel (Kiwicare Corporation, Christchurch)), were supplied during 1997 and stored at room temperature ( $20 \pm 5^\circ\text{C}$ ). Shortly after manufacture and thereafter at intervals of 1, 2, 4, 6, 8, and 12 months, samples of each bait type were taken and the toxin concentration measured.

(b) *During storage of carrot with and without soil residues:* Carrots (4 kg) were harvested and half were washed. One-hundred grams of mud (5% wt/wt) was added to 1900 g of the remaining carrot. Carrot baits (either washed or dirty) were then prepared (with a nominal concentration of 0.15% 1080) by cutting carrot into pieces weighing  $6 \pm 2$  g, then tumbling 1.982 kg of bait in a hand-operated mixer while using a small hand sprayer to apply an aqueous suspension that contained 15 g of 1080 (as a 20% stock solution), 2 g of cinnamon oil (Bush, Boake and Allen, Auckland), and 1.5 g of V200 dye (Bayer Industries, Wellington). The baits were stored in plastic tubs and the 1080 concentration of washed and dirty carrot measured after 0, 3, 7, and 14 days. The amount of 1080 in washed and dirty carrot was compared using a paired t-test.

(c) *Under simulated rainfall:* The effects of simulated rainfall on the leaching of 1080 from carrot, No.7, and RS5 cereal baits have previously been assessed with baits subjected to 5, 10, 20, 50, 100, 150, and 200 mm of "rain" (Bowen *et al.* 1995). During June 1998 these trials were replicated using recently manufactured cereal and carrot baits, and the effect of "rainfall" on the concentration of 1080 in two batches of Pestoff® paste was also evaluated.

Samples of at least 100 baits of each type were placed on the ground alongside garden sprinklers that applied 18 mm of "rain" per hour. Subsamples of eight baits of each bait type were randomly removed after 0, 5, 10, 20, 50, 100, 150, 200 mm of rain and stored in plastic bags at  $-20^\circ\text{C}$  prior to analysis of 1080 concentration. The half-lives of 1080 in different bait types under simulated rainfall were calculated by regression analysis.

### **Variations in toxin concentration during bait preparation**

Recent batches of bait have not been sub-sampled to measure how precisely 1080 is mixed throughout baits. We therefore present historical data from operations during 1990–1995, but recommend future research re-evaluates the variance in 1080 concentration in baits currently used for possum control.

Samples of No.7 cereal baits were collected from 34 bags of bait used during control operations at Puketi (1992), Waipoua (1990), and Titirangi (1992) forests. Baits from each sample were crushed with a mortar and pestle, homogenised into a uniform mix, and the 1080 concentration measured.

Samples of carrot bait from three control operations (Hauhungaroa Forest 1994, Pureora Forest 1995, Tahorakuri Forest 1995) were collected at different times during bait preparation, and the 1080 concentrations from each batch were compared with the nominal concentration by paired t-test.

### 5.3 Bait palatability as a cause of sublethal poisoning

The palatability of baits was tested in standard two-choice tests (Grote & Brown 1971), in which paired trays containing 100 g of test bait and 100 g of control bait (i.e., recently manufactured 1.5 g RS5 bait as a palatable industry standard) were presented overnight to 20 individually caged possums. Palatability of test materials was then calculated from the weight of test bait eaten expressed as a percentage of total (i.e., test and control) bait eaten. A palatability value less than 50% therefore indicates that the test bait was less palatable than the control, and a value greater than 50% that the test baits were more palatable than the control. Differences between test and control baits were estimated by analysis of variance.

A total of 192 possums were fed *ad libitum* on baits containing 0.15% 1080, non-toxic baits, and alternative foods for 18 h from late afternoon. The different batches of bait used in these trials were made from different ingredients, and consequently differed in palatability. The weight of toxic and control baits eaten by each possum, and the palatability of different batches of toxic bait was calculated next day. The amounts of 1080 bait eaten (for baits pooled into palatability groupings of 0–10%, 10–20%, 20–30%, 30–40%, and 40–50%) were tested by analysis of variance. All possums were observed periodically during the following 3 weeks, or until their death, and their fate recorded. The proportion of caged possums surviving baits in different palatability groupings were compared by chi-square tests.

#### Factors affecting the palatability of baits to captive possums

As bait palatability is critical in determining the percentage of possums that survive control (Henderson & Morriss 1996), factors that may affect the palatability of different bait types were assessed using two-choice tests with 20 individually caged possums). The palatability of bait was calculated for individual possums, and unless stated otherwise, these palatabilities were then tested by analysis of variance to determine the significance of each bait factor.

(a) *Bait type:* The relative palatabilities of nine bait types used to control possums were assessed on at least 20 captive possums for each type. The baits purchased during 1997 were Agtech cereal bait (used as a bait base in Talon®; Cropcare Holdings Ltd., Richmond), the original formulation of No.7 cereal bait with 16% moisture, a new improved formulation of No.7 cereal bait with 14% moisture, RS5 cereal bait, Kiwicare gel bait, Pestoff® paste bait, swede, apple, and carrot.

(b) *Carrot variety and size:* Following consultations with Yates N.Z. Ltd (Christchurch), seven varieties of carrot frequently cultivated by commercial growers were identified (Chantenay red-cored, Royal Chantenay, Spring Market, Lancer hybrid, Manchester Table, Topweight, and Egmont Gold). Seeds of these varieties were sown in December 1996, and 4-kg samples of each type were harvested at 3, 4, and 5 months after sowing. All carrots were washed to remove any dirt, and a minimum of 40 weighed before being diced into baits of  $6 \pm 2$  g. Palatability was measured in two-choice trials in which 200 g of diced carrot and 100 g of non-toxic RS5 were presented in paired trays

to 15 individually caged possums. The palatability of carrot to each possum, and for all varieties at 3, 4, and 5 months were compared by analysis of variance. Royal Chantenay carrots were subsequently harvested at 7, 9, 11, and 12 months after planting and palatability measured on 20 individually caged possums at each time point.

The weight of carrots was measured to ensure that they were not too small for carrot cutters, and that yields from each variety would make them commercially viable for growers. At least 40 carrots of seven varieties were weighed 3, 4, and 5 months after sowing.

(c) *Palatability of carrot with and without soil residues:* Approximately 25 kg of carrots (var. Manchester Table) were harvested and half were washed before being cut into baits of  $6 \pm 2$  g. Baits with a soil residue were made by adding 600 g of soil and 300 g of water to 11.4 kg of carrot and then tumbling the baits in a mixer. The baits were stored in buckets and 4-kg samples of washed and dirty carrot taken 1, 3, 7, and 14 d after manufacture to measure palatability. Palatability was tested by presenting paired trays containing 200 g of carrot and 100 g of RS5 cereal bait to individually caged possums. Data were analysed to assess whether washed carrot was more preferred than dirty carrot, and to determine whether palatability of cut carrot declined during the 2 weeks after manufacture.

(d) *Carrot after freezing:* The effect of freezing on carrot baits was assessed for 2 weeks after carrots were defrosted, by monitoring changes to bait palatability and 1080 concentration. Royal Chantenay carrots (24 kg) were harvested and washed. Half were retained whole and the remainder made into baits containing 0.15% 1080. Bait palatability and 1080 concentration were measured and the entire carrot baits were stored frozen in 2-kg lots for 60 h before defrosting. On days 1, 2, 7, and 14 after defrosting, the entire carrots were cut into baits weighing  $6 \pm 2$  g and the palatability measured. The 1080 concentration was measured for baits stored in an open container on days 0, 1, 2, 7, and 14 after defrosting.

(e) *Storage of baits:* The palatability of five types of manufactured bait was measured during 12 months storage. The baits manufactured during 1997 and assessed in storage trials were RS5 cereal bait, the original formulation of No.7 bait, a new formulation of No.7 bait, Pestoff® paste, and Kiwicare gel bait. Baits were stored at room temperature ( $20 \pm 5^\circ\text{C}$ ) in conditions of low humidity (i.e., optimum storage conditions). Palatability was measured at 0, 1, 2, 4, 6, 8, and 12 months using methods described in section 5.3.

In autumn 30 kg of Royal Chantenay carrots were harvested and stored outside in a heap covered by a jute sack. At harvest and after 1, 2, and 4 weeks, 4 kg of carrots from the heap were cut into baits weighing  $6 \pm 2$  g and palatability assessed. Pairwise comparisons were made using Fisher's LSD test.

(f) *Effects of moisture on cereal baits:* The immediate effects of moisture on the palatability of non-toxic cereal baits was determined by adding water to freshly made batches of RS5 bait so they contained 0%, 5%, 8%, 10%, 25%, and 50% added moisture. The palatability of each batch of bait was measured within 12 hours of manufacture.

To evaluate the effects of absorbed moisture on the palatability of broadcast bait, 16 batches of RS5 bait were manufactured with 6–8% added water (i.e., a total moisture content of 18–20% wt/wt) and stored at  $20 \pm 5^\circ\text{C}$ . The palatability of these baits was then measured 1–16 d after manufacture. Regression analysis was used to test whether the palatability of baits to individual possums was correlated with the time baits had been damp.

The effect of moisture and micro-organisms on the palatability of cereal baits during storage was determined to estimate the shelf-life of baits. The moisture content of a loose mix of RS5 bait was determined by weighing two samples, oven drying them at 105°C for 18 h, cooling, weighing and reheating for a further hour to a constant weight. The moisture content was calculated as the percent loss of weight during heating. Water was then added to make three batches of loose mix with 12%, 14%, and 16% moisture content. The loose mix was then pelleted into 1.5-g cereal baits. After baits had cooled to air temperature, approximately 10 kg of each bait type was sealed in plastic bags (to prevent them losing or absorbing moisture) and stored at 20±5°C. Baits were subsequently measured for palatability and moisture content at 0, 1, 2, and 4 months after manufacture; at the same times samples of bait were analysed for counts of yeast, mould, and bacteria (Grayson Laboratories, Auckland). Counts of micro-organisms recorded as colony-forming-units per gram of bait (cfu/g) were log-transformed before data analysis. Samples of bait were pooled into groups of 10–20%, 20–30%, 30–40%, 40–50%, and >50% palatability, and these tested to assess whether mould and bacteria had any effect on palatability. The growth of colonies of micro-organisms during storage in relation to the moisture content of baits was also tested by analysis of variance.

(g) *Effects of cinnamon and orange masks on palatability:* Cinnamon oil was mixed into carbopol (Landcare Research, Lincoln), snow-white petrolatum (Shell Chemicals, Wellington), and carrot (var. Manchester Table) at concentrations of 0–20% before these materials were tested for palatability against plain RS5 pellets. Cereal loose mixes of RS5 and the original formulation of No.7 were supplied, and cinnamon blended into different batches at 0–20% before they were pelleted. Batches of bait were then tested for palatability.

Two types of orange (Jaffa orange, Bush, Boake and Allen, Auckland; Firmenich 502.805/T orange, Terry Holdings, Auckland) were blended into batches of RS5 loose mix at concentrations of 0–20% wt/wt before being pelleted. Jaffa orange was also added to Pestoff® paste at concentrations of 0–0.5% wt/wt. All batches of cereal and paste bait were then tested for palatability.

(h) *Cinnamon loss from cereal and carrot baits:* Two commercial batches of RS5 bait (1.5 g) were trialled that nominally contained either 0.1% or 0.5% cinnamon oil. Carrot bait containing 0.1% cinnamon was prepared by chopping carrots into pieces weighing 6 ±2 g, and then tumbling them in a mixer into which a 10% aqueous suspension of cinnamon was slowly dripped. Baits were stored in opened bags containing a plastic liner (cereal baits) or an open plastic ice-cream container (carrot bait) at room temperatures (20±5°C). Samples of bait were taken for analysis at intervals of 0, 2, 7, and 14 d after manufacture for carrot bait; and at intervals of 0, 1, 2, 4, 8, 16, and 24 weeks after manufacture of cereal baits. The cinnamon concentration at each time point was measured by gas liquid chromatography. Because carrot baits dehydrate during storage, the measured cinnamon concentration was adjusted for natural changes in bait weight.

(i) *Bait hardness:* Baits (weighing 1.5 g or 6 g) were manufactured at Landcare Research or Animal Control Products (Waimate), that ranged in hardness from soft crumbly and fell to pieces when handled, to extremely hard and unable to be broken by the teeth of an animal. The baits were measured for hardness using a FT 3-27 fruit penetrometer (Hi Volt Services Ltd, Hastings) fitted with a pointed 2-mm diameter probe. The apparatus measured the pressure (kg) that had to be applied to the side-walls of cereal pellets before a pointed probe penetrated or fractured cereal baits. Bait hardness was measured on 30 individual baits (per batch) from nine batches of small (1.5 g) and nine batches of large (6 g) RS5 bait. The number of baits required for precise estimates of hardness was determined as the number of measurements that gave 95% confidence intervals within ±1 kg of the mean pressure. The palatability

of test baits was measured in two-choice trials against standard RS5 baits to assess the affect of hardness on palatability.

The hardness of bait is also likely to affect the percentage of bait that breaks as it passes over the spinner on a sowing bucket. The relative ease with which the test baits fragmented was therefore assessed. Approximately 2–3 kg of bait of each type was sieved on a 9.5-mm screen to remove small pieces. Two kilograms of bait was then tumbled in a concrete mixer for 5 min. The tumbled bait was sieved again, and the weight of intact baits and fragments measured. The proportions of intact and fragmented bait for different batches of bait was tested by chi-square tests.

(j) *Dyes*: The green dyes applied to possum baits have changed during 40 years of possum control in response to the availability and price of different products. To evaluate the effects of dyes on palatability, five types of dye that are currently available were added to cereal and citrus paste baits at concentrations that coloured baits to the specified shade of green (colour range 261–267 of New Zealand Colour Specification 7702 (1989)). Baits were then tested for palatability against the control bait and the palatability calculated for individual possums.

