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THE TOXICITY OF BROMADIOLONE TO THE MALAYSIAN WOOD RAT *RATTUS TIOMANICUS*

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RINGKASAN

Percubaan penggunaan racun melalui mulut 'Oral intubation' dan pemakanan tanpa pilihan bagi penentuan keracunan dan keupayaan bromadiolone membunuh *Rattus tiomanicus* yang diperangkap dari kawasan tanaman koko-kelapa di Hilir Perak telah dijalankan di makmal. 'Single dose oral LD 50' yang didapati ialah 2.27 (1.89–2.67) mg/kg manakala jangkamasa yang berkesan untuk membawa maut LFP 50 ialah 1.29 (0.79–1.68) hari. Hitung panjang harian ke maut selepas perlakuan ialah lima hingga tujuh hari. Masa yang paling singkat ialah dua hari dan yang lama sekali ialah 11 hari. Memandangkan tiada tanda-tanda keracunan yang bergerak cerdas (acute poisoning) kesiluan umpan (bait-shyness) tidak akan berlaku.

Kajian ini menunjukkan terdapat perbezaan kepekaan tikus kepada racun. Ini menunjukkan ada kemungkinan pemilihan ketahanan ke atas bromadiolone oleh tikus sekiranya racun ini digunakan dengan lama. Takaran maut LD 95 dan jangkamasa yang berkesan membawa maut, LFP 95, pada paras keyakinan 95% paras atas, boleh digunakan sebagai asas untuk menentu/mengesan ketahanan tikus kepada racun tersebut. Sifat 'anti-coagulant' bromadiolone membolehkan ia digunakan dengan selamat-nya. Dengan pemakanan umpan yang tinggi, kebaikan dari segi maut lepas sekali makan dan masa maut yang singkat, bromadiolone adalah suatu racun rodensia yang berkesan untuk mengawal *R. tiomanicus* dan adalah suatu tambahan kepada racun-racun rodensia yang sedia ada.

INTRODUCTION

Bromadiolone is one of the few new rodenticides developed in the wake of warfarin-resistant rats being encountered in Europe and U.S.A. The compound, though anticoagulant in nature, is far more potent than the conventional anticoagulants like warfarin and diphacinone. A single dose is lethal enough and death occurs after a few days. Complete death in this way has been obtained on *Rattus norvegicus* by MARSH (1977), and REDFERN and GILL (1980). Such rodenticidal action gives effective and efficient control of rats without inducing bait shyness.

The introduction of such highly active anticoagulants into Malaysia has been very recent. This may be to capitalise their usefulness over conventional rodenticides. In areas where warfarin has been in use for a long time where there is a possibility of the development of resistant species (LAM, 1980; LEE, MUSTAFA, SOH and MOHAN, 1982), these new rodenticides could perhaps serve as alternatives. In the light of bromadiolone's introduction into the country and its effectiveness against other species elsewhere, this paper presents laboratory studies on the toxicity of bromadiolone to the Malaysian wood rat *Rattus tiomanicus*, the most predominant and serious pest in plantation crops.

MATERIALS AND METHODS

Rattus tiomanicus were live-trapped from cocoa-coconut fields in Hilir Perak and conditioned in the laboratory in individual cages (45 x 45 x 30 cm) for two weeks prior to the study. The test conditions were similar to that reported by LEE and MUSTAFA (1982).

i) Determination of Oral LD 50 toxicities

Oral intubation of bromadiolone liquid concentrate (0.25% a.i.) was administered by gavage to non-starved animals. The volume administered to the animals ranged from 0.05–0.50 ml. For each dosage 20 animals (10 males and 10 females) were used.

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The animals were maintained on laboratory pellets, fresh copra and ripe bananas with water *ad libitum* throughout a 30-day observation period. Days to death were recorded and dead animals were autopsied for poisoning symptoms.

ii) Feeding tests

The tests carried out was no-choice feeding. The poison bait was prepared commercially in wax-cube maize-base with bromadiolone at 0.005% concentration. Five groups of animals (10 males and 10 females each) were prebaited for two days with plain baits before they were allowed unrestricted feeding upon the poison baits for 1, 2, 3, 4 and 5 days respectively. At the end of the prescribed feeding period for each animal group, laboratory pellets, fresh copra and ripe bananas were given as food and observed for a further 30 days beginning from the first day of feeding with the poison baits. Bait consumption was measured (to the nearest 0.1g) daily and mortality recorded. Dead rats were autopsied for poisoning symptoms. The results obtained in the oral intubation and feeding tests were used to determine the LD 50 and LFP 50 by probit analysis.

RESULTS

The oral toxicity of bromadiolone to both male and female *R. tiomanicus* is as shown in *Table 1*. Animals were observed to die from three to ten days after ingestion of a lethal dose. The duration to death decreased with increase in concentration and the shortest period was three days. The single dose oral LD 50 for males and females were 2.37 (1.78–3.02) mg/kg and 2.18 (1.66–2.72) mg/kg respectively. For LD 95 the values were 7.47 (5.17–16.95) mg/kg and 6.10 (4.43–11.83) mg/kg for males and females respectively. The difference between both the sexes was statistically non-significant and the pooled data had an LD 50 of 2.27 (1.89–2.67) mg/kg. The LD 95 within 95% fiducial limits was 6.75 (5.20–10.46) mg/kg with the slope of the probit line being 3.48 (S.E. ± 0.52).

