University of Nebraska - Lincoln
DigitalCommons@University of Nebraska - Lincoln

Proceedings of the 8th Vertebrate Pest Conference (1978)

Vertebrate Pest Conference Proceedings collection

March 1978

RODENTICIDAL ACTIVITY OF BROMADIOLONE—A NEW ANTICOAGULANT

Adrian P. Meehan
Chief Biologist, Rentokil Limited, Felcourt, East Grinstead, Sussex, England

Follow this and additional works at: https://digitalcommons.unl.edu/vpc8

 Part of the Environmental Health and Protection Commons

Meehan, Adrian P., "RODENTICIDAL ACTIVITY OF BROMADIOLONE—A NEW ANTICOAGULANT" (1978). *Proceedings of the 8th Vertebrate Pest Conference (1978)*. 31.
https://digitalcommons.unl.edu/vpc8/31

This Article is brought to you for free and open access by the Vertebrate Pest Conference Proceedings collection at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Proceedings of the 8th Vertebrate Pest Conference (1978) by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

RODENTICIDAL ACTIVITY OF BROMADIOLONE—A NEW ANTICOAGULANT

ADRIAN P. MEEHAN, Chief Biologist, Rentokil Limited, Felcourt, East Grinstead, Sussex, England

ABSTRACT: Bromadiolone, a new anticoagulant rodenticide, has been evaluated against laboratory rats and mice. Kill of both species was excellent, even when bromadiolone was offered for only one day in the presence of alternative food. Effectiveness against homozygous warfarin resistant rats was also demonstrated. Limited field trials on farms showed that after baiting for seven days with bromadiolone, warfarin resistant rat populations were substantially reduced although subsequent re-infestation occurred. Longer or continuous periods of baiting with bromadiolone would almost certainly prevent population increase, but government restrictions on the amount of bait approved for the trials did not allow this to be demonstrated.

INTRODUCTION

The introduction of anticoagulants stands out as the major development in rodent control in the last twenty-five years. But in 1958, a population of Rattus norvegicus, in Scotland, became resistant to the most commonly used anticoagulant, warfarin (Boyle 1960) - a problem which was subsequently recognized in other parts of Britain. Since then anticoagulant resistance has been found in a number of rodent species in various parts of the world, including the U.S.A. This has been well-documented and Kaukeinen (1977) has produced an extensive bibliography. Generally there has been cross-resistance, to a greater or lesser degree, to all of the more established anticoagulants.

Recently, however, new anticoagulants with the ability to control these resistant animals have been synthesized. The first to be marketed was difenacoum and Bull (1976) has reviewed the properties of this anticoagulant. More recently, Rennison and Dubock (1978) have field tested brodifacoum, an analogue of difenacoum.

Bromadiolone, is another similar type compound, which is also capable of controlling warfarin resistant rats and mice. Marsh (1976) and Grand (1976) have shown that bromadiolone has activity against a wide range of rodents, including gophers, ground squirrels, various "field" mice and voles.

Bromadiolone is a hydroxy coumarin with the full chemical name: - 4 - hydroxy - 3 - coumarinyl - 3 - phenyl - 3, 4 - bromo 4 - biphenyl, - 1 - proponal - 1 (Fig. 1).

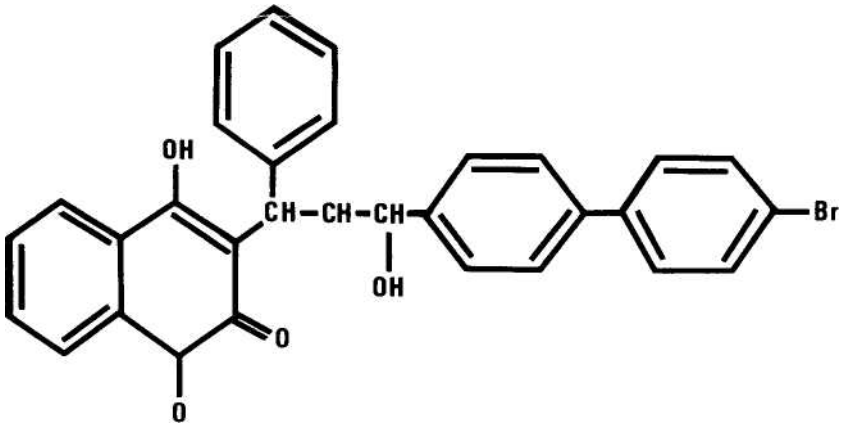

Fig. 1. Structure of bromadiolone.

