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Comparative effect of the three rodenticides warfarin, difenacoum and brodifacoum on eight rodent species in short feeding periods

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SUMMARY

Short laboratory feeding tests were carried out with the anticoagulants warfarin, difenacoum, and brodifacoum on a number of European rodent species: *Clethrionomys glareolus*, *Microtus agrestis*, *M. arvalis*, *Apodemus flavicollis*, *A. sylvaticus*, *Mus musculus*, *Rattus rattus* and *R. norvegicus*. It was found that the toxicity to all species was highest with brodifacoum and lowest with warfarin, and that only 0·005% brodifacoum would give a complete mortality in most species after one day's feeding. The potential of this compound for the control of microtine field rodents is suggested.

INTRODUCTION

The synthesis of the two potent anticoagulants difenacoum (3-(3-p-diphenyl-1,2,3,4,tetrahydronaphth-1-yl)-4-hydroxycoumarin) and brodifacoum (3-(3-(4′-bromobiphenyl-4-yl)-1,2,3,4-tetrahydronaphth-1-yl)-4-hydroxycoumarin) has been a great improvement in the efforts to control warfarin-resistant brown rats (*Rattus novegicus*) and house mice (*Mus musculus*) (Redfern, Gill & Hadler, 1976). Recent laboratory tests of brodifacoum and difenacoum against *Mastomys natalensis* have given very promising results (Gill & Redfern, 1979), and preliminary studies indicate that these anticoagulants are highly effective against *Bandicota bengalensis* (Brooks, Htun & Naing, 1980).

One hundred per cent mortality can only be achieved in susceptible house mice after more than 21 days' feeding on 0·025% warfarin, but can be obtained after only one or two days' feeding on 0·005% difenacoum or brodifacoum (Redfern *et al.* 1976). To investigate the potential advantages of these anticoagulants for the control of various other rodent pest species in short feeding periods, a comparative study of the relative toxicity of warfarin, difenacoum and brodifacoum was undertaken.

MATERIALS AND METHODS

All the rodents used were laboratory bred and belonging to the following species: Brown rat (*Rattus norvegicus*) (warfarin-susceptible strain), roof rat (*Rattus rattus*), house mouse (*Mus musculus*) (warfarin-susceptible or warfarin-resistant strain), wood mouse (*Apodemus sylvaticus*), yellow-necked mouse (*Apodemus flavicollis*), continental vole (*Microtus arvalis*), short-tailed field vole (*Microtus agrestis*) and bank vole (*Clethrionomys glareolus*).

In all tests the rodents were singly caged and supplied with water from a bottle on top of the cage. The mice and voles had access to a small bottle with cotton as nest material. After being starved for approximately 24 h the rodents were offered an unrestricted amount of the poison bait as the only food for one, two, or in a few cases, three days. In tests with rats the amount consumed each day was measured, for all the other species the amount consumed was measured when the rodent was transferred to a clean cage at the end of the test. For an observation period of at least two weeks the rodents were fed ordinary laboratory diet. Dead rodents were examined for internal bleeding.

The three anticoagulants were all presented in coarsely ground rolled oats. The warfarin bait was prepared from a technical compound (NDH 469-58) dispersed in wholemeal flour, difenacoum from a 2 % concentrate and brodifacoum from a 0·1 % concentrate thoroughly mixed with the rolled oats. Brodifacoum was, as probably the most potent material, offered at two different concentrations.

RESULTS

Summarized results of the feeding tests with the three anticoagulants are given in tables 1, 2, 3, and 4.

With 0·025 % warfarin (table 1) the highest mortality was obtained in *Microtus agrestis* and *M. arvalis*, *Apodemus sylvaticus* and *R. norvegicus*, but it never exceeded 50 % after two days' feeding. In several cases no mortality was seen after an intake of substantial amounts of warfarin. The two closely related species *A. flavicollis* and *A. sylvaticus* differed considerably in susceptibility.

