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# EVALUATION OF SYSTEMIC INSECTICIDES MIXED IN RODENTICIDE BAITS FOR PLAGUE VECTOR CONTROL

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**Abstract.** Rodenticide baits containing systemic insecticides were evaluated in the laboratory for their palatability to the house rat *Rattus rattus* and for their toxicity against the oriental rat flea *Xenopsylla cheopis* - both animals are important vectors of plague in Africa. The test bait and a non-poisonous alternative were given to the rats for four days. The evaluation of the effectiveness was based on mortality and poison bait intake in percent of the total consumption. Different concentrations of technical material and different types of encapsulation of the three insecticides phoxim, fenthion and dimethoate were used in the tests. The rodenticide used was 0.005% bromadiolone. For all three insecticides, a reduced intake of the poisonous bait was observed compared with the test of bromadiolone without insecticide. Based on the acceptance of the baits, the dimethoate encapsulated with beef tallow only was considered as the most promising candidate. The formulation was tested on flea infested rats and after four days, a raised flea mortality was observed.

*Key words*: Rodenticide bait, systemic insecticides, *Rattus rattus*, *Xenopsylla cheopis*.

## INTRODUCTION

Several systemic insecticides have been evaluated in field trials for their effectiveness in flea control when mixed into non-poisonous bait given to rodents (*e.g.* MILLER *et al.*, 1977a, 1977b, 1978). Especially organophosphorus compounds have been reported as having a potential. However, an increase in the acceptibility to the rodents would be desirable, as higher concentrations of insecticides may be used. For this purpose, (micro-)encapsulated formulations of the insecticides could be suitable.

The objective of the present study was to develop rodent baits containing systemic insecticides along with the rodenticide for plague vector control. In the present paper we report on laboratory experiments to 1) evaluate potential insecticides in palatability tests with groups of rats (*Rattus norvegicus* [Berkenhout, 1769] and *Rattus rattus* [L.,1758]) and 2) evaluate the efficacy of these insecticides in the baits on the flea *Xenopsylla cheopis* (Rothschild, 1903) occurring on *Rattus rattus*.

## MATERIAL AND METHODS

### Rodenticide

The rodenticide chosen for the study was the anticoagulant bromadiolone. Bromadiolone was chosen because it is palatable to many rodent species and because it is being used worldwide (MARSH, 1977; BUCKLE, 1994). A concentration of 0.005% bromadiolone in the bait formulation was used as it is the standard concentration for practical control purposes (BUCKLE, 1994). Bromadiolone is a slow-acting anticoagulant and although one single feeding may be enough for the rodent to ingest a lethal dose, several days will pass before the rodent starts showing symptoms and dies (thus there is time enough for the fleas to die before the death of the rat).

### Insecticide

The insecticides and concentrations used in the trials reported here were 0.24% phoxim, 0.15% and 0.3% fenthion and 0.6% dimethoate.

To test whether the acceptability of these compounds to the rodents could be increased, different encapsulated formulations were also used. A commercially available microencapsulated fenthion product (Baytex ME 35%) was tested along with the following formulations made specifically for this project: fenthion and phoxim coated with hydrogenated beef tallow, dicalcium phosphate dihydrate, silicium dioxide and PVP 30 (formulation C), and two formulations of dimethoate - one having a coating of hydrogenated beef tallow alone (formulation X) and another with a coating of hydrogenated beef tallow, dicalcium phosphate dihydrate and PVP 30 (formulation Y). The encapsulated formulations were tested in the concentrations of 0.15% fenthion, 0.24% phoxim and 0.6% dimethoate, respectively.

### Rodents

The rodents used for the experiments were a Danish Pest Infestation Laboratory (DPIL) strain of the brown rat *Rattus norvegicus* and a Tanzanian and a DPIL strain of the roof rat *Rattus rattus*. (The Tanzanian strain of *R. rattus* was obtained from T. Mbise, TPRI, Arusha, Tanzania.) The strains of both species are wild type and susceptible to anticoagulants. Although *R. rattus* was the main test species of the project, *R. norvegicus* was chosen for some preliminary tests as this species is rather particular about what it eats and, furthermore, in the first part of the project only a limited number of *R. rattus*, primarily males, were available.

