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The susceptibility of *Bandicota bengalensis* from Rangoon, Burma to several anticoagulant rodenticides*

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SUMMARY

The baseline susceptibility of the lesser bandicoot rat, *Bandicota bengalensis*, from Rangoon, Burma, to five anticoagulant rodenticides was established with no-choice feeding in the laboratory. The susceptibility of lesser bandicoots to the several poisons (brodifacoum, difenacoum, diphacinone, coumatetralyl, and warfarin) was such that they were offered at a 0·001 % concentration. *B. bengalensis* was most susceptible to brodifacoum, and in descending order, difenacoum, coumatetralyl, diphacinone and warfarin. In comparison with *Rattus norvegicus* on warfarin at 0·005 %, *B. bengalensis* proved more susceptible. Feeding tests at 0·005 % concentration indicated that a 1-day feeding on brodifacoum and difenacoum would result in complete mortality, whereas coumatetralyl and warfarin would require 4 days feeding to a 100 % kill. Brodifacoum and difenacoum are recommended at 0·002–0·005 % bait concentrations and coumatetralyl at 0·005–0·01 % concentrations for the control of *B. bengalensis* in the field in Rangoon. The use of any anticoagulant material in rat control should be alternated with acute toxicants to retard the possible development of anticoagulant resistance.

INTRODUCTION

The lesser bandicoot rat, *Bandicota bengalensis*, is the most common and ubiquitous commensal small mammal throughout urban and suburban environments in Rangoon, Burma. Even though it is a large ground-living rat with well-developed burrowing habits, it also readily enters households, shops, bazaars, grain-storage warehouses, and numerous other man-made structures. In Rangoon, this species has been found naturally infected with plague (Brooks *et al.* 1977) and carries large numbers of fleas of the genus *Xenopsylla* on its body (Walton & Tun, 1978). Its burrowing activities are extensive and damaging to building foundations, sidewalks and storm-water drains. Because of its potential for spreading human disease and causing extensive damage and economic losses, control of this species is especially desirable.

Very little has been published on the susceptibility of *B. bengalensis* to anticoagulant rodenticides. Deoras (1964, 1965, 1967) reported on the susceptibility

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to warfarin of *Bandicota* from Bombay but omitted important details of methods and results, thus making it difficult to assess his conclusions. In a later paper Deoras *et al.* (1972) indicated that *B. bengalensis* was highly susceptible to warfarin. In contrast, Renapurkar *et al.* (1973) reported that this species showed considerable tolerance to a warfarin diet but, as observed by Greaves & Rehman (1977), this was most likely due to low warfarin dosage rather than to physiological tolerance. Greaves & Rehman (1977) found that *B. bengalensis* from Lower Sind, Pakistan, was less susceptible to warfarin than suggested by the earlier reports. They also gave some data on susceptibility of lesser bandicoot rats to difenacoum.

Because of this paucity of information and the somewhat contradictory observations, the Rodent Control Demonstration Unit of the World Health Organization, in cooperation with the Ministry of Health of the Socialist Republic of the Union of Burma, undertook studies of the susceptibility of certain candidate anticoagulant rodenticides against *B. bengalensis* from Rangoon, Burma.

MATERIALS AND METHODS

The rats

Lesser bandicoot rats, *B. bengalensis*, were trapped alive from residential and commercial areas in Rangoon. In the laboratory they were anaesthetized with chloroform and combed for ectoparasites, or were simply dusted with insecticide, and then sexed, weighed and measured. They were individually housed in cages measuring approximately $15 \times 20 \times 30$ cm. Food was provided in glass cups secured at the front of each cage. Water was available *ad lib*. Most animals were held a minimum of 3 weeks in order to ensure thay were in good health and to acclimate them to cage conditions and the maintenance diet. Animals tested ranged in weight from 109 to 542 g. Males and non-pregnant females only were used in the tests.

Diet

The bandicoot were maintained and tested on a locally milled diet consisting of 10 parts dried whole fish meal, 17 parts pressed peanut meal, 42 parts crushed rice, 15 parts corn meal, 15 parts rice bran and one part oyster shell. Rats fed quite well on this diet, ensuring that they received an adequate daily voluntary dosage of the several anticoagulant rodenticides.

