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LABORATORY AND FIELD INVESTIGATIONS WITH DIFENACOUM, A PROMISING NEW RODENTICIDE

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ABSTRACT: Difenacoum is a new rodenticide recently introduced on the British market; it is one of the most potent of a series of hydroxycoumarin-based anticoagulants. Difenacoum is effective against laboratory rats and mice resistant to conventional anticoagulants and has a marked selectivity in favour of non-target species. Vitamin K_1 is an effective antidote and the hazard of secondary poisoning is minimal. Laboratory and field trials confirm difenacoum's efficacy against wild resistant strains. Further work is in progress to evaluate the efficacy of difenacoum against other rodent pest species.

INTRODUCTION

The rapid introduction of warfarin into the United Kingdom during the early 1950's provided a much needed boost to the control of rats and mice and there was a short period during which rodent control was easier but at the same time, more effective. This Utopian situation did not last long. Rats resistant to both warfarin and diphacinone were soon confirmed in the Scottish Lowlands (Boyle, 1960); anticoagulant resistance was also noted in mice (Dodsworth, 1961). Since then, the incidence of resistance has increased considerably, not only in the UK but in other countries, notably the United States (Brooks and Bowerman, 1975; Jackson et al., 1975).

In contrast with the comparative abundance of alternative pesticides in agriculture, there was no immediate replacement for warfarin and other anticoagulants. Since 1960, much effort has been expended in the UK by Government (Rowe et al., 1970) and private companies (Bull, 1967) to find alternative compounds.

Routine screening of toxic materials rejected by the pharmaceutical industry for human use proved time consuming and few compounds reached even the field trials stage (Rowe, 1970). Routine synthesis of new compounds in the pharmaceutical industry has thrown up very few promising new rodenticides, notably norbormide, parachlorphenyl silatrane (Beiter et al., 1970) and a series of compounds from Rohm & Haas (Peardon, 1974). The two former compounds have not fulfilled their initial promise (Rennison et al., 1968) and are now little used, while only one of the Rohm & Haas series (787) has reached registration and marketing in the United States.

A more fundamental approach was adopted by Hadler and Shadbolt (1975) who, after carefully examining the current evidence on anticoagulant resistance and coagulation theory, proceeded to synthesize a series of hydroxycoumarin-based anticoagulants, many of which showed exceptional potency to both normal and anticoagulant resistant rats. One of the most potent of the series is 3-(3-P-dipheny1-1,2,3,4-tetrahydronaphth-1-yl)-4-hydroxycoumarin, the proposed British Standard Common name being difenacoum. The present paper examines the properties and toxicity of difenacoum and reviews laboratory and field performance in the UK and other parts of the world.

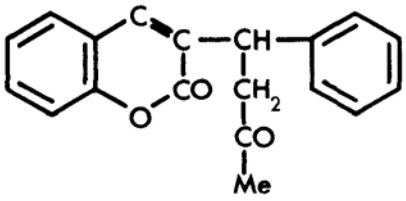
STRUCTURE OF DIFENACOUM

The anticoagulant coumatetraly1 has a higher activity than warfarin against resistant strains of Rattus norvegicus (Lund, 1972) possibly related to the size and complexity of the groups attached to the 4-hydroxycoumarin. The rationale of Hadler and Shadbolt (loc. cit) in synthesizing compounds with substituted phenyl groups attached by a three carbon chain to the 4-hydroxycoumarin moiety is illustrated in Figure 1. Fuller details of the chemistry and structure/activity relationships are given in the original paper.

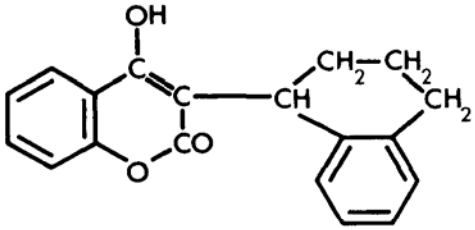
TOXICITY

a) Acute

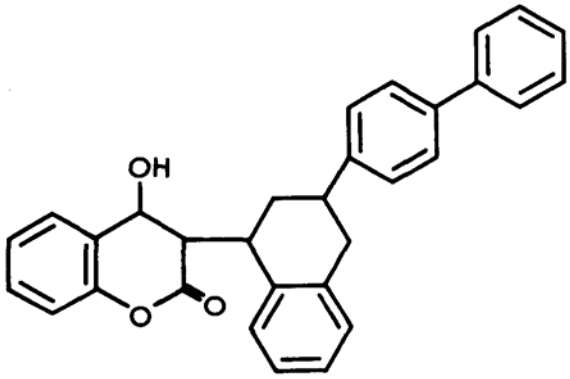
Difenacoum is highly active against a number of important rodent species (Table 1). In contrast with warfarin, difenacoum is more toxic to the Mus musculus than to Rattus norvegicus. Although the data are limited, the susceptibilities of Rattus rattus and its



Warfarin



Coumatetralyl



Difenacoum

Table 1. The acute oral toxicity of difenacoum to some rodents and domestic animals.

