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The toxicity of 0·025% warfarin to wild 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

The most extensively used rodenticide in Britain over the last 10 years has been warfarin [3-(α-acetonylbenzyl)-4-hydroxycoumarin]. During this time a considerable amount of information has been accumulated, both in the laboratory and in the field, on its toxicity to rats, and in particular to *Rattus norvegicus* Berk. In contrast, the toxicity of warfarin to the wild house-mouse (*Mus musculus* L.) has been less well investigated. However, recent improvements in rat control methods have resulted in the house-mouse supplanting the rat as the major pest in some habitats. Furthermore, in the last 2 years there have been reports of some house-mouse populations proving extremely difficult to kill with warfarin. For these reasons a laboratory investigation of the toxicity of warfarin to mice was undertaken and the results of this are described below.

MATERIALS AND METHODS

The mice used in the tests were drawn from thirteen different localities. The majority were hand-caught in corn-ricks at threshing time; only ricks in which no warfarin treatment had been carried out were used. After capture, the mice were kept in metal pens, 6 ft. square, erected in an unheated building. Animals from the same source were penned together in colonies of fifty or less. They were given excess whole wheat and water, and hay for nesting material, and were provided with wooden nest-covers.

At least 2 weeks before a test, each mouse was put individually in a metal cage (14 in. × 11 in. × 6 in.) containing a metal food-pot, a water bottle and a wooden nesting box (3 in. × 1 in. × 1 in.). During this period it was supplied with a diet of mixed whole wheat and pinhead oatmeal and with water *ad lib.*

The method employed in all tests was to offer each mouse excess amounts of a bait containing 0·025% warfarin for a fixed number of days. This warfarin concentration is normally used against *Mus musculus* in Britain and is based on early work carried out in the United States of America. The poison was dispersed thoroughly in fine oatmeal to give a master-mix containing 0·5% warfarin. One part of the master-mix was then added to nineteen parts of a bait-base consisting of pinhead oatmeal, castor sugar and a technical grade 'white oil' in the proportions

by weight of 17:1:1, respectively. Before poison was administered the mice were fed on the plain bait-base for 4 days. The total amount of bait-base eaten in this period was recorded, paper placed in the tray underneath each cage facilitating the collection of spilled food. Animals that failed to eat adequately during this period were discarded. Wherever possible an equal number of each sex and animals of comparable weight were chosen for the toxicity tests. None of the females was pregnant. In a majority of the tests the amount of warfarin bait eaten was recorded daily and the food pots were replenished each day with fresh poison bait. Occasionally in tests exceeding 14 days, the poison bait was left unchanged for a single 2- or 3-day period. The time of death and weight of mice that died was recorded and the dead animals were autopsied. Mice still alive at the conclusion of the test period were given a wheat/oatmeal diet until 30 days had elapsed from the beginning of the test. Animals still surviving at the end of this period were killed, weighed and autopsied.

RESULTS

The results of the toxicity tests are summarized in Table 1. In all, 345 mice were tested over feeding periods ranging from 4 to 28 days. The data show that there was considerable variation in the susceptibility of individual mice to warfarin at 0·025 %. The lowest fatal dosage was 26·1 mg./kg. of body weight. The animal concerned, a male, which weighed 13·4 g., ate only 1·4 g. of 0·025 % warfarin bait and died with normal warfarin symptoms on the fourth day of a specified 6-day test period. At the other extreme, a female weighing 11·9 g. survived the equivalent of 1067·2 mg./kg. of body weight after eating 50·8 g. of 0·025 % warfarin bait over 21 days. It is noteworthy that a complete kill was not obtained in any feeding test of less than 18 days' duration and five mice fed for 21 days survived total dosages of 666·7, 671·9, 689·0, 790·2 and 1067·2 mg./kg., respectively. These five animals were taken from three different localities, as also were the four that survived 14 days. The time required to kill the first mouse in each test was similar—between 2 and 4 days. Not unexpectedly, because of the mode of action of warfarin,

