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Toxicity tests on suspected warfarin resistant house mice (*Mus musculus* L.)*

By F. P. Rowe and R. Redfern
Infestation Control Laboratory, Ministry of Agriculture, Fisheries and Food, Tolworth, Surbiton, Surrey

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INTRODUCTION

Infestations of house mice (*Mus musculus* L.) are generally more difficult to control with warfarin [3-(α-acetonyl)-4-hydroxycoumarin] than those of common rats, *Rattus norvegicus* Berk. This is not surprising for two reasons. First, although warfarin is included in bait against mice at a concentration of 0·025% (or five times the strength normally employed in the control of rats), it is still comparably less toxic to *Mus musculus*. Secondly, the mouse is a more diffuse and sporadic feeder than the rat and thus has a greater tendency to feed irregularly on warfarin baits. Prolonged warfarin treatments are therefore sometimes necessary before the effective control of mice is obtained.

Recently, however, Dodsworth (1961) reported mouse infestations that had proved impossible to control with warfarin even with treatments lasting several months. These treatment failures occurred despite the use of adequate numbers of baiting points and continuously high levels of poison bait consumption. As a result of these reports and others made later elsewhere, live-trapping campaigns were carried out in the premises concerned. The results of laboratory tests on the toxicity of 0·025% warfarin to the trapped animals are presented below and compared with similar information obtained on mice drawn from habitats not previously treated with warfarin (Rowe & Redfern, 1964).

MATERIALS AND METHODS

In all, 108 mice (49 males, 59 females) caught in Longworth live-traps at fifteen different localities were tested in the laboratory. For a minimum period of 2 weeks before it was tested each animal was isolated in a cage measuring 14 in. × 11 in. × 6 in. and supplied with a mixed diet of whole wheat and pinhead oatmeal, a wooden nesting-box and water *ad lib.*

The toxicity tests were conducted in the same manner as those reported earlier (Rowe & Redfern, 1964). Each mouse was fed on a sugar/oil/coarse oatmeal bait (hereafter referred to as SOCO) for 4 days before poisoning, when an excess amount of the bait-base containing 0·025% warfarin was offered to it for a limited number of days. Except for occasional 2- or 3-day periods, the amount of warfarin bait eaten was recorded daily.

The first mice tested were offered warfarin bait for 10 days and, after a recovery period, the survivors were re-tested over 12 days. In later trials mice were offered warfarin bait for a single 21-day period. The time of death and the weight of the mice that died were recorded and dead animals were autopsied. Animals still alive at the end of a test were given the wheat/oatmeal diet again and kept under observation.

Further toxicity studies were undertaken with mice reared under laboratory conditions. Six pairs of mice that had survived 21 days feeding on 0·025 % warfarin bait were allowed to breed in metal pens (1–6) supplied with nesting boxes, whole wheat, diet 41 B and water *ad lib*. The litters were isolated from their parents when they were between 5 and 6 weeks old. The young (F_1) mice were then caged individually and raised on the same diet as the parent animals until they were 12 weeks old, when the diet 41 B was removed. Four weeks later, each mouse was fed SOCO for 4 days and then offered the same bait-base containing 0·025 % warfarin for 21 days.

After a recovery period lasting several months, the four surviving males and four of the eight surviving females from one of the pens (pen 3) were paired; their progeny (F_2) were isolated, maintained on the same diet and tested in the same manner as the F_1 mice reported above.

In a third breeding experiment, a further five F_1 male mice (one from each of pens 1, 2, 4, 5 and 6) that had survived feeding on 0·025 % warfarin bait for 21 days, were each mated with several L.A.C. Grey (non-inbred laboratory strain) female mice. Earlier work had indicated that this strain was susceptible to warfarin poisoning (thirty-five males fed 0·025 % warfarin bait from 4 to 10 days all died and twenty-seven out of thirty females were killed after feeding on 0·005 % warfarin bait for from 5 to 10 days). Each breeding pair was maintained on diet 41 B and water *ad lib*. The F_1 hybrid litters produced from these crosses were removed from their parents when they were about 4 weeks old and the sexes separated. One week before they reached the age of 16 weeks the mice were housed separately; at the end of this period, the diet 41 B was withdrawn and the SOCO bait-base was offered to each mouse for 4 days, followed by the bait-base containing 0·025 % warfarin for 21 days. The fourteen parent L.A.C. females were similarly tested several weeks after their last litters had been removed.