Animal and Strain	Acute Oral LD_{50} (mg/kg)
(Rattus norvegicus)	
Wistar male	1.8
Wistar female	2.5
Wild male	2.5
Wild female	3.5
(Rattus rattus mindanensis)	
Wild male	7.0
Wild female	2.5
(Rattus argentiventer)	0.7
(Mus musculus) LAC male	0.8
Rabbit (Dutch male)	2.0
Dog (Beagle)	50
Cat	100
Pig (large white)	80-100
Guinea pig	50
Chicken (Leghorn male)	50

Figure 1. Comparative structures of three rodenticides.

sub-species are of the same order as that of R. norvegicus. This again contrasts with warfarin since R. rattus has required a concentration about five times more than that for R. norvegicus (Bentley and Larthe, 1959).

For non-target species, difenacoum is much less toxic; in particular the pig is far less susceptible than to warfarin (Papworth, 1958) for which a dose of 1 mg/kg has been recorded as lethal.

b) Chronic

Groups of male Wistar rats and male LAC mice were given a graded series of doses of difenacoum on five consecutive days to establish its cumulative toxicity vis-a-vis warfarin. The chronic oral LD_{50} was established as 5 x 0.15 mg/kg for rats and 5 x 0.07 mg/kg for mice. In each instance the cumulative dose was about half of the acute. A similar test on homozygous resistant (Welsh strain) R. norvegicus gave 5 x 0.54 mg/kg, from which a resistance factor of about x 3 can be deduced in comparison with non-resistant animals.

At first sight, the activity of difenacoum seems to differ from warfarin for which the ratio of acute to chronic dose is taken to be large, i.e. > 50 mg/kg: 5 x 0.5 mg/kg (Hayes and Gaines, 1950). Reviewing other work on warfarin, Bentley and Larthe (1959) list acute oral LD_{50} values ranging from 1.3-323 mg/kg the most widely quoted being 60 mg/kg. Because of these disparities and to allow valid comparison of warfarin with difenacoum, new

determinations have been made. The oral LD_{50} of high purity warfarin in glycol solution to female rats was 8.5 mg/kg, 95% confidence limits 4-15 mg/kg (M.Hadler, personal communication). The LD_{50} of an aqueous suspension of warfarin was 6.9 mg/kg, 95% confidence limits 4.3-ll (R. Parkinson, personal communication).

These data on warfarin indicate that the ratio of chronic to acute is less than has been widely accepted and that the safety margin may be less than has been supposed. Further work is in progress to resolve the apparent discrepancy.

c) Hazards to domestic animals

High potency per se is sometimes taken out of context and without reference to the end-use concentration. Data is therefore included on various rodenticide to compare their hazards to non-target species (Table 2). The calculations for warfarin are based on early published work and take no account of the data given in the previous paragraph. The margins of safety offered by difenacoum need no further comment.

Table 2. Amount of bait (grams) required to deliver 1 acute LD_{50} dose of various rodenticides to different species.

Species & Body weight (kg)	Rodenticidal baits					
	ZN_3P_2 (2.5%)	1080 (0.25%)	RH787 (2.0%)	Vit. D_2 (0.1%)	Warfarin (0.025%)	Difenacoum (0.005%)
Rat 0.25	0.45	0.25	0.15	14	58	9
Mouse 0.025	-	0.17	0.12	0.75	37	0.4
Rabbit 1.0	-	-	>15.0	-	3,200	40
Pig 50	40-80	60-80	>125.0	-	200-1,000	40,000
Dog 5	4-8	0.12-0.4	>250	15	400-5,000	5,000
Cat 2	1.6-3.2	0.24-0.4	<10	-	8-320	4,000
Chicken 1	0.8-1.2	4-12	36	-	>4,000	1,000
References	5, 7	6, 7	1	4, 8	2, 3	

(1) Peardon, 1974. (2) Hagan & Radomski, 1953. (3) Papworth, 1958.
(4) Yendt, 1970. (5) Martin, 1974. (6) Spector, 1959.
(7) Garner, 1967. (8) Ward Blenkinsop, 1975.

TOXICITY OF IMPURITIES PRESENT IN DIFENACOUM

Analysis shows two main impurities in difenacoum; 4-hydroxycoumarin and 2-p-diphenyl -1,2-dihydronaphthalene. Oral dosing of these compounds in polyethylene glycol to mice gave acute oral $LD_{50's}$ of >200 mg/kg and 180 mg/kg respectively. The corresponding LD_{50} for difenacoum against mice is 0.8 mg/kg and since neither of the impurities is present at more than 2-3% in technical difenacoum they can be ignored as toxic components.

ANTICOAGULANT ACTIVITY

The structure, method of synthesis and mode of action of difenacoum are all characteristic of a classical indirect anticoagulant. The activity of difenacoum was compared with that of some other commonly used anticoagulants against Wistar Rats (Fig. 2); the most active of the warfarin isomers (s-) was the least effective compound tested and difenacoum the most effective with the other three compounds being intermediate. For homozygous resistant (Welsh strain) rats, differences between compounds were enormous (Fig. 3). The resistance factors between the strains varied from x 2 for difenacoum to about x 227 for diphacinone (Hadler, 1975a).