With 0·005 % difenacoum (table 2) high mortality was obtained in most species after only one day's feeding, if a few individuals with a very low intake are left out. In this study the two *Apodemus* species and *R. norvegicus* were less susceptible than the other rodents. Warfarin-resistant house mice were not affected after one day's feeding, but showed a mortality of 80 % after two days.

Brodifacoum at the lowest concentration (0·0005 %) (table 3) was very close in its effect to 0·005 % difenacoum except for warfarin-resistant house mice, where no mortality was obtained after two days' feeding. In the three microtine species a complete mortality was obtained with the lowest concentration of brodifacoum.

However, 0·005 % brodifacoum (table 4) was significantly better than the others, giving a complete, or almost complete, kill in all species after one as well as after two days' feeding.

For difenacoum and brodifacoum the day of death may be very much delayed (up to 27 days with 0·005 % brodifacoum in warfarin-resistant house mice), but in all cases typical signs of anticoagulant poisoning were found at the autopsy.

DISCUSSION

In the present study brodifacoum at 0·005 % was the only compound showing a very uniform, high toxicity to all species tested, and the only anticoagulant which could be called a single-dose rodenticide.

Such laboratory findings cannot always be transferred to the field situation as has recently been demonstrated by Rowe, Swinney & Plant (1978) in house mice

Table 1. *The effect of* 0·025 % *warfarin on various rodent species*

Species	Sex M/F	Mean weight (g)	Feeding period (days)	Mortality (%)	Average no. of days to death (range)	Average amount consumed (range) mg/kg	
						lethal	non-lethal
Clethrionomys glar.	6/4	19·5	1	0	—	—	30·7 (23·8–42·5)
	7/3	20·6	2	0	—	—	69·5 (54·2–113·8)
Microtus agrestis	4/6	36·4	1	10	3	2·8	25·5 (11·9–35·9)
	6/4	25·7	2	50	7·2 (3–10)	57·4 (19·0–94·2)	55·1 (27·7–85·6)
Microtus arvalis	5/5	21·7	1	10	5	26·3	36·1 (15·6–48·9)
	5/5	21·8	2	30	3·7 (3–4)	47·4 (12·9–77·7)	71·5 (56·9–91·7)
Apodemus flavicollis	10/0	34·9	1	0	—	—	44·8 (25·0–51·3)
	6/4	29·5	2	0	—	—	88·7 (43·6–125·0)
Apodemus sylvaticus	5/5	25·6	1	10	11	17·5	23·6 (14·4–35·7)
	5/5	23·3	2	40	5·3 (3–8)	41·3 (23·2–61·1)	51·3 (33·8–62·5)
Mus musculus	5/5	20·2	1	0	—	—	56·2 (41·7–69·4)
(warfarin sensitive)	5/5	19·1	2	0	—	—	101·1 (56·9–134·7)
Mus musculus	10/0	20·4	3	0	—	—	82·9 (57·9–109·1)
(warfarin resistant)							
Rattus rattus	4/6	188·7	1	0	—	—	11·8 (4·9–18·2)
	5/5	230·0	2	0	—	—	16·7 (7·0–41·2)
	5/5	206·7	3	30	4·7 (3–6)	66·4 (48·9–90·0)	58·6 (38·0–78·2)
Rattus norvegicus	8/2	304·1	1	10	6	14·9	11·2 (7·1–14·3)
	7/3	345·0	2	40	6·3 (5–8)	21·8 (14·8–25·4)	19·4 (7·3–29·2)

