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THE COMPARATIVE RODENTICIDAL EFFICIENCY OF FIVE ANTI-COAGULANTS

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INTRODUCTION

In a previous communication (Bentley & Rowe, 1956) an account was given of the comparative rodenticidal efficiency of warfarin* [3-(1-phenyl-2-acetyl)-4-hydroxycoumarin] and 'Pival' [containing pindone* (2-pivalyl-1,3-indandione)] when these are incorporated in food baits. The present paper extends consideration to five more anti-coagulants for which claims have been made. These are: fumarin* [3(1-furyl-2-acetyl furfuryl)-4-hydroxycoumarin], manufactured by C. F. Spiess and Son, Kleinkarlbach, Germany; coumachlor* [3(1-*p*-chlorophenyl-2-acetylethyl)-4 hydroxycoumarin] manufactured by Geigy and Co., Basle, Switzerland; 2-naphthyl-1,3-indandione—which has been marketed under the name of 'Radione' by Lyonnaise Industrielle Pharmaceutique, Lyon, France; diphacinone [2-diphenylacetyl-1,3-indandione] developed as a rodenticide by the Niagara Chemical Division of Food Machinery and Chemical Corporation, Middleport, N.Y., U.S.A.: and 2-isovaleryl-1,3-indandione.

METHODS

An outline of the methods used in the toxicity and comparative acceptance tests, the results of which are given below, may be found in the earlier paper (Bentley & Rowe, 1956)—which also contains a brief account of the mode of action and desirable characteristics of anti-coagulant rodenticides and their efficiency *vis-à-vis* acute poisons. The toxicity tests with mice were conducted in a similar manner to those with rats. In all tests, approximately equal numbers of singly caged males and non-pregnant females were used. Each poison was dispersed in fine oatmeal to give a master-mix twenty times stronger than the required final concentration. One part of master-mix was then added to nineteen parts of bait base. In most of the toxicity tests with *Rattus norvegicus*, this base consisted of pinhead oatmeal, castor sugar and a technical grade 'white oil' in the proportions by weight of 16:2:1, respectively. In a few instances, indicated below, the mineral oil was replaced by glycerine. In the toxicity tests with *R. rattus* and *Mus musculus* the bait base usually consisted of the same three ingredients in the proportions of 17:1:1 by weight, respectively, but again, on occasions, glycerine was used instead of oil. At no time was there any indication that varying the bait base influenced mortality.

* British Standard recommended common name.

TOXICITY TESTS

(*a*) *With* Rattus norvegicus

The results of feeding warfarin and each of the five candidate poisons, in oatmeal baits, to singly caged wild *R. norvegicus* for a limited number of days are summarized in Table 1. The figures for warfarin, which may be taken as a standard, include the data given in Bentley & Rowe (1956). Since warfarin at 0·005 % gives a nearly complete kill of all common rats that have fed exclusively on it for 96 hr., and since it will probably be agreed that it would be a retrograde step to replace this poison by another calculated to prolong control treatments, Table 1 would appear to contra-indicate the use of coumachlor at 0·05 % or less, 2-naphthyl-1,3-indandione at 0·025 or 0·05 % and 2-isovaleryl-1,3-indandione at 0·005 %. The last named at 0·025 % seems to be reasonably effective but further examination of its toxicity for *R. norvegicus* was stopped when it failed to show up well in palatability tests (see below).

The rodenticidal properties of coumachlor have been reported upon by Reiff & Wiesmann (1951). These authors fed *R. norvegicus* on the poison until death occurred. As this method overestimates the period required to ingest a lethal dose, it is not possible to compare their figures with ours. However, the data in Table 1 do not confirm their statement (Reiff & Wiesmann, 1951, p. 111) that after 3 days of feeding on 0·05 % coumachlor, a 200 g. rat (presumably *R. norvegicus*) would acquire a lethal dose. The lowest dosage that killed in the present assays was 7 mg./kg. of body weight (0·005 % for 3 days), but animals survived dosages of 133 and 126 mg./kg. (0·05 % for 4 and 5 days, respectively).

A feature of the data on coumachlor is that at 0·005 %, both for 3 days and for 4 days, the poison was almost as lethal as at ten times the dosage. This was probably partly the result of heterogeneity among the test animals; but experience suggests that increasing the dosage level of anti-coagulants may not always bring about increased toxicity—especially if the interval between successive doses is short.

Some data by Molho on the toxicity of 2-naphthyl-1,3-indandione to *R. norvegicus* are given by Lhoste (1955). Lhoste does not make it clear whether or not the experimental animals were fed on the poison until they died—but also reaches the conclusion that it is less efficacious than warfarin.

The results of toxicity tests with 2-isovaleryl-1,3-indandione on *R. norvegicus* have been presented by Kabat, Stohlman & Smith (1944). In their experiments they fed the poison at 0·005, 0·01 and 0·1 % to young albino rats until death occurred and again, therefore, a strict comparison cannot be made with our own results. In general, however, the two sets are in agreement.

