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Some properties of calciferol as a rodenticide*

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SUMMARY

The potentiality of calciferol (alone and combined with warfarin) for the control of commensal rats and mice has been examined in the laboratory. Nearly all animals fed on 0·1 % calciferol for 2 days died. Though illness usually reduced food intake after the first 24 hr. there was no sign of aversion to the poison at 0·1 % – which is considered to be the lowest concentration suitable for use against *Rattus norvegicus*, *R. rattus* and *Mus musculus* in the field. There was some indication that resistance to warfarin in *R. norvegicus* may be correlated with susceptibility to calciferol. Toxicity tests with calciferol combined with warfarin indicated an additive effect between the compounds. No evidence for synergism was found however, although elsewhere there is some evidence for this.

INTRODUCTION

One of the problems that has confronted agriculturalists, public health authorities and others in Britain during the last decade has been the development of populations of rats and mice resistant to the widely used anticoagulant rodenticides such as warfarin (Greaves, 1971). In an attempt to solve the problem this laboratory has pursued collaborative research with the chemical industry with the object of identifying and developing new rodenticides. The present paper describes a laboratory investigation of calciferol (vitamin D_2). Study of this compound was prompted by data presented by Mr M. R. Hadler of Sorex (London) Ltd. regarding its promise against warfarin-resistant rats and mice and by the knowledge that his company would soon be marketing it in a formulation that also contained warfarin.

The action of calciferol is to raise blood calcium levels by stimulating the absorption of calcium from the intestine and mobilizing skeletal reserves. This takes many hours to build up to an effective level and the period of latency between the ingestion of calciferol and the development of hypercalcaemia has particular relevance to the effectiveness of the compound as a rodenticide.

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METHODS

Wild rodents were used in nearly all the tests to be described and they included warfarin-resistant and non-resistant representatives of the three important commensal species, the Norway rat (*Rattus norvegicus*), the ship or roof rat (*Rattus rattus*) and the house mouse (*Mus musculus*). Males and females were employed in approximately equal numbers. Warfarin-resistant Norway rats were trapped on farms in Montgomeryshire, Shropshire and Kent and non-resistant rats caught by hand in a refuse destructor. Ship rats, both resistant and non-resistant, were trapped in dockland areas of Merseyside and London. Resistant house mice were caught by hand in agricultural premises in Hampshire and Nottingham, while non-resistant mice were bred in captivity from wild-caught parents. The 'non-resistant' animals were actually only presumed to be such on the basis that they were drawn from populations where to the best of our knowledge warfarin-resistance was not present. Resistant animals were classified as such after survival of a test exposure to warfarin, viz. a single subcutaneous dose of 200 mg./kg. of warfarin in dimethyl formamide (Norway rat), or unrestricted feeding on a sole diet of oatmeal containing 0·025 % warfarin for 21 days (house mouse) or 28 days (ship rat). As these methods of classification were empirical and to some extent arbitrary, it is possible that a few animals, particularly among the resistant Norway rats and the non-resistant ship rats may have been wrongly characterized.

Except where stated, animals were caged singly and maintained on Diet 41B for at least 2 weeks before being weighed within a few days of testing. Resistant animals were permitted to recover from their warfarin pre-treatment for a minimum of 2 weeks before being given calciferol. Feeding tests were of two kinds. First, there were tests in which the rodents were allowed to feed without restriction on a diet solely of poisoned bait for a specified number of days, after first being given the same bait unpoisoned in the experimental food pots for a few days. Second, there were tests in which animals were presented with a choice between plain and poisoned baits; fresh food pots were provided and the positions of the two baits interchanged each day. Any animal that rejected both of the baits on the first day was eliminated from the test. In all feeding tests bait consumption was measured regularly, usually each day, and fresh bait provided. Animals were maintained on Diet 41B for a 2-week observation period after withdrawal of the experimental baits and mortality recorded daily.

