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Field trials of second-generation anticoagulants against difenacoum-resistant Norway rat populations

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SUMMARY

Trials of rodenticidal baits containing 50 p.p.m. difenacoum, 50 p.p.m. bromadiolone or 20 p.p.m. brodifacoum were carried out on farmsteads against populations of *Rattus norvegicus* containing difenacoum-resistant individuals. Six difenacoum treatments failed in 14–42 days of baiting. Two treatments with bromadiolone succeeded in 23 and 33 days, but four further treatments lasting 35–56 days failed to eradicate the populations. Brodifacoum gave virtually complete control of six populations in 21–73 days and of the ten residual populations left behind by the other two compounds, after baiting for a further 11–85 days. The performance of both bromadiolone and brodifacoum was well below that reported by previous investigators, indicating the possibility of low-grade resistance to these compounds in the difenacoum-resistant strain.

INTRODUCTION

In a previous report we presented observations showing that resistance to the anticoagulant rodenticide, difenacoum, was widespread in populations of the Norway rat, *Rattus norvegicus* in an area in Southern England (Greaves, Shepherd & Gill, 1982). The resistance is potentially a serious problem since difenacoum, introduced in 1975, is now the compound most commonly used in Britain for the control of rodents resistant to warfarin and other long-established anticoagulant rodenticides. More recently, two potent new anticoagulants, brodifacoum and bromadiolone, have been introduced for the control of warfarin-resistant infestations (Rennison & Dubock, 1978; Richards, 1981) and these two compounds, together with difenacoum have come to be known as the 'second-generation' anticoagulant rodenticides.

In preliminary laboratory tests both brodifacoum and bromadiolone appeared to be effective against the difenacoum-resistant strain (Greaves, Shepherd & Gill, 1982) and it was decided to attempt to confirm this in the field trials reported here. Difenacoum was also included in the trials for comparative purposes and to further characterize the response of infestations containing resistant individuals to treatment in the field. As expected, all of the difenacoum treatments failed. To

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our surprise, however, brodifacoum and bromadiolone were also relatively unsuccessful when judged against the high efficacy of these compounds as reported by previous investigators.

METHODS

The experimental treatments were carried out during January–August 1981 on 18 farmsteads near Basingstoke, Hampshire, in the area where resistance to warfarin and difenacoum was known to be present in the rat population (Greaves, Shepherd & Gill, 1982). The farmsteads were assigned at random to three groups treated with bait containing 50 p.p.m. difenacoum, 50 p.p.m. bromadiolone or 20 p.p.m. brodifacoum, the concentrations at which these compounds are registered for use as rodenticides in Britain. The baits were prepared by mixing cereal-based concentrates at 5% with a medium oatmeal bait base. Where, however, it was suspected that consumption of this bait by rats might be limited by their preference for alternative food, a more highly palatable bait was substituted, based on whole wheat that had been made succulent by soaking overnight in water.

Each farmstead, including its nearby fields was surveyed to determine the full extent of infestation and, at the same time, plastic bait trays were laid in appropriate positions. Natural cover was generally used to protect the bait points from the weather and from non-target animals but, where no cover was available, wooden bait boxes or drain pipes were provided. Three to five days later, on a Monday (day 0) the bait was laid in surplus amounts and inspected and replenished on each subsequent Wednesday, Friday and Monday (days 2, 4, 7, etc.). Where it appeared necessary to place bait closer to the rats than could be achieved by the use of containers it was placed directly into the burrow entrances; since such 'hole baits' could not be reliably inspected afterwards, they were omitted from the recorded results.

The treatments continued until bait takes and other signs of rat activity ceased or, where this did not occur, until it was apparent that the treatments had ceased to progress satisfactorily, subject to a minimum treatment length of 14 days for difenacoum and 35 days for bromadiolone. When treatments with these two compounds were terminated with infestation still present, a follow-up treatment was instituted immediately with brodifacoum which, by this time, appeared to be the most effective of the three rodenticides.

