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Laboratory evaluation of WBA 8119 as a rodenticide for use against warfarin-resistant and non-resistant rats and mice

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SUMMARY

Feeding tests were carried out in the laboratory to evaluate WBA 8119 as a potential new rodenticide against wild common rats (*Rattus norvegicus*), ship rats (*R. rattus*) and house mice (*Mus musculus*). The results obtained are compared with data previously obtained for difenacoum, another member of the same series of 4-hydroxycoumarin anticoagulants.

With warfarin-resistant and non-resistant common rats, complete kills were obtained using a concentration of 0·0005% for 2 days, or 0·001% for 1 day: a 1-day test at 0·0005% killed 6 out of 10 and 17 out of 20 of the two types respectively. At 0·005% complete kills of resistant ship rats were obtained after 2 days exposure and of resistant house mice after 1 day, but at 0·002% for 2 days there was some survival. Non-resistant ship rats and house mice were all killed after 2 days feeding on 0·002% bait.

In 2-day palatability tests, *R. norvegicus* showed no significant aversion to the poison at 0·002% and 100% mortality was obtained. The poison was significantly unpalatable to *R. rattus* at 0·005% and to *M. musculus* at 0·005% and 0·002%, although with the last species these concentrations gave complete kills.

It is concluded that WBA 8119 has greater activity than other known anticoagulants against the three commensal species examined. The laboratory results suggest that concentrations between 0·0005% and 0·002% would be suitable for field use against common rats, and between 0·002% and 0·005% for ship rats and house mice.

INTRODUCTION

The difficulty of controlling warfarin-resistant infestations of *R. norvegicus* was eased considerably by the development of difenacoum, one of a series of novel 4-hydroxycoumarin derivatives with high anticoagulant activity (Hadler & Shadbolt, 1975). Although difenacoum was shown to be very effective against *R. norvegicus*, results with *R. rattus* and *M. musculus* were less favourable (Hadler, Redfern & Rowe, 1975; Rennison & Hadler, 1975).

The present paper describes the laboratory evaluation of another member of the series, 3-(3-[4′-bromobiphenyl-4-yl]-1,2,3,4-tetrahydronaphth-1-yl)-4-hydroxycoumarin, for which the British Standards Institution proposed common name is 'bromfenacoum'. The compound is not yet commercially available.

The initial toxicological studies on laboratory rats and mice were carried out by Sorex (London) Ltd, and the work with wild rodents by P.I.C.L.

METHODS

Oral toxicity to laboratory strains of rodents

Determinations of the acute (single dose) and chronic (5 consecutive daily doses) oral toxicities were performed on adult Wistar rats and LAC Grey mice. The compound, dissolved in polyethylene glycol 300, was administered by intubation to non-starved animals at a rate of 0·1 ml/100 g body weight. The animals were maintained on FFG(M) pellets (E. Dixon (Ware) Ltd) and water *ad lib.* throughout the test, and were observed for 21 days after intubation.

Laboratory feeding tests on wild rodents

Feeding tests were carried out on individually caged *R. norvegicus*, *R. rattus* and *M. musculus*, using both warfarin-resistant and non-resistant representatives of each species. All *R. norvegicus* were caught in the wild: non-resistant individuals were obtained from a Midlands refuse destructor with no history of resistance, while resistant rats were trapped in central Wales and subsequently subjected to a 6-day feeding test on 0·005% warfarin bait (Drummond & Wilson, 1968). A few *R. rattus* and *M. musculus* were wild-caught, but the majority were first generation laboratory-bred descendants of wild resistant or non-resistant parents. *R. rattus* were classified as resistant if they had survived a 28-day feeding test on 0·025% warfarin bait (Greaves, Rennison & Redfern, 1973), or non-resistant if they were derived from ports where resistance to warfarin had not been found. The criterion for resistance in *M. musculus* was survival of a 21-day feeding test on 0·025% warfarin bait (Rowe & Redfern, 1965).

Before testing, all animals were maintained on diet 41B (Oxoid Ltd, London) and water *ad lib.* Individuals were weighed before the start of a test.

Two types of feeding experiment were carried out, 'no-choice' and 'choice' tests. In the former the unpoisoned bait-base was given for several days until the rodents were feeding freely on it, at which time it was replaced by the toxic bait. In 2-day tests fresh bait was given daily. In choice tests, food pots containing the bait-base with and without the toxicant were placed symmetrically in the cages. After 24 hr exposure the positions of the two foods were interchanged and fresh baits and clean pots given. The contents of food pots were weighed daily, and the intake of bait recorded. At the end of a test, animals were given diet 41B again and observed for 3 weeks. Days to death were recorded, and some animals autopsied.

