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Laboratory evaluation of bromadiolone as a rodenticide for use against warfarin-resistant and non-resistant rats and mice

By R. REDFERN and J. E. GILL
Ministry of Agriculture, Fisheries and Food, Tolworth Laboratory, Hook Rise South, Tolworth, Surbiton, Surrey

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SUMMARY

Laboratory feeding tests were carried out to determine the efficacy of the anticoagulant rodenticide bromadiolone against *Rattus norvegicus*, *R. rattus* and *Mus musculus*. Using 0·005% bromadiolone, complete kills of *R. norvegicus* and *R. rattus* not resistant to warfarin were obtained after exposure to the poison for 1 and 5 days respectively. Warfarin-resistant *R. norvegicus* were all killed in 4 days, and resistant *M. musculus* in 12 days. In general, the results resembled those obtained with difenacoum. Acceptance of bromadiolone was very good.

INTRODUCTION

During the last decade there has been a considerable increase in the research effort put into developing new rodenticides. The impetus for this was the discovery of resistance to anticoagulants, first in Scotland (Boyle, 1960) and later in Wales, other European countries and the USA. Results of this work include the use of calciferol as a rodenticide (Greaves, Redfern & King, 1974), and the development by Sorex (London) Ltd of difenacoum and brodifacoum, two of a group of unusually potent anticoagulant compounds that are effective against warfarin-resistant rats and mice (Hadler, Redfern & Rowe, 1975; Redfern, Gill & Hadler, 1976).

Bromadiolone, 3-[3-(4′-bromo[1,1′-biphenyl]-4-yl)-3-hydroxy-1-phenylpropyl]-4-hydroxy-2H-1-benzopyran-2-one, another very active anticoagulant, was developed by Lipha (Lyon, France), and marketed in France in 1977. Little work has been published so far on the response of wild rats and mice to bromadiolone. Marsh (1977) obtained complete mortality in the laboratory with non-resistant *R. norvegicus* after 1 day's feeding, but *M. musculus* appeared considerably more resistant to the chemical. Grand (1976) states that first results indicated a marked activity on rats 'said to be resistant'.

This paper describes the laboratory evaluation of the poison against wild rodents, and compares its performance with other new anticoagulants.

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METHODS

Feeding tests were carried out on wild, individually caged *R. norvegicus*, *R. rattus* and *M. musculus*. The *R. norvegicus* were caught on farms in the warfarin-resistance area of central Wales; the other two species were the laboratory-bred descendants of wild stock. The *R. rattus* were derived from infestations where there was no history of resistance to anticoagulants, and were therefore presumed to be non-resistant. All *R. norvegicus* used had previously been classified as warfarin-resistant or not by examination of the blood-clotting activity 24 h after an injected dose of warfarin and vitamin K-oxide (Martin, Steed, Redfern, Gill & Huson, 1979). All *M. musculus* were survivors of a 21-day warfarin feeding test, the criterion for resistance in this species (Rowe & Redfern, 1965). Test animals were maintained on a standard laboratory food (diet 41B, Oxoid Ltd, London) and water *ad lib.*

Bromadiolone was presented in either medium oatmeal (95%) or pinhead oatmeal (90%)/corn oil (5%). To each of these bait-bases was added a 'master-mix' (5%), made up of the appropriate amount of pure active ingredient dispersed in wholemeal flour. In 'no-choice' tests unpoisoned bait was given for several days before the poison was introduced, thus ensuring that the rodents were eating properly before the test began. In 'choice' tests naïve animals were used: these were fed on diet 41B until the start of the test. The positions of the baits were interchanged, and fresh baits and clean food pots given daily (EPPO, 1975).

Autopsies were carried out to confirm that deaths were due to anticoagulant poisoning.

Survivors were kept under observation for at least 2 weeks after exposure to poison.

A sample of technical grade bromadiolone was supplied by Lipha.

RESULTS AND DISCUSSION

No-choice feeding tests

(i) R. norvegicus

Preliminary work with *R. norvegicus* showed that 0·002% bromadiolone gave kills of 18/20 and 20/20 with non-resistant rats after 1 and 2 days feeding, but with warfarin-resistant animals 0/10 after 2 days. The results for non-resistant rats are very similar to those obtained with 0·002% brodifacoum (44/50 and 20/20 for 1 and 2 days respectively); with resistant rats, however, brodifacoum killed 59/60 after 2 days (Redfern *et al.* 1976).

In all subsequent tests, bromadiolone was used at 0·005%, the concentration recommended by the manufacturer. The results (Table 1) again show a clear indication of cross-resistance between warfarin and bromadiolone in this species. In non-resistant rats there was a complete kill after 1 day's feeding (although only 95% after 2 days), whereas with warfarin-resistant animals the kill was only 28%. The results for the non-resistant group preclude any statistical treatment, but when the mortality data for resistant rats are subjected to probit analysis, values obtained for the median lethal feeding period (LFP 50) and LFP 98 (with

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the corresponding 95% fiducial limits) are 1·45 days (1·27–1·62) and 4·21 days (3·52–5·50). The slope of the probit line was 4·43 (S.E. ± 0·50). With 0·005% difenacoum, Redfern & Gill (1978) found LFP 50 and 98 values of 1·19 (0·96–1·37) and 3·80 days (3·17–5·09) for warfarin-resistant rats: the slope of the corresponding probit line was 4·06 (S.E. ± 0·56). The relative potency of bromadiolone to difenacoum is estimated to be 1·07, indicating that the two anticoagulants are equally effective against warfarin-resistant *R. norvegicus*.

