# THE ECOTOXICOLOGY OF RODENTICIDE USE ON FARMS

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# ABSTRACT

**The Ecotoxicology of rodenticide use on farms**

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This study investigated anticoagulant rodenticide consumption by wild brown rats (*Rattus norvegicus*) on 18 farms in the UK. A first-generation anticoagulant rodenticide (coumatetralyl) was compared with a second-generation compound that is limited to use indoors (brodifacoum). Field trials were carried out on 9 farms in central southern England where physiological resistance to anticoagulant rodenticides is widespread, and on a further 9 farms in the east midlands where resistance was believed not to occur. The two anticoagulants were formulated to contain 98 $\mu g\ g^{-1}$ hexachlorobiphenyl (HCBP), which was used as a stable marker compound to allow the bait consumption levels by individual rats to be assessed. Rodent carcasses that resulted from the rodenticide treatments were collected from the farms and the guts, feet and tails were removed. The rodent bodies were then extracted to produce a sample of HCBP that could be analysed using gas chromatography with mass spectrometry (GCMS). On farms where the presence of physiological resistance prevented rodent control, Fenn trapping was used to obtain bodies for comparative HCBP analyses. Further tissue from carcasses and trapped rats was also extracted for residues of anticoagulant rodenticide using high performance liquid chromatography (HPLC). Infra-red video photography at two sites enabled detailed observation of population and individual behaviour of rats at feeding points.

The major findings of the study were as follows:

1. Rats ate significantly more coumatetralyl than brodifacoum, and rats in an area of physiological resistance (central southern England) ate greater quantities of rodenticide than rats in the east midlands. Coumatetralyl failed to achieve rat control on farms in central southern England.
2. Physiological resistance was suspected on two farms in the east midlands (near Lincoln) where rats ate excessive quantities of coumatetralyl and the control programme was unexpectedly extended.
3. GCMS analyses performed on extracts of 169 whole rodents revealed that some rats had eaten levels of coumatetralyl that far exceeded a lethal dose for susceptible animals. Excessive bait consumption occurred mostly in the area of physiological resistance, but also on the two sites in the east midlands where resistance was suspected. Brodifacoum consumption by some rats was also high, but complete control was usually achieved with brodifacoum and there was no evidence of any resistance to brodifacoum.
4. HPLC analyses carried out on 10 rats from coumatetralyl sites (five from each region), revealed that trapped, physiologically-resistant animals are capable of carrying 50 times the $LD_{50}$ of coumatetralyl without any obvious ill effect. Rats from the east midlands carried a significantly lower load of coumatetralyl.
5. Video observations gave no evidence to support a bait point monopoly theory. Interactions at feeding sites were common.
6. This study has revealed that the potential exposure of non-target predators and scavengers to rodenticides is considerably higher in areas where rats show physiological resistance. This fact is discussed with reference to the regulation and monitoring of pesticides in the UK.

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# CHAPTER 1 INTRODUCTION

## 1.1 THE BIOLOGY AND NORMAL BEHAVIOUR OF RATS AND MICE ON FARMS

The brown rat (*Rattus norvegicus,* Berk.), sometimes called the Norway rat, has been in the U.K for over 250 years and it was first classified by Berkenhout in 1769. Like the black rat (*Rattus rattus,* L.), it is widespread throughout most of the world. It lives in urban and rural areas and, in the U.K., is absent only from some islands and in exposed mountainous regions.

This species (from now on referred to simply as "the rat") continues growth throughout its normal life span of 1.5-2 years and has an adult weight range of 275-600g. Males are generally heavier than females and weight is an important factor for determining social dominance (see later). Rats are mainly nocturnal and use their sense of touch (via whiskers and tactile hairs in their fur) to orientate themselves. They use their tactile senses far more than *Rattus rattus* which orientates visually (Sokolov *et al.*, 1992). They move in close contact to cover (Hardy and Taylor, 1979) or vertical objects such as walls and fences (thigmotaxis) and along well-defined runways that are defined by olfactory cues (e.g. Galef and Buckley, 1996) and possibly maintained by urine marking (Mallick, 1992). Smell is also an important sense used for recognising relatives and the presence of predators (Whishaw and Dringenberg, 1991), for locating food (Stetter *et al.*, 1995) and for discovering females in oestrus (Natynczuk and Macdonald, 1994). Rats also have excellent hearing, which extends into the ultrasonic range, and their sight is specially adapted to allow good night vision.

Rats usually live near to food supplies, in burrows or within banks and hedgerows. On farmsteads, they will often nest or make dens in hay stacks or amongst bagged feed. They may also tunnel under the floor of sheds and barns or live within the roof space or among stored equipment. They are able to climb well. As they require a daily supply of water, they are also often found in the vicinity of slurry lagoons or dykes and they are good swimmers. Rats frequently move their home site (Taylor, 1978), on average once a week for males and once a fortnight for females.

Rats are omnivorous, but they favour protein-rich and starchy foods. In different environments they have been observed eating seabirds (Bertram and Nagorsen, 1995; Fitzgerald *et al.*, 1991) and a range of intertidal invertebrates and seaweed (Navarette and Castilla, 1993). On farmland, rats are often found in potato fields (Cooke *et al.* 1996) and in kale (Hardy and Taylor, 1979). They may consume invertebrates or small vertebrates as well as cereals and seeds (Hardy and Taylor, 1979). Females generally feed more frequently and take less at each bout than males (Inglis *et al.*, 1996), although the overall amount consumed is more or less equal for males and females. Shepherd and Inglis (1987), however, showed that feeding is very variable between individuals; a pregnant female, for instance, may eat far more than either a male or a juvenile. Rats can use their forelimbs and even digits to catch and manipulate prey (Ivanco *et al.*, 1996) and they may also be cannibalistic. They often hoard food or take it elsewhere to eat (Nakatsuyama and Fujita, 1995; Whishaw and Dringenberg, 1991; Whishaw and Whishaw, 1996) and this tendency increases with the size of the food items. Food is carried less where the journey involved is long or hazardous (with regard to both predators and other rats, which try to steal the carried food; Whishaw and Whishaw, 1996) and instead the rats eat more at the food source. Whishaw and Whishaw also noted that subordinate rats will often try to carry food in order to avoid aggressive encounters at bait points. The presence of a predatory odour halts food carriage completely (Whishaw and Dringenberg, 1991).

Rats are known to show wariness of new foods or objects and this behaviour is termed "neophobia". They are observed sampling new food sources (e.g. Berdoy and Macdonald, 1991) until, it is believed by researchers, they are sure that the food is not causing them any illness. Once accepted as a reliable resource, the rats will then take much larger quantities of food at each bout (Buckle *et al.*, 1986; Berdoy and Macdonald, 1991). Considerable neophobia to new food containers lasting several days if not weeks has been observed, but a new food within a familiar container is more readily accepted (Shepherd and Inglis, 1987; Brunton, 1995; Inglis *et al.*, 1996). Juveniles show the least neophobic behaviour (Nott, 1988; Shepherd and Inglis, 1987), but there is also considerable variation in neophobic response between different adults (Inglis *et al.*, 1996). Interestingly, Nott and Sibly (1993) noted that dominant rats excluded subordinates from a novel food during cage trials from as early as the first day of the novel food arriving.

Many studies have shown that inexperienced or juvenile rats will copy older or established rats with their choice of food source (Chou and Richerson, 1992; Galef and Whiskin, 1995a; Galef and Whiskin, 1995b; Heyes *et al.* 1994; Stetter *et al.*, 1995) especially where their own food intake is poor or protein deficient (Galef *et al.*, 1991). The learning process that allows a better quality (higher protein) food to be discovered and utilised appears to be quicker among grouped rats as opposed to isolated individuals (Galef and Wright, 1995) implying that social interaction is important for food selection. Galef *et al.* (1994) and Stetter *et al.* (1995) suggested that odours play an important role in the social interactions of food selection, but this odour-instigated copying is not observed with regard to taking cover or nest building.

Rats can breed throughout the year if they have sufficient food and the climate is mild. This is usually the case for farmstead rats (e.g. Bishop and Hartley, 1976). Populations living in fields may have two breeding peaks during the year, in spring and in autumn (e.g. Butler and Whelan, 1994; Cooke *et al.*, 1996). Females reach sexual maturity in 8-12 weeks after birth and gestation lasts for about 3 weeks. Litters contain up to 15 pups. There is therefore a great potential for fast population growth. Male sperm production may, however, be inhibited if the weather is very cold or if food is limited and females have the ability to reabsorb their foetuses into the womb. Conversely, if the population has been reduced through predation or control, rats have the ability to increase their reproduction rate. These facts imply that the populations are controlled by density-dependence. Butler and Whelan (1994) discovered that the population density of wild rats in County Kildare, Ireland was maintained by a complex social structure. The limiting resource was the number of reproducing females. The heaviest males and females were the only breeders and the sex ratio for the entire population was approximately 1:1. Kataranovski *et al.* (1991), studying rats in Belgrade, calculated that 71% of the population was adult, about 20% was subadult and the rest were juveniles. They too found the sex ratio to be 1:1, although among adult rats there were slightly more females than males. Bacon and McClintock (1994) found that different factors affect the sex ratio of new born litters. Where females had a large litter for instance, post-partum-conceived litters had a tendency to be female-biased. In contrast, the presence of suckling pups during gestation brought the sex ratio up to parity.

The female rears the pups without the male and is responsible for defending them from intruders. She may even move her young if a nest is disturbed. Colonies develop from a pair of rats or a single pregnant female and, where numbers are low, all the rats cohabit harmoniously. Unrelated intruders are repelled by the residents of a colony (usually the males) and may even be killed (Adams and Boice, 1983). As the population size increases, a population hierarchy develops with dominance almost entirely linked to size. Adams and Boice (1983) observed that there is always an "alpha" male that wins contests against other males and is active in patrolling the habitat and attacking intruders. Subordinate to him, but still active and more dominant than other rats, are the beta males. If the alpha male should disappear, the beta males will contest each other for dominance. The shyest "omega" males lose in contests and have inferior access to food. Omega males often become weak and diseased and may die. Butler and Whelan (1994) found that the largest animals fought most often and Lucion *et al.* (1996) noted that heavier rats had the most testosterone. Agonistic behaviour is usually between animals of the same size (Robitaille and Bovet, 1976; Berdoy *et al.*, 1995) and, in natural environments, the loser will escape unharmed (Robitaille and Bovet, 1976). Berdoy *et al.* (1995), however, discovered that dominance was more closely linked to age than weight. High-ranking individuals have precedence at feeding sites and the survival of their pups is therefore increased (Adams and Boice, 1983). Dominant males also have a monopoly of oestrous females (Robitaille and Bovet, 1976) although Berdoy *et al.* (1995) argue that the acceptance of subordinate status by some rats implies that such a monopoly of mates and food cannot occur.

Juvenile males were found to play-fight frequently, but any hierarchy was highly unstable (Adams and Boice, 1983). The subordinates, at this stage in development, are the smaller rats (Kahana *et al.*, 1997). Hole (1991) observed that play amongst juveniles did not represent practice for serious contests of dominance or monopoly of resources in later life. Smith *et al.* (1996) showed that castrated juveniles did not develop dominance relationships with maturation as intact rats do.

Females form a hierarchy once sexually mature and dominance is usually decided by posture such as "passing" rather than fighting (Adams and Boice, 1983; Ziporyn and McClintock, 1991). The alpha female is most active socially and in fighting off intruders and her male offspring usually become dominant themselves, probably because they are

born first and so are both larger and older than their peers. The beta females are also highly active (Adams and Boice, 1983).

Rats are active throughout the night, using this relatively safe time for feeding (Berdoy and Macdonald, 1991; Whishaw *et al.*, 1992; Shepherd and Inglis, 1987), copulating (Logan and Leavitt, 1992), play and other movements (Robitaille and Bovet, 1976; Hardy and Taylor, 1979). Peak activity occurs around sunset and continues for a few hours and then a smaller peak occurs before sunrise (Taylor, 1978; Nieder, 1985). Subordinate individuals may be confined to feeding at less safe times of the day (Taylor, 1978; Hardy and Taylor, 1979; Shepherd and Inglis, 1987; Berdoy and Macdonald, 1991). Likewise, if the population is large or if there is little disturbance during the day or high predation at night, rats may become active by day instead of or as well as by night and feed at regular intervals (Shepherd and Inglis, 1987; Shekarova *et al.*, 1995). Field-living rats will travel up to 1 km routinely to an abundant food supply (Taylor, 1978; Hardy and Taylor, 1979; Fenn *et al.*, 1987). They usually move along hedgerows rather than across open spaces (Taylor, 1978; Hardy and Taylor, 1979). Over these longer distances, Benhamou (1997) stated that rats are unlikely to use spatial memory as a route-finder. Instead, rats are likely to rely on olfactory cues (e.g. Galef and Buckley, 1996) to repeat their nightly excursions. On farmsteads, rats will not need to move far to access food and water supplies and so their home range is greatly reduced (Hardy and Taylor, 1979). Farm rats will have one or two safe places, sometimes termed home bases (Golani *et al.*, 1993) from which they will make forays and then return. Investigative behaviour seems to increase as rats reach maturity (Renner *et al.*, 1992) and males are more investigative than females or juveniles (Shepherd and Inglis, 1987).

In some places, rats will move to the farmstead in winter for shelter and food and return to the fields and hedgerows in the summer to feed on the growing crops. This seasonality is not found, however, if the farm has a constant food supply throughout the year. Thus on poultry and pig farms for example, where animals may be housed indoors all the time, there will often be rats making use of the reliable food supply. Likewise, if a farm is not maintained well so that feed and carcasses are left around without being cleared up, rats may take advantage and will live within the farm all year round. If the farm rats are killed, others that live in the surrounding fields are likely to immigrate (Hardy and Taylor, 1979; Taylor, 1978). The daily home range of field rats may well

include the farmstead and they are often only unable to live there permanently because of hostility by the resident population of farm rats.

The house mouse (*Mus musculus domesticus,* Rutty) is also a target for rodent control in the U.K. The house mouse has an adult weight of 12-22g with females being slightly heavier than males on average. House mice use the same sensory organs as rats in order to orientate themselves and communicate with each other but olfactory cues play a major role in their social structure (Meehan, 1984). The introduction of a male into a group will trigger synchronous oestrus among the females in the group, and a strange male scent can induce spontaneous abortion in what is termed the Bruce effect. A breeding pair and their adult offspring will use urine to coat everything within their environment and by doing so they mark out their family territory (Macdonald and Fenn, 1994). They live in small family groups and attack intruders. At moderate densities, one male may live with two or more females in a firmly held territory (Meehan, 1984; Hanney, 1975). If the population density increases, subordinate animals may be excluded and will not mate or hold territories. Feral mice have breeding peaks in May and June, but farmstead populations may breed all year round. They reach sexual maturity at about 40 days, have a gestation period of 19 days and have 5-10 litters of 5-7 young on average per year (Hanney, 1975). Their life span is about 18 months maximum in the wild. House mice are opportunistic colonisers that can tolerate a high level of inbreeding and can even breed at -10° Celsius. This adaptability means that the population can rapidly grow and house mice can reach pest status quickly if a food supply is abundant.

House mice inhabit a range of buildings but can also live among hedgerows and in fields, although they rarely leave cover. There is some degree of movement between the fields in summer and farmsteads during winter months. Like the rat, house mice are omnivorous but they show a preference for cereals and are often found in fodder stores (Cooke *et al.*, 1996). Unlike the rat, house mice can live without a daily supply of water. Mouse feeding appears to be very random with many feeding sites visited each night if supplies allow. Mice are not at all neophobic and even seem to show a preference for new foods. The average daily consumption of house mice is 3.5g and they forage during the night and are least active during daylight when they are usually asleep.

## 1.2 THE NEED FOR RODENT PEST CONTROL

The competition imposed by rats and mice on man and his resources has led them to be termed "commensal rodents", literally meaning that they are rodents that eat at the same table. Both species are considered to be pests because of the economic damage that they produce and the hygiene problems that result from their presence. Rodents in tropical and subtropical regions of the world cause considerable damage to a range of crops, in particular to rice, coconuts, sugar cane, cacao, cereals, groundnuts and oil palm (reviews by Meehan, 1984; Wood, 1994) and plagues of mice or rats in some countries have been known to destroy all vegetable material in their path (Hanney, 1975; Brown *et al.*, 1997; Mwanjabe and Morner, 1997). In the UK, as with other temperate regions, direct damage to standing crops is a far smaller problem. It is non-commensal rodents that cause the damage in most cases, *e.g.* injury to young trees by voles (Lund, 1984; Myllymaki, 1987; MacVicker and Trout, 1994) and wood mouse destruction of sugar beet crops (Pelz, 1987; Pelz, 1989). A more important issue on UK farms is the considerable structural damage that can occur to buildings and other property by gnawing and burrowing of commensal rodent species. Building collapse, flooding, electrical faults and even fire can be caused by rodent activities. Rats can ruin bagged silage if they expose it to the air and they consume and spill sacked animal feed. Grain heaps and fodder can be contaminated by urine, faeces and hairs and young chicks and eggs may be taken (Meyer, 1994).

Rats and mice are also held responsible for acting as reservoirs and vectors for dangerous diseases such as Leptospirosis, Salmonella, Rickettsia, Plague, Yersiniosis, Meningitis, Typhus, Toxoplasmosis, Emmonsia lung fungi, Listeria, Lyme disease, Foot and Mouth, Encephalitis and Herpes plus various endo and ectoparasitic diseases, and they are therefore considered to pose a risk to human health (Taylor *et al.*, 1991; Vlcek, 1991; Badi *et al.*, 1992; Matuschka *et al.*, 1997, Webster, 1994; Webster and Macdonald, 1995a and b; Webster *et al.*, 1995; Inoue *et al.*, 1992; reviews in Meehan, 1984 and Gratz, 1994). Other researchers have found that, in fact, the pathway between rat and human is insignificant compared to the route *via* other animals such as dogs and cats (Frenkel *et al.*, 1995; Davoust *et al.*, 1997; Koshimizu *et al.*, 1993) but the obvious possibility of infection still exists while unwanted rodents are present on a farm. More likely is the spread of disease and parasites to livestock and also pet animals, which may

cause both economic and emotional loss as well as a more definite risk of disease spreading to man *via* direct contact.

As a result of these concerns, mice and particularly rats are labelled as unwanted vermin, to be removed from the premises as soon as they are evident. There are several options for rodent control. Long-term, the cause of an infestation needs to be ascertained and means taken to prevent it recurring. The most cost-effective method is to change site management to separate possible harbourage from food storage areas and to clean-up waste that may have accumulated. Rodent-proofing of buildings can also be achieved although it can be expensive to guarantee complete exclusion. Introduction of predators, particularly cats, may provide additional protection (Smith, 1994).

Short term measures to remove the unwanted rodents may include trapping, shooting and use of ferrets or terriers. Chemical control is, however, the most rapid and effective method and can be achieved using fumigants or rodenticidal baits of which the anticoagulant is the most popular choice on UK farms (Olney *et al.*, 1991).

## 1.3 ANTICOAGULANT RODENTICIDES AND THEIR ACTION

The first rodent control with rodenticides involved the use of "acute" poisons. Substances such as thallium sulphate and zinc phosphide were being used to control rats in the UK from the 1920s and others, such as 1080 and 'Red Squill', became popular in the 1940s. These acute poisons were fast-acting, sometimes within 15 minutes (Meehan, 1984), and generally all resulted in death within 24 hours. Symptoms varied, but included heart failure, respiratory difficulties, paralysis and muscle seizure. There were a few drawbacks associated with the use of acute poisons. First, no specific antidotes existed, so the use of these poisons was often unsafe. Second, they were largely unpalatable (Meehan, 1984) and so sometimes the target rodents did no take a lethal dose. Third, conditioned bait-aversion became a common occurrence. Rats that took a sub-lethal dose of bait would suffer the effects of toxicosis but would recover. In such cases, rats form an association between the bait and the effects of toxicosis and develop "conditioned bait-aversion" (Naheed and Khan, 1989; Berdoy and Macdonald, 1991; Shepherd and Inglis, 1993; Boakes *et al.*, 1997). This can last a considerable time and means that effective control is prevented.

Anticoagulant rodenticides started to replace the formerly-used acute toxicants in the 1950s and nowadays acute poisons account for less than 1% of poisons used for rodent control on farms in the UK (Olney *et al.*, 1991). The first anticoagulant rodenticide was warfarin, which was developed initially as an agent to counteract thrombosis in humans. Warfarin seemed to provide an answer to all the problems associated with acute rodenticides; it had a specific antidote that could completely counteract the effects of accidental poisoning in humans and non-target animals, and no bait aversion was associated with its use.

Anticoagulant rodenticides act on the vitamin K cycle in the vertebrate liver (Figure 1.1). Anticoagulants are generally thought to inhibit competitively the enzymes vitamin K epoxide reductase and vitamin K reductase and so prevent the recycling of vitamin K and the formation of clotting factors (Buckle, 1994). Clotting factors are needed for the production of thrombin, which aids the conversion of the soluble fibrinogen (circulating in the blood plasma) to the insoluble fibrin and is necessary for the formation of blood clots. After a few days of consuming anticoagulant, the body's

clotting factors are depleted and the animal is liable to haemorrhage (Buckle, 1994). Haemorrhage is mostly internal rather than by external bleeding through wounds. The rodent will then die. In accidental poisoning cases, an excess of vitamin K can be administered to act as an antidote to the anticoagulant.

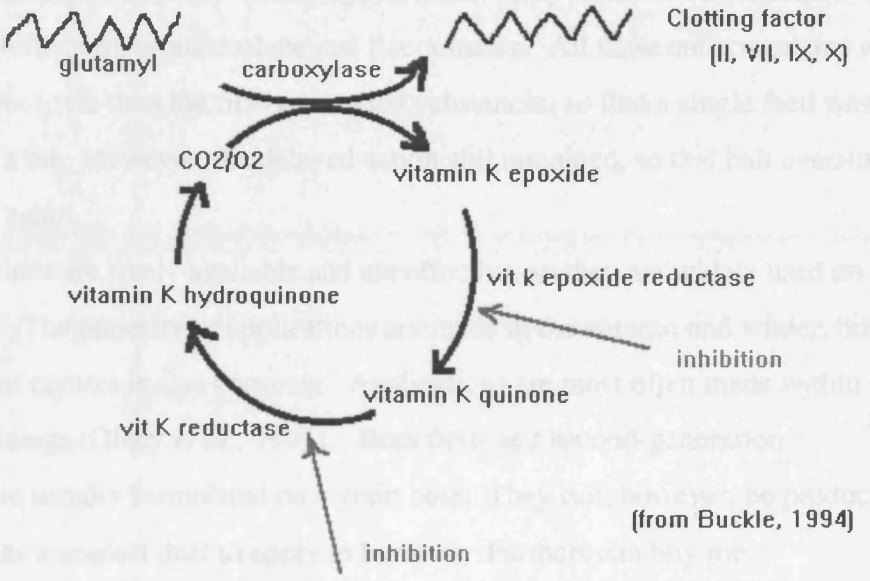



Figure 1.1 The site of action for anticoagulant rodenticides: the vitamin K cycle in the liver. The anticoagulant blocks the vitamin K cycle and thus prevents the γ-carboxylation of glutamyl residues to form the blood clotting factors II, VII, IX and X.

The delayed onset of haemorrhage, while clotting factors are naturally depleted, has been the key to success for the anticoagulants, because the rodent ingests a lethal dose before it feels any effect. Inevitably this means that control with anticoagulants tends to be far more effective than when acute poisons are used, because no bait aversion develops. The high efficacy of anticoagulants, along with the presence of an antidote, have made their use very popular with farmers. These early anticoagulants, including those produced up until the 1970s, are termed "first-generation compounds". They include the chemical group indane-diones, such as chlorophacinone and diphacinone, and

the hydroxycoumarin group, such as warfarin, coumachlor and coumatetralyl. These compounds are not toxic enough to cause death from a single feed and must be repeatedly ingested, over a number of days.

By the late 1950s, resistance to some of these first-generation anticoagulants had arisen. This led to a search for alternative compounds that could continue to produce effective rodent control. By the early 1970s, various related anticoagulant compounds had been discovered, which were effective over both susceptible and resistant rodents. They were collectively termed the "second-generation" compounds and included difenacoum, brodifacoum, bromadiolone and flocoumafen. All these anticoagulants were considerably more toxic than the first-generation substances, so that a single feed was sufficient to kill a rat. However, the delayed action still remained, so that bait aversion was avoided yet again.

Rodenticides are freely available and are effective, so they are widely used on farms in the UK. The majority of applications are made in the autumn and winter, but year-round rodent control is also common. Applications are most often made within and around farm buildings (Olney *et al.,* 1991). Both first- and second-generation anticoagulants are usually formulated on a grain base. They can, however, be produced as liquid bait or as a contact dust to apply to burrows. Farmers can buy the anticoagulants freely and apply them themselves. The exception is for the second-generation compounds brodifacoum and flocoumafen, which cannot be bought or applied by farmers. Instead, their use is limited to application by professional pest controllers and they must be applied indoors only.

The efficacy of the first-generation compounds relies on rodents having continuous access to the bait over a period of many days to weeks. "Saturation baiting" is therefore used, where bait is replenished frequently enough and in sufficient quantity to ensure that it never runs out throughout the whole control period (Dubock, 1984). Saturation baiting is not necessary with the second-generation compounds and may in fact lead to overdosing. Instead, "pulsed baiting" is recommended, where the poison is put out in smaller doses and replenished intermittently (Dubock, 1982; Dubock, 1984).

## 1.4 EFFICACY OF ANTICOAGULANT USE AGAINST TARGET SPECIES

Anticoagulants have been successfully used since their introduction in the 1940s to control a variety of rodent species across the world. The successful use of anticoagulants against brown rats has been reported for a variety of situations such as on islands (*e.g.*Taylor and Thomas, 1989; Moors, 1984), in crops (*e.g.* Kumar *et al.*, 1996; Smith and Nott, 1988; Tongtavee *et al.*, 1987) and in urban environments (*e.g.* Jackson, 1984). In the UK, the majority of rat eradication is from farmsteads where the rats are apparent in and around farm buildings (*e.g.* Smith *et al.*, 1993; Quy *et al.*, 1992a; Cox, 1991). House mouse control in the UK is usually a problem associated with the urban environment (Meehan, 1984), although control is also often required on farms (*e.g.* Rowe, 1987).

Laboratory studies have revealed that rats and mice will succumb to poisoning by the first-generation anticoagulants, such as warfarin and coumatetralyl, within 5-14 days (Buckle, 1994). The second-generation compounds, such as bromadiolone and brodifacoum are more toxic, but their action is the same and so it still takes a few days for a poisoned rodent to die. The difference is that a far smaller amount of bait needs to be consumed for death to result, which can occur after just a single feed (Buckle, 1994).

First-generation compounds must be fed on over a period of a few days to achieve a lethal dose. If a sub-lethal amount is ingested, the rodent may feel the effects of toxicosis without dying and this may lead to bait aversion. This is a common problem with acute rodenticides (Naheed and Khan, 1989; Shepherd and Inglis, 1993; Boakes *et. al*, 1997). The delayed action of anticoagulants should enable a lethal dose to be consumed before any ill effects set in and the rat stops feeding. Nevertheless, bait aversion has been noted with sub-lethal consumption of anticoagulants (Smith *et al.*, 1994a) with a change in behaviour as early as the first day of treatment. Brunton *et al.* (1993) even suggested that rats have an ability to detect and recognise poison (an ability also apparently found with acute poisons by Inglis and Shepherd, 1994) and this may lead to increased neophobia and hence bait aversion. There have been no other reports of bait aversion developing against anticoagulants, however, and it is generally believed that conditioned bait aversion does not occur, certainly to the extent noted with acute rodenticides.