Table 1 shows that both diphacinone at concentrations of 0·0025 % and upwards and fumarin at 0·025 % are at least as toxic as warfarin at 0·005 % when fed to the common rat over 1–4 days. Fumarin at 0·025 % appears to be slightly less effective than warfarin at the same strength but the difference may not be significant. On the other hand, more data would probably show that fumarin at 0·025 % is rather more toxic than warfarin at 0·005 %.

Table 1. *Mortality to* Rattus norvegicus *after unrestricted feeding on anti-coagulants for a limited number of days*

Poison	Concentration (%)	No. of days feeding	Mortality	Dosage range that killed (mg./kg.) of body wt.	Highest dosage survived (mg./kg.)	Range of days until death	Day by which approx. 90 % mortality reached
Warfarin	0·005	1	5/12	3–5	5	5–9	—
Warfarin	0·025	1	9/12	12–32	24	5–10	—
Fumarin	0·025	1	8/12	17–39	22	4–11	—
Diphacinone	0·0025	1	7/12	1·4–2·5	2·5	5–7	—
Diphacinone	0·005	1	10/12	2·0–4·2	4·5	5–9	—
Warfarin*	0·005	2	35/56	5–11	38	5–9	—
Warfarin	0·025	2	21/23	20–53	55	4–10	10
Fumarin	0·005	2	4/11	7–12	12	5–6	—
Fumarin	0·025	2	20/22	22–48	40	5–8	8
Diphacinone	0·005	2	8/11	2–8	10	6–11	—
Diphacinone	0·0125	2	11/11	7–22	—	3–8	7
Warfarin	0·005	3	16/18	4–12	10	4–10	10
Warfarin	0·025	3	12/12	21–50	—	4–7	7
Fumarin†	0·005	3	8/9	7–12	7	5–11	11
Fumarin‡	0·025	3	20/23	20–67	44	4–12	12
Diphacinone	0·0025	3	18/19	3–6	5	4–11	11
Diphacinone	0·005	3	12/12	8–13	—	5–10	9
Coumachlor	0·005	3	2/11	7–11	15	5–10	—
Coumachlor	0·025	3	5/12	38–59	59	4–9	—
Coumachlor	0·05	3	2/11	55–62	94	6–7	—
Isovaleryl ind.	0·005	3	0/12	—	15	—	—
Isovaleryl ind.	0·025	3	10/12	41–69	51	5–8	—
Warfarin	0·0025	4	10/12	3–8	7	6–10	—
Warfarin§	0·005	4	28/29	7–17	13	4–11	9
Warfarin	0·025	4	17/17	27–79	—	4–10	7
Fumarin	0·005	4	29/33	3–21	10	3–11	11
Fumarin‖	0·025	4	22/22	21–93	—	4–14	8
Diphacinone	0·0025	4	10/10	4–7	—	3–9	8
Coumachlor	0·005	4	4/11	7–15	13	6–8	—
Coumachlor	0·025	4	14/23	16–63	65	5–8	—
Coumachlor	0·05	4	6/12	63–120	133	4–7	—
Naphthyl ind.	0·05	4	7/11	40–107	127	4–7	—
Fumarin	0·005	5	10/11	8–18	18	4–9	9
Coumachlor	0·05	5	9/10	63–130	126	4–18	18
Naphthyl ind.	0·025	5	6/12	27–48	60	4–7	—

* Twenty-three rats had oatmeal baits and twenty-three had oatmeal baits plus mineral oil and sugar. Ten rats had glycerine in place of the oil.

† All the rats had bait containing glycerine instead of mineral oil.

‡ Eleven rats had bait consisting of medium oatmeal.

§ Fourteen rats had bait consisting of medium oatmeal.

‖ Twelve rats had bait consisting of medium oatmeal.

The comparative toxicity of fumarin and warfarin to *R. norvegicus* has been investigated by Steiniger (1953) who administered single doses (calculated in terms of mg./kg. of body weight) for 5 consecutive days to arrive at a chronic LD_{50}. He concluded that fumarin (LD_{50} about 1·4 mg./kg.) was at least twice as toxic as

warfarin (3·4 mg./kg.). This result does not necessarily contradict the data of Table 1. For it does not follow that the relative chronic toxicity of two fairly similar anti-coagulants remains the same whatever the level and frequency of the dosing. And because there is evidence (see below) that large single doses of fumarin are less toxic than similar single doses of warfarin, Steiniger's figures imply that there is some intermediate point, between acute dosing and 5-day administration of small doses, at which fumarin and warfarin produce roughly the same mortality. If this is so, the importance is underlined of choosing a method of assay of chronic rodenticides that approximates to the conditions of their use in the field. In this respect a method based on only 50 % mortality to *R. norvegicus* after 5 days of dosing is perhaps not entirely satisfactory.

