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THE TOXICITY OF FOUR ANTICOAGULANT RODENTICIDES TO *RATTUS RATTUS MINDANENSIS* MEARNS

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UNIVERSITY OF THE PHILIPPINES AT LOS BANOS, IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS
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MASTER OF SCIENCE
(ENTOMOLOGY-APPLIED ZOOLOGY)

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The thesis attached hereto, entitled "The Toxicity of Four Anticoagulant Rodenticides to *Rattus rattus mindanensis* Mearns", prepared and submitted by Mrs. Melanda Mariano-Hoque, in partial fulfillment of the requirement for the degree of Master of Science (Entomology-Applied Zoology), is hereby accepted.

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ABSTRACT

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Four anticoagulant rodenticides were tested on *Rattus rattus mindanensis* to determine the acute median lethal dose, effect of concentration on the acceptance of bait, and the interaction between concentration and exposure time.

The acute oral LD50 in mg/kg for males and females are diphacinone- 1.13, 0.75; pival- 99.49, 92.60; warfarin- 705.00, 807.10; racumin- 477.00, 587.00, respectively. Except for diphacinone, the LD50 values were higher than the previously reported LD50's of these compounds to *Rattus norvegicus*. Thus, *R. rattus mindanensis* appeared to be less susceptible to pival, warfarin and racumin.

Diphacinone had the highest acceptance index followed by pival, warfarin, and racumin. It was observed that as the acceptance index increased the percentage mortality increased.

The interaction of chemical, feeding level, concentration and sex was insignificant with respect to bait and chemical consumption and mean days required for 100 per cent mortality of rats.

There was a decrease in bait consumption beyond 0.01% for diphacinone and 0.025% for pival, warfarin, and racumin, however, the total amount of chemical consumed increased with the increase in concentration. A slightly greater amount of bait and chemical was consumed by the rats in continuous and intermittent feeding than in three-day feeding.

There were no differences in the number of days required to obtain 100 per cent mortality of males and females. Most of the rats died from 4th to 8th day after exposure to the bait.

INTRODUCTION

Rodenticides have been widely used in the control of various rodent pests. The rodenticides in common use are either chronic or acute type. The acute type rodenticides, commonly known as single dose, act rapidly on the heart, nervous system, gastro-intestinal tract or excretory organs. The chronic types, on the other hand, are anticoagulant compounds which, when introduced into the body, prevent coagulation of blood. Their effect mostly develop within a period of 3 to 9 days (Schoof and Maddock, 1968). Chemically, the anticoagulants fall within two groups: coumarin derivatives, such as warfarin, fumarin, racumin, coumachlor, and dicoumarin (Schoof and Maddock, 1968); and the indandione groups, such as pival, diphacinone, radione, valone, and chlorophacinone. The anticoagulants differ in rodenticidal activity, the concentrations vary from 0.005% to 0.05% depending upon the compound. Although the acute type rodenticides are fast acting, more bait shyness is observed in the acute types than in the chronic types. Morover, the chronic type rodenticides are relatively safer for domestic animals and man (Rowe et al., 1970).

These properties of the anticoagulants have led to their wide use in the developed countries. In Asia,

however, anticoagulants have not been widely used in agricultural situations despite the persistent problems with rodent pests, mainly because of incomplete understanding of their use (Brown, 1970). In the Philippines, although both types of rodenticides are already employed in the control of rodent pests, the anticoagulants are mostly confined to pest control operators due to the same reason. Morover, while some information are available on the toxicity of anticoagulants on some species of rats, little information is available on Rattus rattus mindanensis Mearns which is an endemic sub-species widespread throughout the Philippines. These situations thus call for studies to obtain first hand knowledge about the use of anticoagulants. With the prospect of increased usage of these materials in the country, there is an urgent need to obtain basic information about these materials relative to R. rattus mindanensis, an important pest of agricultural crops.

In the light of the above circumstances, the present studies were undertaken with the following objectives: a) to determine the acute median lethal doses of four anticoagulant rodenticides, b) to determine the relationship between concentration and acceptance of baits, and c) to study the interaction between concentration and

exposure time. The information gathered here may provide the necessary basic toxicological information to determine appropriate commercial concentrations for field use and to set minimum number of days of regular feeding for effective field use against the Philippine field rat.

