PALATABILITY OF RODENTICIDE BAITS IN RELATION TO THEIR EFFECTIVENESS AGAINST FARM POPULATIONS OF THE NORWAY RAT

ROGER J. QUY, DAVID P. COWAN, COLIN MORGAN, and **TOM SWINNEY,** Central Science Laboratory (MAFF), London Road, Slough, Berkshire, SL3 7HJ, United Kingdom.

ABSTRACT: The palatability of 12 rodenticide baits, formulated to vary from poorly accepted to well accepted, was measured in laboratory choice tests against Wistar and wild-caught Norway rats. The baits, derived from six bait bases and two active ingredients, difenacoum and bromadiolone, were simultaneously tested in the field against 24 farm infestations (2/formulation) in order to investigate the relationship between palatability and efficacy. Bait acceptance in laboratory tests, with EPA meal as the challenge diet, varied from 7.0 to 50.6% for Wistar rats and 3.7 to 85.1% for wild rats. Changing the challenge diet to a ground-up laboratory animal food significantly increased the apparent palatability of three selected baits to Wistar rats, although the relative palatabilities between the formulations remained the same. Bait acceptance, as measured in the laboratory, was unrelated to the degree of control achieved in farm treatments. The presence or absence of alternative food and whether the baits were placed in containers or applied directly into rat burrows appeared more likely to determine the outcome and overwhelmed any influence due to bait palatability. The combined effect of container- and burrow-baiting reduced the rat populations by an average 96.8% with 16 of the 24 populations tested completely eradicated. The least palatable baits dispensed into burrow entrances controlled rats on all farms, including those with abundant food sources.

KEY WORDS: Norway rats, *Rattus norvegicus*, commensal rodents, baits, bait acceptance, efficacy, field tests, rodenticides, anticoagulants

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INTRODUCTION

The optimum concentration of active ingredients in anticoagulant rodenticide baits is determined by their toxicity and the likelihood of the target animals ingesting a lethal dose in a reasonable time. The longer the time required to receive a lethal dose, the more important it is that the bait should be palatable, especially when alternative foods are available. Ideally, baits should be equally, or more, palatable than the usual food source. Conventionally, the palatability of poison baits is determined in the laboratory, either by testing whether the presence of the active ingredient significantly reduces the amount of bait consumed (Bentley 1958) or how successfully the test formulation will compete in a choice test against the rodents' normal diet (Palmateer 1979). For the latter test, the "normal" food will often be a laboratory-made unpoisoned bait which consists of ingredients that commensal rodents may consume in the wild. Formulations which show poor palatability in these tests are unlikely to go forward to field trials. There has been some controversy over the level of palatability which is considered acceptable (Miller 1974), particularly since the outcome of any treatment depends on a wide range of factors. Prior to this study, the palatability of a bait, although critical in the development of a rodenticide, has been of unknown practical importance in the field.

Without knowing the relationship between palatability in laboratory trials and effectiveness in the field, it is difficult to assess new formulations during the early stages of development. When resistance to the anticoagulant warfarin developed in Norway rats (*Rattus norvegicus*), more potent compounds with the same mode of action were introduced. It was soon realized that these new "second-generation" anticoagulants produced a considerable overkill when tested against susceptible rats which ate far more than was necessary to kill them. The concept of "pulsed baiting" was introduced (Dubock 1979), which sought to limit the amount of bait a rat consumed and thus, incidently, had environmental benefits by reducing any toxic residue in carcasses. Furthermore, as rats needed to eat less bait to get a lethal dose, palatability could be reduced, enabling the use of formulations which were less attractive to non-target wildlife. Thus, relatively unpalatable baits may be as efficacious as palatable baits with the same active ingredient, provided that the less palatable bait does not encourage individuals to completely avoid it.

In this study the authors sought to establish whether a link between the palatability of baits and treatment efficacy existed by measuring the palatability of 12 rodenticide baits to Norway rats in the laboratory and then testing each formulation in the field. The baits were formulated to give a range of palatabilities.