Lhoste (1955) also gives some results of toxicity tests with fumarin against *R. norvegicus*. He used white rats and fed the poison to them at 0·025, 0·0125 and 0·005 %. At the first concentration, of six rats given access to the bait for 2 days, five died (cf. 20/22 in Table 1). At 0·005 % he obtained kills of one out of six and five out of nine in 2 and 3 days feeding, respectively—a result somewhat less favourable to fumarin than the figures in Table 1. However, the number of animals concerned is small and, in any case, further data given by Lhoste for 0·025 % warfarin suggest that his strain of albinos may be more resistant to anti-coagulants than our wild *R. norvegicus*. Thus it is not possible to challenge his conclusion that there is no great difference between the toxicity of warfarin and that of fumarin to common rats.

The duration of an efficient rat control operation with a chronic poison largely depends on the period during which the population continues to feed. Information on how long this is likely to be is not forthcoming from assays in which poisoning is limited to a few days. Since, however, with most anti-coagulants, it happens that feeding usually continues to within a day or two of death, the next best thing is to note when this occurs. Steiniger (1953) observed greater uniformity in the range of time to death in his fumarin-poisoned rats than in the animals he used earlier in tests with warfarin. But greater uniformity is only an asset when the upper end of the range is thereby lowered. In any case, the data in column 7 of Table 1 do not suggest that there is any obvious difference in the range of time to death between warfarin and fumarin at the dosage levels used in our tests. Similarly, no marked difference between the two poisons is discernible when the alternative criterion of the point in the assay when 90 % of the animals have died is considered (Table 1, column 8).

We know of no satisfactory published information on the chronic oral toxicity of diphacinone to rats. Saunders, Heisey, Goldstone & Bay (1955) note that a daily dose of 0·1 mg./kg. of diphacinone administered intraperitoneally to white rats for 7 days gave 39 % mortality while 0·5 mg./kg. of warfarin produced 83 % mortality; and go on to say that 'a series of tests... via the oral route gave similar results'. However, there is general agreement (Dykstra, 1956; Gates, 1957) that diphacinone is more toxic to rodents than most other well-known anti-coagulants, including warfarin. Table 1 shows that it kills *R. norvegicus* in 2- and 3-day tests at perhaps less than half the concentration in bait that is required when using warfarin.

Thus, 0·0025 % diphacinone is roughly equivalent in killing power to 0·005 % warfarin. At 0·0125 % it appears to be lethal after only 2 days feeding. As far as can be seen from the data on killing time, at concentrations that give comparable mortality, it is similar to warfarin and fumarin in its speed of action.

(*b*) *With* Rattus rattus

The results of similar toxicity assays with *R. rattus* are given in Table 2 in which the figures for warfarin at 0·025 % for 5 and 7 days are repeated from Bentley & Rowe (1956). No tests were done with 2-naphthyl-1,3-indandione or 2-isovaleryl-1,3- indandione.

Table 2. *Mortality to* Rattus rattus *after unrestricted feeding on anti-coagulants for a limited number of days*

Poison	Concentration (%)	No. of days feeding	Mortality	Dosage range that killed (mg./kg.)	Highest dosage survived (mg./kg.)	Range of days until death	Day by which approx. 90 % of the mortality had occurred
Fumarin	0·025	3	1/9	44	54	5	—
Diphacinone	0·0125		6/11	22–37	26	5–12	—
Fumarin	0·025	4	5/9	18–57	69	4–8	—
Diphacinone	0·0125		4/12	37–48	57	5–14	—
Warfarin	0·025	5	1/12	105	76	8	—
Fumarin	0·025		11/16	64–80	94	2–9	—
Diphacinone	0·0125		4/9	30–58	69	9–14	—
Coumachlor*	0·025		0/11	—	102	—	—
Warfarin	0·1		2/11	348–497	481	6–12	—
Diphacinone	0·0125	6	22/24	26–79	48	5–14	8
Warfarin	0·025	7	10/12	33–110	113	5–12	—
Fumarin*	0·025		14/22	44–131	140	7–11	—
Coumachlor*	0·025		7/10	53–96	106	5–7†	—
Diphacinone	0·005	8	12/13	10–24	21	4–13	13
Diphacinone	0·0125		11/11	28–83	—	3–10	9
Warfarin	0·025	9	9/12	42–144	151	7–15	—
Fumarin*	0·025		7/12	90–151	166	8–20	—
Diphacinone	0·0025	10	12/12	3–14	—	4–14	11
Warfarin	0·025		9/12	62–149	151	6–12	—
Warfarin*	0·025	12	11/11	64–170	—	7–17	13
Fumarin*	0·005		9/16	28–41	43	9–17	—
Fumarin*	0·025		11/12	60–112	138	7–12	12

* The bait base in these tests contained glycerine instead of oil.

† The seven deaths occurred over a week-end and the exact day was not noted.