Bromadiolone acts as a classical indirect anticoagulant, i.e. it interferes with Vitamin K activity, but its potency is about 10 times greater than that of warfarin in rabbits (Grand loc. cit.). This author also describes laboratory and field tests with bromadiolone against rats carried out in France.

The present paper gives details of tests with bromadiolone against laboratory strains of Rattus norvegicus and Mus musculus particularly in feeding tests. Preliminary results from field trials in England against warfarin resistant R. norvegicus are also given.

MATERIALS AND METHODS Laboratory Studies

T.O. mice and Wistar rats were used for both the oral dosing and feeding studies. Mice weighed approximately 24-26g and rats 150-200g and equal numbers of males and females were used in all tests.

Homozygous warfarin resistant rats were bred by ourselves from a small number originally obtained from the laboratories of Ward-Blenkinsop Limited.

Acute Oral Toxicity

Bromadiolone was administered to groups of ten mice and rats as a suspension in 0.6% gum tragacanth at five concentrations ranging from 0.2-3.2mg/kg. The dose was administered in 0.5ml of the suspension to mice and in 1.0ml to rats. Gum tragacanth alone was used as control. LD_{50}'s were determined by the method of Weil (1952).

Feeding Studies

The feeding tests were of two types - the "no-choice" test and "paired preference" test.

The "no-choice" test involves feeding a poisoned bait alone and is designed simply to assess the toxicity of a rodenticide when incorporated in a bait. Likewise this is the primary objective of the "paired preference" test, but this also measures the relative acceptance of a similar unpoisoned (alternative) food, when offered in competition with the poisoned bait.

In both types of test, bromadiolone was formulated in a medium oatmeal/sugar base at 0.0025%, 0.005% and 0.01%, with a small amount of oil as a binder. The alternative food, when provided, was medium oatmeal/sugar. As a basis for comparison, a proprietary formulation of warfarin at 0.05% on whole wheat, Biotrol^{R}, was used. Each concentration of test bait was offered to groups of twenty mice, caged in pairs, and to ten rats caged singly, with or without alternative food.

Food consumption was assessed daily and hopper positions alternated to avoid place preference. After one, two and four days the experimental diets were replaced with a standard laboratory diet. Kill was scored for at least 14 days after the diets containing bromadiolone were withdrawn.

Water was available at all times. The mean ambient temperature was 20°C ±_2°C and the relative humidity varied between 40% and 60%.

A "paired preference" test against rats and mice was also carried out with a liquid concentrate of bromadiolone impregnated into whole wheat at 0.005%. This formulation was also tested against homozygous warfarin resistant rats in comparison with the standard warfarin formulation. Only four rats could be used in each test because of the small numbers available.

Field Trials Against Rattus norvegicus

It was not possible to carry out tests against wild rodents in the laboratory, but a small number of preliminary field trials have been completed.

Eight sites infested with Rattus norvegicus in the warfarin resistant area of Kent and Sussex, England were used. All the premises were farms and all had a long-standing rat control problem.

Before the start of the trial, a bait containing 0.05% warfarin was laid daily for 7 days at each site. Large quantities of this bait were taken by rats and census baiting and visual observations showed that large numbers survived the treatment, confirming their resistance to warfarin. Bromadiolone, at 0.005% on whole wheat, was then laid at each site for seven days. This was followed by a post-treatment census.

All census treatments were with unpoisoned oatmeal. A "rest period" of 1 week was interposed between all poison and census treatments to avoid "pre-baiting".

