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PERFORMANCE OF BROMADIOLONE AS A RODENTICIDE AGAINST *RATTUS ARGENTIVENTER*

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RINGKASAN

Racun tikus bromadiolone didapati sangat berkesan terhadap *Rattus argentiventer*. Takaran tunggal (oral) LD_{50} bagi tikus jantan ialah 0.49 mg/kg dan tikus betina pula 0.59 mg/kilogram. Kepekatan racun 0.001% berupaya membunuh tikus jantan dalam tempoh purata (LFP_{50}) 1.43 hari dan tikus betina dalam 1.67 hari. LFP_{50} dan LFP_{99} tergembleng (pooled) bagi kedua-dua jantina ialah 1.55 hari dan 4.53 hari. Dalam ujian pemakanan sehari tanpa pilihan didapati bromadiolone pada kepekatan 0.005% dan 0.01% menyebabkan 80% dan 100% kematian ke atas kedua-dua jantina tikus. Purata hari hidup selepas memakan racun bagi tikus jantan ialah 7.5 hari dan tikus betina pula 7.9 hari. Ujian-ujian perasa menunjukkan bahawa bromadiolone diterima baik hingga ke kepekatan 0.02 peratus. Berdasarkan kepada ujian-ujian di atas, bromadiolone pada kepekatan antara 0.005% hingga 0.02% boleh disyorkan untuk mengawal *R. argentiventer*.

INTRODUCTION

Bromadiolone, 3–[3–(4'–bromo[1,1'–biphenyl]–4–yl) – 3 – hydroxy – 1 – phenylpropyl] – 4 – hydroxy–2H – 1 – benzopyran – 2 – one, is a new potent hydroxycoumarin derivative. Laboratory tests have shown that bromadiolone is highly toxic to *Rattus norvegicus* (Berkenhout), *Rattus rattus* (Linnaeus) and *Mus musculus* (Linnaeus) (GRAND, 1976; LUND, 1977; MARSH, 1977; MEEHAN, 1978; REDFERN and GILL, 1980) and it has also shown good rodenticidal properties against other rodent species (GRAND, 1976; MARSH, 1977; MARSH, HOWARD and JACKSON, 1980). In field trials against warfarin-resistant *R. norvegicus* infestations, 0.005% bromadiolone achieved complete control with unrestricted surplus baiting (RICHARDS, 1981). This paper describes the performance of bromadiolone against *Rattus argentiventer* (Robinson & Kloss).

MATERIALS AND METHODS

Bromadiolone of technical grade with 99.8% a.i. was used in the experiments. All doses of bromadiolone are expressed as mg/kg which refer to milligrammes of bromadiolone per kilogramme of body weight. Determination of the single-dose oral LD_{50} and feeding tests under 'no-choice' and 'choice' conditions were conducted against *R. argentiventer* from an out-bred laboratory colony. Test conditions and methods were as given in LAM (1980) and largely followed the WORLD HEALTH ORGANISATION (1982) tests procedures.

For the feeding tests, a master-mix containing 0.25% bromadiolone was first prepared by mixing the technical grade material with powdered rice. The toxicant was presented in a bait-base as described by LAM (1979). The dosage/mortality data were analysed by probit analysis (FINNEY, 1971), using DAUM's (1970) computer programme.

RESULTS

Single-dose Oral LD_{50}

Results of oral intubation with various levels of bromadiolone are given in *Table 1*. Probit analysis of the dose/mortality data *(Table 1)* are summarized in *Table 2*. The LD_{50} for males and females, with 95% fiducial limits, were 0.49 mg/kg (0.45–0.57

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Table 1. Results of oral intubation with various levels of bromadiolone against *Rattus argentiventer*

Sex	Mean body weight (g)	Dose (mg/kg)	Mortality (dead/treated)	Days to death	
				Mean	Range
M	136.2	0	0/10	–	–
M	136.1	0.33	0/10	–	–
M	136.3	0.40	1/10	17.0	–
M	137.3	0.50	5/10	5.0	6–17
M	137.4	0.70	10/10	8.3	5–11
M	131.1	1.00	10/10	8.0	5–10
F	122.2	0	0/10	–	–
F	122.0	0.33	0/10	–	–
F	122.4	0.50	3/10	13.7	6–18
F	121.5	0.70	7/10	7.9	3–11
F	122.1	1.00	10/10	8.3	4–15

mg/kg) and 0.59 mg/kg (0.50–0.69 mg/kg) respectively *(Table 2)*. The probit dosage/response line for males and females did not contradict the hypothesis of parallelism and using the females as reference, the estimated relative potency (with 95% fiducial limits) was 1.2 (1.0–1.4).