The poison was presented in a bait-base consisting of pinhead oatmeal (90%)

Table 1. *Results of oral intubation tests with laboratory rodents*

Type of animal	Dose (mg/kg)	Mortality	Days to death
(*a*) Acute (single dose)			
Wistar rat (male)	5·0	5/5	4–5
	2·0	5/5	4–6
	1·0	10/10	3–9
	0·5	10/10	4–6
	0·2	1/10	8
	0·1	0/10	—
LAC Grey mouse (male)	2·0	10/10	4–9
	1·0	10/10	4–12
	0·5	7/10	7–10
	0·2	0/10	—
	0·1	0/10	—
(*b*) Chronic (5 daily doses)			
Wistar rat (male)	0·2	10/10	5–11
	0·1	10/10	6–11
	0·05	2/10	9
	0·02	0/10	—
	0·01	0/5	—
Wistar rat (female)	0·5	5/5	5–7
	0·2	5/5	6–9
	0·1	0/5	—
	0·05	1/5*	3
	0·02	0/5	—
	0·01	0/5	—

* Death probably due to faulty technique.

and corn oil (5%), to which was added a 'master-mix' (5%), consisting of the finely divided active ingredient dispersed in wholemeal flour.

RESULTS AND DISCUSSION

Oral toxicity to laboratory strains

The results of the oral intubation experiments are summarized in Table 1(*a*) and (*b*). The acute LD 50 values for male Wistar rats and male LAC Grey mice, obtained by best line of fit, are 0·26 mg/kg (95% confidence limits 0·20–0·37) and 0·40 mg/kg (0·30–0·63) respectively. Corresponding figures for difenacoum are 1·3 mg/kg (0·9–1·7) and 0·8 mg/kg (0·3–1·8) (M. R. Hadler, unpublished).

The chronic LD 50 for male Wistar rats was 0·06 mg/kg/day × 5 (95% confidence limits 0·04–0·08) compared with 0·18 mg/kg/day × 5 (0·13–0·23) for difenacoum.

Toxicity to wild rodents

Initial no-choice feeding tests were done using the active ingredient at 0·002% for *R. norvegicus* and 0·005% for *R. rattus* and *M. musculus*; later tests were done at lower concentrations. Feeding periods of 1 and 2 days were used.

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Table 2. *Mortality and bait consumption of wild rodents given a sole diet of WBA 8119 in pinhead oatmeal/corn-oil bait*

Sex	Mean body weight (g)	Concentration of poison (%)	No. of days	Mortality	Mean daily bait intake (g)		Lethal dose of active ingredient (mg/kg)		Survived dose of active ingredient (mg/kg)		Days to death	
					Prebait	Poison	Mean	Range	Mean	Range	Mean	Range
					Rattus norvegicus Warfarin-resistant							
M	281	0·002	2	10/10	15·1	16·9	2·2	1·2–2·8	—	—	5·3	4–6
F	220	0·002		10/10	12·6	14·3	2·6	1·6–4·1	—	—	6·1	4–11
M	275	0·001	2	10/10	14·5	14·0	1·2	0·9–1·5	—	—	6·4	4–10
F	191	0·001		10/10	12·5	13·9	1·6	1·0–3·3	—	—	5·3	3–9
F	190	0·001	1	10/10	12·7	11·5	0·7	0·4–1·0	—	—	5·3	4–9
M	236	0·0005	2	4/4	16·4	15·2	0·7	0·4–0·8	—	—	6·0	6
F	210	0·0005		5/5	13·6	12·9	0·6	0·4–0·8	—	—	7·0	4–11
M	375	0·0005	1	3/5	20·7	19·9	0·3	0·02–0·4	0·2	0·01–0·3	8·3	7–10
F	255	0·0005		3/5	11·6	14·9	0·3	0·3	0·3	0·3	7·0	6–8
					Non-resistant							
M	272	0·01	1	5/5	18·8	18·1	6·8	5·7–8·3	—	—	3·2	2–6
F	155	0·01		5/5	11·0	12·7	8·2	6·3–9·8	—	—	3·1	1–6
M	269	0·002	2	10/10	16·0	14·3	2·3	2·0–2·6	—	—	6·3	5–9
F	199	0·002		10/10	12·7	13·5	2·8	1·9–4·1	—	—	7·2	6–10
M	286	0·001	2	10/10	16·8	17·0	1·2	1·0–1·4	—	—	6·6	5–12
F	235	0·001		10/10	13·2	14·7	1·3	0·9–1·8	—	—	6·0	4–8
M	178	0·0005	2	10/10	14·0	14·7	0·8	0·6–1·0	—	—	6·1	5–7
F	207	0·0005		10/10	16·4	14·6	0·7	0·6–0·9	—	—	7·2	6–10
M	197	0·0005	1	8/10	12·7	16·7	0·5	0·2–0·9	0·3	0·2–0·3	7·2	5–10
F	177	0·0005		9/10	13·1	15·2	0·5	0·3–0·6	0·3	0·3	5·6	4–7