RESULTS

Toxicity tests with Mus musculus *caught in the field*

The data from the feeding experiments are summarized in Tables 1–4. Fifty-two mice (26 males, 26 females) were offered warfarin bait for 10 days (Table 1). After 30 days, the period during which mice tested in earlier work had been kept under observation (Rowe & Redfern, 1964), the mortality was 22/52 (13/26 males and 9/26 females). Ten mice died between day 31 and day 64 (no. 466), three (nos. 426, 446 and 447) without normal warfarin symptoms. The death of some animals with poison symptoms several weeks after the end of a single test period showed that the effects of warfarin in mice can be extremely prolonged. Jaques (personal

Table 1. *Effect of feeding warfarin to* Mus musculus *for* 10 *days*

Mouse no.	Sex	Locality	Weight (g.)	Amount of bait eaten (g.)	Warfarin dose (mg./kg.)	Day of death
422	M	Leeds (A)	10·4	20·5	492·8	28
423	M		13·9	14·0	251·8	Survived
424	F		10·1	24·8	613·9	Survived
425	F		10·6	14·0	330·2	9
426	M		8·3	20·4	614·5	35*
428	F		15·3	19·0	310·5	Survived
440	F		16·4	22·5	343·0	Survived
441	M		8·3	18·7	563·3	34
462	M		9·8	8·7	221·9	7
443	F		8·1	16·8	518·5	Survived
444	F		9·4	20·7	550·5	Survived
434	M	Leeds (B)	12·5	23·4	468·0	27
445	F		7·5	2·9	96·7	5
446	M		8·3	17·4	524·1	42*
447	F		11·6	22·6	487·1	49*
429	M	Harrogate (A)	12·7	29·0	570·9	Survived
430	M		14·6	9·9	169·5	6
431	M		13·6	26·0	477·9	25
432	F		11·6	9·3	200·4	5
433	M		15·4	13·8	224·0	7
450	M		11·4	14·9	326·8	10
451	M		10·4	14·7	353·4	14
452	F		12·0	13·8	287·5	Survived
453	F		12·0	20·5	427·1	35
454	F		12·2	25·8	528·7	Survived
455	F		10·5	24·1	573·8	Survived
476	F		18·3	11·1	151·6	7
435	F	Harrogate (B)	15·1	20·8	344·4	9
471	F		19·9	32·1	403·3	Survived
472	F		14·9	15·2	255·0	Survived
473	M		11·4	23·3	511·0	Survived
474	M		9·9	22·9	578·3	49
475	M		17·7	17·7	250·0	Survived
439	F	Harrogate (C)	8·0	17·6	550·0	9
448	F		19·2	25·9	337·2	12
449	F		9·3	9·0	241·9	6
436	F	Harrogate (D)	11·6	25·2	543·1	Survived
437	M		13·8	18·8	340·6	13
456	M		8·7	23·1	663·8	27
457	M	Norwich (A)	12·7	30·0	590·6	Survived
458	F	Huddersfield (A)	15·9	19·0	298·7	7
459	M		11·3	10·7	236·7	7
460	F		21·7	32·7	376·7	Survived
461	M		19·5	27·7	355·1	Survived
463	M		23·5	37·7	401·1	Survived
464	M		20·8	15·8	189·9	7
465	F		16·4	20·2	307·9	Survived
466	F		19·0	30·6	402·6	64
467	M		22·4	36·1	402·9	59
468	M	Huddersfield (B)	11·8	18·6	394·1	49
469	M		16·9	16·6	245·6	10
470	F		11·6	18·0	387·9	32

* No warfarin symptoms.

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communication) observed similar delayed toxic effects in rabbits treated with dicumarol.

The lowest fatal dosage was 96·7 mg./kg. of body weight ingested by a female (no. 445) that weighed 7·5 g. and which ate 2·9 g. of 0·025 % warfarin bait, and died on the fifth day. The highest total dose survived by any animal was 613·9 mg./kg. of body weight consumed by a female (no. 424) that weighed 10·1 g. and which ate 24·8 g. of poison bait.

