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## Original article

# Difethialone (LM-2219) : a new anticoagulant rodenticide for use against warfarin-resistant and -susceptible strains of *Rattus norvegicus* and *Mus musculus*

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**Summary** — Data on the efficacy of difethialone, a new anticoagulant rodenticide derived from 4-hydroxybenzothioipyranone against rats and mice, are reported.

After one day of feeding with 25 mg/kg of bait, 100 and 85%, respectively, male and female warfarin-susceptible *Rattus norvegicus* were killed. With the resistant strain, mortality was 90 and 94% for males and females, respectively. 100 and 96% of warfarin-sensitive males and females, respectively, were killed after 3 days of feeding with the same bait. Those of the resistant strain were respectively, 100 and 93%.

In *Mus musculus*, after one day of feeding, mortality was 97, 94, 95 and 93% for males and females of susceptible and resistant strains, respectively, and 100, 97, 100 and 91% after 3 days of feeding.

No significant difference in bait intake was observed between 25 mg/kg difethialone bait and a control bait.

Compared to other anticoagulant rodenticides, these results show that difethialone is a promising rodenticide.

**difethialone — anticoagulant — rodenticide — warfarin — *Rattus norvegicus* — *Mus musculus***

**Résumé** — La diféthialone (LM-2219) : un nouveau rodenticide anticoagulant actif contre les souches sauvages (résistante ou sensible au coumafène) de *Rattus norvegicus* et *Mus musculus*. Nous décrivons les résultats de l'efficacité, obtenus dans les conditions de laboratoire, d'un nouveau rodenticide anticoagulant, la diféthialone, dérivé de la famille de hydroxy-4 benzothioipyranone.

Chez le rat, *Rattus norvegicus*, après une période de consommation d'un jour avec un appât de blé titrant 25 mg/kg de diféthialone, 100% des mâles et 85% des femelles sensibles au coumafène sont morts, et 90% des mâles et 94% des femelles de la souche résistante. La mortalité était respectivement 100 et 96% chez les mâles et les femelles sensibles après une période de consommation de 3 jours. Chez la souche résistante, elle était de 100 et 93%.

Chez la souris, *Mus musculus*, après un jour de consommation, la mortalité était 97, 94, 95 et 93% des mâles et femelles des souches sensible et résistante respectivement, et 100, 97, 100 et 91% après 3 jours de consommation.

La comparaison de la consommation de l'appât contenant 25 mg/kg de diféthialone avec celle de l'appât seul, montre que la diféthialone est bien acceptée par le rat et la souris.

*Comparée aux autres anticoagulants, les résultats obtenus montrent que la diféthialone est un rodenticide prometteur.*

**diféthialone — rodenticide — anticoagulant — coumafène — *Rattus norvegicus* — *Mus musculus***

## Introduction

Since the appearance of warfarin-resistant strains of *Rattus norvegicus* in Europe and the USA (Lund, 1964; Greaves and Ayres, 1967; Jackson *et al.*, 1975), considerable progress in the search for new rodenticides has been made. In the 70's three new anticoagulant rodenticides were commercialized : difenacoum (Hadler and Shabdolt, 1975), bromadiolone (Grand, 1976) and brodifacoum (Redfern *et al.*, 1976).

Many studies concerning the efficacy of these rodenticides, against a wide variety of rodents, and especially against warfarin-resistant strains of *Rattus norvegicus* and *Mus musculus*, have been published (Dubock and Kaukeinen, 1978; Richards, 1981).

About 10 years after the commercialization of these compounds, their intensive use as alternatives of "first generation" anticoagulant rodenticides led to the appearance of resistant strains of *Rattus norvegicus* and *Mus musculus*. Lund (1984) reported some cases of resistance to these compounds.

The present paper reports data on the efficacy against rats and mice of a new anticoagulant rodenticide, LM-2219 (dife-thialone) : [(bromo-4'-(biphenyl-1-1')-yl-4) 3-tetrahydro-1, 2, 3, 4- naphthyl-1] 3-hydroxy-4 2H-1 benzothiopyrane-one, which belongs to a family other than that of the compounds described before. It is the first representative of the family of 4-hydroxy-benzothiopyranone.

This compound was synthesized by LIPHA (Lyon, France) and is not yet commercialized. The initial data on its anticoagulant activity were described by Lechevin in 1986.

## Materials and Methods

### Animals

Feeding tests were carried out on wild, individually caged *Rattus norvegicus*. The susceptible strain was reared in a terrarium; neither the parents, nor their progeny used for the tests, had ever been in contact with rodenticides. The resistant strain was warfarin-resistant, reared in a terrarium, where selection for resistance (6 days 250 mg/kg of warfarin bait) had been continued for 6 years. The resistance ratio in the population was more than 77%. Prior to tests, a 21 day period of acclimatization was observed.

Both strains of *Mus musculus* (susceptible and warfarin-resistant) were reared in the laboratory. The resistant strain was issued from parents who had survived a 21 day test with 250 mg/kg of warfarin bait. A test to evaluate resistance of progeny was performed periodically to insure that the resistance ratio remained over 50%.

