March 1984

RESISTANCE TO THE SECOND-GENERATION ANTICOAGULANT RODENTICIDES

Mogens Lund
Danish Pest Infestation Laboratory, Skovbrynet 14, 2800 Lyngby, Denmark

RESISTANCE TO THE SECOND-GENERATION ANTICOAGULANT RODENTICIDES

MOGENS LUND, Danish Pest Infestation Laboratory, Skovbrynet 14, 2800 Lyngby, Denmark

ABSTRACT: The second-generation anticoagulants, difenacoum, bromadiolone and brodifacoum, have taken over a considerable part of the rodenticidal market during the last six to eight years. This is partly due to the higher efficiency against a larger spectrum of rodent pest species and partly to the increasing problem of physiological resistance to the older anticoagulants. Resistance of practical importance has, however, now been encountered to difenacoum and bromadiolone in Europe, i.e., UK and Scandinavia. Brodifacoum, in spite of the evidence of a somewhat increased tolerance in some commensal rodent populations, still must be considered a highly effective rodenticide against almost all important rodent pest species.

Research leading to the synthesis of similar potent anticoagulant molecules or other slow-acting rodenticides should be encouraged in order to cope with the possible development of resistance also to brodifacoum in the future.

INTRODUCTION

Rodent resistance to warfarin and other "first-generation anticoagulants" has now been known for 25 years, the first population of warfarin-resistant brown rats (Rattus norvegicus) being discovered in Scotland in 1958 (Boyle 1960). Today resistance to these anticoagulants has been established in all the three major commensal species in UK and USA (Greaves et al. 1973, Dodsworth 1961, Greaves et al. 1976, Jackson et al. 1972, Jackson et al. 1979); in brown rats in Denmark (Lund 1964); in brown rats and house mice (Mus musculus) in Holland (Ophof et al. 1969; pers. comm. 1982); in both rat species in France (Desideri et al. 1978); in roof rats (R. rattus) in Australia and Malaysia (Saunders 1978, Lam et al. 1982); and in house mice in Sweden, Belgium, Finland and Canada (Lund et al. 1978, pers. comm. 1982, Cronin 1979, Morgan et al. 1979).

Typically, the rodent populations have shown cross--resistance to the other anticoagulants in use up to 1975, and the "level" of resistance seems to have been inversely closely correlated with the relative toxicity of the compounds in question.

The more potent "second-generation" anticoagulant--difenacoum, bromadiolone and brodifacoum--were registered and marketed in the mid-seventies primarily as an attempt to improve this situation. But after a very short time the initial success of two of these compounds was shaken by reports indicating that rodent populations had also shown resistance to difenacoum and bromadiolone.

RESISTANCE TO DIFENACOUM

Difenacoum (3-(3-p-diphenyl-1,2,3,4-hydronaphth-l-yl)-4-hydroxycoumarin) was synthesized by Sorex Ltd (Hadler et al. 1975) and marketed by ICI in 1975. The acute LD_{50} for R. norvegicus varies in the literature between 1.8 mg/kg (Bull et al. 1976) and 3.5 mg/kg (DuboCK 1980). Already in 1976 the first case of resistance was detected in UK where 6 of 72 brown rats from five farms survived a 6-day feeding test (Redfern et al. 1978). In 1980 resistant populations were established in a larger area in Southern England (Hampshire). No more than a 33% reduction in rat numbers could be achieved in a two weeks' treatment in six farmsteads (Greaves et al. 1982a). Of 202 rats trapped on 42 farms in this district 85% were resistant to warfarin and 14% to difenacoum, and only those resistant to warfarin also showed difenacoum-resistance (Greaves et al. 1982b). If the concentration in the bait was raised from 0.005% to 0.025% a complete kill was obtained, and the resistance factor is presumed to be around five. Difenacoum-resistance has not been detected in warfarin-resistant rat populations in Wales and Scotland, confirming the view that different genetic types of resistance occur. It is suggested (Greaves et al. 1982a) that either the penetrance of difenacoum-resistance is lower than that of warfarin-resistance or that only animals homozygous for the warfarin-resistance gene are also resistant to difenacoum. Other reports indicate resistance in roof rats in France (Desideri 1979) and in UK (Redfern et al. 1978). House mice from suspected resistant field populations in UK survived a 21-day feeding test on 0.005% difenacoum consuming doses up to 147 mg/kg (Rowe et al. 1981).