The mortality among mice from Leeds (A) [5/11] and Harrogate (B) [2/6] was particularly low. The twelve mice from Leeds (A), Harrogate (A), Harrogate (D), and Norwich (A) that survived the 10-day warfarin test were re-tested (after a recovery period of 39 days) over a 12-day feeding period (Table 2). Ten of the twelve mice died; one (no. 444) from Leeds (A) died after only one day but without

Table 2. *Effect of feeding warfarin for* 12 *days to* Mus musculus *that had survived a* 10*-day test*

Mouse no.	Sex	Locality	Weight (g.)	Amount of bait eaten (g.)	Warfarin dose (mg./kg.)	Day of death
423	M	Leeds (A)	16·1	17·5	271·7	Survived
424	F		12·5	33·0	660·0	56
428	F		15·0	13·5	225·0	7
440	F		16·3	33·7	516·9	Survived
443	F		9·1	2·9	79·7	4
444	F		9·3	0·8	21·5	1*
429	M	Harrogate (A)	13·2	11·4	215·9	7
452	F		11·5	10·6	230·4	7
454	F		11·2	33·4	745·5	35
455	F		9·5	18·4	484·2	11
436	F	Harrogate (D)	10·9	2·5	57·3	3
457	M	Norwich (A)	12·5	35·7	714·0	14

*No warfarin symptoms.

warfarin symptoms. Five of the nine mice with symptoms died within 10 days, the time of the test period that they had survived previously. The last death (no. 424) occurred on day 56; both survivors (nos. 423, 440) came from Leeds (A).

The results of feeding 0·025 % warfarin bait for a 21-day period to the latter two animals and to the mice from Harrogate (B) and Huddersfield (A) that had survived a single 10-day poisoning period are shown in Table 3. One of the two mice from Leeds (A) died on the 20th day of the test period; the other died, apparently from natural causes, 10 weeks after the end of the test period. All eight mice from Harrogate (B) and Huddersfield (A) died, but only two did so within 30 days; the last death of a mouse showing symptoms of warfarin poisoning occurred on day 75. Evidence of warfarin poisoning was not found in the one animal that died later (no. 475; day 126).

The toxicity of warfarin to the remaining fifty-six mice captured (23 males, 33 females) was determined over a 21-day feeding period. The data in Table 4

show that there was considerable individual variation in susceptibility to warfarin poisoning. The death of some mice occurred as early as the fourth day. However, the mortality at the end of 30 days was only 32/56 (14 males, 18 females). As in the earlier tests, some mice died a considerable time after the end of the test period. Of eight animals that died after the 30th day, three had no visible warfarin symptoms. The highest dose of warfarin survived by any animal was 1129·1 mg./kg. of body weight consumed by a female (no. A 124) that ate 55·1 g. of poison bait.

Table 3. *Effect of feeding warfarin to* Mus musculus *(survivors of* 10- *and* 12-*day tests) for* 21 *days*

Mouse no.	Sex	Locality	Weight (g.)	Amount of bait eaten (g.)	Warfarin dose (mg./kg.)	Day of death
423	M	Leeds (A)	15·1	19·8	327·8	20
440	F		17·4	36·2	520·1	Survived
460	F	Huddersfield (A)	19·2	68·6	893·2	49
461	M		16·4	23·8	362·8	10
463	M		20·9	53·7	642·3	21
465	F		15·3	48·5	792·5	71
471	F	Harrogate (B)	16·8	61·3	912·2	75
472	F		12·8	37·7	736·3	60
473	M		12·1	51·1	1055·8	35
475	M		14·8	58·2	983·1	126*

* No warfarin symptoms.

Toxicity tests with Mus musculus *bred in the laboratory*

Each of the six breeding pairs of mice produced three or more litters. In all eighty-nine F_1 mice were fed 0·025 % warfarin bait over a 21-day period. After 30 days, the combined litter mortalities obtained were 10/16 (3 litters), 4/15 (4), 4/16 (3), 1/8 (3), 5/18 (3) and 9/16 (4) in pens 1–6 respectively giving a mortality of 33/89 (37·1 %). A further four mice died between days 41 and 80. More males (20/38, 52·6 %) were killed than females (13/51, 25·5 %), the difference being significant at the 0·05 level [$\chi^2 = 5{\cdot}76$; $P = 0{\cdot}01$–$0{\cdot}02$].