### Fleas

The strain of the rat flea *Xenopsylla cheopis* used in this study originates from Sokoine University of Agriculture, Morogoro, Tanzania. By modifying the rearing technique for rearing the squirrel flea, *Ceratophyllus sciurorum sciurorum* (Schrank, 1803), (LARSEN, 1994), we were able to rear *X. cheopis* on Danish rodent species. Two species of mice,

long-tailed field mouse *Apodemus sylvaticus* (L.,1758) and yellow-necked field mouse *Apodemus flavicollis* (Melchior,1834), were used for this purpose.

Newly emerged fleas and the mouse were put into the nest box (Fig. 1). One table spoon of dried bovine blood and one table spoon of sand were placed in the filter paper tray. The blood was added to ensure adequate food for the flea larvae. Sand was used because both larvae and adult *X. cheopis* fleas like to "hide" in sand (Frank Clark, *pers. comm.*). The environmental conditions in the room housing the flea cultures were 21°C and 80% RH. A photo period of 19:5 hour light:dark was used. This encouraged the mice to spend more time in the nest box with the fleas, because both species of mice used are most active during night (GELMROTH, 1969, 1970).

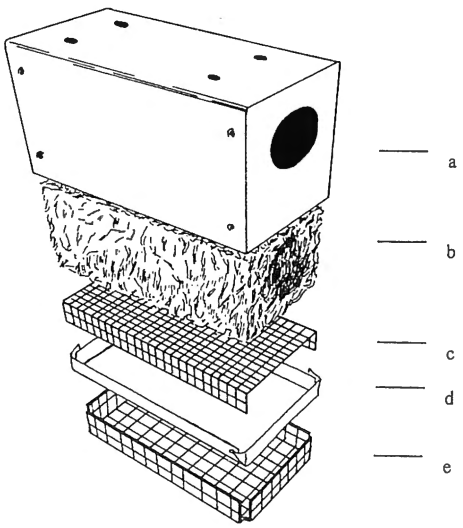


Fig. 1. – Mouse nest box.
a = stainless steel box,
b = straw material,
c = nest box floor (metal grid),
d = filter paper tray and
e = metal grid protecting tray.

When fleas were needed for supplementation of the cultures, flea eggs and larvae were obtained by sieving the nest box material plus the material of the filter paper tray in a sieving tower of a vibratory shaker (Retsch Laboratory Sieving Machine (VIBRO); Retsch, Germany). The eggs and larvae were collected from the sieved material and placed in tissue culture flasks with filter caps (Nunclon Flasks, Nunc, Denmark) containing larval food. The flasks were placed at 23°C and 80% RH.

When the adults emerged from the cocoons in the flask, they were transferred to a mouse nest. It has been experienced that sieving the nest material every second week will keep down the number of mites nourishing on flea eggs. The cultures produced about 300 adult fleas per week per mouse when the adult flea population in the mouse nest was kept on approximately 50 in number.

**Palatability tests**

The palatability tests were carried out as choice tests in a special test room at the DPIL (Fig. 2). This room was about 13 $m^2$ and equipped with nest boxes, water ad lib, and during

the test period a group of 12-20 trays in the centre of the floor. Before the start of a test, a group of 5 or 10 rats were introduced into the room and allowed to acclimatise for a period of at least three days. In this period the rats were offered normal laboratory food (Altromin pellets) on the floor; the trays in the centre of the floor were empty and placed as shown in Fig. 2. When the acclimatisation period was over, the non-poisonous Altromin pellets were removed and the rats were given the choice between the mixtures A and B alternating in the central trays. Mixture A was a bait base with 0.005% bromadiolone mixed with the insecticide in question or in some tests with *R. rattus* without insecticide. Mixture B was either 0.005% bromadiolone in the actual bait base (in tests with *R. norvegicus*) or the bait base alone as a non-poisonous placebo to mixture A (in tests with *R. rattus*). Tests with bromadiolone in both mixtures should give an indication of the effect on palatability of adding an insecticide, whereas tests with a non-poisonous alternative should simulate a field situation. The bait base used for *R. norvegicus* was the so-called EPA bait base (containing 65% ground yellow corn, 25% steamed rolled oats, 5% powdered sugar and 5% corn oil [MARSH, 1977]) whereas for *R. rattus* the bait base was crushed wheat.