METHODS

Laboratory Trials

Six bait bases in combination with two anticoagulants, difenacoum (D) and bromadiolone (B), were tested, giving 12 formulations in total. The six bait bases were:

1) pinhead oatmeal and corn oil (PHCO)
2) pinhead oatmeal, corn oil and caster sugar (PHCOCS)
3) medium oatmeal (MO)
4) 1:1 mixture of maize (corn) meal and barley meal (MMBM)
5) cut wheat and corn oil (CWCO)
6) whole wheat (WW)

Corn oil and caster sugar were added, where appropriate, at 2.5% and 5% by weight, respectively. Each active ingredient was dissolved in 1:100 triethanolamine: polyethylene glycol 200 to make a liquid concentrate which was added to each bait base at 2.5% by weight. The final concentration of difenacoum or bromadiolone in each bait was 0.005%. Commercially available formulations replaced the cut wheat/corn oil/difenacoum (CWD) and whole wheat/bromadiolone (WWB) combinations. Two untreated challenge diets were used in the choice tests: EPA OPP rat and mouse challenge diet (EPA 1982) consisting of maize (corn) meal (65% by weight), ground rolled oat groats (25%), corn oil (5%) and sugar (5%) and, in a series of supplementary tests, three formulations were tested against a proprietary laboratory pelleted animal diet (GRK3 R20 diet, SDS Ltd., Witham, Essex, U.K.) which was ground into a fine powder.

The test baits were prepared three to four days before the test began, sealed in polythene bags and stored at room temperature. The cereal ingredients of the EPA meal were sieved and weighed at the same time, but were not mixed with the sugar and corn oil until the first day of the test period. As it has been reported that the palatability of EPA meal may vary from batch to batch (Johnson and Prescott 1994), the EPA tests were divided into ten replicates for laboratory rats and five for wild rats with each of the 12 bait formulations offered to a pair (one male, one female) of animals in each replicate. Similarly, for the supplementary tests, in which three baits, MMBMB, CWCOB and PHCOCSB, were offered to laboratory rats with ground SDS as the challenge diet, each bait was offered to five pairs in each of two replicates. Each of the two commercial baits was bought from an agricultural supplier with sufficient quantity in one batch for all replicates.

Each test bait was offered to 20 laboratory (Wistar strain) and 10 wild-caught rats with equal numbers of each sex included. The laboratory rats were healthy adults ranging in weight from 204 to 294 g three days before the test period began. The wild rats were caught in live-traps baited with whole wheat on three farms from an area of southern England where most rats were thought to be susceptible to first-generation anticoagulants. Only healthy adults were brought to the laboratory where they were treated with an insecticide to kill ectoparasites and allowed to acclimatize to laboratory conditions for a minimum of three weeks. As expected, the body weights varied considerably when the animals were weighed three days before the tests began: males 210 to 503 g and females 129 to 422 g.

All rats were caged singly and the cages were arranged on the racks such that the sexes alternated vertically and horizontally. Water was available at all times. Two food pots were placed symmetrically at the front of each cage and filled with a ground laboratory diet one week before the test period (but after the acclimatization period for the wild rats); all other food was removed. During this pre-test period, the amount of laboratory diet eaten by each rat was recorded on four consecutive days to ensure that all rats were eating normally from the pots. On the first day of the test, clean pots were substituted and one was filled with about 50 g of the challenge diet and the other with the same amount of the test bait. On each of the next three days, the amount of food eaten from each pot was recorded to the nearest 0.1 g and any remaining food was discarded. Clean pots were filled with fresh bait and replaced in the cage with the positions of the test and challenge diets interchanged to cancel the effect of place preferences. On the fifth day, the amount of food eaten was recorded and the rat was humanely killed. Post-mortem body weights were recorded.

The palatability (acceptance) of each formulation was calculated as the total amount of test bait eaten expressed as a percentage of the total amount of food consumed.

Field Trials

The infested farms used in this study were located in areas of southern England where the majority of rats were thought to be susceptible to warfarin (MacNicoll et al. these proceedings). Each formulation was tested twice on separate farms, giving a total of 24 field trials. The treatments were carried out over a 12-month period commencing in March 1994 with the test baits allocated in turn as the farms became available. Each farm was surveyed to assess the extent of the infestation by looking for rat signs such as runs, fresh droppings and active burrows. Farms were classified according to the type of stored food available to rats as: 1) no obvious food source identified; 2) cereals, such as wheat or barley; 3) commercial or farm-prepared animal feeds; and 4) maize silage (often burrowed into by rats especially where the clamps were lined with straw bales or railway sleepers). Wooden bait containers with metal lids were set out at least one week before the treatments began to enable rats to get used to them. On the first day of each treatment, 100 g of the test bait was placed into each container. Thereafter, all bait points were inspected each weekday, the remaining bait weighed and replenished sufficiently to maintain a surplus until the next inspection. However, during the first three trials most rats failed to take bait from the boxes. Container-baiting was, therefore, terminated after three weeks in these and all subsequent trials and the bait redistributed, if the infestation still persisted, to the entrances of active rat burrows. (No burrows were baited during the first three weeks of each trial.) When baiting burrows, the bait was laid as far into each burrow as possible and the entrance was lightly blocked with any suitable material. Such hole-baits could not be reliably inspected but the number of burrows baited was recorded on 11 farms. Hole-baiting was continued until all evidence of rat activity had gone, or for a maximum of three weeks.