The four established breeding pairs of F_1 survivors (mice that were born in pen 3) produced a total of eighty-nine young. The mortality obtained after feeding these F_2 mice 0·025 % warfarin bait for 21 days was 48/89 (53·9 %). The kills obtained with mice from each of the four pens were similar—21/37 (5 litters), 9/19 (4), 8/17 (4) and 10/16 (4) respectively. No mice died after day 30. Although, as with the F_1's, more males (34/55, 61·8 %) than females (14/34, 41·2 %) were killed, the difference is not significant [$\chi^2 = 2{\cdot}82$; $P = 0{\cdot}05$–$0{\cdot}10$].

Of the 176 hybrid F_1 progeny (87 males, 89 females) produced by crossing five wild male and fourteen L.A.C. Grey female mice, all but two (one male, one female) died after feeding on 0·025 % warfarin bait in a 21-day test (days to death 2–23). All fourteen parent L.A.C. females, similarly tested, were dead by day 9.

Table 4. *Effect of feeding warfarin to* Mus musculus *for* 21 *days*

Mouse no.	Sex	Locality	Weight (g.)	Amount of bait eaten (g.)	Warfarin dose (mg./kg.)	Day of death
558	M	Norwich (A)	16·0	64·3	1004·7	62
559	M		17·9	55·4	773·7	23
560	M		15·6	17·4	278·8	8
561	M		15·3	63·3	1034·3	82*
563	F		13·6	50·5	928·3	40
565	F		12·5	47·2	944·0	82*
567	F		13·4	40·4	753·7	62
568	F		9·4	20·1	534·6	16
570	M		12·5	18·9	378·0	12
572	M		16·3	47·9	734·7	27
573	F		19·0	22·6	297·4	10
574	M		14·9	18·6	312·1	8
575	F		11·5	40·1	871·7	19
576	F		8·6	5·3	154·1	7
577	F	Norwich (B)	15·2	17·1	281·3	13
578	F		17·9	49·6	692·7	36*
579	F		13·4	57·8	1078·4	23
580	F		14·1	49·8	883·0	Survived
582	F	Norwich (C)	12·2	44·3	907·8	56
583	M		15·0	14·0	233·3	8
A 120	F	Leeds (C)	18·9	32·9	435·2	15
A 121	M		17·0	12·9	189·7	7
A 122	M		20·0	11·3	141·3	6
A 123	F		22·4	27·4	305·8	13
A 124	F		12·2	55·1	1129·1	Survived
A 125	M		14·4	47·8	829·9	Survived
A 126	M		14·7	10·2	173·5	7
A 127	F		11·1	43·1	970·7	Survived
A 129	M		10·1	46·3	1146·0	39
A 130	F		14·3	8·9	155·6	6
A 572	M	Kidbrooke (A)	16·4	46·0	701·2	Survived
A 573	F		16·7	52·6	787·4	Survived
A 574	M		13·2	50·4	954·5	Survived
A 575	M		16·4	47·3	721·0	Survived
A 576	F		16·9	24·0	355·0	15
A 601	F		10·0	43·3	1082·5	Survived
A 602	M		11·8	8·8	186·4	4
A 603	F		14·4	23·1	401·0	15
A 604	F		14·0	47·6	850·0	Survived
A 605	F		13·4	8·2	153·0	5
A 612	M		8·7	32·3	928·2	20
A 613	F		16·5	22·1	334·8	14
A 614	F		10·4	18·0	432·7	10
A 838	F	Kidbrooke (B)	19·9	3·9	49·0	11
A 839	F		10·8	42·3	979·2	Survived
A 840	F		14·6	27·3	467·5	14
A 841	M		16·4	6·1	93·0	5
A 842	F		14·9	44·9	753·4	Survived
A 843	F		18·2	31·8	436·8	Survived
A 844	M		13·8	0·5	9·1	4
A 845	F		8·7	34·8	1000·0	Survived
A 846	F		11·0	3·2	72·7	4
B 166	M	Wakefield	13·4	33·0	615·7	Survived
B 167	M		15·7	52·7	839·2	Survived
B 168	M		12·5	12·8	256·0	14
B 169	F		12·7	30·1	592·5	17

* No warfarin symptoms.

