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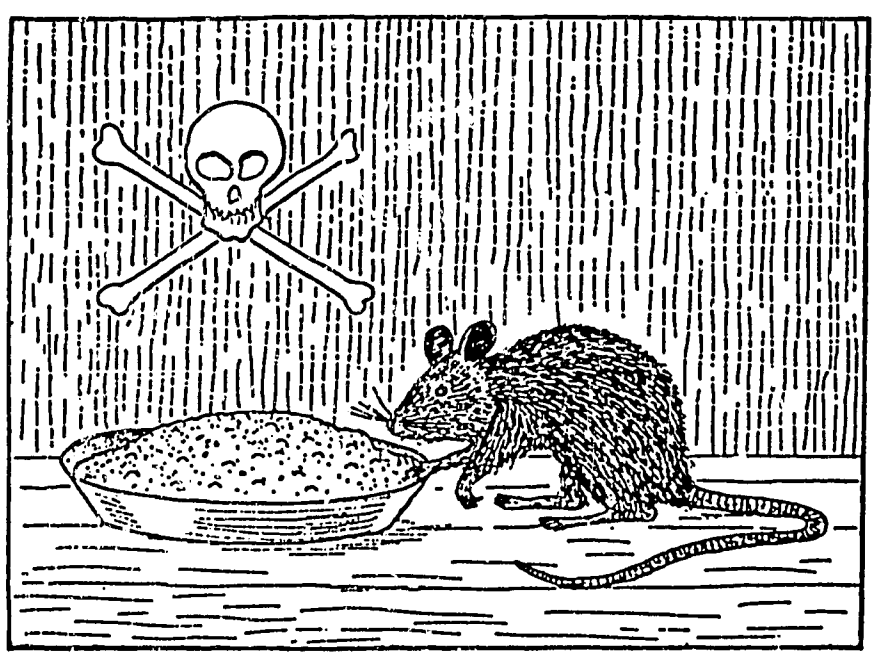

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THE CHRONIC ORAL TOXICITY OF SEVERAL ANTICOAGULANT RODENTICIDES TO *RATTUS RATTUS*

Joe E. Brooks*, Ejaz Ahmad* and Iftikhar Hussain*

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*Team Leader, Research Specialist and Scientific Officer, respectively, GOP/USAID/DWRC Vertebrate Pest Control Project, National Agricultural Research Centre, Islamabad, Pakistan.

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

By Joe E. Brooks[1], Ejaz Ahmad[2] and Iftikhar Hussain[2]

Abstract: A new method is described for determining the comparative toxicities of anticoagulant rodenticides against susceptible rodent species. The method uses a no-choice 4-day feeding test with small groups of rodents. By varying the concentration of the active ingredient, a value for the 4-day LC_{50} (lethal concentration that kills 50% of the test animals after 4-days feeding) can be statistically estimated from the mortality data using probit analysis. The 4-day LD_{50} can be estimated in the same way from the mean amounts of active ingredient consumed by each test group. The derived comparative toxicities of brodifacoum, bromadiolone and coumatetralyl to *Rattus rattus* from Rawalpindi, Pakistan, for 4-day LC_{50}s were 1.8 ppm, 2.1 ppm and 19.6 ppm, respectively, for the three compounds. The 4-day LD_{50}s were 0.41 mg/kg, 0.51 mg/kg and 4.4 mg/kg, respectively. These values indicate that *R. rattus* from Rawalpindi are reasonably susceptible to the three anticoagulants tested and that the usual recommended field concentration of each active ingredient are more than enough to give complete mortality in four days feeding or less.

INTRODUCTION

The new, "second generation" anticoagulant rodenticides, difenacoum, brodifacoum and bromadiolone, have created a large amount of interest in rodent control programs because they are so much more toxic than the older anticoagulants. Brodifacoum, being the most toxic of the group, has been proposed as a one shot poison, to be used repeatedly against a rodent population only once per week for 2 or 3 treatments (Dubock 1982). It was found that brodifacoum can kill as well in only a one night's feeding as the more commonly used acute poison, zinc phosphide.

Basic toxicity information about the "second generation" anticoagulants, with the exception of brodifacoum, has been sketchy and sometimes acute oral LD_{50} determinations have been used to compare anticoagulant materials. These are not appropriate when comparing slow-acting chronic poisons (Ashton et al. 1987). Several protocols have been used by other investigators to compare the toxicities of anticoagulants. The World Health Organization's (1975) protocol for determining the baseline susceptibility of rodents to various anticoagulants has been used but it is time consuming and requires a large number of test animals. Other researchers have fed groups of rats one or two concentrations of several anticogulants for periods of 1 to 7

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1 Team Leader, DWRC, Vertebrate Pest Control Project, NARC, Islamabad, Pakistan.

2 Research Specialist and Scientific Officer, Vertebrate Pest Control Project, NARC, Islamabad, Pakistan.

days but, in many cases, all the test animals are killed after 2 days of feeding, or sometimes even with one day of feeding, so the information gained about the comparative toxicity of the several materials is limited.

