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A. P. Buckle
ICI Agrochemicals, Fernhurst, Haslemere, Surrey, UK

D. E. Kaukeinen
ICI Americas Inc., Agricultural Chemicals Division, Biological Research Centre, Goldsboro,

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A. P. BUCKLE, ICI Agrochemicals, Fernhurst, Haslemere, Surrey, UK, GU27 3JE

D. E. KAUKEINEN, ICI Americas Inc., Agricultural Chemicals Division, Biological Research Centre, Goldsboro, NC 27533-0208, USA

ABSTRACT: Assessments of the palatability of rodenticide baits are usually conducted in the laboratory but little is known of the value of such tests as determinants of the potential performance of formulations in the field. Field bait acceptance tests conducted earlier were either unduly time-consuming or failed to take account of aspects of rodent behavior in relation to baiting regimes which make the interpretation of results difficult. This paper describes a novel, cost effective technique for assessing the palatability of baits in the field and the use of the new method to compare the acceptance of three commercial formulations, containing either difenacoum or brodifacoum, with that of an EPA approved challenge diet. No statistically significant differences were found in the acceptance of the three baits and the challenge diet at three farmsteads harboring infestations of Rattus norvegicus. Similar results were obtained in equivalent laboratory choice tests conducted following an established protocol. A comparison of results from the two environments bestows confidence both in the practical value of the laboratory test method and in the likely performance of the baits when used for rodent control operations. Wider possible uses of the field test methodology are discussed.

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The palatability of rodenticide baits is usually assessed in the laboratory where test procedures are easily standardized and, within normal limits of experimental error, reproducible results are obtained. Standard protocols for such tests have been published both in Europe (EPPO 1982) and the USA (Palmateer 1974, Anonymous 1977), and these have been widely adopted by laboratories in the government and private sectors to generate data used in the development of rodenticide formulations, to support registration submissions and to determine the quality of commercial products. In these tests, singly-caged rodents are offered a choice between a rodenticide formulation and a standard challenge diet. Results are usually expressed as the percentage of total bait consumption contributed by the test bait (see Palmateer 1981).

It is difficult, however, to estimate the value of such data as an indication of the practical performance of rodenticide baits in the field because laboratory and field conditions are very different and, at present, no simple, statistically valid field technique is available for the verification of laboratory findings. Dubock and Rennison (1977) described field trials of different baits but their method, amounting to replicated efficacy tests of each candidate formulation, was very demanding both in the number of trial sites required and the time and effort needed to conduct the evaluations. A number of more economical designs have been proposed in which several formulations are offered together at a single trial site (e.g., Howard and Marsh 1977). The results obtained are often difficult to interpret, however, being much influenced by infestation levels; differences in the acceptance of baits that are apparent when rat numbers arc low to moderate are lost when infestation is heavy (Richards pers. comm.).

This paper describes a field test method, based on a balanced latin square design (Cochran and Cox, 1957), which allows the palatability of different baits to be compared when offered at the same trial site, but which takes account of a number of potentially confounding factors. These factors arc: (1) that rats may show preferences for certain feeding areas at trial sites, (2) that, when baits are changed at a given baiting point, one bait may have a residual effect on the uptake of the one that follows it, and (3) that there may be differences in the daily levels of bait uptake during the period of the trial. The results obtained in a field test comparing the palatability of three commercial formulations with that of a challenge diet are presented alongside results from equivalent choice tests conducted in the laboratory.

METHOD

Field Trial

Three sites were chosen for the trial, each comprising an isolated group of farm buildings. Two of these farmsteads were mixed livestock/arable holdings near Shrewsbury, Shropshire, UK and the third was an intensive pig-rearing facility near Andover, Hampshire, UK. The farms harboured moderate to heavy infestations of Norway rats (Rattus norvegicus).

Each site was surveyed by a single, experienced rodent control operator and the number of bait points required to bait the infestation and their positions were determined, according to the distribution and density of signs of rat activity, as for a normal control treatment. In laying the bait points the only concession to the experimental design (three balanced latin squares of which each site was a square) was that the number of bait points used at each farm was a multiple of four. Thus, at each site, the four baits (Table 1) could be put out on each of four nights at one of four different

Table 1. The three commercial formulations and the challenge diet used in laboratory and field palatability tests.

Name	Active Ingredient	Description
A. 'Ratak'*	Difenacoum (50 ppm)	Cereal-based pellets
B. 'Ratax' S*	Difenacoum (50 ppm)	Whole wheat grains
C. 'Talon' G**	Brodifacoum (50 ppm)	Cereal-based pellets
D. EPA Meal		Maize grits, rolled oat groats, maize oil, sugar

*Trademark of ICI PLC
**Trademark of ICI Americas, Inc.

groups of baitpoints of equal number (see Table 2). Bait point groups covered, where possible, a single farm building or a distinct group of buildings.