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DISCUSSION

In an earlier laboratory investigation of the toxicity of 0·025 % warfarin to so-called 'normal' mice (animals that had not been subjected to warfarin previously), the most significant aspect was the high poison dosage that some animals could withstand (Rowe & Redfern, 1964). Even after 21 days' continuous feeding on warfarin bait, five of the fifty-three mice tested (9·4 %) survived. As it is likely that these animals would have been equally difficult to kill with warfarin in the field, it could be said that from the control viewpoint there are probably some mice 'resistant' to 0·025 % warfarin in any sizeable population.

With this in mind, the degree of resistance to warfarin of the mice tested in the present study can be assessed by comparing the data on mortality and days to death given in Tables 1 and 4 with the information obtained previously for 'normal' mice over the same two test periods. At the end of 30 days, the mortality figures of the mice fed for 10 and 21 days were 22/52 and 32/56 respectively compared with 31/37 and 48/53 for 'normal' mice (Rowe & Redfern, 1964). These differences in mortality are highly significant ($\chi^2 = 13{\cdot}8$; $P = 0{\cdot}001$ for 10 days, and $\chi^2 = 13{\cdot}9$; $P = 0{\cdot}001$ for 21 days). In both test periods, furthermore, the mean time to death of the mice killed by warfarin was considerably longer for allegedly resistant mice than it was for normal animals. In the 10-day test shown in Table 1 the mean time of death of twenty-two mice killed within 30 days was $11{\cdot}7 \pm 7{\cdot}7$ days compared with the $6{\cdot}3 \pm 2{\cdot}2$ days for thirty-one 'normal' mice. Student's '*t*' test applied to the data gives a probability of less than 0·001 that this was due to chance. The difference between the mean time of death of $11{\cdot}9 \pm 6{\cdot}1$ days for the thirty-two mice shown in Table 4 and the $8{\cdot}9 \pm 3{\cdot}9$ days of forty-eight 'normal' mice killed in 21-day tests is also significant ($P < 0{\cdot}01$).

Most of the samples of trapped mice used in the present study are small (and it is for this reason that the information on them has been combined), but it was noticeable that in all samples some individuals succumbed readily to warfarin poisoning and in one at least (Harrogate C) the mice were all fairly easily killed. It must be presumed that these mice survived the field treatments because of inadequate feeding on the poison bait. In general, however, it is clear that the proportion of mice resistant to warfarin in the premises concerned was higher than would normally be expected.

As stated above, the house mouse is rather more prone than the rat to the ingestion of sublethal doses of poison during control treatments. One possible explanation therefore for the increased resistance to warfarin found in some mouse populations is that resistance has been 'acquired' following sublethal dosing with warfarin. Direct observation and examination of the daily bait consumption figures of a few of the mice tested over 21 days in the laboratory suggested that these individuals may in fact have developed some sort of tolerance to warfarin. Mouse no. A 843, for example, fed normally on warfarin bait for 7 days, but in the next 3 days it ate only 0·3 g. The bloody droppings voided during this time indicated that in this animal at least the prothrombin level was lowered, but on the eleventh day it fed normally again and continued to do so for the remainder of

the test period. In contrast, however, some of the mice re-tested with warfarin succumbed within the time of the test period that they had survived previously, suggesting that the earlier sublethal feeding period had rendered them more rather than less susceptible to subsequent poisoning.

While the possibility that mice may become more tolerant to warfarin following the repeated ingestion of sublethal doses of the poison is being investigated further, present evidence suggests that resistant populations are more likely to have arisen as a result of selection, susceptible animals being killed off during poison treatments, leaving the more resistant forms to reproduce. If this is so, resistant populations would be most likely to develop after long or frequent warfarin treatments. Unfortunately there is little detailed information available concerning the history of most of the infestations from which the samples were trapped. However, according to the rodent control workers concerned, several of the treatments extended over a period of months and all appeared to become less effective the longer they were continued, although the amount of poison bait consumed increased.