The size of each rat population was assessed using a tracking plate method (Quy, Cowan and Swinney 1993) in the week before baiting began, then again after three weeks of container baiting, but before hole-baiting started. A final assessment was made in the week following the cessation of hole-baiting. In the analysis of results, any treatment in which the size of the population had increased between the pre-treatment census and the end of container baiting was considered to have 100% of the original population remaining alive. Weekly estimates of the size of the rat population present on each farm were obtained by linear interpolation between successive

census estimates. Dividing the average daily amount of bait consumed by these weekly estimates gave an estimate of the take by each rat. Additionally, a tracking plate was placed on one side of each bait container to detect visits by rats whether or not any bait had been taken; plates were inspected each time the bait was checked and scored as being marked or not.

In analyses relating bait take and efficacy to the palatability of the various baits, the data for palatability is the percentage bait acceptance obtained for each bait from the tests on Wistar rats rather than wild rats because the sample size of the former was greater. In all statistical tests percentages were transformed to arcsine square roots to stabilize variances. Untransformed means together with their standard errors are given in the text.

RESULTS

Laboratory Trials

The percentage bait acceptance for the test baits offered to Wistar rats varied from 7.0 ± 1.99% (MOD) to 50.6 ± 5.38% (PHCOCSB) ($F_{11, 216}$ = 19.1, P = <0.001, Figure 1). There was no difference in bait acceptance between the sexes (F < 1.0). EPA meal was preferred to all baits (paired t-tests, P <0.001 – P <0.01) except for WWB and PHCOCSB where no preference was detected. The acceptance of each bromadiolone bait was greater than its equivalent difenacoum bait (t-tests, P = 0.05 – P <0.001) except for cut wheat baits where there was no difference. The comparisons involving cut wheat and whole wheat bases should be treated with caution as, in each case, a commercial formulation was included which contained additional unspecified ingredients. Changing the challenge diet to ground SDS for three selected baits increased the measured palatability of the test baits: for MMBMB acceptance increased from 13.2 ± 2.33% to 26.1 ± 3.25% ($F_{1, 36}$ = 18.1, P = <0.001), for CWCOB from 31.9 ± 3.21% to 62.0 ± 4.16% ($F_{1, 36}$ = 47.3, P <0.001) and for PHCOCSB from 50.6 ± 5.38% to 81.2 ± 2.56% ($F_{1, 36}$ = 25.4, P <0.001). However, there was a significant interaction between the sex of the rat and the type of challenge diet for MMBMB (P = 0.003) and CWCOB (P = 0.011). The acceptance of MMBMB by female Wistar rats with SDS as the challenge diet was greater (38.0 ± 2.94%) than males (14.3 ± 2.13%, t_{18} = 6.54, P <0.001); similarly, the acceptance of CWCOB by females (76.0 ± 2.49%) was greater than that by males (48.0 ± 4.81%, t_{18} = 5.12, P <0.001).

The percentage acceptance of the 12 test baits offered to the wild rats varied from 3.7 ± 1.65% (MMBMD) to 85.1 ± 6.09% (PHCOCSB) (Figure 1). Within each group there was considerable variation in acceptance of the same bait: for MOD, MMBMD, PHCOD, MOB, MMBMB and PHCOB the minimum percentage acceptance recorded was <2.0%, while a maximum acceptance >98% was recorded for WWD, PHCOCSD, CWD, MOB, PHCOB, PHCOCSB and WWB. The mean percentage acceptance for seven baits exceeded 50% (range 52.1 to 85.1%), but there was no significant difference between them ($F_{6, 56}$ = 1.74, P = 0.13) and none related to the sex of the rat (F <1.0). The mean percentage acceptance of the other five baits (range from 3.7 to 43.3%) varied significantly ($F_{4, 40}$ = 5.54, P = 0.001), and the mean acceptance by females consistently exceeded that of males ($F_{1,40}$ = 5.09, P = 0.03). In paired t-tests comparing each test bait with EPA meal, WWD and WWB (P <0.05) and PHCOCSB (P <0.001) were preferred to the challenge diet. EPA meal was preferred to both baits containing maize meal/barley meal (P <0.001). There was no preference shown with the other seven test baits. Statistical analysis (by t-tests) indicated that adding bromadiolone or difenacoum to the baits did not influence the preference of wild rats for the different bait bases.