#### 5.4 Amounts of bait eaten

Because the amounts of bait eaten by possums are likely to affect percentage kills, bait consumption by captive and wild possums was monitored. Bait consumption from bait stations was measured by attaching a "Coontrol" bait station to an electronic scale (Sartorius QS; Biolab Scientific Ltd., Lower Hutt) and recording the combined weight ( $\pm 1$  g) of the feeder, bait, and possum (when present) at 10-second intervals with a Psion datalogger (Pocket Solutions, Wellington). Because possums typically feed in bouts of 6–14 mins (MacLennan 1984; Hickling *et al.* 1990), the equipment was used to record the time after sunset that each feeding bout occurred, the duration of each bout, the amount eaten in each bout, the number of bouts each hour, the per capita amounts eaten within 30 and 60 min of when they first commenced eating bait, and the weight of individual possums eating bait. The baits offered were recently manufactured RS5 and Agtech baits containing 0.15% 1080 or non-toxic RS5 and Agtech bait. These baits were chosen because they were manufactured baits that contrasted in palatability. The RS5 bait was of high palatability ( $\approx 50\%$ ) and the Agtech bait was of low palatability ( $\approx 18\%$ ). All bait consumption data were transformed to normality using the natural log scale before being analysed. Different baits and strategies for their use in ground-level bait stations were compared by analysis of variance unless otherwise stated.

(a) *Relative amounts eaten by captive and wild possums*: To assess whether existing data on bait consumption by captive possums could be used for modelling, the per capita amounts of RS5 and Agtech bait eaten from bait stations by penned and wild possums were compared. Penned possums used in this study were exposed to a variety of novel foods that constituted the diet of captive possums (Duckworth & Meikle 1995). In comparison, wild possums (that had not been controlled for at least 4 years) were eating natural foods (i.e. foliage and grasses) and had not previously been exposed to cereal pellets.

(b) *Bait consumption from conventional and scale-mounted bait stations*: Preliminary studies were conducted to check if the electronic scales deterred wild possums. At 32 sites the consumption of bait from a conventional "Coontrol" bait station and from one mounted on an electronic scale were compared. To ensure the two types of bait station were independent, they were located

approximately 150 m apart. Each bait station contained a measured amount of RS5 bait containing 0.15% 1080. The amounts of bait eaten from the two bait stations over 3 nights were compared by an unpaired t-test.

### **Consumption of baits from bait stations by wild possums**

Wild possums were offered three types of recently manufactured (i.e., stored no longer than 3 months) bait containing 0.15% 1080 (carrot, RS5, Agtech) and two types of non-toxic bait (RS5 and Agtech). The amounts of RS5, Agtech, and carrot eaten by wild possums was compared for toxic and non-toxic bait. Factors evaluated that may have influenced bait consumption were presence of toxin, bait palatability, location, season, and prefeeding.

(a) *Presence of toxin:* Possums eating bait from bait stations consume most bait in an initial feeding bout of 6–14 min (Henderson & Hickling 1997). The mean time to loss of appetite for possums ingesting large doses of 1080 (>4 mg/kg) is 41 min (Henderson *et al.* in press). Therefore, the consumption by wild possums of non-toxic and toxic baits was compared over 30 min to assess whether 0.15% 1080 in baits affected the amounts eaten.

(b) *Bait palatability:* The effects of bait palatability on the consumption of non-toxic Agtech and RS5 baits were compared. Similarly, the consumption of toxic carrot, RS5, and Agtech bait were also compared.

(c) *Site factors and season:* The effect of site factors on bait consumption was determined by positioning bait stations outfitted with remote-sensing equipment at eight different locations in Canterbury for 12 months. Habitats varied and included plantations of mature pines (2 sites), remnant beech/podocarp forest (4 sites), beech forest (1 site) and a mix of exotic deciduous and evergreen trees (1 site). At all locations there was no known history of recent control. The amounts of four different bait types eaten (toxic and non-toxic RS5 and Agtech) at the eight locations were compared.

Toxic RS5 bait was the bait type eaten most often by wild possums. Consumption of this bait throughout four seasons was tested for seasonal effects.

(d) *Prefeeding:* The use of prefeed to increase bait consumption was determined using scales and dataloggers that compared four methodologies for possum control: RS5 with prefeed, RS5 without prefeed, Agtech with prefeed, and Agtech without prefeed. Data was downloaded to a laptop computer from dataloggers every 3 days. At all locations there were low to moderate densities of possums, so individual animals were readily identified by their bodyweight. The number of nights on which each animal had previously consumed prefeed, the time of its visit (in minutes after sunset), the amount of non-toxic bait consumed within 60 min of when it first started eating, and the weight of the possum were entered into a database. The amounts of toxic RS5 and Agtech bait eaten without prefeed and after 1–8 nights of prefeed were compared. The percentage of possums eating small sublethal amounts of RS5 or Agtech bait (i.e., 3 g of bait or less) was also estimated, and the proportions that were small eaters were compared by chi-square tests after 0, 1, 2, 3, 4, 5, and >5 nights of prefeeding.

## **5.5 Predictive modelling of population kills at bait stations**

The percentage of wild possums likely to be killed by existing baits at bait stations was modelled by randomly combining data on possums' susceptibility to 1080, variation of 1080 content in baits, possum



bodyweights, and possums' consumption of baits. The amounts of 1080 bait eaten from bait stations was assessed for RS5 and Agtech bait with and without prefeed. For possums that ate bait the average probability that each would die was predicted, and the predicted kills estimated as the overall mean for the probabilities of death.

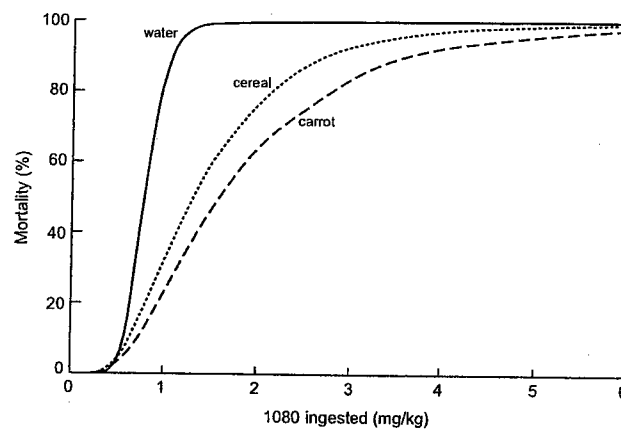
## **5.6 The efficacy of baits used during aerial control**

The effects of bait type (palatability), 1080 concentration, and prefeeding on the efficacy of aerial baiting were determined from the measured kills following aerial control. During the last 20 years there have been several databases of aerial operations compiled where operational factors have been documented and the percentage kills have been monitored by a validated methodology (Batcheler 1978; Spurr, 1993; Brown & Arulchelvam 1995; Morgan *et al.* 1997; Henderson *et al.* 1998; Henderson *et al.* in press). These were combined into a database of 209 operations and analysed to assess the bait factors (i.e., palatability, 1080 concentration, prefeeding) that significantly contributed to the percentage kills measured. As there may have been confounding influences of different operational factors, data were sorted and tested by analysis of variance to assess the independent effects of bait type, 1080 concentration, and prefeeding.

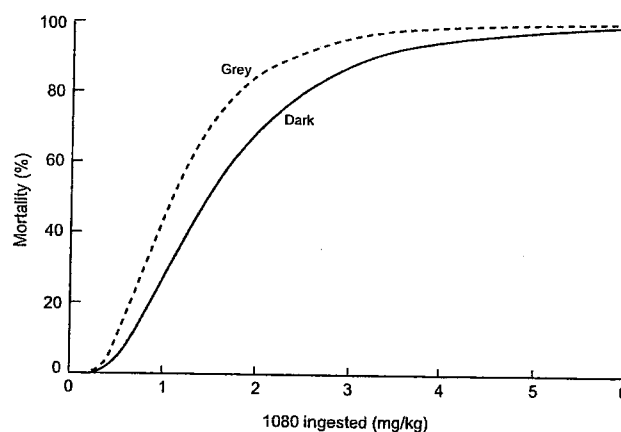
## 6. Results

### 6.1 Acute toxicity

The susceptibility of female and male possums to 1080 was similar ( $\chi^2=0.71$ , d.f.=2,  $P=0.702$ ). The carrier significantly affected the acute toxicity of 1080: it was more toxic as an aqueous solution than in cereal ( $\chi^2=38.61$ , d.f.=2,  $P<0.001$ ) or carrot ( $\chi^2=43.69$ , d.f.=2,  $P<0.001$ ) baits, and cereal baits containing 1080 were more toxic than carrot baits ( $\chi^2=6.66$ , d.f.=2,  $P=0.036$ ) (Fig. 1). Grey possums were more susceptible to 1080 than dark possums ( $\chi^2=6.58$ , d.f.=2,  $P=0.037$ ; Fig. 2).



**Fig. 1** Probit curves demonstrating differences in the acute toxicity of 1080 in water, cereal baits, and carrot baits.



**Fig. 2** Probit curves demonstrating differences in the acute toxicity of 1080 to grey and dark possums.

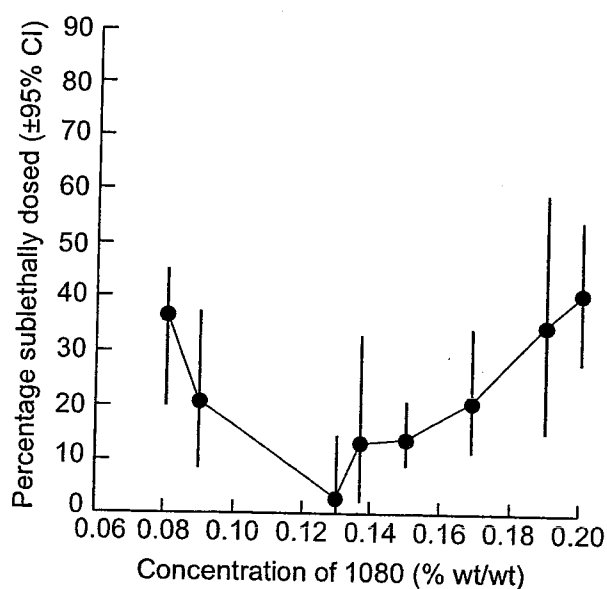
Although the variable number of possums given different bait types ( $n=24-576$ ) did not markedly influence the accuracy or precision of  $LD_{50}$  estimates, the only precise estimates for the  $LD_{95}$  of 1080 to possums were obtained for cereal ( $n=576$ ) and carrot ( $n=573$ ) (Table 1). For all bait types, the  $LD_{95}$  of 1080 ranged from 3.6–4.7 mg/kg (Table 1).

**Table 1** The acute toxicity of 1080 (mg/kg) in different bait types to captive possums (95% C.I. in parentheses)

Bait type	Lethal dose estimates		
	LD <sub>50</sub>	LD <sub>90</sub>	LD <sub>95</sub>
Apple (n=24)	1.6 (1.0 - 2.2)	3.3 (2.4 - 10.7)	4.1 (2.8-18.5)
Swede (n=35)	1.7 (1.4 - 2.7)	3.4 (2.3 - 13.0)	4.1 (2.6-20.8)
Gel (n=60)	1.5 (0.9 - 2.1)	3.4 (2.4 - 10.7)	4.4 (2.8-18.7)
Paste (n=167)	1.4 (1.0 - 1.8)	3.6 (2.8 - 5.5)	4.7 (3.5-8.1)
Carrot (n=573)	1.6 (1.4 - 1.7)	3.6 (3.2 - 4.3)	4.6 (4.0-5.7)
Cereal (n=576)	1.3 (1.2 - 1.5)	2.9 (2.5 - 3.5)	3.6 (3.0-4.5)

## 6.2 Toxin concentrations as a cause of sublethal poisoning

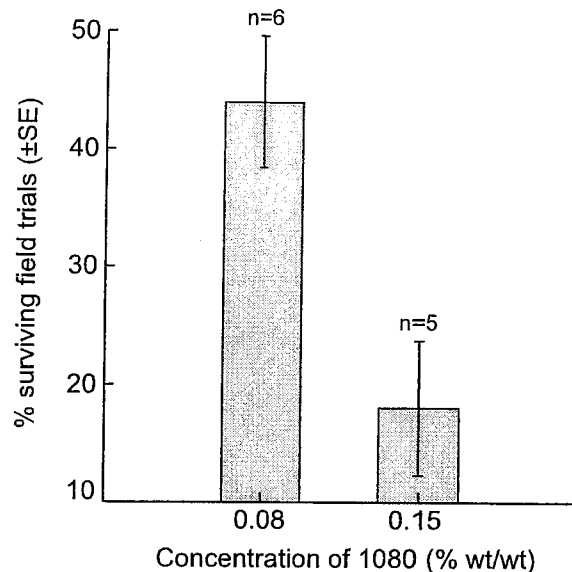
The percentage of 494 captive possums that were sublethally poisoned by 1080 baits was dependent on 1080 concentrations ( $\chi^2=21.8$ , d.f.=4,  $P<0.001$ ) (Fig. 3). As 1080 concentrations increased from 0.08% to 0.13% wt/wt, most possums ingested a higher toxic dose (mg/kg) when feeding on baits, and the percentage sublethally poisoned declined from 37% to 3%. However, at concentrations higher than 0.13% the number sublethally poisoned progressively increased. This is attributed to an increasing aversion to 1080 that reduced the amounts of bait eaten ( $F_{4,489}=7.6$ ,  $P<0.001$ ). Fewer captive possums survived baits containing 0.15% 1080 (14%) than baits containing 0.08% 1080 (37%).



**Fig. 3** Percentage of captive possums sublethally poisoned after feeding *ad libitum* on baits containing different concentrations of 1080.

In replicated field trials, the percentage of possums surviving bait station control was affected by the concentration of 1080 in RS5 baits (Fig. 4). As with captive possums there were fewer possums that survived baits containing 0.15% 1080 (18%) than baits with 0.08% 1080 (44%) ( $F_{1,10}=13.09$ ,  $P=0.006$ ).

Prefeeding did not significantly reduce the number of possums surviving bait station control with baits containing 0.15% 1080 ( $F_{1,10}=2.90$ ,  $P=0.11$ ); a result that is mainly attributed to one prefeed operation only killing 50% of possums. On average, prefeeding bait stations killed 90% of possums ( $n=6$ ) and stations not prefeed killed 82% of possums ( $n=5$  field trials).



**Fig. 4** Percentage of possums surviving bait station control that used baits containing 0.08% and 0.15% 1080.

### Stability of 1080 in baits

(a) *During storage of manufactured baits:* The measured 1080 concentration in four types of commercial possum bait stored for up to 12 months is shown in Table 2.