The results confirm the findings of Marsh (1977) that bromadiolone kills non-resistant *R. norvegicus* after 1 day's feeding. Lund (1977), however, infers from his results that the concentration of the poison needs to be raised to 0·05% to give a complete kill of warfarin-resistant rats in Denmark, although no indication of the

Table 1. *Mortality and bait consumption of wild rodents given a sole diet of* 0·005% *bromadiolone in a cereal bait*

No. of days feeding	Mean body weight (g)	Sex	Mortality	Lethal dose of active ingredient (mg/kg)		Survived dose of active ingredient (mg/kg)		Days to death	
				Mean	Range	Mean	Range	Mean	Range
				Rattus norvegicus Warfarin-resistant					
1	346	M	10/30	2·7	1·1–3·8	2·7	1·7–4·0	7·6	5–14
	259	F	7/30	3·1	1·7–3·9	2·9	1·2–4·5	9·7	4–14
2	346	M	24/30	5·6	1·1–7·2	5·1	1·1–6·4	5·7	2–9
	234	F	14/30	5·5	3·3–7·1	6·3	4·6–11·6	6·4	3–9
3	301	M	29/30	8·7	2·8–12·8	8·7	—	6·1	4–12
	242	F	27/30	9·4	4·6–16·4	8·2	7·7–9·2	7·2	2–16
4	313	M	30/30	10·2	5·4–18·4	—	—	5·8	4–8
	265	F	30/30	10·8	4·2–14·3	—	—	7·3	3–12
				Non-resistant					
1	313	M	40/40	3·4	1·7–5·0	—	—	6·1	3–9
	201	F	40/40	3·8	1·2–5·8	—	—	7·1	4–12
2	277	M	30/30	6·9	3·6–13·7	—	—	5·9	3–8
	185	F	29/30	8·0	5·1–13·0	12·5	—	6·9	5–14
3	307	M	20/20	10·3	2·2–15·3	—	—	6·3	4–10
	205	F	20/20	11·6	8·2–15·6	—	—	7·0	4–10
4	297	M	20/20	10·5	4·1–15·3	—	—	6·5	4–10
	190	F	20/20	12·2	4·4–18·8	—	—	5·9	3–12
				Rattus rattus Non-resistant					
1	183	M	8/15	3·2	2·7–4·1	3·2	2·7–4·6	9·4	5–11
	130	F	6/15	4·3	3·5–5·3	4·4	1·1–6·5	7·5	6–11
2	150	M	14/15	8·0	5·8–11·1	8·3	—	8·4	4–11
	137	F	13/15	8·6	6·8–12·1	8·4	8·1–8·7	10·1	6–13
3	205	M	15/15	10·3	7·3–13·1	—	—	8·3	6–11
	175	F	12/15	10·9	7·4–16·8	9·9	8·4–11·0	9·8	6–15
4	164	M	15/15	14·3	8·6–18·2	—	—	8·6	5–12
	134	F	14/15	16·4	11·1–20·2	13·4	—	8·0	7–10
5	120	F	15/15	18·9	12·6–23·0	—	—	7·9	5–12

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Table 1 (*continued*)

No. of days feeding	Mean body weight (g)	Sex	Mortality	Lethal dose of active ingredient (mg/kg) Mean	Lethal dose of active ingredient (mg/kg) Range	Survived dose of active ingredient (mg/kg) Mean	Survived dose of active ingredient (mg/kg) Range	Days to death Mean	Days to death Range
				Mus musculus Warfarin-resistant					
1	20	M	0/10	—	—	9	7–11	—	—
	13	F	1/10	8	—	11	8–16	11·0	—
2	18	M	7/10	18	13–23	19	18–21	9·0	6–11
	15	F	3/10	20	16–24	17	13–23	8·7	7–11
3	19	M	5/10	32	28–37	26	18–30	8·8	6–10
	13	F	4/10	33	30–37	28	24–32	7·0	7–10
4	19	M	8/10	34	26–46	35	27–42	6·8	6–13
	13	F	7/10	37	29–48	42	38–46	7·0	5–8
5	18	M	9/10	41	29–50	59	—	7·2	4–10
	15	F	9/10	41	24–49	42	—	8·7	5–13
6	17	M	8/10	43	32–48	48	45–52	8·7	6–11
	16	F	9/10	36	6–55	41	—	6·6	3–9
7	17	M	7/10	59	35–83	45	37–50	9·6	6–12
	16	F	7/10	37	0·7–54	43	42–44	7·9	3–11
8	18	M	8/10	36	13–50	48	47–48	7·4	4–11
	18	F	10/10	46	23–62	—	—	10·1	5–17
9	18	M	8/10	40	3–75	69	67–71	9·1	3–13
	15	F	7/10	46	23–59	67	60–73	8·6	6–12
10	21	M	10/10	47	37–61	—	—	8·8	7–14
	17	F	6/10	51	25–64	64	48–77	11·5	6–18
11	20	M	10/10	53	34–84	—	—	9·8	8–12
	18	F	9/10	50	39–64	88	—	8·2	5–12
12	17	M	10/10	57	39–92	—	—	9·0	6–11
	15	F	10/10	49	32–65	—	—	9·3	4–15
21	17	M	10/10	63	26–117	—	—	9·2	5–17
	15	F	10/10	46	21–80	—	—	8·8	5–14