Feeding Tests

No-choice feeding tests

No-choice feeding tests with bromadiolone at concentrations of 0.001%–0.01% were conducted. Results of no-choice tests are summarized in *Table 3*. Bromadiolone at 0.002% and 0.005% caused 100% mortality in both sexes in two-day tests. At 0.01% bromadiolone caused 100% mortality in both sexes in one-day tests. The lowest lethal dose in males was 0.30 mg/kg and the highest dose survived was 1.07 mg/kg *(Table 3)*. In the case of females the lowest lethal dose was 0.36 mg/kg and the highest dose survived was 1.34 mg/kg *(Table 3)*.

Mean days to death (combined data from no-choice tests) for males was 7.5 days (range 4–14 days) and females 7.9 days (range 4–14 days). No significant difference was detected in the mean days to death between the sexes ($t = 1.2538$; d.f. = 222; $P>0.05$). There was no evidence that bromadiolone at higher concentrations caused death more rapidly *(Table 3)*. Probit analysis of the dose (feeding period)/mortality data is given in *Table 4*. The median lethal feeding periods (LFP_{50}), with 95% fiducial limits, for male and female *R. argentiventer* were 1.43 days (1.13–1.71 days) and 1.67 days (1.36–1.96 days) respectively *(Table 4)*. There was no significant difference in the LFP_{50} between males and females and the data were pooled for probit analysis. The pooled LFP_{50} of 0.001% bromadiolone against *R. argentiventer* was 1.55 days (1.34–1.75 days) and the pooled LFP_{99} was 4.53 days (3.66–6.34 days) respectively *(Table 4)*.

Choice feeding tests

Choice feeding tests were conducted with bromadiolone at concentrations of 0.002%–0.05% and the results obtained are summarized in *Table 5*. In general, rats did not show significant aversion to the bromadiolone baits up to the 0.02% level. Under choice conditions 0.005% bromadiolone caused 80% mortality in the males and 100% mortality in the females. Bromadiolone at 0.01% caused 90% mortality in males and 100% mortality in females. Bromadiolone at 0.02% and 0.05% caused 100% mortality in both sexes. Both male

Table 2. Probit analysis of data from oral intubation tests with bromadiolone against *Rattus argentiventer*

Sex	Regression equation[1]	Chi-sq.	d.f.	LD_{50} (mg/kg)	95% fiducial limits of LD_{50} (mg/kg)	LD_{95} (mg/kg)	95% fiducial limits of LD_{95} (mg/kg)	LD_{99} (mg/kg)	95% fiducial limits of LD_{99} (mg/kg)
M	Y = 15.83X − 5.96 (4.81)*	0.26	2	0.49	0.45−0.57	0.63	0.55−0.97	0.69	0.59−1.23
F	Y = 9.53X − 2.34 (2.63)	0.58	2	0.59	0.50−0.69	0.88	0.74−1.44	1.03	0.83−2.03

*Figures in parentheses denote the standard error of the slope of regression line.
[1]Lines did not contradict the hypothesis of parallelism (Chi-sq. 2.2; 5 d.f.), using females as reference, the relative potency was 1.2 (95% fiducial limits 1.0−1.4).

Table 3. Results of no-choice feeding tests with 4 concentrations of bromadiolone against *Rattus argentiventer*