Anticoagulant rodenticides

The several anticoagulant rodenticides either were supplied as technical products or as commercial concentrates by the manufacturers. Brodifacoum was supplied as the technical material, a white granular powder of 94 % purity. The difenacoum used was a 0·1 % concentrate of experimental material and was a light greyish powder. Diphacinone was used as the commercially available 0·1 % concentrate and was a white powder. Warfarin was a white crystalline technical powder of 98 % active ingredient of the special WARF Lot 53 as used by Brooks & Bowerman (1974) and Jackson *et al.* 1975 in their anticoagulant resistance studies in the

United States. Coumatetralyl was supplied as the technical material and was a pure white powder. All anticoagulants were mixed dry by hand into the maintenance diet as described above to give the appropriate concentrations used in the tests.

Testing procedure

Rats were preconditioned to the test diet from the time they were first caged, since the maintenance diet and test diet were the same. Daily food consumption was measured for 2 days before the test to ensure that all animals were feeding normally. Animals not showing normal daily food consumption for their body weight were excluded.

The anticoagulant diet was then offered no-choice for periods ranging from 1 to 6 days to groups of rats (WHO, 1970). Spillage from food cups was caught on papers placed below each cage and the wastage was accounted for in computing daily consumption. The post-test observations covered a 15-day period after the last day of feeding on the anticoagulant. Animal condition was noted daily and the first signs of haemorrhage were recorded.

Animals were weighed at the end of the 2-day pre-test period. This initial weight was used in computing the dosages of toxicant consumed by each animal. A terminal weight was determined only on animals dying during the trial period. The day of death was noted and all dead animals were autopsied to verify anticoagulant effects. Dose–response curves were calculated using the method described by Litchfield & Wilcoxon (1949).

RESULTS

Mortality

The results of free-feeding (no-choice) by *B. bengalensis* upon several anticoagulant rodenticides are given in Table 1. In order to directly compare relative response to the several materials, all were offered at 0·001 % concentration in the diet. The response to the several materials varied considerably. Whereas complete mortality was obtained with 4 days feeding on brodifacoum and difenacoum, even 6 days feeding did not suffice to obtain a complete kill using diphacinone or warfarin. Coumatetralyl was found to be considerably more toxic than either warfarin or diphacinone.

When the concentration of anticoagulant in the diet was increased to 0·005 % by weight, the results as given in Table 2 were observed. The superiority of brodifacoum and difenacoum over coumatetralyl and warfarin is quite significant; both the former gave complete kills on 1-day feeding (no-choice) while 4 days were required to obtain 100 % mortality with coumatetralyl and warfarin. The sex difference in mortality was not significant with any material.

Speed of rodenticidal action

The speed of rodenticidal action was measured by noting the day on which rats ate less than half the quantity eaten on day 1. At 0·001 % concentration little

Table 1. *Mortality in* B. bengalensis *when offered baits containing anticoagulants at* 0·001 % *concentrations*