Takes of bait from each baiting point were assessed daily and where necessary, baits were replenished. When a bait was completely taken, twice the amount was replaced. A numerical system of measuring "takes" was used as weighing was impractical.

RESULTS AND DISCUSSION

Laboratory Studies - Acute Oral Toxicity

Bromadiolone is somewhat more toxic to males than females (Table 1). It is also somewhat more toxic to the strains used by Rentokil than those tested by Grand (loc. cit.) who determined an acute oral LD_{50} of 1.125mg/kg for rats and of 1.75mg/kg for mice. The difference may also be due to the vehicle used for administering bromadiolone.

Laboratory Studies - Mortality

Kill of the test animals was good, and far superior to that found with warfarin baits. Only the occasional animal survived. Mice (Table 2) appeared somewhat more tolerant than rats (Table 3) but this is experienced with most anticoagulants.

The feeding regime made little difference to results: kill was similar whether alternative food was present or not and regardless of the number of days poison was fed. It would therefore appear that in the laboratory, bromadiolone is capable of killing rats and mice after feeding for only one night even at concentrations as low as 0.0025%. This supports the results of Marsh's work with Norway rats in one day “no. choice” tests.

Table 1. The acute oral toxicity of bromadiolone (mg/kg) to rats and mice when orally dosed.

Species	Acute total LD_{50} ♂	Acute total LD_{50} ♀	Acute total LD_{50} Mean	95% Confidence Limits
Wistar Rats	0.57	0.75	0.65	0.57 - 0.73
T.O. Mice	0.86	1.13	0.99	0.88 - 1.10

Table 2. Food consumption and kill of T.O. mice offered bromadiolone or warfarin with and without alternative food.

	Days Feeding	% Concentration in bait	Total bait intake(g) Poison	Total bait intake(g) Alternative	Mean active ingredient intake (mg/kg)	Kill	Days to Death Mean	Days to Death Range
Bromadiolone (Oatmeal base)	1	0.0025	61	20	3	19/20	6.6	3-10
			82	-	4	20/20	6.8	4-8
		0.005	62	23	6	16/20	7.2	5-10
			88	-	10	20/20	6.8	5-11
		0.01	64	12	14	19/20	7.2	6-10
			82	-	16	20/20	6.5	3-10
	2	0.0025	139	41	7	20/20	7.0	5-9
			176	-	9	20/20	6.4	5-8
		0.005	105	47	10	20/20	7.3	3-13
			173	-	17	20/20	7.3	4-11
		0.01	149	27	29	19/20	7.0	3-11
			172	-	33	18/20	7.4	4-15
	4	0.0025	223	66	11	20/20	6.9	5-10
			287	-	14	20/20	8.0	4-14
		0.005	240	90	23	19/20	7.0	4-12
			295	-	28	20/20	7.5	5-10
		0.01	268	28	52	20/20	7.4	4-11
			296	-	57	20/20	8.4	6-13
Warfarin (Whole wheat base)	1	0.05	43	42	4	0/20	-	-
			18	-	8	0/20	-	-
	2	0.05	90	107	9	0/20	-	-
			176	-	18	2/20	4.5	4-5
	4	0.05	142	170	14	15/20	6.3	3-8
			304	-	30	16/20	6.9	3-9

The interval between feeding and death was not influenced significantly by any parameter, including the amount of poison eaten. The mean time to death is more than 6 days for both rats and mice and only one animal (a mouse) died in less than four days. Some animals continued to die up to 15 days from the start of experiments.

The smallest amount of bromadiolone eaten was by the test group of animals given the lowest concentration of bromadiolone for one day only. This was 3 and 2mg/kg respectively for mice and rats, but was still well in excess of the respective LD_{50}'s. Excellent kill was achieved at even these levels.