To study the possibility of synergism between warfarin and calciferol, a comparison was made of the subacute toxicity of the compounds administered separately and in combination. Male LAC Grey mice were grouped five to a cage and the compounds administered as four daily doses by stomach tube, using 5 % gum acacia as the vehicle. Dosage levels and the ratio of the two compounds in the mixture (warfarin: calciferol = 7:4) were chosen on the basis of preliminary assays, in such a manner that if the hypothesis that the toxicities were merely additive were correct, the 4-day log LD50 of the mixture would lie between those of the constituent compounds (see Finney, 1971).

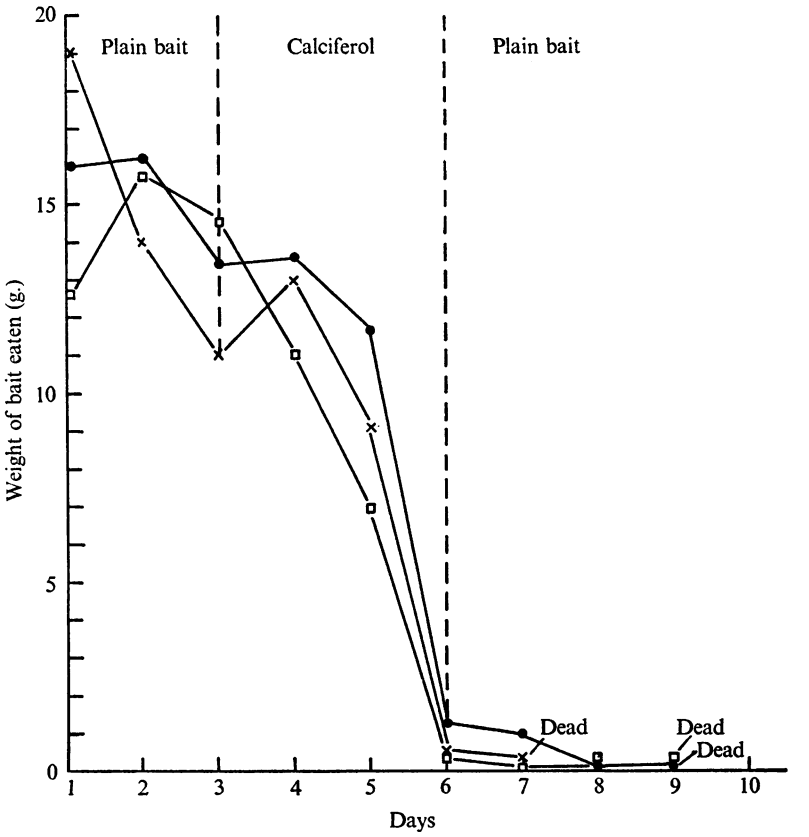

Fig. 1. Individual feeding figures for 3 wild non-resistant *R. norvegicus* given 0·1 % calciferol in pinhead oatmeal/corn oil bait for 3 days.

In the majority of feeding tests the poisoned bait was prepared by dispersing a wheat flour premix of calciferol (99 % purity, B.P. grade) and/or warfarin (99 % purity) at 5 % in medium oatmeal. In tests of a proposed field formulation a proprietary warfarin premix (fine oatmeal containing 0·5 % warfarin and 1·0 % Chlorazol Sky Blue FF200) was added to an equal amount of a corn oil solution of technical grade calciferol (67 % purity) and the resulting slurry thoroughly mixed at 10 % with medium oatmeal. Other baits in which corn oil was included were prepared in a similar manner. Plain bait was prepared in the same way as the poisoned bait for each test, the proprietary warfarin premix being replaced by wheat flour.

