ACTIVITY OF LM 2219 (DIFETHIALONE), A NEW ANTICOAGULANT RODENTICIDE, IN COMMENSAL RODENTS

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ABSTRACT: Preliminary studies completed on commensal rodents with the new anticoagulant rodenticide difethialone showed very good efficacy, such that 25 ppm baits could be used effectively. New test results presented in this publication confirm the activity as shown under laboratory conditions in choice tests, which represent more severe conditions, as well as its effectiveness against rodents that are resistant and non-resistant to warfarin. In tests where the palatability was only fair the chemical activity resulted in excellent mortality. In a field test against a large population of Mus musculus the results proved very satisfactory. Difethialone is toxic to birds and fish. However, it seems to be better tolerated by dogs and pigs, animals that are frequently on the list of accidental poisonings. Difethialone is stored over a prolonged period in the liver but the risk to non-target species consuming rodents having ingested the compound does not seem to be high. For reasons attributed to the mode of action, difethialone must be handled with precautions as other anticoagulants for which Vitamin K_1 is the antidote. In the event of an accidental poisoning, an antidotal therapy plan is proposed. The lower levels of active ingredient in finished baits (25 ppm) should pose a low risk to non-target species.

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INTRODUCTION

LM-2219, the proposed common name difethialone, is the first representative of a new chemical family called hydroxy-4 benzothiopyranones. The pharmacological and toxicological properties of this compound were published by Lechevin (1986), as well as activity on the compound in commensal rodents (Lechevin 1986, 1987b) and on several field species (Lechevin 1987a).

The chemical modification involving the replacement of oxygen by sulfur in the 1'hydroxy-4 coumarin (Figure 1) has generated interesting improvements in toxicology. Difethialone also possesses reduced oral subchronic toxicity, weaker than its oxygenated homologue, brodifacoum and equally less active than warfarin in the pig (Table 1).

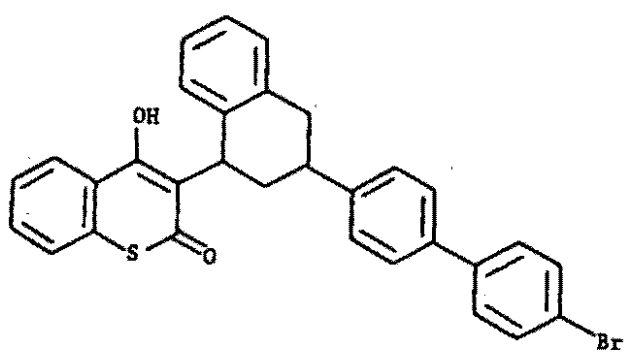

Fig. 1. Structure of difethialone.

The differences in toxicity as shown in Table 1 are clear if one considers the level of difethialone in baits (25 ppm). In these conditions the maximum tolerated dose in the dog of 10 kg in weight, a dose level which might produce clinical signs in the animals but not mortality, is 400 grams for difethialone and 40 to 100 grams for brodifacoum (Table 2).

In the area of commensal rodent efficacy, difethialone is characterized as rapid in action and very palatable. In the

Table 1. Comparative oral subchronic toxicity in the pig (Lorgue 1986).

	Dose administered (mg/pig/day)	Results
Brodifacoum	1	Mortality 14, 15 and 18 days after administration
Difethialone	1	No mortality 30 days after administration
Warfarin	5	Mortality 8, 9 and 10 days after administration

Table 2. Acute oral toxicity in the dog.

	Maximum tolerated dose in grams for 10 kg dog
Brodifacoum bait (50 mg/kg)	40 - 100 g[1]
Difethialone bait (25 mg/kg)	400 g[2]

[1]Godfrey et al. 1981
[2]Lorgue et al. 1986

laboratory, it is possible to obtain 100% mortality in both non-resistant or warfarin-resistant rodents presented bait for 24 hours at 25 mg/kg (Table 3).

Table 3. Toxicity of 25 ppm difethialone baits to rodents offered in no-choice tests for 24 hours (Lorgue 1986).

Species		Mortality		Time until death (days)	
Rattus norvegicus	N	42/44	95.5%	3-11	Mean 7.2
	R	33/33	100%	4-10	Mean 7.0
Rattus rattus	R	25/27	92.6%	4-16	Mean 7.5
Mus musculus	N	66/69	95.7%	7-18	Mean 9.9
	R	78/83	94%	4-16	Mean 8.1

N = Non-resistant
R = Resistant

The palatability of commercial baits developed in France (whole wheat for Rattus, semolina for Mus) is excellent because the consumption of toxic bait is always in excess of 45% of the total consumption (Table 4).