Concentration of poison (%)	Sex	Mean body weight (g)	Feeding period (days)	Mortality (dead/treated)	Bait intake (g)		Lethal dose of poison (mg/kg)		Survived dose of poison (mg/kg)		Days to death	
					Last day of prebait	First day of poison	Mean	Range	Mean	Range	Mean	Range
0.001	M	213.1	1	5/20	8.65	8.37	0.45	0.30−0.66	0.39	0.19−0.60	8.0	4−10
	F	142.9	1	3/20	5.78	5.18	0.41	0.36−0.50	0.35	0.20−0.54	7.0	4−10
	M	208.0	2	14/20	7.92	7.99	0.84	0.47−1.04	0.65	0.40−0.90	8.1	5−14
	F	150.7	2	12/20	6.32	6.10	0.89	0.55−1.17	0.76	0.42−1.19	8.7	5−12
	M	208.5	3	19/20	8.02	7.54	1.19	0.72−1.83	0.64	−	8.3	6−12
	F	153.6	3	18/20	5.83	5.63	1.11	0.62−1.55	0.71	0.63−0.79	8.3	6−12
	M	197.7	4	20/20	8.11	7.30	1.43	0.76−2.21	−	−	7.4	4−12
	F	136.5	4	20/20	4.82	4.31	1.22	0.27−2.23	−	−	8.3	5−11
0.002	M	214.1	1	5/10	8.91	7.76	0.83	0.34−1.28	0.67	0.29−1.07	9.2	8−11
	F	142.3	1	7/10	6.99	6.58	1.00	0.66−1.37	0.88	0.60−1.34	7.0	5− 9
	M	177.8	2	10/10	9.34	8.91	1.94	1.03−2.99	−	−	7.5	4−10
	F	139.8	2	10/10	6.00	6.86	1.91	1.51−2.90	−	−	8.8	4−14
0.005	M	252.7	1	8/10	6.81	5.81	1.47	1.17−2.14	0.02	−	7.0	5−10
	F	154.9	1	8/10	5.01	4.22	1.77	1.07−2.56	0.01	0 −0.01	9.0	4−12
	M	192.7	2	10/10	7.22	7.42	3.72	1.90−5.40	−	−	6.4	4−12
	F	133.8	2	10/10	5.84	4.71	3.61	2.19−5.47	−	−	6.1	4− 9
0.01	M	160.4	1	10/10	9.22	8.29	5.20	2.47−7.38	−	−	5.3	4− 8
	F	141.4	1	10/10	5.57	5.63	4.00	3.21−5.03	−	−	8.3	6−11

Table 4. Probit analysis of data from no-choice feeding tests with 0.001% bromadiolone against *Rattus argentiventer*

Sex	Regression equation	Chi-sq.	d.f.	LFP_{50} (days)	95% fiducial limits of LFP_{50} (days)	LFP_{95} (days)	95% fiducial limits LFP_{95} (days)	LFP_{99} (days)	95% fiducial limits of LFP_{99} (days)
M	Y = 4.89X + 4.24 (0.93)*	0.81	2	1.43	1.13–1.71	3.11	2.49–4.67	4.28	3.21–7.63
F	Y = 5.20X + 3.84 (0.93)	0.94	2	1.67	1.36–1.96	3.46	2.81–5.01	4.68	3.58–7.84
(M+F)	Y = 4.99X + 4.05 (0.65)	1.75	2	1.55	1.34–1.75	3.31	2.82–4.21	4.53	3.66–6.34

(M+F) = Pooled data of males and females.

*Figures in parentheses denote the standard error of the slope of regression line.

Table 5. Bait consumption and mortality of *Rattus argentiventer* given a choice between plain and bromadiolone baits for 2 days

Sex	Mean body weight (g)	Concentration of poison (%)	Mean daily bait intake (g)		No. of rats preferring plain[1]	t value	Mortality (dead/treated)
			Plain	Poison			
M	215.2	0.002	4.12	4.45	4/10	−0.76N.S.	7/10
F	163.0	0.002	2.81	2.59	6/10	0.34N.S.	5/10
M	190.7	0.005	3.36	4.59	4/10	−1.42N.S.	8/10
F	135.9	0.005	3.40	3.44	5/10	−0.06N.S.	10/10
M	168.1	0.01	4.20	2.79	7/10	1.66N.S.	9/10
F	143.3	0.01	2.99	3.24	5/10	−0.33N.S.	10/10
M	196.7	0.02	3.88	4.20	5/10	−0.44N.S.	10/10
F	161.3	0.02	3.06	3.36	3/10	−0.62N.S.	10/10
M	224.2	0.03	6.83	1.94	9/10	4.39**	8/10
F	162.9	0.03	3.52	1.80	7/10	2.65*	8/10
M	233.4	0.05	5.42	3.18	9/10	2.95*	10/10
F	187.9	0.05	3.25	2.40	5/10	0.93N.S.	10/10

*Significant at $P<0.05$

**Significant at $P<0.01$

N.S. = Not significant at $P>0.05$

[1]Number of rats which consumed a greater amount of plain baits.

and female rats showed significant aversion ($P<0.05$) to the 0.03% bromadiolone baits. However, at 0.05% only the males showed significant aversion ($P<0.01$) to the bromadiolone baits *(Table 5)*.