Eight days prior to tests, mice weighing 15-30 g were housed in a glass terrarium (30 x 60 x 40 cm) in groups of 10-15 individuals of the same sex.

All animals were maintained on a laboratory diet (UAR-04) and water, *ad libitum*, up to bait distribution without any fasting period.

### Baits

Liquid colored concentrates, supplied by LIPHA, containing 1.25 g/l of difethialone, and a control bait were used for bait preparation. 20 ml of concentrate were added to 1 kg of wheat

or semolina in order to obtain baits containing 25 mg/kg of the active ingredient.

### Tests

Two types of tests were carried out : The efficacy test was a "no choice" test. Baits were offered daily, in a trough, for periods of 1 or 3 days, after which normal laboratory diet was redistributed *ad libitum*. Bait consumption and losses were measured daily; animals were observed for a period of 21 days.

The palatability test was a "choice" test which consisted of measuring the ratio of poisoned bait consumed in comparison to the whole bait intake (poisoned and control bait). Equal quantities of each bait were offered daily, in two troughs, for a period of 4 days for rats and 7 days for mice. For rats, bait consumption was measured daily, while for mice, it was measured on days 1, 2, 3 and 7. All animals were sacrificed at the 4th or 7th day.

Consumption of poisoned and control bait were compared using the Student's *t*-test.

Rats were weighed, in both tests, the day of bait distribution, whereas the mice were only counted.

### Results

Following a one day feeding period, mortality was for male and female rats 93 and 92% in the warfarin-susceptible and resistant-strains, respectively (Table I).

Analysis of individual data shows that all males of the susceptible strain died after a one day feeding period. The lethal dose of active ingredient absorbed was  $1.65 \pm 0.5$  mg/kg (range 0.46-2.67). Four females who consumed 0.96, 0.93, 0.71 and 0.55 mg/kg of active ingredient survived the test, while the lethal dose for the remaining females was  $1.86 \pm 0.55$  mg/kg (range 1.08-2.83).

In the resistant strain, the lethal dose for males was  $1.49 \pm 0.33$  mg/kg (range 1.00-2.28). Two males which consumed, 0.12 and 0.14 mg/kg respectively, of

active ingredient survived. The quantities taken by the latter were inferior to the minimal dose which killed warfarin-susceptible males. Sixteen out of 17 warfarin-resistant females were killed with a lethal dose of  $1.77 \pm 0.85$  mg/kg (range 0.1-4.20).

After a three day feeding period (Table I), mortality was 98 and 97% for warfarin-susceptible and -resistant strains, respectively. For the warfarin-susceptible strain, in the first day of the test, rats ingested a dose of active ingredient of  $1.75 \pm 0.49$  and  $2.05 \pm 0.63$  mg/kg for males and females, respectively. Warfarin-resistant males and females ingested,  $1.79 \pm 0.59$  and  $1.55 \pm 0.17$  mg/kg respectively. These doses were similar to the lethal doses in the one day feeding test. The surviving warfarin-susceptible females ingested 0.68 mg/kg on day three only. Resistant strain females consumed 0.23 mg/kg on day one only.

The comparison of lethal doses ingested the first day on the tests with the  $LD_{50}$  (Lechevin, 1986) shows that the former were superior and that when rats fed normally on the bait, death could occur.

With male and female mice, 96 and 94% mortality for the non-resistant and resistant strains were obtained after a one day feeding period (Table II). The mean daily intake of bait was 3.1 and 4.1 g/mouse for non-resistant males and females, respectively. In the resistant strain, daily intake was 3.1 and 3.3 g/mouse for males and females, respectively.

After three days of feeding, male and female deaths reached 98 and 95%, respectively, for the susceptible and resistant strains.

The "choice feeding" test (Table III) show that 25 mg/kg of difethialone bait was well accepted by rats and mice. No

**Table I.** Bait consumption and mortality of *Rattus norvegicus* fed 25 mg/kg of Difethialone bait.

Sex	Mean body weight (g)	Bait consumption (g/kg) <sup>a</sup>			No. of rats refusing bait <sup>b</sup>	Mortality <sup>c</sup> (%)	Days to death (range)
		day 1	day 2	day 3			
Warfarin susceptible							
1 day of feeding							
M	310	65.9 ± 20.1	—	—	2	29/29 (100)	3-9
F	230	67.8 ± 25.6	—	—	2	23/27 (85)	4-10
3 days of feeding							
M	311	70.1 ± 19.8	73.1 ± 18.3	82.3 ± 42.5	0	26/26 (100)	6-10
F	207	82.1 ± 25.3	95.3 ± 45.0	101.0 ± 57.2	0	23/24 (96)	3-9
Warfarin resistant							
1 day of feeding							
M	396	54.3 ± 20.9	—	—	2	18/20 (90)	4-11
F	284	67.2 ± 36.0	—	—	1	16/17 (94)	2-10
3 days of feeding							
M	364	71.5 ± 23.4	79.3 ± 23.6	65.7 ± 19.0	0	15/15 (100)	4-8
F	310	62.1 ± 6.7	71.2 ± 23.0	67.5 ± 19.2	0	13/14 (93)	4-12

<sup>a</sup> Daily bait consumption (mean ± SD).