RESULTS AND DISCUSSION

Speed of rodenticidal action

In preliminary tests in which doses of 100 mg./kg. of calciferol were given on one or more days by stomach tube, laboratory rats and mice became visibly ill within 3 days. In feeding tests the onset of illness tended to curtail bait intake slightly on the 2nd day and severely on the 3rd day. Few animals ate more than a fraction of a gramme of bait on each subsequent day, and deaths usually ensued

Table 1. *Mortality and bait consumption by wild rodents given a sole diet of calciferol in medium oatmeal for 2 days*

Species	Type of animal	Mean body weight (g.)	Sex	Concentration of calciferol (%)	Mortality	Mean daily bait intake (g.)		Lethal dose of active ingredient (mg./kg.)		Survived dose of active ingredient (mg./kg.)		Days to death	
						Pre-bait*	Poison	Mean	Range	Mean	Range	Mean	Range
Rattus norvegicus	Non-resistant	250	M	0·1	5/5	16·2	11·8	95	82–104	—	—	4·2	4–5
		198	F		5/5	12·3	11·8	115	84–153	—	—	6·2	4–10
		244	M	0·05	4/5	17·4	16·0	65	53–75	67	—	5·5	5–7
		175	F		5/5	13·9	12·6	74	52–104	—	—	5·4	4–7
		260	M	0·025	1/5	15·7	14·2	37	—	26	20–33	8·0	—
		198	F		3/5	16·8	13·0	29	26–32	43	35–51	5·3	4–6
		271	M	0·0125	0/5	20·0	17·5	—	—	16	16–18	—	—
		178	F		0/5	13·5	13·6	—	—	20	11–24	—	—
		267	M	0·00625	0/5	14·6	13·1	—	—	6	5–8	—	—
		185	F		0/5	14·8	13·5	—	—	9	8–11	—	—
	Resistant	251	M	0·1	5/5	16·9	14·1	101	18–193	—	—	4·4	4–6
		247	F		5/5	16·1	13·0	106	83–133	—	—	4·6	4–6
		169	M	0·05	5/5	11·3	11·4	76	50–109	—	—	4·0	3–5
		142	F		3/5	13·5	10·8	74	61–90	85	84–85	5·0	4–6
		268	M	0·025	7/10	16·0	15·0	30	23–48	28	22–40	4·6	4–5
		217	F		5/10	15·8	13·9	33	27–41	32	25–42	5·6	4–10
		214	M	0·0125	1/5	15·6	16·8	18	—	21	17–25	6·0	—
		165	F		0/5	13·3	11·9	—	—	19	10–26	—	—
		196	M	0·00625	2/5	12·2	13·1	7	6–9	10	7–12	4·0	4
		223	F		3/5	17·0	14·7	7	6–9	12	10–13	4·7	3–7
Rattus rattus	Non-resistant	153	M	0·1	5/5	12·2	8·2	112	89–141	—	—	6·6	5–9
		107	F		5/5	11·2	8·0	156	89–201	—	—	5·8	5–7
		194	M	0·05	5/5	16·7	15·1	92	48–185	—	—	4·4	3–5
		178	F		4/5	20·6	13·6	69	59–83	100	—	3·3	2–4
		194	M	0·025	4/5	13·4	13·0	33	21–40	34	—	4·0	3–7
		115	F		3/5	13·0	11·8	49	36–68	67	62–73	4·3	3–6
		177	M	0·0125	5/5	15·8	14·2	21	15–26	—	—	6·4	4–9
		188	F		2/5	18·4	13·1	16	15–16	19	16–23	5·0	4–6
		203	M	0·00625	0/4	13·8	13·3	—	—	9	6–9	—	—
		144	F		0/5	12·3	11·5	—	—	10	9–11	—	—

* Last day only.