RESISTANCE TO BROMADIOLONE

Bromadiolone (3(3-(4'-bromo(l,1'-biphenyl)-4-yl)-3-hydroxy-l-phenyl-propyl)-hydroxy-2H-l-benzopyran-2-1) was developed by the Lipha Company in Lyon, France, in the mid-seventies and is now one of the preferred anticoagulants for the control of the commensal species in many countries. The acute LD_{50} for R. norvegicus varies from 0.65 mg/kg (Meehan 1978) to 1.125 mg/kg (Grand 1976). Recently, brown rat populations in UK are reported to be slightly resistant to this compound in spite of its being effective against difenacoum-resistant strains (Greaves et al. 1982c). Field tests resulted in only 14% mortality after 14 days of baiting and 83% after 35 days compared with the complete kill typically obtained with 0.005% warfarin in similar treatments of susceptible rat populations (Drummond et al. 1973). Similarly, house mouse populations in UK--difficult to control efficiently—were sampled and tested in the lab revealing a substantial resistance, as no more than about 57% mortality could be

achieved in a 21-day feeding test on 0.005% bromadiolone. Individuals survived doses up to 410 mg/kg (Rowe et al. 1981). In Canada evidence of increased tolerance to bromadiolone has been found in house mice from Toronto (Siddigi et al. 1982).

RESISTANCE TO BRODIFACOUM

Brodifacoum (3-(3-(4'-bromobiphenyl-4-yl)-l ,2,3,4-tetrahydronaphth-l-yl)-4-hydroxycoumarin) is the newest and most potent of the second-generation anticoagulants, synthesized in 1977 by Sorex Ltd., and recently marketed in several countries by ICI for the control of a wide range of commensal as well as field rodent species. The LD_{50} value for Rattus norvegicus is reported to be 0.22 mg/kg. At present no resistance of practical importance has been encountered in any country, and very few of the world's most important pest species have shown a natural tolerance to this compound (e.g., Acomys cahirinus and Meriones shawi). Previous reports have indicated a high degree of efficiency of 0.0005% brodifacoum against populations of warfarin-resistant brown rats in UK and USA (Rennison et al. 1978, Apperson et al. 1981), but lately British experts have found it difficult to explain that baiting periods up to 73 days have been needed in order to obtain complete mortality with 0.002% brodifacoum against brown rats on five farmsteads in Hampshire (Greaves et al. 1982c). Tests at the Tolworth Laboratory have revealed that difenacoum-resistant brown rats show a two to three times higher tolerance to brodifacoum than susceptible laboratory strains (Res. Dev. Rep. 1983). In roof rats and house mice a complete kill is usually achieved after only one day's feeding on 0.005% brodifacoum (Dubock et al. 1978), but recently house mice have shown increased tolerance to this compound in laboratory tests in Canada (Siddiqi et al 1982).

RESULTS OF RESISTANCE TESTS WITH SECOND-GENERATION ANTICOAGULANTS IN DENMARK

In Denmark warfarin-resistance in brown rat populations was detected in 1962, and since that time rats from 30 municipalities have survived the WHO standard resistance test. Coumatetralyl, another hydroxycoumarin anticoagulant, was quite effective for four to five years and was the only anticoagulant used in the western part of the country from 1972 to 1976. Today resistance to coumatetralyl is found in 19 municipalities and in 32% of the rats trapped in this region, compared with 41% to warfarin (Table 1). Difenacoum was introduced in 1976, but has only been used in small quantities and particularly where satisfactory results could not be achieved by means of calciferol. Bromadiolone, introduced in 1979, soon became the preferred anticoagulant in most parts of Denmark.