Transformation of the values of bait acceptance to z-scores, and testing by analysis of variance, indicated that the relative palatability of the 12 baits was the same for both Wistar and wild rats. There was no significant interaction between the 12 baits and the two rat strains (P = 0.43).

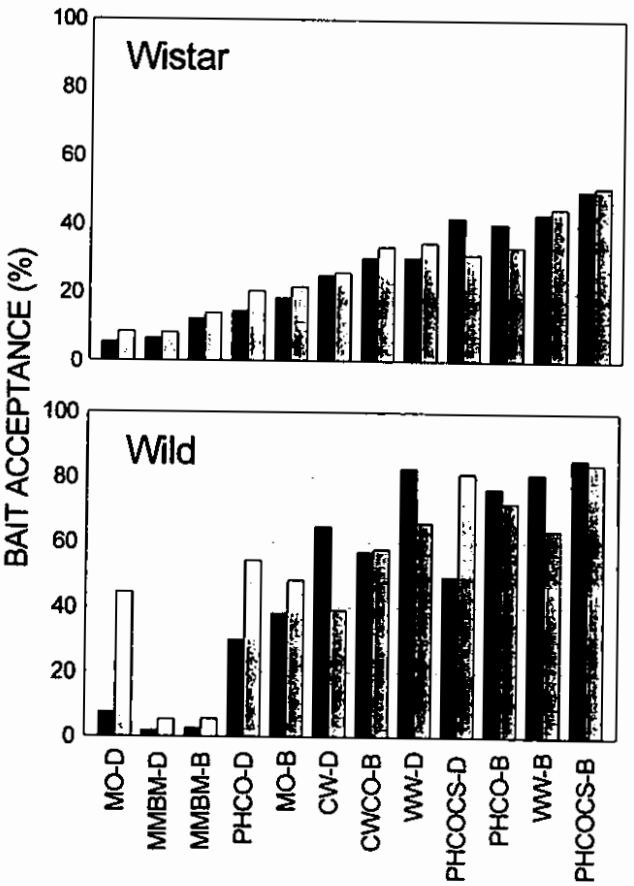

Figure 1. Choice tests using Norway rats between 12 rodenticide baits and EPA challenge diet: black bars, males; grey bars, females. MO, medium oatmeal; MMBM, maize meal/barley meal; CW, cut wheat with CO corn oil; WW, whole wheat; PH, pinhead oatmeal with CO corn oil CS caster sugar; D, difenacoum; B, bromadiolone.

Field Trials

There was no correlation between percentage bait acceptance, as determined in the laboratory tests, and the estimated percentage reduction in the population during

the first three weeks of the treatment when the bait was laid in boxes (Pearson correlation coefficient r = -0.368, df 22, P = 0.08). Excluding the four farms where there was no stored food, r increased to -0.430 (P = 0.06). The estimated mean size of the populations at the start of each treatment was 49.0 ± 9.2 (range 10 to 215) rats. The estimated percentage reduction in the population following container baiting was 37.1 ± 7.1. The estimated mean take of bait during the first week of each treatment was 2.5 ± 51 g rat/day (Figure 2), but varied from 6.4 ± 2.99 g for four farms with no stored food, 2.3 ± 0.82 g for eight cereal farms, 1.8 ± 0.58 g for nine animal-feed farms, to 0.0 g for three farms with stores of maize silage. Within each farm type, there was no correlation (Spearman rank correlation test) between the estimated mean daily take by each rat during the first week and the palatability of the bait. The estimated mean take during the second and third weeks of each treatment was 1.6 ± 0.6 g (range 0 to 13.8 g) and 1.6 ± 0.56 g (range 0 to 11.3 g) rat/day, respectively. After a further three weeks of hole-baiting, the populations were finally reduced by an estimated mean 96.8%, with 16/24 infestations completely eradicated (Figure 3). On the 11 farms where the number of hole-baits was recorded, there were in total 267 bait containers, of which 181 (67.8%) were "active" i.e., a take was recorded or rat footprints were found at least once on the adjacent tracking plate. The total number of holes baited was 300 (mean 1.66 holes/active bait box), but varied on individual farms from 0.5 to 6.0 holes/active bait box.