Table 1 (*cont.*)

Species	Type of animal	Mean body weight (g.)	Sex	Concentration of calciferol (%)	Mortality	Mean daily bait intake (g.)		Lethal dose of active ingredient (mg./kg.)		Survived dose of active ingredient (mg./kg.)		Days to death	
						Pre-bait*	Poison	Mean	Range	Mean	Range	Mean	Range
	Resistant	175	M	0·1	3/5	12·0	10·1	120	101–145	118	103–133	5·0	5
		161	F		4/5	6·2	5·4	83	40–149	12	—	5·5	4–7
		197	M	0·05	1/5	10·0	15·1	60	—	32	20–44	4·0	—
		160	F		2/5	10·0	11·0	58	58–59	25	9–42	6·5	3–10
		199	M	0·025	2/5	8·9	9·9	22	18–24	29	28–30	4·0	4
		156	F		2/5	9·3	9·4	33	29–38	30	25–35	7·0	5–9
		193	M	0·0125	0/5	7·3	9·7	—	—	13	7–21	—	—
		153	F		1/5	9·4	12·6	21	—	19	10–26	4·0	—
		161	M	0·00625	0/5	11·2	8·9	—	—	9	3–18	—	—
		175	F		0/5	7·3	5·7	—	—	4	2–6	—	—
Mus musculus	Non-resistant	21	M	0·1	5/5	2·5	2·6	252	212–277	—	—	6·2	4–11
		14	F		5/5	2·1	2·2	312	279–369	—	—	6·0	5–8
		19	M	0·05	5/5	2·2	2·7	138	128–159	—	—	6·0	6
		13	F		4/5	2·1	2·2	162	139–173	186	—	6·0	6
		16	M	0·025	4/5	1·7	2·1	67	53–84	65	—	6·5	6–7
		11	F		4/5	1·9	1·8	94	83–103	50	—	6·0	6
		19	M	0·0125	0/5	3·0	3·4	—	—	44	42–47	—	—
		12	F		1/5	2·4	2·2	39	—	50	44–61	6·0	—
		16	M	0·00625	0/5	2·7	3·0	—	—	25	20–28	—	—
		11	F		0/5	2·4	2·4	—	—	28	23–39	—	—
	Resistant	18	M	0·1	5/5	3·9	2·9	330	265–400	—	—	4·2	4–5
		14	F		5/5	3·6	3·6	500	346–587	—	—	4·4	3–7
		17	M	0·05	2/5	3·7	3·2	188	181–195	184	122–234	7·0	5–9
		11	F		5/5	2·6	2·5	240	217–265	—	—	4·6	4–5
		16	M	0·025	0/5	3·5	2·9	—	—	91	91–103	—	—
		13	F		1/4	3·5	3·2	110	—	129	129–145	6·0	—
		16	M	0·0125	0/5	4·0	3·2	—	—	50	44–58	—	—
		11	F		0/5	2·7	2·5	—	—	56	49–66	—	—

* Last day only.

by the 7th day. The typical pattern is illustrated by the data for three rats in Fig. 1.

Illness induced by a toxic diet sometimes predisposes animals to avoid that diet subsequently – a phenomenon usually termed 'bait shyness' or 'poison shyness'. A theoretical consequence in field treatments with calciferol could be that, if some rodents failed to ingest a lethal dose within the first 2 days, they might then tend to turn to an alternative, non-toxic diet. In the laboratory such considerations make the interpretation of results of prolonged feeding tests problematical, and it was therefore decided to limit the majority of tests to a duration of 2 days.

Toxicity of bait containing calciferol only

The results of feeding tests in which animals were offered calciferol bait for 2 days are summarized in Table 1. As a result of the gradual onset of illness towards the end of the 2nd day the mean daily consumption of calciferol bait, particularly at the highest concentration of 0·1 % was usually rather less than the last day's consumption of unpoisoned pre-bait. However, it can be seen from the last two columns but one of Table 1 that survivors often ate more calciferol than did animals that died, indicating that survival was not associated with any aversion to the compound.