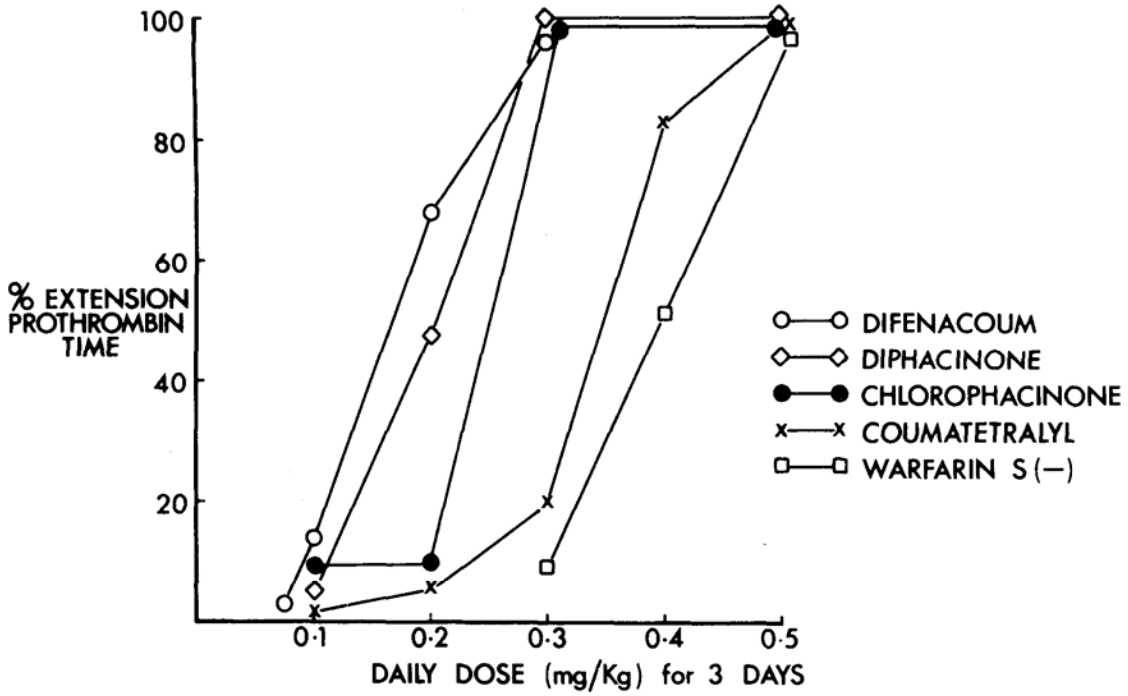


Figure 2. The effect on the extension of male Wistar rats prothrombin times following three daily oral administrations of various rodenticides.

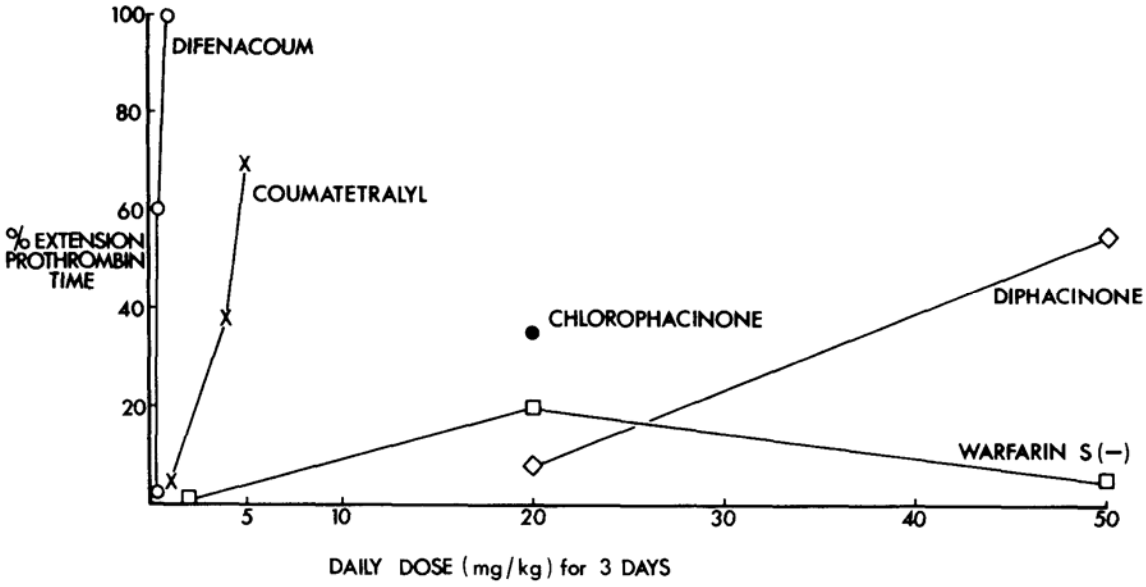


Figure 3. The effect on the extension of prothrombin times of homozygous resistant (Welsh strain) male rats following three daily oral administrations of various rodenticides.