To begin the trial, wooden bait trays were set out on Thursday or Friday (prior to the first placement of bait on the following Monday) to condition the rodents to these novel objects. Had it been necessary to use bait boxes, a longer conditioning period would have been advisable. When first laid, the quantity of bait put down at each point, 100 or 200g, was judged to avoid the occurrence of complete takes. The four baits were exposed in their original positions (Table 2) for 24 hours and the quantity of bait remaining at each point at the end of this time was recorded to the nearest 5g. The baits were then changed, the sequence of rotations being arranged so that each bait followed every other bait three times, once at each of the three occasions when changes took place (Table 2). Once again, the quantity of the new baits put down at each bait point was judged to ensure that bait was available for consumption by rats throughout the exposure period.

Laboratory Tests

Laboratory tests were conducted to determine the acceptance of each of the three rodenticide formulations in comparison with that of EPA challenge diet. Test methodology followed established guidelines (Palmateer 1974, Anonymous 1980) except that the duration of the test, as in the field, was 4 days. Essentially, the protocol adopted involved the presentation to individually caged test animals (albino R. norvegicus) of two food pots, each containing weighed amounts of either the test or challenge diet. Bait consumption was measured daily, to the nearest O.lg, and palatability expressed as the percentage of total bait consumption contributed by the test formulation.

Table 2. The sequence of presentation of the different formulations at each trial site and the quantities (g) of each eaten by rats. Letters in brackets refer to the baits as in Table 1.

Site 1

	Area I	II	III Bait Points	IV	
Day	1-15	16-30	31-45	46-60	Totals
1	(D) 260	(A) 625	(B) 515	(C) 425	1825
2	(C) 240	(B) 1560	(A) 990	(D) 590	3380
3	(B) 530	(C) 3110	(D) 1840	(A) 925	6385
4	(A) 360	(D) 2460	(C) 1905	(B) 1790	5915
Totals	1370	7755	4650	3730	17505

Site 2

	Area I	II	III Bait Points	IV	
Day	1-15	16-30	31-45	46-60	Totals
1	(B) 165	(C) 620	(A) 340	(D) 35	1160
2	(D) 300	(A) 1000	(C) 595	(B) 185	2080
3	(C) 470	(B) 1095	(D) 695	(A) 140	2340
4	(A) 225	(D) 440	(B) 850	(C) 20	153
Totals	1160	3095	2480	380	7185

Site 3

	Area I	II	III Bait Points	IV	
Day	1-15	16-30	31-45	46-60	Totals
1	(A) 395	(D) 705	(B) 730	(C) 370	2200
2	(D) 1300	(A) 365	(C) 810	(B) 900	3375
3	(B) 2120	(C) 695	(A) 1380	(D) 1705	5900
4	(C) 1865	(B) 650	(D) 2780	(A) 1705	7010
Totals	5710	2415	5680	4680	18485

RESULTS

Field Trial

The weights of the four different baits consumed by rats (Table 2) were summed separately for each of the three trial sites (Table 3). The results thus obtained showed that there was considerable variation in the relative performances of baits among farms. For example, EPA challenge diet was the best accepted bait on two of the farms but was poorly accepted on the other and the apparent superiority of this bait (table 3) was almost entirely due to data from site 3.

The raw data were subjected to an analysis of variance (Table 4) which allows a number of treatment effects to be partitioned (Cochran and Cox 1964). There was significant variation (Table 4, line 2) in the total consumption of bait among the twelve groups of bait points used at the three sites. Such variation was anticipated and was related to differences

in the intensity of infestation both among the three farmsteads and among the different areas within each farm. There was also significant variation in bait uptake on the four days of the experiment (line 3). Bait takes were lowest on the first day of the trial (Table 2), because rats were initially suspicious of the novel foods. Bait consumption increased, however, during the next two days and decreased overall on the fourth and final treatment day, presumably because of morbidity and mortality caused by toxicants in three of the tested formulations.

Table 3. The consumption (g) of the four formulations at the trial sites.

Formulation	Site 1	2	3	Total
A. 'Ratak'	2900	1705	3845	8450
B. 'Ratax' S	4375	2235	4400	11010
C. 'Talon' G	5080	1705	3770	10555
D. EPA Meal	5150	1470	6470	13090
Totals	17505	7115	18485	43105

Table 4. Analysis of variance of bait consumption data (Cochran and Cox, 1964).

Line	Source of Variation	DF	Sums of Squares	Mean Square	F
1	Total	47	24983787	531570	
2	Between areas	11	13134031	1194003	7.67**
3	Days within squares	9	7377289	819699	5.27**
4	Treatments	6	1203743	200624	
5	Direct (unadjusted)	3	905706	301902	
6	Residual (adjusted)	3	298037	99346	0.64 n.s
7	Direct (adjusted)	3	870635	290212	1.86 n.s
8	Residual (unadjusted)	3	333107	111036	
9	Error	21	3268725	155654	

**significant at 99% level.

Variation in the consumption of the four different baits by rats was segregated into residual and direct effects. However, the residual effect of one bait on the consumption of the bait that followed it at the same bait point was not found to be significant (line 6), although this parameter has been found to have a significant effect in tests of other formulations (Buckle unpublished data).