Anticoagulant	Feeding period (days)	Sex	Weight (g) (mean ± S.E.)	Mortality	Anticoagulant consumed mg/kg (mean ± S.E.) Dead	Survived	Mean day of death
Brodifacoum	1	M	340·3 ± 23·8	5/16	0·6 ± 0·06	0·6 ± 0·05	5·8
		F	308·4 ± 13·0	8/16	0·7 ± 0·03	0·6 ± 0·05	5·4
	2	M	287·3 ± 33·6	9/10	1·3 ± 0·1	1·3	5·0
		F	261·3 ± 16·5	9/10	1·3 ± 0·08	0·9	5·9
	3	M	419·0 ± 28·1	9/9	1·6 ± 0·1	—	6·3
		F	308·6 ± 19·9	9/10	1·8 ± 0·1	1·3	7·5
	4	M	—	—	—	—	—
		F	260·6 ± 22·9	11/11	2·7 ± 0·2		7·0
Difenacoum	1	M	271·2 ± 29·8	3/11	1·2 ± 0·2	0·8 ± 0·1	7·3
		F	339·4 ± 9·5	3/11	0·7 ± 0·1	0·6 ± 0·03	8·3
	2	M	304·0 ± 35·2	9/11	1·6 ± 0·1	1·0 ± 0·2	7·2
		F	273·3 ± 26·5	11/13	1·6 ± 0·1	1·2 ± 0·04	6·1
	3	M	370·2 ± 32·1	10/10	1·8 ± 0·1	—	6·6
		F	304·6 ± 29·8	9/11	1·9 ± 0·2	1·3 ± 0·02	7·5
	4	M	348·3 ± 44·3	9/9	2·4 ± 0·2	—	7·1
		F	250·1 ± 26·9	10/10	2·2 ± 0·2	—	8·3
Coumatetralyl	1	M	348·2 ± 36·0	0/10	—	0·7 ± 0·04	—
		F	333·5 ± 23·4	0/11	—	0·5 ± 0·04	—
	2	M	305·9 ± 26·8	12/15	1·4 ± 0·1	1·3 ± 0·1	6·8
		F	296·2 ± 21·4	5/12	1·2 ± 0·06	1·1 ± 0·08	6·4
	3	M	361·3 ± 19·0	9/10	1·9 ± 0·1	1·4 ± —	6·4
		F	359·6 ± 17·8	8/10	1·6 ± 0·1	1·5	7·1
	4	M	314·7 ± 34·0	10/10	2·7 ± 0·2	—	6·8
		F	279·0 ± 13·9	9/10	2·0 ± 0·1	1·8 —	7·9
Diphacinone	2	M	367·2 ± 32·5	1/10	1·1	1·2 ± 0·1	8·0
		F	297·7 ± 24·9	4/12	1·2 ± 0·3	1·2 ± 0·2	6·7
	3	M	333·1 ± 19·9	5/9	2·0 ± 0·1	1·6 ± 0·1	9·4
		F	293·4 ± 11·9	8/10	1·8 ± 0·1	1·5 ± 0·06	7·0
	4	M	326·5 ± 34·0	12/12	2·7 ± 0·2	—	7·9
		F	302·7 ± 19·4	7/9	2·6 ± 0·1	2·6 ± 0·3	9·4
	5	M	341·6 ± 18·1	9/10	2·5 ± —	2·5 —	7·9
		F	314·1 ± 28·6	9/12	2·7 ± 0·1	2·7 ± 0·1	6·4
	6	M	315·3 ± 26·8	9/9	3·6 ± 0·2	—	7·4
		F	310·7 ± 16·9	10/11	3·0 ± 0·3	3·4 —	8·6
Warfarin	3	M	295·6 ± 23·1	4/11	1·9 ± 0·2	1·5 ± 0·3	5·4
		F	300·7 ± 21·4	1/12	2·7 ±	1·6 ± 0·1	5·0
	4	M	355·0 ± 18·1	5/10	2·0 ± 0·2	2·4 ± 0·1	6·2
		F	300·0 ± 18·8	4/10	2·2 ± 0·2	1·9 ± 0·2	6·0
	5	M	384·9 ± 23·6	11/12	2·5 ± 0·1	2·0 —	6·8
		F	294·4 ± 19·3	10/11	3·1 ± 0·2	2·5 —	8·4
	6	M	316·4 ± 38·3	9/10	3·3 ± 0·4	3·0 —	6·3
		F	294·8 ± 19·4	10/11	3·5 ± 0·3	2·7 —	7·5

difference was seen between warfarin and coumatetralyl, while difenacoum and, particularly, brodifacoum affected the rats earlier (Table 3). In no case did any rat show effects before the third day. With brodifacoum, all rats had decreased

Table 2. *Mortality in* B. bengalensis *when offered baits containing anticoagulants at* 0·005 % *concentration*