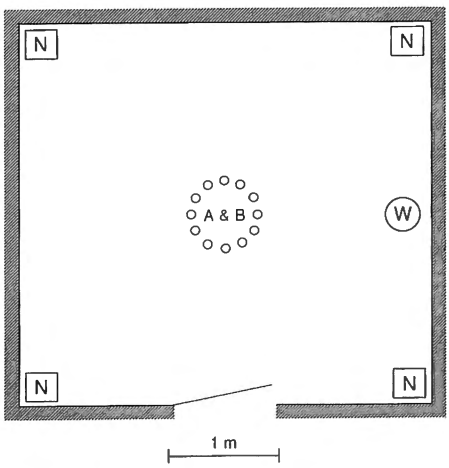


Fig. 2. – Ground plan of rodent test room. N=nest box, W=water, A and B=trays for the two mixtures alternating in a circle.

The palatability was tested over a four-day period. The four-day period was found suitable because death caused by bromadiolone often occurs from the fourth day in laboratory tests. The amounts consumed were recorded each day and the trays were refilled and the position of A and B interchanged. When the actual palatability test period was finished, it was followed by a three-week observation period for registration of any possible symptoms of poisoning and days to death. During this period, the rats were given Altromin pellets and/or rye bread. Dead rats were removed successively.

The tests with the DPIL *R. norvegicus* strain and with the Tanzanian *R. rattus* strain included both males and females whereas tests with the DPIL *R. rattus* strain were on males only.

The palatability of the baits was measured from the bait consumption and the mortality of the rats. The percentage bait acceptance (P) was computed by use of the formula $P = 100T/(T + S)$, where T is the weight of the treated bait consumed and S is the weight of the untreated bait consumed (THOMPSON *et al.*, 1972).

**Flea toxicity test**

The effectiveness of an insecticide given in a bait was tested on *R. rattus* infested with the rat flea *X. cheopis*. The DPIL strain of *R. rattus* was used. The rats were taken from the breeding colony and placed singly in glass terrariums. The terrarium was equipped with a metal nest box with nest material, a food tray and a water bottle. Each rat had an acclimatisation period in the terrarium of at least three days during which the food was non-poisonous organic crushed wheat. The nest material was renewed with fresh nest material at the end of the acclimatisation period and then followed a) a two-day period with daily recordings of consumed amounts of non-poisonous crushed wheat, b) a one-day starvation period, and c) a 4-, 5- or 6-day period with the combined bait preparation of the rodenticide and the insecticide to 3 or 4 rats in each test series (non-poisonous crushed wheat was given to a control rat). After the first day, each rat should have consumed at least 1 g of bait per 100 g body weight to be allowed to continue in the test. If consumption was satisfactory, 100 newly emerged unfed fleas were introduced into the nest box to each of the rats. The consumption of poisonous or non-poisonous bait respectively was recorded daily. As no rats were killed in the test due to the rodenticide intake, the rats were killed by $CO_2$ and frozen before examination for occurrence of fleas. The effect of the systemic insecticide was evaluated by collecting and counting the fleas alive in the nest material and from the rats themselves. Fleas found on the rats that had been frozen were recorded as live fleas.

Only unfed fleas newly emerged from the cocoon (less than 24 hours before the test) were used. This is important because a level of mortality (dehydration) can often be observed for fleas kept away from a host (especially those who already have been feeding on a host). Used in efficacy evaluations like the present, such weakened fleas would give an artificially high mortality as they are more susceptible to insecticides (own observation and EL-GAZZAR *et al.*, 1988) and are probably removed more easily by the grooming activities of their host.

## RESULTS

The preliminary palatability tests where *R. norvegicus* was used (Table 1) showed that the least accepted bait was the one containing 0.24% phoxim followed by baits containing 0.3% and 0.15% fenthion, respectively. The commercially available microencapsulated fenthion product (Baytex ME 35%), which was tested in a concentration of 0.15%, was the best accepted insecticide formulation in the baits. In all tests using *R. norvegicus*, no alternative non-poisonous food was offered to the rats and a 100% mortality was observed.