ANTIDOTE STUDIES

Vitamins K_3 and K_1 were tested as possible antidotes to difenacoem. As expected, Vitamin K_3 was almost inactive; Vitamin K_1 was much more effective. A single dose of 2 mg/kg difenacoum killed 8/10 male mice (Fig. 4) while only 2/10 died when protected by daily injections of 10 mg/kg Vitamin K_1. More detailed examination of the optimum dose for rats (Hadler, in preparation) has shown that doses of 10 mg/kg Vitamin K_1 will provide effective protection against difenacoum at all levels up to 50 mg/kg. This compares with work on warfarin for which 1 mg/kg Vitamin K_1 will protect against any level (Lowenthal and Macfarlane, 1964; Lowenthal and Birnbaum, 1969). The higher level of Vitamin K_1 required for difenacoum may only reflect the higher potency of the rodenticide but the data also indicate the prolonged action of difenacoum and underline the need for careful monitoring of antidotal measures taken in instances of accidental poisoning. However the delay of up to three days before death occurs is more than adequate time for instigation of effective prophylactic measures.

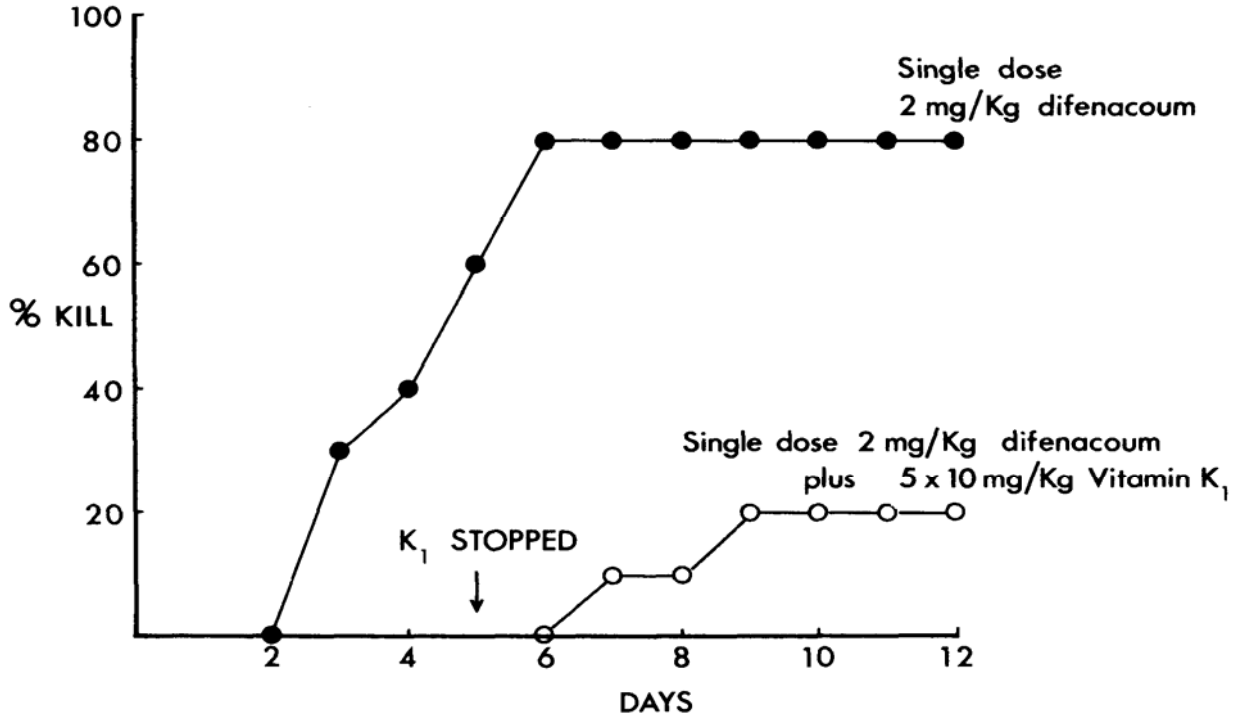


Figure 4. The effect of Vitamin K_1 in reversing the anticoagulant action of a single dose of difenacoum given to LAC mice.

SECONDARY TOXICITY

Anticoagulants in general have a good record regarding toxicity to predators feeding on poisoned rodents. The possible hazard of difenacoum was elevated by feeding poisoned mice to rats for either one or three days.

Determination of rat prothrombin times 24 hours after completion of feeding showed slightly greater elevation with warfarin than with difenacoum for the one day feed (Table 3); the situation was reversed for the three day feed, probably the result of the more prolonged

action of difenacoum. However the results are sufficiently close to suggest that difenacoum is unlikely to offer significantly greater hazard than warfarin to non-target species.