REVIEW OF LITERATURE

The anticoagulants have been available since the early 1950's (Dykstra, 1951). Dykstra mentioned that these materials were originally considered to provide final solutions to rodent infestations. However, many problems have limited their effectiveness. A current problems in some areas in Europe and the United States of America, where anticoagulants have been used intensively for several years, was the development of genetically resistant populations of rodents (Greaves, 1970, 1971; Jackson, 1969; Jackson et al., 1971). In addition to this problem, it is possible that ineffective control with these materials could result from a variety of causes. However, these problems have not precluded the continued use of the anticoagulants.

Comparison of the efficacy of different anticoagulants has been made by Hayes and Gaines (1959). These workers showed that warfarin and diphacinone were more toxic to

Rattus norvegicus Berkenhout as compared to pival. At 50 ppm, warfarin and diphacinone had a slightly greater bait refusal than pival. In test against the Roof rat (*Rattus rattus*), Hayes and Gaines (1959) reported that at a dose of either 18.1 mg/kg for warfarin or 17 mg/kg for pival 100% mortality was obtained. Bentley and Larthe (1959), as cited by Schoof and Maddock(1969), reported diphacinone at 0.0125% concentration to be less acceptable than warfarin at 0.025% concentration. Diphacinone was equal or superior to warfarin, but Bentley and Larthe (1959) considered diphacinone to offer a slightly greater risk to domestic animals. In fact, it was the anticoagulant most toxic to rodents. Jackson (1969) reported that racumin was a toxic as warfarin and other anticoagulants. Further, it has higher levels of bait acceptance than other anticoagulants (Jackson, 1969).

MATERIALS AND METHODS

Anticoagulants

The anticoagulants tested were: warfarin 98% (3/alpha-acetonyl 3 benzyl/4-hydroxycoumarin), racumin 60% (4-hydroxy-3/1,2,3,4,-tetrahydro-1-napthyl/coumarin), pival 100% (2-pivalyl, 1,3-indandione), diphacinone 100% (2-diphenyl-1,3-indandione)

Test Animals

Field collected male and female rats were used in this study. Certain conditions were followed prior to and during the test as suggested by Drummond (1970). The animals were provided with suitable accomodations, where disturbance were kept to a minimum of one week before they were tested. During each test the animals were caged and given the appropriate diet. Water was made available at all times. Mature (not pregnant) and healthy (not bearing recently healed wounds) animals were used. In all the tests, no control animals were used since a preliminary study showed that field collected rats caged in similar cages as used in this tests do not die for as long as five months.

Determination of LD50

he LD50, the dose lethal to 50% of the test population, was determined for each of the four rodenticides. A single dose of the poison formulated at mg/ml using corn oil as carrier was administered by gavage to each of the test animals (Kverno, 1970). Eight animals (4 males and 4 females) were treated with each of the four geometrically spaced dosage levels. The animals were fasted for 12 to 24 hours before administering the toxicant. The animals were observed daily for 14 days after

treatment. The animals were maintained on water and pellets as feeds, all offered ad libitum. The data obtained were then used to compute acute oral LD50 values by the method of Thompson (1947) and Thompson and Weil (1952).

Determination of Acceptance of Treated Baits

After the determination of LD50 for each of the four chemicals, acceptance test were conducted. The animals (5 males and 5 females) whose weight did not vary more than 20% from the mean animal weight were used. Three days before the test, 18 grams of polished rice was given to each animal. The amount of rice consumed during this period was recorded to determine the average daily consumption. The dry poisoned baits were prepared by mixing appropriate amounts of the chemical with rice. One LD50(previously determined) was mixed gradually with one-tenth of the average daily consumption. The poison was mixed gradually with the amount of rice and sticker (paraffin liquid) needed in a beaker and was stirred up with a spoon until thoroughly mixed. The animals were offered free-choice of unpoisoned pellet, and rice containing an appropriate concentration of the rodenticide being tested. The two feeds were offered so as not to force the animals to feed on the treated

bait. The animals were fed for three days and were observed for 14 days. Everyday the animals were given more food than what can be actually consumed during the following 24 hours. On each subsequent day of feeding the unconsumed baits (including any spillage) was removed and a weighed amount of freshly prepared bait was given to the animals. After three days of feeding on the treated bait, the animals were fed with the ordinary laboratory diet which was used as the alternate food during the test. The acceptance index was computed by dividing the total bait consumption during the first 24 hours of feeding by one-tenth of the daily diet then divided by the number of animals used.