Laboratory support for the genetical control of warfarin resistance in mouse populations is to be found in the low kills obtained with the progeny of resistant animals. Only 33/89 F_1's died after being fed with 0·025 % warfarin bait for 21 days compared with 48/53 'normal' mice treated in the same manner [$\chi^2 = 36{\cdot}63$, $P = 0{\cdot}001$]. Although proportionally more F_2's were killed (48/89) compared with the F_1 stock from which they were derived (mortality 4/16), the difference is not significant ($\chi^2 = 3{\cdot}46$; $P = 0{\cdot}05$–$0{\cdot}10$) and the mortality of the F_2 mice was still much lower than that obtained with 'normal' animals [$\chi^2 = 18{\cdot}72$; $P = 0{\cdot}001$].

The absence of clear-cut monofactorial ratios and the failure to maintain resistance in crosses with the warfarin susceptible L.A.C. strain, suggest two possible explanations of the inheritance of warfarin resistance in house mice. Either resistance is under polygenic control, determined by the interaction between genes showing no dominance—in which case it differs from the resistance shown by *Rattus norvegicus*, for in the two areas in Britain where resistant rat populations are widespread a single dominant mutant gene is believed to be responsible (Sheppard & Drummond, personal communications)—or it is controlled by a single major gene whose expression is strongly affected by the rest of the genotype, that is, influenced by modifiers.

There is as yet no satisfactory explanation of the physiological mechanism or mechanisms involved in the resistance to 0·025 % warfarin in the house mouse. Abnormal supplies or requirements of vitamin K, poor absorption of warfarin from the gastro-intestinal tract or its rapid excretion or metabolic transformation, and altered enzymic activity resulting in a general decrease in sensitivity to warfarin may all play some role in determining resistance.

In more recent tests, the comparative response of the progeny of warfarin resistant mice to 0·025 % warfarin, 0·10 % warfarin, 0·025 % pival (2-pivalyl-1,3-indandione), 0·025 % chloradione (2-bis (para-chlorophenyl) acetylindane-1-3-dione), and 0·025 % warfarin plus 0·025 % sulphaquinoxaline has been determined. Sulphaquinoxaline has been reported to potentiate the action of warfarin

by reducing the bacterial flora in the gut and thereby diminishing vitamin K synthesis (Derse, 1963). The mortalities obtained (Table 5) indicate that mice unresponsive to warfarin at 0·025 % are likely to be little if any more responsive to warfarin at 0·10 %, to other anti-coagulant poisons, and to the potentiating effects of sulphaquinoxaline.

Table 5. *Mortality of* Mus musculus *after unrestricted feeding on anti-coagulants for* 21 *days*

Poison	Concentration (%)	Mortality	Dosage range that killed (mg./kg.)	Highest dosage survived (mg./kg.)	Range of days until death
Warfarin	0·025	9/23	164–361	1022	7–15
Warfarin	0·10	7/11	255–1282	2702	6–13
Pival	0·025	6/10	94–448	802	7–14
Chloradione	0·025	5/10	127–502	718	9–21
Warfarin Sulphaquinoxaline	0·025 0·025	5/10	294–607	964	14–23

SUMMARY

1. Residual populations of wild house mice (*Mus musculus* L.) were trapped alive in premises where poison treatments with 0·025 % warfarin bait had been reported to be ineffective.

2. In the laboratory the individually caged mice were fed 0·025 % warfarin in a sugar/oil/coarse oatmeal base. In toxicity tests lasting 10 and 21 days, the total mortality was low and the rate of death slow compared with mice drawn from habitats not treated with warfarin.

3. It is suggested that tolerance to warfarin in *M. musculus* either is polygenically based or is controlled by a single major gene influenced by modifiers and that these hard-to-kill populations have arisen through selection during successive control treatments.

REFERENCES

Derse, P. (1963). Anti-K factor in anticoagulant rodenticides. *Soap, N.Y.*, **39** (3, 4), 82–8.

Dodsworth, E. (1961). Mice are spreading despite such poisons as warfarin. *Munic. Engng Lond.*, no. 3746, 1668.

Rowe, F. P. & Redfern, R. (1964). The toxicity of 0·025 % warfarin to wild house mice (*Mus musculus* L.). *J. Hyg., Camb.*, **62**, 389–93.