Table 2. (*cont.*)

Sex	Mean body weight (g)	Concentration of poison (%)	No. of days	Mortality	Mean daily bait intake (g)		Lethal dose of active ingredient (mg/kg)		Survived dose of active ingredient (mg/kg)		Days to death	
					Prebait	Poison	Mean	Range	Mean	Range	Mean	Range
						Rattus rattus						
						Warfarin-resistant						
M	137	0·005	2	5/5	7·5	6·7	5·7	4·8–6·6	—	—	9·6	6–14
M	138	0·002		3/3	8·8	10·7	2·6	1·5–4·0	—	—	8·3	5–13
F	106	0·002		1/2	10·8	8·4	4·3	—	2·5	—	11·0	—
						Non-resistant						
M	122	0·005	2	10/10	10·2	10·3	9·2	6·5–14·1	—	—	8·7	6–12
F	130	0·005		10/10	8·8	7·2	6·2	2·6–16·6	—	—	8·7	5–12
M	167	0·002	2	5/5	10·8	11·7	2·6	2·0–3·6	—	—	7·5	6–10
F	129	0·002		5/5	10·1	10·8	3·1	2·3–4·3	—	—	11·8	9–17
						Mus musculus						
						Warfarin-resistant						
M	17	0·005	2	5/5	3·5	3·6	24·7	20·0–29·1	—	—	7·0	5–13
F	15	0·005		5/5	2·7	2·8	19·6	13·4–24·1	—	—	8·4	4–12
M	11	0·005	1	10/10	3·7	3.2	9·6	7·4–13·8	—	—	8·5	4–20
F	13	0·005		10/10	3·0	2·7	10·6	7·0–14·2	—	—	7·8	4–19
M	16	0·002	2	10/10	3·2	3·1	7·0	5·4–8·2	—	—	8·1	2–13
F	13	0·002		9/10	2·8	2·8	8·6	5·2–12·9	12·9	—	8·4	3–18
M	17	0·002	1	9/10	2·7	2·9	3·5	2·4–5·3	3·7	—	8·4	5–13
F	13	0·002		10/10	2·7	2·7	4·4	3·4–5·2	—	—	8·2	5–14

Table 3. *Bait consumption and mortality of wild rodents given a choice between plain and poisoned baits for 2 days*

Type of animal	Mean body weight (g)	Concentration of poison (%)	Mean daily bait intake (g)		Significance (P) of Student's t	Mortality
			Poison	Plain		
Rattus norvegicus						
Warfarin-resistant	215	0·002	8·0	12·6	> 0·1	5/5
	188	0·001	6·1	7·3	> 0·5	5/5
	314	0·0005	7·5	11·1	> 0·5	2/5
Non-resistant	233	0·002	8·7	9·7	0·6–0·5	10/10
	207	0·001	9·0	9·1	> 0·9	10/10
	220	0·0005	9·5	8·5	0·6–0·5	10/10
Rattus rattus						
Non-resistant	124	0·005	4·0	6·5	0·05–0·02	16/20
	112	0·002	5·1	6·0	0·4–0·3	9/10
Mus musculus						
Warfarin-resistant	17	0·005	1·3	2·1	0·02–0·01	10/10
	15	0·002	1·3	1·9	0·05–0·02	10/10

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The results (Table 2) show that 2-day tests at the initial concentrations gave complete mortality in resistant animals of all three species. In comparison, difenacoum at 0·005 % (in the same bait-base) gave complete kills with non-resistant *R. norvegicus*, but with resistant individuals kills of 9/10, 5/10 and 9/10 were obtained with *R. norvegicus*, *R. rattus* and *M. musculus* respectively (Hadler *et al.* 1975). With resistant and non-resistant *R. norvegicus* complete kills were obtained with the concentration of poison reduced to 0·001 % (for 2 or 1 day). With a concentration as low as 0·0005 %, a complete kill of both types of rat was again obtained in 2 days, but with a 1-day exposure the mortality was 6/10 and 17/20 with resistant and non-resistant groups respectively. The time to death tended to increase as the concentration of poison was lowered.