Effect of concentration and Feeding Level on the Consumption of Bait and Chemical, and Mortality of Rats

Three days before the test, 18 grams of polished rice was given to each animal. The amount of rice consumed during this period was recorded to determine the average daily consumption. After the average daily consumption was determined, the bait and unpoisoned ordinary laboratory diet were offered. Rice was chosen as the food in which to offer the poison because it was proven to be the most acceptable under field conditions (Hayes and Gaines, 1959). Laboratory diet was used as the

poison-free food because it was the food to which the rat was conditioned before the test began. The dry poison bait was prepared by mixing the anticoagulant at the desired concentration with the bait with a spoon in a beaker and stirred up by hand. Corn oil was used as sticker of the chemical on the bait. The poison was mixed with a small quantity of the rice, adding the rest of the rice gradually to ensure oven mixing of the poison. Sixteen rats per sex per rodenticide were tested. The concentration was selected on the basis of the recommended concentration for R.norvegicus which is 0.025% for pival, warfarin and racumin and 0.005% for diphacinone. Four concentrations per chemical were tested: (1) one-half the recommended dose, (2) recommended dose, (3) double the recommended dose, and (4) quintaple the recommended dose. The feeding levels tested were as follows: (1) continous feeding of the poison for three days, (2) continous feeding of the poison until all rats died, and (3) intermittent feeding (every other day feeding) until all rats died. Each animal was offered the poisoned bait was well as the ordinary laboratory diet (alternate food) in all the tests. The two feeds were offered so as not to force the animals to feed on the treated bait. Animals that were fed three days of the

poison were maintaned on the alternate food after the test. The animals fed continously and intermittently were given the same ordinary laboratory diet as food. During the tests, daily consumption was recorded. On each subsequent day of feeding, the unconsumed baits (including any spillage) was removed and a weighed amount of freshly prepared bait was given to the animals. The animals were observed as to when sympton of poisoning and death occurred.

The amount of chemical (mg/kg) was computed by multiplying the amount of bait consumed by the concentration then divided by the weight of the animal. This was done to determine the relative amount of rodenticidal material taken by an animal until it dies.

The data were statistically analyzed at the Los Banos Computing Center using four factor factorial in completely randomized design.

RESULTS AND DISCUSSION

Determination of LD_{50}

The acute oral LD50 and confidence limits are shown in Table 1. Based on these data the toxicity of the four compounds are in the following order: males-diphacinone is 88x > pival, 624X > warfarin, and 423X > racumin; pival is 7X > warfarin, 4.8X > racumin and

Table 1. The acute median lethal dose and confidence limits of four anticoagulant rodenticides to *Rattus rattus mindanensis*

Compound	Sex	LD50 mg/kg	Confidence Limit (p=0.05) Lower	Upper
Diphacinone	Male	1.13	0.25	1.65
	Female	0.75	0.18	1.13
Pival	Male	99.49	67.89	145.18
	Female	92.60	45.91	105.90
Warfarin	Male	705.00	505.40	963.40
	Female	807.10	528.70	1231.00
Racumin	Male	477.70	326.20	669.80
	Female	587.90	303.20	1140.00

racumin is 1.4X> warfarin; females - diphacinone is 123X> pival, 1076X> warfarin, and 784X> racumin; pival is 9X> warfarin and 6.4X> racumin; and racumin is 1.3X> warfarin. The toxicity of the four anticoagulants in their decreasing order was : diphacinone> pival> racumin> warfarin.

It is clear from Table 1 that the LD50's obtained of Rattus norvegicus as reported by Thomson (1972) were as follows: diphacinone - 3 mg/kg, warfarin - 186 mg/kg, pival - 50 mg/kg, and racumin - 16.5 mg/kg. Similarly, Gaines (1960), as cited by Schoof and Maddock (1968), reported the following LD50 values: diphacinone - 1.4 to 2.5 mg/kg, warfarin 2.3 to 3.8 mg/kg, and pival 223 to 336 mg/kg. The higher LD50 values obtained with warfarin and racumin tend to show that the endemic sub-species, R. rattus mindanensis is less susceptible to these compounds as compared to R. norvegicus. This is further supported by the findings of Decras et al. (1972) and Jackson (1969) who reported that R. rattus is more resistant than R. norvegicus to these anticoagulants. The low LD50 values obtained with diphacinone was in agreement with those reported in previous studies (Thomson, 1972; Schoof and Maddock, 1968). Table 1 also shows that the response

of the individual animals varied considerably as shown by the wide ranges.

It should be pointed out here that the acute toxicity was primarily determined for the purpose of establishing baseline studies which may provide a basis for gauging development of rat resistance with intensive use of these materials, and to determine the acceptance of treated baits.