At a concentration of 0·1 % calciferol surpassed all lower concentrations, producing complete mortality in nearly every group that received it. The concentration of 0·1 % is therefore proposed as the lowest that should be considered for use in rodenticidal bait in the field. Warfarin-resistant ship rats were the only animals to survive feeding for 2 days on 0·1 % calciferol and it may well be expedient to increase the concentration further for use against animals in this category. The fact that mortality in warfarin-resistant ship rats and house mice was generally less than in non-resistant animals of the same species suggests that warfarin resistance in these species may be due to a relatively generalized detoxification mechanism rather than to a change in metabolism that affects warfarin specifically. There is also a suggestion in the greater mortality that occurred among warfarin-resistant Norway rats as compared with their non-resistant counterparts, that the former are the more sensitive to calciferol poisoning, possibly owing to the tendency of these animals to suffer from spontaneous vitamin K deficiency (Greaves & Ayres, 1973). The possibility that resistance to warfarin may be positively correlated with susceptibility to calciferol in the Norway rat is of obvious practical importance and deserves to be investigated further.

Toxicity of mixtures of calciferol and warfarin

The results of various tests conducted with bait containing both warfarin at 0·025 % and calciferol at 0·1, 0·025 and 0·01 % are summarized in Table 2. With calciferol at 0·1 % for 2 days (Groups 1–4) there was complete mortality in all three species except for the survival of a single warfarin-resistant ship rat. With calciferol at 0·025 % for 4 days, 10/10 warfarin-resistant Norway rats also died. But when the concentration of calciferol was as low as 0·01 % complete mortality was not attained with warfarin-resistant Norway rats (Group 6) even after a

Table 2. *Mortality and bait consumption by wild rodents given a sole diet of bait containing calciferol and/or warfarin for a limited number of days*

Type of animal	Group	Mean body weight (g.)	Feeding periods (days)	Concentration of poison (%)		Mortality	Mean daily bait intake (g.)		Days to death	
				Calciferol	Warfarin		Pre-bait†	Poison	Mean	Range
R. norvegicus (resistant)	1*	201	2	0·1	0·025	10/10	—	6·2	4·7	3–6
M. musculus (non-resistant)	2*	15	2	0·1	0·025	10/10	—	1·7	3·2	3–4
M. musculus (resistant)	3*	14	2	0·1	0·025	10/10	—	1·5	3·9	3–6
R. rattus (resistant)	4*	140	2	0·1	0·025	9/10	13·6	7·1	5·8	4–11
R. norvegicus (resistant)	5	135	4	0·025	0·025	10/10	14·8	6·1	4·3	3–7
R. norvegicus (resistant)	6	226	28	0·01	0·025	12/14	18·8	9·2	8·5	4–20
R. norvegicus (non-resistant)	7	185	2	0·0125	—	0/10	10·7	12·6	—	—
		229	2	—	0·003125	2/10	11·9	13·6	7·0	6–8
		203	2	0·0125	0·003125	7/9	9·0	11·6	7·6	6–12
M. musculus (non-resistant)	8	12	2	0·0125	—	1/10	2·4	2·2	5·0	—
		12	2	—	0·003125	3/10	2·9	2·1	4·7	4–6
		11	2	0·0125	0·003125	0/10	2·6	2·1	—	—

* Bait contained 5% corn oil. † Last day only.