<sup>b</sup> Individual daily bait consumption is less than 1 g/rat.

<sup>c</sup> Rats who refused bait were excluded.

**Table II.** Bait consumption and mortality of *Mus musculus* fed 25 mg/kg of difethialone bait.

Sex	Bait consumption <sup>a</sup>			Mortality (%)	Days of death (range)
	day 1	day 2	day 3		
Warfarin susceptible					
1 day of feeding					
M	104.1	—	—	33/34 (97)	6-18
F	144.1	—	—	33/35 (94)	7-18
3 days of feeding					
M	158.7	114.1	97.6	34/34 (100)	6-20
F	150.6	158.4	126.1	28/29 (97)	6-20
Warfarin resistant					
1 day of feeding					
M	127.8	—	—	39/41 (95)	4-14
F	137.1	—	—	39/42 (93)	5-16
3 days of feeding					
M	103.6	108.5 (36) <sup>b</sup>	74.5 (31)	38/38 (100)	2-16
F	148.7	134.3 (42)	107.2 (41)	39/43 (91)	2-15

<sup>a</sup> Total bait consumption of all mice.

<sup>b</sup> Numbers in brackets are the number of mice alive.

significant differences in bait intake was observed during the first three days of the test.

## Discussion

Initial data on the efficacy of difethialone showed that 50 mg/kg of bait killed warfarin-susceptible and -resistant strains after a one day feeding period (39/39, 19/19 rats, respectively). The concentration recommended by the manufacturer is 25 mg/kg, so tests were performed with this concentration.

On the basis of the performance of "second generation" anticoagulant rodenticides (brodifacoum, difenacoum and bromadiolone), the search for new rodenticides is directed towards compounds for which the efficacy is complete after 1 or 2 feedings. One and 3 day feeding periods were used, and the lethal feeding period (LFP<sub>50</sub> and LFP<sub>98</sub>)

estimated as described by Redfern and Gill (1978) had no significance.

Comparison of these results with those from different authors concerning other compounds is very difficult, since various protocols have been used. Hadler *et al.* (1975), Redfern *et al.* (1976) and Redfern and Gill (1980) used protocols for rodenticide testing with pre-baiting periods with the unpoisoned base bait. Such tests increase bait intake. In these present tests, laboratory diet was given up to bait distribution, which decreases bait consumption following bait shyness, so animals could not reach the lethal dose with only one day of feeding. 25 mg/kg of difethialone bait was more potent than 50 mg/kg of bromadiolone, used against warfarin-resistant rats, the latter killed only 17/60 rats after a one day feeding period (Redfern and Gill, 1980). Difenacoum, at the same concentration as bromadiolone, killed 9/10 resistant rats after two days of feeding (Hadler *et al.*, 1975). Data from Redfern *et al.* (1976) concerning brodifacoum

**Table III.** Palatability of 25 mg/kg of difethialone bait compared to the control bait.

Baits	Bait consumption <sup>a</sup>			
	day 1	day 2	day 3	day 4 <sup>b</sup>
<i>Rattus norvegicus</i> <sup>c</sup>				
poisoned	9.3 ± 6.8	9.4 ± 5.8	10.1 ± 6.8	6.6 ± 5.8
control	7.7 ± 6.0	10.1 ± 5.3	10.3 ± 6.3	8.9 ± 6.2
palatability (%) <sup>e</sup>	54 ± 27	47 ± 22	49 ± 23	41 ± 28
(P) Student's t test	0.14	0.54	0.86	0.03
<i>Mus musculus</i> <sup>d</sup>				
poisoned	26.4	21.6	24.0	28.9
control	33.5	30.9	25.4	88.0
palatability (%)	44.1	41.1	48.6	24.7

<sup>a</sup> For mice, total consumption (g); for rats, mean ± SD daily consumption (g of bait).

<sup>b</sup> For mice, consumption from day 4 to day 7.

<sup>c</sup> 37 males and 33 females.

<sup>d</sup> 12 males and 12 females.

<sup>e</sup> Palatability = 100 x poisoned bait consumption/total intake of poisoned and control baits.

coum, showed that 1 day of feeding with 10 mg/kg of bait caused the death of 10/10 warfarin-resistant females. No data concerning males was given. All other data available on brodifacoum employs a 2 days feeding period.

Results for *Mus musculus* show that difethialone is more active than bromadiolone and difenacoum, but appears to have the same efficacy as brodifacoum. The latter, after a 2 day feeding period with 20 mg/kg of bait, killed 19/20 mice (Redfern *et al.*, 1976; Redfern and Gill, 1980).

All anticoagulant rodenticides cited in this paper have been shown to be palatable for all species under laboratory conditions (Hadler *et al.*, 1975; Redfern *et al.*, 1976; Dubock and Kaukeinen, 1978; Redfern and Gill, 1980).

From this results it can be concluded that difethialone is a very potent anticoagulant rodenticide, effective against *Rattus norvegicus* and *Mus musculus*. The data obtained suggest that the efficacy of difethialone is similar to that of brodifacoum. Field tests and hazards for nontarget species could give it an advantage over other rodenticides.

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