Determination of Acceptance of Treated Baits

The acceptance of treated bait containing one LD50 on one-tenth of daily diet by individually caged *Rattus rattus mindanensis* are presented in Table 2. The order of decreasing efficiency of the four compounds were: based on acceptance index - diphacinone > pival > warfarin > racumin, and based on mortality - diphacinone > pival = warfarin > racumin. It shows that the highest and lowest amounts of bait were taken when fed with diphacinone and racumin - treated bait, respectively. The total amount of chemical consumed was highest in warfarin and lowest in diphacinone. Except for racumin, the acceptance index was higher in males than in females in all the animals. Although the concentration of warfarin bait was higher than that of pival, the mortalities were the same since the latter had a slightly higher acceptance

Table 2. Acceptance of treated bait containing one LD50 on 0.1 of daily diet by individually caged *Rattus rattus mindanensis* during three days of feeding

Compound	Sex [a]	Chemical concentration (%)	Total bait consumed (g)	Total chemical consumed (mg/kg)	Acceptance index [b]	Mortality within 14 days (%)
Diphacinone	Male	0.016	32.5	24.33	8.14	100
	Female	0.008	26.0	12.67	7.45	80
Pival	Male	1.100	5.3	323.88	1.58	67 [c]
	Female	1.000	3.1	238.48	1.28	60
Warfarin	Male	6.800	3.6	1215.80	1.28	67 [c]
	Female	8.400	2.2	1920.60	0.83	60
Racumin	Male	5.020	1.6	420.53	0.62	60
	Female	5.560	1.7	737.17	0.75	40

[a] Five animals were used unless specified

[b] Number of LD50's consumed during the first 24 hours of feeding. Computed by dividing the total bait consumption (first 24 hours feeding) by one-tenth of the daily diet then divided by the number of animals used

[c] Six male animals were used instead of five

index. Maybe at higher concentration the bait was less palatable, which would account for the lower acceptance of warfarin as compared to pival.

Warfarin exhibited higher per cent mortality than that in racumin, because warfarin had a higher acceptance index. Although it was reported that acceptance of racumin treated bait by R. norvegicus is better than warfarin bait (Jackson, 1969), R. rattus mindanensis preferred warfarin to racumin.

Effect of Concentration and Feeding Level on the Consumption of Bait and Chemical, and Mortality of Rats

The amount of bait (g/rat) and chemical (mg/kg) consumed as affected by concentration, feeding level, and sex are presented in Table 3. The analyses of variance showed an insignificant interaction of the chemical, feeding level, concentration and sex (Appendix 1 and 2).

There was a decrease in bait consumption beyond 0.01% in diphacinone-treated bait in all feeding levels. In general, there was a decrease in bait consumption beyond 0.025% with pival-, racumin-, and warfarin-treated bait except at 0.05% with warfarin (male) and pival (male and female) at continous feeding. Probably, this variability was due to the differences in size and

Table 3. Amount of bait and chemical consumed per rat until it rats died within 23 days

Concentration[b] (%)	Amount of bait consumed[a]					
	Three days feeding		Continuous feeding until death		Intermittent feeding until death	
	Male	Female	Male	Female	Male	Female
			DIPHACINONE			
0.0025	15.8	38.2	39.6	33.6	39.0	52.1
0.005	25.1	31.6	39.0	50.6	35.0	34.1
0.010	25.8	32.5	58.2	52.9	53.6	35.8
0.025	13.7	25.0	38.5	41.2	32.2	22.1
			PIVAL			
0.0125	20.7	19.7	19.5	34.1	23.7	50.0
0.025	28.2	28.1	51.5	34.2	40.0	27.3
0.050	18.0	18.0	28.1	25.8	39.1	38.0
0.125	10.7	10.5	15.3	15.0	16.8	22.7
			WARFARIN			
0.0125	29.2	22.6	27.6	38.6	37.8	26.1
0.025	34.3	24.1	28.6	30.8	61.3	33.2
0.050	33.6	14.2	49.2	27.6	50.1	20.1
0.125	10.7	10.5	15.0	15.3	16.8	22.7
			RACUMIN			
0.0125	16.5	21.1	16.8	29.5	31.7	48.2
0.025	16.8	25.1	45.0	49.3	21.5	45.1
0.050	21.6	22.8	66.1	62.5	22.3	45.0
0.125	15.7	22.1	35.8	52.1	24.8	34.7