**Table 2** The concentration of 1080 in baits kept in a dark storeroom at  $20\pm 5^\circ\text{C}$ .

Bait type	Moisture content (%)	Months of storage and toxin concentration (% wt/wt)						
		0	1	2	4	6	8	12
RS5	12	0.19	0.14	0.16	0.18	0.17	0.16	0.16
No.7	16.4	0.14	0.11	0.13	0.13	0.13	0.1	0*
Gel bait	24.5	0.12	0.15	0.12	0.14	0.13	0.14	0.14
Paste bait	60.4	0.14	0.15	0.13	0.14	0.15	0.14	0.15

\* the biodegradation of all 1080 in cereal baits containing >16% moisture was confirmed by a second assay (see below)

In RS5 cereal, gel, and paste baits there was no change in the concentration of 1080 during 12 months storage. However, 1080 concentration in the original formulation of No.7 cereal baits that was used until 1998 (containing 16.4% wt/wt moisture) declined to below the limit of detection 8–12 months after bait manufacture. This is attributed to microbial degradation, as mould was evident on the surface of baits after they had been stored for 6 months.

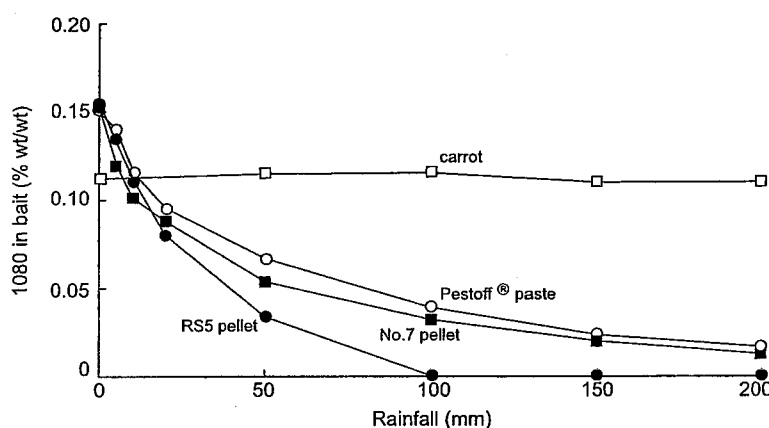
(b) *During storage of carrot with and without soil residues:* The amount of 1080 in washed and muddy carrot baits declined during 2 weeks of storage ( $F_{3,4}=10.02$ ,  $P=0.02$ ), but there was no difference in the rate of loss from carrot with and without soil residues ( $t_3=1.65$ ,  $P=0.20$ ) (Table 3). Carrot baits rotted quickly in the laboratory during these trials; it is therefore probable that the micro-

organisms in the decaying carrot contributed to the biodegradation of some 1080. In comparison, baits in the field that dehydrate with high temperatures and low humidity may remain toxic for many months (G. Wright unpubl. data).

**Table 3** The 1080 concentration in carrots with and without soil residues during 2 weeks storage at room temperature ( $20\pm 5^\circ\text{C}$ ).

Bait type	Days in storage and 1080 concentration (% wt/wt)			
	0	3	7	14
Washed carrot	0.139	0.13	0.14	0.102
Dirty carrot	0.161	0.136	0.138	0.109

(c) *Under simulated rainfall:* During the application of simulated rain there were no differences in the 1080 concentrations in baits used during previous research (Bowen et al. 1995) and similar types of bait exposed to "rain" in 1998 trials ( $F_{1,49}=1.49$ ,  $P=0.17$ ), and data was therefore pooled (Fig. 5). Rainfall significantly affected the 1080 concentration in RS5 baits ( $F_{1,14}=96.2$ ,  $P<0.001$ ), No.7 baits ( $F_{1,14}=30.8$ ,  $P<0.001$ ) and Pestoff® paste ( $F_{1,14}=23.0$ ,  $P<0.001$ ). However, concentrations of 1080 in carrot bait were not affected by exposure to up to 200 mm of rain ( $F_{1,11}=1.38$ ,  $P=0.27$ ) (Fig. 5). The half-lives of 1080 in RS5 (23 mm), No.7 bait (49 mm), and Pestoff® paste (71 mm), suggest that these are the maximum amounts of precipitation that baits containing 0.15% 1080 can sustain without causing significant levels of sublethal poisoning (assuming broadcast baits containing 0.15% 1080 kill similar percentages of possums as those containing 0.08% 1080 (Thomas & Meenken 1995).



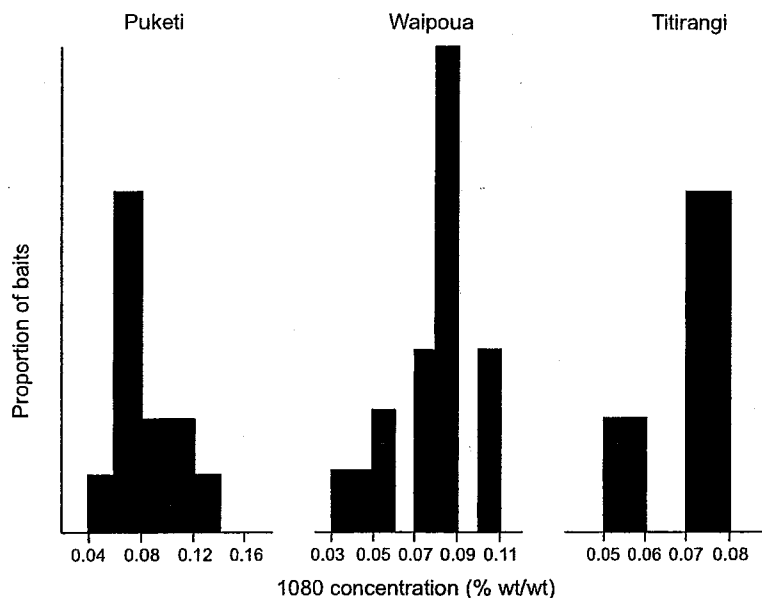
**Fig. 5** Mean concentration of 1080 (% wt/wt) in four different bait types after they were subjected to 0, 5, 10, 20, 50, 100, 150, and 200 mm of simulated rainfall during replicated trials ( $n=2$ ).

Detoxification of carrot will not be facilitated by rainfall leaching 1080 from baits, and is therefore reliant on defluorination of 1080 by naturally occurring micro-organisms (Parfitt *et al.* 1994; King *et al.* 1994; Eason 1997). In areas of low rainfall, the detoxification of cereal and paste baits will also be reliant on breakdown of 1080 by micro-organisms; a factor likely to affect cereal baits more than paste baits. Pestoff® paste has proved very stable during prolonged periods of dry weather. For example, following "normal" withholding periods, domestic deer and other livestock have succeeded in turning

over "spits" and eating the dried rubbery residues of paste, and they subsequently died (Grant Cordwall pers. comm.).

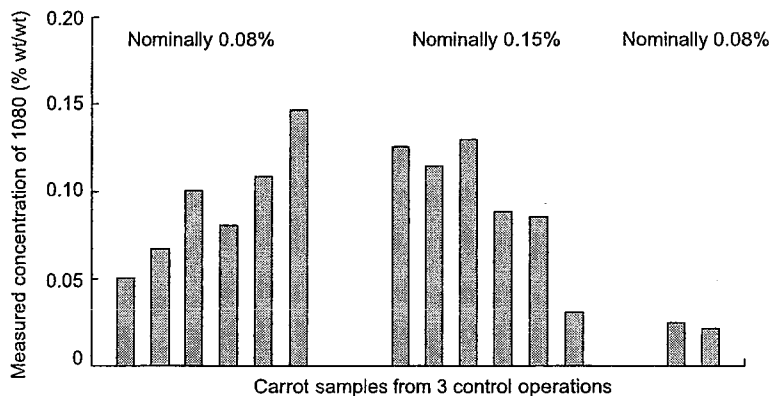
### Variations in toxin concentration during bait preparation

(a) *In cereal bait:* The amount of 1080 in batches of the original formulation of No.7 bait has been measured previously (Frampton *et al.* in press) and was demonstrated to range from 0.03% to 0.13% in batches of bait that nominally contained 0.08% 1080 (Fig. 6). The standard deviations about mean 1080 concentrations for Puketi ( $0.080 \pm 0.022\%$  wt/wt,  $n=12$ ), Waipoua ( $0.077 \pm 0.018\%$  wt/wt,  $n=18$ ), and Titirangi ( $0.068 \pm 0.008\%$  wt/wt,  $n=4$ ) suggest that 1080 was previously not uniformly mixed throughout many batches of No.7 cereal bait. The accuracy and precision of 1080 concentrations in recent formulations is not known.



**Fig. 6** Distribution of 1080 concentration in baits taken from 34 different bags of No.7 used to control possums at Waipoua, Titirangi, and Puketi forests.

(b) *In carrot bait:* Samples of carrot bait collected during two operations had similar 1080 concentrations ( $F_{1,11}=0.029$ ,  $P=0.87$ ) despite 1080 being nominally applied at 0.08% in one operation and at 0.15% wt/wt in the other (Fig. 7). In the first operation, the overall mean concentration of 1080 (0.093% 1080) was not significantly different to a nominal concentration of 0.08% 1080 ( $t_5=0.91$ ,  $P=0.41$ ), but the mean concentration of 1080 in the second operation (0.096% 1080) was significantly different to the nominal concentration of 0.15% ( $t_5=3.60$ ,  $P=0.02$ ). The 1080 concentration in a third operation

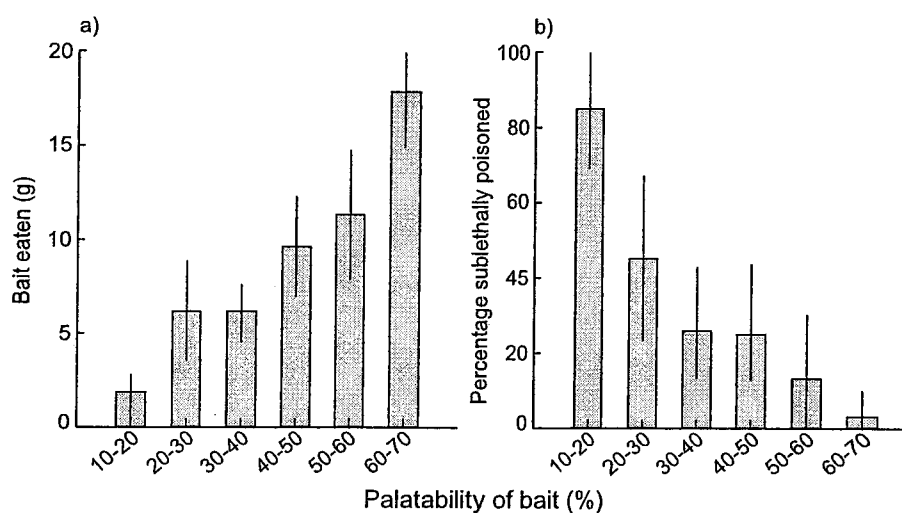


**Fig. 7** Measured 1080 concentration (% wt/wt) in carrot baits at different times of bait preparation during three control operations.

(0.023% 1080) was also significantly different to the nominal concentration of 0.08% 1080 ( $t_1=37.7$ ,  $P=0.02$ ). Imprecise carrot 1080 concentrations during these three control operations are assumed to be a consequence of inadequate monitoring of the amounts of toxic field solution and bait used (Environment Waikato 1997), and poorly calibrated equipment not applying appropriate amounts of 1080 to carrot (Simmons 1996).

### 6.3 Bait palatability as a cause of sublethal poisoning

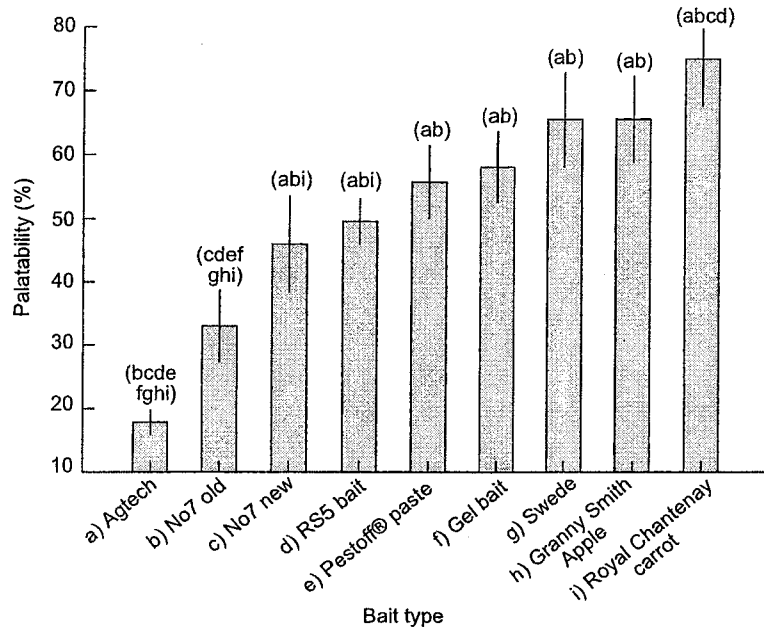
As the palatability of baits increased possums ate significantly more 1080 bait ( $F_{5,186}=17.2$ ,  $P<0.001$ ) (Fig. 8(a)), and consequently ingested a higher dose (in mg/kg) of 1080 ( $F_{5,186}=25.6$ ,  $P<0.001$ ) which resulted in a higher proportion of them dying ( $\chi^2=50.4$ , d.f.=5,  $P<0.001$ ). Caged possums sublethally poisoned by 1080 were mainly those that fed sparingly on baits with a palatability less than 30% (Fig. 8).



**Fig. 8** (a) the mean weight of bait containing 0.15% 1080 eaten by groups of individually caged possums with *ad libitum* access to toxic baits of known palatability and alternative foods, and (b) the percentage sublethally poisoned ( $\pm$  95% C.I. intervals).