Bait aversion may possibly occur in rats exposed to a new bait at the same time as conspecifics are dying. There is some evidence that this effect, termed the "Poisoned Partner Effect" is strongest of all within a family group (Berdoy and Macdonald, 1991). Social interactions play a large role in food choice among rats, with juvenile or subordinate rats being particularly prone to influence by other rats in the vicinity (*e.g.* Galef and Wright, 1995; Galef and Whiskin, 1995b) so an aversion of this nature could affect a group of rats at any one time.

Large, dominant rats have frequently been observed to monopolise bait points (Kenward, 1988; Nott and Sibly, 1993) and to guard them from other rats, sometimes aggressively (Taylor and Thomas, 1989) until they have had what they want. If too little bait or too few bait points are used, this monopoly may result in only the dominant rats being fully affected by the rodenticide. The subordinate rats will either not feed on the bait at all or may consume a sub-lethal amount. Bait-point exclusion may therefore lead to the control programme being unnecessarily extended or even to complete failure to eradicate a percentage of the rats present. The method of pulsed baiting (Dubock, 1982) was developed for use with second-generation compounds, partly to combat the problem of competitive exclusion. A small amount of bait is applied in pulses, perhaps once a week or once a fortnight. Whichever rats eat the bait die and this allows other rats in to feed by the time the next pulse is applied. Monopoly of bait points therefore cannot prevent effective control. Control failure has sometimes been attributed to poor application where either too few bait points or too little bait was used. Buckle *et al.* (1987) found that control was far more effective on a farm where twice the normal density of bait points was used.

Since resistance to anticoagulants first appeared in the late 1950s, certain areas of the UK have had increasing difficulty achieving satisfactory eradication of pest rodents. The problem of resistance has largely been combated by the use of more toxic second-generation anticoagulants (see the following section). Resistance is, however, not the only problem. The abundance of alternative food at some sites has rendered treatments ineffective (Quy *et al.*, 1992b; Brown *et al.*, 1997). Additionally, Brunton *et al.* (1993) found that rats that had regular access to bait points were not being poisoned. Such problems led MacNicoll and Gill (1993) to investigate whether menadione, a form of vitamin K found as a supplement in animal feed, could be acting as an antidote to

anticoagulant action. Their experiments on caged animals with second-generation compounds indicated that in fact there was no effect on survival through including menadione in the diet of rats and mice, unless they exhibited anticoagulant resistance already.

## 1.5 RODENTICIDE RESISTANCE

"Resistance" is the ability to withstand application of a chemical which, in normal concentrations, is designed to kill or inhibit that organism in some way. It is found among a wide range of organisms, *e.g.* bacteria resistant to penicillin, house flies resistant to pyrethroids and groundsel resistant to triazine (Begon *et al.*, 1990). In the rat, resistance is the term used to describe inherited physiological resistance, although other factors such as behavioural traits (Berdoy and Macdonald, 1991) and alternative foods (Quy *et al.*, 1992b) may produce an environment where some rat individuals are apparently resistant to control. In addition, some species of rodents have a naturally high tolerance to certain rodenticides. For example, species such as *Acomys cahirinus, Meriones shawi* and *Mesocricetus auratus* show a naturally high tolerance to various first and second generation anticoagulants (Gill, 1992). In this section however, "resistance" is used to discuss genetically inherited physiological resistance to anticoagulants in the Norway rat and the house mouse.

Resistance was first revealed in the central lowlands region of Scotland in 1958 when a farm population of brown rats failed to be controlled by diphacinone and warfarin (Boyle, 1960). Later cases were reported in the Welsh borders in 1960 and in house mice near Harrogate, Yorkshire. By the mid 1960s there was widespread resistance in these areas and in Kent, based around Maidstone. A Rentokil survey conducted in 1971 revealed that house mouse resistance was very widespread throughout Scotland, particularly in urban areas (Meehan, 1984). A similar survey showed that the spread of resistance among rats, however, was far slower and by the mid 1970s resistance still only occurred within discrete populations. Nowadays, resistance affects many parts of the south of England including Oxfordshire, Berkshire, Hampshire and Kent as well as parts of the West Midlands and Welsh borders and also the Scottish central lowlands. The incidence of phenotypic resistance to first-generation anticoagulants in areas in which it is established in the UK is commonly 25-85% (Greaves, 1994). The average rate of spread of warfarin-resistant populations in rural areas in Britain is reported to be 4.8-7.8 km per year, consistent with the mobility of rats, although in other areas the spread is negligible (Greaves, 1994).

Since the first case in the UK, resistance to first-generation anticoagulants has been reported in many other countries such as the USA, Canada, Denmark, the Netherlands, France and Germany and in Black rats (*Rattus rattus*) in India, Japan and Australia (Meehan, 1984; Lund, 1988; Greaves, 1994; Jackson and Ashton, 1995). Resistance has also been noted in non-commensal rodents such as *Bandicota bengalensis* in Sri Lanka and *Holochilus sciureus* in Guyana (Meehan, 1984).

Some resistance to second-generation anticoagulants has also now developed; difenacoum and bromadiolone resistance has been noted in the south of England (MacNicoll and Gill, 1987; Greaves, 1986; MacNicoll, 1986; Quy *et al.,* 1992a; Gill *et al.* 1994). Resistance to difenacoum and bromadiolone has also been reported in Denmark and Germany (Lund, 1984; Myllimaki, 1995; Pelz *et al.*, 1995). It has been noted that the populations involved were already highly resistant to first-generation compounds (Greaves *et. al,* 1982; Lund, 1984; MacNicoll and Gill, 1987; Pelz *et al.*, 1995). In addition, control failures blamed on physiological resistance have sometimes later been attributed to other practical control problems such as the presence of alternative foods or neophobia (Quy *et al.,* 1992b; Brunton *et al.,* 1993). In contrast, second-generation compounds still appear to be fully effective in Welsh warfarin-resistant populations (Quy *et al.,* 1992a). There have been no reports of resistance to brodifacoum at its full application strength, although a decreased sensitivity and low-level resistance to this compound has been noted (Gill and MacNicoll, 1991; Gill *et al.*, 1992). Quy *et al.* (1992a) found that both brodifacoum and flocoumafen achieved good control of difenacoum-resistant rats in Hampshire. There are still no cases of confirmed resistance to second-generation compounds in the USA (Jackson and Ashton, 1995).

Resistance to second-generation compounds in mice has also been noted in Canada, Denmark and the UK. There have been reports of mice surviving a period of 21 days feeding on bromadiolone and warfarin-resistant mice have shown some tolerance to brodifacoum (Lund, 1984; MacNicoll and Gill, 1987; Jackson and Ashton, 1995).

*Mechanisms & Genetics*: In the brown rat, warfarin resistance is the result of a mutation, $R_w$, on chromosome 1 involving a single gene that controls the enzymes involved in vitamin K metabolism in the liver (Greaves and Ayres, 1967; Greaves and Ayres, 1976). At least three different geographical strains of resistance have been identified in the UK

and slightly different mechanisms of resistance appear to operate in each (Gill *et al.*, 1992; Greaves, 1994; Thijssen, 1988; Thijssen, 1995). In the Welsh and Hampshire strains, the vitamin K-epoxide reductase has both a decreased activity and a decreased affinity for warfarin. The warfarin is therefore inefficient as an inhibitor of the vitamin K cycle. Welsh-type resistance is dominant and the penetrance (proportion of heterozygotes showing the phenotypic trait) is complete. In the Scottish strain, the vitamin K-epoxide reductase is reversibly inhibited by warfarin, whereas in susceptible animals, the inhibition is irreversible. Again, the major resistance allele is dominant, but penetrance is incomplete. In the Berkshire resistance strain proposed by Gill *et al.* (1992), warfarin-resistance is also a dominant trait. There is some evidence that there is also a decreased sensitivity of vitamin K-quinone reductase to warfarin inhibition in some strains (Thijssen, 1995) and the possibility of increased microsomal clearance of anticoagulant acting as a subsidiary mechanism (Greaves, 1994). MacNicoll (1988) has proposed a 3rd mechanism for resistance based on a resistant rat's ability to synthesise menaquinone from menadione (artificial vitamin K found in animal feedstuffs) in the presence of anticoagulant.

Greaves (1994) reported that the gene for resistance may be a closely linked group of loci, which is influenced by modifying genes (Greaves and Ayres, 1976). It is also likely that different alleles are responsible for the different mechanisms of resistance seen. Indications that more than one locus may be involved lie in the occurrence of variation among resistant animals. For example, in rats from Hampshire, warfarin-resistance is dominant, but difenacoum-resistance is recessive or incompletely recessive and there have been reports of both sensitivity and insensitivity to vitamin K (Greaves and Cullen-Ayres, 1988; Gill *et al.*, 1992). In the Berkshire strain, resistance to difenacoum appears to be recessive in males but dominant in females, implying that either the resistance gene itself or one of its modifiers is sex-linked (Gill *et al.*, 1992). Sex-linked modifiers are also apparent in the mechanism for resistance in German rats (Pelz *et al.*, 1995).

In mice, the gene for resistance, War, is a mutation on chromosome 7 in an analogous position to the gene for resistance in rats (Wallace and MacSwinney, 1976). MacSwinney and Wallace (1978) reported that the resistance gene in mice is strongly affected by modifiers and its penetrance is dependent also on age and sex. Some tests on warfarin-resistant mice have implied a physiological mechanism exists that is similar to the Welsh-type strain; decreased sensitivity to warfarin by the vitamin K-epoxide reductase

enzymes in the liver (Greaves, 1994; MacNicoll, 1995). In other populations, however, this mechanism does not seem to operate (MacNicoll, 1995). Instead, MacNicoll (1995) proposed that the mice in certain strains may achieve resistance by having the ability to metabolise and detoxify warfarin in the blood before the anticoagulant reaches the liver.

*Pleiotropic effects*: With warfarin resistance being a dominant trait, the artificial selection imposed by anticoagulant use places resistant animals at an advantage. One would expect, therefore, that the occurrence of resistance in a population would increase. Studies of Welsh resistance, however, have revealed that the very mechanism that makes the vitamin K-epoxide reductase enzyme less sensitive to warfarin also gives it a lower affinity for vitamin K analogues. This means that the animal has difficulty in converting vitamin K epoxide into vitamin K, rendering the rat vitamin K-deficient. It therefore relies on dietary vitamin K to overcome the deficiency, but dietary sources are usually unable to fulfil the greater vitamin K requirement. As a result, coagulation is affected and the animal is liable to haemorrhage. In the heterozygote form, the animal contains both normal type and resistant type enzymes so the deficiency is not so great. As a result, when anticoagulant is not applied to a population containing resistant animals, there is a strong selective pressure against homozygous resistant animals and a weaker one against the heterozygotes. Partridge (1979) studied a barn population of rats in Wales that were not exposed to anticoagulant over an 18 month period. The incidence of resistance went down from 80% to 33% during that time and the fitness estimates for the three genotypes were calculated as 0.46 (homozygous resistant), 0.77 (heterozygote) and 1.00. Greaves *et al.* (1977) also showed that, in the absence of poison, the incidence of resistance in two populations was reduced from 57% to 39% and Bishop *et al.* (1977) noted a similar decrease in a barn population in Wales. Hampshire resistance, which is thought to have a similar mechanism for resistance as the Welsh type, also suffers from vitamin K deficiency (Greaves and Cullen-Ayres, 1988). An additional cost of this type of resistance may be the reduced growth rate caused by a reduction of biosynthesis of vitamin K-dependent calcium-binding enzymes in resistant animals. Smith *et al.* (1991) showed that growth rate in laboratory strains of Scottish- and Welsh-resistant rats was impaired. This effect, if it exists in the wild, has obvious implications for social status of the rats involved if size

is a major factor in determining dominance. Berkshire resistance is apparently not associated with vitamin K-deficiency (Gill *et al.,* 1992).

There may be no disadvantage to resistance in the absence of poisons if sufficient vitamin K can be taken from the diet. Animal feedstuffs are often reinforced with supplements such as menadione, which is an artificial form of vitamin K. Gill and MacNicoll (1993) investigated whether menadione could counteract the adverse effects of vitamin K deficiency in resistant animals. They found that, in caged rats, there was a slight benefit from feeding on menadione in conjunction with any one of the four second generation anticoagulants. This effect was only apparent in resistant rats, however, not in either susceptible rats or in mice. This result would be consistent with the idea that resistant rats suffer from vitamin K-deficiency. Greaves and Cullen-Ayres (1988) showed that heterozygotes of the Welsh strain of resistant rats require twice as much vitamin K as susceptible rats, whereas homozygous resistants need 20 times as much vitamin K to survive.

Where anticoagulant use is fairly constant, natural selection will be opposed by artificial selection. Heterozygotes, which contain both normal and resistant forms of the vitamin K cycling enzymes, will thus be placed at an advantage and so heterozygote advantage should maintain a balanced polymorphism (Bishop *et al.,* 1977). Fitness estimates of the Welsh strain in the presence of anticoagulants were estimated as being 0.37 (resistant homozygote), 1.00 (heterozygote) and 0.68 (Greaves *et al.*, 1977). Indeed, Greaves *et al.* (1977) found that in rat populations regularly exposed to anticoagulant, the incidence of resistance did not rise but remained relatively stable at about 44%. Greaves and co-authors also discovered that there were more heterozygotes in the population than would be expected from the Hardy Weinberg equation for random mating. It was later postulated that either immigration from nearby populations was maintaining the equilibrium of fitness or that there was indeed a self-maintaining heterozygous advantage (Berdoy and Smith, 1993).

In resistance strains other than the Welsh and Hampshire ones, there are no consistent reports of vitamin K deficiency. There may well therefore be other selective pressures steering the relative fitnesses of the three genotypes in the absence of poison. Apparent heterozygotes of a difenacoum-resistant population in Berkshire/Oxfordshire were found to be heavier and more numerous than expected and than either homozygote

(Smith *et al.*, 1993). These apparent heterozygotes were later shown to be socially dominant in the population. (Note: the term "apparent heterozygotes" is used because the blood-clotting test used to genotype the rats was developed in a different context). As social status affects feeding and mating choice, one would therefore expect that these heterozygous individuals would incur a survival advantage for themselves and even their offspring. What exactly it is that maintains the polymorphism in this population is not understood. The genetic complexity of resistance and the difficulty in accurately sampling genotypes from field populations have made it even more hard to assess what factors appear to drive both the physiological mechanism of resistance and its resulting fitness.

*Testing for resistance*: Testing for resistance in the UK is not a particularly regimented process, but instead relies on reports of suspected incidences or on evidence from feeding studies. Since 1982, a test recommended by the World Health Organisation has been used for comparing suspect resistant animals to data on lethal feeding periods for any given rodenticide in susceptible populations. Myllimaki (1995) noted, however, that this test is now dated and is no longer particularly relevant to the various types of resistance that have been discovered. Instead, a two-tier blood-clotting test, which was developed by Martin *et al.* (1979) to test for Welsh resistance, is often used. This in itself may pose some problems. Rats that are not of the Welsh-resistance strain may react slightly differently to this test and so the interpretation of results must sometimes be taken as indicative rather than as absolute (*e.g.* Smith *et al.*, 1993). The first part of the test is used to determine phenotype. *i.e.* it is simply a test for the presence of resistance. A group of rats are removed to the laboratory and injected with a simultaneous dose of 5.0 mg $Kg^{-1}$ of warfarin sodium and 1.0 mg $Kg^{-1}$ of vitamin K epoxide. After 24 hours, clotting activity is tested. In susceptible animals, the blood-clotting time is elongated whereas in resistant individuals, blood-clotting time is around the normal level.

The second stage is a genotype test to see if the resistant rat carries the heterozygous or homozygous genotype. Each animal is dosed with 0.36 mg $Kg^{-1}$ of vitamin K then placed on a vitamin K-deficient diet for 4 days. At the end of this period, clotting activity is measured. Homozygotes, having a greater insensitivity to vitamin K, show a greatly elongated clotting time in comparison to heterozygotes. Thus these two

tests separate out three classes of rat, which are taken as an indication of the three different genotypes.

For assessing resistance to second-generation compounds, this test cannot be used because large overlaps between the different genotypes are found and so they cannot be accurately distinguished. Instead, Gill *et al.* (1993) developed a blood-clotting response test to discriminate between difenacoum-resistant and susceptible rats. The method involves testing blood-clotting activity after administering difenacoum and vitamin $K_3$ simultaneously. A test for bromadiolone resistance, following the same type of method, has also been developed (Gill *et al.*, 1994).

*Eliminating resistance and management*: Greaves (1995) highlighted the lack of structured resistance management in the UK. Where problems with rodent control exist, it is usually left to the farmer to decide what else to try. Pest control companies will have more options available to them, including the use of flocoumafen and brodifacoum, to which there is no full strength resistance. Country-wide, resistance is not seen as a major problem and it is believed that there is always an effective control method available. In Denmark, there is a systematic approach to resistance and testing has been carried out since the 1970s. Warfarin was banned from Jutland as early as 1972 and every year a number of rats in each region is tested. Depending on the degree of resistance, a poison is recommended for use within each municipality. Where resistance to one poison exists, the next poison up the scale, from coumatetralyl through bromadiolone, difenacoum, brodifacoum and flocoumafen to difethialone is chosen. In this way, the Danish government is attempting to tackle the issue of resistance on a nation-wide scale.

Good resistance management, like Integrated Pest Management, is based on the combination of efforts in different areas of the problem. Greaves (1995) suggests that use of anticoagulants to which there is resistance should be halted. This recommendation has, however, been made for the last decade or more without much nation-wide heed being taken (Greaves, 1986; Smith and Greaves, 1987). Greaves (1995) also suggests that animal feedstuffs containing vitamin $K_3$ should not be used for poison bases and, in fact, should not be used at all, especially in areas of bad rodenticide resistance. Lowering the amount of vitamin $K_3$ to 2 mg $Kg^{-1}$ would be sufficient to act as an effective supplement to livestock but would take away the antidotal qualities for resistant rats (MacNicoll and

Gill, 1993). Additional difficulties with making anti-resistance management recommendations result from the fact that, as new discoveries of resistance strains, their mechanisms and their fitness costs arise, different action plans are required. For instance, Smith and Greaves (1987) recommended periodic reduction in the use of anticoagulants in order to decrease the incidence of resistance in a population. They based their recommendation on the fact that Welsh strain resistant rats are less able to survive in an anticoagulant-free environment. As discoveries were made that revealed that Hampshire rats appear to maintain an heterozygous advantage in the absence of poison, it became clear that the recommendation of Smith and Greaves would do nothing to halt the spread of Hampshire-type resistance (Smith *et al.*, 1993). What is clear is that monitoring should continue in a regular way. At present, suspected incidences of resistance are reported to the Ministry of Agriculture, Fisheries and Food and a resulting test of blood clotting activity may then be carried out. It is only by constant monitoring that the nation-wide resistance pattern will truly be recognised and, where necessary, combated. Possible means to eradicate resistance include the use of acute rodenticides or traps or the use of flocoumafen and brodifacoum. Use of more powerful anticoagulants is not possible in many cases however, because these two rodenticides are only licensed to be used indoors by official pest control operators. This option is too costly for many farmers and so they continue to use ineffective poisons. Not only is this in fact costly for them, but it may be environmentally damaging and it only aids the spread of resistance in the area.

## 1.6 OTHER SPECIES ON AND AROUND FARMSTEADS

Alongside the rats and house mice that may exist on farmsteads, other small rodents often found include the wood mouse (*Apodemus sylvaticus*), the field vole (*Microtus agrestis*) and the bank vole (*Clethrionomys glareolus*). Harvest mice (*Micromys minutus*) and water voles (*Arvicola terrestris*) may be found occasionally and the yellow-necked mouse (*Apodemus flavicollis*) is common in the south of Britain but is rarely found further north in the midlands. None of these are considered as pests to the farmer, as they do not usually cause any quantifiable damage (except for vole damage to young trees and isolated cases of wood mouse damage to sugarbeet) and man, rightly or not, does not associate these species with disease in the same way as he does with rats and house mice. Common and pygmy shrews (*Sorex* spp.) are found on farms frequently, but being insectivores they too have no adverse effect on the farmer. Cox (1991) found that common shrews, voles (bank and field) and wood mice were common throughout farmsteads in Berkshire and Townsend *et al.* (1995) noted that wood mice were by far the most common species found on and near to two farmsteads in Sussex and Hampshire with the yellow-necked mouse and bank vole also being common.

In contrast to house mice, which are able to breed all year round (Hanney, 1975), wood mice, voles and shrews breed from late spring to autumn and their populations reach a peak in numbers in the autumn (Churchfield, 1990; Gurnell, 1978; Flowerdew and Gardner, 1978). Shrews are carnivorous, foraging largely underground and eating invertebrates such as earthworms. They are not likely to eat granular bait, although Colvin (1984) found residues of bait in shrews in the USA. Cox (1991) also noted that shrews are likely to walk across bait trays and so may ingest the rodenticide when they clean themselves. In grassland, common shrews can reach densities of 98 per hectare during summer, making them a common prey item. Their natural body odours deter many mammalian predators from eating them (Churchfield, 1990; Erlinge, 1975), but weasels (*Mustela nivalis*), stoats (*Mustela erminea*) and foxes (*Vulpes vulpes*) will predate shrews to a small extent, especially if other food is scarce. Common shrews make up 6-13% of the diet of barn owls, *Tyto alba* (Churchfield, 1990; Morris, 1979) and a smaller proportion of the tawny owl, *Strix aluco* diet (Plesnik and Dusik, 1994b; Southern, 1954). Pygmy shrews are also eaten by owls in small numbers. Other avian predators of shrew

species include kestrels, *Falco tinnunculus* (Churchfield, 1990) and various corvids, *Corvus* spp. (Wilmore, 1977).

Voles inhabit long grass (field vole) or hedgerows and woodland (bank vole) and eat seeds, grain and other vegetation (Flowerdew and Gardner, 1978). Field voles are reported to be the favourite prey of weasels (Eadsforth *et al.*, 1996; Erlinge, 1975; Day, 1968; Tapper, 1979), foxes (Macdonald, 1987), kestrels (Kostrzewa and Kostrzewa, 1994; Plesnik and Dusik, 1994a), tawny owls (Petty, 1994; Plesnik and Dusik, 1994b) and barn owls (Colvin, 1984; Hegdal and Blaskiewicz, 1984). Barn owls and short-eared owls (*Asio flammeus*) will time their breeding season to coincide with an increase in the population abundance of field voles (Southern, 1954). Bank voles are a favoured prey item of tawny owls (Southern and Lowe, 1968).

Wood mice and yellow-necked mice live in areas of scrub and in wood piles and walls around farmsteads. Further out, they will live in the hedgerows and woodland. They are largely seed and grain eaters but may also take small invertebrates (Flowerdew and Gardner, 1978). Colvin (1984) found that white-footed mice (*Peromyscus leucopus*) in the USA had consumed bait placed for rats around farm buildings despite often living within hedgerows some distance away. The provision of supplementary food has also been known to attract wood mice into an area (Flowerdew, 1972; Flowerdew and Gardner, 1978). Wood mice are a major prey item of barn owls (Eadsforth *et al.*, 1996), tawny owls (Southern, 1954; Southern and Lowe, 1968) and of weasels (Day, 1968; Erlinge, 1975).

There are also grain-eating birds that feed around farmsteads and may be attracted to granular bait. These include various passerines, doves and pigeons (*Columba* spp.) and game birds (Edwards *et al.*, 1988) and corvids (Wilmore, 1977). These birds are also prone to predation by barn owls (Eadsforth *et al.*, 1996; Mead, 1987; Colvin, 1984) and weasels (Tapper, 1979) and stoats (Sleeman, 1989) as well as opportunistic scavengers such as badgers, *Meles meles* and foxes (Neal, 1986; Macdonald, 1987).

Mammal and bird species that may feed on granular bait are the ones most likely to be directly affected by rodenticides. Predators and scavengers that feed upon both the target rodents and grain-eating, non-target animals may also be affected. Mammalian predators of target rodents include the weasel (Sleeman, 1989) and stoat (Taylor, 1978; Day, 1968; Sleeman, 1989), especially when field vole numbers are low. Polecats

(*Mustela putorius*) are known to hunt around buildings, especially in winter (Shore *et al.*, 1996) and they have been observed routinely predating rats (Lode, 1994; BBC, 1997). Martens will eat both live rats and carrion (Sleeman, 1989) as may hedgehogs, *Erinaceus europaeus* (Morris, 1983), foxes (Macdonald *et al.*, 1994; Taylor, 1978; Macdonald, 1987) and badgers (Neal, 1986; Kruuk and Parish, 1977). Cats are said to be the main predator of farm rats, although the level of predation is hard to quantify. Cats often kill rats but will rarely eat them (Taylor, 1978; pers. obs., 1997). Instead they leave the carcasses, which are then available for scavengers such as other rats, corvids, foxes, dogs and badgers. Rats are known to be the main food of feral cats, *Felis catus* in some places (Fitzgerald *et al.*, 1991).

Barn owls are known to prey upon sub-adult rats (Eadsforth *et al.*, 1996; Ille, 1991; Harrison *et al.*, 1990; Hegdal and Blaskiewicz, 1984; Glue, 1974; Colvin, 1984) and house mice (Cooke *et al.*, 1996; Eadsforth *et al.*, 1996; Hegdal and Blaskiewicz, 1984; Glue, 1974; Colvin, 1984), especially when other more favoured prey items are low (Colvin, 1984). Barn owls will also take weasels and small stoats (Mead, 1987, Sleeman, 1989), so the possibility of tertiary poisoning exists. Other avian predators of rats and mice include tawny owls (Southern, 1954) and long-eared owls, *Asio otus* (Mead, 1987), herons (*Ardea cinerea*) and kestrels (Hammond and Pearson, 1983) and birds such as corvids, magpies (*Pica pica*), gulls (*Larus* spp.), buzzards (*Buteo buteo*) and kites (*Milvus* spp.) will take both live rats and carrion (Hammond and Pearson, 1983; Wilmore, 1977).

Less common visitors to a farmstead that may consume granular bait include squirrels (*Sciurus* spp.), rabbits (*Oryctolagus cuniculus*) and deer (Cervidae). Although these species are susceptible to rodenticide poisoning (*e.g.* Kenward, 1988), they are far more likely to forage in the fields and woodland around the farm rather than among the farm buildings where the bait is usually laid (Olney *et al.*, 1991).