[a] Average of 4 animals

[b] The concentrations used were ½X, 1X, 2X, and 5X of the diphacinone and 0.025% for pival, warfarin and racumin

died as affected by concentration, feeding level, and sex. All

Amounts of chemical consumed (mg/kg) a					
Three days feeding		Continuous feeding until death		Intermittent feeding until death	
Male	Female	Male	Female	Male	Female
		DIPHACINONE			
2.17	3.85	4.85	7.35	6.67	7.20
2.52	8.70	15.27	16.27	3.35	10.92
12.65	19.02	29.47	38.75	4.52	23.60
20.52	35.67	69.12	78.12	5.37	38.50
		PIVAL			
16.42	29.32	11.10	24.75	14.30	35.90
20.72	40.72	17.60	47.67	58.82	70.65
60.12	60.22	61.57	76.35	92.25	157.65
106.50	104.42	93.20	171.35	111.35	220.17
		WARFARIN			
16.65	24.47	23.35	28.35	26.67	25.62
48.87	43.60	38.37	61.65	86.60	68.50
74.47	164.67	143.45	100.57	139.70	59.50
234.00	202.80	358.12	304.10	266.62	171.02
		RACUMIN			
11.55	16.67	15.52	25.20	28.55	42.67
42.67	42.67	73.50	81.30	21.92	77.95
62.50	89.37	232.00	216.32	48.55	183.62
122.55	215.05	238.42	473.20	157.85	380.77

recommended concentration for R.norvegicus which is 0.005% for

age of the rats used in the study. It is probable that the decrease of consumption beyond a certain concentration was due to the unpalatability of the bait is due to the increased amount of the active ingredient of the chemical. This agrees with findings of Cornwell (1970) that the palability of bait influences bait consumption by rats. Cornwell reported that warfarin at a concentration of 0.05% had a lower bait intake by rats as compared to an encapsulated bait at the same concentration because the encapsulated bait was more palatable since the active ingredient could hardly be detected by the rats.

In general, the amount of bait consumed was slightly higher in continuous and intermittent feeding than at three-day feeding. This may be due to shorter exposure of animals to bait at three-day feeding.

Table 3 shows that as the concentration increased there was a corresponding increase in the amount of chemical consumed in all the feeding levels. This is true because more chemical is present at higher concentration. In most cases, there seemed to be no differences in the bait consumption between male and female except in three-day feeding with diphacinone-treated bait at all concentrations. This shows that the lack of significance in the amount of bait consumption between male and female rats is probably due to the differences in size and age of the

animals used in the test. Hayes and Gaines (1959) reported in their study that field-collected Rattus rattus specimens tended to show greater variability of dose-response than field-collected R. norvegicus. They further reported that field-collected R. rattus is less susceptible than field-collected R. norvegicus to warfarin coumachlor, pival and PMP.

Table 4 shows the mean days required to obtain 100 per cent mortality of animals exposed to four concentrations of four rodenticides at three feeding levels. The analysis of variance showed an insignificant interaction of the chemical, feeding level, concentration and sex (Appendix 3) although with diphacinone, the females required more days to obtain 100 per cent mortality than the males except at 0.010% at three-day feeding and 0.025% in continuous feeding. The same variable response has been observed by Hayes and Gaines (1959) on R. rattus to warfarin, pival, coumachlor and PMP. The differences in response is may be due to the differences in the ability of individual animal to produce vitamin K necessary to counteract the action of the anticoagulant. In warfarin, pival, and racumin there seemed to be no differences between male and female rats. The results agree with the studies of Hayes and Gaines (1959) that there were no differences in susceptibility between sexes. However,

Table 4. Mean days required to obtain 100 per cent mortality of rats exposed to four rodenticides at three feeding levels at four concentrations

Concentration[b] (%)	Average days elapsed until death[a]					
	Three days feeding		Continuous feeding until death		Intermittent feeding until death	
	Male	Female	Male	Female	Male	Female
			DIPHACINONE			
0.0025	4.50	10.75	3.40	7.25	5.25	7.25
0.005	4.25	10.75	5.50	6.25	5.50	5.00
0.010	8.25	6.25	6.00	6.25	6.50	8.00
0.025	3.75	5.00	6.00	4.75	4.50	8.00
			PIVAL			
0.0125	5.50	5.50	3.50	7.75	5.50	8.00
0.025	11.75	11.75	5.50	10.00	5.75	7.25
0.050	7.00	7.00	5.75	8.00	5.75	8.50
0.125	8.50	8.50	7.00	5.25	6.00	6.00
			WARFARIN			
0.0125	5.75	6.25	3.50	5.00	5.50	8.75
0.025	5.25	6.00	5.50	5.00	14.00	9.75
0.050	4.50	5.00	5.75	5.00	8.50	7.00
0.125	10.50	4.50	7.00	5.00	9.75	8.25
			RACUMIN			
0.0125	8.25	5.50	5.00	4.00	5.25	8.00
0.025	6.50	9.75	4.25	6.25	5.00	8.00
0.050	7.25	6.25	5.50	7.00	7.50	9.00
0.125	4.25	7.50	4.50	7.75	8.75	5.75

[a]Average of 4 animals

[b]The concentrations used were ½X, 1X, 2X, and 5X of the recommended concentration for R.norvegicus which is 0.005% for diphacinone and 0.025% fof pival, warfarin, and racumin

the pregnant females are usually more susceptible than the males.

