Pak. j. sci. ind. res., vol. 33, no. 5-6, May-June 1990

THE CHRONIC ORAL TOXICITY OF THREE ANTICOAGULANT RODENTICIDES TO *RATTUS RATTUS*

JOE E. BROOKS, EJAZ AHMAD* AND IFTIKHAR HUSSAIN*

Vertebrate Pest Control Project, Denver Wildlife Research Centre, Denver, Colorado, USA

(Received August 1, 1989; revised June 6, 1990)

This study reports the comparative toxicities of three anticoagulants to *Rattus rattus rufescens* in Pakistan, and describes a simple method to determine the toxicities. A nochoice, 4-day feeding test with small groups (3 or 4 males, 3 or 4 females) of rodents was used. By varying the concentrations of active ingredients, a value for the 4-day LC_{50} and LC_{95} can be statistically estimated from mortality data using probit analysis. The 4-day approximate lethal dose (ALD_{50} and ALD_{95}) also can be derived. Brodifacoum proved the most toxic, followed by bromadiolone and coumatetralyl, giving 4-day LC_{50}'s of 1.8, 2.1 and 19.6 ppm respectively and 4-day LC_{95}'s of 8.4, 10.1 and 126.4 ppm respectively. These values indicated that *R.r. rufescens* from Rawalpindi are susceptible to the three anticoagulants at recommended field concentrations.

Key words: Anticoagulant rodenticides, Toxicity, Lethal concentration.

Introduction

The new "second generation' anticoagulants have stimulated considerble interest for rodent control programmes because they are much more toxic to rodents than the other anticoagulants. Brodifacoum, the most toxic, has been proposed for use against a rodent population once a week for 3-treatments. Dubock [3] found that brodifacoum was as effective as zinc phosphide when offered for one feeding. The amount of anticoagulant bait consumed, however, needs to be much greater than the required for zinc phosphide.

Although studies of the toxicities of several "second generation" anticoagulants to *R.rattus* have been conducted, basic comparative toxicity information is limited. Brodifacoum studies were reviewed by Kaukeinen and Rampaud [8]. Bromadiolone against *R.rattus* was studied by Hoppe and Krambias [7] and Redfern and Gill [10]. Coumatetralyl was studied by Girish *et al.* [6] and Chopra and Parashad [2].

Acute oral LD_{50} determinations sometimes have been done with anticoagulants but these are not always appropriate for slow-acting chronic poisons [1]. The World Health Organization's protocol [11] for determining baseline susceptibility of rodents for anticoagulants is time consuming and requires a large number of test animals. Feeding "second generation" anticoagulants at recommended field concentrations quite often results in complete mortality with one or two days feeding, rendering the comparative toxicity information of limited value.

The method used here was to compare the toxicities of brodifacoum, bromadiolone, and coumatetralyl to *R.rattus refescens* using a progression of very small concentrations (1.25, 2.5, 5, 10, 20 ppm etc.) of the poisons. These were offered no choice for 4 days, a time period during which rodents usually exhibit normal daily food intake.

Materials and Methods

Wild roof rats, *R.r. rufescens* were captured from grain shops in Rawalpindi, Pakistan. They were individually caged and acclimated to laboratory conditions for 3 weeks. Rats were provided a diet of poultry mix and free access to water. Rats were weighed to the nearest 0.1 g at the start of the trial. Test groups of three or four males and three or four females were formed. On test day, they were given broken rice mixed with 1% corn oil and the anticoagulant rodenticide. Following testing the rats were observed daily for 30 days for bleeding and mortality. All dead animals were necropsied to verify death from hemorrhage.

Concentrated anticoagulant was as supplied by the manufacturers: Coumatetralyl as a 0.75% powdered concentrate, brodifacoum as a 0.25% concentrate in propylene glycol, and bromadiolone as a 1% powdered concentrate. These were diluted to the desired concentrations and mixed into the broken rice with 1% (by weight) of corn oil. These diets were offered to the rats in a no-choice test for 4 days. Each anticoagulant was given in concentrations half or double that of the previous offering, depending upon mortality. Concentrations were offered until a level was reached that resulted in 100% mortality. Food cups were weighed to the nearest 0.1 g at the beginning and end of the 4-day exposure period. All spillage was recovered and weighed. At the end of the test, animals were returned to a plain rice diet. The 4-day LC_{50} and LC_{95} was determined using probit analysis [5]. Likewise, a 4-day approximate lethal dose (ALD_{50} and ALD_{95}) was determined by the same method using the mean intake of active ingredient by each test group.

Results

Data on the observed mortalities, mean doses of active ingredients consumed, and mean day to death are summarized in Table 1. Brodifacoum clearly was the most toxic of the three materials offered to *R.r. rufescens*. Based on 4-day LC_{95}'s and 4-day ALD_{95}'s brodifacoum was approximately 1.2 times more toxic than bromadiolone and 9 to 15 times more toxic than coumatetralyl (Table 2 and 3). The mean day of death was delayed for brodifacoum and bromadiolone at lower doses, with some animals dying up to 26 days from first

*Vertebrate pest control project, NARC, Islamabad, Pakistan.