Again, warfarin may be used as a standard by which to judge the other rodenticides. At present warfarin is almost everywhere used against *R. rattus* at 0·025 %—at which concentration, to bring about a near 100 % mortality, it needs to be ingested for well over a week. Since this is undoubtedly slower than is desirable, it

is obvious that chronic poisons less toxic than 0·025 % warfarin have no future against *R. rattus*; in fact, a rodenticide that is more toxic is needed. In the event, as Table 2 shows, both coumachlor and fumarin at 0·025 % were fairly similar to warfarin in their effect on the test animals, while diphacinone was rather more toxic than any of the other three. At 0·0025 % it killed every one of twelve rats that were fed on it for 10 days—a kill comparable to that obtained with warfarin at 0·025 %. At 0·0125 %—half as strong as the latter—it gave a complete kill of eleven rats in a test lasting 8 days and killed twenty-two rats out of twenty-four in a 6-day test.

It is possible to analyse the data in Tables 1 and 2 by one of the various methods developed for the elucidation of dosage-mortality relationships. The main difficulty is in deciding how to transform the data. For mortality resulting from the ingestion of a particular concentration of an anti-coagulant for varying periods may not be normally distributed in time. The regression line obtained by plotting the figures for 0·0125 % diphacinone against *R. rattus* in terms of probit mortality and days (or log days) appears to be significantly non-linear when tested by the χ^2-method (Finney, 1952). This is largely due to the high mortality (6/11) in the 3-day test—which, however, may not be repeatable. For when the data on 0·025 % warfarin and 0·025 % fumarin are transformed into probits and log time the corresponding regression lines show much less departure from linearity. The line for warfarin crosses, and is steeper than that for fumarin. The 'lethal feeding period' corresponding to a 50 % kill with warfarin (the 'LFP_{50}', cf. LD_{50}) is 6·8 days compared with only 4·7 days for fumarin; but the respective LFP 95's are 13 days and about 20 days. Present indications therefore are that in controlling *R. rattus* (if possible differences in bait acceptance are ignored) warfarin can be expected to give a nearly complete kill sooner than fumarin.

Consideration of the data of Table 2 from the standpoint of how soon death occurs shows that diphacinone is at least as quick to act as warfarin or fumarin and may be quicker. When fed at 0·0125 % for 8 days diphacinone killed all the test animals in 3–10 days. Ship rats fed on warfarin and fumarin at 0·025 % for 12 days died in 7–17 and 7–12 days, respectively, while one rat fed on fumarin at the same strength for 9 days died on the 20th day. A similar comparison is obtained by considering the period that elapsed before 90 % of the test animals succumbed.

(*c*) *With* Mus musculus

The results of a small number of toxicity tests with warfarin, fumarin and diphacinone against *Mus musculus* are given in Table 3. The figures in parentheses refer to laboratory mice and the remainder to wild mice. The concentration of warfarin employed normally against *M. musculus* is 0·025 %—as for *R. rattus*. Table 3 shows that this needs to be ingested for at least a week to give a complete kill. Fumarin at the same strength would appear to need a day or two longer. The results of three tests with diphacinone at one-tenth of the concentration (0·0025 %) were equivocal but were generally similar to those obtained with fumarin at 0·025 %. When diphacinone is fed to house-mice at 0·0125 % a complete kill seems to be obtained in about the same time as with warfarin at 0·025 %.

Table 3. *Mortality to* Mus musculus *after unrestricted feeding on anti-coagulants for a limited number of days*

Poison	Concentration (%)	No. of days feeding	Mortality	Dosage range that killed (mg./kg.)	Highest dose survived (mg./kg.)	Range of days until death	Day by which approx. 90 % mortality reached
Diphacinone	0·0125	4	9/11	96–265	186	6–21	—
Diphacinone	0·0025	6	9/10	17–42	20	5–21	21
Diphacinone	0·0125		15/17	40–137	91	3–9	9
Warfarin	0·025		9/10	114–236	169	6–8	8
Diphacinone	0·0025	7	6/12	18–38	27	7–8	—
Diphacinone	0·0125		(8/8)	75–440	—	5–8	—
Warfarin	0·025		11/11	57–101	—	7	—
Fumarin	0·025	8	(8/9)	106–277	189	6–9	9
Diphacinone	0·0025	10	9/11	18–55	53	7–11	—
Fumarin	0·025		11/12	124–613	391	4–10	10
Fumarin	0·025	12	12/12	233–509	—	5–9	—

Figures in parentheses relate to tests with a laboratory strain of mouse.

ACCEPTANCE TESTS

(*a*) *With* Rattus norvegicus

Table 4 shows the results of a number of 2-day acceptance tests using singly caged wild *R. norvegicus*. In some of the early trials the poison master-mix was added to nineteen parts of a medium grade oatmeal. Subsequently, the medium oatmeal was replaced by the same coarse oatmeal/sugar/oil mix (16:2:1) that was used in the toxicity tests with the common rat—since this is the bait base that is at present recommended for control.

Three tests were carried out with warfarin at 0·005 % versus coumachlor at 0·025 %. In two of these both master-mixes were commercial formulations containing a dye. They also may have differed in other ways. In the third test the poisons were obtained in pure form and mixed in the laboratory by the standard method. In all three tests there was a significant preference for warfarin—which, however, was least marked in the test based on the pure poisons.