Studies of the toxicity of several "second generation" anticoagulants to *R. rattus* have been done in a half dozen countries during the past decade. Brodifacoum studies have been reviewed in detail in Kaukeinen and Rampaud (1986). These mainly consist of no-choice feeding tests of caged animals for varying numbers of days and at several concentrations. The majority of the studies showed that *R. rattus* was susceptible to brodifacoum in concentrations ranging from 0.00125% to 0.005%. All rats died at two to seven days feeding. Some *R. rattus* from India even gave complete mortality on one day's feeding on brodifacoum at 0.005% in food baits (Jain, Saxena and Nag 1982; Chopra and Parshad 1985) and Rai, Lal and Srivastava (1984) achieved complete mortality with one day's feeding at a 0.001% concentration. Lund (1981), working on Danish *R. rattus*, found 100% mortality when they fed only one day on 0.005% brodifacoum in rolled oats. Hoppe and Krambias (1984) reported complete mortality in *R. rattus frugivorous* from Cyprus when offered brodifacoum at 0.005% for one day in food baits. The conclusion to be drawn from these studies is that *R. rattus* is extremely susceptible to brodifacoum in concentrations ranging from 0.001 to 0.005%.

Efficacy studies of bromadiolone against *R. rattus* are not as extensive as those of brodifacoum. Hoppe and Krambias (1984) found that one day feeding no-choice on 0.005% bromadiolone gave 100% mortality of *R. rattus frugivorous* from Cyprus. Redfern and Gill (1980) found that bromadiolone at 0.005% was highly acceptable to *R. rattus* but that it took 5 days feeding on this concentration to give a complete kill.

Coumatetralyl studies on *R. rattus* are very limited. Girish, et al. (1972) in studies of *R. rattus* from Hapur, India, found that complete mortality resulted from feeding coumatetralyl at 0.025% when fed for 13 days with no-choice. The intake of coumatetralyl was the highest of the five anticoagulants tested, possibly indicating it was the most palatable. Chopra and Parshad (1985) have compared the efficacy of coumatetralyl against warfarin, brodifacoum and bromadiolone and found that coumatetralyl required 10 days of feeding to give a complete kill whereas brodifacoum killed all in one day of feeding and bromadiolone required 2 days for 100% mortality.

We utilized a new and simpler laboratory procedure that uses a progression of anticoagulant concentrations that is increased or decreased by a factor of two: 1.25, 2.5, 5, 10, 20 ppm, etc. (Sultana, et al. 1981). Groups of 6 animals, 3 of each sex, are offered these concentrations in food baits, without choice for 4 days. This time period is used because rodents usually have a normal daily food intake for 4 days when feeding on anticoagulants before food intake drops rapidly. Two or three day feeding periods could be used, but the longer period tends to provide

better data if one or more animals are slow to respond to the diet. Brodifacoum, as the wax block formulation, and coumatetralyl, as the 0.75% concentrate and the ready made bait, are the only anticoagulant rodenticides registered for use in rodent control in Pakistan. We were interested in the toxicity of bromadiolone also since this was the only anticoagulant we had on hand up to late 1986. This paper reports our findings on the comparative toxicities of brodifacoum, bromadiolone and coumatetralyl to *R. rattus* from Rawalpindi, Pakistan.

METHODS AND MATERIALS

Wild roof rats, *R. rattus*, were captured in live-capture traps from grain shops in Rawlapindi, Pakistan. They were individually caged in the laboratory and acclimated to laboratory conditions for three weeks. Rats were provided a diet of poultry mix and free access to water. On the day of the test they were given broken rice mixed with 1% corn oil and the anticoagulant rodenticide.

Anticoagulants used in this study were the concentrates 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 along with the 1% by weight of corn oil.

These diets were offered no-choice for 4 days. Each anticoagulant was given in several low concentrations, each concentration half or double that of the previous offering, depending upon mortality. Concentrations were offered until a level was reached that gave 100% or near complete mortality. Food cups were weighed to the nearest 0.1 g when first placed and were again weighed at the end of the 4-day exposure period. All spillage was recovered and accounted for. After the last poison offering, animals were returned to a plain rice diet.

Rats were weighed to the 0.1 g at the start of the trial. Each test group consisted of 3 males and 3 females. They were observed daily for 30 days for bleeding and mortality. All dead animals were necropsied to verify death from hemorrhage.

The 4-day LC_{50} (lethal concentration in ppm to kill 50% of the test animals following a 4-day feeding) was determined using a probit analysis computer program obtained by DWRC from the Swedish Environmental Protection Board. Likewise, it was possible to determine the 4-day LD_{50} of each material by the same method using the mean intake of active ingredient by each test group.

RESULTS

The observed mortalities, mean doses of active ingredients consumed and mean day to death are detailed in Table 1. Brodifacoum clearly is the most toxic of the three materials to

R. rattus, followed closely by bromadiolone and distantly by coumatetralyl.

The calculated 4-day LC_{50}s and 4-day LD_{50}s are given in Table 2. Brodifacoum is approximately 1.2 times more toxic than bromadiolone and 11 times more toxic than coumatetralyl based upon lethal concentration required for 50% mortality.