Table 1. *Mortality of* Mus musculus *after unrestricted feeding on bait containing* 0·025 % *warfarin for a limited number of days*

No. of days feeding	Mortality	Total dosage range that killed (mg./kg.)	Average total lethal dose (mg./kg.)	Highest total dose survived (mg./kg.)	Range of days until death
4	6/30	90·4–152·9	120·4	189·8	4–23
5	16/35	113·3–251·8	167·7	220·0	3–30
6	23/33	26·1–303·6	157·0	322·0	3–10
7	36/46	41·0–439·9	190·6	319·6	3–13
8	35/41	67·3–253·6	164·0	329·0	4–14
10	31/37	68·3–471·6	192·2	557·5	4–12
14	41/45	106·2–571·4	248·2	642·2	4–30
18	12/12	68·2–307·2	174·7	—	2–17
21	48/53	104·7–805·7	268·6	1067·2	3–20
28	13/13	82·4–238·1	164·4	—	4–10

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there was considerable variation in the time to death of mice in the same test; for example, in a 5-day test the first death occurred on day 3 and the last on day 30. The lethal feeding period corresponding to a 95% kill (LFP 95) with 0·025% warfarin, calculated from a regression line obtained by transforming the data into probit mortality and log(days − 3·85), was about 22 days.

Table 2. *Mortality of* Mus musculus *taken from one rick site after unrestricted feeding on bait containing* 0·025% *warfarin*

No. of days feeding	Mortality	Total dosage range that killed (mg./kg.)	Average total lethal dose (mg./kg.)	Highest total dose survived (mg./kg.)	Range of days until death
4	3/11	115·2–152·9	128·0	158·7	5–23
5	7/14	118·0–184·7	151·5	220·0	3–7
6	8/14	26·1–181·8	120·7	322·0	3–8
7	10/14	92·8–199·2	146·3	218·2	3–8
8	12/14	67·3–212·8	147·8	287·6	4–11
10	10/12	110·4–206·5	152·2	250·0	4–9
14	12/14	106·2–494·2	256·2	642·2	5–30
18	12/12	68·2–307·2	174·7	—	2–17
21	18/20	104·7–545·1	242·1	671·9	4–18
28	13/13	82·4–238·1	164·4	—	4–10

Table 3. *The mortality of male and female house-mice in each of four weight classes after unrestricted feeding on* 0·025% *warfarin for periods of from* 4 *to* 28 *days*

Weight class (g.)							
10·0		10·1–15·0		15·1–20·0		20·1	
M	F	M	F	M	F	M	F
3/3	10/11	59/70	52/72	55/71	60/80	14/18	8/20

Examination of the toxicity data indicated no obvious difference in the susceptibility of mice from different localities, but some of the samples were small. By far the largest sample (138 mice) came from three wheat ricks standing on one site. The data for these animals have been abstracted from Table 1 and are set out in Table 2. The lowest lethal dose for the sample was 26·1 mg./kg. of body weight spread over 4 days (see above), and the highest dose survived by any animal was 671·9 mg./kg. spread over 21 days. This mouse, a female, which weighed 11·2 g., ate 30·1 g. of 0·025% warfarin bait. One other female survived a feeding period of 21 days.

The data are indicative of a slight difference in susceptibility between the sexes. Of 211 animals (92 males, 109 females) tested over 8 days or longer (Table 1) five males survived (5·4%) compared with sixteen females (14·7%) [$\chi^2 = 3{\cdot}62$; $P = 0{\cdot}05$–$0{\cdot}1$]. In all but two of the test periods (18 and 28 days, in which complete kills were obtained), the average time to die of animals killed by warfarin was longer for females than for males. The greatest difference occurred in the two shortest feeding periods (4 and 5 days), the figures for males being 4·5 and 5·8 days,

and for females 10·8 and 8·9 days, respectively. There was little obvious difference in susceptibility based on weight. Females weighing 20·0 g. or more appeared to be the most difficult to kill (Table 3) but the sample was small and the range in weight of mice surviving 8 days feeding or longer was considerable (11·6–19·1 g. for males and 10·0–21·9 g. for females).