## 1.7 POSSIBLE RISKS TO NON-TARGET SPECIES WHEN ANTICOAGULANTS ARE USED

There have been many reports of adverse effects of pesticides on wildlife species (*e.g.* Johnson *et al.*, 1991). Greig-Smith (1988) reviewed the incidents of wildlife poisoning due to treated seed and granules in England between 1980-1987 and found 100-125 incidents involving vertebrates each year. Anticoagulants affect any animal with a vitamin K cycle, and use of anticoagulant rodenticides could produce non-target casualties. Indeed, there have been reports of accidental poisoning of wildlife (*e.g.* Hegdal *et al.*, 1984; Tongtavee *et al.*, 1987; Cox, 1991; Shore *et al.*, 1996). In a five year period from 1982-1986, there were 18 reported cases of accidental poisoning by rodenticides in the UK (Brown *et al.*, 1988). Others have placed blame on anticoagulants for the continuing fragility of animal populations such as barn owls (Mead, 1987), though this view is not universally accepted. Many cases of accidental mortality in small birds and mammals may go unnoticed or unreported (Kjolholt, 1990) whereas incidents involving large or rare species are far more likely to be noticed. Shore *et al.* (1996) noted rodenticide residues of second-generation anticoagulants in the liver or stomach walls of 31% of polecats found dead. All animals had been run over, found dead or accidentally trapped. Newton *et al.* (1990) analysed the livers of barn owls that had been found dead. Residues were detected in 15/145 birds. Hegdal *et al.* (1984) found six out of 38 radio-collared Eastern screech owls (*Otus asio*) and a long-eared owl (*Asio otus*) dead after brodifacoum was used to control orchard voles. In other cases, the possibility of secondary poisoning has been reported. Kenward (1988) noted that poisoned squirrels were picked up by foxes, rats, buzzards and a sparrowhawk.

A number of studies have been undertaken to investigate the possibility of non-target poisoning, particularly for protected or high profile species such as barn owls. Many of these have shown that non-target wildlife species are *not* placed at significant risk from rodenticide treatments. For example, Eadsforth *et al.*(1996) found that only 3% of pellets they analysed from barn owls living within one mile of rodenticide treatments contained a detectable level of rodenticide residue, and Colvin (1984) found that a very low percentage of prey found in barn owl pellets was marked with a bait residue. Hegdal and Blaskiewicz (1984) and Hegdal *et al.* (1984) stated that, from their

studies, brodifacoum poisoning could not be implicated in barn owl mortality; traces of brodifacoum were found in one electrocuted owl, but young were successfully fledged from sites where bait was used and breeding pairs were evident post-treatment. Similarly, Townsend *et al.* (1981) noted that tawny owls were unlikely to obtain a lethal dose from consuming warfarin-contaminated mice in woodland where grey squirrel poisoning had been carried out. Cox (1991) showed that voles, though common throughout baiting areas on farmsteads, were found not to be poisoned during outdoor baiting trials with bromadiolone.

Many other studies have revealed a more definite hazard to wildlife. When considering the fate of pesticides in the environment and the hazard to non-target species, the likely risk to any species is a function of both the *toxicity* of the pesticide to that species and the *exposure* of the species to the pesticide (Urban, 1990; Kjolholt, 1990; Cox and Smith, 1990). Thus the risk of accidental primary poisoning (*i.e.* non-target animals eating the anticoagulant directly) depends not only on the tolerance of the animal to the poison but also depends on the bait formulation and positioning and the foraging behaviour of the feeder as well as its interactions with the target rodents. Secondary or tertiary poisoning is again dependent not only on the tolerance to the poison, the residue of poison in the prey and the proportion of poisoned prey in the diet, but also upon the behaviour of poisoned animals, and the habitat use and prey preference of the predatory or scavenging animals (Kaukeinen, 1993).

*Toxicity*: For rodenticides, the only possible route of exposure to a non-target animal is via the oral pathway; either by consuming the bait directly or by preying upon other animals contaminated by the bait. Feeding-toxicity studies in the laboratory have shown that a number of the non-target species found around farmsteads (mentioned in the previous section) are affected adversely by anticoagulant rodenticides. Shrews are very susceptible to poisoning on low doses of warfarin and they have a 28 times lower tolerance than wood mice (Churchfield, 1990). Mendenhall and Pank (1980) fed barn owls with rats dosed with bromadiolone, brodifacoum and difenacoum. Sub-lethal haemorrhaging occurred with consumption of difenacoum-dosed rats, and death occurred after feeding on bromadiolone and brodifacoum-dosed rats. Newton *et al.* (1990) also performed laboratory toxicity trials on barn owls and found that, when fed poisoned mice

for one day (0.002% brodifacoum), four out of six owls died between six and 17 days later. The two survivors also withstood three days and six days of feeding on poisoned mice, but they haemorrhaged for 30 days afterwards. Barn owls appear to regurgitate much (25-44%) of the anticoagulant consumed within poisoned prey items (Harrison *et al.*, 1990; Newton *et al.*, 1990; Gray *et al.*, 1994a; Newton *et al.*, 1994). This possibly enables owls to withstand a certain amount of poisoned-prey consumption without dying. Indeed, Gray *et al.* (1994b) showed that barn owls survived a 15 day period of eating an equivalent of two 25g mice containing 1 mg $Kg^{-1}$ of brodifacoum, difenacoum or flocoumafen per day and Newton *et al.*, (1994) found four out of five barn owls fed a cumulative dose of 0.78-1.25 mg $Kg^{-1}$ of flocoumafen in dosed mice over six days survived.

Godfrey (1984) reported lethal dose values for brodifacoum poisoning in target animals in New Zealand (including the rabbit, possum, wallaby and hare) and also for dogs and sheep. Kaukeinen (1982) reported the values for comparative lethal doses of warfarin, brodifacoum and acute rodenticides 1080 and zinc phosphide in rabbits, pigs, dogs, cats and chickens. Krambias and Hoppe (1986) reported toxicity studies of bromadiolone and difenacoum on partridges.

*Exposure:bait formulation and application.* Positioning of bait is an important consideration if non-target hazards are to be minimised. Cox (1991) found that outdoor baiting was detrimental to wood mice whereas indoor baiting was not. This is presumably because few wood mice venture into farm buildings (Arnold, 1993). Predatory species, such as foxes, polecats, barn owls and cats, do hunt within buildings (Macdonald, 1987; Mead, 1987; Shore *et al.*, 1996), so they are placed at risk if this behaviour exposes them to poisoned target rodents. Shore *et al.* (1996) found that residues of the anticoagulants brodifacoum and flocoumafen (that are legally used indoors only) were present in far fewer polecat carcasses than difenacoum and bromadiolone, which can be used outdoors (3% compared to 38%). Krambias and Hoppe (1986) found that, despite a relatively high toxicity of anticoagulants to partridges, the risk of accidental poisoning in their studies was low because the bait was placed out of reach of the partridges. Olney *et al.* (1991) reported that most rodenticide in the UK is applied inside buildings. This may therefore reduce the risk to non-target feeders. The same report also noted, however, that more

than half the weight of brodifacoum that was applied in 1989 (40% of occurrences) was outside. This action not only is illegal, but poses a greater risk of accidental poisoning to non-target feeders than if the bait is used indoors as it should be.

Bait formulation is also likely to have an effect on exposure levels to non-target animals. Field voles were found to accumulate a far greater dose of brodifacoum rodenticide when application concentrations were higher (Merson *et al.,* 1984) and the link with secondary poisoning of screech owls was implicated within the same study. Edwards *et al.* (1988) found that passerines are less likely to eat anticoagulants formulated in wax blocks than as loose grain, whereas Cox (1991) found that the opposite was true for wood mice; they were far more likely to take wax blocks away and cache them. Wood mice are a prey item for many predators in the UK, so the use of wax blocks may be detrimental to predatory species too. Taylor and Thomas (1989) noted rats invariably removing wax blocks to eat elsewhere, but exposed residues of bait were very rare as a result.

Pulsed baiting is argued to pose less of a risk to non-targets (Dubock, 1984; Cox and Smith, 1990; Cox, 1991) because the poison is available for much shorter intervals and there is less likelihood of excessive bait consumption by a few dominant rats. Harrison *et al.* (1990) noted that residue levels in rodent carcasses where pulsed baiting was used were less than half the level where saturation baiting had been employed. With pulsed baiting, however, more bait points are used and this may in fact lead to higher residues in the rats (Buckle *et al.*,1986). Fenn *et al.* (1987) controlled a farm population by placing bait at one place only and argued that this limited the exposure to non-target animals.

The timing of bait application is also important. Most rodent problems are treated during the winter (Olney *et al.*, 1991) but many farms use bait year-round. Cox (1991) found that wood mice were low in number in the late winter and early spring and so outdoor baiting was far more detrimental on wood mouse survival during this time than during the summer and autumn. Gurnell (1978) measured peaks in wood mouse populations in the winter and noted low numbers during the summer. Gurnell argued that the provision of food (or bait) during the summer would therefore attract wood mice. Colvin (1984) stated that most barn owl foraging is done in spring when the nestlings need feeding, yet most rat poisoning occurs in winter, therefore there is a low risk of

poisoning the owls at the nest. The winter is also when vole numbers are at a low, however, (Arnold, 1993), so predators that usually eat voles may need to switch to rats and house mice. Weasels exhibit this change if voles are scarce (Erlinge, 1975) and barn owls are also known to consume a higher proportion of rats in winter (Colvin, 1984). Thus, just when the use of rodenticide is at a maximum, the predation of rats increases. This means that the potential risk of secondary poisoning is worsened.

*Foraging behaviour of feeders/habitat use/prey preferences*: Cox (1991) noted that shrews are inquisitive and may therefore walk across bait trays and become accidentally poisoned when their body becomes contaminated. Shrews may also eat carcasses, showing a preference for the liver, the site at which anticoagulants accumulate (Churchfield, 1990). Neal (1987) noted that badgers are very opportunistic feeders and often consume unhealthy prey because they are easier to catch. Badgers will also eat carrion, so the likelihood of them picking up poisoned rodents may be high. Foxes also eat carrion and they will cache excess food (Macdonald, 1987). It is possible that foxes may therefore be adversely affected by an intensive poisoning programme which produces many carcasses. Other predators have more specific tastes. For example, Erlinge (1975) noted that female weasels hunt underground in vole tunnels, whereas male weasels hunt above ground. The position of a poisoned carcass may thus affect which sex of weasel is likely to be poisoned. Many predators will only take rats or house mice if their favoured prey, such as field voles, is scarce (Colvin, 1984; Sleeman, 1989). This means that in a low vole year, the chance of secondary poisoning will be greater because the rodents that are targeted in control programmes become the prey. Even then, barn owls are known only to select prey up to about 80g in weight (Ille, 1991; Colvin, 1984; Morris, 1979) so this excludes all adult rats. Additionally, Colvin (1984) argued that the smaller rats that barn owls select might not contain poison if larger, dominant rats prevent the smaller ones from feeding (Dubock, 1982). Bishop and Hartley (1976) calculated that rats of less than 100g accounted for only 12% of poisoned individuals from a farm rat population. In contrast, Tawny owls are bigger than barn owls and can therefore take larger prey (Morris, 1979). The proportion of a poisoned rat population 'available' to tawny owls is thus greater. Although tawny owls usually prey on wood mice, they are known to take rats (Southern & Lowe, 1968). They are also "sit and wait" predators. This behaviour

could make them more exposed to rodents that exhibit the effects of poisoning, such as staggering in the open (Cox and Smith, 1992). Mead (1987) stated, however, that tawny owls are a woodland species primarily so they should be relatively unaffected by farm-rodent control. Both barn owls and long-eared owls forage over grassland (Colvin, 1984, Mead, 1987) and therefore should not encounter many poisoned rodents either. Similarly, Hegdal and Colvin (1988) noted that barred owls were unaffected by a brodifacoum treatment that targeted orchard voles in the USA because they avoided hunting over the orchards. Eastern screech owls were affected because the orchards were a preferred hunting ground.

*Proportion of the diet that is poisonous*: This is a difficult measurement to make and has been widely ignored by many researchers. Kenward (1988) calculated that 24% of poisoned squirrels posed a secondary poisoning risk to scavengers and Eadsforth *et al.*(1996) found that only 3% of barn owl pellets contained a detectable level of rodenticide residue. Animals with a small home range are more at risk than those with a large one if the poisoning incident occurs in their territory. Tawny owls are strongly territorial and will only take prey in their patch (Southern, 1954). Hegdal and Colvin (1988) found that there was a high risk of poisoning (minimum 58% deaths) where more than 20% of the home range of Eastern screech owls was affected by brodifacoum treatments. Where just 10% of the home range was treated, deaths among the screech owls were calculated to be 17%.

*Behaviour of poisoned animals*: Many poisoned animals are said to die under cover (Fenn *et al.*, 1987). Kenward (1988) found that 43% of poisoned squirrels died in their dreys and Harrison *et al.* (1988) noted that only 4% of poisoned rodents were found after anticoagulant treatments targeted them. Before death, many animals show behavioural changes as a result of being poisoned. Hooper *et al.* (1990) noted that Bobwhite quail had difficulty maintaining covey-affiliation and were more prone to predation after ingesting insecticide. Kjolholt (1990) reported an observation by the Danish Game Biology Station that brown hares were confused and had eye irritation on fields treated with dinoseb or DNOC. Residues could not be detected in any of the hares, yet their behavioural changes surely made them more susceptible to predation. Before death,

rodents may exhibit behaviour that will either enhance or decrease their predation (Cox and Smith, 1992). For instance, Cox (1991) showed that poisoned rats were less active and spent more time away from cover and in daylight compared to unpoisoned rats. Ille (1991) found that barn owls select for active rather than inactive prey and long-eared owls, badgers and foxes, being nocturnal, would possibly avoid much contact with poisoned animals. Other species may, however, be placed at risk. Kotler *et al.* (1988) found that tawny owls predated desert rodents in the open far more than under cover, so tawny owls in the UK may be more likely to prey upon rats exhibiting behavioural changes. Metzgar (1967) stated that screech owls were far more prone to prey upon transient white-footed mice than residents.

*Population effects and the ability to recover*: Hooper *et al.* (1990) noted that starlings had a lower nesting success in an area exposed to pesticide (in this instance, insecticide) and the fledglings were weaker and more prone to predation. Cox (1991) found that wood mouse decline was worst of all when anticoagulant was used during winter months when the population was already low and could not quickly recover. For predatory species, if their prey item is removed, they may be adversely affected and breeding will suffer. Barn owls apparently do not show any decline in breeding (Hegdal *et al.*, 1984), perhaps because they are a r-selected species and can therefore recover quickly from any incident (Colvin, 1984). The chances of being adversely affected by farm-rodenticide treatments over a long period were therefore considered to be small. Townsend *et al.* (1981) noted that tawny owls could metabolise out much of the rodenticide consumed in poisoned mice and were therefore able to avoid long-term effects.

*Pets and livestock*: Pigs and dogs may be particularly opportunistic scavengers and so could consume poisoned rats and mice. Other livestock, such as chickens, are obviously likely to eat granular bait if it is within their reach. Great care must therefore be taken when positioning bait to avoid any risk of accidental poisoning and rodent carcasses should be buried or burned to avoid scavengers picking them up. In the event of a case of poisoning, the vitamin $K_1$ antidote can be administered. This has successfully saved the lives of dogs in the past (McInnes, 1993).

## 1.8 JUSTIFICATION AND OVERALL AIMS OF THE PROJECT

Smith *et al.* (1990) developed a compartment model to describe the fate of rodenticides in the environment. They pooled data from various sources to develop the model, but a paucity of data in some areas of study was highlighted; scavengers, non-target bait feeders and individual bait-consumption by rats, the target rodents. There were also few or no data on the metabolism of rodenticides in rats and the levels of residue found in carcasses.

The fate of any chemical in the environment can be accurately predicted only if enough is known about its transport between different environmental compartments. Much work has been done on the toxicity of rodenticides to high-status predatory animals such as barn owls (Harrison *et al.,* 1990; Gray *et al.*, 1994a), but little is known about what residues are likely to be available within carcasses or live rodents in the field. Similarly, work focusing on rat feeding behaviour and dose responses to rodenticides has been carried out largely in the laboratory or in an artificial environment such as an enclosure (Shepherd and Inglis, 1987; Gill and MacNicoll, 1991; Cox and Smith, 1992). These studies have produced much useful and necessary information, yet the similarity between captive rats and those living freely in the wild is not known.

Physiological resistance is an area of much interest to rat researchers, particularly because the occurrence of resistance is now widespread across parts of the UK (Greaves, 1995). The fate of rodenticides in the environment where resistant rats are present has not been studied in any detail, yet the effects of resistance are likely to have an impact on rodenticide fate and environmental hazards.

The aim of this project was to provide information on aspects of rat ecology and rodenticide ecotoxicology that were previously unresearched or needed further study (as outlined above). Specifically, the project was organised to study rodenticide fate from the compartment of the target rodent and to investigate the following:

**1.** Individual bait consumption by the target rodents (particularly rats).
**2.** Residue levels of rodenticide in **a.** live target rodents during a control programme and **b.** poisoned, dead target rodents.

**3.** Feeding behaviour of wild, free rats on farms.

**4.** Behavioural changes of individual rats and population changes occurring as a result of anticoagulant rodenticide application.

**5.** The effects of physiological resistance on 1 - 4 above.

# CHAPTER 2: PROGRESSION OF RODENT CONTROL

## 2.1 INTRODUCTION

### 2.1.i Aims of this study

The main aspect of this section of the project concerned field trials using anticoagulant rodenticides. In particular, two hypotheses were being tested: First, to discover whether bait-take and population patterns through a control programme differ between areas where anticoagulant resistance is common and where it is absent. Second, to find whether bait-take and rat population levels are the same when a first generation anticoagulant is used or when an indoor-use second generation anticoagulant is applied. In addition to these two main investigations, the farm trials also allowed data to be collected for behavioural studies (Chapter 3) and for carcass residue analyses (Chapter 4).

### 2.1.ii The anticoagulants used

Coumatetralyl (a first generation anticoagulant) and brodifacoum (a second-generation anticoagulant registered for use indoors only) were used as the rodenticides in this project. The chemical structures of the two compounds are shown in Figure 2.1. Coumatetralyl is manufactured by Bayer and has a chemical formula $C_{19}H_{16}O_3$. It was introduced to the UK in 1956 and is sold under the trade name "Racumin". Olney *et al.* (1991a) reported that coumatetralyl is used on 10% of farms in England that carry out rodent control with chemicals. Coumatetralyl is usually formulated on granular bait at 375 $\mu g\ g^{-1}$ or alternatively it can be applied as a contact dust. It has an acute/single lethal dose to 50% of Norway rats ($LD_{50}$) of 16.5 mg $Kg^{-1}$ (Johnson & Prescott, 1994), but a daily dose of only 0.39-0.5 mg $Kg^{-1}$ for 5 consecutive days produces 50% mortality in wild male and female rats (Ashton *et al.*, 1987). Greaves and Cullen-Ayres (1988) found that the $LD_{50}$ values are somewhat elevated in anticoagulant-resistant strains. They tested the standard Welsh and Scottish warfarin-resistant strains and found that on average 29.0 and 219.0 mg $Kg^{-1}$ (for males and females respectively) of coumatetralyl were necessary to produce an $LD_{50}$ in the Welsh strain, and in the Scottish strain of resistance, values of 29.2 and 73.1 mg $Kg^{-1}$ were recorded. This compared to values among susceptible rats of 0.86 and 1.3 mg $Kg^{-1}$.

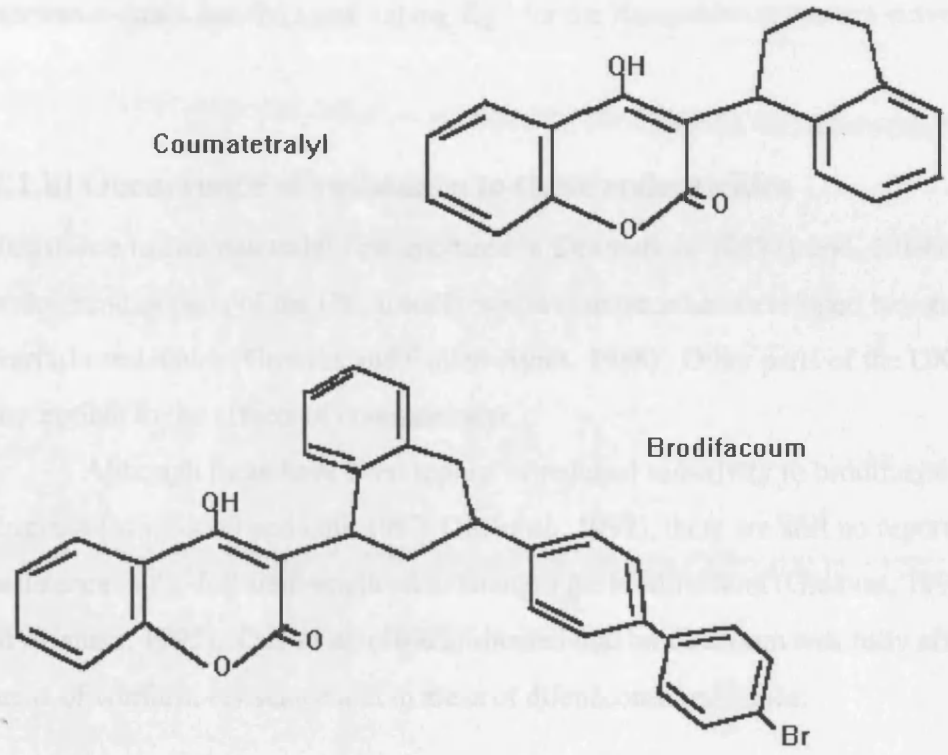



Figure 2.1 The chemical structures of coumatetralyl and brodifacoum

Brodifacoum is a second-generation anticoagulant and has the chemical formula $C_{31}H_{23}BrO_3$. It was first available in the UK in 1982 and is sold under the brand names of 'Klerat', 'Talon', 'Havoc', 'Ratak Super' and 'Matikus'. Brodifacoum is much more potent than coumatetralyl, so is formulated in granular bait at a concentration of 50 $\mu g\ g^{-1}$. Brodifacoum has a higher toxicity to birds than earlier anticoagulants (Lund, 1984) and so it is only licensed for indoor use by professional pest-control operators. Despite this, Olney *et al.* (1991b) reported that in 40% of cases where brodifacoum was used on farms in England and Wales in 1989, bait was placed outside. The overall use of brodifacoum is low, however, being used as a poison on just 3% of farms. Brodifacoum has an acute oral $LD_{50}$ in the rat of 0.22-0.3 mg $Kg^{-1}$ (Lund, 1984; Johnson and Prescott, 1994). This is enough to produce mortality after just one feed. Greaves and Cullen-Ayres (1988) reported a slight elevation in the $LD_{50}$ values required for anticoagulant-resistant rats. The Welsh-resistance strain $LD_{50}$ was 0.42 and 0.56 mg $Kg^{-1}$ for males and females

respectively, with corresponding values of 0.98 and 1.3 mg $Kg^{-1}$ for the Scottish-resistance strain and 0.81 and 1.0 mg $Kg^{-1}$ for the Hampshire-resistance strain.

## 2.1.iii Occurrence of resistance to these rodenticides

Resistance to coumatetralyl first appeared in Denmark in 1969 (Lund, 1984). It is now widespread in parts of the UK, usually where resistance has developed beyond simple warfarin resistance (Greaves and Cullen-Ayres, 1988). Other parts of the UK still remain susceptible to the effects of coumatetralyl.

Although there have been reports of reduced sensitivity to brodifacoum in parts of England (MacNicoll and Gill, 1987; Gill *et al.*, 1992), there are still no reports of resistance at the full field-application strength for brodifacoum (Greaves, 1994; Myllymaki, 1995). Quy *et al.* (1992a) showed that brodifacoum was fully effective in areas of warfarin resistance and in areas of difenacoum resistance.

## 2.1.iv The study areas

The two regions that were selected for this study were the Leicester area, where resistance has not been reported, and the Reading area, where resistance has a very high incidence. Both these regions are similar in that the farms are mostly small to medium in size with the main livestock being pigs, poultry and cattle. A mixture of livestock and arable farming is also common.

## 2.1.v The likely effects on non-target species

When poison is used to eradicate or reduce commensal rodents on a farm site, there is always a possibility that other species will be accidentally poisoned. Any vertebrate with a vitamin K-dependent mechanism of blood clotting may be harmed by anticoagulant rodenticide, so all mammals and birds can be put at risk. Many birds and mammals have been shown to be adversely affected by anticoagulant rodenticides (see section 1.7). Small rodent species and grain-eating birds are the most likely to be poisoned

accidentally, as they may take the grain on which the poison is coated. Predators of grain-eating rodents and birds, and scavengers of their carcasses may also be affected via secondary poisoning (see sections 1.6 and 1.7). The exposure to birds is minimised by partially covering each bait point. Outdoor-use rodenticides are also coloured blue in order to deter birds. Rodents, such as mice and voles, may be highly exposed to the poison. Not only do their habitats overlap with those of rats and hence the baiting areas, but being smaller than rats, they cannot be effectively excluded from bait stations. Scavengers and predators are at risk of secondary poisoning from eating the bodies of poisoned target and non-target animals. The potential exposure of predators to the poisoned rodents may be enhanced by behavioural changes prior to death (Cox, 1991; Cox and Smith, 1992). The exposure of anticoagulants to scavengers may be increased if the poison treatment is effective, because the treatment may result in carcasses that contain poison being discovered by the scavengers.

Coumatetralyl has a lower toxicity to most species than brodifacoum and so it should in theory pose less of a risk to non-target species. The fact that it can be used outside and also that it is less effective than brodifacoum in areas of phenotypic resistance may conversely mean that coumatetralyl is potentially more harmful than brodifacoum (Cox and Smith, 1990; Cox, 1991).

## 2.2 METHODS AND MATERIALS

### 2.2.i The farm sites

A total of 9 farms in the east midlands and 9 farms in central southern England were selected for the rodent control trials. An attempt was made to find sites with an abundance of rats. There is, however, an apparent stigma attached to the presence of rats on one's farm. This meant that sites where the owner openly invited use of the area were gladly accepted as being of use, although some sites really had a very minor commensal rodent 'problem'. Farms were surveyed and previous rodent problems and control history were determined. Suitable sites were then selected and assigned to treatment with one of the two rodenticides, depending on areas of infestation and other considerations such as free-ranging animals.

Site plans for all the farms are shown in the Appendices and the descriptions are given below.

A. In the east midlands, the 9 farms selected were located in the counties of Leicestershire and Lincolnshire.

A1: A 600 acre dairy and beef farm with some sheep and poultry. Buildings were mostly modern. A large maize silage and straw stack was positioned under plastic in the main yard and provided cover and food for rats. The open slurry lagoon and wooden poultry pens and kennels on the outer edge of the yard area were also used by rodents. Voles and wood mice were living in wood piles and scrub around the edge of the main yard area and house mice were evident in the base of the grain mill and in the older brick buildings.

The farm had been maintaining three permanent bait stations with "Slaymor", a bromadiolone-based 2nd generation anticoagulant. The farm had also been applying "Racumin" (coumatetralyl) dusting powder to the burrows within the straw stack. There were no cats in the area and the farm dogs were kept in kennels when not working. There were no free-ranging farm animals. This site was therefore suitable for outdoor baiting.

A2: A modern farm supporting 450 sheep, 40 cattle with the rest laid to arable use. Grain was stored in two large, purpose-built barns. Rat runs could be seen across the

grain piles and also in the vent houses between the barns. Rat faeces, urine and hairs contaminated the grain, which was the farmer's main concern. Other parts of the yard area were used for housing ewes during lambing. There was also a silage and straw stack. Dense hedgerows and long grass with newly planted trees surrounded the main yard area. Small mammals such as wood mice, harvest mice, shrews and voles were supported in this habitat. Dogs and cats were allowed to roam freely across the yard area. This site was selected for indoor-only baiting.