There seemed to be no differences in the number of days required to obtain 100 per cent mortality among the feeding levels in all the chemicals (Table 4). This shows that three-day baiting is enough to obtain 100 per cent mortality, hence it is more economical for field use in terms of bait and labor.

The number of dead animals after exposure to four rodenticides at four concentrations at different feeding levels are shown in Table 5. It shows that most of the animals died from 4th to 8th day. Some animals died beyond 8 days, however, most of these animals took lesser amount of bait than those that died earlier. The result of this study agrees with the report of Schoof and Maddock (1968) that the effect of anticoagulants was observed within a period of three to nine days.

Table 5. Number of dead rats after exposure to four rodenticides

Days after exposure	Three days feeding							
	Male				Female			
	0.0025[a]	0.005[b]	0.010[c]	0.025[d]	0.0025	0.005	0.010	0.025
1	0	0	0	0	0	0	0	0
2	1	0	0	1	0	0	0	0
3	0	0	0	1	0	0	1	0
4	1	3	1	1	0	0	0	0
5	0	0	0	0	2	1	0	4
6	2	0	0	1	0	0	0	
7		1	1		0	0	2	
8			0		0	2	1	
9			0		0	0		
10			0		1	0		
11			2		0	0		
12					0	0		
13					0	0		
14					0	0		
15					0	0		
16					0	0		
17					0	0		
18					0	0		
19					0	0		
20					0	0		
21					0	0		
22					1	1		
23								

[a] One-half the recommended concentration for *R. norvegicus*

[b] The recommended concentration

[c] Twice the recommended concentration

[d] Five times the recommended concentration

at four concentrations at different feeding levels

DIPHACINONE							
Continuous feeding until death							
Male				Female			
0.0025	0.005	0.010	0.025	0.0025	0.005	0.010	0.025
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
2	1	1	0	0	0	0	1
2	1	0	0	0	0	0	0
	0	1	3	1	2	0	1
	0	1	0	1	1	3	1
	1	0	0	1	0	1	1
	1	0	1	0	1		
		1		0			
				0			
				1			

Intermittent feeding until death							
Male				Female			
0.0025	0.025	0.010	0.025	0.0025	0.005	0.010	0.025
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	2	1	0	1	0	0
0	0	0	0	0	1	0	0
3	3	0	3	1	1	0	0
1	0	0		0	0	1	1
	1	0		1	0	0	0
		0		1	1	2	2
		0		1		0	0
		2				1	1

Three days feeding							
Male				Female			
0.0125[a]	0.025[b]	0.050[c]	0.125[d]	0.0125	0.025	0.050	0.125
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	1	0	0	0	1	0
2	0	1	1	2	0	1	1
1	1	1	1	1	1	1	1
0	0	0	0	0	0	0	0
0	1	0	0	0	1	0	0
0	0	0	1	0	0	0	1
1	0	0	0	1	0	0	0
	0	0	0		0	0	0
	0	0	0		0	0	0
	0	0	0		0	0	0
	0	0	0		0	0	0
	0	0	0		0	0	0
	0	1	0		0	1	0
	1		1		1		1
	1				0		
					1		

PIWAL

Continuous feeding until death

Male				Female			
0.0125	0.025	0.050	0.125	0.0125	0.025	0.050	0.125
0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
1	0	0	1	0	0	0	0
1	0	3	2	0	0	0	3
1	2	0	0	0	0	1	0
	0	1	1	1	0	0	0
	1			0	1	0	0
	1			2	0	1	0
				1	1	0	1
					0	1	
					1	0	
					1	0	
						1	

Three days feeding							
Male				Female			
0.0125[a]	0.025[b]	0.050[c]	0.125[d]	0.0125	0.025	0.050	0.125
0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0
0	0	0	0	0	1	0	0
0	2	0	0	0	0	2	3
3	1	2	1	0	0	0	0
0	0	1	1	3	2	2	1
0	0		0	1	0		
1	1		1		1		
			0				
			0				
			0				
			0				
			0				
			0				
			0				
			0				
			0				
			0				
			0				
			0				
			1				