To investigate the feasibility of using WBA 8119 as an acute poison against *R. norvegicus*, a no-choice test was carried out with a concentration of 0·01 % active ingredient for 1 day. The complete kill obtained and the almost identical mean daily bait intake of prebait and poisoned bait suggest that this regimen might be worth investigating for field use.

Because of the shortage of *R. rattus* the numbers allocated to each test were unavoidably small. With resistant and non-resistant ship rats complete kills were obtained in 2-day tests at a concentration of 0·005 %: at 0·002 % one resistant rat out of five survived, compared with a 10/10 kill with non-resistant animals. Difenacoum at 0·005 % for 2 days gave kills of 10/10 and 5/10 for non-resistant and resistant ship rats respectively (Hadler *et al.* 1975). The time to death of resistant ship rats was very similar with both poisons (at 0·005 %), but with non-resistant animals difenacoum (mean 6·1; range 4–8 days) was significantly more rapid ($t = 3{\cdot}31$, $P = < 0{\cdot}005$) than WBA 8119 (mean 8·7; range 5–12 days). WBA 8119 was more toxic to resistant *M. musculus* than difenacoum, with a 1-day exposure to 0·005 % giving complete mortality: difenacoum at this concentration, but for 2 days, gave kills of 9/10 and 7/10 for resistant and non-resistant mice respectively. With WBA 8119 at 0·002 %, kills of 19/20 resistant mice were obtained with 1- and 2-day tests.

The higher toxicity of WBA 8119 compared with that of difenacoum found in the present study confirms the findings of Hadler & Shadbolt (1975), who give values for prothrombin ED 50's (the amount of active ingredient given in three daily doses required to increase the prothrombin time from a resting value of 16 sec. to 112 sec. on the fourth day) of 0·10 and 0·32 mg/kg for WBA 8119 and difenacoum respectively in the resistant HW strain of rat. The comparable ED 50 for warfarin is given as > 50 mg/kg. Resistance indices (called 'resistance factors' by Hadler & Shadbolt, and defined as the ED 50 for resistant rats divided by that for normal rats) for the two compounds are given as 1·2 and 1·9 respectively, compared with values of > 15 and > 166 for the R(+) and S(−) isomers of warfarin. In 10-day no-choice feeding tests with HW rats, the same authors obtained kills of 80 % and 70 % with 0·0002 % WBA 8119 and 0·001 % difenacoum respectively.

Palatability of WBA 8119 to wild rodents

The results of 2-day choice tests are summarized in Table 3. With *R. norvegicus* there was no significant unpalatability at 0·002 %, the highest concentration tested; complete mortality was obtained at 0·001 %, and also at 0·0005 % with non-resistant rats. WBA 8119 was significantly unpalatable to *M. musculus* at 0·005 % ($P = < 0{\cdot}02$) and 0·002 % ($P = < 0{\cdot}05$) although complete kills were obtained in both tests. Although these findings suggest that 0·001 % would be optimal for the control of *R. norvegicus* in the field, and 0·002 % for *M. musculus*, it is possible that if populations were feeding incompletely from the poisoned bait, the higher concentration would be necessary. Hadler *et al.* (1975) found that difenacoum at the recommended concentration of 0·005 % was significantly unpalatable to resistant and non-resistant *R. norvegicus*, but palatable to *M. musculus*. WBA 8119 at 0·005 % was significantly unpalatable to non-resistant *R. rattus*, but at 0·002 % there was no significant preference. At the first concentration a kill of 16/20 was obtained. Hadler *et al.* (1975) showed that difenacoum was significantly unpalatable at 0·005 %, and that with resistant rats the mortality was 4/10 (but in a test period of 4 days instead of 2 in the present study).

The figures for 'mean daily bait intake' (Table 2) show that at the various concentrations used, and with all three species, the amounts of prebait and poisoned bait eaten were very similar, indicating that the compound is sufficiently palatable to avoid a fall-off in food consumption when the poison is first presented.

WBA 8119 appears to be a typical indirect anticoagulant, acting in the same manner as, for example, warfarin, and is therefore antidoted by vitamin K_1 (M. R. Hadler, unpublished).

The laboratory results indicate that in the field the optimum concentration of WBA 8119 would fall between 0·0005 % and 0·002 % for *R. norvegicus*, and between 0·002 % and 0·005 % for *R. rattus* and *M. musculus*. The compound has the advantage of being more effective than other anticoagulants, including difenacoum against *R. rattus* and *M. musculus*, and it is probable that it would be valuable for *R. norvegicus* should resistance to difenacoum develop in this species.

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