A3: This small farm was now uninhabited, but was used for storage and for housing ewes at lambing time. The buildings were brick except for a Dutch barn that covered a hay stack. Straw was stored outside next to a hedgerow with a ditch. Rats moved between the straw and the hedgerow and were known to feed on the field crops (there was no significant food supply on the farm yard). Wood mice and voles lived in the hedgerows and foxes, and stoats and weasels were also evident.

"Drat", a chlorophacinone rodenticide, had previously been used at this site but there had been no need for it over the 6 months prior to the trial. This site was chosen as a control to note feeding activity of rats and non-target rodents in the absence of poison.

A4: A smallholding with sheep, a few cattle and some root crops. The compact yard consisted of a mixture of old brick sheds and some small, modern barns. Young cattle were housed permanently at one end of the yard and the rest of the area had been used for lambing prior to the trial. One barn was also used for potato and beetroot storage. A few hens ranged the area and there were some farm cats and dogs. Rotting potatoes and sheep carcasses were flung out into the yard and seemed to provide a fairly constant food source for rats, which were living in refuse within the brick buildings. Dykes, which surrounded the farm, were also used by rats. Indoor-only baiting was used at this site.

A5: This site was an animal-feedstuffs mill. One large barn was used for processing feed and storing paletted bags. A second large barn was used for loose storage of feeds with some paletted bags also stacked around the edges. A small barn held loose grain and a further double barn housed beef cattle. A straw stack was positioned along the back of one barn. The whole yard was surrounded by scrub, hedgerow and long grass with arable

fields off to one side. Rats were abundant throughout the storage areas of the farm, living within the pallets that supported the feed sacks and also within the cavity walls. In many places there were holes in the walls where nests had been made. It was clear that there had been a rat infestation at this site for many months, if not years. Both indoor and outdoor baiting were feasible at this site.

A6: A small riding establishment, consisting of stables with an attached hay barn. There were also some wooden sheds for storage and a second barn used as a furniture workshop. Rats had been entering the stables and were also nesting in the barn. Wood stacks and scrub in the vicinity harboured wood mice, and housemice were evident in the stables and the furniture workshop. Outdoor and indoor baiting were used at this site.

A7: This purpose-built poultry unit was now used for pigs. Large wooden sheds were separated by areas of grass that had been allowed to over-grow and some had turned scrubby. Drainage dykes ran along two sides of the unit and arable fields lay beyond them. The whole unit was poorly maintained and there was much evidence of rat activity. Pig carcasses were left in the passages of the sheds or piled up outside. Spilled grain was left as it stood and areas of refuse were allowed to build up. The wooden buildings were unlit and provided plenty of safe harbourage for rats, both in the rafters and at ground level. The conditions also provided ample food for the rats. A mixture of indoor and outdoor baiting was used at this site.

A8: This site lay about half a mile from A7 and was managed by the same group. The old poultry sheds were similarly used for pigs, but the general hygiene and level of input to the site maintenance were better. Some sheds lay empty whereas the ones used for pigs had been properly converted. Muck heaps were situated at the back of the site and dykes ran along two edges. One shed was used for storage. Rats were confined mainly to roof spaces and undisturbed storage areas. Indoor and outdoor baiting were used at this site.

A9: This tidy pig farm consisted of modern barns adjacent to old brick sheds. Rats had been seen in the grain store, but that had recently been emptied. Areas of scrub at the

back of the yard area may have provided harbourage for the rats. Rats were also seen frequently in the brick buildings. House mice were living in the machinery store and in the air vents underneath the grain store. Indoor baiting was used at this site.

B. Nine more sites were chosen in central southern England, which were located on the borders of Berkshire, Hampshire and Oxfordshire. One of these sites, B8, was used as a study site by Cox (1991) and was coded as site F.

B1: This was a smallholding that consisted of about 5 acres of scrub and pasture. Free-range chickens, geese and ducks and also goats were kept in the paddocks. Small poultry sheds, which were positioned in each paddock, held a supply of grain for the poultry. The paddocks were surrounded by hedge and woodland with some areas of wood storage and scrub. There was also a small stream to one side. The resident rats were active around and underneath the poultry houses, although it was clear that they also moved to and from the neighbouring property across the road, which had substantially more livestock. Rodenticides used in the past had been mostly ineffective and the farmer now tried to shoot any rats he saw. There were free-ranging animals and bait needed to be carefully placed, but baiting was possible both indoors and outdoors.

B2: This was a large site housing about 300 pigs under cover and 100s more in the surrounding fields. About 50 beef calves were also kept on the yard. Rats were abundant throughout the farm buildings where they fed mainly at the pig troughs. Rats had burrowed under the concrete floor of many of the pig pens and elsewhere sheltered within the thick straw bedding. Rats were frequently seen in the grain storage and milling areas and they had permanent burrows at the periphery of the yard area where the fields began. Clearly neither habitat nor food were limiting factors for this population. Previous control treatments had been carried out unsuccessfully in latter years with anticoagulant rodenticides such as difenacoum and, most recently and partly effectively, with chlorophacinone. Baiting treatments had been carried out more or less constantly for the previous two years. Outdoor baiting was the only practical method to use at this site because of all the penned animals.


Plate 2.1a Farm site B3 showing hay rick and pig pens

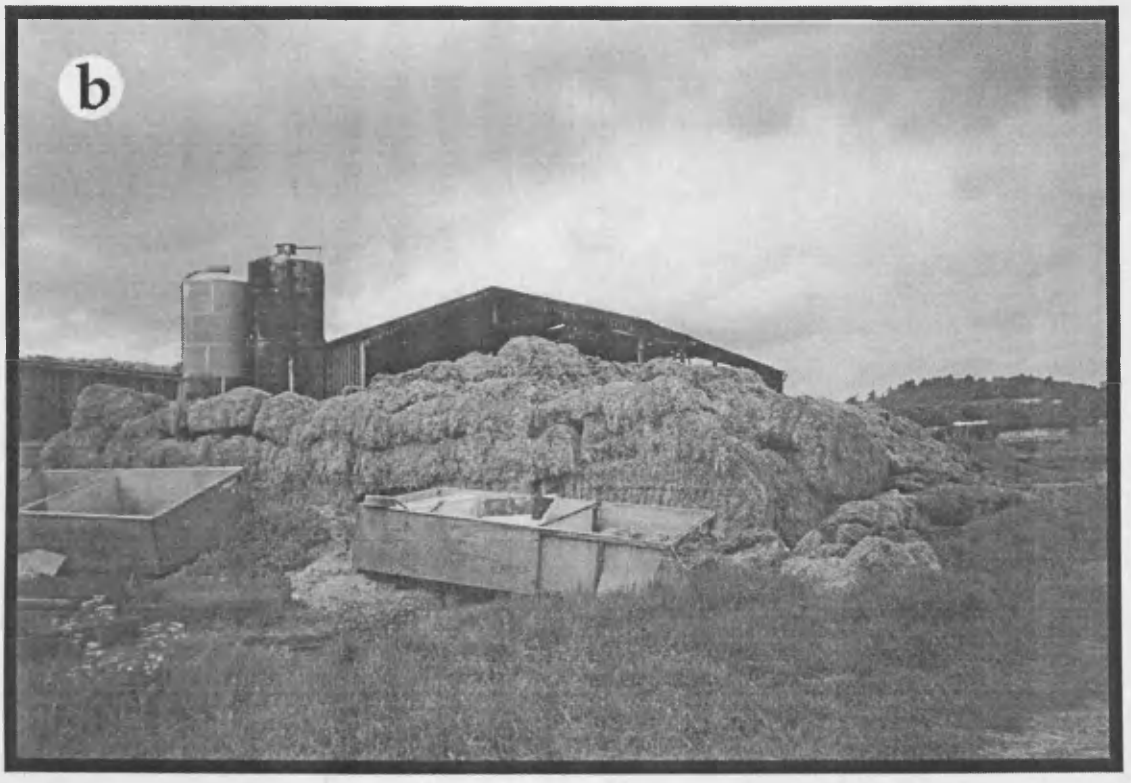



Plate 2.1b Farm site B3 showing good rat harbourage

B3: This site consisted of two barns and 14 small piglet pens and was surrounded by open fields. One of the barns housed pigs exclusively, the other was also used to store straw and sacked feed. Both barns were open on one side. The muck was heaped on the yard adjacent to a straw stack. Rats were using the heap and stack for harbourage and as a limited food supply. From there, they were also running across to the barns and feeding from the automatic pig-feed dispensers. Other rats were living permanently in the banks of the small slurry pool that lay adjacent to one barn. Prior to the trial, large quantities of bromadiolone and zinc phosphide had been heaped at a few permanent bait stations on the site. Although some rats had died, there was clearly a large proportion of the population that had remained unaffected. Outdoor baiting was the most suitable method at this site.

B4: This farm lay 600m up the track from B3, but it appeared to have a quite separate rat population. The site was an old-fashioned yard, now largely unused. The buildings were all mainly wooden and years of rat activity were evident from the greased marks along the rafters and under the wooden doors. There were also several holes in the building structures, and well-used rat runs were evident through the walled paddock that lay between the feed-storage barn and the rest of the farm. The only other food source was provided by the remnants of the previous year's grain harvest, which still lay in an underground bin and around some milling machinery in one of the barns. Rats were frequenting this building to feed on the grain. They were also seen to feed from the compost heap next to the garden and were nesting in the stables and kennels by the house. Heaps of the anticoagulant bromadiolone and also zinc phosphide had been maintained at permanent bait points on the outer side of the farm buildings. There were clearly some rats succumbing to this treatment although there were plenty of others still alive. The farmer reported that very large amounts of bait were consumed each month. Indoor baiting was used at this site.

B5: This clean and tidy farm was a small site consisting of a very localised rat infestation in the now empty grain store. Large fertiliser bags were stacked inside the building and there was some evidence of the rats eating into the bags. Mostly the rats were 'cleaning up' the remains of the previous harvest, which had been swept up into one

corner of the barn. The rats were living outside the barn or somewhere within the roof and were descending one corner of the barn wall to feed. No poison had been used at this site recently. Indoor baiting was suitable for this site.

B6: This dairy unit housed approximately 200 cows. The rat activity was in two areas at opposite ends of the farm yard and appeared to result from two distinct populations. One had permanent burrows in the banks at the end of the yard adjacent to the silage presses. The rats were feeding mainly on the silage, but were also venturing towards the milking sheds where the cattle cake was stored in open mounds. At the other end of the yard there was a block of disused stables that had not been cleared of old bedding and feed. The unit was riddled with rat holes and these rats also travelled across to one of the cow sheds to feed on the powdered milk used for the calves. Bromadiolone had been used in the previous few months, but appeared to have been largely ineffective. Indoor and outdoor baiting were feasible here, but indoor baiting was selected.

B7: This site consisted of a small wooden stable block surrounded by woodland and scrub. A stream ran along the back of the block and a small paddock faced the stables. Rats had been feeding from bread left out for the donkeys in the paddock and they were nesting in the floor of the stable block. Wood mice were common in the area and field voles and shrews were living in the grassy area facing the stables. A variety of anticoagulant rodenticides had been used at this site before but had been largely ineffective. Indoor baiting was selected for this site.

B8 This farm contained two battery-chicken sheds and some open faced barns for calf rearing and straw and hay storage. Three other sheds lay virtually empty except for storage of a small amount of bagged feed and some machinery. The buildings were mostly wooden. Dense hedgerow and scrub surrounded the buildings and other small mammal species such as wood mice were evident. Rats were mostly living in the scrub and burrowing underneath the sheds. They were feeding from the spilled grain and chicken droppings on the floor of the battery units. There were no reports of chickens being killed by the rats, but some eggs were thought to be taken. Rats had been in the area for a number of years with control treatments being made when the rodent numbers

became unacceptable. Control using bromadiolone had often reduced the numbers, but there was always a sizeable part of the population that remained unaffected. Indoor baiting was chosen for this site.

B9: This site consisted of pig-rearing units surrounded by open fields to one side and woodland, scrub and slurry waste on the other sides. Rats had been seen in between the pig sheds and there were holes where they had chewed through to the feed storage area. House mice were more evident and were seen in abundance throughout most of the site. Outside the buildings, banks and hedgerows provided suitable habitat for a range of other small mammal species There were also many predatory mammals and birds in the area. Indoor baiting was therefore chosen for this site.

## 2.2.ii Bait application

Rodent-control treatments were carried out on the 18 farms described over a period of 28 months. In both areas, farms were discovered via contacts in the area and then by word of mouth. Each farm was visited and those that had an obvious rat infestation were assigned to treatment either with coumatetralyl, with baiting allowed both indoors and outdoors, or with brodifacoum where baiting was only allowed indoors (Table 2.1). There were two exceptions: In the east midlands, one derelict farm, A3, without an obvious rat infestation was selected and baited with non-poisonous wheat. In the south of England, one farm, B9, was chosen although mouse infestations, rather than rats, were the main rodent problem.

Table 2.1 The sites assigned to each treatment

| | | | | | | |
|---|---|---|---|---|---|---|
| east midlands | coumatetralyl | A1 | A6 | A7 | A8 | A5 |
| | brodifacoum | A2 | A4 | A9 | | |
| | no poison | A3 | | | | |
| c.s.England | coumatetralyl | B1 | B2 | B3 | B9 | |
| | brodifacoum | B4 | B5 | B6 | B7 | B8 |

Trials took place in the east midlands from January to July 1994. In central southern England, trials were carried out between February and June 1995 and between March and April 1996. This design confounds area with year, but it was not possible logistically to work in the east midlands and central southern England at the same time. As each farm was unique, no attempt was made to pair up treatments. Instead, sites were assessed for the feasibility of baiting indoors or outdoors, depending on the extent of free-ranging animals and the location of rat infestations. The most suitable baiting practice was adopted at each site. In the east midlands, an additional site, A3, was used to assess feeding by rats and other rodents in the absence of poison.

The following materials were used for the farm trials to investigate target rodent bait-take:

- Coumatetralyl anticoagulant rodenticide formulated on cut wheat @ 375 $\mu g\ g^{-1}$ and containing hexachlorobiphenyl (HCBP) @ 98 $\mu g\ g^{-1}$ and later @ 89 $\mu g\ g^{-1}$
- Brodifacoum anticoagulant rodenticide formulated on cut wheat @ 20 $\mu g\ g^{-1}$ and containing 98 $\mu g\ g^{-1}$ HCBP
- Standard white plastic rodent control bait trays measuring approximately 16cm x 11cm, supplied by Kill Germ Chemicals Ltd, Ossett, W. Yorks
- Hardboard rectangles with anti-weather coating on one side and measuring 40cm x 25cm
- Digital kitchen scales graduated in 2g intervals
- A high powered torch
- Sterile medical gloves which were worn as a precaution against disease
- A long handled metal spoon

A search was done at each farm to find areas of high rat activity. Plastic bait trays containing 100g of either coumatetralyl or brodifacoum rodenticide were positioned next to runs in and around these regions, most often alongside linear features such as walls or buildings, and near to holes. Trays were placed every two metres in heavy usage areas and far more widely apart in less used areas. The quantity of trays used at each farm depended upon the suspected size of the rat population present and on the availability of safe, accessible sites to place bait (Table 2.2). It ranged from 7 at site B1 where the

infestation was very small and localised, to 73 at A1 where rats were far more abundant and widespread.

Table 2.2 The number of bait points used at each site

| Coumatetralyl Site | Number of Bait Points | Brodifacoum Site | Number of Bait points |
|---|---|---|---|
| A1 | 73 | A2 | 14 |
| A5 | 37 | A4 | 71 |
| A6 | 11 | A9 | 53 |
| A7 | 54 | B4 | 51 |
| A8 | 49 | B5 | 12 |
| B1 | 7 | B6 | 33 |
| B2 | 54 | B7 | 10 |
| B3 | 40 | B8 | 57 |
| B9 | 20 | | |
| | | Other | |
| | | A3 | 19 |

Brodifacoum was never used outside. Coumatetralyl, in contrast, could be placed both indoors and outdoors. Each bait tray was covered by a numbered hardboard cover, placed at 45 degrees from vertical to allow rats to go underneath to feed from the tray, but birds and other animals could not feed on the bait from above. The covers also prevented rain from spoiling the bait. The covers were usually held down with a brick to prevent them from being knocked or blown away. Care was taken to position the bait in places where other animals could not easily access them A note was made of all the bait-point locations on the site plans.

All bait trays were checked within the first two days. The bait in each tray was weighed and the amount that had been taken was calculated. The bait was topped up to 100g again unless all the bait had gone, in which case 200g was issued. After that, bait points were checked every few days. Where the bait had all gone, the amount was doubled up to a maximum of 400g. The aim was to prevent the bait running out between visits. This method is known as "saturation baiting". Trials continued until the rat population had been reduced to a negligible level or for a period of 6 weeks, whichever occurred first. On a few of the sites, because of various difficulties, trials continued for longer periods. Another problem encountered was that it was sometimes not possible to weigh the contents of all the bait trays at every visit. On occasions, time permitted only

the trays that were in need of more bait to be weighed and replenished. These incomplete data sets are accounted for within the format of the results.

## 2.2.iii Rodent population-abundance indices

Techniques were used to assess the size of the resident rat population while baiting trials progressed. Two methods were used. For the first method, all holes and burrows that could be found were loosely blocked with tissue paper. Cox (1991) showed a good relationship between population size and the number of holes maintained at a site. The locations of the holes were then mapped out on the site plan. After each week of the trial, the holes were revisited and a note was made of which holes had been used, as shown by removal of the tissue. These holes were reblocked each week until the end of the trial. The numbers of active burrows throughout the experimental period were thus noted.

The second method involved using "tracking tiles" as described by Shepherd and Greaves (1984). Pale coloured vinyl floor tiles were cut into squares of 15cm x 15cm and were lightly coated with a suspension of 3% lamp black in methanol, leaving a margin of about 2cm around the edges. These tiles were placed on the ground across the whole baiting area, about 5m apart, usually on rat runs or alongside linear features where rats would be likely to run over the tiles. When a rat moved across a tile, the footprints or tail marks could be seen either as a scuffing of the lamp black or as an imprint of black on the pale borders.

Table 2.3 The number of tracking tiles used at each site

| Coumatetralyl Site | Number of Tracking tiles | Brodifacoum Site | Number of Tracking tiles |
|---|---|---|---|
| A1 | 27 | A2 | 10 |
| A5 | 0 | A4 | 0 |
| A6 | 0 | A9 | 0 |
| A7 | 0 | B4 | 40 |
| A8 | 0 | B5 | 0 |
| B1 | 0 | B6 | 20 |
| B2 | 40 | B7 | 20 |
| B3 | 20 | B8 | 40 |
| B9 | 26 | | |
| | | Other A3 | 12 |

Plate 2.2 Photographs showing rat holes in a hay rick. Note the tracking tile marked by rat footprints

Each week through the trial, the tiles were collected in and scored in a system described by Quy *et al.* (1993). A note was made of whether the tile had been marked or not and, if it had, the percentage of the tile that had been marked was also noted. Tiles with no mark were given a score of 0, those with 1-25% were scored as 1, those with 26-95% were scored as 2 and those with 96-100% were scored as 3. All the scores were tallied to give a total for the site relative to the number of tiles laid out. The total number of marked tiles was also tallied to give a 'percentage marked' value. After the scores had been noted, the tiles were re-coated and replaced.

No other census method was used during these trials. The sampling-graph method may be misleading if some rats within the population do not feed from bait trays or where the presence of alternative foods or phenotypic resistance result in altered feeding behaviour (Cowan and Townsend, 1994). It was therefore decided that census techniques that were independent of feeding would be used.

## 2.2.iv Carcass collection

Searches for dead rats and mice and other non-target animals were made around the farms throughout the baiting periods. It is notoriously difficult to retrieve a carcass of any small animal species from 'the field' (Balcomb, 1986; Butcher, 1965), but rats usually die under cover making it especially hard (Fenn *et al.*, 1981). An effort was therefore made to look beneath bales and slats *etc.* that might have been used for harbourage All rodent carcasses were numbered and weighed, and the sex and stage of development were noted. The carcasses were then de-gutted, from the top of the stomach down, and the feet and tail were removed following the method supplied by MAFF, Central Science Laboratory (unpublished). The carcass was then wrapped in a polythene bag and deep frozen. Bodies of non-target species found at the site were also bagged and frozen as were the bodies of trapped rats and mice. The carcass details were primarily used for later residue analyses. The rat weights also gave an idea of whether the rats were feeding (and dying) according to a dominance hierarchy (Dubock, 1982; Cox and Smith, 1990; Nott & Sibly, 1993) or whether juveniles were the first to be poisoned (Shepherd & Inglis, 1987; Nott, 1988). The outcome may have implications for secondary poisoning.

## 2.2.v Trapping

Carcasses were needed for residue analyses and it was intended to obtain these by carcass collection. Trapping had to be used on sites in central southern England, where the rodenticide treatment was not effectively reducing rodent numbers and there were few carcasses. Fenn Mk IV snap traps (supplied by A. A. Fenn and Co., Redditch) were positioned along runs and in hole entrances and were then secured, via their chains, with tent pegs to the ground. Traps were sometimes positioned along runs on the concrete yards and were then secured by tying the chain with twine to a solid object. Purpose-made hardboard tunnels were positioned over the top to prevent other animal species from being caught in the traps. The tunnels allowed access only from each end. A chicken-wire tunnel was used instead in some places where traps were placed on soft ground and the tunnel was pegged into the ground. Two twigs were also inserted vertically at each end to narrow the entrance width and minimise non-target risks (Quy *et al.*, 1993). When each trap was set, the treadle plate was lightly dusted with soil and with a small amount of grain or pig feed. The traps were checked every day. Any rats that were caught were removed for use in residue analyses. The traps were then re-set and secured at the same position. Trapping was carried out from any day after 3 weeks of the trial up to the end of the trial period.

Longworth traps were set up through and around the buildings on sites where house mice were common. House mice that were caught were transported inside the traps to the laboratory at Reading University and were then killed humanely by placing them in a high flow $CO_2$ chamber (30% $CO_2$ rising to 80%) for 15 minutes. The bodies were saved for residue analyses.

## 2.2.vi Monitoring non-target species

The following materials were used to investigate non-target rodent use of farm sites:

- Longworth traps, some with shrew-release holes
- Hay, castors (blowfly pupae) and crushed oats
- A 50g Salter spring balance graduated in 0.5g intervals

•Blunt-ended, curved scissors

•Plastic bags

Monitoring of non-target small rodent species was carried out using Longworth traps on three of the farm sites in the east midlands and on four of the sites in central southern England. The traps, containing a ball of hay for bedding, a handful of oats and a few blowfly pupae or castors (as food for shrews, which may be accidentally caught) were positioned around the farm area in typical small mammal habitat. This usually constituted bits of scrub or hedgerow, wood and brick piles and areas of long grass, all within a short distance of the baiting areas. The traps were placed in pairs, usually about 10m apart. A week of trapping (4 trap nights) was carried out prior to the start of bait application at each of the sites. Then a further week of trapping was carried out after completion of the trial. The traps were checked each morning. Where a trap had sprung, the contents were emptied into a clear plastic bag. From here, the small mammal was weighed, using a spring balance, and, where possible, sexed. Shrews were released, as, being insectivorous, they were not the main species of concern with regard to accidental poisoning. Mice and voles were picked up by the scruff of the neck and a small patch of fur was clipped off the back or flanks of the body using blunt-ended scissors. The clip removed the outer edge of the guard hairs and revealed the darker undercoat beneath. This type of clip lasts a number of weeks so that a recaptured animal is easily recognisable (Cox, 1991; pers. obs.) A unique fur-clip position was used for each separate night of trapping, with six different marks in total; three for each trapping session. The position of any other clip was also noted as this indicated that an animal had been caught before. After these measurements had been made, the animal was released at the point of capture. The trap was then refilled with dry bedding and more grain and castors and the trap was reset in the same position as before.

The trapping data were pooled and population estimates of the small mammals were made for the week before and the week after the trial using the Petersen Weighted Mean method (Begon, 1979; Cox, 1991). Population changes through the course of the treatment could thus be estimated. This method uses data collected over the four nights to estimate population size. The method assumes that there is no gain to the population through birth or immigration and no loss by death or emigration. It also assumes that

trapping and marking individual small mammals does not affect their chance of being recaught. It also assumes that the marks are permanent. Some of these assumptions, such as birth, death and migration, and possibly the permanence of marking could not be guaranteed between baiting sessions that were up to 7 weeks apart. It was therefore decided that population estimates would be made separately for the two trapping sessions before and after the rodenticide application trials but that the two estimates would not necessarily be tied together.

For the Weighted Mean method, $n_i$ individuals are caught on each day (i) of which $m_i$ are already marked. Unmarked individuals are marked and then $r_i$ marked individuals are released. The population estimate, $\tilde{N}$ is then calculated by the following equation using the pooled data for the four trap nights:

$$\tilde{N} = \frac{\Sigma M_i n_i}{(\Sigma m_i)+1}$$

where $M_i=[\Sigma(r_i-m_i)]$

Bait trays were also checked for signs of non-target feeding. Wood mice, for instance, will husk the bait and leave the remains, as well as droppings, in the tray. An estimate of bait-take by non-target species was made where non-target use was suspected. Other non-target species were not monitored directly, but any evidence of activity in the area was noted. The tracks of other animals could be seen in snow or mud and footprints were occasionally picked up on the tracking tiles. Droppings, or rodent carcasses that had been discarded by predatory species, also gave indications as to what other wildlife was in the area.

## 2.3 RESULTS

### 2.3.i Bait-take by target species in the east midlands

The patterns of bait consumption for each of the two rodenticides are illustrated in Figures 2.3-2.5. The daily bait-take graphs represent the bait-take totals measured at each site visit, divided by the number of days since the last visit. This allows a standardisation between sites, because some farms were assessed more frequently than others. The week by week bait consumption values are listed in Tables 2.4 and 2.5. These show the total amount of bait eaten at the sites during each week. The overall totals, for the whole experimental period, are revealed in Figure 2.6. These figures compare how much bait the target rodents ate at each site.