Table 4 shows that warfarin at 0·005 % and fumarin at 0·025 %, both in oatmeal with oil and sugar, are fairly similar as regards acceptance—and since it is known that the former is slightly unpalatable to *R. norvegicus*, it can safely be concluded that fumarin at 0·005 % would also be as acceptable as warfarin at the same strength.

As stated earlier, 2-isovaleryl-1,3-indandione is less acceptable than warfarin to the common rat. In 2 days twelve animals ate only 18 g. of a medium oatmeal bait containing the former at 0·025 %. During the same period they ate 292 g. of 0·005 % warfarin, which is roughly comparable in toxicity with 0·025 % 2-isovaleryl-1,3-indandione. One animal accounted for 13 g. of the total of 18 g. while ten rats ate less than 1 g. each. In a further test, not shown in Table 4, 2-iso-

valeryl-1,3-indandione was offered at 0·025 %, in the standard oatmeal/oil/sugar bait, to eleven rats which also had access to plain oatmeal. Here on the whole it was equally acceptable—suggesting that it is perhaps about as inferior in acceptance to 0·005 % warfarin as is medium oatmeal to coarse oatmeal plus sugar. However, even in this test two rats completely refused the poisoned bait.

Table 4. *Comparative acceptance of warfarin and four other anti-coagulants to* Rattus norvegicus *over 2 days*

Warfarin bait and amount eaten (g.)	Compared bait and amount eaten (g.)	No. of rats preferring warfarin and total no. in test	Significance level of difference
Proprietary sample at 0·005 %* (323)	Proprietary sample of coumachlor at 0·025 %* (165)	19/23	0·001
Proprietary sample at 0·005 % (711)	Proprietary sample of coumachlor at 0·025 % (157)	22/24	0·001
Pure sample at 0·005 % (407)	Pure coumachlor at 0·025 %* (298)	23/33	0·05
Pure sample at 0·005 % (329)	Pure fumarin at 0·025 % (316)	11/23	Not significant
Pure sample at 0·005 %* (292)	Pure 2-isovaleryl-1,3-indandione at 0·025 %* (18)	11/12	Highly significant
Pure sample at 0·005 % (626)	Pure diphacinone at 0·005 % (392)	23/35	0·01–0·001
Pure sample at 0·005 % (460)	Pure diphacinone at 0·0025 % (439)	10/24	Not significant
Pure sample at 0·025 % (494)	Pure diphacinone at 0·0125 % (308)	18/24	0·05–0·02
Pure sample at 0·025 % (364)	Pure diphacinone at 0·005 % (467)	14/24	Not significant

* In these tests the bait base was medium oatmeal. In all the other tests it was coarse oatmeal, plus sugar, plus oil at 16:2:1.

The data in Table 4 indicate that, strength for strength—and contrary to early reports—diphacinone is less acceptable than warfarin to *R. norvegicus*. However, the difference between them is not marked. When given a choice of both poisons at 0·005 % twelve rats out of thirty-five ate more diphacinone bait and when the concentration of diphacinone was halved (to 0·0025 %) no difference in acceptance could be demonstrated. In a test with 0·025 % warfarin as the standard (since this concentration is recommended, for example, for controlling rats in sewer systems) diphacinone at 0·0125 % was significantly less palatable. At 0·005 %, however, it appears to be as acceptable as 0·025 % warfarin.

(*b*) *With* Rattus rattus

The results of two small-scale tests on the relative acceptance of warfarin and fumarin at 0·025 % to *R. rattus* and of four tests with warfarin and diphacinone are given in Table 5. No tests were done with 2-naphthyl-1,3-indandione, 2-isovaleryl-1,3-indandione or coumachlor.

In one of the tests with fumarin the bait base was the standard oatmeal/oil/sugar mix at 17:1:1. In the other, glycerine was substituted for the oil. In neither case was there any indication that fumarin is less acceptable to the ship rat than warfarin at 0·025 %.

Table 5. *Comparative acceptance of warfarin, fumarin and diphacinone to* Rattus rattus *over 2 days*

Warfarin bait and amount eaten (g.)	Compared bait and amount eaten (g.)	No. of rats preferring warfarin and total in test	Significance level of difference
At 0·025 % in oatmeal/ glycerine/sugar (112)	Fumarin at 0·025 % in oatmeal/ glycerine/sugar (108)	4/9	Not significant
At 0·025 % (59)	Fumarin at 0·025 % (62)	3/7	Not significant
At 0·025 % in oatmeal/ glycerine/sugar (40)	Diphacinone at 0·0025 % in oatmeal/glycerine/sugar (104)	2/8	0·05–0·02
At 0·025 % (332)	Diphacinone at 0·0125 % (348)	13/35	Not significant
At 0·025 % (161)	Diphacinone at 0·025 % (114)	6/12	Not significant
At 0·1 % (39)	Diphacinone at 0·025 % (258)	1/12	Highly significant

Table 5 shows that even in the presence of a masking substance such as glycerine or sugar, 0·0025 % diphacinone is preferred by *R. rattus* to warfarin at ten times its strength (with which it is comparable in toxicity). When the diphacinone level is increased to 0·0125 % there is no significant difference between its acceptance and that of warfarin at the standard concentration. The increasing unpalatability of warfarin at higher concentrations than 0·025 % is brought out in the last test summarized in Table 5.