In a continuous-resistance survey, rats trapped in various parts of the country were--from 1979 as a routine--first tested on 0.005% warfarin or 0.03% coumatetralyl; and after an observation period of at least three weeks, most of the survivors were then submitted to a 6-day test on 0.005% bromadiolone, and, if possible, after another three weeks again on 0.005% difenacoum and eventually on 0.005% brodifacoum. In a few tests the concentration of bromadiolone was increased five times (to 0.01%, in consequence of the fact that baits of this concentration had been registered for the control of especially resistant rats. In a single test brodifacoum was administered at a ten times lower concentration (0.0005%) in order to reveal the possible presence of a slightly increased tolerance to this compound. The results are given in Tables 1 and 2. The first case of difenacoum-resistance was established three years after the introduction (1979), and at the end of 1983 six municipalities appear to have difenacoum-resistant rats.

Survivors from bromadiolone-resistant tests were found one year after introduction (1980), and at the end of 1983 ten municipalities in Jutland and West-Funen had resistance problems. Of 323 rats tested, 95 survived doses up to 47 mg/kg. Some evidence of cross-resistance was seen, as survivors from warfarin/coumatetralyl tests showed 63.4% resistance compared with 15.7% resistance in rats not previously selected by these anticoagulants in the laboratory. Mortality was increased significantly if the concentration in the bait was raised five times; a complete kill could, however, still not be achieved. Of 32 bromadiolone- or difenacoum-resistant rats, none survived a 6-day feeding test on 0.005% brodifacoum; and even at a concentration as low as 0.0005%, no indication of increased tolerance could be observed.

The roof rat is a rare species in Denmark today and no evidence of resistance has been registered.

Prior to the introduction of bromadiolone the house mouse was not routinely controlled by anticoagulants in this country, and no sign of resistance has been detected. House mice trapped in the Swedish town of Mai mo, very close to Copenhagen, where warfarin had been used as the only compound for decades, were, however, found highly resistant when submitted to 21-day feeding tests on 0.025% warfarin at our laboratory in 1977. This strain has been kept at the laboratory since that time and used routinely for testing new anticoagulants. Results of tests with this strain and with mice trapped in Denmark are given in Tables 3-7.

Where 100% mortality could be obtained in 21-day feeding tests on 0.025% warfarin with Danish mice, only 50% of the Swedish mice were killed with survivors consuming up to 700 mg/kg. Bromadiolone (0.005%) produced a lower mortality in Swedish mice after ten; days' feeding than in Danish mice after one day, but doubling the concentration increased the mortality six times. Difenacoum (0.005%) gave a complete kill in tests with Danish mice after four days' feeding, but only 91% mortality in Swedish mice after 21 days. Finally, brodifacoum at a concentration of 0.005% gave complete kill in Danish mice in a 1-day feeding period and the same result in Swedish mice after a 3-day feeding. If the concentration was reduced 10 times, a 100% mortality could still be achieved in Danish mice after a 2-day feeding period, but now all Swedish mice survived. Swedish mice previously tested on bromadiolone also showed a higher tolerance to brodifacoum than those not tested before, indicating some degree of cross-resistance (Table 6 and 7).

Table 1. Showing when and where resistance to various anticoagulants was first detected in Denmark (the names are indicating Danish municipalities)

Year	Type of Anticoagulant			
	Warfarin	Coumatetralyl	Difenacoum	Dromadiolone
1962	Juelsminde			
1965	Hedensted			
1969	Børkop Egtved Horsens Tørring-Uldum Vejle	Hedensted		
1970	Grindsted	Vejle Juelsminde Horsens		
1971	Børkop Christiansf. Fredericia Hørning Kolding Lunderskov	Børkop Kolding Lunderskov Tørring-Uldum		
1972	Egvad Varde Arhus	Christiansfeld Hørning		
1973	Esbjerg Holstebro Odder	Esbjerg		
1974	Herning Skive	Egvad		
1975	Jelling Skanderborg			
1976	Middelfart			
1977	Gedved	Gedved		
1978		Middelfart		
1979		Herning Jelling	Jelling	
1980	Arden Hjørring Nørre Snede	Skanderborg Nørre Snede	Nørre Snede	Herning Horsens Nørre Snede
1981		Hjørring	Horsens	Skanderborg
1982			Tørring-Uld.	Gedved Juelsminde Middelfart Tørring-Uldum
1983	Bogense		Juelsminde Braedstrup	Lunderskov Braedstrup

Table 2. The effect of bromadiolone, difenacoum and brodifacoum on brown rats (Rattus Norvegicus) trapped in various municipalities in western Denmark. Feeding period: 6 days.

