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Trials of the anticoagulant rodenticides bromadiolone and difenacoum against the house mouse (*Mus musculus* L.)

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SUMMARY

Laboratory and field trials were conducted to determine the efficacy of the anticoagulant rodenticide bromadiolone against the house mouse (*Mus musculus*). In laboratory feeding tests, family groups of warfarin-resistant mice maintained in pens and conditioned to feeding on plain foods were offered pinhead oatmeal bait containing bromadiolone at 0·005%. Overall mortality in replicated 21-day poison treatments was 55/58 or 94·8%. Six field trials were carried out, using the same poison bait, against mice infesting farm buildings. Treatment success, estimated from the results of census baitings conducted before and after treatment, ranged between 60·4% and 100%, mean 92·4%. In equivalent field trials using difenacoum, another newly developed anticoagulant rodenticide, the control achieved ranged between 70·2% and 100%, mean 96·0%.

Five field trials, three involving bromadiolone and two difenacoum, were not completely successful and the surviving mice were removed for laboratory examination. In 21-day toxicity tests, each animal was fed the poison bait offered to it earlier in the field. Bromadiolone and difenacoum gave kills of 12/21 (57·1%) and 9/11 (81·8%) respectively. The possible emergence of mouse populations resistant to these anticoagulants is considered.

INTRODUCTION

The problem of resistance to warfarin and to other anticoagulants of longstanding use in rodent control stimulated research into improved rodenticides of this type. In recent years, three new compounds – brodifacoum, difenacoum and bromadiolone, have been developed for rodenticidal use. The efficacy of each of these anticoagulants has now been evaluated in this laboratory against rats, *Rattus norvegicus* and *R. rattus* and mice, *Mus musculus*. Brodifacoum 3-(3-4′-bromobiphenyl-4-yl)-1,2,3,4-tetrahydronaphth-1-yl)-4-hydroxycoumarin, showed the greatest activity in oral dosing and feeding tests (Redfern, Gill & Hadler, 1976) but both the closely related compound difenacoum, 3-(3-*p*-diphenyl-1, 2,3,4-tetrahydronaphth-1-yl)-4-hydroxycoumarin, and bromadiolone, 3-[3-(4′-bromo[1,1′-biphenyl]-4-yl)-3-hydroxy-1-phenylpropyl]-4-hydroxy-2H-1-benzopryan-2-one, were also found to be effective against all three species, including warfarin-resistant animals (Hadler, Redfern & Rowe, 1975; Redfern & Gill, 1980).

Brodifacoum also performed well when it was tested against mouse populations infesting farm buildings (Rowe, Swinney & Plant, 1978). The present paper reports on the results of similar field trials undertaken with difenacoum and bromadiolone. In keeping with earlier work on brodifacoum (Rowe & Bradfield, 1976) and on difenacoum (Rowe & Bradfield, 1975), bromadiolone was also investigated in feeding trials on mice maintained in laboratory pens.

METHODS

Pen trials of bromadiolone

Family groups of wild mice, consisting of sub-adult and adult animals, were used. Each group was reared in a breeding cage from parent stock known to be resistant to warfarin (Rowe & Bradfield, 1975) and the cage was transferred to the nesting area of a metal pen enclosure measuring 9·5 × 2·5 m when two or more litters had been raised successfully. Trays containing plain food (whole wheat grain mixed with powdered laboratory Diet 41B) were placed on either side of the cage and drinking water was also provided *ad lib*. The mice were allowed to escape from the cage and to become accustomed to the environmental conditions for 7 days before they were tested. Four trials were conducted, using bromadiolone at 0·005% in cereal bait. The poison bait, prepared by thoroughly mixing an appropriate amount of the pure compound in corn oil (5%) with pinhead oatmeal (90%) and wholemeal flour (5%), was laid in open trays placed at eight sites outside the nesting area. The sources of plain food were maintained throughout the treatment period (21 days maximum duration) and the total amount of bromadiolone bait eaten was measured daily. Dead mice were also recovered daily and each animal was autopsied to confirm symptoms of anticoagulant poisoning.

Field trials

The six field trials undertaken with both poisons were carried out in granaries, milling sheds, dairy units and workshops on mixed arable/dairy farms. A building was allocated for experimental use after an inspection for fresh rodent signs (faeces, runs, smears and holes) showed that it was infested by mice and not by rats.

A census baiting was conducted in each building before poison bait was laid. For the purposes of a census, a known weight of plain bait, canary seed (*Phalaris canariensis*), was placed in small covered containers which were distributed 1 to 2 m apart throughout the infested area. The containers were examined on each of the next 4 days to ensure that surplus bait was always available at each point and the total amount of canary seed eaten was measured daily. The containers and surplus bait were removed at the end of the census period.