(*c*) *With* Mus musculus

Because of the small daily intake of the house-mouse and its habit of kibbling food, accurate measurement of its food preferences is difficult—though not impossible. For this reason, in examining the effectiveness of new anti-coagulants against *M. musculus*, more reliance is usually placed on the results of field trials. Only two palatability trials are therefore reported here. In these nine and twelve mice were each given a choice between 0·0125 % diphacinone and 0·025 % warfarin (the standard concentration for control). In neither test was any obvious preference shown. The total amount of warfarin bait ingested was 42 g. compared with 37 g. of diphacinone. Eleven of the twenty-one mice ate more warfarin and one showed no preference.

TOXICITY TO NON-RODENTS

In considering the rodenticidal efficiency of a particular poison, its toxicity to non-rodents must be assessed. For obvious reasons, however, this has to be done on the basis of very few assays with domestic or other animals and by extra-

polation from the results of experiments on rats and mice. And since, in most accidents with rat poisons, the victim only gains access to the bait on a single occasion, as much importance attaches to the acute toxicity of an anti-coagulant as to its chronic toxicity.

Unfortunately, the acute oral toxicity of warfarin to *R. norvegicus* has not yet been satisfactorily established. Early reports, unsupported by experimental data—such as those by Heinz (1950) and Hüter (1950), who mention LD_{50}'s of 1·3–1·5 mg./kg. and 1·5–3·0 mg./kg., respectively—can probably be discounted. The figure of 60 mg./kg. that is most frequently quoted (e.g. Meyer, 1952; British Patent Specification, 1955) seems to have originated from Reiff & Wiesmann (1951) who also gave the LD_{50} of coumachlor as 900–1200 mg./kg. Again no experimental details were given. Hagan & Radomski (1953) partly support Reiff & Wiesmann (1951) in that the figure they arrived at for the LD_{50} of warfarin (sodium salt) for male albino rats was 58 ± 18 mg./kg.; but they also found the LD_{50} to females to be 323 ± 70 mg./kg. On the other hand, Wilk (1957) found the LD_{50} of warfarin to male albinos to be 14·5–20 mg./kg.

The acute oral toxicity of fumarin to rats is probably less than that of warfarin, but this cannot be claimed for certainty until more data on the latter poison are forthcoming. The figure given by the manufacturers for the LD_{50} of fumarin is 400 mg./kg. (British Patent Specification, 1955). Our own estimate based on forty male albino rats, is just 200 mg./kg. Because we prefer not to use an oesophageal tube in assays with anti-coagulants, the animals were allowed to feed from measured amounts of thoroughly mixed bait over a morning period of 90 min. The amount of fumarin ingested by each animal was then estimated from the bait residues, and the LD_{50} calculated by the modified probit method recommended by Bliss (1938) for individual mortality records. This method of assay lacks accuracy and because of the considerable heterogeneity observed, no fiducial limits were calculated. It may be noted, however, that the lowest fatal dosage was 45 mg./kg. while one rat managed to survive an estimated 396 mg./kg.

No data on the acute toxicity of fumarin to animals other than rats have been reported, but its chronic toxicity to dogs, cats, rabbits and ducks has been investigated by Steiniger (1953). He used the method of consecutive daily dosing for 5 days and concluded that cats and dogs were much less resistant than rabbits (or ducks) but that, on the whole, fumarin was no more dangerous to use than warfarin.

No information on the acute or chronic toxicity of coumachlor to non-rodents seems to be available. Thus, the risks involved in its use can only be estimated from its chronic and acute toxicity to rats. And since coumachlor is less effective than warfarin as a chronic poison against *R. norvegicus*, the advantage it appears to possess by virtue of its reported high LD_{50} (900–1200 mg./kg.) is largely offset by the need to use it at a higher concentration to give comparable results.

The acute oral toxicity of 2-isovaleryl-1,3-indandione to rats and rabbits has been studied by Kabat, Stohlman & Smith (1944). The LD_{50} for albino rats would appear to lie between 100 and 200 mg./kg. and for rabbits, between 100 and 150 mg./kg. Hagan & Radomski (1953) found the LD_{50} of warfarin for rabbits to

be 800 mg./kg. Their figures for male and female rats have already been quoted above. At concentrations in baits that would give the same degree of control over the common rat, therefore, the little evidence that is available is in favour of warfarin—but not by a large margin.