Table 3. The effect on prothrombin times of rats fed with difenacoum poisoned mice.

Toxicant fed to mice	Rat No.	Mean Prothrombin times (secs)	
		One day feed	Three day feed
0.005% difenacoum	1	19	212+
	2	27	100
	3	19	212+
0.0025% warfarin	1	36	53
	2	21	63
	3	36	35

LONG TERM FEEDING STUDIES

Although all available evidence showed that difenacoum acted only as an indirect anticoagulant, laboratory rats and mice were fed continuously with low concentrations of difenacoum (0.1 ppm and 0.5 ppm respectively) in their diet for 12 weeks to elucidate other possible effects. During this period 3.13 mg/kg was consumed by rats and 1.48 mg/kg by mice (about 2 LD_{50}'s in each case). Four out of 12 male rats died in weeks 10 and 11 and one female in week eleven; only 2/25 male mice died. All deaths were attributable to classical anticoagulant hemorrhaging; moreover, histological examination detected no abnormalities in the organs of animals of either species.

BAIT FEEDING STUDIES

Baits were fed to homozygous resistant R. norvegicus to compare the activity of difenacoum with other rodenticides. Coumatetralyl again showed its superiority over other conventional anticoagulants but was much less effective than difenacoum which gave significant mortality even at 10 ppm (Table 4).

Table 4. Ten day no choice bait trials in female homozygous resistant (Welsh strain R. norvegicus[1].

Anticoagulant	Kill					
	Concentration (ppm)					
	250	200	100	50	20	10
Warfarin	0/5	0/5	-	-	-	-
Diphacinone	0/5	-	-	-	-	-
Chlorophacinone	1/5	-	-	-	-	-
Coumatetralyl	2/5	1/5	-	-	-	-
Difenacoum	5/5	-	5/5	10/10	10/10	7/10

[1] From Hadler (1975b)

More detailed studies at the Toworth Laboratories of the Ministry of Agriculture, Fisheries and Food on 0.005% difenacoum bait were made with R. norvegicus, R. rattus and Mus musculus (Hadler, Redfern and Rowe, 1975). These tests confirmed the efficacy of difenacoum in two day no-choice feeding (Table 5). Non-resistant rats were all killed; 9/10 and 5/10 respectively of the resistant strains succumbed. Mice were less susceptible with 9/10 non-resistant and 13/15 resistant mice being killed. In similar tests with warfarin, a kill of 21/23 was obtained with non-resistant rats (Bentley and Larthe, loc. cit.) whereas no resistant rats would die.

Species	Type[2]	Sex	Mean body weight	Mortality	Mean daily bait intake (g)		Lethal dose of active ingredient (mg/kg)		Survived dose of active ingredient (mg/kg)		Days to death	
					Prebait[3]	Poison	Mean	Range	Mean	Range	Mean	Range
Rattus norvegicus	NR	M	179	5/5	18.2	14.2	8	1-17	-	-	4.4	4-6
	NR	F	149	5/5	16.1	11.9	8	5-10	-	-	6.0	4-10
	R	M	211	5/5	17.6	13.3	7	5-10	-	-	4.8	3-6
	R	F	208	4/5	16.3	12.7	7	4-12	6	-	7.3	6-8
Mus musculus	NR	M	13	4/5	2.4	2.4	18	13-22	20		6.0	5-7
	NR	F	13	3/5	2.1	2.6	20	17-22	21	18-24	7.3	6-9
	R	M	15	5/5	3.1	2.6	18	9-23	-	-	5.4	2-7
	R	F	15	4/5	2.3	2.6	19	12-22	-	-	5.4	2-7
	R	F	15	4/5	2.3	2.6	19	12-22	14	-	7.5	6-10
Rattus rattus	NR	M	124	5/5	8.2	8.6	7	6-8	-	-	6.6	4-8
	NR	F	99	5/5	8.2	8.0	8	5-11	-	-	5.6	4-7
	R	M	158	3/5	12.1	10.6	7	4-10	8	7-8	9.3	9-10
	R	F	122	2/5	9.5	9.1	7	6-7	8	8-9	9.5	6-13

[1] From Hadler, Redfern and Rowe (1975).

[2] NR = non-resistant, R = resistant to warfarin.

[3] Last day only.

Table 6. Bait consumption and mortality in wild R. norvegicus, R. rattus and M. musculus given a choice between plain and poisoned baits[1].

