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Laboratory evaluation of gophacide as a rodenticide for use against *Rattus norvegicus* and *Mus musculus**

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SUMMARY

Laboratory tests were carried out to assess the efficacy of gophacide as a rodenticide against the Norway rat (*Rattus norvegicus*) and the house mouse (*Mus musculus*). Results of feeding tests with wild animals suggest that the compound would be more useful against mice than rats, and that 0·3 % would be a near optimal concentration for field trials for both species.

The hazards of using gophacide as a rodenticide are discussed.

INTRODUCTION

The compound 0,0-bis(*p*-chlorophenyl)acetimidoylphosphoramidothioate was developed by Farbenfabriken Bayer A.G. of Leverkusen as an experimental acute rodenticide under the code number Bayer 38819, and is marketed in the U.S.A. under the trade name 'gophacide' for the control of pocket gophers. It is a powerful cholinergic substance which apparently lacks some of the undesirable features of other organophosphates, including rapidity of action and instability. Ward, Hegdal, Richens & Tietjen (1967) and Richens (1967) describe laboratory and field work with pocket gophers (*Thomomys*, *Geomys* and *Cratogeomys* spp.), and Schroeder (1967) with Ord's kangaroo rat (*Dipodomys ordii*). In all these cases gophacide used in the field was laid in, or at the entrance to burrow systems, and found to be effective.

Little work appears to have been done on rats and mice, although Richens (1967) mentions that while acceptance of the poison by *Rattus rattus* is poor, acceptance by *Mus musculus* is good and the chemical shows promise for this last species.

The present report describes laboratory trials carried out with gophacide on the Norway rat (*R. norvegicus*) and the house mouse (*M. musculus*), as part of our Laboratory's search for new and improved rodenticides (Rowe, Greaves, Redfern & Martin, 1970). The aim of the work was to examine the compound under laboratory conditions, and to determine suitable bait concentrations for use in field trials.

Since the work was completed, Bayer Agrochem Ltd have decided not to develop gophacide for the United Kingdom market.

METHODS

The animals used were male laboratory rats (Wistar-derived, designated TAS) and mice (LAC Grey), a laboratory strain of rat (HS) homozygous for warfarin resistance, and wild rats and mice of both sexes. The wild mice were laboratory-bred first generation descendants of wild-caught mice. The wild rats included warfarin-susceptible animals caught in a Midlands refuse destructor and held in the laboratory for at least 3 weeks before use, and also warfarin-resistant rats. These last had been caught on farms in Wales where resistance was known to exist, and had subsequently survived a laboratory screening test for warfarin resistance – either an injection of 200 mg./kg. warfarin or 6 days feeding on bait containing 0·005 % warfarin in medium oatmeal.

The oral toxicity of gophacide was determined by stomach tube administration to laboratory animals grouped 5 to a cage. The compound was ground finely in a mortar, and suspended in a 5 % solution of powdered acacia B.P. Treated animals were kept under observation and the time of onset of signs of poisoning was recorded.

Feeding experiments were carried out with singly caged laboratory rodents, and later with wild-caught animals. The bait was made up of 90 % pinhead oatmeal, 5 % corn oil and 5 % wholemeal flour into which the active ingredient had been dispersed.

Feeding tests were of 2 types – 'no-choice' tests in which the poisoned food was given alone for 24 hr., and 'choice' tests lasting 48 hr. in which the same bait without the toxicant was also given, with the positions of the two baits being interchanged after 24 hr. and fresh bait being given each day. Mortality and the amounts of bait eaten were recorded daily. Animals that died were autopsied and survivors kept under observation for 7 days after their last exposure to poison.

The concentrations of gophacide used in preliminary 'no-choice' feeding experiments were chosen bearing in mind the LD 50 values of currently used acute rodenticides and the concentrations at which they are used in baits (see Rowe *et al.* 1970).

We are indebted to Bayer Agrochem Ltd for supplying samples of gophacide.