TABLE 1. ANTICOAGULANTS, CONCENTRATIONS USED, AND MORTALITY IN *RATTUS RATTUS* WHEN FED NO-CHOICE FOR 4-DAYS.

Anticoagulant and concentration ppm	Mortality No. dead/ tested	Dose of a.i. consumed (mg/kg) Minimum Mean	Minimum to kill	Maximum survived	Mean day of death
Brodifacoum					
0.625	2/6	0.12	0.13	0.15	25.0
1.25	1/6	0.26	0.28	0.34	15.0
2.5	2/6	0.57	0.56	0.68	23.0
5.0	6/6	1.4	1.06	–	7.2
10.0	8/8	3.2	2.64	–	7.2
20.0	6/6	6.0	3.50	–	6.3
40.0	6/6	9.6	4.70	–	7.0
Bromadiolone					
0.625	1/6	0.13	0.14	0.17	28.0
1.25	2/6	0.27	0.20	0.39	22.5
2.5	2/6	0.61	0.42	0.93	16.0
5.0	5/6	1.4	1.05	1.12	7.6
10.0	6/6	3.3	2.71	–	9.3
20.0	6/6	5.4	4.53	–	8.3
40.0	6/6	11.7	10.60	–	10.0
Coumatetralyl					
5.0	1/6	1.2	0.89	1.86	4.0
10.0	2/6	3.0	3.00	3.36	24.5
20.0	5/12	4.5	2.48	7.34	8.0
40.0	4/6	8.3	4.62	11.84	10.2
80.0	6/6	12.2	8.00	–	10.0

TABLE 2. ESTIMATED FOUR-DAY LETHAL CONCENTRATIONS AND 95% FIDUCIAL LIMIT (PPM) OF THREE ANTICOAGULANT RODENTICIDES FOR *RATTUS RATTUS*.

Anticoagulant		Lethal concentration (ppm)	95% Fiducial limit lower	upper
Coumatetralyl	LC_{50}	19.6	9.8	38.3
	LC_{95}	126.4	54.7	460.8
Bromadiolone	LC_{50}	2.1	1.1	3.7
	LC_{95}	10.1	5.3	63.4
Brodifacoum	LC_{50}	1.8	0.9	3.1
	LC_{95}	8.4	4.,5	47.2

TABLE 3. ESTIMATED APPROXIMATE LETHAL DOSES (ALD) IN mg/kg FOR THREE ANTICOAGULANTS

Anticoagulant		Approx.lethal dose mg/kg	95% Fiducial limit Lower	Upper
Coumatetralyl	ALD_{50}	4.4	2.3	7.8
	ALD_{95}	22.0	–	–
Bromadiolone	ALD_{50}	0.51	0.24	0.94
	ALD_{95}	3.0	1.45	21.3
Brodifacoum	ALD_{50}	0.41	0.19	0.75
	ALD_{95}	2.4	1.15	15.8

exposure with brodifacoum and up to 28 days with bromadiolone. For coumatetralyl, there was delayed death in individual animals at all concentrations except at 5 ppm. One animal died 35 days after the first exposure to the 80 ppm level.

The lowest lethal dose for coumatetralyl, bromadiolone, and brodifacoum was 0.89, 0.14, and 0.13 mg/kg, respectively. The maximum dose survived was 11.84 mg/kg for coumatetralyl, 1.12 mg/kg for bromadiolone, and 0.68 mg/kg for brodifacoum. There was no difference in mortality between sexes when poisoned with coumatetralyl. Males died in greater numbers than females on both brodifacoum (77.3% dead vs. 63.6%) and bromadiolone (76.2% dead vs. 57.1%) but the difference were not statistically significant (x^2= 0.982 and 1.714, respectively).

Discussion

The toxicity to *R.r. refuscens* of the three anticoagulants tested in this study compared favourably to results previously reported in the literature. For brodifacoum, the consumption-derived ALD_{50} value was 0.40 mg/kg and the minimum lethal dose was 0.13 mg/kg. In comparison, Dubock and Kaukeinen [4] reported an acute oral LD_{50} value of 0.65 mg/kg for female *R.rattus* and 0.73 mg/kg for males, and Mathur and Prakash [9] report 0.77 mg/kg for both sexes.

The accuracy of the ALD_{50} values are limited because we had no way of knowing how much more toxicant would have been consumed than was needed to produce death. The method of calculation used here tends to over-estimate the ALD_{50} values. When comparing anticoagulants, the LC_{95} and ALD_{95} are more reliable indicators of the relative differences between rodenticides with regards to field use. This is because baits are designed to kill all the rodents. A comparison of the LC_{95} of brodifacoum with bromadiolone showed it was 1.2 times more toxic. Compared with coumatetralyl, it was 15 times more toxic.