Table 2. *The effect of* 0·005 % *difenacoum on various rodent species*

Species	Sex M/F	Mean weight (g)	Feeding period (days)	Mortality (%)	Average no. of days to death (range)	Average amount consumed (range) mg/kg	
						lethal	non-lethal
Clethrionomys glar.	12/8	20·6	1	100	5·9 (2–10)	8·1 (2·8–18·7)	—
	12/8	20·0	2	100	5·3 (2–10)	17·8 (2·0–32·8)	—
Microtus agrestis	5/5	39·5	1	90	4·6 (3–7)	6·8 (4·5–10·8)	4·8
	9/11	33·4	2	95	5·6 (3–10)	10·4 (7·9–19·2)	0
Microtus arvalis	5/5	21·5	1	90	5·0 (3–6)	6·5 (5·0–9·8)	0·01
	5/5	20·9	2	100	4·5 (2–7)	12·9 (0·4–35·0)	—
Apodemus flavicollis	8/2	38·4	1	20	8·5 (4–13)	5·9 (3·6–8·3)	4·8 (0·5–8·7)
	15/15	33·2	2	67	6·0 (4–10)	11·7 (0·8–21·8)	10·5 (7·5–12·1)
Apodemus sylvaticus	5/5	22·3	1	10	6	5·5	7·6 (5·5–10·8)
	4/6	24·0	2	60	5·5 (3–8)	12·2 (9·8–18·2)	8·6 (5·5–10·8)
Mus musculus	10/5	19·6	1	87	7·4 (4–12)	9·4 (2·8–14·6)	13·2 (4·3–22·1)
(warfarin sensitive)	29/6	20·0	2	97	6·4 (5–17)	18·1 (7·9–23·7)	13·6
Mus musculus	10/0	19·0	1	0	—	—	2·2 (0·3–5·0)
(warfarin resistant)	10/0	19·4	2	80	9·5 (6–13)	17·6 (12·8–22·4)	12·5 (10·0–14·4)
Rattus rattus	7/3	225·0	1	70	7·9 (6–12)	2·1 (1·0–3·6)	1·4 (0·5–2·8)
	6/6	211·0	2	100	8·0 (5–12)	5·6 (1·9–9·2)	—
Rattus norvegicus	5/5	288·5	1	20	4	2·9 (2·8–3·0)	0·9 (0·4–2·0)
	5/5	310·5	2	90	6·9 (4–11)	4·4 (0·8–8·6)	0·3

Table 3. *The effect of* 0·0005 % *brodifacoum on various rodent species*

Species	Sex M/F	Mean weight (g)	Feeding period (days)	Mortality (%)	Average no. of days to death (range)	Average amount consumed (range) mg/kg	
						lethal	non-lethal
Clethrionomys glar.	6/4	19·0	1	100	5·9 (2–10)	0·7 (0·3–1·0)	—
	5/5	18·6	2	100	4·9 (1–8)	1·3 (0·1–1·8)	—
Microtus agrestis	4/6	27·5	1	100	4·8 (3–7)	0·7 (0·4–0·9)	—
	4/6	25·8	2	100	4·7 (3–8)	1·4 (0·3–2·0)	—
Microtus arvalis	10/0	19·0	1	100	4·0 (2–7)	0·5 (0·1–0·9)	—
	10/0	21·0	2	100	5·2 (2–8)	0·8 (0·2–1·9)	—
Apodemus flavicollis	5/5	19·6	1	20	5·5 (5–6)	1·0 (0·9–1·1)	0·9 (0·7–1·2)
	5/5	21·9	2	80	6·1 (4–11)	1·7 (1·2–2·1)	2·1 (1·9–2·4)
Apodemus sylvaticus	5/5	24·2	1	100	7·9 (5–9)	0·6 (0·5–0·8)	—
	4/6	25·1	2	90	5·7 (2–8)	1·2 (0·9–1·6)	1·1
Mus musculus	7/3	16·2	1	50	7·0 (6–10)	0·8 (0·5–1·0)	0·9 (0·75–0·96)
(warfarin sensitive)	8/2	19·0	2	100	9·1 (5–18)	1·4 (1·1–1·6)	—
Mus musculus	10/0	20·1	1	0	—	—	0·5 (0·3–0·7)
(warfarin resistant)	10/0	19·6	2	0	—	—	0·8 (0·6–1·0)
Rattus rattus	5/5	194·2	1	20	11·0 (8–14)	0·3 (0·1–0·4)	0·4 (0·3–0·4)
	6/4	188·2	2	60	8·5 (5–11)	0·8 (0·3–1·2)	0·3 (0·2–0·4)
Rattus norvegicus	7/3	291·6	1	50	8·8 (6–10)	0·3 (0·25–0·34)	0·3 (0·20–0·31)
	7/3	373·4	2	80	7·1 (5–12)	0·5 (0·3–0·7)	0·2 (0·19–0·21)