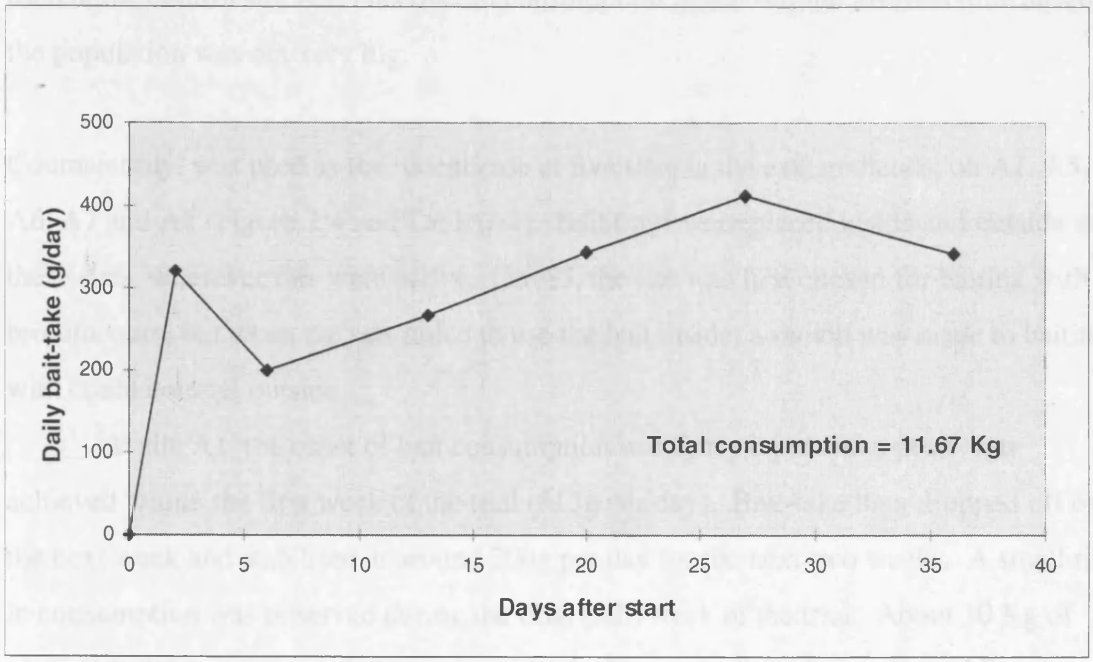



Figure 2.3 Daily bait consumption from a control (no poison) farm in the east midlands

The control farm, A3, was used in this study to give an indication of the effect of supplying food to an area where rodents were present without removing any of the population by poisoning. At A3, the snow that fell during the trial allowed rodent tracks to be followed. It was clear that rats were regularly travelling between the hedges and the

main straw stack where some of the bait points were positioned. Taylor (1978) also noted that rats living in hedgerows moved to nearby farmsteads every night in order to feed. The bait-take graph (Figure 2.3) reveals that consumption levels generally increased through the trial period. This could be because either more rats were feeding or the consumption of individuals was going up. When faced with a new food supply, rats have been shown initially to sample the food in small amounts, then progressively to take more as the food becomes familiar (Shepherd and Inglis, 1987; Buckle *et al.*,1986). A small drop in consumption was noted between day 2 and day 6 and again at the end of the trial. This reveals that day to day variation can exist even in the absence of poisons and may be the result of weather or some other influence, perhaps within the population. It is important, therefore, when assessing the effects of poison on a farm population, to look at the end effect as well as day to day changes. In total, 11.7 Kg of non-poison bait was used at the control site A3. This overall consumption figure is quite low and indicates that the population was not very big.

Coumatetralyl was used as the rodenticide at five sites in the east midlands, on A1, A5, A6, A7 and A8 (Figure 2.4 and Table 2.4). Bait trays were placed inside and outside at these sites, wherever rats were active. On A5, the site was first chosen for baiting with brodifacoum, but when the rats failed to use the bait inside, a switch was made to baiting with coumatetralyl outside.

At site A1, the onset of bait consumption was fairly rapid and a peak was achieved within the first week of the trial (613g per day). Bait-take then dropped off over the next week and stabilised at around 200g per day for the next two weeks. A small rise in consumption was observed during the final (5th) week of the trial. About 10 Kg of coumatetralyl bait was used at this site in total. These results indicate that, while moderate levels of control were achieved, there were still some rodents present at the site after 5 weeks.

At A6, a similar pattern of bait consumption was noted, although far less bait was used at this site (1.3 Kg in total). Bait-take had been reduced to virtually nothing by the end of five weeks, indicating that the target rodents had more or less stopped feeding. This implies that they had been effectively controlled.

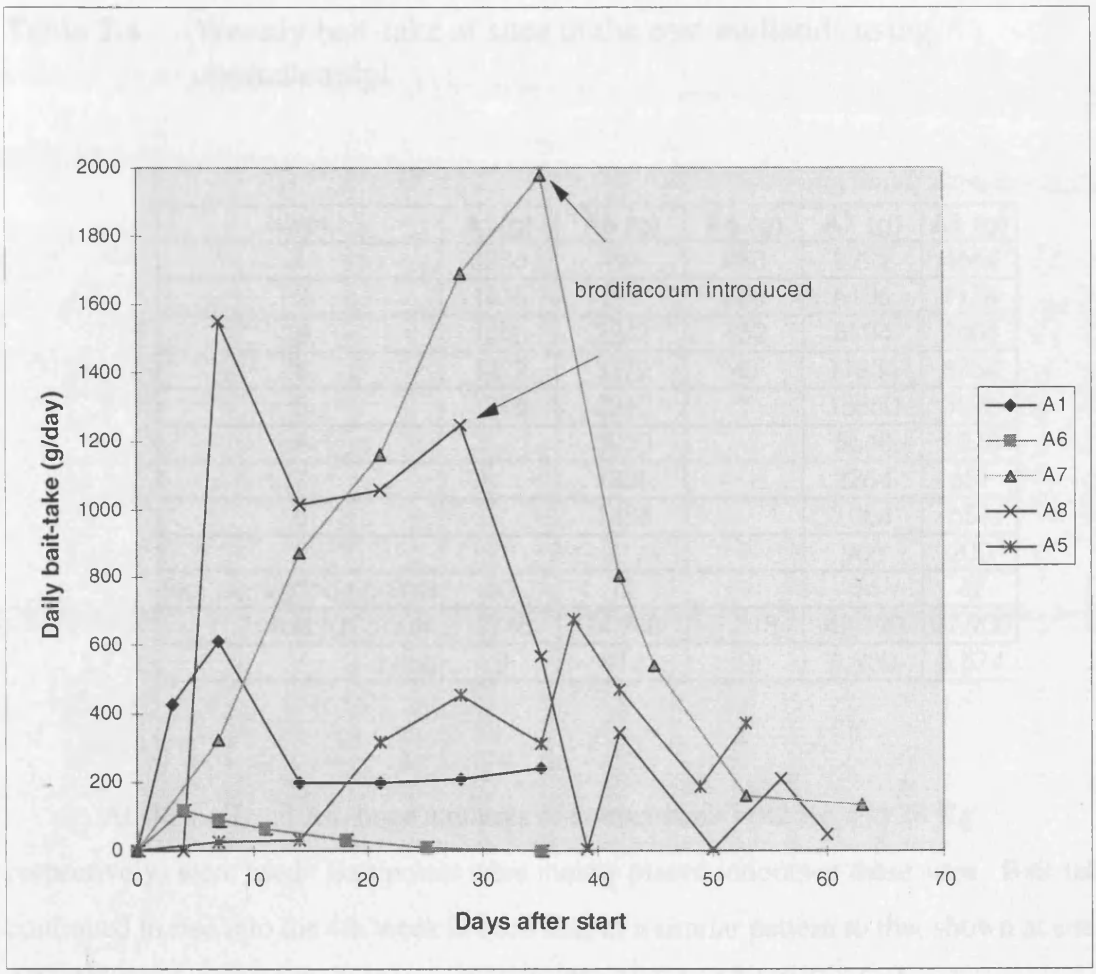



Figure 2.4 Daily bait-take from coumatetralyl sites in the east midlands

At site A5, bait-take was very low in the first two weeks and yet rats were notably abundant. Indoor baiting with brodifacoum was clearly unsuccessful because of all the stored food already present in the buildings (this was an animal-feed mill). Outdoor bait points of coumatetralyl were therefore positioned at points around the buildings and other parts of the yard area. The change was made at 14 days. Usage of coumatetralyl was immediate and frequent. Bait-take rose for 2 weeks before a small drop, then a further rise was measured until a peak at 38 days with 680 g per day taken. After this, a decline was observed until day 49, followed by a further rise until the end of the trial on day 53. Bait-take never approached a negligible level. In total, 400g of brodifacoum and 14.3 Kg of coumatetralyl were used at this site.

Table 2.4 Weekly bait-take at sites in the east midlands using coumatetralyl

| week | A1(g) | A5 (g) | A6 (g) | A7 (g) | A8 (g) |
|---|---|---|---|---|---|
| 1 | 3736 | 194 | 750 | 2292 | 4644 |
| 2 | 1418 | 218 | 353 | 6106 | 7114 |
| 3 | 1406 | 2242 | 162 | 8104 | 7406 |
| 4 | 1472 | 3172 | 43 | 11836 | 8734 |
| 5 | 1716 | 2210 | 7 | 13850 | 3996 |
| 6 | | 3930 | | 5648 | 1078 |
| 7 | | 1306 | | 2264 | 651 |
| 8 | | 1488 | | 1054 | 651 |
| 9 | | | | 966 | 200 |
| No. active bait points | 60 | 12 | 9 | 54 | 42 |
| Totals (g) coum | 9,748 | 14,348 | 1,315 | 42,190 | 27,900 |
| brod | 0 | 412 | 0 | 9,930 | 6,574 |

At sites A7 and A8, huge amounts of coumatetralyl (42 Kg and 28 Kg respectively) were used. Bait points were mostly placed indoors at these sites. Bait-take continued to rise into the 4th week at each site, in a similar pattern to that shown at site A3 where non-poisoned wheat was the bait. Despite the high bait-take levels, rats were still very abundant four weeks into the trial. No traps were available to catch the rats, so an alternative was needed to achieve effective control. All the coumatetralyl was collected in and bait trays containing unmarked (i.e. containing no hexachlorobiphenyl) brodifacoum were placed throughout the buildings instead. Bait-take was still monitored until the end of nine weeks, at which point the trials were ended. The point at which the switch was made at each site is shown in Figure 2.4. Site A7 showed a consistently high increase in bait usage up to 35 days into the trial when brodifacoum replaced the coumatetralyl. Thereafter, a rapid decline was observed until consumption levelled out at about 140 g per day at the end of the 9 weeks. In addition to the coumatetralyl eaten, about 10 Kg of brodifacoum was used at A7.

At A8, no bait had been taken within the first four days. Thereafter there was a sudden and massive onset of consumption to a peak at seven days of more than 1.5 Kg per day. Bait-take then dropped off, but later began to rise to reach a further peak of about 1.2 Kg per day at four weeks into the trial. For the next five weeks, brodifacoum

was used instead of coumatetralyl. Bait-take plummeted to near zero and then fluctuated between near zero and about 250 g per day for the rest of the trial. In addition to the coumatetralyl bait eaten, 6.5 Kg of brodifacoum bait was consumed at A8.

Brodifacoum alone was used as the rodenticide at 4 farms in the east midlands, on A2, A4, A5 and A9 (Figure 2.5 and Table 2.5). At all these farms, the bait trays were placed indoors only.

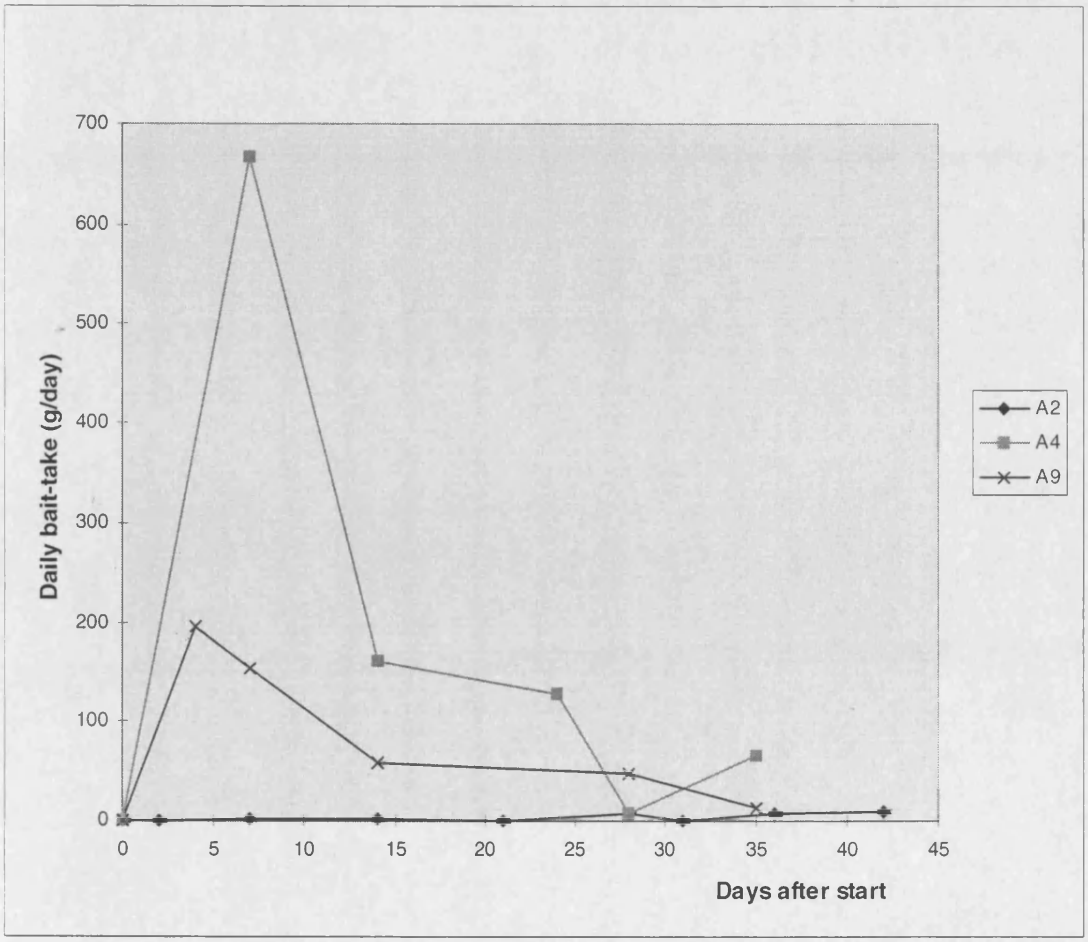



Figure 2.5 Daily bait-take from brodifacoum sites in the east midlands

On A2, the uptake of bait was very slow, with virtually no take at all until the 4th week. After that, only small amounts were taken, until after 6 weeks just 155g had been used in total. This result indicates that there was a problem with achieving satisfactory bait consumption and hence control at this site.

Rats rapidly took the bait at A4. Within the first week, 4.7 Kg had been used. An equally rapid decline in take ensued for the next few days and then bait-take was reduced to virtually nothing by the end of four weeks. Over the final week of the trial, there was a noted re-usage of the bait stations, with consumption measured at about 65g per day. A total of 7.6 Kg of brodifacoum was used at this site.

The onset of bait-take was equally rapid at site A9, with a peak in consumption being reached in just four days. Bait-take then steadily dropped to just over 10 g per day at the end of five weeks. The bait-take total was measured at 2.4 Kg.

Table 2.5 Weekly bait-take from sites in the east midlands where brodifacoum was used.

| week | A2 (g) | A4 (g) | A9 (g) |
|---|---|---|---|
| 1 | 4 | 4675 | 1246 |
| 2 | 4 | 1120 | 414 |
| 3 | 2 | 896 | 334 |
| 4 | 56 | 414 | 334 |
| 5 | 28 | 460 | 88 |
| 6 | 61 | | |
| No. active bait points | 3 | 61 | 29 |
| Totals (g) coum | 0 | 0 | 0 |
| brod | 155 | 7,565 | 2,416 |

The total poison-consumption levels at the experimental sites are shown in Figure 2.6. The mean value for all the farms in this region using coumatetralyl was 19.10 Kg and for those using brodifacoum, including A7 and A8 it was 5.33 Kg. It would therefore appear that coumatetralyl was eaten in greater quantities than brodifacoum. A Mann-Whitney Test was applied to the values for total bait-take per active bait point at each site in the east midlands. (This test was chosen because the bait-take values did not show a normal distribution.) The Mann-Whitney Test showed that consumption of bait per active bait point on coumatetralyl sites was significantly higher ($p= 0.0008$) than on brodifacoum sites. This significant difference must be a result of treatment alone as rodent density was not considered when farms were allocated to a treatment.

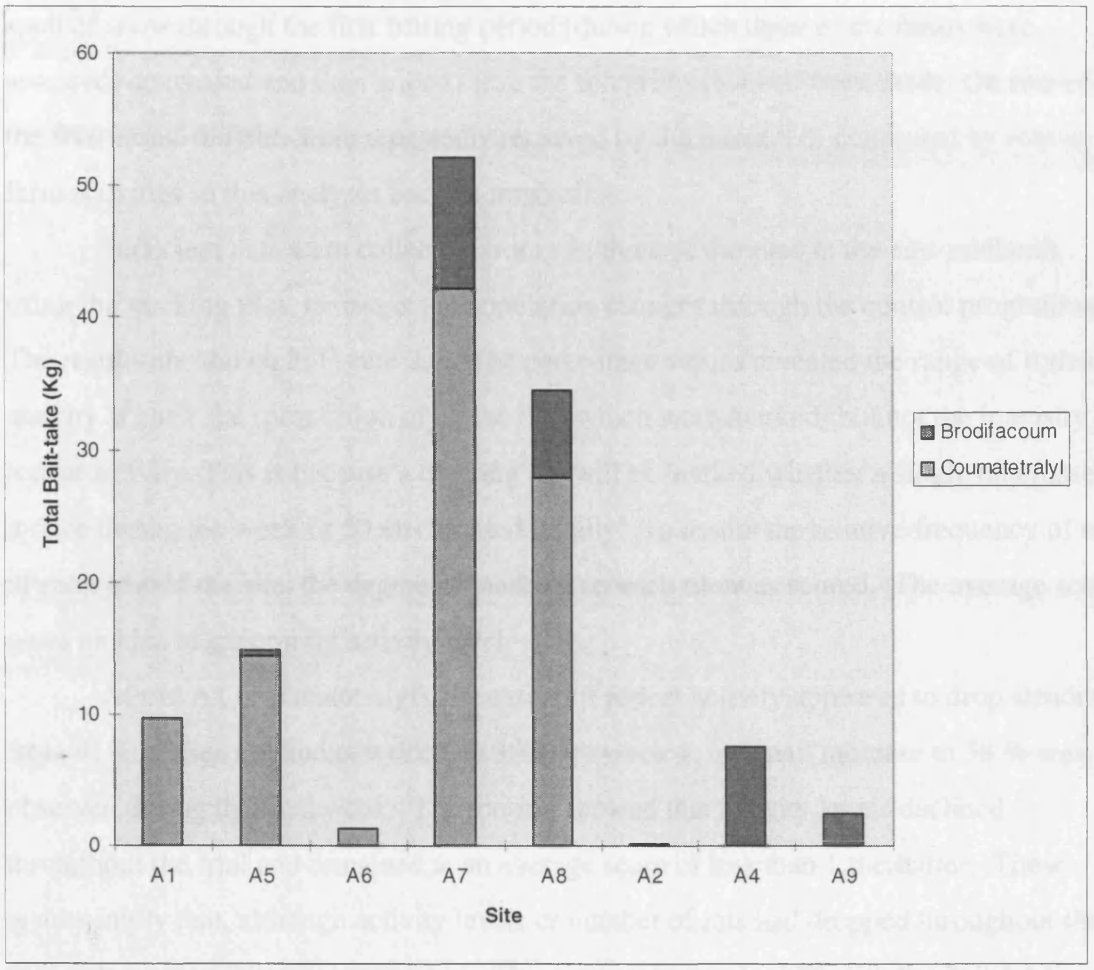



Figure 2.6 Total consumption of bait from farms in the east midlands

## 2.3.ii Population changes as the trials progressed

General observations on rat movements and abundance were made during all the trials. Snow, which fell during some of the trials, facilitated observations of movement as footprints became clearly visible. In addition, new excavations and use of different parts of a site were all noted. These observations proved helpful for understanding the outcome of the control treatments.

Tracking tiles were used on five of the farms in the east midlands to give an estimate of rodent abundance as the control programmes developed. This technique gave some problems: Firstly, the clarity of the tracks was often not good and so it was difficult to distinguish between prints left by target and non-target animals. Secondly, a prolonged

spell of snow through the first baiting period (during which three of the farms were assessed) concealed and then wiped clean the footprints that had been made. On two of the five farms, the tiles were repeatedly removed by the farmers or concealed by routine farm activities so that analyses became impossible.

Sufficient data were collected, from just three of the sites in the east midlands using the tracking tiles, to assess the population changes through the control programmes. The results are shown in Figure 2.7. The percentage values revealed the range of rodent activity at each site (proportion of all the tiles which were marked) but not the intensity of rodent activity. This is because a tracking tile will be marked whether a single rat crossed it once during the week or 50 rats crossed it daily. To assess the relative frequency of use of each part of the site, the degree of marking on each tile was scored. The average score gives an idea of general rat activity level.

At site A1 (coumatetralyl), the extent of rodent activity appeared to drop steadily from 91% of tiles marked at week 2 to 30 % by week 4. A small increase to 38 % was observed during the final week The scoring showed that activity levels declined throughout the trial and remained at an average score of less than 1 thereafter. These results imply that, although activity levels or number of rats had dropped throughout the trial, rats were still present at the end. This appears to correspond with the bait-take patterns observed.

At site A2 (brodifacoum), use of the area fell to start with but then increased to reach a peak of activity during the 4th week. About 2/3 of the tiles were marked at the end of the trial compared to 1/3 at the start. The score values, however, show that the level of activity was never very high. These tracking tile results correlate with the data collected for bait consumption and show that there is no evidence that rats were controlled effectively at this site.

At the control site, A3 (no poison), tracking tile data revealed an increase in use of the site throughout the trial, with both the extent and the frequency of activity going up. This again corresponds to the bait-take patterns seen.

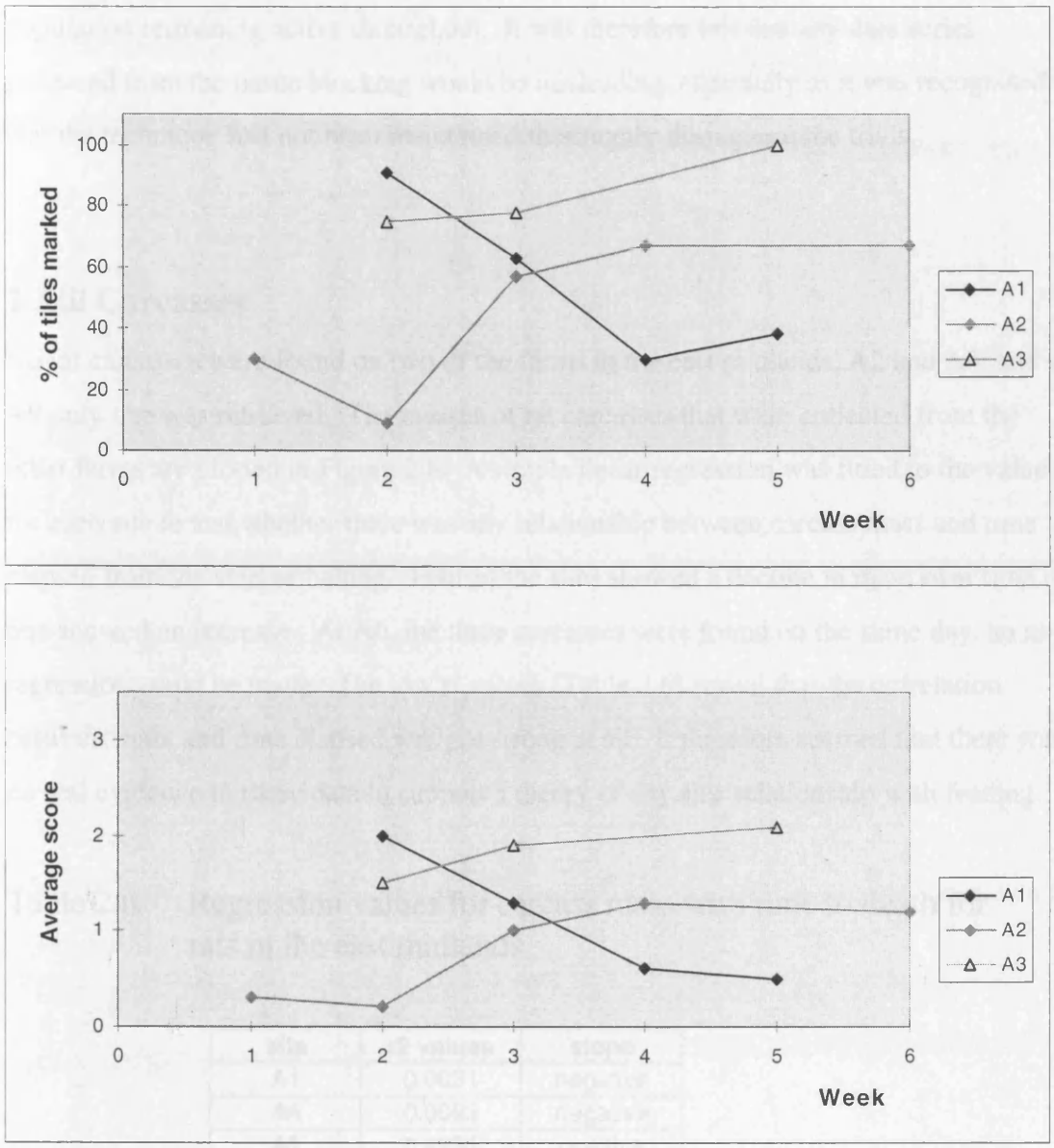



Figure 2.7 Tracking tile results from three farms in the east midlands; A1 = coumatetralyl, A2 = brodifacoum, A3 = no poison

Hole blocking with tissues was also used on some of the farms to indicate rat activity. This method did not work satisfactorily. The large size of the farms and the complexity of the habitat meant that not all the holes in the area were found. The data collected appeared to show no correlation to rat activity in the area. On some sites, the same number of holes were used throughout the trial despite a decline in bait-take and tracking tile marking. On other sites, hole unblocking was very erratic despite the

population remaining active throughout. It was therefore felt that any data series produced from the tissue blocking would be misleading, especially as it was recognised that the technique had not been maintained thoroughly throughout the trials.

## 2.3.iii Carcasses

No rat carcasses were found on two of the farms in the east midlands, A2 and A3, and on A9 only one was retrieved. The masses of rat carcasses that were collected from the other farms are plotted in Figure 2.8. A simple linear regression was fitted to the values for each site to test whether there was any relationship between carcass mass and time elapsed from the start of baiting. Four of the sites showed a decline in mass over time and one showed an increase. At A6, the three carcasses were found on the same day, so no regression could be made. The low $r^2$ values (Table 2.6) reveal that the correlation between mass and time elapsed was not strong at all. It therefore seemed that there was no real evidence in these data to support a theory of any size relationship with feeding.

Table 2.6 Regression values for carcass mass with time to death for rats in the east midlands

| site | r2 values | slope |
|---|---|---|
| A1 | 0.0021 | negative |
| A4 | 0.0093 | negative |
| A5 | 0.4935 | positive |
| A7 | 0.3106 | negative |
| A8 | 0.0139 | negative |

The carcass mass data show a wide spread of mass with juveniles, sub-adults and adults represented. Only one rat weighed more than 500g. A one way analysis of variance showed that there was no significant difference between carcass masses from sites using coumatetralyl and those using brodifacoum ($p=0.789$).