Species	Type[2]	Mean body weight (g)	Duration of test (days)	Concentration (%)	Mean daily bait intake (g)		Significance (P) of Student's 't'	Mortality
					Poison	Plain		
Rattus	NR	190	2	0.3	1.5	15.3	< 0.001	14/20
norvegicus	NR	178	2	0.03	6.0	10.1	< 0.01	18/20
	NR	176	4	0.01	5.6	10.0	< 0.01	29/30
	NR	185	4	0.005	6.7	9.0	< 0.02	29/30
	R	224	2	0.3	4.6	12.1	< 0.01	15/20
	R	289	2	0.03	7.2	10.6	0.1-0.05	13/20
	R	212	4	0.01	5.9	10.6	< 0.001	27/30
	R	246	4	0.005	6.3	9.8	< 0.001	28/29
Mus	NR	13	2	0.3	0.4	2.2	< 0.001	7/10
musculus	NR	13	2	0.03	1.0	1.7	< 0.01	8/10
	NR	12	4	0.01	1.4	1.6	0.2-0.1	19/20
	NR	14	4	0.005	1.5	1.3	0.5-0.4	18/20
	R	14	2	0.3	0.7	2.2	< 0.001	7/10
	R	14	2	0.03	1.1	1.6	< 0.001	7/10
	R	17	4	0.01	1.6	1.8	< 0.02	8/10
	R	17	4	0.005	1.6	1.4	0.6-0.5	19/20
Rattus	NR	127	4	0.005	3.3	7.9	< 0.01	10/10
rattus	R	152	4	0.01	4.3	9.5	0.3-0.2	3/5
	R	156	4	0.005	2.3	6.6	< 0.01	4/10

[1] From Hadler, Redfern and Rowe (1975)

[2] R = resistant, NR = non-resistant to warfarin.

Choice tests were carried out on the three species using different feeding periods with various concentrations (Table 6). High concentrations of difenacoum were clearly unacceptable to M. musculus and R. norvegicus; at 0.005% there was still some discrimination by the latter against difenacoum but kills were excellent. A further test comparing the acceptance of 0.025% warfarin with 0.005% difenacoum showed insignificant preference for difenacoum.

Further tests on house mice in large pens confirmed that longer feeding periods were required to get good control and that slightly higher concentrations might be required (Table 7). However the Cambridge strain of mice used in this experiment is known to have exceptional resistance to anticoagulants; field strains are likely to be more susceptible and more readily controlled by difenacoum than the results above would suggest.

Table 7. Summary of tests on house mice in pens (after Rowe, 1975).

% Difenacoum	No. mice tested	No. killed	% kill	Days to death
0.005%	81	72	89	3-23
0.010%	67	65	97	3-15

FIELD TRIALS

Difenacoum was tested on a number of farms in Wales and in Kent, since the resistant rats in these two areas probably represented different genetic strains (Rennison and Hadler, 1975). Three farms were treated first with 0.025% warfarin and, not unexpectedly, all treatments were unsuccessful; the farms were then treated with 0.005% difenacoum and the rat infestations were eradicated (Fig. 5). Eleven further farms were treated with 0.005% difenacoum and six farms with 0.010% difenacoum. The results showed that difenacoum effectively controlled warfarin resistant rats in both areas; no practical difference could be demonstrated between the two concentrations used and the lower concentration was therefore, chosen for field use. Since Autumn 1974, difenacoum (under the trade name of "NeoSorexa", registered trade mark of Ward Blenkinsop & Co. Ltd.) bait has been commercially available to professional pest control operators, local authorities and large agricultural users and has proved remarkably successful.

TESTS ON OTHER STRAINS AND SPECIES

Difenacoum at 0.01% has also been tested against American strains of anticoagulant resistant R. norvegicus (Table 8) as well as R. r. mindanensis from the Philippines and R. exulans from Hawaii (Scott, DWRC, personal communication). The results were promising and further work is planned to test difenacoum under field conditions. Preliminary tests by Brooks and Bowerman (personal communication) conducted on five resistant Norway rats from Chicago gave 100% mortality with 0.005% difenacoum after a 6 day no-choice feeding test. One hundred percent mortality of eight resistant house mice (Mus musculus) was also obtained after a 21 day no-choice test with 0.005% difenacoum. Jackson (personal communication) has also conducted preliminary laboratory trials with 0.005% difenacoum baits on 10 Chicago resistant rats with complete mortality after a 6 day, no-choice test. In view of these results and preliminary genetic data obtained for American resistant rats by Brooks and Bowerman (loc. cit), it is expected that difenacoum will perform well under American conditions.

Danish resistant rats (Lund, 1975) all died when subjected to the standard 6 day feeding regime (Table 9).

Evaluations of difenacoum against Rattus tiomanicus, an important pest of oil palm, and Rattus argentiventer a rice field rat have shown promising results (B.J. Wood, personal communication). Difenacoum at 0.01% killed all R. tiomanicus whether they ate 20g bait/day or were restricted to 5g (Fig. 6). Even 0.001% under the latter regime killed nearly 90% of rats. Comparative field trials in oil palm have confirmed the promise of laboratory tests (Table 10: B.J. Wood, personal communication). Difenacoum at 0.0055% gave equivalent results to warfarin at 0.05% but 0.0275% difenacoum was more effective in that half the previous number of rounds and baits were needed to obtain control. Further work is planned to determine the optimum concentration for use against R. argentiventer.