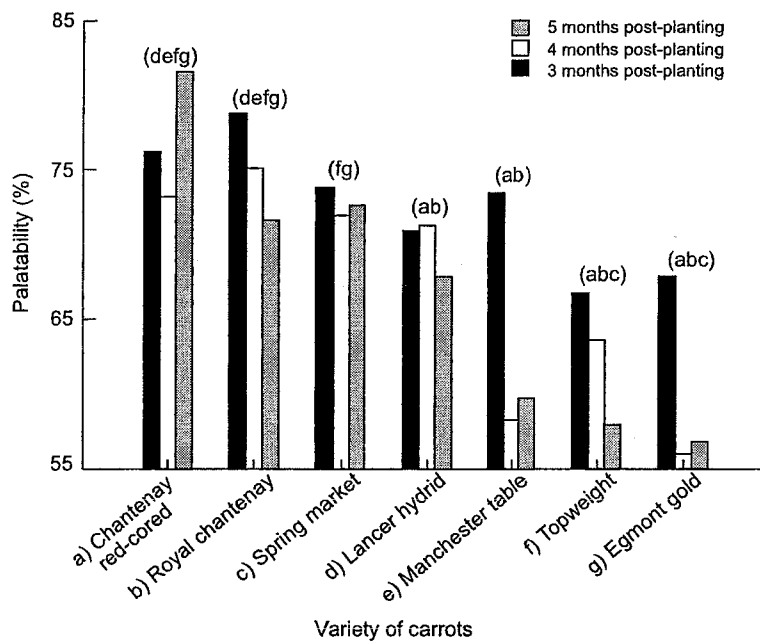
### Factors affecting the palatability of baits to captive possums

(a) *Bait type*: The palatability to caged possums of nine types of bait used for the control of possums was significantly different ( $F_{8,309}=13.9$ ,  $P<0.001$ ) (Fig. 9). Agtech bait was least preferred and carrot (var. Royal Chantenay) most preferred. Although all recently manufactured baits (except Agtech) had a palatability exceeding 40%, the natural baits (swede, apple, carrot) proved to be more palatable to captive possums than synthetic baits.



**Fig. 9** The palatability of nine different types of bait to individually caged possums ( $\pm$  S.E.). The letters in parentheses indicate bait types that had a significantly different palatability (using two-tail Fisher Exact tests).

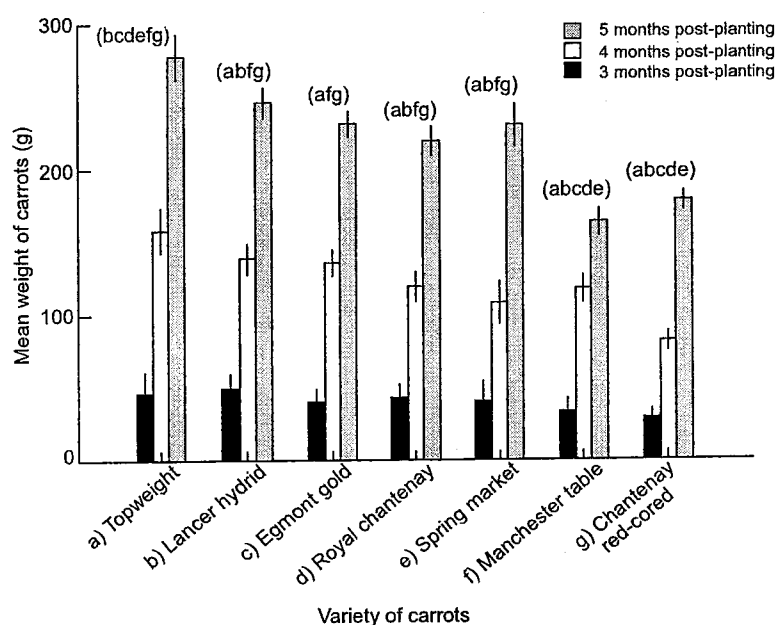
*b) Carrot variety and size:* The palatability of carrots to caged possums was dependent on the variety of carrot ( $F_{6,306}=4.3$ ,  $P<0.001$ ) and the time that had elapsed since seeds were sown ( $F_{2,306}=10.3$ ,  $P<0.001$ ) (Fig. 10). The Chantenay varieties of carrot (Chantenay red-cored and Royal Chantenay) were significantly more palatable than Lancer Hybrid, Manchester Table, Topweight, and Egmont Gold (Fig. 10).



**Fig. 10** The palatability of seven varieties of carrot to caged possums at 3, 4, and 5 months after they were planted. The letters in parentheses indicate carrot varieties that were significantly different in palatability (using two-tail Fisher Exact tests).



There are likely to be differences in the yield (tonnes/hectare) from different varieties of carrot grown under contract, as size is dependent on variety ( $F_{6,919}=39.1$ ,  $P<0.001$ ), and on the time since sowing ( $F_{2,919}=1549.2$ ,  $P<0.001$ ) (Fig. 11). Although Topweight produced carrots that were larger than other varieties at 4–5 months after planting, this variety was amongst those least preferred by possums. Chantenay red-cored produces carrots smaller than all other varieties except Manchester Table. In this trial Royal Chantenay and Spring Market (varieties that are highly palatable to possums) were of intermediate size, producing carrots significantly smaller than Topweight or Lancer Hybrid but larger than Manchester Table and Chantenay red-cored.



**Fig. 11** The mean weight of carrots 3, 4, and 5 months after seed was sown ( $\pm$  95% C.I.). The letters in parentheses indicate carrot varieties with significantly different weights (using two-tail Fisher Exact tests).

The time elapsed since seed was planted ( $F_{9,155}=3.74$ ,  $P<0.001$ ) and mean size ( $F_{6,383}=4.29$ ,  $P<0.001$ ) significantly affected the palatability of Chantenay carrots (Table 4). A trend of reduced palatability of carrots as they mature suggests that they are best harvested within 6 months or before they reach a mean size of 200 g (Table 4).

**Table 4** The size of Royal Chantenay carrots during 12 months growth, and their palatability at different stages of maturity

	Months since planting, mean size (g), and bait palatability.						
	3	4	5	7	9	11	12
Carrot size ( $\pm 95\%$ C.I.)	42.3 $\pm$ 1.9	119.3 $\pm$ 5.1	196.3 $\pm$ 10.5	203.2 $\pm$ 12.5	209.9 $\pm$ 13.2	194.9 $\pm$ 13.7	219.0 $\pm$ 11.5
Bait palatability (%)	78.7	75.1	71.6	63.0	66.1	61.4	55.5

(c) *Palatability of carrot baits with and without soil residues:* There was no difference in the relative palatability of cut carrots with and without soil residues ( $F_{1,114}=0.03$ ,  $P=0.87$ ) (Table 5). Although cut carrot rotted quickly if it was stored at room temperature (by the end of the second week of storage they were too rotten to present to possums to assess palatability), there was no significant change in palatability during the week after carrot was cut ( $F_{2,114}=0.48$ ,  $P=0.62$ ).

**Table 5** The palatability of cut carrot (i.e., baits of  $6 \pm 2$  g) to captive possums during 2 weeks storage with and without soil residues (var. Manchester Table).

	Duration of storage (days) and bait palatability (%)			
	1	3	7	14
With soil residues	64.0	63.8	54.8	NA
Washed carrot	68.8	63.7	54.8	NA

NA= not assessed because carrot was too degraded to feed to caged possums

(d) *Carrot after freezing:* Frozen carrots rapidly lost palatability after they were defrosted ( $F_{3,84}=3.15$ ,  $P=0.02$ ) (Table 6.). After 2 weeks of storage the uncut carrot had putrefied to such an extent it could not be tested for palatability.

**Table 6** The palatability of uncut carrots after freezing (var. Royal Chantenay).

	Time since defrosting (days) and bait palatability (%)			
	1	2	7	14
Before freezing	73.7	73.2	64.7	53.8
				NA

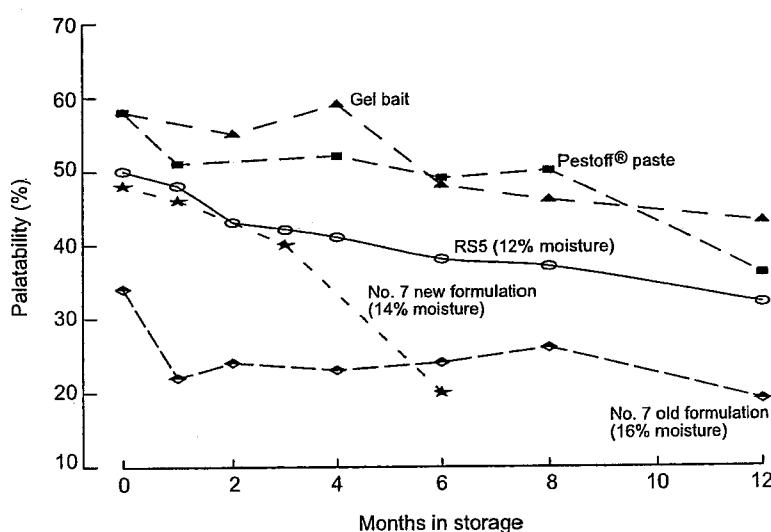
NA= not assessed because carrot was too degraded to feed to caged possums

The freezing of carrot baits caused the release of a large volume of fluid. Surprisingly, when this fluid was decanted from the remnants of the carrot bait, the carrot still contained the same 1080 concentration as it had before freezing (Table 7).

**Table 7** The 1080 concentration of stored carrot bait before freezing and then during the week after it had defrosted

Before freezing (nominally 0.15% wt/wt)	Days since carrot baits defrosted and 1080 concentration (% wt/wt)		
	1	3	7
0.134	0.13	0.133	0.145

e) *Storage of baits:* The palatability of five types of manufactured bait (Fig. 12) was significantly different ( $F_{4,734}=20.5$ ,  $P<0.001$ ). The new formulation of No.7 bait (14% moisture) was more preferred by captive possums than the earlier formulation (16–17% moisture). During the first 3 months of storage the new No.7 formulation was of similar palatability to RS5 bait. All baits became less palatable during storage ( $F_{6,734}=6.37$ ,  $P<0.001$ ), although only cereal baits with moisture contents of 16% and 14% had bait palatabilities that declined to less than 30%. Baits with a palatability less than 30% are those that sublethally poison large numbers of possums (Henderson *et al.* in press). If 40% is assumed the minimum acceptable palatability for baits, the "shelf-life" for Kiwicare gel is 12 months, for Pestoff® paste 8 months, for RS5 (12% moisture) 6 months, and for No.7 (14% moisture) it is 3 months. No.7 bait containing 16% moisture has an unsatisfactory palatability within 1 month of the date of manufacture.



**Fig. 12** Changes in the palatability of five types of commercial possum bait during storage.

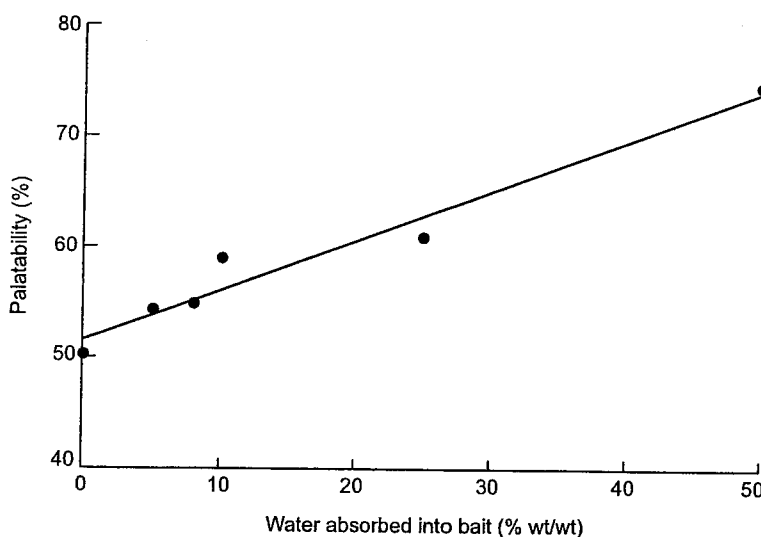
Carrots that were harvested and then stored outdoors in a heap progressively declined in palatability ( $F_{3,68}=3.1$ ,  $P=0.03$ ) (Table 8.). The most significant decline in measured palatability occurred after 1 week's storage ( $P<0.05$  for pairwise comparison of palatability groupings using Fisher's LSD test)

However, after 4 weeks storage whole carrots were still more palatable to captive possums than RS5 cereal bait.

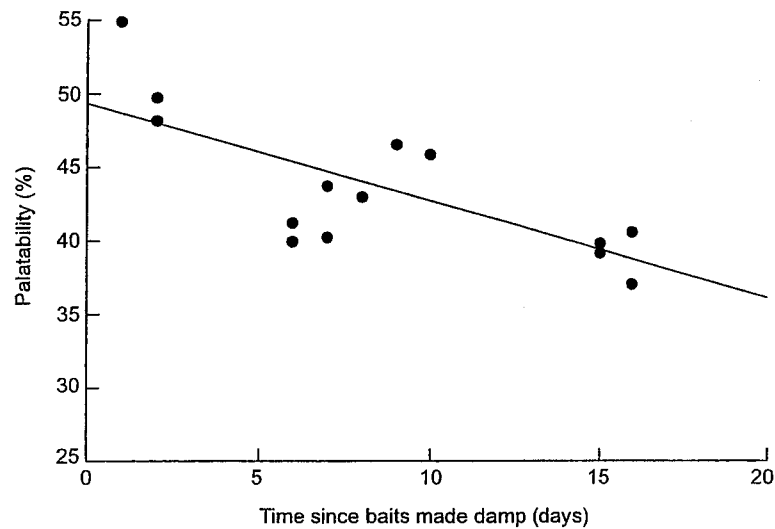
**Table 8** The post-harvest palatability of stored carrots (var. Royal Chantenay).

Duration of storage (weeks) and palatability (%)			
0	1	2	4
79.8	77.9	68.6	66.3

(f) *Effects of moisture on cereal baits:* On the day that dry RS5baits were dampened they became more palatable to captive possums (Fig. 13), and their palatability increased ( $F_{1,115}=6.57$ ,  $P=0.012$ ) as the percentage of added moisture increased from 0% to 50% (at which stage baits were saturated). However, after 2 days damp RS5 cereal baits lost palatability (Fig. 14). The percentage decline was directly correlated with the time that baits had been damp ( $r = -0.19$ , d.f.=299,  $P < 0.001$ ). Within 6–8 days of becoming damp, palatability had on average declined to 43%, but still exceeded the minimum of 40% required to kill most possums. Most (>90%) possums eat bait within 2 days of aerial application (Morgan 1982).



**Fig. 13** The relationship between the amount of water absorbed into RS5 bait and the palatability of baits on the day they became damp.