Most of the survivors showed signs of illness at some time during the test; some produced bloody droppings and ate little or no poison bait for a day or two, but then subsequently recovered and ate normally. The bait consumption of each of the five females that survived 21 days feeding was in fact as great at the end of the test period as it had been at the beginning.

The examination of animals killed by warfarin poisoning showed that haemorrhages occurred at several sites in the body and were most common in the thoracic cavity (51·4 %), digestive organs (24·4 %), central nervous system (18·2 %), lungs (15·4 %) and abdominal cavity (8·4 %). The decrease in body weight in some cases amounted to over 40 %.

DISCUSSION

There is little published information available on the toxicity of warfarin to wild mice. Most of the early work in the United States of America involved offering mice either 0·025 % warfarin bait till death or a choice of warfarin bait and a laboratory diet. A straightforward comparison with the toxicity data of these experiments is therefore impossible. Bonnet, Mau & Gross (1951) fed 0·025 % warfarin bait to fifteen mice till death; fourteen died by the 13th day and the last animal died on the 17th day (mean 8·6, S.E. ± 1·0 days). Hayes & Gaines (1959) offered batches of individually caged mice baits containing warfarin of varying concentrations (between 0·0003 and 0·08 %) together with poison-free food for a maximum of 40 days. They concluded that some individuals were extremely susceptible to warfarin but that there was considerable variation in response. Giban (1958) found that the proportion of small rodents (including house-mice) surviving periods of 0·025 % warfarin bait in tests lasting 30 days amounted to between 10 and 20 %. The data from the present study support these findings; some animals succumbed readily to warfarin, whereas others survived substantial amounts of the poison.

From the control standpoint, the most significant aspect of the results of the present tests is the extent to which some mice were able to survive large doses of poison. While it is probably true to say that free-living mice would be more active than caged animals and might as a result be more easily killed by warfarin, it must be remembered that the animals under test had no choice of food. This kind of situation seldom occurs in the field, where the presence of alternative foods may make it difficult to induce mice to feed regularly at baiting points: and tests by Spencer (1950) indicate that the survival of wild mice is increased considerably if gaps of two or more days occur between periods on warfarin bait. The duration of warfarin treatments against mouse populations then will depend mainly on the susceptibility of the populations to warfarin and on their feeding behaviour. Populations fairly susceptible to warfarin poisoning and readily drawn to baiting

points will probably be controlled within one week, whereas a considerably longer time will undoubtedly be required for the eradication of populations more tolerant to warfarin and feeding irregularly at the baiting points.

The results of laboratory tests (unpublished) on small numbers of mice taken from premises treated periodically with 0·025 % warfarin over several months have shown that in some cases a higher proportion of animals tolerant to warfarin has been present than would normally be expected from the data presented in Table 1. The most likely explanation of these difficult cases is that the individuals in the original populations most susceptible to 0·025 % warfarin were killed off, leaving the more tolerant animals to reproduce. Certainly, current laboratory investigations indicate that tolerance to warfarin is a heritable trait in *Mus musculus*. Thus it would seem that, apart from the demand on time and labour involved in a lengthy warfarin treatment (of, say, more than 6 weeks), the prolonged use of 0·025 % warfarin to control such populations is of doubtful value.

SUMMARY

1. Individually caged wild house-mice (*Mus musculus* L.) were fed 0·025 % warfarin in a pinhead oatmeal/oil/sugar base. In all, 345 mice were tested for a fixed period ranging between 4 and 28 days.

2. There was considerable variation in the susceptibility of individual mice. The lowest effective dosage was 26·1 mg./kg. of body weight in 4 days and the highest dosage survived was 1067·2 mg./kg. eaten over 21 days.

3. The time required to obtain a 100 % kill of house-mouse populations with 0·025 % warfarin under field conditions is discussed.

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