In the no-choice feeding test, 100% mortality of the animals tested was obtained in the five-day feeding period *(Table 2)*. At lower feeding periods, increase in mortality was noted with increase in duration of feeding. The least poison bait uptake was 6.1 g while the highest was 12.4 grammes. This was comparable to the uptake of plain baits

Table 1. Oral toxicity of bromadiolone to *R. tiomanicus*

Sex	Mean body weight (g)	Dose (mg/kg)	Mortality	Days to death mean (range)
M	91.2	1.0	2/10	8.0(6–10)
F	83.9	1.0	1/10	9.0 –
M	110.2	1.5	2/10	7.5(6–9)
F	110.5	1.5	3/10	7.0(4–10)
M	101.4	2.0	4/10	6.0(5–9)
F	99.2	2.0	5/10	7.3(5–10)
M	90.2	3.0	5/10	7.4(4–10)
F	88.6	3.0	6/10	6.0(4–8)
M	84.9	4.0	7/10	6.4(4–8)
F	86.0	4.0	8/10	5.3(4–8)
M	89.0	5.0	9/10	5.4(3–6)
F	89.1	5.0	9/10	4.6(4–6)
M	96.2	6.0	10/10	4.3(3–6)
F	94.8	6.0	10/10	4.7(3–6)

Table 2. Results of non-choice feeding tests where *R. tiomanicus* was given 0.005% bromadiolone in maize base wax cube for different number of days.

Number of feeding days	Sex	Mean body weight (g)	Mean bait intake		Mortality	Lethal dose of bromadiolone (mg/kg) mean (range)	Survived dose of bromadiolone (mg/kg) mean (range)	Days to death mean (range)
			last day prebait (g)	1st day poison (g)				
1	M	107.50	11.0	10.40	4/10	6.38(4.48–8.53)	5.72(1.99–7.64)	6.75 (6–7)
	F	99.95	10.0	7.78	4/10	5.62(4.63–6.69)	2.98(0.86–6.01)	6.25 (4–9)
2	M	103.96	6.8	7.30	7/10	8.07(4.54–11.63)	5.20(4.55–5.56)	6.29 (5–8)
	F	92.13	9.0	8.53	7/10	8.60(6.44–10.73)	6.21(4.45–8.53)	6.57 (4–11)
3	M	91.90	8.2	7.99	9/10	10.76(8.40–13.86)	1.96 –	6.44 (4–10)
	F	92.01	7.8	8.86	7/10	8.10 (4.81–11.97)	7.22(3.72–9.25)	6.50 (3–11)
4	M	117.40	6.1	6.10	9/10	15.15(8.23–22.29)	15.36	6.88 (5–9)
	F	97.74	7.4	6.80	9/10	13.80(10.07–34.43)	12.69	5.40 (2–7)
5	M	125.20	9.3	9.20	10/10	16.28(9.50–23.27)	–	6.20 (4–11)
	F	99.30	9.5	8.60	10/10	15.54(8.29–22.58)	–	5.80 (4–11)

indicating good bait acceptance. Most of the animals tested succumbed to the poison between five to seven days although the shortest recorded time was two days and the longest was eleven days.

Variation in susceptibility of *R. tiomanicus* to bromadiolone poisoning was seen in the feeding tests. The least fatal dose was 4.5 mg/kg and the animal concern was a male weighing 127 g which ate 11.4 g of the bait in one day and dying on the seventh day. In contrast the highest survived dose was 15.36 mg/kg by a male, weighing 140 g and eating 43.0 g of the poison over a 4-day feeding period.

Probit analysis of the data showed that the lethal feeding period corresponding to 50% and 95% mortality within 95% fiducial limits (LFP 50, LFP 95) were 1.26 days (0.54–1.77) and 4.26 days (2.90–13.50) for males while it was 1.34 days (0.48–1.93) and 5.55 days (3.51–28.78) for females. The difference between both the sexes was statistically non-significant ($P>0.05$) and the pooled data had LFP 50 and LFP 95 values of 1.29 days (0.79–1.68) and 4.87 days (3.53–9.72) respectively. The slope of the probit line was 2.86 (S.E. $\pm$ 0.63).

DISCUSSION

In the oral intubation test, the LD 50 of 2.27 mg/kg (2.18 for females; 2.37 for males) established was higher than that found for *Rattus norvegicus* and *Rattus rattus* (GRAND, 1976). However, in view of its high toxicity, a small amount of the bromadiolone bait at 0.005% concentration need be eaten to bring about kill. With good bait acceptance as has also been reported in the US (MARSH, 1977) and Great Britain (REDFERN and GILL, 1980), the amount of bait taken by a rat at a single feeding would exceed the lethal dose required; thus giving rise to a single dose effect.