Rodenticide	Sex	No.	No. Survived	Mortality %	Average Amount Consumed Mg/kg Lethal	Non-lethal
Bromadiolone 0.005%	♂	146	47	67.8	12.9 (2.8 - 27.1)	20.9(5.8-37.5)
	♀	177	48	72.9	16.7 (2.2 - 51.3)	23.8(7.4-47.3)
	Total	323*	95	70.6	14.8	22.4
Bromadiolone 0.01%	♂	18	2	88.9	27.7(9.6-44.9)	24.9(11.8-37.9)
	♀	25	3	88.0	32.2(9.2-51.9)	28.3(17.6-43.7)
	Total	43	5	88.4	29.9	26.6
Difenacoum 0.005%	♂	46	6	87.0	15.3(7.5-31.1)	14.5(9.5-24.6)
	♀	48	7	85.4	16.7(6.5-66.3)	17.5(9.0-26.1)
	Total	94	13	86.2	16.0	16.0
Brodifacoum 0.005%	♂	15	0	100.0	15.9(5.0-27.9)	-
	♀	19	0	100.0	13.5(7.8-22.2)	-
	Total	34	0	100.0	14.7	-
Brodifacoum 0.0005%	♂ + ♀	6	0	100.0	1.5(0.6-2.5)	-

*Of 93 previously tested on warfarin/coumatetralyl, 59 survived (63.4 of 230 not previously tested, 36 survived (15.7%).

Table 3. The effect of warfarin on house mice (Mus musculus) from Denmark or southern Sweden.

Concentration	Origin of Mice	Feeding Period (Days)	No. of Mice	Mortality %	Average Amount Consumed (Range) mg/kg Lethal	Non-lethal
0.025%	Danish	1	10	0	-	56.2(41.7-69.4)
0.025%	Danish	2	10	0	-	101.1(56.9-134.7)
0.025%	Danish	21	25	100	249,2(145.8-510.4)	-
0.025%	Swedish	3	10	0	-	82.9(57.9-109.1)
0.025%	Swedish	21	10	50	387.4(312.7-456.5)	655.0(625.0-700.0)

Table 4. The effect of bromadiolone on house mice (Mus musculus) from Denmark or southern Sweden.

Concentration	Origin of Mice	Feeding Period (Days)	No. of Mice	Mortality %	Average Amount Consumed (Range) mg/kg Lethal	Non-lethal
0.005%	Danish	1	40	95	9.5(5.0-21.4)	7.9(5.0-10.8)
0.005%	Danish	6	20	95	31.9(12.0-58.9)	35.4
0.005%	Swedish	4	10	10	39.6	20.7(7.1-42.7)
0.005%	Swedish	6	10	50	32.6(26.2-37.9)	41.9(32.9-48.6)
0.005%	Swedish	8	10	60	38.2(25.9-47.7)	36.9(25.5-41.0)
0.005%	Swedish	10	10	80	48.5(25.0-104.6)	64.6(57.0-72.2)
0.01%	Swedish	4	10	60	56.5(33.5-84.2)	63.2(36.5-115.8)

Table 5. The effect of difenacoum on house mice (Mus musculus) from Denmark or southern Sweden.

Concentration	Origin of Mice	Feeding Period (Days)	No. of Mice	Mortality %	Average Amount Consumed (Range) mg/kg Lethal	Non lethal
0.005%	Danish	1	15	87	9.4(2.8-14.6)	13.2(4.3-22.1)
0.005%	Danish	2	35	97	18.1(7.9-23.7)	13.6
0.005%	Danish	4	15	100	39.4(22.7-61.4)	-
0.005%	Swedish	1	10	0	-	2.2(0.3-5.0)
0.005%	Swedish	2	10	80	17.6(12.8-22.4)	12.5(10.0-14.4)
0.005%	Swedish	21	11	90.9	62.4(32.4-105.0)	164.1

Table 6. The effect of brodifacoum on house mice (Mus musculus) from Denmark or southern Sweden.