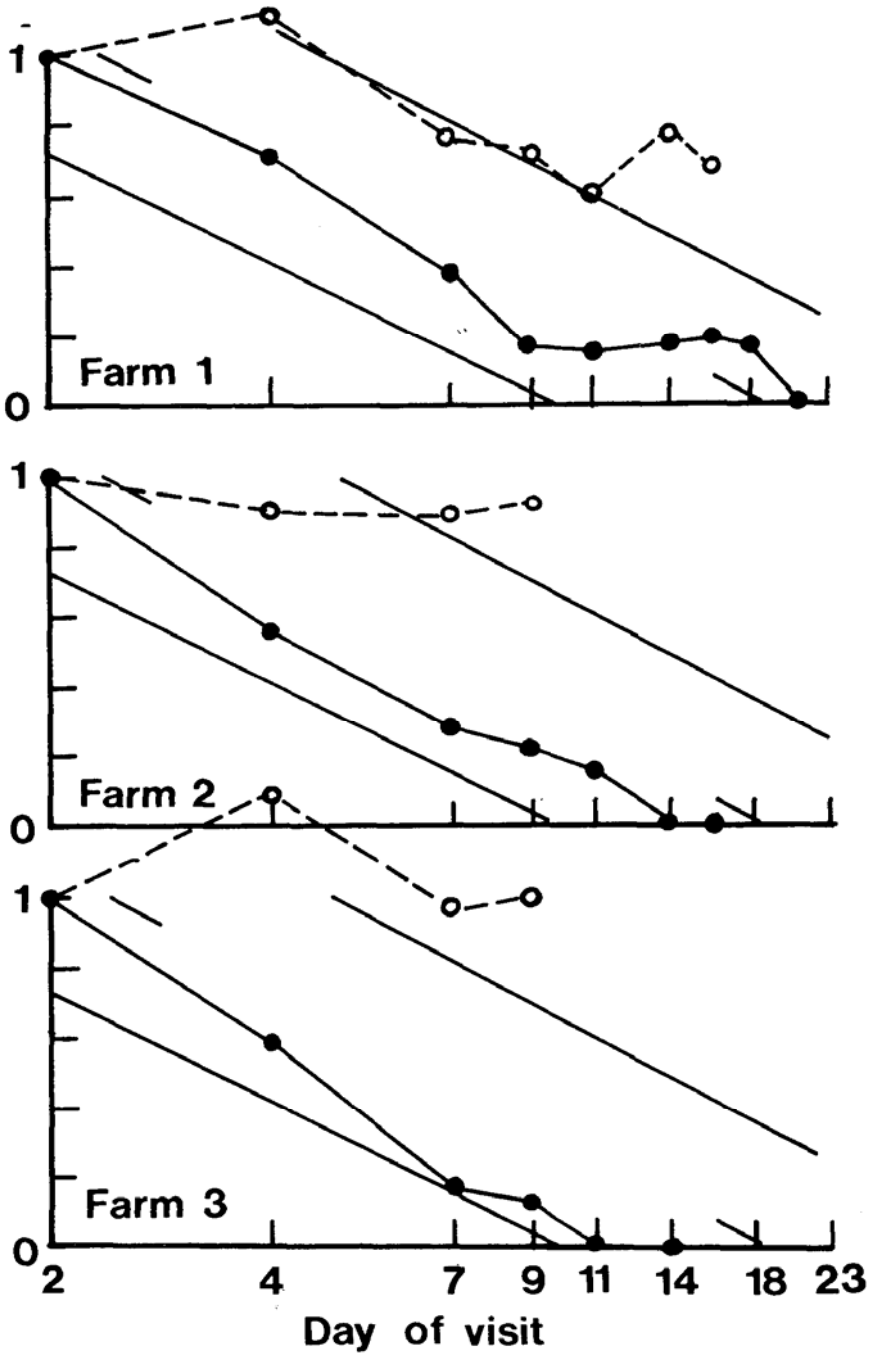


Figure 5. The regression of rat populations on three farms after treatment with 0.025% warfarin (broken lines) and afterwards with 0.005% difenacoum (solid lines). Taken from Rennison and Hadler, 1975.