Table 4. Laboratory studies using difethialone baits (Lorgue 1986).

Species	Number	Consumption of toxic bait as percent of total consumption
Rattus norvegicus	50	45.6
Rattus rattus	10	50.4
Mus musculus	20	51.3

Difethialone's relatively rapid mode of action, coupled with excellent acceptance in the laboratory, were confirmed in field trials in which infestations of Norway rats and roof rats were treated with baits (Table 5).

These preliminary findings obtained in France prompted the development of difethialone in other countries.

Table 5. The results of a field trials with 25ppm difethialone-wheat bait to control Norway rats (Grolleau 1986) and roof rats (Grolleau 1987).

Species	Test condition	Population reduction
Norway	Duck, pheasant and partridge farm infested with about 3400 Norway rats	96.8
Roof rat	Swine farm	97.4

EFFICACY TESTS

In the U.S. a number of laboratory studies were conducted using difethialone against various wild rodent species (Biocenotics 1987).

Tables 6 and 7 present the results against wild Rattus norvegicus and Mus musculus presented with 25 ppm pelleted and meal formulations. The studies followed choice test EPA guidlines over three days of toxic bait presentation. Difethialone when used against Norway rats showed the chemical activity was quite good, even when the palatability was reduced as seen in one pellet formulation (30.1% acceptance). The development of a newer formulation showed results of 59.7 and 56.3% acceptance (Chempar, unpublished). The data presented in Table 7 present the amount of active material required to induce mortality was 0.092 and 0.65 mg/kg.

In studies involving the use of the compound against warfarin resistant mice using a meal formulation, mortality was quite good even though palatability was somewhat mediocre (Table 8: Biocenotics, unpublished data). In the 15-day study, 15 of the 20 animals died within 2 to 10 days after the study was initiated.

Table 6. Laboratory evaluation of difethialone against Norway rats and house mice (Biocenotics 1987).

Bait type	Consumption over 3-day test			Mortality & time until death	Palatability (%)
	Bait	EPA placebo	Total		
Meal	39.9	33.6	73.5	20/20 5-9 days Mean 7.05 days	54.3
Pellets	18.2	42.2	60.4	17/20 3-12 days Mean 7.4 days	30.1

Table 7. Activity and palatability of difethialone baits against house mice (Biocenotics 1987).

Bait type	Consumption Total in grams			Mortality & Time until death	Palatability
	Bait	EPA placebo	Total		
Meal	114.6	69.4	184	19/20 5-13 days Mean 7.5 days	62.3
Pellets	172.4	126.1	298.5	37/40 4-13 days Mean 6.8 days	57.8

Table 8. Efficacy of difethialone 25 ppm meal bait used against warfarin resistant mice (Biocenotics 1987).

Consumption (g) in 15-day study			Mortality and time until death	Palatability (%)
Bait	EPA placebo	Total		
56	164.4	220.4	20/20 2-25 days Mean 8.6 days	25.4

Satisfactory results were obtained in choice and no-choice testing against rats using both resistant and warfarin-resistant strains of rats from Denmark, where rodents are reported to be resistant to bromadiolone (Lund, 1987). The choice test results are summarized in Table 9.

Palatability is better in the rolled oat bait. With the rolled barley bait used against resistant strains, although lower acceptance of 22.1% was noted, mortality was 70%. Resistant Norway rats presented with baits for four days (no-choice) resulted in 100% mortality with the mean time until death at 5.4 days.

A laboratory study against Rattus rattus in a choice test over a five-day period showed good potential of the particular meal formulation (Baker 1987). The results are presented in Table 10.

The oral LD50 of difethialone in three species of commensal rodents indicates there is not a notable difference between sexes nor between warfarin-resistant and non-resistant animals (Lorgue et al. 1987). Table 11 presents the LD50 values for the Norway rat, roof rat, and house mouse.

The ratio: LD50 resistant strains/LD50 non-resistant strains portrays that resistance is never in excess of 1.21 (Mus musculus, females). There is, therefore, no reason to believe that difethialone will not be capable of controlling warfarin-resistant rodents.