Concentration	Origin of Mice	Feeding Period (Days)	No. of Mice	Mortality %	Average Amount Consumed (Range) mg/kg Lethal	Non lethal
0.0005%	Danish	1	10	50	0.8(0.5-1.0)	0.9(0.75-0.96)
0.0005%	Danish	2	10	100	1.4(1.1-1.6)	-
0.0005%	Swedish	1	10	0	-	0.5(0.3-0.7)
0.0005%	Swedish	2	10	0	-	0.8(0.6-1.0)
0.005%	Danish	1	10	100	6.9(2.2-8.8)	-
0.005%	Danish	2	10	100	14.6(11.4-19.2)	-
0.005%	Swedish	1	10	90	4.6(2.1-7.2)	1.1
0.005%	Swedish	2	10	90	21.2(14.2-27.1)	32.9
0.005%	Swedish	3	10	100	12.6(7.6-19.3)	-

Table 7. The effect of 0.005% brodifacoum on Danish house mice (Mus musculus) and Swedish house mice resistant to bromadiolone.

Origin of Mice	Feeding Period (Days)	No. of Mice	Mortality %	Average Amount Consumed (Range) mg/kg Lethal	Non lethal
Danish	1	10	100	9.2 (7.3 - 11.9)	-
Swedish	1	10	60	8.2 (3.3 - 12.0)	7.9 (6.4 - 9.5)

CONCLUSIONS

As resistance to two of the three second-generation anticoagulants, difenacoum and bromadiolone, has become a problem of practical importance in some countries shortly after their introduction, this must call for attention in those countries still fighting resistance "only" to the first-generation anticoagulants. In spite of brodifacoum still being highly effective, certain findings of increased tolerance also to this compound should be taken as a warning not to rely too much on this single rodenticide to solve our resistance problems in the future. It is recommended to concentrate research on the synthesis of similar potent anticoagulants or on the development of other slow-acting rodenticides.

The importance of resistance to the second-generation anticoagulants may be indicated by the situation revealed in British rodent surveys in 1970 and 1980: The proportion of rat-infested farmsteads in Hampshire, where difenacoum had been the rodenticide of choice since 1975, increased in this period from 45% to 95% (Greaves et al. 1982c).

LITERATURE CITED

APPERSON, C. S., 0. T. SANDERS, and D. E. KAUKEINEN. 1981. Laboratory and field trials of the rodenticide brodifacoum against warfarin-resistant Norway rats. Pestic. Sci. 12:662-668.

BOYLE, C. M. 1960. Case of apparent resistance of Rattus norvegicus Berkenhout to anticoagulant poisons. Nature 188:517.

BULL, J. O., and J. C. FRANCIS. 1976. RATAK-a new rodenticide. First Afro-Asian Vertebrate Pest Conference, Cairo:1-15 (Published by ICI).

CRONIN, D. E. 1979. Warfarin resistance in house mice (Mus musculus L.) in Vancouver, British Columbia. Master of Pest Manag. Thesis, Dept. of Biol. Sci., Simon Fraser Univ. 27pp.

DESIDERI, D., R. ALDIGHIERI, M. L. LOVET, and A. TARDIEU. 1978. Note preliminaire sur la mise en evidence, a Marseille, d'une resistance au coumafene chez Rattus rattus. Bull. Soc. Path. exotique 71: 210-215.

DESIDERI, D.,R. ALDIGHIERI, M. L. LOVET, and A. TARDIEU. 1979. La resistance murine aux anticoagulants dans le port de Marseille. Elat de responde au difenacoum. Bull. Soc. Path. exotique 72: 278-283.

DODSWORTH, E. 1961. Mice are spreading despite such poisons as warfarin. Munic. Engng., London 3746: 1668.

DRUMMOND, D. C, and B. D. RENNISON. 1973. The detection of rodent resistance to anticoagulants. Bull. World Health Org. 48: 239-242.

DUBOCK, A. C, and D. E. KAUKEINEN. 1978. Brodifacoum (TALON Rodenticide), a novel concept. Proc. 8th Vertebrate Pest Conference: 127-144.