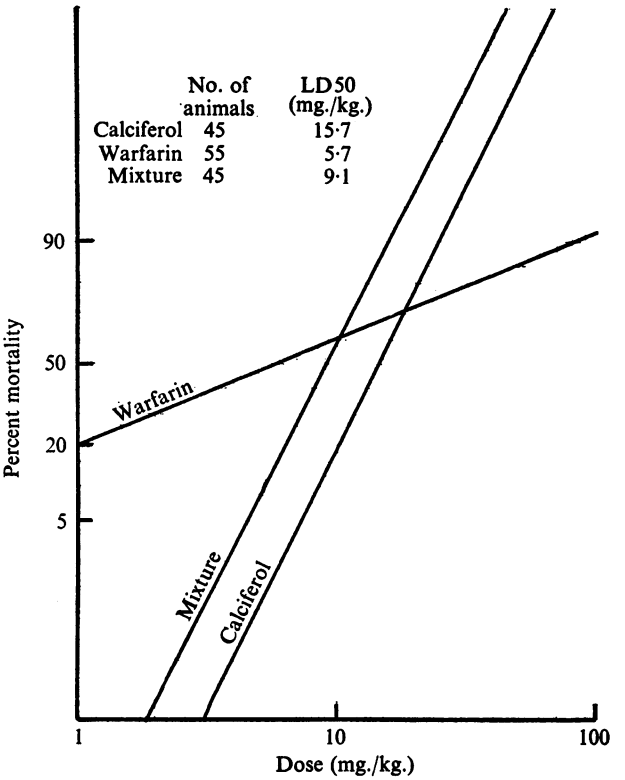

Fig. 2. Subacute oral toxicity of warfarin, calciferol and a 7:4 mixture to LAC Grey mice.

feeding period of 28 days. However, the latter animals (Group 6) were survivors of a pilot field treatment in which exactly the same bait formulation was used as in the laboratory test, and their susceptibility may therefore have been atypically low; their feeding behaviour while on test was of some interest in that the daily bait intake of several rats decreased and increased intermittently as they became ill and recovered in varying degrees. Taken together, the above results indicate that warfarin/calciferol mixtures are no less toxic than calciferol alone.

A not uncommon practical difficulty in field treatments is that rodents may continue to utilize their normal food sources, feeding only partly from the rodenticidal bait that is offered. In an attempt to mimic a low level of bait consumption in the laboratory, bait containing 0·0125 % calciferol and 0·003125 % warfarin (i.e. one-eighth of the candidate field concentration of 0·1 % calciferol and 0·025 % warfarin) was given to non-resistant Norway rats and house mice for 2 days. Two further groups of each species were given bait containing calciferol or warfarin separately, for comparative purposes. The results are given in Groups 7 and 8 of Table 2. The tests with 0·0125 % calciferol alone duplicated the low kills given for this concentration in Table 1, and warfarin also gave low mortalities. In comparison the mixture produced a marked increase in mortality in Norway rats suggesting at least an additive effect between the two compounds.

Table 3. *Bait consumption and mortality in wild* R. norvegicus *and* M. musculus *given a choice between plain and poisoned baits containing* 5 % *corn oil*

Species	Group	Mean body weight (g.)	Duration of test (days)	Concentration of poison (%)		Mean daily bait intake (g.)*		Significance (P) of Student's 't'	Mortality
				Calciferol	Warfarin	Plain	Poisoned		
R. norvegicus	1	253	4	0·025	—	9·0	11·3	< 0·7	3/5
	2	266	4	0·25	—	14·2	6·1	< 0·02	5/5
	3	234	4	0·1	0·025	10·9	6·4	< 0·1	10/10
	4	221	4	0·025	0·025	7·9	5·2	< 0·05	8/10
M. musculus	5	16	4	0·025	—	1·6	1·6	1·0	5/5
	6	14	4	0·25	—	1·2	0·7	< 0·01	5/5
	7	16	4	0·1†	0·025	1·2	0·8	< 0·3	9/9
	8	12	5	0·1†	0·025	1·2	0·8	< 0·2	9/9

* During the first two days of each test.

† Active ingredients in the form of proprietary concentrates.

With house mice however the kill with the mixture was below that obtained with either compound given separately. To follow up this curious observation in conditions more reproducible than could be provided in a feeding test, the compounds were administered separately and together to LAC Grey mice by stomach tube. The results are summarized in Fig. 2. The difference between the slopes of the lines for warfarin and calciferol precludes an analysis of relative potency, though it may be noted that, in terms of slope, the mixture resembles calciferol rather than warfarin. At the LD50 level the mixture is intermediate in toxicity between calciferol and warfarin, indicating an additive effect at this point.

We do not consider that the present results provide a basis for suggesting that any synergistic or other interaction between warfarin and calciferol exists. However, this subject also might repay further investigation, for Mr M. R. Hadler (private communication) reports that in oral intubation tests with *R. norvegicus* heterozygous for warfarin resistance, calciferol at 10 mg./kg. plus warfarin at 20 mg./kg. in polyethylene glycol administered on each of 3 days killed 8/8 animals – whereas the results of administering calciferol at 10 mg./kg. alone and warfarin even at 50 mg./kg. were 0/5 and 1/8 dead, respectively.