The poison treatment, begun 3 days later, was done using baiting points different from those used in the census baiting. Difenacoum or bromadiolone, at 0·005% in pinhead oatmeal/wholemeal flour/corn oil bait, was laid in excess and the amount of poison bait eaten was measured on 4 days of each week (Tuesday to Friday) and then over the next 3 days. A treatment was terminated when the take of poison bait ceased or, alternatively, when there had been regular consumption of bait over a prolonged period (6 to 7 weeks). The excess poison bait and containers were then removed.

The post-treatment census baiting, begun after a further interval of 3 days was conducted in the same manner as the pre-treatment census baiting except that the take of canary seed was measured on days 1 and 4 only. Percentage success in each treatment was estimated from the total amounts of census bait eaten at pre-and post-treatment.

Laboratory feeding tests

Mice which survived treatment with 0·005 % bromadiolone bait in the pens were kept under observation for a further 7 days. The plain food supplies were then removed and the survivors presented with poison bait as sole diet for 21 days.

The take of post-treatment census bait and visual signs of mice indicated that some animals escaped poisoning in the field. Longworth live-traps (Chitty & Kempson, 1949) were laid in the buildings concerned immediately after the completion of each post-treatment census baiting. Captured mice were transferred to the laboratory, individually caged and provided with Diet 41B. After a rest period lasting 2 weeks or longer, each animal was given, without choice, the poison bait that had been tested against it in the field trial. The amount of poison bait eaten was measured daily for a maximum of 21 days. Any survivors were allowed a further rest period before they were re-tested, using double the concentration (0·01 %) of bromadiolone or difenacoum in bait.

RESULTS

Pen trials of bromadiolone

The results of the pen trials are summarized in Table 1. In each treatment, the consumption of 0·005 % bromadiolone bait fell markedly after day 7 and three of the families of mice were killed, one group dying in 8 days. Overall, 55/58 or 94·8 % of the mice under test were killed.

Field trials

The amount of poison bait eaten by mice in each field trial is shown in Table 2. In all 12 treatments, poison bait consumption was highest during week 1 and, thereafter, there was a progressive decline in take in six of the treatments (Trials 2, 5, 8, 9, 11 and 12). A less regular fall in poison bait consumption occurred during four other treatments (Trials 1, 3, 6 and 7) which were also terminated when feeding ultimately ceased. The take of poison bait was more erratic during the course of the two remaining treatments (Trials 4 and 10) and mice were still visiting the baiting points in both buildings when the treatments were abandoned (after 7 and 6 weeks respectively).

Treatment success in the trials employing difenacoum was estimated to range between 70·2 and 100 %, mean 96·0 %; in comparison, bromadiolone gave control ranging between 60·4 and 100 %, mean 92·4 %. Statistical analysis of the data given in Table 2, using the post-treatment census bait takes as a percentage of the pre-treatment census bait takes in an analysis of variance (Huson, 1980), indicated no difference in effectiveness between the two poisons.

Table 1. *The toxicity of bromadiolone* (0·005 %) *to family groups of warfarin-resistant* Mus musculus *in* 21*-day choice feeding tests*

Trial No.	Poison bait eaten (g) Days 1–7	Days 8–14	Days 15–21	Mortality	Mortality (%)	Days to death Range	Mean
1	125·5	27·7	3·2	18/18	100	4–17	7·8
2	77·6	3·7	1·9	14/14	100	4–16	8·7
3	89·4	33·2	21·9	10/13	76·9	3–18	9·1
4	84·3	0·3	—	13/13	100	2–8	5·0

Laboratory feeding tests

Three adult females, members of the same group, survived treatment with 0·005 % bromadiolone bait in one of the pens. In the subsequent no-choice feeding test, they ate 29·1 g (days 1–7), 29·3 g (days 8–14) and 31·3 g (days 15–21) of the same poison bait. One animal survived and the other two died at a late stage (days 20 and 21).

The results of the laboratory feeding tests on mice which survived poisoning in the field are shown in Table 3. Two of the difenacoum treatments were not completely successful but both mice caught following the completion of Trial 4 died before reaching the laboratory. The survivors of the other treatment (Trial 6) were presented with 0·005 % difenacoum bait over a 21-day period; mortality was 9/11 (days to death, 6–20). In the further test, using bait containing difenacoum at 0·01 %, both of the adult male survivors died (on days 6 and 9).

Incomplete control occurred as a result of three of the bromadiolone treatments (Trials 9, 10 and 12) and kills of 2/3, 4/12 and 6/6 respectively were obtained when the survivors were fed 0·005 % bromadiolone bait in the laboratory for 21 days. When the nine survivors were re-tested using bromadiolone at 0·01 % in bait, further deaths resulted; Trial 9 (1/1) and Trial 10 (7/8).

DISCUSSION

Redfern & Gill (1980) concluded from the results of their laboratory tests that bromadiolone and difenacoum were about equally effective against *M. musculus*. This conclusion is supported by the results of the pen and field trials conducted with both poisons. In the present pen trials, bromadiolone gave a kill of 55/58 (94·8 %) and in the comparable trials using difenacoum (Rowe & Bradfield, 1975) a kill of 72/81 (88·9 %) was obtained.