In the feeding tests the LFP 50 of 1.29 days (1.26 for males; 1.34 for females) would result in poison uptake exceeding the lethal dose required. The least fatal dose noted was 4.48 mg/kg. The LFP 50 found was higher than 1.01 days established for *R. rattus* and lower than 1.45 days for *R. norvegicus* resistant to warfarin (REDFERN and GILL, 1980). The highest survived dose of 15.36 mg/kg by a male and 12.69 mg/kg by a female appears to contradict the effectiveness of bromadiolone as these values exceeded the upper limits of LD 95 (10.46 mg/kg) established. On examining the duration of feeding this contradiction is false because such a dose was attained after a 4-day feeding period, whereby such tolerance could possibly be due to the unexplained properties in the metabolism of bromadiolone in the individual animals. This has previously been noted with warfarin in *Rattus argentiventer* (LAM, 1979). This with the variation in susceptibility of the rats to bromadiolone poisoning suggests that its continued use over a long period of time would possibly lead to selection of resistant rats.

The LD 95 of 6.75 mg/kg and LFP 95 of 4.87 days (approximately five days) established conform to the fact that in this study no rats were found to survive an oral intubation dose of 6.0 mg/kg and so was total death attained over a five-day feeding period. In view of the variation in susceptibility and the selection of resistant rats could be possible; the upper limits within 95% fiducial level where the LD 95 of 10.46 $\triangleq$ 11.0 mg/kg and the LFP 95 of 9.72 $\triangleq$ 10.0 days can be used as a baseline for the monitoring/determination of resistance.

In both the oral intubation and feeding tests undertaken, death of the animals was noted by the third day. No acute or immediate death was recorded. Studies elsewhere also indicated that even at very high doses, haemorrhage and death only occurred by the third day (MEEHAN, 1978). This characteristic is highly advantageous to the poison as no bait shyness would be induced. The shorter duration to death (5–7 days, longest 11 days) when compared to that required for conventional anticoagulants (more than one week) also showed that pod losses during control can be minimised too.

The poison being an anticoagulant in nature would render it safe for use.

CONCLUSION

Bromadiolone, a second generation rodenticide is found to have an LD 50 of 2.27 mg/kg and an LFP 50 of 1.29 days for *Rattus tiomanicus*. No acute poisoning was noted and with delayed death, no bait shyness would be induced. A shorter duration to death over conventional anticoagulants also helps to minimise pod losses. With pronounced advantages of good bait acceptance, a single dose effect, non-detectibility (MARSH, 1977) and delayed death, bromadiolone would be an alternative to the current rodenticides available for rodent control.

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SUMMARY

Oral intubation and no-choice feeding tests were conducted to determine the toxicity and efficacy of bromadiolone against *R. tiomanicus* trapped from cocoa-coconut fields in Hilir Perak. The single dose oral LD 50 is 2.27 (1.89–2.67) mg/kg while the mean lethal feeding period LFP 50 is 1.29 (0.79–1.68) days. The mean duration to death was five to seven days with the shortest being two days and the longest was eleven days. No acute poisoning symptoms were seen indicating that bait shyness would not be induced. The variability in susceptibility of the rats to the poison shows the possibility of selection for resistant rats after the poison has been in use for a long time. As such the LD 95 and the LFP 95 within the upper 95% fiducial limits can be used as a baseline to monitor/detect resistance. The anticoagulant nature of the poison renders it safe for use. With good bait acceptance, the advantage of a single dose effect and the short duration to death, bromadiolone is an effective rodenticide for the control of *R. tiomanicus* and would be an addition to the current conventional rodenticides.

REFERENCES

FINNEY, D.J. (1971). *Probit analysis*. 3rd edition Cambridge University Press, Cambridge, 333pp.

GRAND, M. (1976). Experimental results on a new anticoagulant rodenticide-bromadiolone (in French). *Phytiatrie Phytopharmacie*, **25**, 69–88.

LAM, Y.M. (1980). Warfarin resistance – a new problem for the planting industry? MAPPS, Newsletter **4**, 3.

---------- (1979). The toxicity of warfarin to the rice field rat *Rattus argentiventer* (Robinson & Kloss). *Malays. agric. J.* **52**, 177–181.

LEE, C.H. and MUSTAFA, MD. D. (1982). Laboratory evaluation of 0.025% warfarin against *Rattus tiomanicus*. *MARDI Res. Bull.* **11**, 132–135.

LEE, C.H., MUSTAFA, MD. D., SOH, K.G. and MOHAN, E. (1982). Warfarin resistance in *Rattus tiomanicus* (in prep.).

MARSH, R.E. (1977). Bromadiolone, a new anticoagulant rodenticide. E.P.P.O. Bulletin **7**, 495–502.

MEEHAN, A.P. (1978). Rodenticidal activity of bromadiolone – a new anticoagulant. Proc. of the 8th Vertebrate Pest Conf., Sacramento, California, p. 122–126.

REDFERN, R. and GILL, J.E. (1980). Laboratory evaluation of bromadiolone as a rodenticide for use against warfarin-resistant and non-resistant rats and mice. *J. Hyg., Cambridge* **84**, 263–268.

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