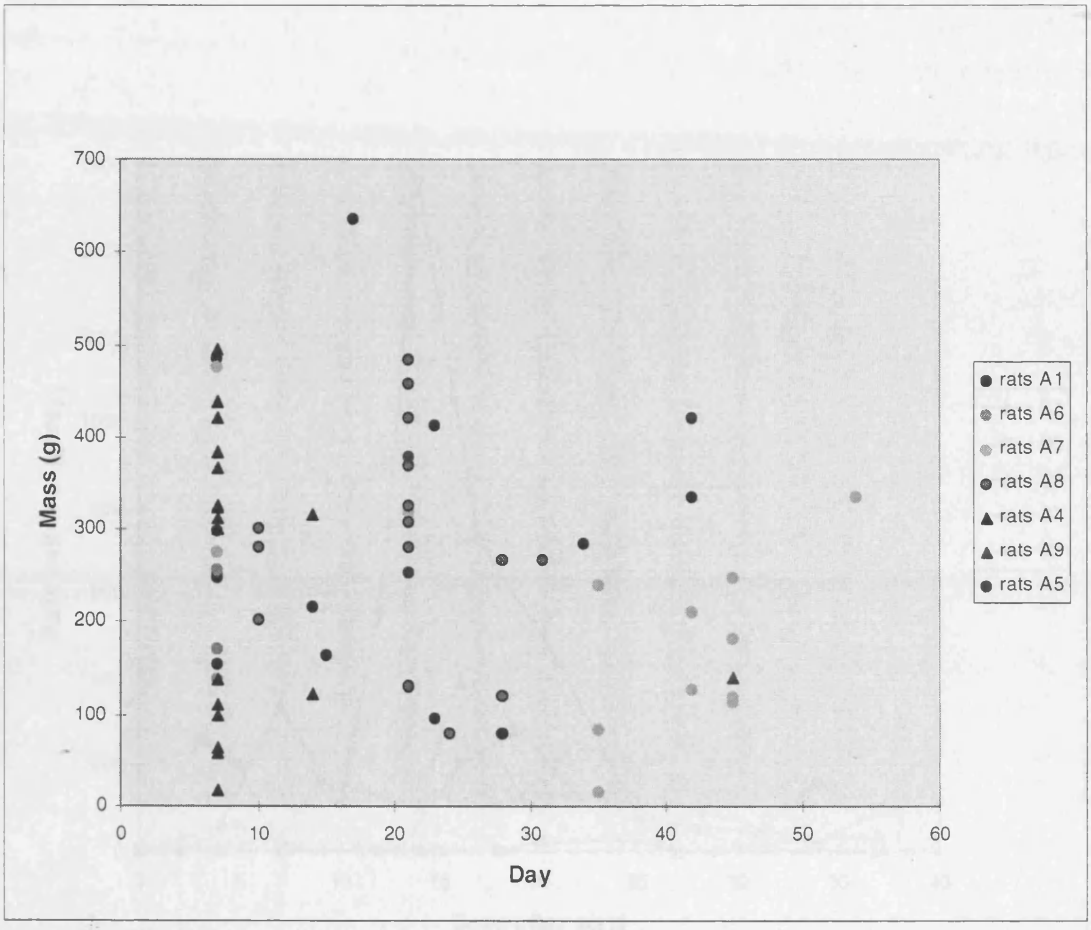



Figure 2.8 Masses of rats found dead on farms in the east midlands Coumatetralyl sites (•) Brodifacoum sites (▲)

Mouse carcasses were found and collected from sites A1, A4, A7, A8 and A9. Some of the rats killed by terriers at site A5 were also collected for residue analyses and their mass data are shown in Figure 2.15.

### 2.3.iv Bait-take by target species in central southern England

Figures 2.9 and 2.10 show the bait consumption patterns for both rodenticides at the sites in central southern England. On five farms brodifacoum was used as the rodenticide and baiting was carried out indoors only (B4, B5, B6, B7 and B8). The bait-take results are shown in Figure 2.9 and Table 2.7.

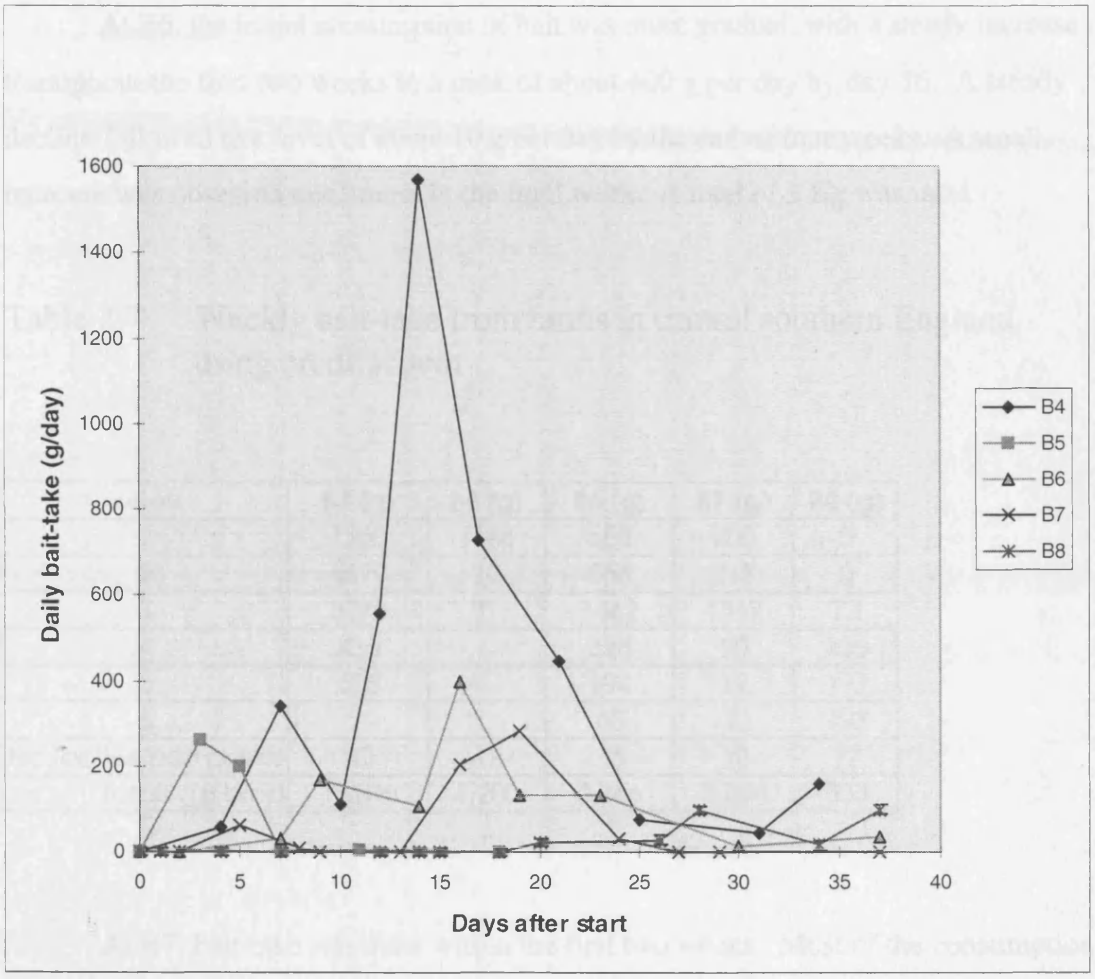



Figure 2.9 Daily bait-take from brodifacoum sites in the central southern England

At B4, bait-take started within a few days and a peak of 1.6 Kg per day was reached by day 14. Thereafter, bait consumption dropped rapidly to just 45 g per day by the end of four weeks. In the final week of the trial, a small rise in bait-take was noted. A total of 10.8 Kg of brodifacoum was eaten at this site. It would appear that the majority of the rat population at this site was effectively controlled, but a few individuals remained at the end of the trial period.

At site B5, initial bait-take was also rapid and a peak was achieved by day 3. The amount eaten then dropped to nil by the end of two weeks. There was then no further bait-take through the trial. Just 1.2 Kg of bait was used in total.

At B6, the initial consumption of bait was more gradual, with a steady increase throughout the first two weeks to a peak of about 400 g per day by day 16. A steady decline followed to a level of about 10 g per day by the end of four weeks. A small increase was observed once more in the final week. A total of 3 Kg was used.

Table 2.7 Weekly bait-take from farms in central southern England using brodifacoum

| week | B4 (g) | B5 (g) | B6 (g) | B7 (g) | B8 (g) |
|---|---|---|---|---|---|
| 1 | 1250 | 1188 | 150 | 200 | 0 |
| 2 | 4579 | 12 | 866 | 218 | 0 |
| 3 | 3976 | 0 | 1462 | 1312 | 72 |
| 4 | 435 | | 326 | 90 | 336 |
| 5 | 605 | | 194 | 12 | 197 |
| 6 | | | 68 | 2 | 198 |
| No. active bait points | 40 | 12 | 15 | 10 | 12 |
| Totals (g) brod | 10,845 | 1,200 | 3,066 | 1,834 | 803 |

At B7, bait-take was slow within the first two weeks. Most of the consumption occurred over the next two weeks, with a peak at around 19 days. By the end of four weeks, bait-take had stopped, although a very small amount of bait was eaten in the last few days of the trial. 1.8 Kg of bait was used at this site.

At B8, the onset of bait-take was very slow. Nothing was eaten at all for the first 18 days. After that, small, localised feeding was observed, which continued until the end of the trial. Only 800 g of bait was taken from this site over the five week period.

Coumatetralyl was used at four of the sites in southern England; B1, B2, B3 and B9 (see Figure 2.10 and Table 2.8). At B1, bait-take was low throughout the trial. A small peak was noted on day 7. Thereafter, bait consumption gradually dropped to reach zero by day 48. Total bait-take at this site was 4.6 Kg.

At B2 the onset of bait consumption was rapid. Bait-take rose sharply for the first week before fluctuating at a level of about 1.6 Kg of bait eaten each day for the rest of the trial. More than 36 Kg of bait was used at this site. Control of the rodent population at this site was clearly not achieved.

On site B3, the initial increase in bait-take was more gradual, but there was then a rapid increase over the third week to reach a peak of about 2.1 Kg per day. The level of bait-take then dropped slightly, although it remained high for the rest of the trial. About 27 Kg of bait was used altogether. Again, the rats at this site had clearly not been removed by the rodenticide treatment.

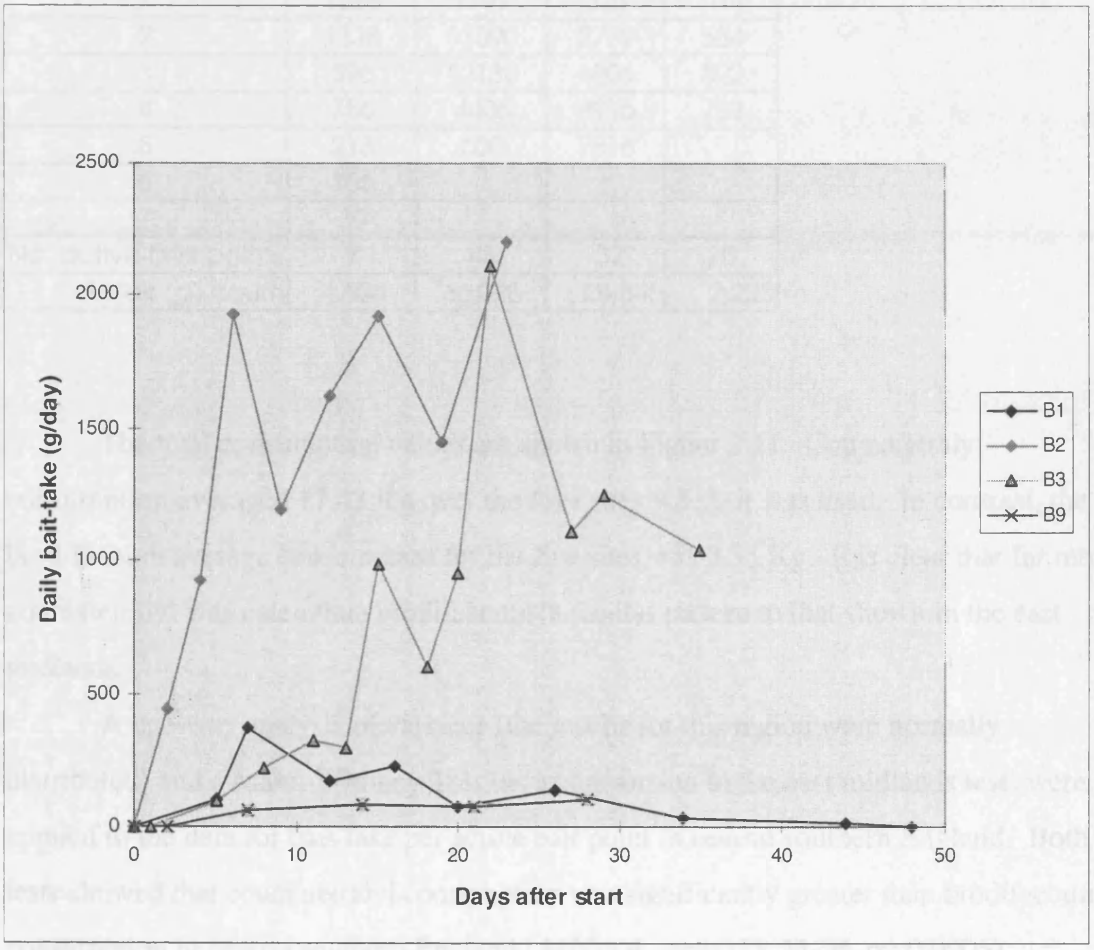



Figure 2.10 Daily bait-take from coumatetralyl sites in the central southern England.

On B9, bait-take very gradually increased to reach a value of about 100 g per day by the end of four weeks. Total bait consumption was measured at 2.2 Kg.

Table 2.8 Weekly bait-take from farms in central southern England using coumatetralyl. Saturation baiting was not maintained at site B2 after the third week, and this explains the apparent drop in bait consumption.

| week | B1 (g) | B2 (g) | B3 (g) | B9 (g) |
|---|---|---|---|---|
| 1 | 1268 | 7799 | 828 | 414 |
| 2 | 1338 | 11090 | 2779 | 584 |
| 3 | 896 | 12130 | 6806 | 522 |
| 4 | 756 | 4406 | 8916 | 708 |
| 5 | 213 | 600 | 7516 | |
| 6 | 105 | | | |
| 7 | 30 | | | |
| No. active bait points | 7 | 48 | 32 | 20 |
| Totals (g) coum | 4,606 | 36,025 | 26,845 | 2,228 |

The total consumption values are shown in Figure 2.11. Coumatetralyl consumption averaged 17.43 Kg over the four sites where it was used. In contrast, the brodifacoum average consumption for the five sites was 3.55 Kg. It is clear that far more coumatetralyl was eaten than brodifacoum, a similar pattern to that shown in the east midlands.

A one-way analysis of variance (the results for this region were normally distributed) and a Mann-Whitney Test (as a comparison to the east midlands test) were applied to the data for bait-take per active bait point in central southern England. Both tests showed that coumatetralyl consumption was significantly greater than brodifacoum consumption in central southern England (ANOVA, $p=0.025$; M-W, $p= 0.0003$).

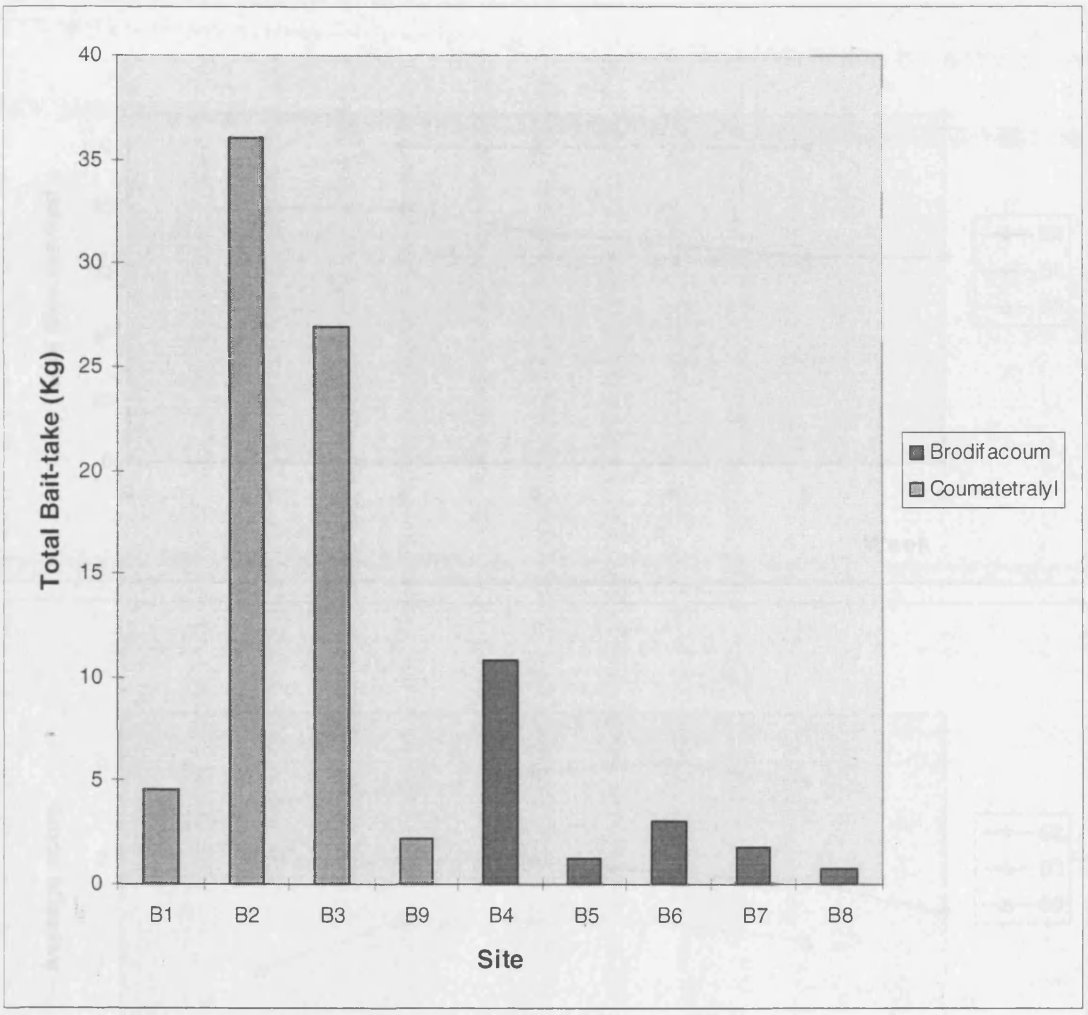



Figure 2.11 Total bait consumption from farm sites in central southern England

## 2.3.v Population changes as the trials progressed

At three of the coumatetralyl sites in this region, B2, B3 and B9, tracking tiles were used to show rodent activity levels through the trials. The results are displayed in Figure 2.12. At B2, the percentage values reveal that activity started at around 80% and gradually dropped through the trial to end at 65%. Score values also remained high throughout the trial. These data show that rats were still very active at the site by the end of the trial and therefore control had not been achieved. This corresponds to the bait-take measurements, which showed high consumption throughout the trial.

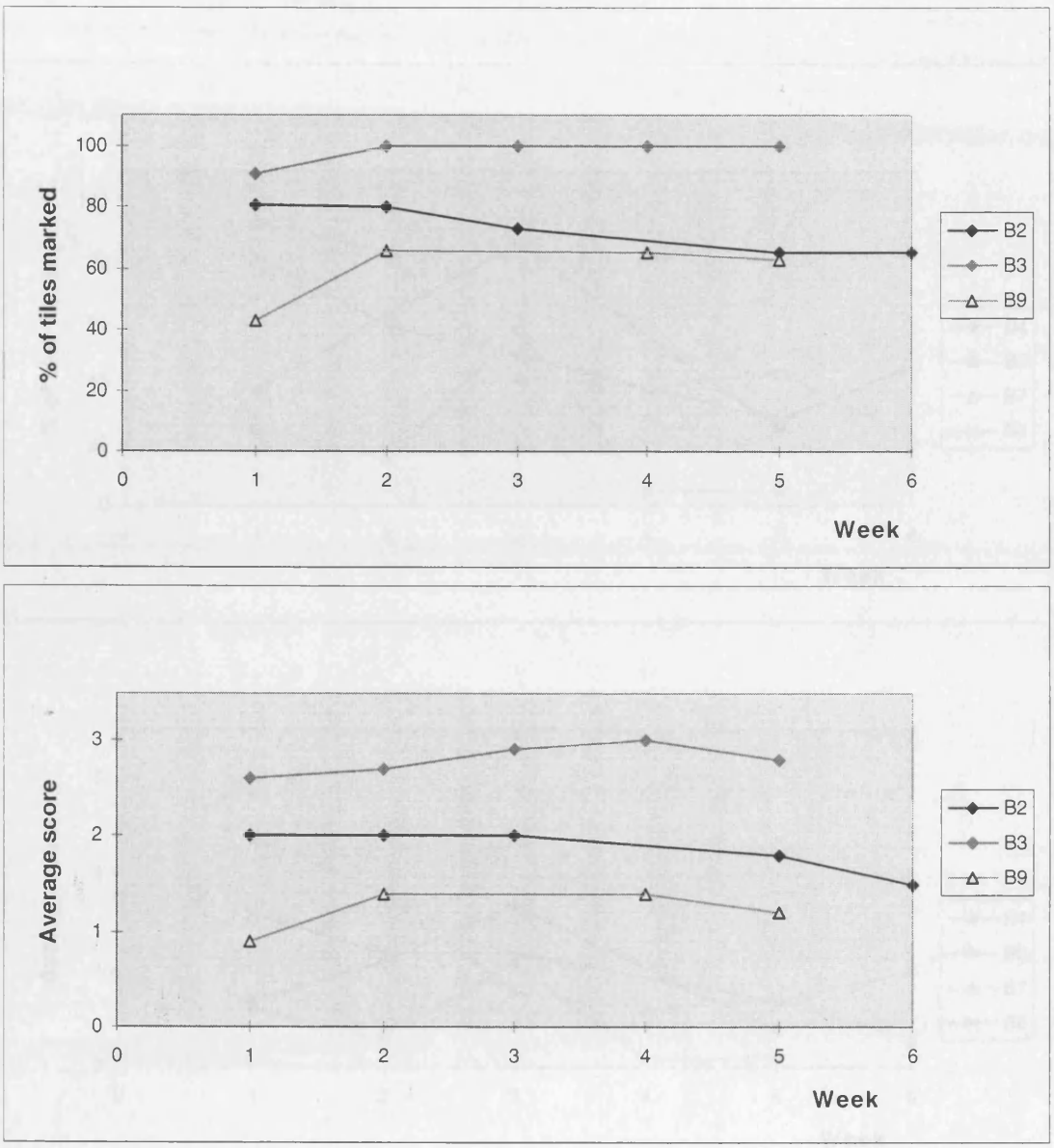



Figure 2.12 Tracking tile results from three farms in central southern England where coumatetralyl was used.

At B3, activity started at 91% and actually rose in the first two weeks of the trial to remain at 100% for the rest of the time. Score values were also quite high at all stages in the trial. These results match the evidence shown from the bait-take measurements; namely that rat activity remained high through the trial and control was not achieved.

At B9, activity rose within the first week and then remained at about 65% and a score average of about 1.4 for the rest of the trial. So at this site also, control was not achieved. This observation supports the bait-take data measurements.

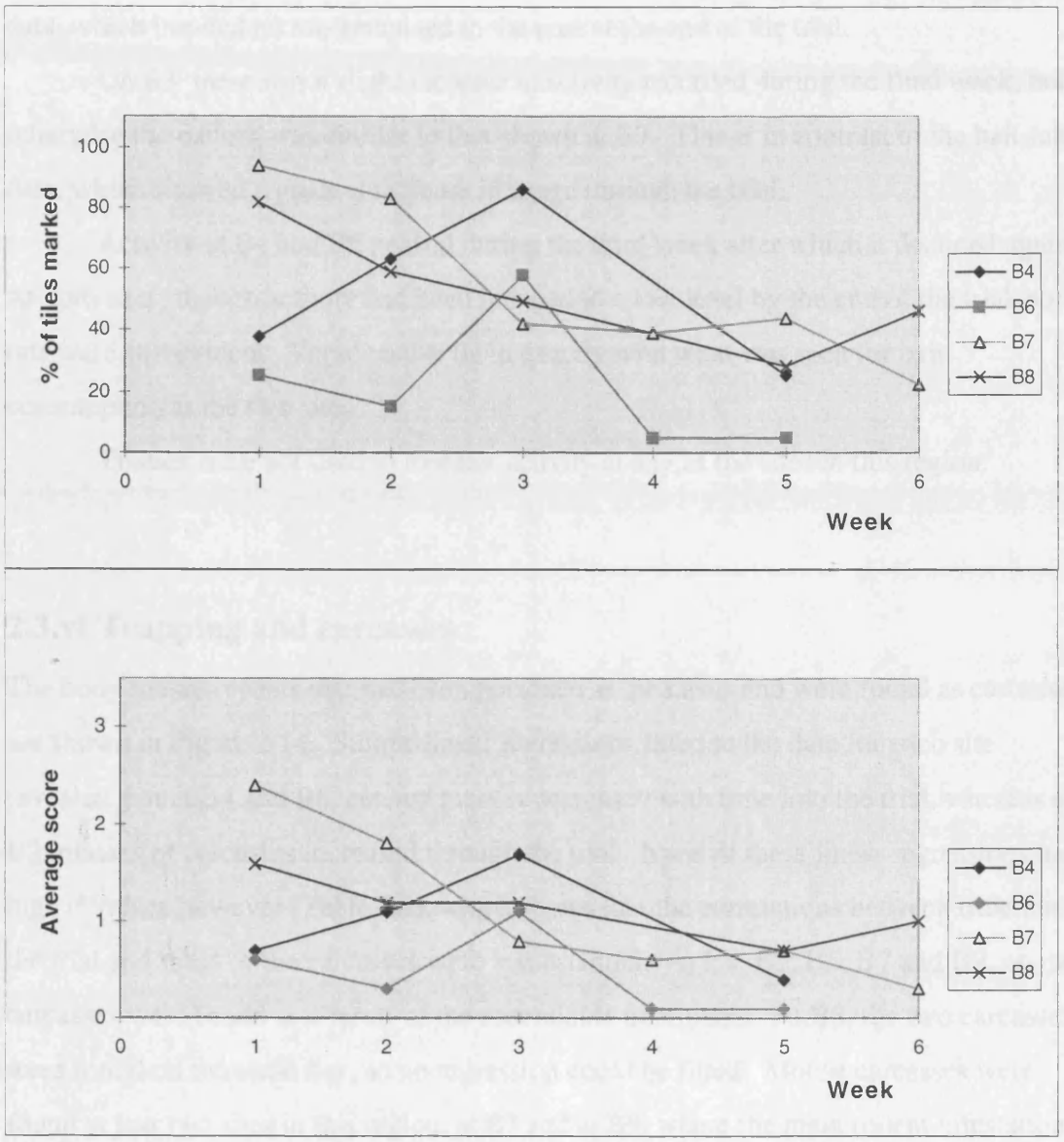



Figure 2.13 Tracking tile results from four farms in central southern England where brodifacoum was used.

Four of the brodifacoum sites in this region were monitored using tracking tiles (see Figure 2.13). At B7, activity levels were high at the start of the trial but steadily dropped over the six week period. By the end of the trial, 22% of the tiles were marked and the average score was 0.3. This implies that there were some rats still present, but

general activity levels were low. These results differ to some extent from the bait-take data, which implied no rats remained in the area at the end of the trial.