RESULTS AND DISCUSSION

Oral intubation tests

The results of oral intubation tests with laboratory animals (Table 1) indicate approximate acute oral LD 50s of 30 and 7–10 mg./kg. for rats and mice respectively. A group of warfarin-resistant HS rats gave an identical result to the TAS rats. Du Bois, Kinoshita & Jackson (1967) quote figures of 7·5 and 3·7 mg./kg. for male and female laboratory rats of unspecified origin.

The first visible signs of illness in rats given 100 mg./kg. appeared between 2 and 4 hr. after intubation, with deaths occurring between 24 and 48 hr. At 10 mg./kg. poison symptoms appeared later, but all rats recovered. With mice at 100 mg./kg. first symptoms appeared between 2 and 4 hr., with three animals dead by 4 hr.; the remaining two died after 24 hr. At 10 mg./kg. no mice appeared sick within 4 hr., but three died between 24 and 48 hr. Gophacide differs from the

Table 1. *Mortality of male laboratory rats and mice after oral intubation tests with gophacide*

Animals	Mean body weight (g.)	Single dose		Four daily doses	
		100 mg./kg.	10 mg./kg.	10 mg./kg.	1 mg./kg.
Rats	103	5/5	0/5	4/5	0/5
HS rats	431	5/5	0/5	—	—
		100 mg./kg.	10 mg./kg.	1 mg./kg.	0·1 mg./kg.
Mice	17	5/5	3/5	1/5	0/5

Table 2. *Results of 24 hr. 'no-choice' feeding of gophacide to male laboratory rats and mice*

Animals	Mean body weight (g.)	Concentration (%)	Mortality	Mean bait intake (g.)	Lethal doses of active ingredient (mg./kg.)		Doses of active ingredient survived (mg./kg.)	
					Mean	Range	Mean	Range
Rats	105	3.0	5/5	1·2	334	257–436	—	—
	107	0·3	5/5	3·3	94	62–142	—	—
	106	0·1	5/5	3·0	28	23–32	—	—
Mice	20	1·0	5/5	0·6	307	250–375	—	—
	20	0·1	5/5	0·9	47	30–63	—	—
	24	0·03	5/5	1·0	13	10–17	—	—

Table 3. *Results of experiments in which male laboratory rats and mice were given a choice for 2 days between plain bait and bait containing gophacide*

Animals	Mean body weight (g.)	Concentration (%)	Mortality	Mean bait intake (g.)		Lethal doses of active ingredient (mg./kg.)		Doses of active ingredient survived (mg./kg.)	
				Poison	Plain	Mean	Range	Mean	Range
Rats	105	0·3	5/5	1·3	1·9	37	12–72	—	—
	103	0·1	2/5	1·5	3·9	24	20–27	8	5–13
Mice	18	0·1	5/5	0·5	1·1	28	14–50	—	—
	24	0·03	4/5	1·2	2·1	13	10–14	23	—

majority of organophosphate compounds in having a relatively slow speed of action, which compares favourably with zinc phosphide, sodium fluoroacetate and fluoroacetamide (Bentley & Greaves, 1960). Richens (1967) states that visual evidence of gophacide poisoning is usually not apparent for 8–12 hr., but the time and intensity of reaction is dependent on dose, sex, age and species.

There was no evidence of sub-acute toxicity in the animals given four successive daily doses. The animals that died all had shown typical poison symptoms after the first dose, although no deaths occurred until the fourth day. However, Du Bois *et al.* (1967) state that the long duration of the effects of gophacide on cholinesterase activity suggests that repeated doses would produce cumulative toxic effects.