duration of the feeding test is given. Meehan (1978) states that three out of four homozygous warfarin-resistant *R. norvegicus* were killed, after a feeding period of 4 days.

(ii) R. rattus

Complete kills of male non-resistant *R. rattus* occurred after 3 days feeding, and of females after 5 days. With 0·005% difenacoum, complete mortality in both sexes occurred after 3 days' feeding (Hadler *et al.* 1975): brodifacoum at 0·005% gave 20/20 mortality in 2 days (Redfern *et al.* 1976). Applying probit analysis to the present results, the LFP 50 and 98 for 0·005% bromadiolone against *R. rattus* are 1·01 days (0·66–1·28) and 4·33 days (3·18–8·12) respectively. The slope of the probit line was 3·25 (S.E. ± 0·64).

(iii) M. musculus

Complete kills of warfarin-resistant males and females were obtained in 10 and 12 days respectively, although heterogeneity of the data prevents a satisfactory

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Table 2. *Bait consumption and mortality of wild rodents given a choice between plain and poisoned bait*

Species	Type	Mean body weight (g)	Duration of test (days)	Mean daily bait intake (g)		No. of rats preferring poison	Significance (P) of Student's t	Mortality
				Poison	Plain			
R. norvegicus	Resistant	257	4 (2)*	8·3	9·8	6/20	< 0·2	15/20
	Non-resistant	189	2	8·5	9·0	8/10	> 0·5	10/10
R. rattus	Non-resistant	169	4 (2)*	5·3	5·7	4/10	> 0·5	10/10
M. musculus	Resistant	17	4 (2)*	1·1	1·3	5/20	< 0·5	17/20
	Non-resistant	16	2	1·3	1·2	4/10	< 0·5	2/10

* Figures in parentheses indicate number of days for which data for bait consumption used in calculations.

Table 3. *Bait consumption and mortality of wild rodents given a choice between bromadiolone and either difenacoum or brodifacoum**

Species	Type	Mean body weight (g)	Duration of test (days)	Mean daily bait intake (g)			No. of rats preferring bromad.	Significance (P) of Student's t	Mortality
				Difen.	Bromad.	Brodif.			
R. norvegicus	Resistant	293	4 (2)†	6·0	8·9		13/20	0·05–0·1	20/20
	Resistant	261	4 (2)†		8·7	7·7	8/20	< 0·4	20/20
R. rattus	Non-resistant	154	4 (2)†	6·5	3·5		3/10	< 0·2	10/10
	Non-resistant	166	4 (2)†		4·5	5·3	4/10	> 0·5	10/10
M. musculus	Resistant	15	4 (2)†	1·4	1·6		6/10	> 0·5	8/10
	Resistant	16	4 (2)†		2·1	2·0	7/10	< 0·2	9/10

* Equal numbers of males and females used throughout.

† Figures in parentheses indicate number of days for which data for bait consumption used in calculations.

probit analysis being carried out. Marsh (1977) obtained 95% mortality after a 15-day feeding period. Bromadiolone appears to be similar to difenacoum in its efficacy to *M. musculus*, but both poisons are markedly less active than brodifacoum, the last giving a complete kill after 1 day's feeding (Redfern *et al.* 1976).

Choice feeding tests

The results of choice tests between poisoned and plain baits (Table 2) show that the acceptance of 0·005% bromadiolone by *R. norvegicus*, *R. rattus* and *M. musculus* was good, and in no test was there a significant difference in bait take. Both difenacoum and brodifacoum have previously been shown to be somewhat unpalatable to one or more of these species in the laboratory (Hadler *et al.* 1975; Redfern *et al.* 1976). However, in choice tests between bromadiolone and either difenacoum or brodifacoum (Table 3), no significant discrimination was shown by any species. Marsh (1977) found that all three species appeared unable to detect bromadiolone at 0·005%.

It is concluded that bromadiolone is another very active anticoagulant rodenticide, effective against *R. norvegicus*, *R. rattus* and to a lesser extent *M. musculus*. The results obtained in the laboratory suggest that it would give a similar performance to difenacoum in the field, and would be effective in dealing with warfarin-resistant infestations. The evidence of cross-resistance suggests that there is a likelihood of resistance to bromadiolone developing in the field, a situation that has already occurred with difenacoum (Redfern & Gill, 1978).

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