The acute LD_{50} of diphacinone was found by Correll, Coleman, Long & Willy (1952) to be 3 mg./kg. for laboratory rats, 35 mg./kg. for rabbits and 340 mg./kg. for laboratory mice. The last figure is surprising—but Table 3 shows that one of our wild mice survived 186 mg./kg. administered over 96 hr. Our own figure for the acute LD_{50} of diphacinone to male white rats of Wistar origin is 17 mg./kg. ($\log LD_{50} = 1{\cdot}2305 \pm 0{\cdot}0029$). Using the pill-feeding method, our assay results were as follows:

Dosage (in mg./kg.)	4	6	9	13·5	20·25	30·375
No. of animals	8	8	13	21	21	13
Kill	1	0	3	7	12	11

Comparing our LD_{50} with the lowest (Wilk's, 1957) estimate for warfarin it seems that the latter and diphacinone in single doses may be about equally toxic to common rats. Diphacinone is, however, twenty times as toxic to the rabbit if the warfarin figure of 800 mg./kg. found by Hagan & Radomski (1953) for that animal is used for comparison.

According to the findings of the Hazleton Laboratories—reported by Gates (1957)—the LD_{50} of diphacinone for dogs and cats lies between 5 and 15 mg./kg. Even assuming the upper figure, this is still about one-tenth of the average lethal dose of warfarin (200–300 mg./kg.) found by Hagan & Radomski (1953) for dogs. On the other hand, Papworth (1958) states that U.S. Fish and Wildlife workers have killed dogs with single doses of 20–50 mg./kg. of body weight of warfarin. Other U.S. Fish and Wildlife Service results quoted by Papworth suggest that the LD_{50} of warfarin for cats is somewhat higher than that for dogs and that, therefore, warfarin is perhaps three or four times less toxic to cats than diphacinone. Jolly (personal communication) informs us that diphacinone and warfarin seem to be fairly similar in chronic toxicity to pigs fed on bait containing 0·0125 and 0·025 %, respectively. Jolly also reports that these concentrations of diphacinone and warfarin were about equally injurious to chickens when fed over a period of 4 days.

Taking the above evidence as a whole therefore, it would seem that, even allowing for its use in rodent baits at half the concentration at which warfarin is used, diphacinone is likely to prove a slightly greater risk to domestic and other animals. However, more information about the toxicity of warfarin and diphacinone to non-rodents, particularly of the comparative kind obtained by Jolly, is needed. In fact, the whole question of the possible effects of such factors as diet, routine and time of year—as well as age and sex—on the acute toxicity of anti-coagulants requires investigation. For the lack of consistency in some of the data discussed above can be due only in small part to the various ways in which death may occur after poisoning with these substances.

DISCUSSION AND CONCLUSIONS

The toxicity and acceptance tests described above were carried out over a considerable period and for a number of reasons. This largely accounts for the varying choice of bait base and the range of poison concentrations covered. Often the latter were initially based on manufacturers' suggestions. Only fumarin and diphacinone have been investigated in any detail. Certain conclusions, however, may be drawn about all five anti-coagulants.

In the first place, 2-isovaleryl-1,3-indandione may be rejected as a rodenticide since though, at 0·025 %, it gave a reasonable kill (10/12) when fed to *R. norvegicus* for 72 hr. (Table 1), its acceptance *vis-à-vis* 0·005 % warfarin was exceedingly poor (Table 4). It does not follow that it will be useless against *R. rattus* but at any rate it is unlikely to be particularly effective.

Even at 0·05 %, 2-naphthyl-1,3-indandione is less effective against *R. norvegicus* than 0·005 % warfarin. It was not tested at higher concentrations, such as 0·1 %, since all anti-coagulants yet examined have proved unpalatable to rats at these levels. While, therefore, its effectiveness against *R. norvegicus* cannot be completely denied, its investigation cannot be said to warrant priority.

Coumachlor, similarly, is less effective against the common rat than is warfarin. Even at 0·05 % it had to be fed for 5 days to give the same mortality as 0·05 % warfarin fed for 3 days. Yet at half this concentration (0·025 %) it was significantly less palatable (Table 4). In two toxicity tests against *R. rattus* (Table 2) coumachlor at 0·025 % gave fairly comparable results to warfarin at 0·025 %. More tests and examination of its palatability may therefore show that in baits it is more useful against *R. rattus* than against *R. norvegicus*. Its effectiveness against mice was not investigated.

It has been shown above that against *R. norvegicus*, fumarin is little, if any, less toxic than, and is as palatable as, warfarin—both at 0·005 and 0·025 %. Against *R. rattus* at 0·025 % it perhaps needs to be ingested for a little longer than 0·025 % warfarin to kill the most resistant animals. On the other hand, it is probably as acceptable. Further, its acute toxicity to rats and non-rodents seems to be less than that of warfarin. On this showing fumarin can be regarded as a reasonable alternative to warfarin against both species of rats. Against the house-mouse present evidence suggests that 0·025 % warfarin is slightly more effective than 0·025 % fumarin. This can probably best be further investigated in the field.