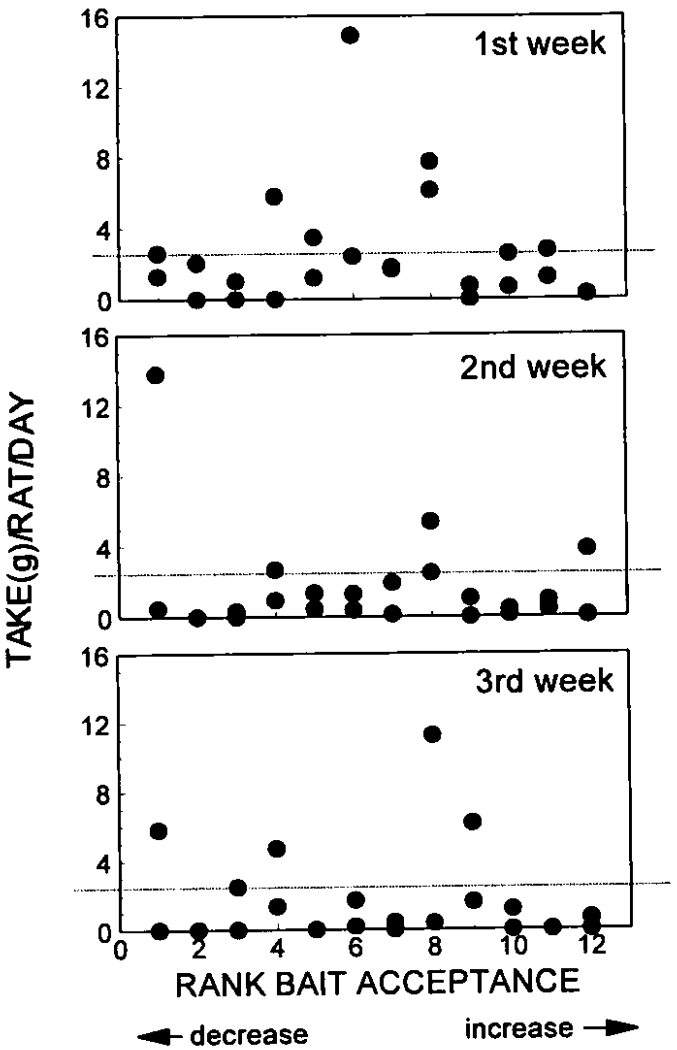

Figure 2. Estimated mean daily consumption by individual rats relative to bait acceptance (laboratory trials with Wistar rats). The dotted line represents the approximate amount of bait that a 250 g rat needs to eat each day for four consecutive days to ingest a LD50 dose of anticoagulant (Greaves and Cullen-Ayres 1988).

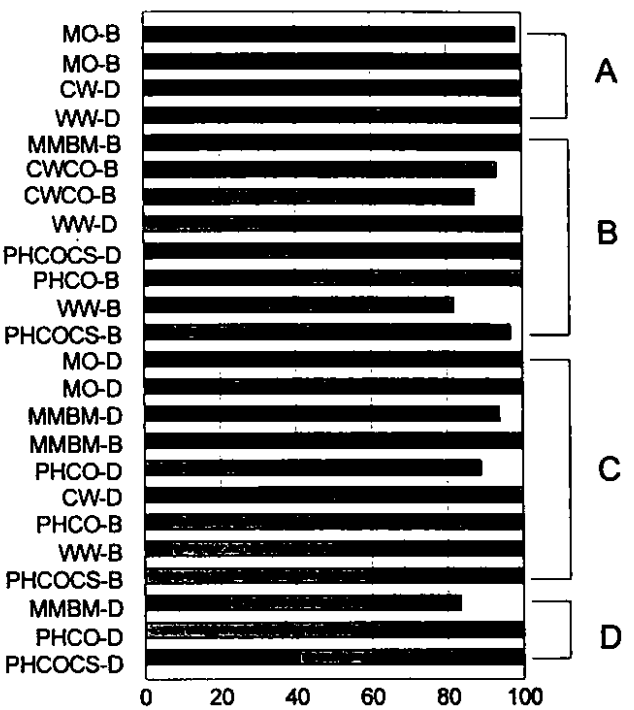

Figure 3. Percentage population reduction after three weeks of container-baiting (black shading) and a further three weeks of hole-baiting (grey shading). The treatments are grouped according to the alternative food available: (A) none; (B) cereals; (C) animal feedstuffs; and (D) maize silage. Within each group the baits are ranked from least to most palatable (top to bottom) according to the results of tests using Wistar rats. The key to the baits is the same as in Figure 1.