The progress of the treatments was assessed at each visit by recording the numbers of points from which bait had been taken by rats and plotting these in standard form on a monitoring graph. This procedure permits the results obtained with different anticoagulants to be compared with a standard curve established for warfarin treatments against normally susceptible infestations, and can yield a provisional indication of resistance in the population (Rennison, 1977). It tends, however, to underestimate mortality during a treatment mainly because, while the takes tend to decrease due to the toxic effects of the bait, they simultaneously tend to increase during the first 10–14 days as more rats locate the bait and overcome their initial reluctance to feed on it (Chitty, 1942). As a control for this factor, six

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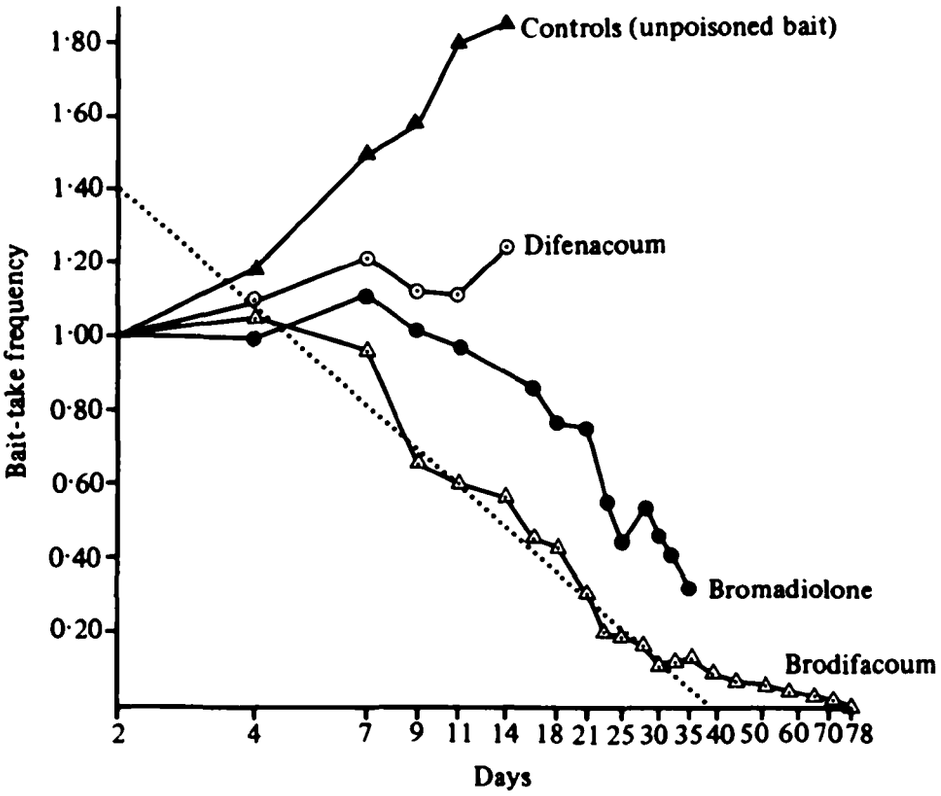

Fig. 1. The results of baiting with unpoisoned bait, 50 p.p.m. difenacoum, 50 p.p.m. bromadiolone or 20 p.p.m. brodifacoum. Each line represents the mean of six treatments. The dotted line is the criterion for resistance to anticoagulant treatment proposed by Rennison (1977).

further farmsteads were treated for 14 days with unpoisoned bait and the numbers of takes recorded were used as a base-line for making indirect estimates of relative population size as the experimental treatments progressed.

RESULTS

The mean values of the bait-take frequency for each set of six treatments are shown in Fig. 1. For this purpose the bait-take frequency is defined as the number of points showing a take by rats at each visit, divided by the number that showed a take at the first bait-replenishment visit, on day 2 of the treatment. Some degree of resistance to all three of the rodenticides was indicated within the first 14 days of the treatments on 17/18 of the farmsteads, when the bait-take frequency exceeded the criterion for resistance (shown as a dotted line in Fig. 1) suggested by Rennison (1977). The single exception was an infestation that was eradicated with brodifacoum in 21 days. It became evident, however, from the ultimate success of two treatments with bromadiolone and 14 with brodifacoum that any resistance to these compounds was incomplete.