Anticoagulant	Feeding period (days)	Sex	Weight (g) (mean ± S.E.)	Mortality	Anticoagulant consumed, mg/kg (mean ± S.E.) Died	Survived	Mean day of death
Brodifacoum	1	M	360·4 ± 30·4	*10/10*	3·1 ± 0·1	—	5·6
		F	319·2 ± 29·6	11/11	3·1 ± 0·2	—	5·9
Difenacoum	1	M	335·7 ± 17·6	10/10	3·5 ± 0·2	—	6·9
		F	286·5 ± 19·3	10/10	2·9 ± 0·2	—	6·1
	2	M	336·6 ± 53·4	9/9	6·7 ± 0·6	—	6·2
		F	332·0 ± 26·7	10/10	5·7 ± 0·6	—	6·8
Coumatetralyl	1	M	359·0 ± 35·2	9/10	3·0 ± 0·1	2·0	6·7
		F	258·3 ± 14·1	8/11	3·6 ± 0·2	2·8 ± 0·2	5·9
	2	M	304·0 ± 34·5	10/10	7·3 ± 0·4	—	5·9
		F	262·2 ± 23·7	12/13	6·4 ± 0·6	6·1	7·0
	3	M	—	—	—	—	—
		F	310·2 ± 16·4	10/11	6·8 ± 0·5	7·8	7·1
	4	M	309·3 ± 34·0	10/10	10·1 ± 0·3	—	7·2
		F	243·2 ± 19·0	11/11	11·1 ± 0·9	—	6·5
Warfarin	1	M	335·2 ± 30·1	6/11	3·6 ± 0·2	3·2 ± 0·3	4·5
		F	321·7 ± 16·3	6/10	3·4 ± 0·2	2·5 ± 0·2	6·0
	2	M	272·1 ± 42·0	10/10	8·8 ± 0·9	—	6·0
		F	337·6 ± 26·1	8/10	4·7 ± 0·3	4·0 ± 0·8	6·6
	3	M	241·9 ± 27·5	9/9	11·5 ± 1·0	—	6·1
		F	315·2 ± 26·9	10/11	7·7 ± 0·7	6·7	6·6
	4	M	—	—	—	—	—
		F	230·4 ± 19·0	10/10	13·0 ± 1·1	—	6·7

Table 3. *Onset of poisoning symptoms of several anticoagulants given no-choice to* B. bengalensis (*per cent of rats eating less than half the amount of food consumed on the first day of the trial*)

Anticoagulant	Conc. (%)	No. of animals	Days since start of poisoning trial 3	4	5	6	7	8	9	10	11	12
Brodifacoum	0·001	58	5	22	52	81	98	100	—	—	—	—
Difenacoum	0·001	63	3	17	43	75	84	92	100	—	—	—
Coumatetralyl	0·001	54	2	4	37	78	93	95	96	100	—	—
Warfarin	0·001	63	2	8	24	67	86	91	97	97	98	100
Diphacinone	0·001	71	0	7	20	52	79	89	97	99	100	—
Brodifacoum	0·005	18	17	56	72	100	—	—	—	—	—	—
Difenacoum	0·005	37	3	19	49	73	95	100	—	—	—	—
Coumatetralyl	0·005	59	5	25	66	81	92	100	—	—	—	—
Warfarin	0·005	69	3	26	55	90	97	100	—	—	—	—

food consumption by day 8 and difenacoum-fed animals by day 9. Some animals on coumatetralyl showed no effects until day 10 and two rats on warfarin became visibly ill only on days 11 and 12.

When the concentration of anticoagulant was increased to 0·005 %, the difference between brodifacoum and the other three materials became obvious (Table 3),

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with all rats on brodifacoum showing decreased food consumption by day 6, while with the other three materials this did not occur until day 8.

Time to death

The mean time to death differed significantly between sexes only in the case of brodifacoum and warfarin: females took longer to die (Table 4). There was also a significant difference between the several anticoagulants, with brodifacoum giving the shortest mean times to death and diphacinone the longest. Among males, the time to death from brodifacoum differed significantly from coumatetralyl ($P = 0{\cdot}02$), difenacoum ($P = 0{\cdot}02$) and diphacinone ($P = 0{\cdot}001$). Among females, brodifacoum produced significantly shorter mean times as compared to warfarin ($P = 0{\cdot}05$) and diphacinone ($P = 0{\cdot}05$). Diphacinone-fed males showed significantly longer times to death compared with warfarin ($P = 0{\cdot}001$), coumatetralyl ($P = 0{\cdot}01$) and brodifacoum.