Overall, the amounts of the four baits consumed by the rats at the three sites varied from a low 8450g for the difenacoum pellets to a high of 13090g for EPA challenge diet (Table 3). However, the variance ratio (line 7) for direct treatment effects approached, but did not exceed, the value required for statistical significance at the 95% level. There was no justification, therefore, in proceeding to the determination of standard errors of the differences between two direct effect means, and thereby least significant differences, which is the final stage of the Cochran and Cox (1957) analysis.

Laboratory Tests

The results of the laboratory palatability tests were analyzed by means of paired 't'-tests. The level of consumption of the test baits did not differ significantly from that of EPA challenge diet (Table 5). Palmateer (1981) considered rodenticide baits to be satisfactory when the acceptance of the test formulation is not significantly lower than 33%. Clearly, all three of the rodenticide products tested fulfil this criterion.

DISCUSSION

No statistically significant differences were demonstrated in the palatability of the four baits tested, either in the laboratory or in the field. A direct comparison of the laboratory and field data (Table 6) shows considerable agreement between results from the two environments and affords confidence, both in the practical value of palatability data generated in the laboratory and, more generally, in the efficacy of the poisoned baits for rodent control.

The inability to demonstrate statistical significance between apparently large absolute differences in bait consumption observed in the field trial was largely a product of the variability of results between sites. In this respect, the results from site 3 were of particular interest as there was a clear preference for EPA challenge diet (a finely paniculate meal) which was not apparent at the other sites. This may have been due to the fact that rats infesting this farmstead were conditioned to feed on the cereal meals provided as food for pigs whereas, at the other two sites, the foods available for rats were predominantly whole grains and animal feed pellets. In several trials conducted subsequently using the design described, in which more homogeneous sites were chosen, relatively small differences in the acceptance of a range of formulations, including wax block, pelletized and whole grain baits were found to be statistically significant (Buckle in preparation). Thus, in conducting field choice tests where it is of paramount importance to identify small differences in palatability between baits as statistically significant, trials sites should be chosen that are as similar as possible. In this case, however, any preferences observed may be site-specific. Alternatively, if an objective is to assess palatability under some of the varied conditions encountered in practical rodent control operations, sites should be chosen with differing characteristics, particularly with respect to the prior feeding experience of the target rodents.

A disadvantage o f the experimental design is that no data are produced with respect to rodent mortality. Clearly, such information is meaningless when individual infestations arc exposed to a variety of bails and toxicants. However, the potential efficacy of candidate formulations may be pre-

Table 5. Results of laboratory tests in which groups of ten albino IL norvegicus were offered a free choice of one of three rodenticide formulations and EPA challenge diet for four days.

Test diet		Challenge diet		% acceptance of test diet (±1 S.D.)	paired 't' test
Formulation	Total consumption (g)	Formulation	Total consumption (g)		
A 'Ratak'	411.0	EPA meal	515.9	46.8 ± 17.3	t=1.02 n.s.
B 'Ratak' S	313.7	EPA meal	396.5	43.2 ± 21.4	t=1.51 n.s.
C 'Talon' G	410.2	EPA meal	327.7	55.6 + 19.9	t=0.88 n.s.

Table 6. A comparison of laboratory and field evaluations of the palatability of three rodenticide formulations and that of EPA challenge diet. The quantity of test bait consumed is expressed as a percentage of the combined consumption of the test and challenge formulation.

	% acceptance	
Formulation	laboratory	field
A 'Ratak'	46.8 ± 17.3	39.2
B 'Ratak' S	43.2 + 21.4	45.7
C 'Talon' G	55.6 + 19.9	44.6

dieted from their palatabilities if a formulation of established effectiveness is included among those tested and the candidate baits contain active ingredients of either equivalent or superior potency. This drawback is more than offset, however, by the economical nature of the test procedure. The method allows the field comparison of four baits to be completed at three experimental sites during the course of a working week. It therefore provides a convenient method for the validation of laboratory-based testing programs and a useful intermediate step between the laboratory and full-scale field efficacy evaluations.

The design could be adapted for use with other species (e.g., Mus musculus) and latin squares of smaller or larger size could be used to permit fewer or more baits to be tested. The scheme should probably not be used over longer than six days, however, because the toxic effects of chronic active ingredients would then begin to exert a significant influence on bait uptake. The test is unlikely to be of value in the assessment of the palatability of fast-acting rodenticides. The method has, however, proven appropriate for field tests of bait stations. In this case, the same bait formulation is offered from candidate bait stations and the effects of the stations' designs on bait uptake is examined.

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