The same data are plotted in Fig. 2, transformed into percentages of the mean values recorded for unpoisoned bait on the six control farmsteads, in order to indicate the decreases in population size attributable to treatment with the rodenticides. Clear differences in efficacy among the three rodenticides are apparent.

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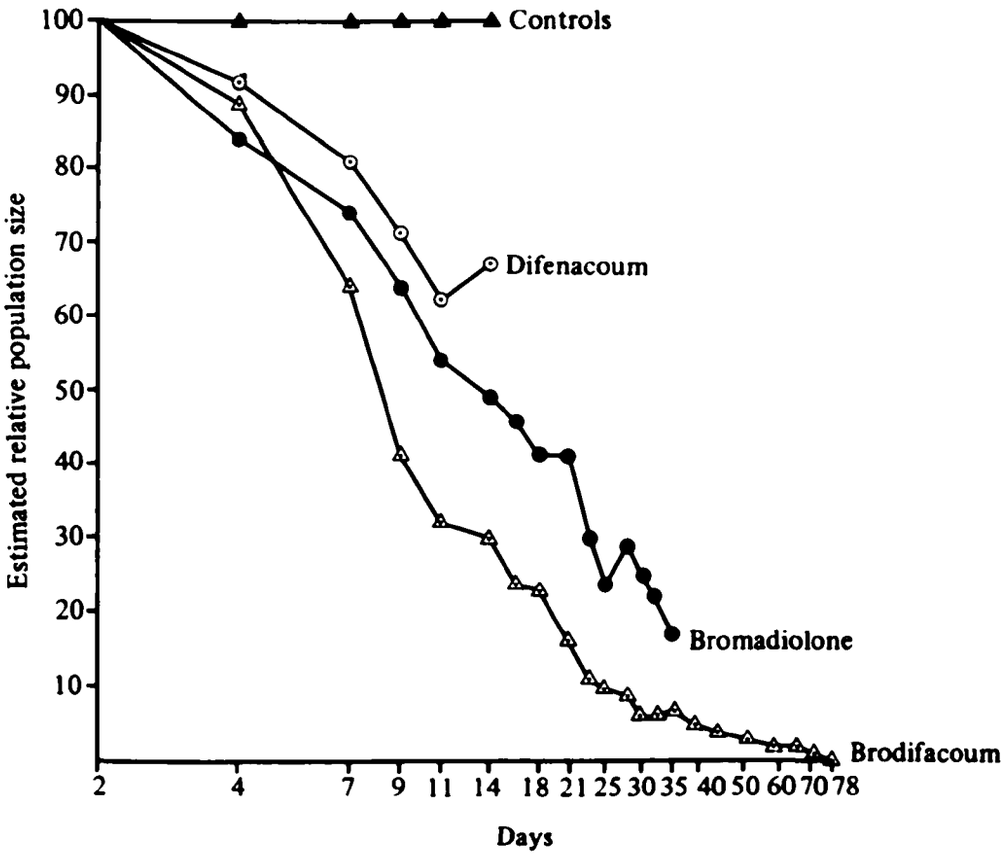

Fig. 2. Estimated relative population sizes during treatments with bait containing 50 p.p.m. difenacoun, 50 p.p.m. bromadiolone or 20 p.p.m. brodifacoum. Each line represents the mean of six treatments. The estimates are derived from the data of Fig. 1 by dividing the mean bait-take frequencies for each rodenticide by that for the controls. The control value is assumed to be constant from day 14 onwards.

Difenacoum gave the poorest result, with a mean estimated kill of only 33% in 14 days, and with the numbers of takes generally increasing during this time. Three treatments in which obvious mortality was occurring were continued for 35, 42 and 42 days and gave, respectively, kills of 74%, 66% and 49%.