Considerable variation apears to exist in the susceptibility of *R.rattus* to bromadiolone. In our study, the minimum lethal dose of bromadiolone was 0.14 mg/kg and the maximum dose survived was 1.12 mg/kg, with 100% mortality achieved at 0.001% and 0.002%. Redfern and Gill [10] found that *R.rattus* from the United Kingdom survived doses of bromadiolone ranging from 1.1 mg/kg to 13.4 mg/kg, given over 1 to 4-days periods and achieved 100% mortality only after 5 days, feeding on 0.005% bromadiolone. Hoppe and Krambias [7] reported 100% mortality of *R. rattus* after 1 day's feeding on 0.005% bromadiolone in Cyprus.

R.r. rufescens from Rawalpindi appear to be more susceptible to coumatetralyl than the same species in India. In our study , coumatetralyl gave 100% mortality in 4-days feeding at the 80 ppm concentration, well below its field use recommendation of 375 ppm. The field concentration is based on the least susceptible pest rodent, which is the house mouse. It is not surprising, therefore, that *R. rattus* is susceptible to lower levels. In contrast, in India, Girish *et.al.* [6] achieved

100% mortality only after 13 days of no-choice feeding on 250 ppm poison and Chopra and Parshad [2] obtained 100% mortality after 10 days of feeding on coumatetralyl at 375 ppm.

R.r. rufescens from Rawalpindi appeared to be quite susceptible to the three anticoagulants tested. Rodenticidal baits containing 50 ppm of brodifacoum and bromadiolone and 375 ppm of coumatetralyl should be more than adequate for field use in Pakistan to control *R. rattus* in households, stores, and godowns.

This study indicated that simple and rapid test protocols can be used to compare toxicities of anticoagulants to rodents. The feeding test protocol used here is not nearly as accurate as stomach intubation of graduated doses but doses not require the skills and sophisticated equipment. Also, it can be done using rodenticide concentrates instead of technically pure rodenticides. This does not imply that this method can replace the use of stomach intubation, because it does not have the accuracy of stomach gavage, which is a much better method where accuracy is desired.

The test method yields minimum doses needed to kill and maximum doses survived. If larger numbers of animals had been used per group, the minimum dose to kill would be smaller and the maximum dose survived would be larger. Accuracy would be improved with larger number of animals, but this partly defeats the simplicity of the test protocol.

The method gives the concentration of the active ingredient needed to achieved complete mortality in 4 nights of feeding. The testing could be shortened into a 2 or 3 day test, but a 4 day period tends to provide better data if animals are slow to respond to the diet. The results would differ from those presented here in percent mortality and mean intake of active ingredient, because of the shorter feeding periods. All that is required is to define whether a 2, 3 or 4 day LC_{50} and LC_{95} is described.

Acknowledgements. We thank Rick Engeman, Research Support Branch, Denver Wildlife Research Centre, for providing us the Probit analysis computer program from the Swedish Environmental Protection Board. Special appreciation is extended to Dale Kaukeinen, Imperial Chemical Industry Americas, Inc., and Melvyn V. Garrison, Peter Savarie, and Richard L. Bruggers, Denver Wildlife Research Centre (DWRC), for reviewing the manuscript. This study was financially supported by the U.S. Agency for International Development under the project "Food Security Management, Vertebrate Pest Control, PASA IPK-0491-P-IF-5017-05. We appreciate the support of the National Agricultural Research Centre (NARC), Islamabad and the Pakistan Agriculautral Research Council for providing laboratory and office space at NARC.

References

1. A.D. Ashton, W.B. Jackson and H. Peters, *Control of Mammal Pests,* CGJ Richards and T.Y. Ku, Eds., (Taylor and Francis, London, 1987), pp. 187.
2. G. Chopra and V.R. Parshad, Indian J. of Agric. Sci., **55**, 125 (1985).
3. A.C. Dubock, Proc. Vertebrate Pest Conf., **10**, 123 (1982).
4. A.C. Dubock and D.E. Kaukeinen, Proc. Vertebrate Pest Conf., **8**, 127 (1978).
5. R.M. Engeman, D.L. Otis and W.E. Dusenberry, J. Stat., Computation and Simulation, **25**, 237 (1987).
6. G.K. Girish, K. Singh, P.K. Srivastava and K. Krishnamurthy, K. Bull. Grain Tech., **10**, 113 (1972).
7. A.H. Hoppe and A. Krambias, A. In Proc. Conf., *Organization and Practice of Vertebrate Pest Control,* A.C. Dubock, Ed., (Imperial Chemical Industries PLC, Plant Protection Division, Fernhurst, UK, 1984), pp. 335.
8. D.E. Kaukeinen and M. Rampaud, Proc. Vertebrate Pest Conf., **12**, 16 (1986).
9. R.P. Mathur and I. Prakash, J. Hygiene **87**, 179 (1981).
10. R. Redfern and J. E. Gill, J. of Hygien, **84**, 263 (1980).
11. WHO, WHO/VBC/75.595 (1975),pp. 7.