Table 4. *Results of experiments in which wild rodents were given a choice for 2 days between plain bait and bait containing gophacide*

Type of animal	Mean body weight (g.)	Sex	Concentration (%)	Mortality	Mean bait intake (g.)		Lethal doses of active ingredient (mg./kg.)		Doses of active ingredient survived (mg./kg.)	
					Poison	Plain	Mean	Range	Mean	Range
Rat (non-resistant)	274	M	0·9	8/10	0·7	9·4	34	9–80	10	9–10
	193	F	0·9	10/10	2·2	3·1	104	16–220	—	—
	315	M	0·3	4/10	2·4	8·5	36	12–86	12	7–17
	215	F	0·3	8/10	3·0	6·7	48	26–78	9	4–14
	289	M	0·1	3/10	4·1	7·9	16	7–25	13	8–23
	171	F	0·1	9/10	3·6	7·3	23	10–44	10	—
	345	M	0·05	1/5	6·1	13·4	14	—	8	5–9
Rat (resistant)	214	M	0·9	7/10	1·5	8·7	101	23–277	15	6–21
	207	F	0·9	7/10	1·3	8·2	78	15–186	14	9–21
	187	M	0·3	6/10	2·7	5·7	65	36–118	19	8–38
	186	F	0·3	9/10	3·3	9·7	44	11–111	19	—
	179	M	0·1	4/10	2·7	3·8	24	16–27	9	0–25
	155	F	0·1	5/10	3·7	5·4	39	32–47	11	1–18
Mice	19	M	0·3	9/10	1·6	1·2	22	0–41	30	—
	15	F	0·3	10/10	0·7	0·5	14	0–46	—	—
	19	M	0·1	9/10	0·4	0·3	31	10–25	20	—
	15	F	0·1	8/10	0·3	0·3	21	12–36	18	13–23
	15	M	0·05	4/5	0·6	1·7	19	14–25	19	—
	18	M	0·03	2/10	1·5	2·5	26	24–28	25	16–35
	16	F	0·03	3/10	0·8	1·2	14	8–21	15	3–26

Table 5. *Analysis of variance using arcsin transformation of the mortality data given in Table 4*

Animals	Source of variation	*F*	D.F.	*P*
Rats	Sex	18·8	1	0·01–0·05
	Concentration	6·4	2	N.S.
	Resistance	2·3	1	N.S.
	Concentration × sex	0·5	2	N.S.
	Sex × resistance	5·5	1	N.S.
	Concentration × resistance	3·9	2	N.S.
Mice	Sex	0·5	1	N.S.
	Concentration	15·7	2	N.S.

N.S. = Not significant ($P > 0·05$).

Feeding tests with laboratory rodents

In preliminary no-choice feeding experiments, the results of which are given in Table 2, complete mortality was obtained at all concentrations. Choice tests were performed using the lowest concentration of poison that gave complete mortality in the no-choice tests, i.e. 0·1 % for rats and 0·03 % for mice. When these failed to give complete kills, further tests were carried out at 0·3 % for rats and 0·1 % for mice, both of which gave complete kills (see Table 3).

Feeding tests with wild rodents

Choice tests were initially carried out at 0·3 % for wild rats and 0·1 % for wild mice, but with both species incomplete kills were obtained. Further tests were performed at concentrations higher and lower by a factor of 3, in an attempt to find the optimum balance between concentration and palatability.

The results (Table 4) suggest that gophacide would be more effective against mice than rats. At 0·3 % the mortality for mice was 19/20. The total amounts of poisoned and plain foods eaten were 2·3 and 1·7 g. respectively, suggesting that the poison was sufficiently palatable and slow in action to avoid sublethal dosing and the development of poison shyness. The one mouse that survived the test ate an equal quantity (0·1 g.) of plain and poisoned bait on the second day. Six of the 20 mice tested ate no detectable quantity of poisoned bait, and five of these apparently ate no plain bait either. However, Greaves, Redfern & Tinworth (1974) comment that apparent failure to eat may be caused by baits absorbing atmospheric moisture, thereby counteracting the detection of small bait takes. At 0·1 % the mortality for mice was 17/20; total amounts of food eaten were 6·7 g. of poisoned and 5·6 g. of plain food. At 0·3 % and 0·1 % all deaths occurred within 24 hr. An analysis of variance using arcsin transformation of the percentage mortalities in the mouse data showed that mortality was not significantly affected by sex or concentration (Table 5). The fact that mortalities of 95 % and 85 % were obtained in the laboratory with concentrations of 0·3 % and 0·1 % respectively without pre-baiting, suggest that these would be near optimal concentrations for field trials against house mice. These mortalities compare favourably with 87 % for zinc phosphide at 3 %, the concentration considered optimal under the same conditions.