The number of homozygous resistant rats fed in the laboratory was small. However, when bromadiolone was fed to these, three out of four were killed, whereas warfarin failed to kill any. This indicates that even highly resistant individuals such as these are capable of being controlled by bromadiolone. Grand (loc. cit.) also states that bromadiolone is active against resistant rats.

Palatability

The palatability of bromadiolone to both rats and mice was excellent when incorporated with an oatmeal base (Tables 2 and 3). Baits offered to mice contained a small amount of oil as a binder, and it was probably this which made the poison material many times more palatable than the untreated alternative.

At the two lowest concentrations used, rats are unable to detect bromadiolone and in fact preferred the treated diet. At the highest concentration (0.01%), rats could just detect the bromadiolone, whereas mice even at this level, continued to eat more treated diet than alternative.

Table 3. Food consumption and kill of Wistar rats offered bromadiolone or warfarin in an oatmeal base with and without alternative food.

	Days Feeding	% Concentration in bait	Total bait intake(g) Poison	Alternative	Mean active ingredient intake (mg/kg)	Kill	Days to Death Mean	Range
Bromadiolone (Oatmeal base)	1	0.0025	145	48	2	10/10	7.1	4-11
			179	-	3	10/10	6.7	4-10
		0.005	127	61	4	10/10	6.2	4-10
			163	-	0	10/10	7.1	4-11
		0.01	87	99	6	10/10	6.6	4-9
			172	-	11	10/10	5.4	4-8
	2	0.0025	295	69	5	10/10	7.4	5-12
			331	-	5	10/10	6.3	4-12
		0.005	256	116	9	10/10	6.4	4-12
			306	-	10	9/10	6.0	4-11
		0.01	187	179	12	10/10	7.1	5-10
			363	-	23	10/10	5.0	4-7
	4	0.0025	513	112	8	10/10	6.0	4-7
			570	-	9	10/10	6.7	4-14
		0.005	494	87	15	10/10	6.1	4-12
			485	-	16	10/10	5.8	4-7
		0.01	276	354	17	9/10	6.8	5-8
			581	-	36	10/10	8.1	4-13
Warfarin (Whole wheat base)	1	0.05	81	119	24	1/10	6.0	-
	2	0.05	162	242	52	6/10	5.7	5-7
	4	0.05	308	382	103	7/10	6.4	4-10

Table 4. Food consumption and kill of Wistar rats and T.O. mice offered 0.005% bromadiolone formulated on whole wheat and fed in competition with alternative food.

		Amount eaten (g) Days 1	2	3	4	Mean active ingredient intake (mg/kg)	Kill	Days to Death Mean	Range
Wistar Rats	Poison	163	128	103	84	5	20/20	5.8	4-9
	Alt	275	373	284	208				
T.O. Mice	Poison	65	42	17	19	25	19/20	7.5	4-15
	Alt	21	43	72	58				

Changing the bait base to wheat (Table 4) slightly reduced the palatability of bromadiolone to both rats and mice, but it was still more palatable than warfarin in similar baits. But despite the somewhat reduced palatability excellent kills were obtained viz. 100% and 95% for rats and mice, respectively.

Field Trials

The tests were carried out in and around farm buildings in the autumn/winter of 1977. The results from all eight sites were similar: progress at three, typical sites is therefore reported (Fig. 2).

After seven days baiting with 0.005% bromadiolone the rat populations judged by the takes of bait were minimal at all the three sites. An average reduction of over 90%. This was confirmed by visual observation and live trapping. However, after a 7 day interval without bait and a further 7 days post-treatment census with oatmeal, the populations again appeared to increase. This was caused by re-infestation of the treated buildings from surrounding land - a common occurrence in rural areas, particularly where food and harborage is sought by rats in the autumn. The amount of bait approved by the U.K. government (MAFF) for these trials was limited. Thus if sufficient bait had been available to