In similar tests with *Mus musculus* calciferol plus warfarin, both at 10 mg./kg., killed 10/10 animals although the two poisons separately killed 4/10 and 0/10 respectively. A complete kill (10/10) was also obtained with the two poisons each at 5 mg./kg. – compared with kills of 2/10 and 1/10 respectively when calciferol and warfarin were used alone at the same concentration.

It may be noted in passing that partial reversal of prothrombin deficiency by vitamin D has been reported (Elliott, Isaacs & Ivy, 1940); contamination with vitamin K is a possibility which must be considered in relation to this report, but taken at its face value it suggests an antagonistic action between warfarin and calciferol. Against this, it seems possible that the vascular lesions familiar in chronic hypervitaminosis D (Hass, Trueheart & Hemmens, 1960) may increase the likelihood of haemorrhage in the presence of warfarin. In the brief post-mortem examinations performed in the present study, though haemorrhages were occasionally seen in animals that had received warfarin, the pale liver and massive bleeding typically associated with death from anticoagulant poisoning was something of a rarity, and it was concluded that hypercalcaemia, occasionally made apparent by gross calcification of the aorta, was more often the cause of death.

The palatability of calciferol baits

The results of tests in which Norway rats and house mice were given a choice for 4 or more days between plain and poisoned bait are summarized in Table 3. Only the first 2 days' bait consumption were taken as an unbiased measure of palatability, since thereafter the toxic effects of calciferol reduced feeding differentially, depending upon the degree of preference each individual animal had shown for the poisoned bait earlier in the test. Bait containing 0·025 % calciferol was well accepted by both rats and mice (Groups 1 and 5), and even when the concentration was increased tenfold (Groups 2 and 6) still accounted for about a third of the bait consumed, confirming that calciferol is not particularly unpalatable at rodenticidal

concentrations. Bait containing 0·1 % calciferol and 0·025 % warfarin was also reasonably well accepted by both species, accounting for rather more than a third of the bait consumed (Groups 3, 7 and 8).

CONCLUSIONS

The above results indicate that the minimum concentration at which calciferol, either alone or combined with warfarin, is likely to give good results in the field against rats or mice is 0·1 %. One pre-eminent advantage that calciferol has is that it is toxic to warfarin-resistant rodents. A second advantage is that it kills more rapidly, within 1 week instead of the 1–3 weeks that are often required with anticoagulants, particularly against the ship rat and house mouse. This more rapid action means however that the time available for the target species to ingest a lethal dose (i.e. before the onset of illness curtails feeding) is limited to little more than 2 days. Misjudgements in making the initial bait placements in field treatments with calciferol would therefore be more likely to lead to sublethal dosing of the rodents. Considerable field experience may be necessary to ascertain whether this would be a significant disadvantage in practice and, indeed, whether calciferol should best be employed as a rodenticide in its own right or in conjunction with anticoagulants. One argument that has been advanced for the latter practice is that where two poisons are used the likelihood is less that resistance to either one of them would develop. This assumes that resistance to calciferol would include the non-development of the fall-off in feeding that the compound normally induces after about 24–48 hr.

Warfarin is generally regarded as a particularly safe rodenticide, largely because it is a cumulative poison and the successive doses required to build up lethal levels are not easily acquired by accident. To the extent that calciferol is cumulative in action it may be considered to have the same advantage from the standpoint of safety in use. However, regular users of rodenticidal formulations of calciferol should be alert to the danger of chronic hypervitaminosis D that could result from careless handling.

As a final practical point it may be noted that calciferol tends to decompose in the presence of air and moisture, and the toxicity of damp calciferol baits has been found to decrease significantly within a week. The long-term stability of corn oil solutions is satisfactory however and, contrary to what had been supposed previously, dry cereal/corn oil bait retains its rodenticidal activity for some months.

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