DISCUSSION

Bromadiolone showed very good rodenticidal properties against *R. argentiventer,* with a single-dose oral LD_{50} of 0.49 mg/kg and 0.59 mg/kg for males and females respectively *(Table 2)*. Females appeared to be less susceptible to bromadiolone than the males. However, there was no significant difference in the LD_{50} and LFP_{50} values between the sexes. Compared with the other two second generation anticoagulant rodenticides, brodifacoum and difenacoum, bromadiolone is about three times less toxic than brodifacoum (LD_{50} – 0.17 mg/kg; LAM, 1980) but more toxic than difenacoum (LD_{50} – 0.82 mg/kg for males and 0.68 mg/kg for females; LAM, 1984a) against *R. argentiventer*. As found in brodifacoum (LAM, 1980) and difenacoum (LAM, 1984a), bromadiolone could induce death with a single feeding. In one-day no-choice tests, bromadiolone at 0.005% and 0.01% respectively caused 80% and 100% mortalities in both sexes *(Table 3)*.

There was considerable variation in the susceptibility of *R. argentiventer* to bromadiolone. One male survived a dose of 1.07 mg/kg which exceeded the estimated LD_{99} of 0.69 mg/kg for males and similarly one female survived a dose of 1.34 mg/kg which also exceeded the estimated LD_{99} of 1.03 mg/kg for females. When these values were compared with the lowest lethal doses of 0.30 mg/kg for males and 0.36 mg/kg for females, it could be seen that the above surviving rats were at least 3.0 to 4.5 times inherently more tolerant than those highly susceptible. It could be expected that this inherent tolerance could give rise to the prospect of resistance to bromadiolone after prolonged use. Resistance to warfarin has since been reported in *Rattus rattus diardii* (LAM, LEE, TAN and MOHAN, 1982; LAM, 1984b) and to coumatetralyl in *Rattus tiomanicus* (HO and LAM, 1983). For the detection of resistance to bromadiolone, a five-day feeding on a sole diet containing 0.001% bromadiolone, based on the LFP_{99}, is a suitable screening test.

Palatability (choice) tests indicated that bromadiolone was highly acceptable to *R. argentiventer* up to a concentration of 0.02 per cent. Results of the choice feeding tests also indicated that the optimum concentration for achieving 100% mortality in both sexes under choice *ad libitum* condition was 0.02% *(Table 5)*. Even at concentrations of 0.005% and 0.01% bromadiolone caused 80% – 90% mortalities in the males and 100% mortality in the females. Compared with brodifacoum (LAM, 1980) and difenacoum (LAM, 1984a), bromadiolone is more palatable to *R. argentiventer*. Significant preference for plain baits was evident at 0.005% for brodifacoum (LAM, 1980) and at 0.001% for difenacoum (LAM, 1984a). Bromadiolone has also been shown to be highly palatable to *R. norvegicus, R. rattus* and *M. musculus* at 0.005% in choice feeding tests (MARSH, 1977; MEEHAN, 1978; REDFERN and GILL, 1980) and results have indicated that *R. norvegicus* could just detect bromadiolone at 0.01% (MEEHAN, 1978).

In conclusion, bromadiolone is a very potent anticoagulant and it is a valuable addition to the arsenal of rodenticides against *R. argentiventer* and other rodent pests of agricultural crops. Bromadiolone at 0.005%–0.02% would be suitable for use against field populations of *R. argentiventer*. However, field trials would be required to evaluate its efficacy in controlling wild populations of *R. argentiventer*.

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ABSTRACT

Bromadiolone showed very good rodenticidal properties against *Rattus argentiventer*. The single-dose oral LD_{50} for males and females were 0.49 mg/kg and 0.59 mg/kg respectively. The median lethal feeding period (LFP_{50}) of 0.001% bromadiolone against males and females were 1.43 days and 1.67 days respectively. The pooled LFP_{50} of 0.001% bromadiolone against both sexes was 1.55 days and the pooled LFP_{99} was 4.53 days. Bromadiolone was found to have a single-feed action. In one-day no-choice tests, bromadiolone at 0.005% and 0.01% caused 80% and 100% mortalities in both sexes respectively. The mean days to death for males and females were 7.5 days and 7.9 days respectively. Palatability tests indicated the bromadiolone was palatable up to 0.02 per cent. Bromadiolone at 0.005% – 0.02% could be used in the field for the control of *R. argentiventer*.

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