On B8 there was a slight increase in activity recorded during the final week, but otherwise the pattern was similar to that shown at B7. This is in contrast to the bait-take data, which showed a gradual increase in usage through the trial.

Activity at B4 and B6 peaked during the third week after which it declined again. At both sites, though activity had been reduced to a low level by the end of the trial, some rats were still evident. These results tie in exactly with what was seen for bait consumption at the two sites.

Tissues were not used to monitor activity at any of the sites in this region.

## 2.3.vi Trapping and carcasses

The body masses of rats that had been poisoned at the farms and were found as carcasses are shown in Figure 2.14. Simple linear regressions fitted to the data for each site revealed that at B4 and B6, carcass masses decreased with time into the trial, whereas at B2, masses of carcasses increased through the trial. None of these linear regressions had high $r^2$ values however (Table 2.9), which shows that the correlations between time into the trial and mass of the carcasses were insubstantial. At B1, B3, B5, B7 and B9, no rat carcasses were found as a result of the rodenticide treatments. At B8, the two carcasses were found on the same day, so no regression could be fitted. Mouse carcasses were found at just two sites in this region, at B7 and at B9, where the main rodent infestation involved mice.

Trapping was carried out at sites B1, B2, B3, B8 and B9, all of which showed evidence that the rodenticide treatment was not achieving control of the rodents. The trapping at B9 involved mice, rather than rats. Trapping was started at any stage after about three weeks and was continued until the end of the trial period. The body mass data for rats caught by trapping are shown in Figure 2.15.

It can be seen from Figure 2.14 that there was a paucity of rats found dead that had a mass between 150g and 250g. There were also only three rat carcasses that were heavier than 350g. These results are in contrast to both the data from the east midlands and the data for trapped-rat masses from central southern England (Figure 2.15). There

were many rats that were trapped, especially at sites B2 and B3, that weighed more than 400g. An analysis of variance (general linear model) was used to compare trapped-rat masses with carcass masses for data within central southern England. The tests showed that there was no significant difference between mass of rats found dead or trapped on coumatetralyl sites compared to brodifacoum sites (p=0.560) and trapped rats were not significantly heavier than rats found as carcasses (p=0.486). There was also no interactive significance (p=0.741). It would therefore seem apparent that trapping does not target rats that are significantly different in weight to rats that succumb to poisoning.

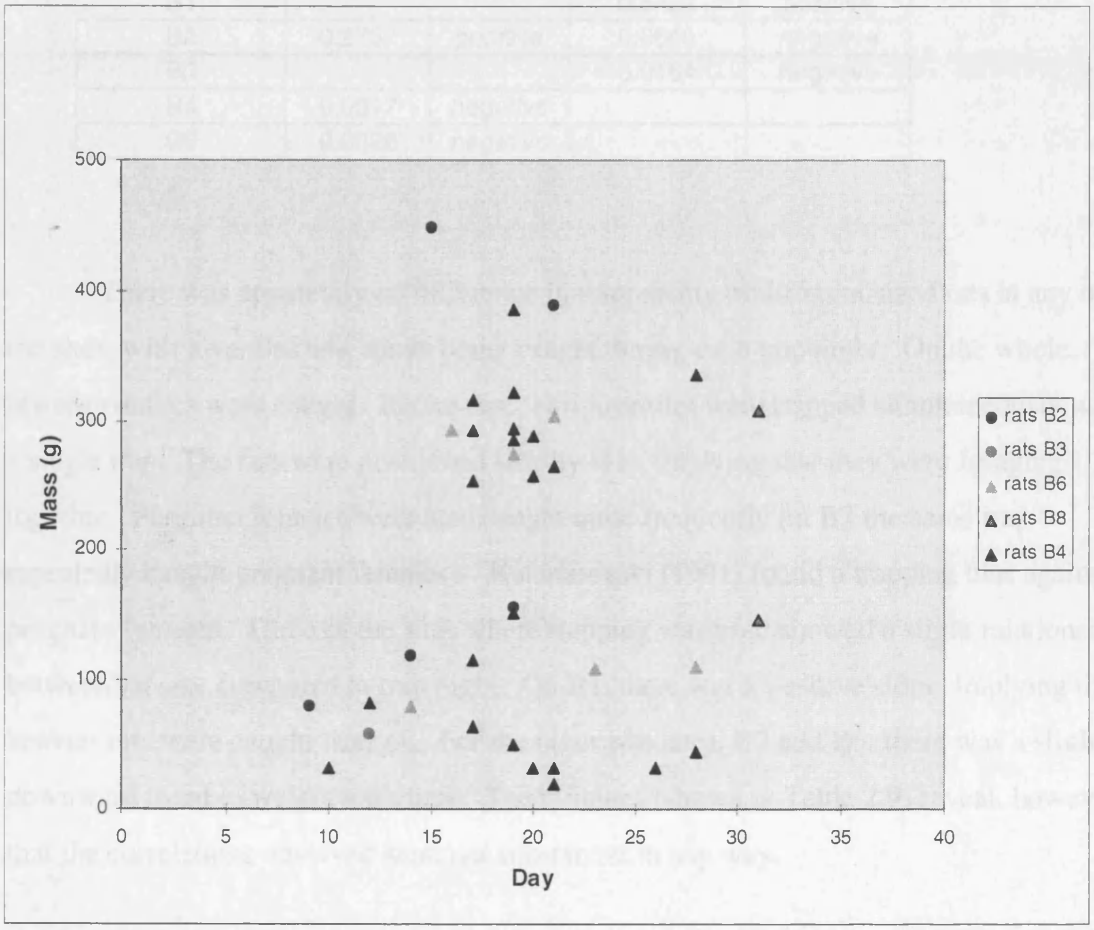



Figure 2.14 Masses of rats found dead on farms in the central southern England.Coumatetralyl sites (•) and Brodifacoum sites (▲)

A comparison of rat masses in the two regions was also made using an analysis of variance general linear model. It was found that rat mass in the east midlands did not differ significantly from rat mass in central southern England (p=0.928).

Table 2.9 Regression values for rat weights by trapping and carcasses found on farms in central southern England.

| site | r2 values poisoned | slope | r2 values trapped | slope |
|---|---|---|---|---|
| B1 | | | 0.5422 | positive |
| B2 | 0.2737 | positive | 0.0645 | negative |
| B3 | | | 0.0164 | negative |
| B4 | 0.0017 | negative | | |
| B6 | 0.0526 | negative | | |

There was apparently no difference in trappability of different sized rats at any of the sites, with juveniles and adults being caught during each trap night. On the whole, fewer juveniles were caught. In one case, two juveniles were trapped simultaneously with a single trap. The rats were positioned side by side, implying that they were foraging together. Pregnant females were also caught quite frequently (at B2 the same trap repeatedly caught pregnant females). Kataranovski (1991) found a trapping bias against pregnant females. Three of the sites where trapping was used showed a slight relationship between rat size compared to trap night. On B1, there was a positive slope, implying that heavier rats were caught later on. For the other two sites, B2 and B3, there was a slight downward trend in weight with time. The $r^2$ values (shown in Table 2.9) reveal, however, that the correlations observed were not substantial in any way.

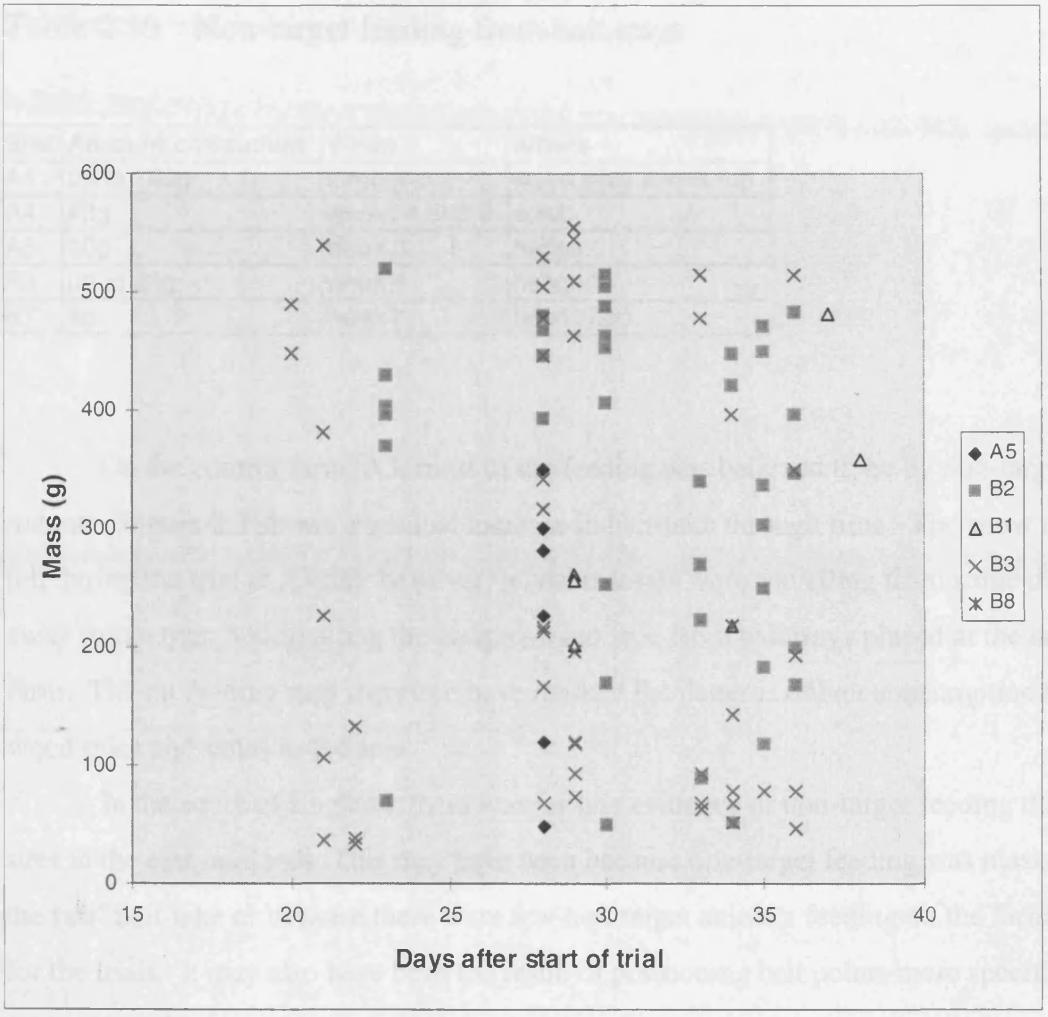



Figure 2.15 Body masses of rats caught by trapping at four farms in southern England and of rats caught by terriers at one site in the east midlands (A5).

## 2.3.vii Non-target use of baiting areas

It was possible to determine whether non-target rodents, especially wood mice, had been eating the bait because they leave the husks of grain and also droppings in the bait trays. Non-target bait-take was recorded when such signs were evident (Table 2.10). It was, however, not easy to estimate the amount of bait that had been consumed by non-targets, because usually the bait trays involved also had rats removing the grain. A descriptive result, rather than a quantitative one, is therefore given for the places where non-target feeding was observed.

Table 2.10 Non-target feeding from bait trays

| Site | Amount consumed | When | Where |
|---|---|---|---|
| A1 | up to 120g | throughout | wood piles and scrub |
| A4 | 43g | weeks 4 and 5 | scrub |
| A5 | 10g | week 3 | hedge |
| B3 | up to 20g | week 4 | hedge |
| B7 | 8g | week3 | open shed |

On the control farm, A3, most of the feeding was believed to be by non-target rodents. Figure 2.3 shows a gradual increase in bait-take through time. The snow that fell during the trial at A3 did, however, reveal that rats were travelling from some distance away (more than 500m) along the hedgerows to feed from bait trays placed at the derelict farm. The rat feeding may therefore have masked the patterns of bait consumption by wood mice and voles in the area.

In the south of England, there was far less evidence of non-target feeding than at sites in the east midlands. This may have been because non-target feeding was masked by the rats' bait-take or because there were few non-target animals feeding on the farms used for the trials. It may also have been the result of positioning bait points more specifically for rats as experience was gained through the study.

The Longworth trapping results are shown in Table 2.11. Trapping was carried out during the week prior to the start of baiting and for a further week at the end of the trial at each site that was used. Three sites were used in the east midlands; A1 was a coumatetralyl site, A2 was a brodifacoum site and A3 was the control (no poison) site. In central southern England, only one wood mouse was trapped prior to the trial at site B4, despite there being a vast amount of suitable small mammal habitat at the site. This site was therefore not used for further investigation of small mammal populations. Sites B7 and B8 were farms where brodifacoum was used and site B9 was a coumatetralyl farm.

Numbers of small mammals at all of the sites were quite low with a high proportion of recaptures occurring. This is a common observation on farmsteads (Cox, 1991). At all sites, vole (both field and bank) numbers were especially low. It may therefore be misleading to ascertain patterns of population change relating to baiting treatments with such small samples. Wood mice were caught in much greater numbers on

the whole. At all sites except A1 (where baiting was done outdoors) and B8 (where baiting was done indoors), numbers after the trial were higher than before. There were no obvious detrimental effects on the farms except for A1 where only 1 small mammal was trapped in 192 trap nights after the trial.

Table 2.11 Small mammal population estimates from farms in the east midlands (A) and in central southern England (B) before and after rodenticide treatment.

| Site | No. Traps | Ist session | Wood mice | Voles | 2nd session | Wood mice | Voles |
|---|---|---|---|---|---|---|---|
| A1 | 48 | early Feb | 7 | 4 | end March | 0 | 1 |
| A2 | 58 | early Feb | 9 | 3 | end March | 13 | 0 |
| A3 | 40 | early Feb | 6 | 5 | end March | 25 | 6 |
| B4 | 60 | early May | 1 | 0 | | | |
| B7 | 18 | early March | 6 | 1 | mid April | 12 | 0 |
| B8 | 40 | early March | 17 | 0 | mid April | 12 | 1 |
| B9 | 26 | early March | 6 | 1 | mid April | 11 | 2 |

Mann-Whitney tests were performed on the wood mouse data to compare population estimates before and after treatment on coumatetralyl farms and on brodifacoum farms. The Mann-Whitney tests revealed that there was no significant difference between population estimates before and after the trials on either coumatetralyl or on brodifacoum sites. The relative changes shown in Table 2.11 may, therefore, be indicative of how rodenticide treatments affect non-target small mammal populations, but at the low sample numbers used, no detrimental patterns can be proved. This result is in contrast to that shown by Cox (1991) where wood mice were found to be adversely affected by rodenticide treatments outdoors.

Other species that were caught included the common shrew (all sites), pygmy shrew (A2) and a single harvest mouse (A2). No yellow-necked mice were caught. On both sites A1 and B9, house mice were frequently trapped. House mice were the most common small mammal species to be trapped at A1 during the second trap session (after the trial). House mice were found only within the farm buildings during the first trap session, but were trapped in scrub and grassland up to 200m from the nearest building during the second trap session. Tattersall (1992) suggested that house mice may be

confined to buildings as a result of competition with wood mice. The lack of wood mice and voles caught during the second trap session may account for the sudden spread of house mice outside the buildings.

There were a few carcasses recovered that had been accidentally poisoned or trapped at the sites used for this study; a hooded crow was found with haemorrhaging from the beak at site B3. However, this occurred on only the 5th day of the trial and none of the bait points appeared to be accessible. There was no certainty that anticoagulant poisoning was the cause of death. A thrush was found at B4 at the end of the trial. The cause of death was unknown, but the bird was removed for *post mortem* analyses. A wood mouse was found dead in a Longworth trap at B8. Food in the trap was still plentiful, so poisoning was suspected. However, later analyses showed that this was not the case.

Trapping also caused non-target casualties; another thrush at B2 and a blackbird at B3 were found dead in a trap. The traps were placed away from food supplies, so the reason why these birds should venture under the trap covers is unknown.

## 2.4 DISCUSSION

### 2.4.i Discussion of trials in the east midlands

Access to farm sites near Leicester was limited as the majority of land owners claimed to have no rodent problem and farms visited gave every appearance of having few or no rats. As a result, sites near Newark and Lincoln were used. Pig and poultry farms are very common in this area. Both these types of farm make use of automatic feeders for the livestock, which are usually housed indoors for at least part of their rearing. Such a farm therefore provides a non-stop supply of food for rats if they infest the site. Many of the farms in the Lincoln area also had dykes positioned along one or two sides of the yard and these acted as natural corridors along which rats could infest the site.

The trials in the Lincoln/Newark area were carried out in conjunction with a local pest control operator who had to be present at most of the farm visits. This meant that some sites could be visited only once a week whereas other sites were checked more frequently. Subsequent time constraints meant that often only a measurement of bait-take and a carcass search could be made. Rat activity indices were therefore not monitored as often as they should have been at the majority of these sites. Hole-blocking was insufficiently maintained and tracking-tile data sets were incomplete. Despite this, the tracking-tile data for the sites where activity was monitored seem to show clear patterns of change with time.

Neophobia, the fear of new objects that rats have been shown to exhibit (Shepherd and Inglis, 1987; Brunton, 1995; Inglis *et al.*, 1996), was not apparent at many of the sites in this region. At all sites except A2 and A8 (and possibly A7 where no measurement was made until day 7), bait had definitely been consumed within the first 2-3 days. Sampling behaviour (as judged by very low bait-take at a bait point), is also often exhibited by rats to allow them to test out the quality or risk of new or different food types (Shepherd and Inglis, 1987; Berdoy and Macdonald, 1991). There is little evidence to show that rats in the east midlands exhibited this behaviour. If they did, then there were many rats sampling a small amount each (Buckle *et al.*, 1987) to produce such a high bait-take or sampling was limited to the first day or two only. Bait was rapidly utilised at most of the sites in the east midlands. A peak of consumption was achieved within the first week at

five of the eight sites, irrespective of how quickly control was eventually achieved. This implies that the rats very quickly overcame any neophobic instincts they may have had.

At sites A5 and A2 where bait-take did seem slow or non existent to start with, the most probable explanation was the presence of alternative foods. Quy *et al.* (1992b) reported that "stored cereal significantly reduced treatment effectiveness". At A2 the entire rat problem centred around some grain barns and at A5 the rats were living among the stored and sacked feed at the mill. The wheat-based rodenticide was therefore not attractive to the rats at either site since they already had a very ample food supply of which they were sure. A liquid-based rodenticide would have been more suitable in these situations, because water was a limiting factor at both sites. For the purposes of these trials, however, liquid bait was not an option. It was the inability to attract the rats to the bait within the buildings at A5 that led to a switch to outdoor baiting instead. There was little alternative food for the rats outside and they subsequently made full use of the bait that was provided. No such measures were taken at A2 and so the control programme failed. Enough bait had been taken by the end of the trial to kill some rats, but no bodies were found and the evidence from the tracking tiles and presence of fresh droppings *etc.* showed that rats were still very much present.

The presence of so much alternative food, in the form of pig carcasses, rubbish and spilled grain from the automatic feeders, at A7 and A8 may have explained why control of the rats was not achieved within nine weeks. Myllymaki (1986) stated that clearing up rubbish alongside a baiting regime was the best strategy for controlling urban rats. Not only did these conditions attract rats in to start with and then provide an alternative food to the bait, but they continued to attract rats into the area throughout the trial. Taylor (1978) considered rats that fed at a farmstead but lived outside it to be potential immigrants and Quy *et al.* (1992a) reported that immigration led to a persistence of bait-take on farmsteads in their study. Immigrating rodents would eventually be expected to take up residence permanently if suitable habitat existed near to the food supply. Reinfestation or immigration of rats was observed at A7 in particular. Areas of the farm that had ceased to show evidence of rat activity after an initial phase of bait consumption were suddenly in use again with apparently healthy and hungry rats. New excavations became apparent around the same time. Most cases where these observations

were made involved buildings on the periphery of the site where dykes and hedgerows would have allowed an easy access for new immigrants.

Immigrants generally can move on to a farmstead only after the area has been vacated by the resident rats. This is because intruders are attacked by the rats already present (Adams & Boice, 1983). Rodent control must therefore be effective, at least to some extent, before reinfestation can occur otherwise immigrants will be deterred. Reinfestation could happen very rapidly, however, with such a pressure from outside for rats to move onto a site to feed and with so much suitable habitat within the site. Rowe (1987) noted that house mice rapidly recolonised cleared buildings and Sullivan (1986) stated that small rodents are very adept at recolonising.

Reinfestation was the probable cause of a renewed uptake of bait seen at the end of the trials on some of the sites such as A4, A5 and A8. The tracking-tile data from A1 also show an increase in activity after apparent reduction of the rat infestation after week 4 (Figure 2.7). Longworth trapping did, however, reveal that house mice at this site, rather than immigrant rats, spread throughout the farm area during the final week of the trial. It would appear that the baiting programme had targeted the rats at the site and, once they were removed, the area was free for house mice to inhabit. The removal of competition from wood mice may, alternatively, explain the spread in house mice after the trial (Tattersall, 1992). The house mice were originally confined to the drying tunnels in the grain barn and were largely unaffected by the rodenticide. The Longworth trapping data, in contrast, revealed a lack of wood mice on the farmstead at the end of the trial.

Eighty-three target rodent carcasses were retrieved from the sites used in the east midlands during this study. At some sites no bodies were found, but at other sites many carcasses were found and retrieved. Most of the bodies that were found were located inside buildings, which implies that carcasses are quickly scavenged from outside. Balcomb (1986) and Butcher (1965) observed that it is notoriously difficult to retrieve any carcass from 'the field'. Harrison *et al.* (1988) calculated that only 4% of a rodent population were found as carcasses and Taylor and Thomas (1989) found no bodies at all. The fact that most carcasses were found indoors may alternatively mean that the rats that died outside were more hidden, and therefore harder to find, than rats that died indoors. Fenn *et al.* (1981) noted that rats usually die under cover making it especially hard to locate them. The carcass mass data were used to test whether any pattern of size-related

feeding exists in wild populations of rats. Temporary food supplies mean that there may not be enough food for a whole rat population to eat at ease. It is therefore not surprising that rats defend food supplies territorially (Adams & Boice, 1983). Defence is usually carried out by the dominant, larger rats (Dubock, 1982; Adams and Boice, 1983). There has been some debate as to whether the dominant rats within a social group are also the first to feed at a *new* food source (Nott & Sibly, 1993) or whether the subordinate rats do most of the early sampling (Shepherd & Inglis, 1987; Nott, 1988). The carcass data revealed no conclusive evidence to determine whether the larger or smaller rats were eating (and therefore dying) first. Cox (1991) found a downward trend of carcass mass with time into the trial during her study on rodenticide treatments on farms. Butler and Whelan (1994), in contrast, discovered that the mean rat weight after rodenticide treatments had increased, implying that the smaller rats had died.

Some kind of feeding pattern appeared to occur towards the end of the trial at site A8. Most of the rat activity was centred around a small part of the site by this stage; a storage shed that had been left undisturbed prior to the trial. Bait-take from this shed had been high throughout the trial and the rats had even been eating the bait trays and other material around the shed. Consumption of bait suddenly dropped to zero before rising again and then it fluctuated for the rest of the trial. During the cessation in feeding, rats were still evident within the room and in the roof space overlooking it; every time the shed was entered there was a mass of activity. There are four possible hypotheses to explain the feeding patterns and presence of rats observed: First, the dominant rats may have defended the bait points and prevented access by other rats, as suggested by Dubock (1982). The dominant rats then died and there was a cessation in feeding. With new bait being supplied (there was no other food in this room), the subordinate rats could move in to feed, so bait-take resumed. Alternatively, all the local rats fed from the bait points with equal opportunity. They all died and reinvasion from outside populations occurred. These new rats were then responsible for the further food consumption. The third hypothesis is that the irregularity of site visits may have led to saturation baiting not being maintained. Some rats may therefore have only consumed sub-lethal amounts of poison, leading to bait aversion rather than death (Smith *et al.*, 1994a). This would explain why rats were still present but were not touching the bait. Finally, some rats (whether dominant or subordinate) may have fed from the bait points and died. There was a gap of

a week in some cases before the next experimental visit to the site, and some carcasses might have been lying around undisturbed for a few days. Other rats in the vicinity, which had not yet gained proper access to the bait points, may have observed the dead bodies, associated them with the new food and thus developed bait aversion. This "Poisoned Partner Effect" is considered in reviews of rat behaviour (Berdoy and Macdonald, 1991; Berdoy and Smith, 1993).

On A5, even though dead rats were found and activity seemed to decrease, the evidence from Figure 2.4 implies that in fact the consumption rate remained high. It is possible that, at this site, movement of rats was actually from inside the buildings. This would seem strange when there was an abundant food supply in the barns. However, it may have occurred as there was a lot of disturbance in the milling sheds during weeks 3-6 of the trial. Terriers were used to clear the rat infestation on three occasions. Sixty rats were killed in this way and the rest of the population was likely to have been disturbed by this. For the short term, rats may have moved out of the buildings to relatively safer habitats. Alternatively, it may be that, with all the alternative food present, the rats were eating sub-lethal amounts of the poison and a longer time period was needed in which to achieve effective control.

The other possibility for the lack of control at this and two more of the sites, A7 and A8 was the presence of rodenticide resistance. Certainly, the consumption of huge quantities of bait (Figure 2.4) without any apparent decrease in rodent abundance was a cause for concern. It was due to this that a switch to brodifacoum was made at sites A7 and A8 after a few weeks of the trial, as there has been no reported resistance to brodifacoum in wild rats. Very soon after the change was made, dead rats were found at both sites. At A8, control appeared to be especially successful. At the other site, the treatment switch also appeared to be working initially. The later increase in consumption may therefore have been the result of reinvasion. At all the other sites using coumatetralyl, control was effective. Resistance was not therefore suspected elsewhere.

Comparing the general treatment effectiveness of farms using coumatetralyl and those using brodifacoum, two things are clear. First, control was achieved more definitely on brodifacoum farms (shown by the reduction of bait-take in Figures 2.4 and 2.5 ). Second, far less bait was required with the brodifacoum formulation than with coumatetralyl (Figure 2.6). As mentioned earlier, the farms were allocated to the

treatments on the basis of suitability for baiting indoors or outdoors. Rodent density was not considered at all and allocation of farms to treatments was randomised with respect to density. The lower levels of brodifacoum required to achieve control therefore indicate an effect of the treatment itself and not the infestation level. It may be that indoor baiting is more efficient. The effective control at site A6 where coumatetralyl bait was placed mostly indoors would appear to support this argument. The majority of bait points at A7 and A8 were also inside however and at these there was a huge amount of bait-take without much control of the population. It is therefore more likely that the higher efficiency of treatment observed on the brodifacoum farms was due to the bait itself.

Brodifacoum is highly toxic and it is possible for rats to ingest a lethal dose in one feed. Johnson and Prescott (1994) calculated that an average sized rat (250g) needed to consume only 11g of brodifacoum bait to receive a lethal dose. Even in the presence of alternative food, an average of 8.7g over 2 days is sufficient to kill 100% of rats (Redfern *et al.*, 1976). Brodifacoum is fully effective against rats that are warfarin-resistant (Quy *et al.*, 1992a). In contrast, the coumatetralyl must usually be consumed more than once to achieve control and it is not properly effective against warfarin-resistant rats (Greaves and Ayres, 1969). The infrequency of visits made to some of the sites may have prevented true saturation baiting from occurring. As a result, rats may have taken a day or two between consecutive feeds on the poison. While this would have had no effect on brodifacoum feeders (Dubock,1982, recommended pulsed baiting with brodifacoum), it may have prolonged the time required for control to be achieved on the coumatetralyl sites. It may also have meant that rats consumed a sub-lethal dose and developed bait aversion although there has still been little evidence of this in field trials of anticoagulants.