It was concluded from earlier pen trial work on difenacoum (Hadler *et al.* 1975) that this poison would be unlikely to control all mouse populations resistant to warfarin and other anticoagulant rodenticides. The same conclusion can be drawn concerning bromadiolone as a result of the present pen trials. Similar to the findings on difenacoum, there was evidence of considerable variation in susceptibility to bromadiolone poisoning. Some members of the warfarin-resistant families of mice tested died as early as day 2 after feeding on 0·005 % bromadiolone bait (Table 1) but the three survivors were not readily killed in the 21-day feeding test on the same poison bait alone.

Table 2. *The results of difenacoum and bromadiolone poison treatments against infestations of* M. musculus

Poison	Trial No.	Pre-treatment census bait eaten (g)	Amount of poison bait eaten (g) Week 1	2	3	4	5	6	7	Post-treatment census bait eaten (g)	Estimated success (%)
Difenacoum	1	338	306	113	97	106	98	85	26	0	100
	2	110	52	12	1	—	—	—	—	0	100
	3	53	24	3	3	—	—	—	—	0	100
	4	516	501	329	249	266	175	89	104	154	70·2
	5	2591	2049	692	339	83	47	18	—	0	100
	6	626	634	184	164	48	58	—	—	17	97·3
Bromadiolone	7	954	797	321	85	100	97	40	—	0	100
	8	138	175	106	68	47	—	—	—	0	100
	9	110	88	8	3	—	—	—	—	24	78·2
	10	366	178	59	50	101	73	92	—	145	60·4
	11	929	1549	287	75	72	28	—	—	—	100
	12	282	212	140	133	45	4	—	—	43	84·8

Table 3. *The results of 21-day laboratory feeding tests on the survivors of difenacoum and bromadiolone field treatments*

Trial no.	Poison	Concentration (%)	Mortality	Survived dose of active ingredient (mg/kg)	Range of days to death
6	Difenacoum	0·005	9/11	144–147	6–20
		0·01*	2/2	—	6–9
9	Bromadiolone	0·005	2/3	213	7–12
		0·01*	1/1	—	16
10	Bromadiolone	0·005	4/12	118–217	7–15
		0·01*	7/8	410	5–16
12	Bromadiolone	0·005	6/6	—	8–19

* Tests on the survivors of the 0·005% poison treatments.

The field data provided further evidence of differential susceptibillty among *M. musculus* to both 0·005% difenacoum and to 0·005% bromadiolone. Dead mice were commonly found during the first week of each treatment but in the laboratory tests on the survivors, two individuals survived doses of difenacoum equivalent to 144 and 147 mg/kg (Table 3) and the nine bromadiolone survivors ingested poison doses ranging between 118 and 217 mg/kg. One animal, furthermore, was found to withstand 21 days feeding on 0·01% bromadiolone bait, consuming 55·8 g of poison bait (410 mg/kg).

Clear evidence of cross-resistance between warfarin and bromadiolone was found as the result of Trial 10. The mouse population infesting the small seed potato store used for this trial had been treated with 0·025% warfarin bait for several weeks before the building was inspected. The warfarin treatment appeared to have been unsuccessful however for no dead mice were found, fresh faeces and smears were abundant and there was recent, extensive damage to polystyrene insulation material. The population was sampled immediately before the bromadiolone treatment was begun. The five animals caught all fed well on 0·025% warfarin bait in the laboratory and survived the 21-day feeding period. There is likelihood therefore that the resident mouse population was resistant to warfarin. A further feeding test on the five mice, using 0·005% bromadiolone bait, gave a kill of 4/5 (days to death 11–21) over a 21-day feeding period, suggesting that some members of the mouse population were also resistant to bromadiolone.

The bromadiolone treatment failed to eradicate the infestation of mice. Poison bait consumption fell until week 3 but it fluctuated at a higher level thereafter (Table 2) and 11 of the 13 dead mice found were recovered during the first three weeks of the six-week treatment period. There was indication therefore that the treatment was largely ineffective in its latest stages. Support for this viewpoint was evident in the low kill that was obtained when the survivors were fed 0·005% bromadiolone bait in the laboratory (mortality 4/12; 33·3%). The laboratory findings and field data arising from this trial strongly suggests that selection, favouring bromadiolone-resistant animals, occurred during the course of the treatment.

In the laboratory, brodifacoum was found to be more active against *M. musculus* than either difenacoum or bromadiolone (Redfern & Gill, 1976). In pen trials using

brodifacoum (Rowe & Bradfield, 1976), a kill of 62/63 (98·8%) was obtained and brodifacoum also performed marginally better than difenacoum or bromadiolone when it was examined in the field (Rowe, *et al.* 1978) the success of the six treatments ranging between 92·7% and 100% mean 98·9%.

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