In rats sex had a significant effect on mortality, with males being more tolerant to the poison. Mortality was not influenced by resistance to warfarin or concentration. Combining results for both sexes and for resistant and non-resistant rats, the mortalities obtained were 80 %, 67·5 % and 52·5 % at concentrations of 0·9 %, 0·3 % and 0·1 % respectively. In comparison zinc phosphide at 3 % gave a kill of 65 % (13/20). Inspection of the bait consumption figures suggests that in the field gophacide at 0·9 % would not be eaten by rats, and that 0·3 % would probably be near the optimum concentration.

One important attribute of a candidate rodenticide must be its safety in use. Acute poisons, such as gophacide, are at a disadvantage in comparison with chronically acting compounds because of, amongst other things, their generally rapid speed of action after a single dose, and frequent lack of a satisfactory antidote.

Gophacide is a relatively slow-acting anticholinesterase; Richens (1967) states that atropine sulphate, protopam chloride and 2-pyridine aldoxime methiodide (2-PAM) are readily available antidotes. On the other hand, Du Bois *et al.* (1967) describe experiments in which they tested the effectiveness of antidotes: neither atropine sulphate nor 2-PAM, given separately or in combination, provided significant protection.

In the U.S.A. gophacide is registered for use against pocket gophers, the bait being laid in natural or artificial burrow systems. Ward *et al.* (1967) state that gophers invariably die below ground and therefore would not present a serious secondary poisoning hazard; surface use of the poison against jackrabbits, however, would constitute a secondary risk to eagles and other raptoral birds, as well as a primary one to many non-target species. In Britain the greatest need for a new rodenticide is for use against house mice, a species in which warfarin resistance is general and widespread, and Norway rats in the more clearly defined areas of resistance. The restrictions that would have to be imposed on the use of gophacide in urban situations would preclude its use for effective mouse control. The majority of resistant rat infestations are found in small, poorly maintained farmsteads where the risks of primary and secondary poisoning accidents would severely restrict its use.

REFERENCES

Bentley, E. W. & Greaves, J. H. (1960). Some properties of fluoroacetamide as a rodenticide. *Journal of Hygiene* **58**, 125–32.

Du Bois, K. P., Kinoshita, F. & Jackson, P. (1967). Acute toxicity and mechanism of action of a cholinergic rodenticide. *Archives Internationales de Pharmacodynamie et de Thérapie, Brussels* **169**, 108–16.

Greaves, J. H., Redfern, R. & Tinworth, H. (1974). Laboratory tests of 5-*p*-chlorophenyl silatrane as a rodenticide. *Journal of Hygiene* **73**, 39–43.

Richens, V. B. (1967). The status and use of gophacide. *Proceedings of the 3rd Vertebrate Pest Conference* (San Francisco), 118–25.

Rowe, F. P., Greaves, J. H., Redfern, R. & Martin, A. D. (1970). Rodenticides – problems and current research. *Proceedings of the 4th Vertebrate Pest Conference* (Sacramento), 126–8.

Schroeder, M. H. (1967). Gophacide, a candidate for control of Ord's kangaroo rat. *Journal of Wildlife Management* **31**, 339–41.

Ward, A. L., Hegdal, P. L., Richens, V. B. & Tietjen, H. P. (1967). Gophacide, a new pocket gopher control agent. *Journal of Wildlife Management* **31**, 332–8.