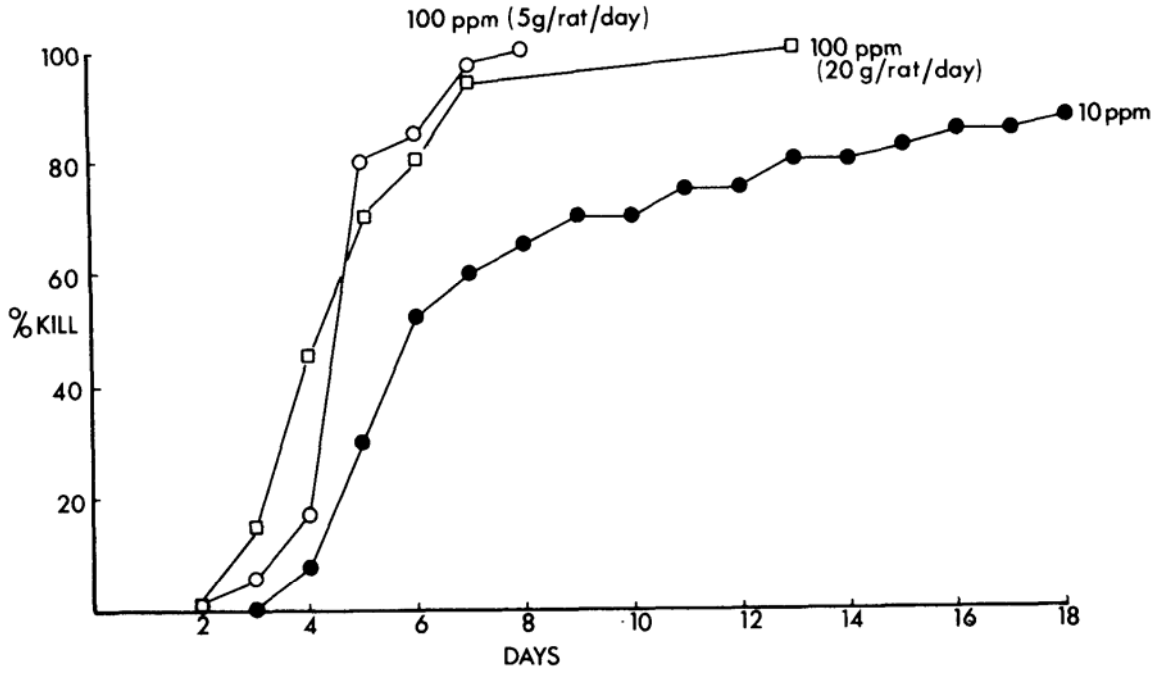


Figure 6. Kill of Rattus tiomanicus after various regimes of no-choice feeding on 10 and 100 ppm difenacoum.

Table 8. Effects of feeding 0.01% difenacoum to various strains and species of rat.

Species & Strain	Type of feeding	Kill	Time to death	Acceptance Ratio	Mean wt. chemical eaten
Rattus norvegicus (N. Carolina resistant)	No choice	10/10	3-9	-	8 mg/kg
Rattus norvegicus (Denver)	Free choice	19/20	4-11	0.50	7 mg/kg
Rattus rattus mindanensis (wild)	Free choice	10/10	5-14	0.57	13 mg/kg
Rattus exulans (wild)	Free choice	10/10	4-13	0.63	13.5mg/kg

Table 9. Effects of feeding rodenticides to Danish anticoagulant resistant rats.

	Rodenticide & Conc	No. of Rats	No. killed	Av. days to death	Av. amount of bait eaten g/kg
Six day test on resistant Rattus norvegicus[1]	0.01% difenacoum	10	10	5.8 (3-8)	247 (91-491)
	0.025% warfarin	10	5	6.8 (5-10)	283 (181-423)
	0.01% difenacoum	6	6	7.2 (5-12)	275 (145-471)
	0.005% difenacoum	16	16	5.9 (3-9)	241 (103-384)

[1]After Lund (1975)

Table 10. Results of field trials with difenacoum-based wax cubes against Rattus tiomanicus under oil palms.

Treatments	No. of rats trapped		Bunch Damage Index		Dead rats seen during treatment	No. of rounds required to reach < 20% 'take'	No. of baits used per palm
	B[1]	A[1]	B	A			
Difenacoum 0.0275%	8.0	0	4.1	0	64	4.5	2.6
Difenacoum 0.0055%	8.0	0	6.4	0	46	8.0	4.8
Warfarin 0.05%	8.0	0	1.7	0	56	8.5	5.0
Warfarin 0.01%	8.5	0	2.6	0	53	10.0	7.7
Control	8.5	6.0	5.1	1.2	1	10.0[2]	10.0[2]

[1] B - Before treatment, A - After treatment.

[2] Trial closed because all treatments have achieved 20% 'take'.

Arvicanthis niloticus (Nile, grass or harsh furred rat) is a serious agricultural pest in Africa and parts of the Arabian peninsula. From comparative laboratory tests (Gill and Redfern, Pans in press) it appears that there is a factor of about x 7 in toxicity of 0.005% difenacoum over 0.025% warfarin; 100% kill was achieved after 2-3 days no choice feeding compared with 5-6 days with warfarin. Overall, the susceptibility of Arvicanthis to difenacoum appears to fall between that of R. norvegicus and R. rattus (Anon., 1970). Gill and Redfern conclude that all the rodenticides tested, in suitable bait formulations, would be effective as practical control agents but rightly stress the need for practical field evaluation before definite conclusions can be drawn.

CONCLUSIONS

Difenacoum is still in an early stage of development and much work remains to be done but the product appears to be one of the most exciting developments in rodent control for many years. It has all the benefits of the 'classical' anticoagulants and a wide spectrum of action against rodents, with the additional advantages of being active against anticoagulant resistant rats and having a marked degree of selectivity towards non-target species.

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