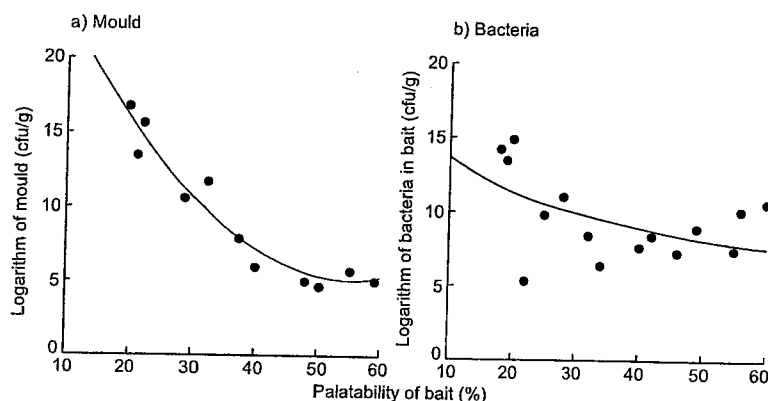
**Fig. 14** Changes in the palatability of baits containing 8% added water during a 2-week period following manufacture.

When RS5 baits were stored with 12%, 14%, and 16% moisture, their palatability was dependent on the duration of storage ( $F_{3,10}=24.4$ ,  $P<0.001$ ) and moisture content ( $F_{2,10}=10.14$ ,  $P=0.004$ ) (Table 9).

**Table 9** Changes in the palatability of RS5 baits stored with 12%, 14% and 16% moisture.

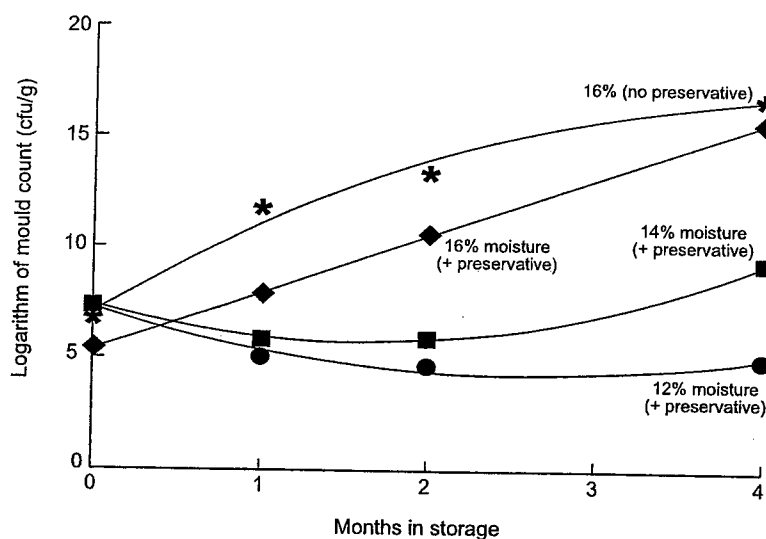
Moisture content	Months in storage and bait palatability (%)			
	0	1	2	4
12%	55	49	43	41
14%	56	46	34	22
16%	56	32	25	20

The palatability of RS5 baits stored with 12%, 14%, and 16% moisture were significantly affected by mould ( $F_{4,15}=21.4$ ,  $P<0.001$ ; Fig. 15(a)) and to a lesser extent by bacteria ( $F_{4,15}=7.5$ ,  $P=0.004$ ; Fig. 15(b)). Some batches of bait had up to 18 million mould units (cfu) per gram of bait after they had been stored with 16% moisture for 4 months. The palatability of stored RS5 cereal baits in these trials was below 30% (a threshold at which many possums are sublethally poisoned; Henderson *et al.* in prep.) when mould counts exceeded 3000 cfu/g, and were below 40% when the mould counts exceeded 400 cfu/g.



**Fig. 15** Correlation between (a) mould counts, and (b) bacterial counts and the palatability of RS5 bait.

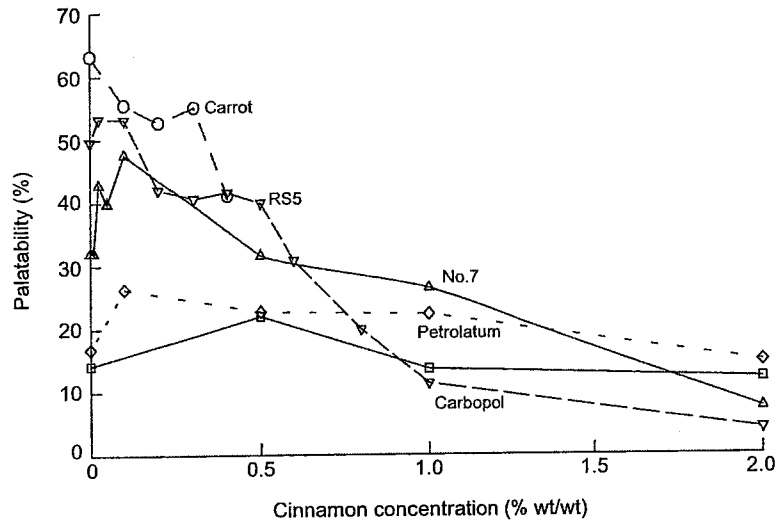
Because palatability is strongly correlated with the amount of mould in bait, we assessed the effect of the moisture content of baits on mould growth. Growth of mould in cereal baits was significantly affected by the combined effects of moisture content and the duration of storage ( $F_{3,8}=15.49$ ,  $P=0.001$  for interaction of storage time and moisture) (Fig. 16). Baits with a moisture content of 16% grew mould during storage irrespective of whether they contained a preservative or not (Fig.16). Baits containing a preservative and 14% moisture had mould counts that exceeded 1000 cfu/g within 4 months storage, which caused palatability to decline from 50% to 22%. There was no increase in microorganisms in baits containing 12% moisture, and consequently baits were still palatable to possums after 4 months storage (i.e., palatability > 40%).



**Fig. 16** The effects of 12%, 14%, and 16% moisture in promoting mould growth in RS5 baits (that contained a preservative). Mould growth was also measured in a batch of RS5 bait containing 16% moisture that did not contain preservative.

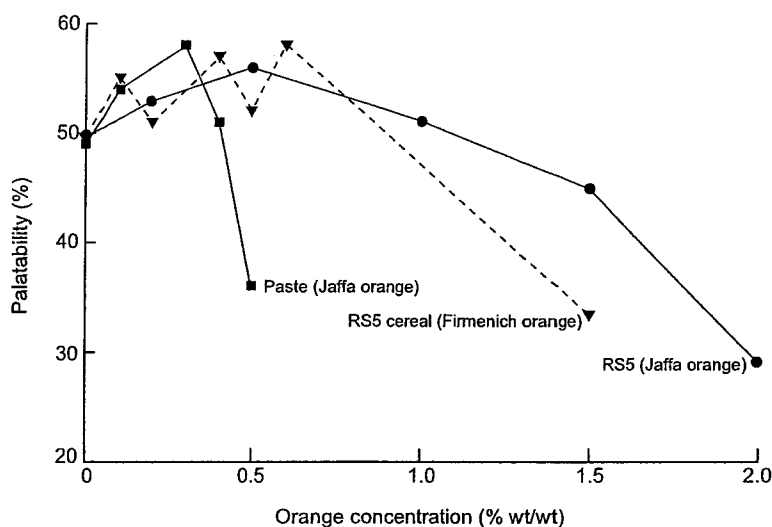
(g) *Effects of cinnamon and orange masks on palatability:* Palatability is significantly affected by the concentration of cinnamon in bait (Fig. 17). Cinnamon concentrations >0.5% wt/wt in RS5 bait markedly impair palatability, causing significant differences in the palatability of batches of bait containing 0% to 0.8% cinnamon ( $F_{7,220}=4.02$ ,  $P<0.001$ ). Concentrations of cinnamon in a range from 0.01% to 0.10% enhanced the palatability of No.7 bait, but concentrations of cinnamon >0.5%

reduced palatability ( $F_{8,164}=4.64$ ,  $P<0.001$ ). The variable response of individual possums to increased cinnamon concentrations on carrot resulted in no significant differences in palatability of batches of bait containing 0–0.4% cinnamon ( $F_{4,104}=1.26$ ,  $P=0.29$ ). However, a trend of reduced palatability with cinnamon concentrations  $>0.3\%$  suggests research should further assess the maximum levels of cinnamon that can be used on carrot. Petrolatum and carbopol are materials which are very unpalatable to caged possums; consequently cinnamon concentrations of 0–20% made no differences to the palatability of either petrolatum ( $F_{5,66}=0.39$ ,  $P=0.39$ ) or carbopol ( $F_{3,54}=0.35$ ,  $P=0.42$ ).



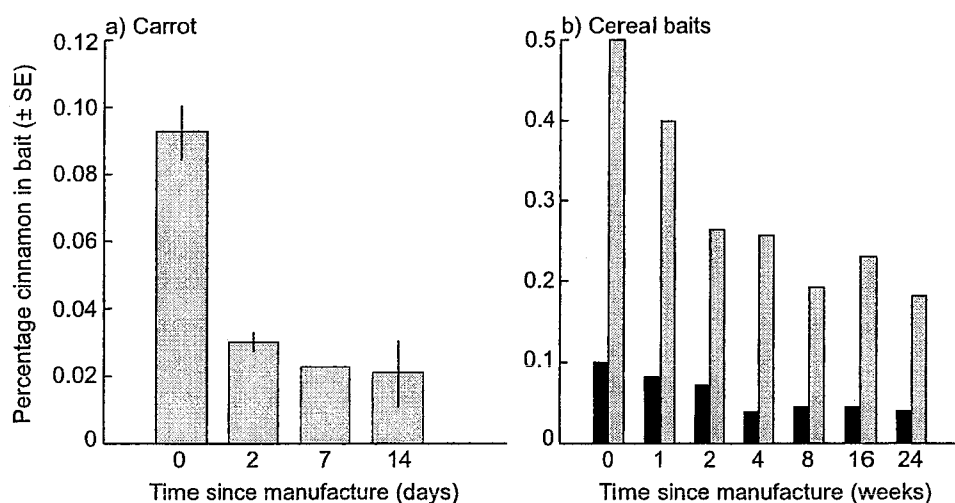
**Fig. 17** The palatability to captive possums of five types of bait containing different concentrations of cinnamon.

The palatability of RS5 and the Pestoff® paste were significantly affected by different concentrations of two types of orange ( $F_{2,275}=9.50$ ,  $P<0.001$ ) (Fig. 18). Concentrations of Jaffa orange  $>1.5\%$  in RS5 cereal baits and  $>0.4\%$  in Pestoff® paste significantly reduced palatability; Firmenich orange in RS5 at  $1.5\%$  wt/wt also reduced palatability ( $P$ -values all  $>0.05$ ).



**Fig. 18** The palatability of RS5 cereal bait and Pestoff® paste with different concentrations of two types of orange flavour.

(h) *Cinnamon loss from cereal and carrot baits:* The amount of cinnamon in carrot baits was significantly correlated with the time that had elapsed since bait manufacture ( $r = -0.87$ ,  $d.f. = 5$ ,  $P = 0.02$ ), with most cinnamon lost during the first 2 days (Fig. 19(a)). Loss of cinnamon from cereal baits was also significantly correlated with time since manufacture ( $r = -0.75$ ,  $d.f. = 7$ ,  $P = 0.05$ ) (Fig. 19(b)). Rates of loss of cinnamon from cereal baits were similar irrespective of whether the initial concentration was 0.1% wt/wt or 0.5% wt/wt. The higher loss on carrot is attributed to cinnamon being lost more rapidly from the surface of carrot bait than from the inside of a cereal bait. During the time since bait manufacture, the cinnamon concentrations declined to asymptote at either 25% of the nominal concentration after 2 days (carrot), or 40% of the nominal concentration after 8 weeks (cereal). These results suggest that cinnamon concentrations should be increased threefold on carrot and twofold in cereal for cinnamon to remain effective as a mask for 1080 during the shelf- and/or field-life of these bait types.



**Fig. 19** Cinnamon concentration (% wt/wt) at different times after manufacture of (a) carrot and (b) cereal baits.

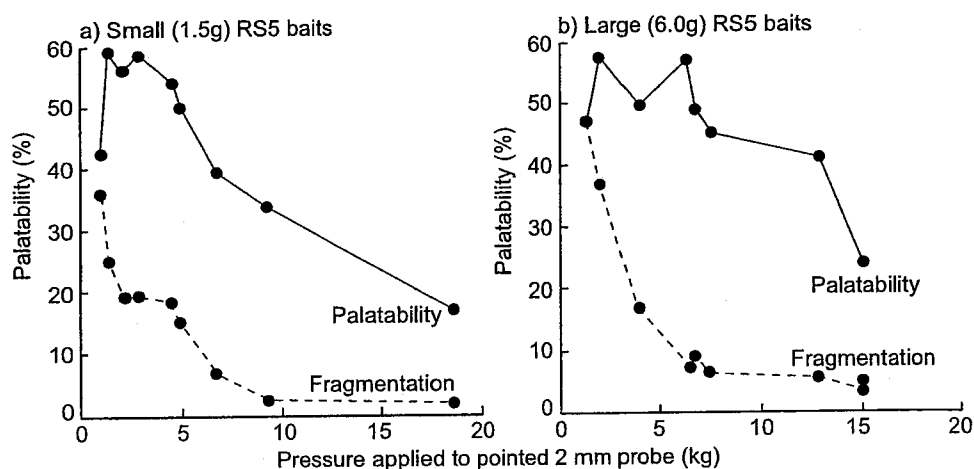


(i) *Bait hardness*: There were significant differences in the hardness of recently made cereal pellets ( $F_{16,578}=80.83, P<0.001$ ). The number of baits required to estimate bait hardness is dependent on whether the precision (i.e., 95% confidence interval) on hardness is required within  $\pm 1$  kg or  $\pm 2$  kg of an estimated mean, and whether the baits being measured are soft (i.e., baits that fracture with less than 6 kg pressure) or hard (i.e., baits that fracture with  $>12$  kg pressure) (Table 10).

**Table 10** Number of baits required to precisely estimate hardness using a pointed 2-mm probe fitted to a fruit penetrometer.