Table 4. *The effect of* 0·005 % *brodifacoum on various rodent species*

Species	Sex M/F	Mean weight (g)	Feeding period (days)	Mortality (%)	Average no. of days to death (range)	Average amount consumed (range) mg/kg	
						lethal	non-lethal
Clethrionomys glar.	5/5	18·5	1	100	4·8 (3–8)	9·9 (6·6–11·5)	—
	5/5	20·1	2	100	5·0 (2–11)	18·8 (2·3–21·6)	—
Microtus arvalis	10/0	23·4	1	100	4·5 (3–6)	4·6 (0·9–8·6)	—
	10/0	23·0	2	100	5·6 (3–9)	10·8 (3·5–12·8)	—
Apodemus flavicollis	13/7	25·1	1	100	6·7 (4–10)	8·2 (4·7–14·0)	—
	9/11	28·7	2	100	6·3 (4–11)	13·6 (4·1–20·3)	—
Apodemus sylvaticus	5/5	27·6	1	100	7·9 (4–15)	5·4 (3·0–8·1)	—
	6/4	26·1	2	100	6·0 (2–10)	10·9 (4·0–14·8)	—
Mus musculus	8/2	18·5	1	100	6·6 (4–11)	6·9 (2·2–8·8)	—
(warfarin sensitive)	8/2	17·3	2	100	6·1 (3–9)	14·6 (11·4–19·2)	—
Mus musculus	10/0	21·9	1	90	8·9 (6–15)	4·6 (2·1–7·2)	1·1
(warfarin resistant)	10/0	19·3	2	90	8·8 (5–17)	21·2 (14·2–27·1)	32·9
	10/0	20·7	3	100	11·4 (6–27)	12·6 (7·6–19·3)	—
Rattus rattus	7/3	226·0	1	100	7·1 (6–9)	2·0 (0·6–3·1)	—
	5/5	174·9	2	100	8·7 (3–13)	7·5 (5·5–9·8)	—
Rattus norvegicus	5/5	392·0	1	100	5·1 (4–7)	2·3 (1·0–3·6)	—
	8/8	337·5	2	94	7·1 (5–9)	3·5 (0·7–5·7)	0·6

and by Rennison & Dubock (1978) in brown rats, due to the behaviour and the social structure of the populations.

The results obtained with microtine rodents may be of particular interest, as these species generally prefer herbs to seeds and grain. The most acceptable bait material seems to be apple or carrot slices, and as such bait deteriorates very rapidly, it is important that the rodents can be killed after a single intake or two. This could theoretically be achieved by using a 0·0005% concentration of brodifacoum.

REFERENCES

BROOKS, J. E., HTUN, P. T. & NAING, H. (1980). The susceptibility of *Bandicota bengalensis* from Rangoon, Burma, to several anticoagulant rodenticides. *Journal of Hygiene* **84**, 127–35.

GILL, J. E. & REDFERN, R. (1979). Laboratory tests of seven rodenticides for the control of *Mastomys natalensis*. *Journal of Hygiene* **83**, 345–52.

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**, 419–26.

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.

ROWE, F. P., SWINNEY, T. & PLANT, C. (1978). Field trials of brodifacoum (WBA 8119) against the house mouse (*Mus musculus* L.). *Journal of Hygiene* **81**, 197–201.