With bromadiolone the mean estimated mortality was 51% in 14 days and 83% in 35 days. Two treatments were completely successful in 23 and 33 days. The remaining four treatments gave kills of 66%, 71%, 86% and 97% respectively in 35, 35, 56 and 56 days.

Brodifacoum was the most effective of the three compounds, giving mean kills of 70% in 14 days and 93% in 35 days. Five of the infestations were eradicated in 21, 35, 51, 71 and 73 days. The sixth treatment had to be terminated after 44 days with small amounts of bait still being taken from two points beside a silage clamp, to avoid the risk of accidentally poisoning cattle that were about to be given free access to the silage. The ten additional treatments with brodifacoum, of the residual infestations left behind by difenacoum or bromadiolone are not strictly comparable with the first six treatments and are therefore omitted from the figures. However, nine led to complete eradication of the residual infestations in 11–85 days, 36 days on average. The tenth treatment ended with bait still being taken from six points in a grain store after 53 days, at which time a large influx of fresh grain stocks prevented the treatment from continuing.

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DISCUSSION

The notable feature of most of the experimental treatments was their very protracted nature, even where the infestations were eventually eradicated. The performance of difenacoum was even worse than in our preliminary trials (Greaves, Shepherd & Gill, 1982), adding weight to the evidence of widespread resistance to this compound in Hampshire. The indifferent results obtained with brodifacoum and, more especially, bromadiolone, contrast strongly with previous reports of the efficacy of these compounds against anticoagulant-resistant Norway rat infestations. Thus, in treatments with 20 p.p.m. brodifacoum on nine farmsteads in Powys and Shropshire, the bait-take frequency decreased by almost 90 % (as against 40 % here) in 11 days, and virtually complete control was obtained in 11–25 days, even when the concentration was reduced to 5 p.p.m. (Rennison & Dubock, 1978). Similarly, in nine trials with 50 p.p.m. bromadiolone, a better than 80 % reduction in the bait-take frequency (as compared with only 3 % here) occurred in 11 days, complete kills being obtained again in 11–25 days (Richards, 1981).

Prolonged bait consumption by rodents in anticoagulant treatments can have several causes but it is rarely possible, in the field, to be sure which ones are operating. One frequently cited is the immigration of rats from outside the treated area. Though this can never be discounted completely we do not think it was significant: special care was taken to treat the infestations thoroughly and in their entirety and, where treatments were protracted, the bait was generally taken continuously from the same bait points, not intermittently or from varied locations as might be expected if new animals were entering areas from which the residents had been eliminated. Furthermore, on inspection of the farmsteads 3–8 months later, the majority were found still to be relatively free of infestation, contrary to what might have been expected if immigation had been occurring continuously.

It is more difficult to exclude the possibility that bait consumption may have been inadequate owing to the competing attractions of other foodstuffs on some of the farms. Bait uptake appeared, however, to be generally more than adequate in relation to other signs of rat activity and, even in the later stages of treatments, appreciable quantities were being consumed wherever a take was recorded. It seems very improbable that any rat consumed less, in total, than the 10·0 g of brodifacoum bait or 50·0 g of bromadiolone bait that, on the basis of published toxicity data should be lethal to the vast majority of rats (Redfern, Gill & Hadler, 1976; Redfern & Gill, 1980). Remarkably little is known, however, of the amounts of bait ingested by individual rats during treatments. Rennison & Dubock (1978) suggest that many individuals eat little or no bait during the first week or so of a typical anticoagulant treatment and bait consumption may therefore often be closer to the minimum required for success than is generally believed.

There remains the possibility of some degree of resistance to brodifacoum and bromadiolone. The evidence for this is scant, for a preliminary laboratory test with difenacoum-resistant rats from the area gave little or no indication of cross resistance to either compound (Greaves, Shepherd & Gill, 1982). On the other hand, the pattern of bait takes in the experimental treatments (Fig. 1) for brodifacoum and, more especially, bromadiolone is very characteristic of what might be

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expected with low levels of resistance. Also, the brief control histories provided for us by the farmers suggested that the infestations that we controlled most easily were those where previous use of anticoagulants had been least intensive. We suggest, therefore, that the difenacoum-resistant strain has a level of resistance to bromadiolone and brodifacoum which, though low, is enough to confer significant protection when a substantial part of the diet is derived from sources other than the rodenticidal bait. A more detailed study of the difenacoum-resistant strain in the laboratory is required to test this hypothesis.