Bait type	Number of baits required to precisely estimate hardness	
	95% CI within $\pm 2$ kg of applied pressure	95% CI within $\pm 1$ kg of applied pressure
Soft baits (penetration with $<6$ kg pressure)	11	41
Hard baits (penetration with $>12$ kg pressure)	6	24

The hardness of small (1.5 g) RS5 baits significantly affected their palatability ( $F_{9,185}=3.38, P<0.001$ ), as did the hardness of large (6 g) RS5 baits ( $F_{8,164}=3.46, P<0.001$ ) (Fig. 20). Bait hardness also determined the proportion of small ( $F_{1,8}=14.3, P=0.005$ ) and large ( $F_{1,7}=11.6, P=0.01$ ) bait that fragmented when 2-kg samples of bait were tumbled in a concrete mixer for 5 min (Fig. 20). The optimal hardness (i.e., highest palatability and least fragmentation) occurs when probe pressures range from 2 to 7 kg for small (1.5 g) baits, and 5 to 12 kg for large (6 g) baits.



**Fig. 20** The palatability of (a) small 1.5-g RS5 baits, and (b) 6-g RS5 baits of different hardness. A decline in the percentage of baits fragmenting into small pieces is also demonstrated as the hardness of baits is increased.

*j) Dyes:* There were no significant differences in the palatability of cereal bait coloured with different dyes ( $F_{4,245}=0.46, P=0.77$ ) (Table 11). However, the palatability of citrus paste was significantly affected by the type of dye used ( $F_{1,78}=8.52, P=0.005$ ) suggesting Levanyl Green (a pigment dye) is better used in this type of bait matrix than Bayer V200 dye (a food colouring).

**Table 11** The palatability of different types of dye in cereal and citrus paste. Concentrations used met the legally required shade of green.

Type of dye	Concentration (% wt/wt)	Bait type	Palatability	Number of possums
Acid brilliant green (ABG)	0.020	cereal	48.7	97
Bayer V200	0.075	cereal	51.0	97
Bayer V200	0.075	citrus paste	40.4	20
Acid Green 16	0.040	cereal	49.9	20
WS Green	0.030	cereal	47.5	20
Levanyl Green (LGGZ)	0.200	cereal	46.6	17
Levanyl Green (LGGZ)	0.200	citrus paste	47.6	60

#### 6.4 Amounts of bait eaten

The numbers of penned and wild possums that fed on baits in bait stations mounted on electronic scales are summarised in Table 12.

**Table 12** Numbers of possums recorded eating baits (1.5 g) presented with and without prefeed from 'Coontrol' bait stations mounted on electronic scales.

Bait type	Use of prefeed	Captive possums	Wild possums
Non-toxic RS5 cereal	N	164	143
Non-toxic RS5 cereal	Y	NA	230 <sup>1</sup>
Toxic RS5 cereal	N	NA	198
Toxic RS5 cereal	Y	NA	126
Non-toxic Agtech	N	99	117
Non-toxic Agtech	Y	NA	259 <sup>1</sup>
Toxic Agtech cereal	N	NA	162
Toxic Agtech cereal	Y	NA	94
Toxic carrot	N	NA	38

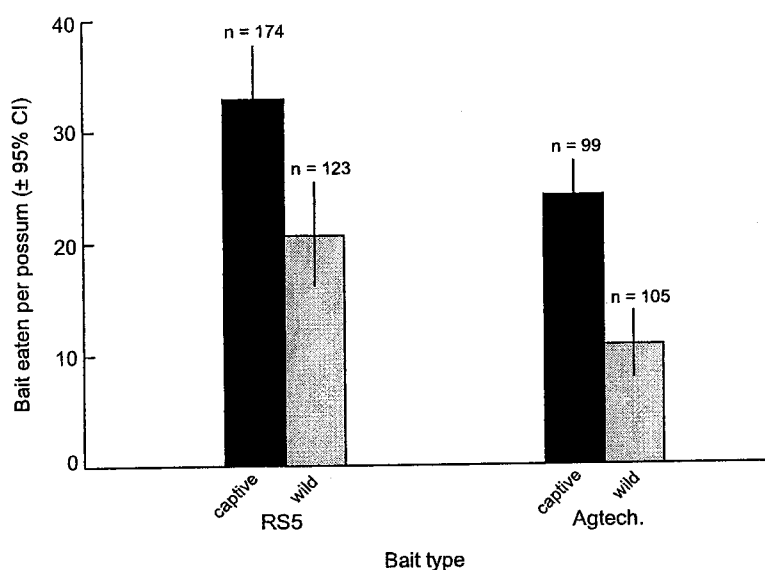
<sup>1</sup> Includes the same possums recorded on different nights

NA = not assessed

### a) Relative amounts eaten by captive and wild possums

The bodyweight ( $F_{4,297}=1.58$ ,  $P=0.18$ ) and gender ( $F_{1,146}=1.92$ ,  $P=0.15$ ) of captive possums did not affect the amounts of non-toxic RS5 bait eaten. However, the consumption of bait from ground-level bait stations was significantly higher than that from bait stations elevated 1.5 m above ground ( $F_{1,65}=7.16$ ,  $P=0.009$ ).

Wild possums ate significantly less bait from ground-level bait stations than captive possums (Fig. 21). During the first 60 min that possums fed on baits presented in bait stations the per capita consumption of non-toxic Agtech bait by wild possums (11 g) was only about half that of captive possums (24 g) ( $F_{1,205}=31.89$ ,  $P<0.001$ ). Similarly, the per capita amounts of non-toxic RS5 eaten by wild possums (21 g) were significantly lower than those eaten by captive possums (34 g) ( $F_{1,275}=41.6$ ,  $P<0.001$ ). To describe the amounts of bait eaten by possums for predictive modelling of population kills we have therefore only used the bait consumption by wild possums.



**Fig. 21** The mean amounts of non-toxic RS5 and Agtech bait eaten (without prefeeding) during a 60-min period by captive and wild possums feeding from bait stations mounted on electronic scales. Higher per capita consumption by captives is attributed to their previous exposure to different types of "novel" foods.

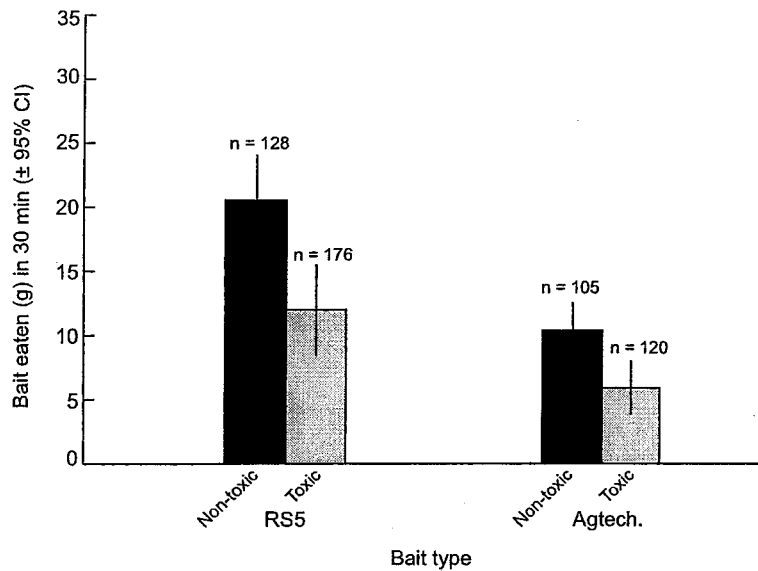
### b) Bait consumption from conventional and scale-mounted bait stations

Similar amounts of bait were eaten by wild possums from conventional "Coontrol" bait stations (25.6 g per station) and "Coontrol" bait stations mounted on electronic scales (28.6 g per station) ( $t=0.82$ ,  $d.f.=62$ ,  $P=0.45$ ). This indicates that the use of electronic scales and remote-sensing equipment for monitoring bait consumption had no effect on the amounts of bait that possums ate.

### Consumption of baits from bait stations by wild possums

a) *Presence of toxin:* The amounts of non-toxic bait eaten in the first 30 min after possums began feeding on baits exceeded the amounts of 1080 bait eaten by possums (Fig. 22). The differences in consumption of toxic and non-toxic Agtech bait (during a 30-min feeding period) were significant ( $F_{1,225}=7.02$ ,  $P=0.009$ ). However, the amounts of RS5 bait eaten without prefeed was extremely variable

(S.D.=20.4 g), and consequently the differences in consumption of toxic and non-toxic RS5 bait were not significantly different ( $F_{1,285}=1.24$ ,  $P=0.167$ ). The lower than "normal" consumption of 1080 bait is attributed to the aversive taste and smell of 1080 in baits (see 6.2.).



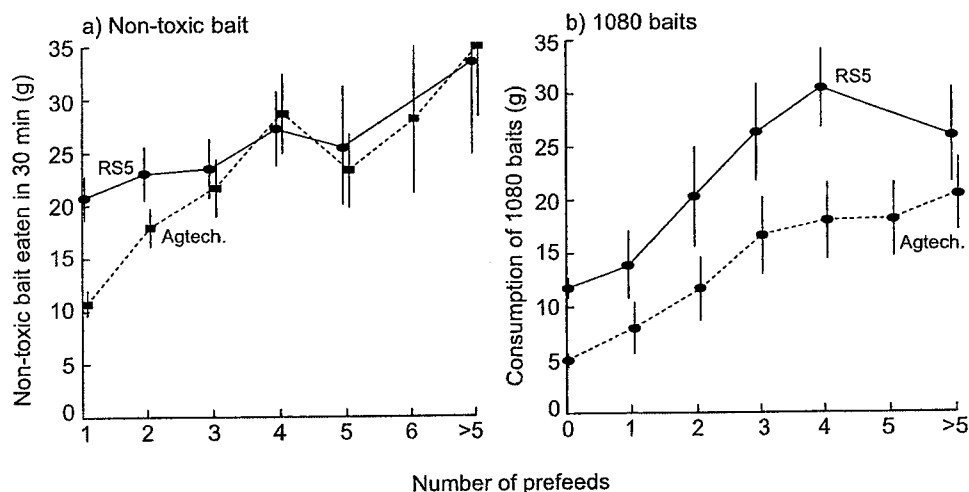
**Fig. 22** The mean amounts of non-toxic and 1080 baits eaten (without prefeeding) from bait stations during a 30-min period by wild possums. The differences in consumption are attributed to inadequate masking of 1080 in toxic baits that had lost cinnamon during 1–4 months storage.

(b) *Bait palatability*: The palatability of freshly made carrot (palatability  $\approx 55\%$ ), RS5 cereal (palatability  $\approx 45\%$ ), and Agtech cereal (palatability  $\approx 18\%$ ) influenced the amounts eaten from bait stations. When possums that refused bait were excluded from analysis, the amounts of toxic carrot ( $16.2 \pm 4.0$  g), RS5 cereal ( $13.7 \pm 2.2$  g) and Agtech cereal ( $6.6 \pm 1.6$  g) baits eaten from bait stations (that were not prefeed) were significantly different ( $F_{2,327}=30.3$ ,  $P<0.001$ ). Similarly when non-eaters were excluded, the mean amounts of non-toxic Agtech ( $11.4 \pm 2.6$  g) and RS5 ( $21.9 \pm 4.4$  g) bait eaten without prefeed were also significantly different ( $F_{1,237}=14.4$ ,  $P<0.001$ ).

(c) *Site factors and season*: Although at all locations there was no reported history of recent control with poisons (i.e., there should have been no residual bait shyness), there were significant differences between sites in the amounts of non-toxic RS5 ( $F_{4,119}=6.80$ ,  $P<0.001$ ), non-toxic Agtech ( $F_{3,94}=4.48$ ,  $P=0.006$ ), toxic RS5 ( $F_{4,155}=5.81$ ,  $P<0.001$ ) and toxic Agtech ( $F_{3,109}=3.99$ ,  $P=0.010$ ) baits eaten. These site differences in bait consumption were in part attributed to the season ( $F_{3,167}=3.09$ ,  $P=0.029$ ), with consumption of toxic RS5 bait highest in winter (18 g) compared to spring (12 g), summer (10 g), and autumn (11 g). The consumption of other toxic and non-toxic baits were not significantly affected by season ( $P$  values = 0.06–0.36), because of smaller sample sizes, and possibly because a combination of possum density, habitat type, and previous exposures to novel foods also affect the amounts of bait that possums ate at different locations.

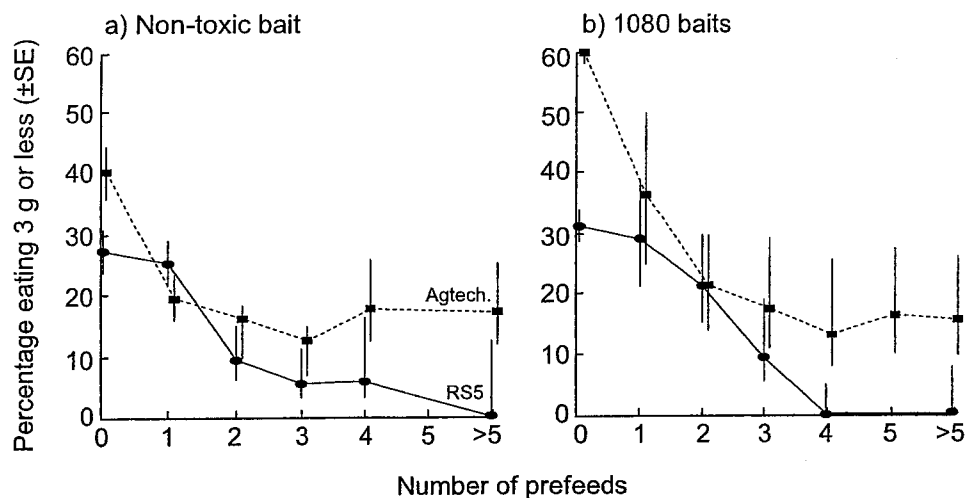
(d) *Prefeeding*: The amounts of bait eaten from bait stations by possums progressively increased with successive exposures to non-toxic Agtech bait over a period of 5 days ( $F_{5,347}=8.20$ ,  $P<0.001$ ). With a more palatable bait (i.e., RS5) the increases in bait consumption following exposures to prefeed were not as great, but they were significantly different over a period of 8 days ( $F_{7,343}=2.08$ ,

$P=0.031$ ). Increased consumption of toxic bait after prefeeding was more significant than that recorded for non-toxic bait; the amounts of Agtech cereal bait eaten increased about 3-fold over that eaten without prefeed ( $F_{1,210}=55.81$ ,  $P<0.001$ ) and consumption of RS5 bait containing 0.15% 1080 more than doubled after exposure to prefeed on 3 successive nights ( $F_{1,290}=25.57$ ,  $P<0.001$ ).