TABLE 1

*Bait acceptance and mortality for* Rattus norvegicus *(DPIL strain, 5 males and 5 females) for baits containing different insecticides and 0.005% bromadiolone (BR). Alternative bait with 0.005% bromadiolone present.*

| *Bait* | *Number of animals* | *Bait acceptance %* | | *Mortality %* | |
|---|---|---|---|---|---|
| | | *Mean* | *Range* | *Mean* | *Range* |
| Phoxim 0.24% + BR | 2 x 10 | 5.2 | 3.2 - 7.2 | 100 | - |
| Fenthion 0.15% + BR | 2 x 10 | 16.4 | 11.8 - 20.9 | 100 | - |
| Fenthion (ME) 0.15% + BR | 2 x 10 | 23.3 | 10.8 - 35.8 | 100 | - |
| Fenthion 0.3% + BR | 1 x 10 | 7 | - | 100 | - |

Besides phoxim and fenthion, several kinds of dimethoate formulations were included in the tests with *R. rattus* (Table 2). The combined preparation of phoxim and bromadiolone gave a rather low intake clearly illustrated by the fact that the poisonous intake did not exceed 3.7% of the total consumption. No rats died in the phoxim experiments. The bait mixtures containing 0.15% fenthion were better accepted by *R. rattus* than those with 0.24% phoxim. The mean total intake over four days was 67.2 mg fenthion/kg body weight (b.w.). The use of 0.6% dimethoate in the bait mixture showed that it was possible to raise the bait acceptance level in general and the rat mortality level to 80%. The mean daily bait intake gave a dimethoate intake of 49.3 mg a.i./kg b.w.

Based on the observation in the palatability tests with *R. norvegicus* that the bait acceptance could be increased by using an encapsulated insecticide, encapsulated phoxim and fenthion were tested along with two types of encapsulated dimethoate (Table 2).

TABLE 2

*Bait acceptance and mortality for* Rattus rattus *(DPIL strain, males) for baits containing different insecticides and formulations and/or 0.005% bromadiolone (BR). Alternative non-poisonous bait present*

| *Bait* | *Number of animals* | *Bait acceptance %* | | *Mortality %* | |
|---|---|---|---|---|---|
| | | *Mean* | *Range* | *Mean* | *Range* |
| Phoxim 0.24% + BR | 1 x 5 | 4.6 | - | 0 | - |
| Phoxim (C) 0.24% + BR | 2 x 5 | 2.5 | 1.2 - 3.7 | 0 | - |
| Fenthion 0.15% + BR | 1 x 5 | 13.4 | - | 40 | - |
| Fenthion (C) 0.15% + BR | 2 x 5 | 20.7 | 7.2 - 34.2 | 60 | 20 - 100 |
| Dimethoate 0.6% + BR | 1 x 5 | 24.4 | - | 80 | - |
| Dimethoate (X) 0.6% + BR | 3 x 5 | 34.4 | 19.0 - 48.3 | 86.7 | 60 - 100 |
| Dimethoate (Y) 0.6% + BR | 2 x 5 | 19.5 | 17.6 - 21.3 | 70 | 60 - 80 |
| BR | 4 x 5 | 29.6 | 6.5 - 53.9 | 75 | 20 - 100 |

The test results showed that for only one of the dimethoate formulations (marked X) it was possible to raise the mean bait acceptance above what was found for the non-encapsulated formulation as well as above the level found for the bait containing bromadiolone only. The increased bait acceptance was also reflected in a higher rat mortality. This dimethoate formulation was chosen for further palatability testing on the Tanzanian *R. rattus* strain.

The test of baits containing the encapsulated dimethoate on the Tanzanian strain of *R. rattus* gave a remarkable difference in the bait acceptance between males and females, viz. 12.1% and 3.9% respectively (Table 3). The observed mean mortality was 95% for males and only 25% for females. There was thus a much lower bait acceptance for *R. rattus* males of the Tanzanian strain than for the tested males of the DPIL strain but a higher mortality rate for a lower total intake of rodenticide. The mean total intake of rodenticide over the four-day test period was 3.3 (DPIL strain) and 2.3 (Tanzanian strain) (in mg bromadiolone/kg b.w.). This indicates that the Tanzanian strain of *R. rattus* could be a bit more susceptible to the rodenticide than the DPIL strain.