Table 1. Anticoagulants, concentrations used and mortality in *Rattus rattus* when fed no-choice for 4 days

Anticoagulant and Concentratation (ppm)	Mortality No. Dead/Tested	Dose of a.i. Consumed (mg/kg) Mean	Min. to Kill	Max. 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

The mean day of death was quite prolonged in brodifacoum and bromadiolone as the lower doses were approached, with some animals dying up to 26 days from first exposure with brodifacoum and up to 28 days with bromadiolone. In the case of coumatetralyl, there was prolonged death in individual animals at all

Table 2: Four-day LC50's (ppm) and 4-day LD50's of three anticoagulant rodenticides for *Rattus rattus*

	LC_{50} (ppm)	95% Confidence Limits	LC_{50} (mg/kg)	95% Confidence Limits
Coumatetralyl	19.6	9.8 - 38.3	4.4	2.3 - 7.8
Bromadiolone	2.1	1.1 - 3.7	0.51	0.24 - 0.94
Brodifacoum	1.8	0.9 - 3.1	0.41	0.19 - 0.75

concentrations except for the lowest, 5 ppm. One animal died 35 days after the first exposure to the 80 ppm level.

The lowest dose that killed was 0.89 mg/kg for coumatetralyl and 0.14 mg/kg and 0.13 mg/kg, respectively, for bromadiolone and brodifacoum. The maximum dose survived was 11.8 mg/kg for coumatetralyl, 1.1 mg/kg for bromadiolone and 0.68 mg/kg for brodifacoum. There was no difference in mortality between sexes when poisoned with coumatetralyl. Males were more susceptible to both brodifacoum (17 dead vs. 14) and bromadiolone (16 dead vs. 12) than were females.

DISCUSSION

The comparative toxicities of the three anticoagulants are similar to those found in the literature. Brodifacoum is the most toxic to *R. rattus* but only by a slight edge over bromadiolone. The 4-day LD_{50} value of 0.4 mg/kg compares favorably with the acute oral LD_{50} value of 0.65 mg/kg (for female *R. rattus*) and 0.73 mg/kg for males (Dubock and Kaukeinen 1978) and the 0.77 mg/kg for both sexes as given by Mathur and Prakash (1981). The minimal amount of 0.13 mg/kg to kill individual *R. rattus* helps explain the high degree of susceptibility of this species to brodifacoum. The 4-day feeding tests gave 100% mortality at a concentration of 5 ppm, indicating that the commonly used 50 ppm in prepared baits should be more than enough to give adequate kills of *R. rattus* in the field.

Bromadiolone was only slightly less toxic for *R. rattus*. It was 8 to 9 times more toxic than coumatetralyl. The minimum dose needed to kill was 0.14 mg/kg and the maximum dose survived was 1.1 mg/kg. Redfern and Gill (1980) found that *R. rattus* from the U.K. survived doses of bromadiolone ranging from 1.1 mg/kg to 13.4 mg/kg, given over 1 to 4 day periods. They achieved a complete kill of their test rats only after 5 days feeding on bromadiolone at 0.005%. Hoppe and Krambias (1984) reported that one day's feeding on bromadiolone at 0.005% gave 100% mortality in Cyprus *R. rattus*. We obtained complete kills in 4-day's feeding on bromadiolone at 0.001% and 0.002%. It is obvious that there is considerable geographic variation in the susceptibility of *R. rattus* to this material.

Coumatetralyl gave 100% mortality at the 80 ppm concentration, well below the 375 ppm as recommended for field use of this rodenticide. Individual animals survived doses as high as 11.8 mg/kg in 4-days intake. These findings, however, indicate that *R. rattus* from Rawalpindi, Pakistan, is much more susceptible to coumatetralyl than the same species from Hapur, India, which needed 13 days of no-choice feeding on the poison at 250 ppm to achieve 100% mortality (Girish et al. 1972). Similarly, Chopra and Parshad (1985) needed 10 days of feeding on coumatetralyl at 375 ppm to obtain 100% mortality in house rats from Ludhiana, India.

The data reported here indicate that *R. rattus* from Rawalpindi is quite susceptible to the three anticoagulants tested. When as little as 5 ppm of brodifacoum, 10 ppm of bromadiolone and 80 ppm of coumatetralyl will give complete mortality when the rats are allowed to feed no-choice for 4-days, it indicates that rodenticidal baits containing these anticoagulants at 0.005% and 0.0375% should be more than adequate for field use in Pakistan in control of *R. rattus* in households, stores and godowns.

This new test method provides a simpler and quicker means of deriving comparative toxicities of several anticoagulants against a given rodent species. It is a feeding test, similar to intubation of graduated doses by stomach tube, but not requiring the skills and sophisticated equipment needed for intubation. It is much easier, less time-consuming and requires fewer test animals than the WHO (1975) procedure. The test method yields minimum doses needed to kill and maximum doses survived. It gives the concentration of the active ingredient needed to achieve complete mortality in 4 nights of feeding. The testing could be shortened into a 2- or 3-day test, if desired. 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} is described.

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