Intermittent feeding until death							
Male				Female			
0.0125	0.025	0.050	0.125	0.0125	0.025	0.05	0.125
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	2	1	1	0	0	0	0
2	0	0	0	2	0	0	2
0	0	1	1	0	1	0	0
1	0	0	1	0	0	0	0
0	1	1	0	0	0	2	0
1	0	1	1	0	3	1	2
	0			0		0	
	1			0		0	
				1		0	
				0		1	
				1			

WARFARIN							
Continuous feeding until death							
Male				Female			
.0125	0.025	0.050	0.0125	0.0125	0.025	0.050	0.125
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
2	0	0	1	1	0	0	0
2	2	0	3	1	1	3	1
	0	3	1	1	1	0	2
	0	0	0	0	0	0	1
	2	0	1	0	0	0	
		1	0	1	1	1	

Intermittent feeding until death							
Male				Female			
0.0125	0.025	0.050	0.125	0.0125	0.025	0.050	0.125
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1
2	0	0	0	0	0	0	0
0	0	0	0	0	0	2	0
0	0	0	0	0	0	0	0
2	0	1	3	2	0	0	0
	1	1	0	0	0	1	0
	0	1	0	1	0	0	0
	0	1	0	0	1	1	3
	0		0	0	1		
	0		0	1	0		
	0		0		1		
	0		0		1		
	0		0				
	0		0				
	3		1				

Three days feeding							
Male				:	Female		
0.0125[a]	:0.025[b]	:0.050[c]	:0.125[d]	:0.0125	:0.025	:0.050	:0.125
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	1	0	1	0
0	1	0	3	1	0	1	1
2	0	0	1	0	2	1	1
0	0	0		1	0	0	0
0	2	3		0	0	0	0
0	1	1		0	0	0	0
0				1	0	0	1
0					0	0	0
0					0	0	0
0					0	0	1
0					0	1	
0					1		
0					1		
1							
1							

RACUMIN

Continuous feeding until death

Male				Female			
0.0125	0.025	0.050	0.125	0.0125	0.025	0.050	0.125
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
1	3	0	1	0	0	0	0
0	0	2	0	4	1	0	0
2	0	0	3		1	0	0
1	0	1			1	2	2
	0	0			0	1	0
	1	1			0	0	0
					0	1	1
					1		

Intermittent feeding until death							
Male				Female			
0.0125	0.025	0.050	0.125	0.0125	0.025	0.050	0.125
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	1
1	4	0	1	0	0	0	1
0		3	0	2	0	0	1
0		0	1	1	4	2	1
0		0	0	0		0	
1		0	0	0		0	
		0	0	1		2	
		0	1				
		1	1				

SUMMARY AND CONCLUSION

At the Rodent Research Center, University of the Philippines at Los Banos, four anticoagulant rodenticides were tested on Rattus rattus mindanensis to determine the acute median lethal dose of the rodenticides; effect of concentration on the acceptance of baits; and interaction between concentration and exposure time.

The acute oral LD50 was determined through single dose by gavage. The LD50's in mg/kg were as follows: males- diphacinone 1.13, pival 99.49, warfarin 705.00, racumin 477.70; females- diphacinone 0.75, pival 92.60, warfarin 807.10 and racumin 587.90. Based on the acute LD50, the toxicity of four rodenticides in their decreasing order was: diphacinone > pival racumin warfarin. R. rattus mindanensis was considered to be less susceptible to pival, warfarin and racumin as compared to R. norvegicus.

The acceptance of treated bait by individually caged R. rattus mindanensis was assessed. It was found that diphacinone has the highest acceptance index followed by pival, warfarin and racumin. It showed that as the acceptance index increases the percentage mortality also increased.

The effect of four concentrations and three feeding levels were studied. The statistical analyses

showed that there was no significant interaction of the chemical, feeding level, concentration, and sex with respect to bait and chemical consumption and mortality of rats. In general, there was a decrease in bait consumption beyond 0.010% with diphacinone and 0.025% with pival, warfarin, and racumin. As the concentration increases there was a corresponding increase in the amount of chemical consumed in all the feeding levels. A slightly higher amount of bait and chemical was consumed by rats at continuous and intermittent feeding than in three-day feeding.

There were no differences between males and females in the number of days required to obtain 100 per cent mortality. Most of the animals died from 4th to 8th day.