Field efficacy test

Field trials were completed in France against house mice on a rural farm heavily infested with rodents. Previous baiting with difenacoum and bromadiolone did not give satisfactory results (Grolleau et al. 1986). The test protocol used was that designed by the Societe Francaise de Phytiatrie et de Phytopharmacie. Two applications of 25 ppm wheat semolina bait were made, the results of which are given in figure one. Five days after the initial application the population reduction was only 78.8%, therefore a second application was performed. The net reduction in rodents was satisfactory, at 92.5% (Figure 2). The inability to obtain complete eradication after a single application is not surprising even though excellent laboratory results were obtained. As rodent control experts agree, the extension of control is more than likely due to the presence of large numbers of house mice which are traditionally more difficult to control with anticoagulants. As a result, many recommend prolonged treatment for at least 15 consecutive days. One should note that in field trials for the control of house mice where populations are low, such as farms and sheds, a single application of bait over five days should eliminate the rodents (Groupement de Defense Sanitaire du Finistere, 1986). The palatability of wheat semoline bait is very good because after the adjustment to the bait over a period of only one day, the consumption of the toxic bait represented on day two was 97.3% of the initial first day level of consumption of placebo bait.

Toxicity to non-target species

Difethialone is relatively well tolerated by the dog (Table 2), since a single ingestion of 400 grams of bait would not produce mortality. As noted in Table 12, two other mammal species, notably the cat and pig, have a relatively moderate acute oral toxicity level for the compound. The maximum tolerated dose in the hare is low but it is well known as with the rabbit that this species is particularly sensitive to

Table 9. Activity and palatability of baits against Norway rats in Denmark (Lund, unpublished data).

Warfarin resistant or non-resistant & length of test	Bait at 25 mg/kg	Total consumption (g) Bait	Placebo	Total	Mortality and time until death	Palatability (%)
Non-resistant 4-days	Rolled barley	190.1	268.6	458.7	10/10 5-12 days	41.4
Non-resistant 4-days	Rolled barley	125.8	273	600.9	10/10	20.9 54.5
	Rolled oats	202.1			4-7 days	33.6
Resistant 6-days	Rolled barley	142.9	503.9	646.8	7/10 5-8 days	22.1

anticoagulant products.

Difethialone is relatively toxic to birds tested and is an important consideration in rat control, particularly in poultry houses (Table 13).

The situation is similar with regard to Daphnia magna and fish (see Table 14).

Table 10. Laboratory efficacy against the roof rat (Baker, unpublished data).

Total consumption (g) over 5 days			Mortality and time until death	Palatability (%)
Bait	EPA placebo	Total		
860	1,385	2,245	20/20 6-13 days Mean 8.2 days	38.3

11.

Table 12. Difethialone LD50 values in wild rodents (Lorgue et al., unpublished).

Species		Oral LD50 (mg/kg)		
		Male	Female	Combined sexes
Rattus norvegicus	N	0.62	0.42	0.51
	R	0.27	0.39	0.29
Rattus rattus	R	0.39	0.36	0.38
Mus musculus	N	0.52	0.43	0.47
	R	0.46	0.52	0.48

N = Non-resistant
R = Warfarin-resistant

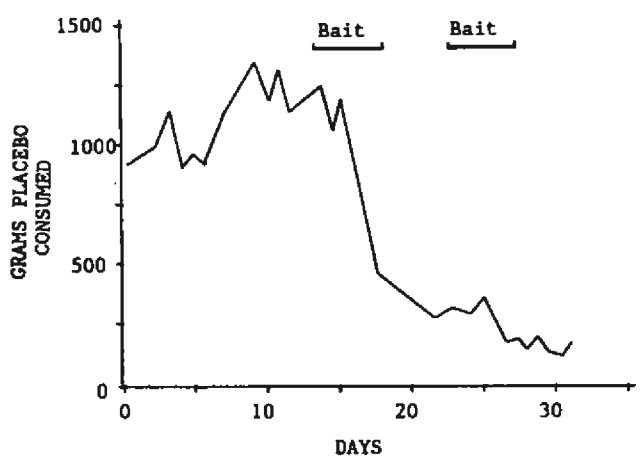

Fig. 2. Results of field trial using difethialone to control house mice in France (Grolleau et al. 1986).

Table 12. Acute oral toxicity of difethialone in non-rodent species (Lorgue et al, unpublished data).

Species	LD50 mg/kg	Maximum tolerated dose	
		mg/kg	gm Bait/animal (weight in kg)
Cat	--	> 16	2560 / 4 kg
Hare	0.75	0.25	26 / 2.6 kg
Pig	2 - 3	0.9	1080 / 30 kg

Table 13. Difethialone acute and subchronic toxicity in birds.

Species	LD50 (mg/kg)	LC50 (ppm)
Mallard duck	--	1.94
Bobwhite quail	0.264	0.56

Table 14. Difethialone toxicity to aquatic organisms.