Against the common rat the greater toxicity of diphacinone over that of warfarin is offset by its greater unpalatability (Table 4). It is doubtful if the advantage to be gained in reducing the 95 % lethal feeding period from 3 to 2 days by using 0·005 % diphacinone instead of 0·005 % warfarin would compensate for the loss of acceptability of the bait and the possible greater risk to non-rodents. A reasonable compromise is to regard 0·0025 % diphacinone as a good alternative to 0·005 % warfarin. In situations where 0·025 % warfarin is recommended (such as in sewer rat control) 0·005 % diphacinone will probably give comparable results.

Against *R. rattus* the much shorter feeding period required to give a kill with 0·0125 % diphacinone compared with, say, 0·025 % warfarin or 0·025 % fumarin

(Table 2) is an advantage that cannot be overlooked—especially as no loss of palatability is involved (Table 5). The possibility that 0·0125 % diphacinone is slightly more dangerous to non-rodents is of less importance here, since in Britain and in several other countries *R. rattus* is commonly found only in buildings at the ports. Nevertheless, if 0·0125 % diphacinone comes to be used in place of warfarin in such circumstances it might be wise to take extra precautions against secondary poisoning of cats.

Against the house-mouse, diphacinone at 0·0125 % is about as acceptable as warfarin at 0·025 % and appears to be about as toxic. More work is needed to show whether it is still as acceptable at higher concentrations—where it may be expected to give quicker results.

FIELD OBSERVATIONS

For reasons that have been stated elsewhere (Bentley, 1958) it is much easier to predict the field performance of anti-coagulants from laboratory observations than in the case of acute rodenticides. And, in view of the time and expense involved in carrying out large-scale field trials according to scientific standards of accuracy, a strong case can be argued for confining field examination of chronic rodenticides that have shown up well in the laboratory to a relatively small number of control treatments. This course has been followed by us in the case of fumarin and, to a limited extent so far, with diphacinone; and since judgement of the results is a subjective process in which past experience of similar trials with warfarin is involved, full details will not be given.

In brief, eleven control treatments have been carried out with 0·025 % fumarin against *R. norvegicus* in different parts of England and Scotland—with successful results. In most cases it was considered that warfarin would have been no more effective. On three occasions it was thought that it would have given a quicker kill: but in each case there were special circumstances—such as disturbance of the environment—to cloud judgement. In three treatments against *R. norvegicus* with 0·005 % fumarin, apparently complete control was obtained in 7, 8 and 9 days. In simultaneously conducted parallel treatments with warfarin at 0·005 % similar results were obtained in 4, 8 and 7 days, respectively. In two further treatments with 0·005 % fumarin clearance was achieved in 8 and 10 days.

These results, as far as they have gone, are about what might be expected from the laboratory findings and are also in accord with the results of field trials carried out under somewhat different conditions in the U.S.A. (Crabtree, 1955).

Eight field trials with diphacinone have been completed to date. In two, with 0·0125 % diphacinone against *R. rattus*, very good results were finally obtained. In one of these it was necessary to change the bait base (to *damp* wheat) before most of the population showed interest in it. In the other six treatments, with 0·0025 % diphacinone—against *R. norvegicus*—complete clearance seemed to have been achieved in 7–11 days. Further trials are in hand.

SUMMARY

1. Results are presented of palatability tests and chronic toxicity tests with five anti-coagulant rodenticides at a variety of concentrations against wild *Rattus norvegicus*, *R. rattus* and *Mus musculus*. These results are compared with similar data for warfarin. Estimates are also given of the acute oral toxicity of two of the five, fumarin and diphacinone, to white rats.

2. The toxicity of the compounds *vis-à-vis* warfarin is examined from the standpoint of the risks to domestic animals involved in their use as rodenticides.

3. Coumachlor, 2-isovaleryl-1,3-indandione and 2-naphthyl-1,3-indandione are considered to be less effective than warfarin against *R. norvegicus*. Their effectiveness against *R. rattus* remains to be demonstrated.

4. Fumarin appears to be about as good as warfarin against *R. norvegicus* and *R. rattus* at similar concentrations. It appears to be slightly less effective against *M. musculus*.

5. Diphacinone at 0·0025% is regarded as a good alternative to 0·005% warfarin against *R. norvegicus*. At 0·0125% it is probably more effective against *R. rattus* than is 0·025% warfarin. The two poisons are about equally effective at this strength against *M. musculus*, but diphacinone is probably slightly more dangerous for domestic animals.

6. The results of a small number of field trials with fumarin and diphacinone are briefly summarized.

So many of our colleagues and others have helped at some stage or other of our investigations that it would be tedious to mention them all by name. We must, however, single out for our special thanks Mrs M. Rowe and Miss E. Taylor who carried out a large number of the laboratory tests, Mr A. Taylor on whose chemical experience and facilities we have freely drawn and Mr C. H. B. Worrall, Miss B. Jones, Mr S. R. Surtees and Mr J. H. Cuthbert (Department of Agriculture for Scotland) who carried out the field trials.

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