**Fig. 23** The mean amount of non-toxic ( $n=715$  possums) and 1080 bait ( $n=504$  possums) eaten following successive nights of prefeeding. The 95% confidence intervals about means are also shown.

Prefeeding also reduced the numbers of possums eating small sublethal amounts of bait. Fewer possums ate small amounts of non-toxic bait (i.e., 3 g or less) after they were prefed by RS5 ( $\chi^2=9.4$ , d.f.=1,  $P=0.002$ ) or Agtech ( $\chi^2=23.6$ , d.f.=1,  $P<0.001$ ) bait (Fig. 24(a)). Similarly, a smaller proportion of possums ate small amounts of toxic RS5 ( $\chi^2=16.0$ , d.f.=1,  $P<0.001$ ) or Agtech ( $\chi^2=41.0$ , d.f.=1,  $P<0.001$ ) after they were prefed (Fig. 24(b)). With a bait of high palatability (i.e., RS5 bait) the numbers of possums eating small amounts ( $\leq 3$  g) after 3 nights exposure to prefeed was negligible; whereas with a less palatable bait (i.e., Agtech) the percentage of possums eating small amounts declined, but only as low as 17% of possums. Therefore if baits are palatable to possums, prefeeding should mitigate almost all neophobia and cautious feeding behaviour.



**Fig. 24** Changes in the percentage of possums eating small amounts of (a) non-toxic bait (n=739) and (b) 1080 bait (n=580) during the first 60 min they fed on baits. The decline in neophobic behaviour to baits is presented for baits of moderate palatability (RS5 bait) and low palatability (Agtech bait).

## 6.5 Predictive modelling of population kills at bait stations

The computer-generated kill for possums eating RS5 without prefeed (82%) is the same as the mean percentage kill in five field trials (82%); and following prefeed the predicted (96%) and measured (90%) kills were similar despite a kill of only 50% at one field site (Table 13). There was, however, a difference of 10% in the predicted (66%) and measured (56%) kills following application of baits containing 0.08% 1080 (Table 13). Therefore, although limited data existed for the amounts of Agtech and RS5 bait eaten by possums with (n=66–122) and without (n=121–171) prefeed, and for the variation in 1080 concentration in baits (n=18), the model provided predicted kills that closely approximated the observed kills during bait station control.

The percentage of populations predicted to be killed after feeding on baits at bait stations was influenced by toxin concentration, bait palatability, and whether prefeed was applied before toxic bait (Table 13). Prefeeding was most effective if possums were exposed to baits for more than 3 nights.

**Table 13** Predicted kills (from modelling) and observed kills (in replicated field trials) by bait station control following the application of baits containing either 0.08% or 0.15% 1080 (with and without prefeed).

Bait type used for control	% 1080 in bait	Nights exposed to prefeed	Mean probability of each dying	Predicted kill	Measured kill in field trials (range of kills)
Agtech bait (palatability $\approx$ 18%)	0.15	0	0.59	59%	NR
	0.15	1-7	0.85	85%	NR
	0.15	3-7	0.88	88%	NR
RS5 bait (palatability $\approx$ 45%)	0.15	0	0.82	82%	82% (70-100) (n = 5) <sup>1</sup>
	0.15	1-7	0.90	90%	NR
	0.15	3-7	0.96	96%	90% (50-100) (n = 6) <sup>2</sup>
RS5 bait (palatability $\approx$ 45%)	0.08	0	0.66	66%	56% (44-75) (n = 6) <sup>3</sup>
	0.08	1-7	0.80	80%	NR
	0.08	3-7	0.88	88%	NR

NR = not recorded

<sup>1</sup> Henderson *et al.* 1997 and Henderson unpubl. data

<sup>2</sup> Thomas & Meenken 1995, Thomas 1998, Warburton 1998 (unpubl. report)

<sup>3</sup> Henderson & Morriss 1996.

## 6.6 The efficacy of baits used during aerial control

From 209 aerial operations there were 163 that provided information on three important bait factors: the type of bait used, the concentration of 1080 in baits, and whether operations were prefed. Operational factors reported included whether GPS was used during bait application, and the sowing rate. Percentage kills were significantly affected by the use of GPS ( $F_{1,100}=9.24$ ,  $P=0.003$ ), but not by sowing rate (e.g.,  $F_{13,62}=1.11$ ,  $P=0.37$  for carrot).

### Bait palatability

For aerial operations that were not prefed, the percentage kills were significantly affected by bait type ( $F_{2,100}=4.68$ ,  $P=0.011$ ) (Table 14), apparently because of differences in palatability. The mean kill with RS5 bait (50% palatability) was higher than that of the original No.7 bait formulation (33% palatability). The mean kills with carrot (a highly palatable bait) were lower than for RS5 bait, due to higher aversions to 1080 on carrot baits.

**Table 14** The percentage kills during operations that used different bait types.

Bait type	Percentage kill with and without GPS ( $\pm 95\%$ C.I.) (number of operations in parentheses)	
	GPS used	GPS not used
Prefed carrot	92.7 $\pm$ 2.4 (9)	87.8 $\pm$ 5.3 (15)
RS5 cereal bait	91.8 $\pm$ 6.3 (6)	84.2 $\pm$ 8.1 (7)
No.7 cereal bait	84.0 $\pm$ 4.1 (23)	70.4 $\pm$ 8.4 (25)
Carrot without prefeed	80.4 $\pm$ 62.5 (2)	73.8 $\pm$ 4.9 (18)
Mapua cereal bait	NR	67.6 $\pm$ 3.1 (5)

NR = not reported

### 1080 concentration

For aerial operations that were not prefeed the overall percentage kills with 0.15% 1080 (82%, n=64) and 0.08% 1080 (78%, n=60) were similar. The concentration of 1080 in baits had no influence on the percentage kills with cereal baits that were applied with GPS ( $F_{1,56}=0.07$ ,  $P=0.79$ ). Percentage kills with carrot baits (applied without prefeed and without the use of GPS) were not influenced by concentrations of 0.08%, 0.15%, and 0.20% 1080 in baits ( $F_{2,15}=0.079$ ,  $P=0.92$ ). Because 1080 concentration does not affect kills it seems likely that possums are eating more than one bait when baits are broadcast at a sowing rate of 5 kg/ha (which is consistent with the findings of Frampton *et al.* in press.). Although field trials indicate that a rate of 2 kg/ha is effective (Morgan *et al.* 1997), factors such as the density of the forest understorey and abundance of other pests, particularly rats, may require the application of bait at higher rates.

### Prefeeding

Almost all reported prefeeding done before aerial control used cut carrot which was then followed by the application of carrot baits containing 0.08% 1080. For these operations prefeeding significantly increased kills ( $F_{1,35}=14.383$ ,  $P=0.001$ ) (Table 15).

**Table 15.** The percentage kills ( $\pm 95\%$  C.I.) and residual trap catch (RTC) for aerial operations broadcasting 1080 baits with and without prefeed.

Bait type	Not prefed (number of operations)		Prefed baits (number of operations)	
	% kills	RTC	% kills	RTC
RS5 cereal	88.1 $\pm$ 3.1 (27)	1.8 $\pm$ 1.2 (9)	NR	NR
No.7 cereal	79.6 $\pm$ 3.4 (81)	4.2 $\pm$ 1.5 (13)	96.0 (n=1)	NR
Carrot bait (0.08% 1080)	77.3 $\pm$ 6.5 (16)	5.4 (1)	91.3 $\pm$ 8.4 (n=23)	1.3 $\pm$ 0.7 (4)
Carrot bait (0.15% 1080)	84.0 $\pm$ 18.6 (4)	NR	99.3 (1)	1.3 $\pm$ 1.8 (4)

NR = not reported



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## 7. Conclusions

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The most effective way of avoiding bait shyness is to ensure that control operations utilise baits of highest quality. The efficacy of baits used for possum control is a combination of the four key facets of bait quality that have formed the focus of this study. The main conclusions that can be drawn from our assessment of these are as follows:

### 7.1 Acute toxicity of 1080

- The LD<sub>95</sub> of 1080 in different bait types ranged from 3.6 to 4.7 mg/kg. Therefore, 3-kg possums need 11–14 mg of 1080 for 95% of them to be killed. A 6-g bait with a concentration of 0.15% 1080 contains 9 mg of 1080. Some possums will therefore need to consume more than one bait to die. However, at low sowing rates (i.e., <5 kg/ha; Morgan *et al.* 1997) many possums are likely to encounter only single baits before they become inappetent; suggesting that a concentration of 0.20% 1080 may be required in some baits used for aerial control. However, before a higher concentration is used, the effects of 0.20% 1080 on non-target species would need to be assessed. At this stage operators should distribute bait at a sowing rate not less than 3 kg/ha to optimise the chances of large possums finding more than one bait.
- Possums are more susceptible to 1080 in cereal than in carrot baits. Provided rain does not leach the 1080 from cereal bait and the cereal baits used are highly palatable, they are likely to kill more possums than 1080 carrot baits (when both baits are not preferred).
- Grey possums are more susceptible to 1080 than dark possums. Control over rainforests where most possums have a dark melanic coat (Brockie 1992; Pracy & Coleman 1998) is therefore likely to kill fewer possums than control in locations with a dry climate (where grey possums are common).
- Possums are less susceptible to 1080 in baits (LD<sub>50</sub> = 1.3–1.7 mg/kg for different bait types) than to 1080 administered as an aqueous solution by oral gavage (LD<sub>50</sub> = 0.8 mg/kg; Bell 1972; Rammell & Fleming 1978; McIlroy 1981; McIlroy 1983). Initial concentrations of 0.08% 1080 were based on estimates of acute toxicity by oral gavage (LD<sub>50</sub> = 0.8 mg/kg) and therefore underestimated the amount of 1080 required for a single bait to be lethal.

### 7.2 Toxin concentrations

- At present many possums are likely to survive control either because baits contain sublethal concentrations of 1080 (i.e., <0.13% wt/wt), or because of aversions to high 1080 concentrations (i.e., ≥0.15% wt/wt) that are not adequately masked.
- Percentage kills with cereal baits containing 0.08% 1080 are lower when baits are presented in bait stations (56% kill, n=6) than when baits are broadcast on the ground (80% kill, n=54). These differences are attributed to possums (that are not preferred) eating small amounts of bait (≤10 g) less frequently from the ground (26% of possums) than from bait stations (50% of possums).
- The concentration of cinnamon oil that is currently used on 1080 bait is inadequate to mask 1080 because it declines to 25% of the nominal concentration on carrot bait within 2 days of manufacture, and to 40% of the nominal concentration in cereal within 8 weeks of manufacture. Cinnamon loss from carrot broadcast for 2 or more days is also likely to contribute to non-target interference with baits (Spurr 1993b; Hickling 1997). To ensure baits contain 0.1% cinnamon

when they are eaten by possums (Morgan 1990), baits should contain higher cinnamon concentrations at the time of manufacture.

- Biodegradation of 1080 in stored cereal bait may contribute to sublethal poisoning, particularly if the baits contain a high moisture (>16%) content. The concentration of 1080 on carrot bait may also decline when micro-organisms are causing carrot to rapidly rot. The 1080 concentrations in Pestoff® paste, RS5 cereal bait (12% moisture), and gel baits did not change during 12 months storage.
- Sodium monofluoroacetate is leached from baits exposed to rainfall at differential rates, which are ranked: RS5cereal >> No.7cereal > Pestoff® paste, >> carrot (Bowen *et al.* 1995, this study).
- Inadequate mixing of toxins throughout manufactured baits and poor calibration of equipment applying 1080 to carrot has previously resulted in a large variation in the 1080 concentrations of baits. The consequences of variable amounts of 1080 in individual baits are twofold: some animals will encounter individual baits with high 1080 concentrations and these baits will be aversive to possums, while other possums are likely to be sublethally poisoned by eating single baits containing low concentrations of 1080.

### 7.3 Bait palatability

- Palatability is a critical factor in determining the percentage of possums that eat sublethal amounts of bait in both ground-based and aerial operations.
- Chantenay carrot (var. Royal Chantenay) is the most suitable variety of carrot for possum control as it is the most palatable (of seven varieties tested) and produces good yields. The palatability of this type of carrot declines after 6 months of growth or when the mean weight of carrots exceeds 200 g.
- Soil residues on carrots do not reduce palatability or exacerbate the rate of biodegradation of 1080.
- Carrots exposed to freezing conditions rapidly became less palatable when they have defrosted. Pest controllers should therefore cover carrot "dumps" with a tarpaulin if there is a risk of overnight frosts.
- During the trials reported here uncut carrots were stored for a month with minimal loss of palatability, but in warm temperatures and high humidity carrots are likely to rot more quickly.
- Carrot cut into baits is likely to remain palatable to possums for up to 1 week.
- Cereal baits that absorb moisture in the field will have a higher palatability than dry baits for the first 1–2 nights; thereafter increased microbial activity in damp baits is likely to cause palatability to decline by 5–8% during the first week they are damp, and 10%–16% within 2–3 weeks of baits becoming damp (Henderson & Morriss 1996, this study). This suggests that changes to palatability during the week after cereal baits are broadcast (which is a period when almost all possums feed on bait; Morgan 1982) are unlikely to have a significant effect on percentage kills during aerial control.
- Cereal baits are likely to lose palatability during storage if they have a moisture content >14% wt/wt, because this amount of moisture in bait promotes mould growth. Indicators of potential palatability problems in stored cereal baits are mould counts >400 cfu/g and moisture contents >14% wt/wt.
- Cereal baits used in bait stations absorb moisture and lose palatability. These baits should not be reused in future control.
- On the basis of existing data on bait palatability and cinnamon half-lives in cereal and carrot baits, the shelf-life of 1080 baits containing 0.2% cinnamon and stored at room temperatures are as follows:

No.7 cereal bait (14% moisture)	- 3 months
No. 7 cereal bait (16% moisture)	- <1 month
RS5 cereal bait (12% moisture)	- 6 months
Pestoff® paste	- 8 months
Gel bait (Kiwicare Corp.)	- 12 months

- The hardness of baits affects both palatability and bait breakage. Hardness can be accurately measured with a pointed 2-mm probe fitted to a fruit penetrometer. Baits (6 g) of optimal hardness were fractured when 5–12 kg pressure was applied to the pointed probe. Baits were either too soft if the probe penetrated baits with <5 kg pressure, or too hard if baits were not penetrated by >12 kg pressure.
- Although the food colours currently used in possum baits do not affect palatability, water soluble food colourings (e.g., Bayer V200 dye) provide only a patchy colouring of carrot bait. Previous research has also demonstrated that rain causes a loss of green colour from carrot bait (Munday *et al.* 1995).