DUBOCK, A. C. 1980. The development and practical use of the novel anticoagulant rodenticide brodifacoum. Plant Prot. Bull. (Taiwan, R.O.C.)22:223-238.

GRAND, M. 1976. Experimental results on a new anticoagulant rodenticide-bromadiolone. Phytiatrie/ Phytopharmacie 25: 69-88.

GREAVES, J. H., B. D. RENNISON, and R. REDFERN. 1973. Warfarin resistance in the ship rat in Liverpool. Int. Pest Control 15(2): 17.

GREAVES, J. H., and P. B. AYRES. 1976. Inheritance of Scottish-type resistance to warfarin in the Norway rat. Genet. Res., Camb.28: 231-239.

GREAVES, J. H., D. S. SHEPHERD, and J. E. GILL. 1982a. An investigation of difenacoum resistance in Norway rat populations in Hampshire. Ann. appl. Biol.100: 581-587.

GREAVES, J. H., and P. B. AYRES. 1982b. Multiple allelism at the locus controlling warfarin resistance in the Norway rat. Genet. Res., Camb. 40: 59-64.

GREAVES, J. H., D. S. SHEPHERD, and R. QUY. 1982c. Field trials of second-generation anticoagulants against difenacoum-resistant Norway rat populations. J. Hyg., Camb. 89: 295-301.

HADLER, M. R., R. REDFERN, and F. P. ROWE. 1975. Laboratory evaluation of difenacoum as a rodenticide. J. Hyg., Camb. 74: 441-448.

JACKSON, W. B., and D. E. KAUKEINEN. 1972. Resistance of wild Norway rats in North Carolina to warfarin rodenticide. Science 176: 1343-1344.

JACKSON, W. B., and A. D. ASHTON. 1979. Present distribution of anticoagulant resistance in the United States. In: J. W. Suttie (Ed.). Vitamin K metabolism and vitamin K-dependent proteins. Univ. Park Press, Baltimore, 572pp.

LAM, Y. M., A. K. LEE, Y. P. TAN, and E. MOHAN. 1982. A case of warfarin resistance in Rattus rattus diardii. MARDI Res. Bull. 10,3: 378-383.

LUND, M. 1964. Resistance to warfarin in the common rat. Nature 203: 778.

LUND, M., and J. LODAL. 1978. Resistance in the house mouse. Danish Pest Inf. Lab. Ann. Rep. (1977): 73-74.

MEEHAN, A. P. 1978. Rodenticidal activity of bromadiolone - a new anticoagulant. Proc. 8th Vertebrate Pest Conference: 122-126.

MORGAN, D. G., and M. L. PETRAS. 1979. Variability in response to warfarin in natural populations of the house mouse. Genetics 91(4): 84.

OPHOF, A. J., and D. W. LANGEVELD. 1969. Warfarin resistance in the Netherlands. In: Proc. Rodent Biol. Contr. Meet., Berlin, Schr. Reihe Ver. Wass.-Boden-u.-Lufthyg.32: 39-48.

REDFERN, R., and J. E. GILL. 1978. The development and use of a test to identify resistance to the anticoagulant difenacoum in the Norway rat (Rattus norvegicus). J. Hyg., Camb., 81:427-431.

RENNISON, B. D., and A. C. DUBOCK. 1978. Field trials of WBA 8119(PP581, brodifacoum) against warfarin-resistant infestations of Rattus norvegicus. J. Hyg., Camb. 80: 77-82.

RESEARCH AND DEVELOPMENT REPORT. 1983. Resistance to rodenticides. Reference book of the Min. of Agriculture, Fisheries and Food., London, 1982: 30-31.

ROWE, F. P., C. J. PLANT, and A. BRADFIELD. 1981. Trials of the anticoagulant rodenticides bromadiolone and difenacoum against the house mouse (Mus musculus L.). J. Hyg., Camb., 87: 171-177.

SAUNDERS, G. R. 1978. Resistance to warfarin in the roof rat in Sidney, NSW. Search 9: 39-40.

SIDDIQI, Z., and W. D. BLAINE. 1982. Anticoagulant resistance in house mice in Toronto, Canada. Pest Management 1982: 10-14.