Species	LD50 (ug/litre)				No effect level ug/litre
	24 h	48 h	72 h	96 h	
Daphnia magna	>5	4.4	--	--	3 - 48 hours
Bluegill sunfish	180	110	80	75	48 - 96 hours
Rainbow trout	86	67	56	51	22 - 96 hours

Kinetics and Metabolism

The summary of tests completed on laboratory rats with radio labeled ^{14}C is given in Table 15. Difethialone is characterized by a short half-life in blood, a longer half-life in the liver, and elimination (essentially fecal) with an almost complete absence of metabolism. It was not possible to calculate the half-life in blood at 5 mg/kg because of mortality in the test animals six days after administration. The prolonged half-life in liver (108 days for the 0.5 mg/kg dose level) should not pose a threat of secondary hazard potential to non-target species because of the level of the active ingredient in the finished product.

In the study conducted at the 5 mg/kg dose level, we were able to calculate the amount of residual difethialone in the rat, then extrapolate the data to examine the potential risk to non-target species. In a routine rodent control operation for five consecutive days, a Norway rat of 350 grams in body weight would consume approximately 25 grams of bait per day, or 3.125 mg of difethialone (8.93 mg/kg). In considering information presented in this paper, the quantity of difethialone residue six days after the start of toxic bait ingestion would be approximately 958 ug. Although this is not a

negligible amount, when examining the maximum tolerated doses for the cat, dog and pig, one may assess the risk to be relatively low (see Tables 2 and 12).

Table 15. Kinetics and metabolism of difethialone (Belleville 1986).

Dose Administered mg/kg	Half-life in days		Elimination
	Plasma	Liver	
0.5	2.3	108	fecal 47% in 3 days
5	2.8	--	fecal 83.1% in 4 days

CONCLUSIONS

In the area of commensal rodent control, difethialone is characterized by a relatively rapid mode of action for an anticoagulant, and superior efficacy against both warfarin resistant and non-resistant rodent species. Because of its inherent activity, there is a built-in risk at the use level of any products containing this compound. As with any pesticide it is imperative that the users of difethialone products take the necessary precautions as with handling any other anticoagulant rodenticide. Difethialone does have the antidote Vitamin K_1 which is always effective when the treatment is done correctly. An antidotal therapeutic method is proposed following a dog study (Lechevin 1986). Since 25 ppm baits are proposed end use products, the projected non-target species secondary hazard potential should be low.

LITERATURE CITED

BAKER, R.O. 1987. Efficacy of 0.0025% LM-2219 (Difethialone) on Rattus rattus under laboratory conditions. Unpublished report prepared for Lipha Chemicals, Inc. 15 pp.

BELLEVILLE, M. 1986. Toxi-Kinetics and Metabolism of LM-2219. Unpublished report. 145 pp. (In French).

BIOCENOTICS. 1987. Laboratory efficacy trials with LM-2219. Unpublished report prepared for Lipha Chemicals, Inc.

GODFREY, M.E.R., REID, T.C., and H. J.F. MCALLUM. 1981. The acute oral toxicity of the anticoagulant brodifacoum in dogs. New Zealand J. of Exper. Ag. 9:147-149.

GROLLEAU, G. and J. PANCIROLI. 1986. The acute oral toxicity of the anticoagulant brodifacoum to dogs. New Zealand J. of Exper. Ag. 9:147-149.

GROUPEMENT DE DEFENSE SANITAIRE DU FINISTERE. 1986. Unpublished report. Quimper France.

LECHEVIN, J.C. 1986. Preliminary experimental results obtained with a new anticoagulant rodenticide LM-2219. Parsitis. Geneva, Switzerland (In French).

LECHEVIN, J.C. 1987a. Use of LM-2219, new anticoagulant rodenticide, in campaigns against field rodents. Conference on Rodents (OEPP and FAQ). Rome, Italy. (In French).

LECHEVIN, J.C. 1987b. Contribution of LM-2219, a new anticoagulant rodenticide, in commensal rodent control campaigns. Simposio Sulla Difesa Antiparassitaria Nelle Industrie Alimentari. Piacenza, Italy. (In French).

LORGUE, G. 1984. 1986/1987. Studies with LM-2219. Eco-toxicology Laboratory (INRA-ENVL). National Veternarian School. Lyon, France. Unpublished reports. (In French).

LUND, M. 1987. Studies with LM-2219. Unpublished report. Lyngby, Denmark.