#### 7.4 Amounts of bait eaten

- The size of baits determines the number of baits that must be eaten for a lethal dose (Frampton *et al.* in press.); the amount of bait that possums discard (Henderson & Hickling 1997); the 1080 concentration in carrot baits (Batcheler 1982, Staples 1969); and the risk that carrot baits present to non-target species (Spurr 1993a). High concentrations of 1080 on small carrot baits are likely to make these baits very aversive to possums, particularly following the partial loss of cinnamon.
- Baits of variable size from the Gibson cutter are likely to sublethally poison possums; baits from other cutters must be adequately screened to remove chaff and small pieces (Batcheler 1996).
- Cereal baits should comply with existing size specifications. Modelling suggests that an optimal size for aerially broadcast baits is 6 g (Frampton *et al.* in press.). Baits used in bait stations should weigh 1.5 g to minimise the amounts dropped by possums, and to allow baits to flow to the opening of the bait station (Henderson & Hickling 1997).
- Captive possums repeatedly exposed to a variety of novel foods that constitute their normal diet (Duckworth & Meikle 1995) were more willing to eat a new novel food (i.e., baits) than were wild possums who routinely eat only natural foods. Potential new technologies for possum control developed during empirical studies on captive possums (e.g., new baits and solutions to bait shyness) should therefore be evaluated in replicated field trials before being adopted by management.
- Bait consumption by captive possums is not affected by bodyweight or the gender of the animal, but was reduced when bait stations were elevated 1.5 m above ground. The amounts of bait eaten from ground-level bait stations by wild possums were significantly affected by site factors, the season, the palatability of bait, aversions to 1080, and prefeeding. Modelling of percentage kills indicates that prefeeding, palatability, and 1080 concentrations all affect the efficacy of baits used in bait stations.
- The efficacy of baits used for aerial control were significantly affected by bait palatability and prefeeding. Although concentrations of 0.08% and 0.15% 1080 in bait had no effect on the percentage kill, this is presumed to be a consequence of inadequate masking of high 1080 concentrations and most possums eating more than one broadcast bait (Frampton *et al.* in press.).
- Prefeeding increases the per capita amounts of bait eaten by possums, reduces neophobic responses to bait, and increases the percentage of possums killed during control. The risks to non-target species from prefeeding before aerial control are not known.
- Prefeeding overcomes neophobic behaviour at baits (this study), and causes a progressive increase in consumption of bait from bait stations by shy possums (Ogilvie *et al.* in press).

Therefore, prefeeding an unfamiliar bait (i.e., a different bait to the type that caused shyness) may be a very cost-effective means to mitigate shyness.

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## 8. Recommendations

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- That the revised specifications recommended in the Appendices be adopted as industry standards.

### 8.1 Management

To meet the updated specifications developed through this research:

(a) *Manufacturers of commercial baits should be advised to consistently produce:*

- cereal baits with a cinnamon concentration of 0.2% wt/wt;
- individual baits with 1080 concentrations within  $\pm 25\%$  of the nominal concentration;
- baits with a palatability exceeding 40% compared to recently manufactured RS5 baits. (Note: palatability trials should always compare baits of equivalent size);
- a label on all possum baits that clearly shows the date of manufacture and a recommended use-by date. (Note: baits are likely to be of substandard palatability after gel bait has been stored for 12 months, Pestoff® paste has been stored for 8 months, RS5 cereal bait (12% moisture) has been stored for 6 months, and when No.7 cereal bait (14% moisture) has been stored for 3 months);
- cereal baits with a moisture content that does not exceed 14% wt/wt;
- cereal baits of a hardness whereby a pointed 2-mm probe will penetrate baits when 2–7 kg pressure is applied to small (i.e., 1.5 g) and 5–12 kg pressure applied to large (i.e., 6 g) baits.

(b) *Control operation managers should be advised to:*

- increase the concentration of cinnamon oil used on carrot baits to 0.3% wt/wt;
- use bait with 0.15% 1080 and routinely measure the toxin concentration of baits;
- use Royal Chantenay carrot in carrot operations, and ensure that they are harvested before they weigh 200 g or within 6 months of sowing;
- store unwashed carrot in a heap for no more than 1 month and, if overnight frosts are likely, ensure that carrots are covered by tarpaulins;
- do not use the Gibson carrot cutter for preparation of baits;
- adequately screen carrot baits;
- use prefeed before bait station control with 1080, cholecalciferol, and cyanide.

## 8.2 Research

In order of priority, future research is needed to assess:

- the persistence of 0.20% and 0.40% cinnamon in cereal and carrot baits; the effectiveness of higher cinnamon concentrations at masking the standard 1080 concentration (i.e., 0.15%1080) and baits such as carrot that on occasions are inadvertently manufactured with a higher concentration (e.g., 0.20% 1080); the effects of packaging on the persistence of cinnamon in baits; the amount of non-target interference (e.g., captive and wild birds) with baits (carrot and cereal) containing higher cinnamon concentrations;
- factors influencing the quality and efficacy of baits containing brodifacoum, cholecalciferol, and cyanide, and to then develop specifications for these products;
- the long-term cost/benefits of prefeeding before aerial and ground control;
- the effectiveness of carrot at sowing rates lower than those currently used (c. 11.5kg/ha);
- the non-target risks when aerial control with 1080 baits follows a previous application of prefeed;
- the accuracy and precision of 1080 concentrations in individual RS5, No.7, and carrot baits that are currently used during possum control;
- the effectiveness of prefeeding unfamiliar baits (e.g., carrot and apple) to mitigate bait shyness caused by sublethal amounts of cereal bait;
- dyes with greater persistence than Bayer V200 for use on carrots to ensure baits comply with colour specifications;
- the efficacy of elevated bait stations (i.e., out of reach of livestock), following the application of prefeed;
- the reasons for naive possums eating different per capita amounts of bait at different locations;
- the proportions of baits of different hardness that fragment on the spinner of a sowing bucket.

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## 11. Appendices

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Specifications for 1080 baits have previously been developed by the Animal Health Board in collaboration with Landcare Research scientists (AHB 1992). These specifications have been updated following the recent evaluations of bait quality.

### 11.1 Recommended specifications for carrot bait

#### Cinnamon-masked 1080 baits

*Efficacy:* When 40 individually caged possums are presented paired trays containing 100 g of bait containing 0.15% 1080 and 100 g of non-toxic RS5 bait, at least 90% of possums shall eat a lethal amount of toxic bait. To ensure most animals are killed quickly and humanely, it is recommended that a minimum of 35 of 40 caged possums eat at least 8 g of bait (i.e., the amount of bait that administers 4 mg/kg of 1080 to a 3-kg possum).

#### Baits

*Specifications for carrot growers:* Carrots supplied will be:

- Royal Chantenay;
- harvested at a time when 90% of carrots weigh 100–200 g;
- clean-pulled within 4 days prior to requested date of delivery of the consignment;
- topped;
- free of carrot worm, stem rot, woody pith, mould, bruising, weed and weed seed, stones and other foreign objects;
- washed so that the consignment contains 99% carrot by weight.

On arrival at the airstrip carrots should be:

- covered by tarpaulins if there is a risk of overnight frosts;
- stored for no longer than is absolutely necessary. If delays occur because of weather then the period of storage will depend on temperature and humidity, but should not exceed 1 month in ideal weather conditions (i.e., low humidity, cool temperatures);
- free from signs of decay (heat, smell, or softness).

*Palatability:* The palatability of toxic bait should exceed 40% compared to recently manufactured non-toxic RS5 bait (6 g).

*Bait size and chaff:*

- Carrot baits shall have a mean weight of 6 g and 95% of baits by weight shall weigh between 3 and 10 g.
- Chaff (pieces of carrot less than 0.5 g) shall make up less than 1.5% by weight of useable bait.
- Chaff as a byproduct will make up less than 40% by weight of the pre-processed carrot.

*Toxin:* The 1080 used in baits shall be at least 93% pure sodium monofluoroacetate and contain less than 0.25% inorganic fluoride. The pH of a 0.1% aqueous solution of the 1080 powder shall be 6.5 or less. The 1080 stock solution will be 20% ( $\pm 0.5\%$ ) sodium monofluoroacetate.

*1080 concentration:* Sodium monofluoroacetate will be surface-applied to carrot baits such that the concentration of 1080 in samples of 10 baits should be  $1.5 \pm 0.22$  mg/g (i.e., all samples should have a concentration within 15% of the nominal concentration). The means of 10 or more such samples shall lie within  $\pm 5\%$  of the nominal concentration. The concentration in 90% of 10 individual baits shall be within  $\pm 25\%$  of the nominal concentration.

*Colour:* A green colour shall be incorporated into the bait to ensure it has a colour range of 221–267 by the New Zealand Standard Specification 7702 (section 23, Standards Act 1965). Surface colour shall be 98% or more of the surface area when tested by intercepts on a dot grid of 1 cm  $\times$  1 cm over a random sample of not less than 100 baits.

*Masks:* Food-grade cinnamon flavour (Bush, Boake and Allen, Auckland; Product No:02-7780) shall be used to mask 1080 by mixing it into baits at 0.3% wt/wt. The lure mixture shall be made by adding 3 litres of flavour concentrate to approximately 16 litres of soya bean or peanut oil, and applying 2 litres of this mix per tonne of cut bait. Alternatively, 300 mls of cinnamon lure should be mixed in 700 mls of monopropylene glycol, and added to the spray tank containing 1080 solution at the rate of one litre per tonne of carrot. Cinnamon concentrations following bait preparation should never be less than 0.1% or more than 0.4% wt/wt.

*Stability:* If, because of storage, uncut carrots start to become soft or ferment, the carrots should not be used for manufacture of baits. Carrot baits may remain palatable for a week after manufacture (i.e., palatability to 20 individually caged possums will be  $\geq 40\%$ ). The 1080 concentration in a sample of stored carrot bait should be within  $\pm 15\%$  of the nominal concentration.

*Leaching:* Detoxification of carrot is reliant on biodegradation of 1080 by micro-organisms as baits rot, therefore intact baits must be analysed for traces of 1080 before livestock are introduced back into control areas.

## 11.2 Recommended specifications for cereal bait

### Cinnamon-masked 1080 pellet baits

*Efficacy:* When 40 individually caged possums are presented paired trays containing 100 g of bait containing 0.15% 1080 and 100 g of non-toxic RS5 bait at least 90% of possums shall eat a lethal amount of toxic bait. To ensure most animals are killed quickly and humanely, it is recommended that a minimum of 35 of 40 caged possums eat at least 8 g of bait (i.e., the amount of bait that administers 4 mg/kg of 1080 to a 3-kg possum).

### Baits

*General:* Baits shall be grain-based, regular in shape, poisoned with monofluoroacetate (1080), masked with cinnamon, and coloured green. The cereal grain for bait manufacture should contain no more than 13% moisture, and have less than 5% screenings. Before pelleting the grain shall be milled with a screen not exceeding 3 mm, so most particle sizes fall in a range from 0.25–0.50 mm. An approved biscuit grade of wheat is recommended for optimal binding of pellet ingredients. To prevent "sweating" of recently manufactured cereal baits, they must be cooled to a temperature of no more than 8°C above ambient room temperature before they are packaged.

*Palatability:* The palatability of toxic bait should exceed 40% compared to recently manufactured non-toxic RS5 bait (of similar size).

*Fracture/breakage/ dust and bait hardness:* Dust and fragments (i.e., pieces less than 1 g) shall comprise no more than 5% by weight.

*Size:* Baits used during aerial control operations shall have a mean weight not less than 6 g. The standard deviation of 50 individually weighed baits should not exceed 1 g (one gram), with 95% of baits by weight weighing more than 4 g.

*Hardness:* A pointed 2-mm diameter probe shall penetrate baits when the mean pressure applied to the side-walls of 40 large (6 g) baits is 5–12 kg; or when 2–7 kg is applied with a pointed probe to the side-walls of 40 small (1.5 g) RS5 baits. The standard deviation of 40 baits shall not exceed  $\pm 5$  kg pressure, with 95% of baits penetrated with 2–15kg of pressure on the probe.

*Toxin:* The toxin, 1080, used in baits shall be at least 93% pure sodium monofluoroacetate and contain less than 0.25% inorganic fluoride. The pH of a 0.1% aqueous solution of the 1080 powder shall be 6.5 or less.

*1080 concentration:* The concentration of 1080 in samples of 10 baits shall be  $1.5 \pm 0.22$  mg/g (i.e., all samples should have a concentration within 15% of the nominal concentration). The means of 10 or more such samples shall lie within  $\pm 5\%$  of the nominal concentration. The concentration in 90% of 10 individual baits shall be within  $\pm 25\%$  of the nominal concentration.

*Colour:* A green colour shall be incorporated into the bait to ensure it has a colour range of 221–267 by the New Zealand Standard Specification 7702 (section 23, Standards Act 1965). Surface colour shall be 98% or more of the surface area when tested by intercepts on a dot grid 1 cm × 1 cm over a random sample of not less than 100 baits.

*Masks:* Food grade cinnamon flavour (Bush, Boake and Allen, Auckland; Product No:02-7780) in monopropylene glycol with a specific gravity of 1.05 shall be mixed into baits at 0.2% wt/wt to mask the taste and odour of 0.15% 1080. Cinnamon concentrations should never be less than 0.1% or more than 0.5% wt/wt.

*Stability:* Baits shall be stored for no longer than 6 months with a moisture content of 12 %, 3 months with a moisture content of 14%, and less than a month with a moisture content of 16%. Baits shall have a mould count less than 400 cfu/g . Bait should be stored in a cool, dry storeroom containing few micro- and macro-organisms.

*Leaching:* Baits shall retain 80% or more of their toxic loading after 5 mm of rainfall over 24 hours.

#### **Storage and stacking of 1080 pellets**

Bait shall be stored in a clean, dry, locked enclosure until it is used. Pallets of bait shall be stacked no more than two high during transport and storage.