Table 4. *Mean day of death for* B. bengalensis *feeding no-choice on foods containing several anticoagulants at* 0·001 % *concentration* (mean ± 1 S.D.)

	Males		Females		*t*-test significance
Anticoagulant	*n*	Day of death	*n*	Day of death	($P =$)
Brodifacoum	20	5·6 ± 1·7	37	6·5 ± 1·8	0·001
Coumatetralyl	31	6·7 ± 1·4	22	7·3 ± 2·2	0·2–0·3
Difenacoum	25	7·0 ± 2·2	26	7·2 ± 2·2	NS
Diphacinone	36	8·0 ± 2·1	37	7·8 ± 3·1	NS
Warfarin	32	6·3 ± 1·4	25	7·5 ± 2·0	0·01–0·02

Baseline susceptibilities

The calculated LFP50 (Lethal Feeding Period to obtain 50 % mortality) and LFP98 for the several anticoagulants given at 0·001 % concentration by weight are summarized in Table 5. The susceptibility of the lesser bandicoot rat to brodifacoum, difenacoum and coumatetralyl was such that all required less than 2 days feeding for an expected 50 % mortality and between 3 and 5 days to obtain a 98 % kill. Lesser bandicoots were only slightly more susceptible to diphacinone than warfarin.

A comparison of the susceptibility of *B. bengalensis* and *Rattus norvegicus* to

Table 5. *Baseline susceptibility of* Bandicota bengalensis *from Rangoon to several anticoagulants at* 0·001 % (95 % *confidence limits in days given for each lethal feeding period*)

Anticoagulant	No. of rats	Slope function (S)	LFP50 (days)	LFP98 (days)
Brodifacoum	82	1·68	1·12 (0·87–1·44)	3·26 (2·02–5·25)
Difenacoum	86	1·55	1·42 (1·19–1·69)	3·45 (2·33–5·11)
Coumatetralyl	88	1·62	1·81 (1·49–2·20)	4·90 (3·11–7·72)
Diphacinone	101	1·69	2·55 (2·12–3·06)	7·50 (4·03–13·9)
Warfarin	87	1·29	3·90 (3·49–4·35)	6·50 (4·90–8·61)

warfarin at 0·005% concentration (data from Brooks & Bowerman, 1974) is given in Table 6. These observations indicate that *B. bengalensis* from Rangoon are even more susceptible to warfarin than are normal warfarin susceptible *R. norvegicus* from the United States, United Kingdom and the Federal Republic of Germany.

Table 6. *Comparison of the baseline susceptibility of* Bandicota bengalensis *from Rangoon to* Rattus norvegicus *from several areas to warfarin at* 0·005% (95% *confidence limits in days in parentheses*)

	Slope function (S)	LFP 50 (days)	LFP 98 (days)
B. bengalensis Rangoon, Burma	1·92	0·91 (0·61–1·36)	3·35 (1·52–7·37)
R. norvegicus			
Merrick, N.Y.	1·72	1·35 (1·05–1·73)	3·80 (2·14–6·72)
Midlands, Eng.	1·42	1·44 (1·26–1·64)	3·00 (2·29–3·93)
Borkum, Ger.	1·29	1·97 (1·79–2·17)	3·36 (2·77–4·06)
Berlin, N.Y.	1·34	2·58 (2·28–2·92)	4·82 (3·79–6·13)

DISCUSSION

The finding that lesser bandicoot rats from Rangoon are even more susceptible to warfarin than are *R. norvegicus*, marks this animal in Burma as the most susceptible rodent species known. These findings are consistent with other toxicological data on lesser bandicoots rats that we have published, where we found the acute oral LD 50 dose of pyrinuron to be 6·7 mg/kg (Brooks & Htun, 1978); for zinc phosphide an acute oral LD 50 of 25·0 mg/kg was estimated (Htun & Brooks, 1979), and of scilliroside extracts, an acute oral LD 50 for females was calculated at 0·5 mg/kg and, for males, 0·8 mg/kg (unpublished reports, RCDU). The value for pyrinuron is similar to that for *R. norvegicus* (Peardon, 1974), while zinc phosphide and scilliroside are more toxic orally to the lesser bandicoot rat than to the Norway rat.