Whatever the reasons, it may be noted that the performance of the rodenticides in these trials was not only poorer than has been reported hitherto, but poor by any normal standard. Thus, Drummond & Rennison (1973) using warfarin, now a traditional anticoagulant rodenticide, found that complete control of normally susceptible Norway rat infestations is typically obtained after baiting for about 19 days, at which time the mean estimated kills in the present trials were only 59 % for bromadiolone and 80 % for brodifacoum. Even zinc phosphide, an acute poison often regarded as being mainly of historical interest for Norway rat control, has been reported to do better, with an average kill of 84 % when applied for 24 h after prebaiting with unpoisoned bait for 5 days (Rennison, 1977).

It is possible that the use of bait containing higher concentrations of the rodenticides might prove to be more effective. The strength of bromadiolone can apparently be raised to 500 p.p.m. without much loss of palatability and that of brodifacoum to at least 50 p.p.m., concentrations that have been advocated by Lund (1977) and Dubock & Kaukeinen (1978). The advantage of formulations capable of giving complete control of the pest with a reasonable expenditure of effort needs no emphasis, though their use might well present increased hazards for non-target species. It is interesting to note that, on the basis of rat acute LD50 estimates given by Dubock & Kaukeinen, (1978) the baits used in the present study were already up to 275 times more toxic than warfarin at 50 p.p.m., which was formerly sufficient to give efficient control of *Rattus norvegicus* (Drummond & Rennison, 1973). This suggests that rodent control is now well along the path, familiar in other areas of pest control, where successive losses of pesticidal efficacy are combated by the use of increasingly toxic formulations.

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REFERENCES

CHITTY, D. (1942). A relative census method for brown rats (*Rattus norvegicus*). *Nature, London* **150**, 59–60.

DRUMMOND, D. C. & RENNISON, B. D. (1973). The detection of rodent resistance to anticoagulants. *Bulletin of the World Health Organization* **48**, 239–242.

DUBOCK, A. C. & KAUKEINEN, D. E. (1978). Brodifacoum (Talon rodenticide), a novel concept. *Proceedings of the 8th Vertebrate Pest Conference, Sacramento, California*, 127–137.

https://doi.org/10.1017/S0022172400070820 Published online by Cambridge University Press

GREAVES, J. H., SHEPHERD, D. S. & GILL, J. E. (1982). An investigation of difenacoum resistance in Norway rat populations in Hampshire. *Annals of Applied Biology* **100**, 581–587.

LUND, M. (1977). New rodenticides against anticoagulant-resistant rats and mice. *Bulletin of the European and Mediterranean Plant Protection Organization* **7**, 503–508.

REDFERN, R. & GILL, J. E. (1980). Laboratory evaluation of bromadiolone as a rodenticide for use against warfarin-resistant and non-resistant rats and mice. *Journal of Hygiene* **84**, 263–268.

REDFERN, R., GILL, J. E. & HADLER, M. R. (1976). Laboratory evaluation of WBA 8119 as a rodenticide for use against warfarin-resistant and non-resistant rats and mice. *Journal of Hygiene* **77**, 419–426.

RENNISON, B. D. (1977). Methods of testing rodenticides in the field against rats. *Pesticide Science* **8**, 405–413.

RENNISON, B. D. & DUBOCK, A. C. (1978). Field trials of WBA 8119 (PP 581, brodifacoum) against warfarin-resistant infestations of *Rattus norvegicus*. *Journal of Hygiene* **80**, 77–82.

RICHARDS, C. G. J. (1981). Field trials of bromadiolone against infestations of warfarin-resistant *Rattus norvegicus*. *Journal of Hygiene* **86**, 363–367.

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