DISCUSSION

In laboratory trials, a more variable response to the baits was observed with wild rats compared with the Wistar rats. This was to be expected, partly because of the difficulty in defining particular age/weight groups for wild-caught rats and the unpredictability of supply. Thus, variation due to age could not be measured. The strong preferences of some individuals for the test baits and total

rejection of EPA meal may have been related to previous experiences. The rats were trapped on farms where they had access to cereals and, thus, EPA meal may have been sufficiently unfamiliar in taste and texture to cause avoidance. In contrast, the laboratory trials showed that both maize meal/barley meal baits were apparently less acceptable to wild rats at only 4% bait acceptance, yet in the field an average reduction of 94% on four farms was achieved with those baits. Of course, the measured palatability of test baits may vary by changing the challenge diet or the strain of rat, but in these tests the relative palatabilities of the 12 baits remained more or less the same.

No relationship was found between the palatability of the baits tested and the degree of control obtained in the field. None of the 12 baits achieved less than an overall 81% reduction of an infestation despite the abundant supplies of alternative food on most farms. In containers, a medium oatmeal/difenacoum bait with an acceptance of 7% reduced a rat population by 78%, while a pinhead oatmeal/corn oil/caster sugar/bromadiolone bait with an acceptance of 51% gave no control at all. Both results were obtained on similar farms with supplies of animal feeds. In this study, the most important factor determining the outcome of a treatment appeared to be the bait application method, but only when there was alternative food available. Quy et al (1992, 1994) considered the impact of unprotected stored foods on the effectiveness of poison treatments and suggested that undermining the predictability of the rats' environment would encourage greater control because, presumably, the rats would be less wary about taking bait from containers in situations where there was constant change. In contrast, where there was little change but alternative food was limited, as on the four farms with no stored food, rats readily consumed baits from containers and any influence on the outcome due to bait palatability was lost. Thus baits, with an average acceptance of 24.5%, reduced infestations by 85.2% in three weeks (category A farms in Figure 3) and only one infestation required hole-baiting. Over the same period on the other farms (categories B, C, D), infestations were reduced by 24.2% with baits whose average acceptance was 27.4% and 19/20 required hole-baiting.

All of the field trials were carried out on farms where, to the best of the authors' knowledge, the majority of rats were susceptible to warfarin and, hence also to the more potent anticoagulants. Thus, the effects on efficacy of the poor palatability of some of the baits might have been offset by increased potency. With this relationship, there might be a fine line between treatment success and failure with difenacoum or bromadiolone. Palatability might, therefore, have more influence on treatment outcome for the less potent anticoagulants. It is quite likely that in conditions ideal for maximum treatment efficiency, many rats may be persuaded to eat apparently unpalatable baits, but such situations are not the norm and pest controllers should expect that their baits will compete with other foods for the rats' attention.

In this study, dispensing baits directly into rat burrows was the most effective means of control when abundant alternative food was present. This technique, although not new, may enable rats to be more easily intercepted between their nest sites and their food supply, especially around maize silage clamps, where the distance between a nest and food can be very short. Substantial reductions in rat numbers were apparent after two weeks of hole-baiting on most farms even with the least palatable baits. There were, on average, more burrows baited than containers and, naturally, the distribution of hole-baits more closely matched the distribution of the rats. For each rat, a choice, in theory, could be made between the benefits of obtaining food with less expenditure of energy and less exposure to predators against the cost of a bait that was relatively unattractive. However, hole-baiting, as a practical technique, can be time-consuming and laborious, particularly when finding all the burrows in thick undergrowth and the bait takes are very difficult to monitor. Moreover, uneaten bait cannot easily be recovered at the end of a treatment and bait spilled as burrows are baited or bait kicked out by rats reopening a burrow may increase the risk to non-target animals.

In these trials against anticoagulant-susceptible rats, any influence that the palatability of the bait had on the outcome was too subtle to be measured. The availability of alternative food and the baiting technique used overwhelmed all other factors. This might not be true in trials to control anticoagulant-resistant rats, if the degree of resistance was sufficiently high such that significantly larger quantities of poison bait had to be consumed to provide a lethal dose.

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