Our observations with warfarin are consistent with those reported for *B. bengalensis* from Bombay (Deoras, 1967; Deoras *et al.* 1972), but do not agree with those of Greaves & Rehman (1977) using *B. bengalensis* from Karachi, Pakistan. They found that complete mortality was not obtained until after 41 days of exposure to a diet containing 0·025% warfarin. These differences could be due to geographical variation in warfarin susceptibility of populations of *B. bengalensis*.

The baseline susceptibility of male *B. bengalensis* to diphacinone at 0·001% was very similar to data on albino male *R. norvegicus* to the same poison at the same concentration on 3- and 4-day exposures (Kusano, 1974). At 3 days feeding, Kusano obtained 60% mortality with a mean intake of 1·8 mg/kg, whereas we observed 55·6% mortality and a mean intake of 2·0 mg/kg. At 4 days we both observed a complete kill in the respective rodent species at mean intakes of 2·7 and 2·8 mg/kg.

Coumatetralyl was considerably more toxic to *B. bengalensis* than either warfarin or diphacinone. This was consistent with the observations of Hadler & Shadbolt (1975), who found the response to coumatetralyl and difenacoum to be quite similar in extending the prothrombin time in homozygous resistant (Welsh strain) male Norway rats, while warfarin and diphacinone gave little response. They coined the term *resistance index* (the dose of anticoagulant required to produce a mean increase in prothrombin time in warfarin-resistant rats divided by the dose of the same anticoagulant needed to produce a mean increase in prothrombin time in susceptible rats). They found a resistance index of 1·9 for difenacoum, about 14 for coumatetralyl and about 227 for diphacinone (Hadler, Redfern & Rowe, 1975). They suggested that the high anticoagulant activity in coumatetralyl might be due to the structure of the tetralin moeity, in which the four carbon atoms of the saturated ring are held adjacent to the unsaturated ring.

The superior toxicity of brodifacoum and difenacoum to lesser bandicoot rats is to be expected since this species responds very similarly to *R. norvegicus* (Hadler *et al.* 1975; Redfern, Gill & Hadler, 1976). Our observations that only 1 day of feeding on either material at 0·005% gives a complete kill (Table 2), indicates that *B. bengalensis* in Rangoon are more susceptible to anticoagulants than the same species in Karachi since Greaves & Rehman (1977) using 0·005% difenacoum, obtained only an 80% mortality in female lesser bandicoots and 91% in males.

Susceptibility tests for the development of anticoagulant resistance in *B. bengalensis* in Rangoon, following any extensive use of anticoagulants, should be carried out with warfarin or coumatetralyl at 0·005% for 6 days (these are feeding periods lying outside the upper 95% confidence limits at the 98% mortality level). Any animal surviving these feeding periods would be cause for additional testing.

These observations suggest that brodifacoum, difenacoum and coumatetralyl could be expected to give useful results against *B. bengalensis* in the field in Rangoon. Coumatetralyl, used at concentrations ranging from 0·005 to 0·01%, could be recommended, while brodifacoum and difenacoum should be useful at concentrations of 0·002–0·005%. Field trials of these three materials at several concentrations against urban populations of *B. bengalensis* and other commensal small mammals will be reported upon shortly.

A word of caution is needed, however. The survival of individual animals in the lower baseline susceptibility tests (0·001%) when using coumatetralyl, diphacinone and warfarin, would indicate that *B. bengalensis* in Rangoon shows considerable individual variation in susceptibility to anticoagulants. The widespread use of anticoagulants as the sole means of control of lesser bandicoots rats in Rangoon could lead eventually to pockets of anticoagulant-resistant animals. Fortunately, the lesser bandicoot rat is quite susceptible to both zinc phosphide and pyrinuron. The use of these acute toxicants in alternation with periods of poisoning with anticoagulants should slow the development of resistance and prolong the usefulness of anticoagulants in long term control programmes directed against *B. bengalensis* populations.

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