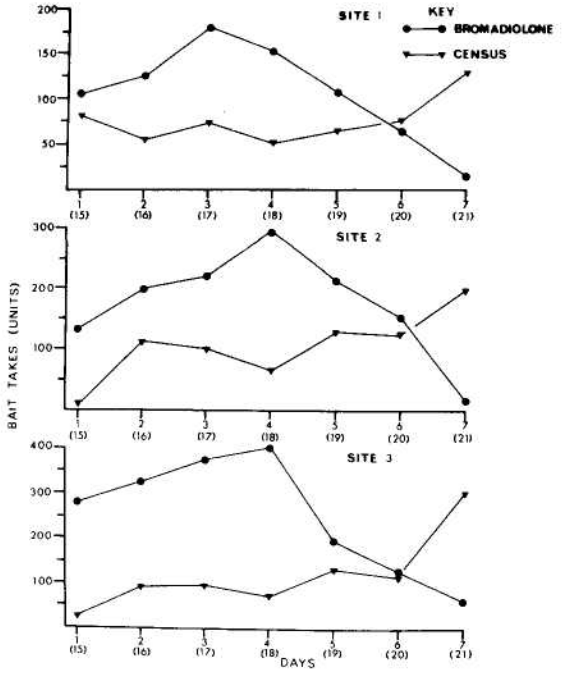

Figure 2. Intake of 0.005% bromadiolone bait and unpoisoned oatmeal post treatment census bait on three typical farms infested with warfarin resistant R. norvegicus. (There is a seven day delay between the treatment and census).

leave it down for longer periods or continuously, complete control would have been obtained. Rennison and Dubock (loc. cit.) made similar conclusions when testing brodifacoum. One, four and seven-day feeds gave only partial control, whereas baiting for up to 25 days gave complete control.

No untoward health incidents occurred during the tests with bromadiolone and none have been reported from France, where more widespread trials have been conducted. More extensive trials with bromadiolone are planned as soon as Government clearance is obtained.

The greatest poisoning risk with rodenticides is to cats and dogs in the U.K. The toxicity of bromadiolone to these animals is lower than that of warfarin. The maximum tolerated single oral dose to dogs is lOmg/kg and to cats 25mg/kg. A dog would thus have to eat 20% of its body weight of 0.005% bait before clinical symptoms appear. The chronic toxicity to pigs is significantly lower than that of warfarin, but strangely bromadiolone is more toxic to chickens. Bromadiolone would thus seem to present no greater hazard in use, than other anticoagulants.

CONCLUSIONS

Bromadiolone clearly shows potential as a replacement for warfarin and other anticoagulants which require repeated feedings to kill. It is active against resistant rats and has a good safety margin to most non-target species. The optimum conditions of use by pest control personnel in the U.K. has now to be documented.

ACKNOWLEDGMENTS

I would like to thank Lipha of France, the manufacturers of bromadiolone for the supply of the material and other help. I would also like to thank S.T. Bohills and S.P. Leonard who carried out much of the routine work, particularly in the field trials.

LITERATURE CITED

BOYLE, C.M. 1960. A case of apparent resistance of Rattus norvegicus Berkenhaut to anticoagulant poisons. Nature (London) 168(47-49):5-7.

BULL, J.O. 1976. Laboratory and field investigations with difenacoum, a promising new rodenticide. Proc. 7th Vert. Pest Conf., Monterey, California, 72-84.

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

KAUKEINEN, D.E. 1977. Bibliography - Rodent Resistance to Anticoagulant Rodenticides. Environmental Studies Center, Bowling Green State University, Bowling Green, Ohio.

MARSH, R.E. 1976. Bromadiolone, a new anticoagulant rodenticide. Joint FAO/WHO/EPPO Conference on Rodents of Agricultural and Public Health Concern. Geneva, Switzerland. June 15-18.

RENNISON, B.D. and DUBOCK, A.C. 1978. Field trials of WBA8119 (PP581, brodifacoum) against warfarin-resistant infestations of Rattus norvegicus. J. Hyg. Camb. 80:77-82.

WEIL, C.S. 1952. Tables for convenient calculation of medium effective dose (LD_{50} or ED_{50}) and instructions in their use. Biometrics 8(3):249-263.