TABLE 3

*Bait acceptance for* Rattus rattus *(Tanzanian strain) for baits containing insecticide and 0.005% bromadiolone (BR). Alternative non-poisonous bait present. M = males and F = females.*

| *Bait* | *Number of animals* | *Bait acceptance %* | | *Mortality %* | |
|---|---|---|---|---|---|
| | | *Mean* | *Range* | *Mean* | *Range* |
| Dimethoate (X) 0.6% + BR | 2 x 10 (M) | 12.1 | 9.8 - 14.4 | 95 | 90 - 100 |
| Dimethoate (X) 0.6% + BR | 1 x 5,1 x 10 (F) | 3.9 | 2.4 - 5.3 | 25 | 0 - 50 |

The 0.6% dimethoate formulation X was chosen for the flea toxicity tests. The intake of this bait formulation by the rats raised the mortality of the fleas compared to the rats given untreated bait (Table 4).

TABLE 4

*Number of fleas found alive on the rats and in the nest material (N = number of rats)*

| *Group* | *N* | *Mean* | *Std Dev* | *Minimum* | *Maximum* |
|---|---|---|---|---|---|
| Treated | 13 | 25.5 | 16.9 | 1 | 50 |
| Untreated | 4 | 59.5 | 9.2 | 53 | 73 |

## DISCUSSION

According to CLARK & COLE (1971), who studied the effect of phoxim on *X. cheopis*, a single dose of 100 mg a.i./kg b.w. was the lowest effective dose given to guinea pigs. In the present tests, such a high value was not obtained as the mean total amount of phoxim

eaten over four days by the rats was about 15.6 mg phoxim/kg b.w. In a later study in outdoor pens (CLARK & COLE, 1974) a 0.24% phoxim bait in bait stations was found to give 99.3% reduction in flea index seven days after treatment. MILLER *et al.* (1975, 1977a, 1977b) obtained effective control of different flea species on native rodents in field tests also with 0.24% phoxim in the bait. An obvious reduction in the flea burden of the rodents was observed as early as 36 hours after treatment. In spite of these relatively positive results, our phoxim palatability experiments gave results that did not encourage further studies on the use of phoxim as the intake of this bait formulation was low and no rats died.

CLARK & COLE (1968) found that a mean daily intake of 4.1 mg fenthion/kg b. w. was the lowest dosage giving 100% kill of *X. cheopis* on hooded white rats. Thus, if the effect of the rodenticide had been higher than the 60% mortality we observed, fenthion would have been a good candidate for use *e.g.* in field trails.

In field tests with 0.24% and 0.36% dimethoate, MILLER *et al.* (1978) obtained a significant flea reduction on *Dipodomys* spp. and *Sigmodon hispidus* Say & Ord (1825). However, CLARK & COLE (1968) did not find dimethoate sufficiently effective against *X. cheopis* because the bait was not readily consumed. The daily intake was in their experiments over a fortnight up to 12.8 mg a.i./kg b.w. with hooded white rats. The lowest daily intake observed in our study was 33 mg a.i./kg b.w. so the 0.6% dimethoate in the X-formulation was chosen for the flea toxicity test.

The difference found in the flea mortality for the rats given the bait containing 0.6% dimethoate (X-formulation) plus 0.005% bromadiolone compared to those given untreated bait (74.5% and 40.5%, respectively) should be regarded as the minimum difference. In the former some of the fleas collected from the body of the rats as well as in the nest had probably taken a bloodmeal recently, having thus consumed the dimethoate. To be able to evaluate how this affects the fleas, the rats should not have been frozen at the end of the test period. The fleas collected then plus those collected from the rat nest material should have been observed for further 24 hours and the effect of any consumed dimethoate could then be demonstrated. Another question to be raised in this test is whether the fleas in the treated group of rats survive because the fleas had not taken a bloodmeal during the test period or just have not got a deadly dosage of the dimethoate from the blood of the host. Further testing is needed to clarify this. The relatively high mortality observed for the fleas in the control group, is probably due to the grooming activities of the rats.

The reduction in the flea population observed in the flea toxicity tests indicates that with the present formulation of 0.6% dimethoate as the systemic insecticide, it is possible to reduce the number of fleas on rodents along with the use of a rodenticide. However, as seen from the palatability tests, the amount of consumed insecticide/rodenticide bait drops when alternative food is available. Whether this intake of insecticide and rodenticide will be high enough to obtain a successful control of the rodent as well as the flea population in the field has to be tested. In the future we plan to continue using bromadiolone as the rodenticide, because it is an efficient slow-acting rodenticide. Concerning the choice of insecticide, many new types of insecticides have been developed in the last few years and some of these may have a potential for increasing the bait palatability and/or the flea mortality.

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