Based on the amount of bait and chemical consumption and 100 per cent mortality, three-day feeding could be recommended for field use, since the result of this study showed that three-day feeding required lesser amount of bait and chemical than in continuous and intermittent feeding.

Based on the mean days required for 100 per cent mortality, there was no significant differences between concentrations, thus for economical use the lowest concentration could be recommended.

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Appendix 1. The analysis of variance for the amount of bait taken by R.rattus mindanensis fed with anticoagulant rodenticides [a]

ANOVA

SV	DF	SS	MS	F
Replication	3	10.765	11.675	0.922 ns
Feeding level (F)	2	17970.833	8985.416	41.403 *
Sex (S)	1	22.912	22.912	0.105 ns
F X S	2	88.535	44.267	0.203 ns
Chemical (H)	3	6182.721	2060.907	9.496 **
F X H	6	4530.862	755.143	3.479 **
S X H	3	6260.846	2086.948	9.610 **
F X S X H	6	3281.541	546.923	2.520 *
Concentration (C)	3	5178.211	1726.070	7.953 *
F X C	6	3488.920	581.486	2.679 *
S X C	3	3220.814	1073.604	4.947 **
F X S X C	6	704.347	117.391	0.540 ns
H X C	9	4872.305	541.367	2.494 **
F X H X C	18	4908.353	383.797	1.768 *
S X H X C	9	1524.153	169.350	0.780 ns
F X S X H X C	18	1711.509	95.083	0.438 ns
Error	285	62501.797	217.020	
Total	383	128448.800		

[a] 4 rats/concentration/feeding level/chemical

ns- Not significant

*- Significant at 5% level of significance

**- Highly significant at 1% level of significance

Appendix 2. The analysis of variance for the total chemical (mg/kg) consumed by R.rattus mindanensis fed with anticoagulant rodenticides [a]

ANOVA

SV	DF	SS	MS	F
Replication	3	0.000062	0.000062	0.007579 ns
Feeding (F)	2	0.001250	0.000625	21.008239 * *
Sex (S)	1	0.000255	0.000255	8.576095 * *
F X S	2	0.000038	0.000019	0.644953 ns
Chemical (H)	3	0.005555	0.001851	62.216263 **
F x H	6	0.000915	0.000152	5.126954 * *
S x H	3	0.001086	0.003620	12.163356 * *
F x S x H	6	0.000383	0.000063	2.145631 *
Concentration (C)	3	0.014080	0.004693	157.705090 * *
F x C	6	0.000385	0.000139	4.676419 * *
S x C	3	0.000236	0.000078	2.646613 *
H x C	9	0.004341	0.000482	16.207812 * *
F x S x C	6	0.000096	0.000016	0.541865 NS
S x H x C	9	0.001155	0.000128	4.315350* *
F x H x C	18	0.000852	0.000047	1.590544 NS
F x S x H x C	18	0.008482	0.000029	0.587160 NS
Error	285	0.008482	0.000029	
Total	383	0.039946		

[a] 4 rats/concentration/feeding level/chemical
* - Significant at 5% level of significance
** - Highly significant at 5% level of significance
NS- Not significant

Appendix 3. The analysis of variance for mean days required to obtain 100 per cent mortality of *R. rattus mindanensis* exposed to anticoagulant rodenticides [a]

ANOVA

SV	DF	SS	MS	F
Replication	3	0.403307	0.134435	0.464641 ns
Feeding level (f)	2	5.967877	2.983938	10.313184 **
Sex (S)	1	3.096886	3.098686	10.703558 **
F x S	2	0.476512	0.238256	0.823468 ns
Chemical (H)	3	0.652585	0.217528	0.751828 ns
F x H	6	5.842979	0.973829	3.365781 **
S x H	3	0.652585	1.113605	3.848865 *
F x S x H	6	1.666907	0.277817	0.960202 ns
Concentration (C)	3	2.240518	0.746839	2.581250 ns
F x C	6	1.621367	0.270227	0.933997 ns
S x C	3	1.355738	0.451912	1.561915 ns
H x C	9	4.029849	0.447761	1.547566 ns
F x S x C	6	1.632344	0.272057	0.940293 ns
S x H x C	9	4.412817	0.4903133	1.694635 ns
F x H x C	18	7.087115	0.393728	1.360817 ns
F x S x H x C	18	7.341466	0.407859	1.409656 ns
Error	285	0.824597	0.289332	
Total	383	133.618000		

[a] 4 rats/concentration/feeding level/chemical
* - Significant at 5% level of significance
** - Highly significant at 10% level of significance
ns - Not significant