## 2.4.ii Discussion of trials in central southern England

All of the sites used in central southern England reported problems of repeated or constant rodent infestations and unsuccessful baiting programmes. At some sites, partial reduction in the rat populations had been achieved in the past. At others, the farmers felt that rats were simply becoming more and more abundant and there was nothing that would work to reduce their numbers. Clearly "resistance" was widespread in this region, but its prevalence and the number of different poisons that it nullified were variable. Sites

used in this region ranged from small holdings to large scale pig units. There seemed to be no pattern to the presence of rats on any site, although most of the farms found to have rats were either poorly maintained or made use of automatic feeders for their housed livestock.

Unlike the east midlands, a degree of neophobia was observed on the sites in central southern England. On all sites except B5, bait-take was much slower in the first week than subsequently. The absence of neophobia at this site was almost certainly because pre-baiting with non poisonous wheat was carried out prior to the start of the trial. This was done to ascertain exactly where the rats were feeding, but it had the effect of producing immediate bait uptake once the switch to poison was made. Why the rats at the other sites were so neophobic probably has something to do with the fact that, in this area, rats are constantly bombarded with different poisons to try to control the infestations. Rats may therefore be very wary of any bait as, with each application, there will be a small percentage of the population that dies.

A certain amount of reinfestation was observed at two of the sites; B4 and B6. Despite very successful rodenticide treatments at these farms, the rats outwith the resident population were untouched. Once the site was vacated due to death of the residents, the outsiders started to move in. Certainly at B4, runs that had become overgrown, indicating that rat numbers had dwindled, were suddenly in full use again at the end of the trial. Bait-take data also revealed an increase in rodent activity on the site during the last week. Tracking tiles may also have done so (if the previous week's record was not missing) but the reinfestation was in such a localised part of the site that the overall activity scores possibly would not reveal the new influx.

As found at some of the sites in the east midlands, the presence of alternative food was a cause for delayed uptake of bait on some of the farms in central southern England. For example, at B7, a pile of bread provided daily food for the donkeys but was also a main attraction for rats in the area. During the first 13 days of the trial, the bait supplied in the bait trays was barely touched. The farmer was asked to remove the bread and he did so on day 13. Thereafter, bait uptake was immediate.

A similar case was that at B4 where rats were entering one of the barns to feed from the remnants of grain in the underground pit and from around the grain rolling machine. Rats were very active in this area and 13 rats were seen leaving the pit one day

in the mid afternoon. Bait trays positioned around that barn were barely touched until the day (day 11) that all the old grain was removed. From that point, the rats turned immediately to feed from the bait trays instead. Tracking-tile results showed that activity also increased during the 2nd and 3rd weeks, probably coinciding with the sudden use of bait stations. Effective control was achieved within two weeks of the barn being cleared. Not only did bait-take drop, but general activity levels were shown to be much lower.

At site B8, alternative food was such a problem that the whole control programme was rendered useless. The rats at this site were feeding from spilled grain and chicken droppings within two battery hen sheds. The rats were living in burrows in the sandy soil underneath the wooden sheds. The vast majority of activity was therefore concentrated in these areas as the rats had all they needed around them. Despite the positioning of bait trays next to the runs and holes being used daily by the rats, the bait was not touched. Eventually, small localised feeding occurred from day 20 onwards, but very little bait was taken. It was clear that the rats would continue to feed primarily from the chicken lines, although an extended baiting regime might have killed a portion of the population eventually.

Apart from at this site, brodifacoum appeared to be a very successful rodenticide. On all the farms where it was used, rodent control, based on the drop in bait-take and the reduction in rat activity shown by the tracking tiles, was achieved within about four weeks of the trial. Additionally, overall bait consumption was quite low (average value for the brodifacoum sites was 3.5 Kg). In contrast, the coumatetralyl sites showed a lack of efficient rodent control and bait use was much higher (average value was 17.4 Kg). On three of the four sites, control was not achieved and bait-take remained high, even increasing at the end of the trial. At both B2 and B3, alternative food may have hampered effective control (Quy *et al.*, 1992b). However, by the large amounts of bait consumed it is clear that, had the bait been effective, some degree of population reduction would be expected. The tracking-tile data at both sites show that that was clearly not the case. The one site, B1, that apparently did show control unfortunately had insufficient tracking-tile data to back up this observation. Personal observation, however, revealed that rats were still very much present at the site although they stopped feeding after about day 35. It is possible that some of the rats ingested a sub-lethal dose of the poison bait and, instead of dying, experienced discomfort that led to later bait aversion (Smith *et al.*, 1994a). This

theory, however, would imply that the rats at this site were not resistant to the bait, for if they were, they would not have suffered any discomfort at all from eating the bait. An alternative may be that the rats, probably resistant, developed bait aversion through some other cause, such as the poisoned partner effect (Berdoy and Macdonald, 1991). None of the rats at this site were found poisoned, but there may have been some that were undiscovered. There were also rats caught through trapping, which could possibly have provoked the same reaction amongst the other rats.

At sites B2 and B3 rats were eating the bait within the first night of each rebaiting. In addition, they were eating the trays and dragging them towards their holes. In many cases, bait was spilled in the process. Placing bricks against the boards that covered the bait trays helped the problem to some extent, but on many occasions the cover had also been knocked away. Supply of bait at these sites was clearly not meeting demand. Quy *et al.* (1995) described a situation where saturation baiting could not be maintained because of the high demand at the site. There was concern for non-target animals at both sites B2 and B3, however . At B2 the farmer was also angry that the rats were being fed rather than killed. As a result, the amount of bait issued was limited after day 23 at B2 and day 22 at B3. A small amount of baiting was continued at B2 after this point in order to facilitate trapping, but monitoring of bait stations did not include bait-take measurements. The consumption figure seen is therefore less than it might have been under saturation baiting. At B3, monitoring did continue, but saturation baiting was not maintained. So again, even more bait could have been used than the value recorded.

It seems apparent from the data collected from the coumatetralyl sites that resistance was indeed prevalent. As there is no known resistance to full application strength brodifacoum in the field at present (Greaves, 1994), the sites where brodifacoum was used were unaffected by the occurrence of resistance. Redfern *et al.* (1976) found that an average intake of 8g of brodifacoum bait over just 2 days was sufficient to produce 100% mortality in warfarin-resistant rats, even with other food present. This would explain the high efficiency of control that brodifacoum produced during these trials. Control at the coumatetralyl sites was, in contrast, seemingly impossible to achieve. Greaves and Ayres (1969) found that in coumatetralyl-resistant rats, coumatetralyl could only achieve a 66% kill at maximum, even when as much as 94g was eaten by an average 250g rat over a 6 day period. The situation 30 years on is apparently

far worse. When the carcasses were dissected, it was clear that many of the rats trapped at coumatetralyl sites had been eating the bait. In the majority of cases, however, their organs looked very healthy and certainly showed none of the normal symptoms of anticoagulant poisoning such as a pale liver or internal haemorrhaging. In contrast, those bodies found at brodifacoum sites often contained very pale livers and kidneys and haemorrhaging was usually apparent either internally or at the feet, nose and anus.

Carcass data again revealed no pattern of feeding status with size of animal, as found by Cox (1991). This may be because there was no feeding hierarchy at these sites or it may be that not enough carcasses were retrieved to find a true sample. No carcasses were found at all at B5, and at B7 the farm workers unfortunately threw away the only carcasses that were discovered. Despite this 39 poisoned carcasses and 104 trapped target rodents were retrieved in total from sites in central southern England.

Contrary to expectations, the removal of so many rats by trapping failed to have much effect on the overall population size at sites B2 and B3. The tracking-tile data revealed that the extent of activity remained high throughout the trials at both sites. The level of activity did drop slightly at the same time that trapping was carried out, but it was clear that there were many tens of rats left in each population at the end. Buckle *et al.* (1986) noted that 4.1% of a population of rats failed to feed from bait trays during a rodenticide treatment. Since the traps were positioned within the baiting area, some rats may have also avoided being trapped. Brunton *et al.* (1993) suggested that physiologically-resistant rats may show a lower likelihood of feeding from bait trays than susceptible rats due to enhanced neophobia. This may also make them less easy to trap.

## 2.4.iii Comparison of the two regions

The main difference noted between the two regions was emphatic confirmation of the presence of established physiological resistance within central southern England. Coumatetralyl failed to control rats and mice in central southern England, although brodifacoum did achieve effective reduction on all farms except one. At that farm, B8, rats were failing to feed from the bait properly and it was due to this, rather than failure of the bait itself, that control was not achieved. Coumatetralyl achieved control on some of the sites in the east midlands, but on others the baiting programme was extended well

beyond the five weeks that was necessary to achieve effective rodent reduction elsewhere. It seems likely that low-level or perhaps newly established resistance was present at the two sites where control was inefficient. An alternative explanation for the poor control may be that rapid and persistent immigration occurred. Quy *et al.* (1992a) reported that immigration led to a persistence in bait-take. In comparison to central southern England, the coumatetralyl baiting produced more carcasses in the east midlands, implying that many rats were still susceptible.

In both regions it was found that the coumatetralyl was consumed in significantly higher quantities than brodifacoum. Data from the two regions were pooled in an analysis of variance and also produced a significant result ($p=0.007$). The occurrence of resistance in the south of England, and on a few of the sites in the east midlands, would partly account for this, because the coumatetralyl was eaten without taking effect. Saturation baiting could not be maintained at coumatetralyl sites in central southern England, however, because of creating unnecessary hazards to non-target species in the process. This effectively curbed the quantity of bait that was consumed, where in reality perhaps far more would have been eaten.

Bait-take in the two regions was compared using analysis of variance (general linear model). There was no significant difference for total bait-take per active bait point in the east midlands compared to central southern England ($p=0.991$). This is surprising and may have been biased by the presence of suspected resistance in the east midlands. A repeated analysis of variance with the suspect sites removed revealed that there was in fact a significant difference between the two regions ($p=0.036$). The time to peak bait-take was also tested. One may expect that in an area where resistance is widespread, effective control (as measured by a reduction in bait-take) might be delayed. The peak in bait-take usually occurs immediately before the reduction, so it follows that the time to peak may also be delayed. An analysis of variance (general linear model) fitted to the data for both regions revealed, however, that there was no significant difference ($p=0.898$). Even the removal of the suspected resistance sites in the east midlands produced no real change ($p=0.352$). There was also no significant difference in the time to peak for the two different poisons ($p=0.856$). This is slightly surprising as one would expect that the brodifacoum may take effect slightly earlier than the coumatetralyl (because only 1-2 feeds are necessary to achieve control). The explanation probably lies in the fact that,

despite having consumed a lethal dose, the rats at brodifacoum sites would still continue to feed until affected by the poison. This would make the time to peak comparable with the data from coumatetralyl sites.

Treatment failures are often wrongly attributed to "resistance" when the real reasons for failure may be the result of factors such as the use of insufficient bait points (competitive exclusion) or bait point maintenance (saturation baiting not maintained), continual immigration, or inability to attract the rodents to the bait, usually because of alternative foods. Where resistance does exist, however, it can cause very real problems for rodent control. Farmers in severely affected areas, such as the borders of Berkshire, Hampshire and Oxfordshire, are often left without any really effective means with which to remove the rodents. Many of the anticoagulants are ineffective and those that would be (such as brodifacoum and flocoumafen) cannot be obtained unless the farmer is willing to pay for a professional pest control operator to maintain the site. The presence of resistant rats across a wide area in the counties mentioned means that immigration from neighbouring farms is likely. The use of a professional to eradicate the rats may therefore prove very costly as it is likely that frequent visits will be necessary. It is perhaps time to look for alternative strategies to use in heavily resistant areas. The removal of rodent harbourage is essential, but in many cases this will in fact be impractical as the rats make use of essential farmstead objects (such as straw ricks, animal pens *etc.*) Perhaps a complete alternative like the use of sterilants is the way forward. The sterilised individuals will prevent immigration yet they will be unable to reproduce and spread (Smith and Greaves, 1987). The short life span of farm rats (1.5-2 years) means that sterilising the population would only be a temporary measure as the dominance structure and stability of the population against immigrants would disintegrate as the older rats died off.

## 2.4.iv Non-target exposure

Farmers who are keen to remove rats or house mice from their property will usually be keen to apply rodenticide in sufficient quantity to "do the job". Sufficient bait may, however, be perceived as quantity supplied at bait points rather than the number of different bait points or frequency of repeated visits. An effort to achieve rodent control had been made at most of the farms used in this study prior to the trials. Control was

carried out by the farmer rather than by professional pest control operators in most cases. The farmers would usually apply rodenticide when they noticed that rats were present, but a few maintained permanent bait stations all year round. The largest number of bait points used by farmers was three, compared to a maximum of 71 bait stations used during these trials. The sites that used a professional to apply the bait had a similarly high frequency of bait stations and these were usually visited once a week or once a fortnight. It is evident, therefore, that there are considerable differences between the baiting regimes used and the potential hazard to non-target wildlife species is likely to differ accordingly. There has been some debate as to whether a minimal risk is created by using a few bait points (Fenn *et al.*, 1987) or many (Dubock, 1984). Perhaps more important than the number of bait points is the positioning of bait. Farmers are likely (personal observation) to place bait wherever they believe that rats are living. This may include hedgerows and scrub and other habitat that is utilised by non-target wildlife. Many incidents of accidental feeding on poison bait by non-target animals have occurred where bait has been positioned well away from buildings in this way (Cox, 1991; Townsend *et al.*, 1995). The noticeable reduction in non-target small mammals at site A1 during this study may have also resulted from bait positioning away from buildings. Only one non-target animal, a field vole, was caught during the whole of the second trap session. At this farm, many of the coumatetralyl bait points were positioned around wood stacks and bales. This is because there was evidence of rats in those areas, but wood mice and perhaps voles were also present. One site in central southern England, B4, may also have been affected by the previous baiting regime The farmer at this site maintained three permanent bait stations with a mixture of zinc phosphide and bromadiolone. The bait was literally heaped at each one. Resistance was evident at this site and the farmer complained that he had to apply "bucket loads" of poison each week. One of the bait points was completely uncovered, despite being positioned outdoors, and the others were only loosely covered by sheets of corrugated metal. Flowerdew (1972) reported that supplementary feeding increases the chances of small mammal immigration into an area. Longworth trapping at this site before the trial produced only one captured wood mouse within the whole of the first trap session (240 trap nights). This result was very surprising as there was an abundance of small mammal habitat of varied nature surrounding the farm yard area. It is possible that the lack of small mammals resulted from the baiting regime maintained at the site. The

abundance of rats may provide an alternative explanation; rats may prevent wood mice from becoming established as a result of competition or predation.

Longworth trapping results revealed an increase in wood mice after the baiting period at the non-poison site, A3. A general rise in small mammal numbers was also observed at the brodifacoum sites, A2 and B7. The rodent control at A2 was unsuccessful and there was very little bait-take (~200g only). It is not surprising, therefore, that no apparent poisoning of non-target primary feeders occurred. The fact that the same pattern arose at B7 implies, however, that the bait positioning was safe. Cox (1991) found no adverse affects on non-target small mammals when baiting was carried out indoors. Wood mice are not generally observed to go in to buildings (Arnold, 1993). Olney *et al.* (1991) reported that most rodenticide in the UK is applied inside buildings. This may therefore reduce the risk to non-target feeders. The same report noted, however, that more than half the weight of brodifacoum that was applied in 1989 (40% of occurrences) was outside. This action is not only illegal, but may pose a greater risk of accidental poisoning to non-target feeders because of the higher toxicity of brodifacoum compared to poisons that are legally used outdoors.

Grain-eating birds are also likely to be adversely affected by misplaced or excessive bait. Pheasants and partridges were observed near to bait points at B2 during this trial and hens tried to access bait points at B1 and B2. Quy *et al.* (1995) noticed that chaffinches were feeding near to bait points and some were found dead afterwards. Other passerines and a jackdaw were also noted to be looking ill.

*Secondary poisoning*

No evidence of secondary poisoning was found during this project, although rodent carcasses were frequently found in the open. Dozy, live rats that had clearly been poisoned were also found away from cover. There was, therefore, direct evidence that the opportunity exists for exposure of predators and scavengers to poisoned rodents. Indeed, some rodent carcasses had clearly been partly eaten by other animals.

Newton *et al.* (1990) found that 6% of dead barn owls around the country had brodifacoum residues in them. Mead (1987) considered that rodenticides could be the cause of persistently low numbers of barn owls in the UK. Shore *et al.* (1996) noted

rodenticide residues of second generation anticoagulants in 31% of polecats found dead in the UK. Many of these had been run over, so perhaps sub-lethal dosing via contaminated prey adversely affects predatory behaviour. Rodenticides have been proved to affect adversely the survival of predatory species during caged trials, but similar effects in the field have rarely been quantified. Predatory birds were monitored following brodifacoum application to orchards to control field voles in the USA (Hegdal and Colvin, 1988). Screech owls were severely affected, particularly where 20% or more of their home range was covered by the treatment. The barred owl, in contrast, avoided hunting over orchards and thus remained unaffected. Trials of this type, where the survival of local predatory species is closely monitored, have not apparently been conducted in the UK. Carcass searches have sometimes been made. Quy *et al.* (1995) found 1 weasel and some grain-eating birds dead after a farm rodenticide treatment. Kenward (1988) noted that poisoned squirrels were picked up by foxes, rats, buzzards and a sparrow hawk. However, the risk of poisoning scavengers remains largely unknown.

Any effect that rodenticide has on rat or mouse behaviour may alter the usual food chains observed. Behavioural changes in rats may include staggering in the open and in daylight (Cox, 1991; Cox and Smith, 1992; personal observation). Metzgar (1967) found that transient rodents were more prone to predation by owls and Kotler *et al.* (1988) observed that barn owls mostly predated rodents that were in the open. Rats may therefore be more prone to predation if they start to move in the open. Barn owls are, however, a nocturnal species, so a change in diurnal pattern by the rats may exclude barn owls from being placed at risk. Daytime predators and scavengers may instead be targeted. Rats are a highly mobile species (Taylor, 1978). They will regularly travel from hedgerows and woodland to a farm to feed and may potentially pick up poison before returning. Woodland is the preferred habitat of tawny owls (Southern & Lowe, 1968), foxes (Macdonald, 1987) and badgers (Neal, 1987). These species may therefore be exposed to rodenticide well beyond the perimeter of the farmstead buildings.

The timing of a rodenticide treatment is also likely to be important for determining the secondary poisoning hazard. Olney *et al.* (1991) reported that most rodenticide applications are made in the winter. This is when vole numbers are at a low (Arnold, 1993), so predators that usually eat voles may need to switch to rats and house mice. Weasels exhibit this change if voles are scarce (Erlinge, 1975) and barn owls are also

known to consume a higher proportion of rats in winter (Colvin, 1984). Thus, just when the use of rodenticide is at a maximum, the predation of rats increases. This means that the potential risk of secondary poisoning is worsened.

## 2.5 CONCLUSIONS

Data on bait consumption, population densities and individual masses were collected from 18 farm sites; 9 farms in the east midlands and 9 farms in central southern England. On each farm, brodifacoum (indoor use, highly toxic) bait or coumatetralyl (indoor and outdoor use, less toxic) bait was applied. The farms varied from each other considerably, but some definite patterns were revealed from the data that applied to the majority of the sites:

1. Bait consumption (per active bait point) of coumatetralyl was significantly greater than consumption of brodifacoum on farms in both regions ($p=0.007$).
2. Bait consumption (per active bait point) was significantly greater on farms within known areas of physiological rodenticide resistance than consumption on sites where there was no resistance ($p=0.036$).
3. Tracking-tile and bait consumption indices showed that coumatetralyl failed to achieve rodent control in areas of physiological resistance. Rodent control was achieved within non-resistant areas.
4. Brodifacoum achieved control of rodents in the regions where resistance was common as well as in the region showing no resistance.
5. Resistance was indicated on two farm sites near Lincoln where no previous report of resistance had been made. This putative resistance has not yet been confirmed by blood clotting tests.
6. On some sites in both regions, control was not achieved because alternative foods were accessed in preference to the rodenticide.
7. On two farm sites, the "Poisoned Partner Effect" or some other cause of bait aversion may have been responsible for prolonging the treatment period and even failing to achieve control.
8. Reinfestation of peripheral areas of farm sites was common within the five-six week treatment periods used.
9. Fenn Mk IV traps effectively captured rats of all sizes and conditions with no apparent bias against pregnancy or weight. The traps were, however, ineffective at killing the larger rats.

10. The individual mass data for rats found poisoned revealed no pattern of change with time into the treatment period; rats of all sizes were found throughout the control period. This result suggests that on a farm-wide scale where mutiple bait points are used, there is no effective bait point exclusion by larger rats and no differences in neophobic response for rats of different ages. This result contradicts other studies that have suggested that larger rats access bait points first.

11. There was no significant difference between the masses of rats that were trapped and those that were found poisoned.

12. There was no significant difference between the masses of rats in the east midlands compared to those in central southern England.

13. Rats on sites where brodifacoum was applied were not significantly different in mass to those on farms where coumatetralyl was applied.

14. There was no evidence of neophobia among rats on many of the farm sites; significant quantities of bait were consumed within the first two days. At other sites, neophobia was more apparent.

15. Non-target species evidently fed from bait points directly. Small mammal populations were much lower on some of the farm sites after a few weeks of rodenticide treatment, but due to the small samples used, this result was not significant.

16. Rodent carcasses were frequently found in the open as well as under cover, so the opportunity for scavengers or predators to pick up exposed rats exists.

# CHAPTER 3: BEHAVIOURAL EFFECTS OF RODENTICIDE USE

## 3.1 INTRODUCTION

A number of studies have shown that pesticide treatments cause effects on the behaviour of vertebrate species (Hart, 1990; Hooper *et al.*, 1990). More specifically, rats have been shown to change their behaviour during the pre-lethal phase of a rodenticide treatment (Cox, 1991; Cox and Smith, 1992). Previous behavioural studies on rats have involved either cages or enclosures, yet many authors have agreed that these situations may not suitably mimic what happens in the wild. The aim of this part of the study was to monitor rat-feeding behaviour before and during rodenticide treatments on farms. The hypotheses being tested were:

1. That "normal" behaviour involves maximum use of cover and thigmotaxis and a preference for activity at night.
2. That bait point exclusion by dominant rats causes subordinate rats to feed at less favourable bait points or at less safe times of the day.
3. That behaviour changes as a result of rodenticide poisoning.

### 3.1.i Normal feeding behaviour of rats

Rats are known to choose to move under cover or in close contact to potential cover such as walls (Hardy and Taylor, 1979). They are also nocturnal by preference (Berdoy and Macdonald, 1991; Whishaw *et al.*, 1992), but may be forced to feed during daylight hours if they are prevented from feeding sufficiently at night (Shepherd and Inglis, 1987; Berdoy and Macdonald, 1991). Shepherd and Inglis (1987) also noted that diurnal feeding is more frequent with bigger colony sizes. Rats may also feed by day if there is little disturbance or predation (Shekarova *et al.*, 1995). Eating times are shorter generally in exposed environments than in dark or covered areas (Whishaw *et al*, 1992).

Feeding at bait points is likely to be affected by other rats in the vicinity. Whishaw and Whishaw (1996) noted that aggression around food sources was high and Cox (1991) noted that indoor bait points near to cover were actively defended. Adams and Boice (1983) found that dominant rats had a monopoly of feeding points and Shepherd and Inglis (1987) observed that subordinate individuals were confined to feeding at less

popular times. Shepherd and Inglis (1987) also found that juveniles fed mostly with an adult from the family group rather than alone, whereas adults never fed together at the same bait point. Young rats have often been observed copying from older rats during cage trials (Chou and Richerson, 1992; Galef and Whiskin, 1995a, 1995b; Stetter *et al.*, 1995), so it is likely that young rats will copy more established rats in a variable farm environment. Subadult rats were observed exploring more as they got older (Renner *et al.*, 1992)

Rats exhibit neophobia in response to new foods (Shepherd and Inglis, 1987; Berdoy and Macdonald, 1991; Inglis *et al.*, 1996) but progressively take more food as it becomes familiar (Buckle *et al*, 1987; Berdoy and Macdonald, 1991). There may be considerable variation between individuals with regard to neophobic response (Inglis *et al.*, 1996) and juveniles are thought to be less neophobic than adults (Shepherd and Inglis, 1987; Nott, 1988). Females generally eat in smaller, more frequent bouts than males (Inglis *et al.*, 1996) although there may be considerable variation between individuals (Shepherd and Inglis, 1987; Whishaw *et al.*, 1992). Rats are prone to socially induced preferences in food choice (Galef and Whiskin, 1994) but social influence breaks down if food is always available (Galef and Whiskin, 1997).

## 3.1.ii Changes in behaviour caused by anticoagulant rodenticides

Rat behaviour during anticoagulant baiting has been studied with rats in cages and in enclosures (Shepherd and Inglis, 1987; Cox, 1991; Cox and Smith, 1992; Smith *et al.*, 1994a; Inglis *et al.*, 1996). Some of these studies showed that rat behaviour does change in response to consuming anticoagulant (Cox, 1991; Cox and Smith, 1992; Smith *et al.*, 1994a). The changes in behaviour can occur within 24 hours of consuming the poison (Smith *et al.*, 1994a) and may last until the rat dies, about four to five days later if second-generation anticoagulants are used (Shepherd and Inglis, 1987; Cox, 1991). Behavioural changes may include a temporal shift in general activity and feeding from night time to day time and an increased use of open areas rather than remaining under cover (Cox and Smith, 1992). Cox (1991) noted that male rats may stagger, while females become drowsy. All rats affected by poison during the studies reported by Cox and Smith (1992) changed their response to fear; from bolting to freezing.

There is a possibility that rats exposed to rodenticide may develop an enhanced neophobia (Brunton *et al.*, 1993), or an alternative mechanism for conditioned bait aversion (Berdoy and Macdonald, 1991). Most aversive behaviour regarding rat poison has, however, referred to treatment with acute rodenticides and there has been only one reported case of rats developing conditioned aversion to anticoagulants (Smith *et al.*, 1994a).

### 3.1.iii Studying behaviour with video photography

Video-monitoring of animal behaviour is a useful tool that is still being developed. Videos allow non-stop observation of animals that are within view of the camera and videos are able to run overnight, over the weekend or even for longer (Shepherd and Inglis, 1987). This factor means that the dedicated field biologist no longer has to move around in the dark to observe nocturnal animals such as rats, and can sift through the video footage at leisure at a later time. This in itself places video-monitoring at an advantage over traditional methods, such as observation with infra red binoculars, or radio-telemetry (Macdonald, 1987; Fenn *et al.*, 1987; Berdoy and Macdonald, 1991), but a further advantage is that the video data can be viewed and reviewed a number of times until behaviour is accurately recorded. This also means that different behaviours are likely to be noted in a more objective fashion than when the observer is making instant notes at the scene of activity. The addition of time-lapse recording with video photography means that many hours of photography can fit onto one video tape. Frame by frame analysis of the tapes allows viewing of interactions and swift events that may otherwise be missed.

Video monitoring places little disturbance on the subject animals. Rats may be aware of human presence by a person's smell even if the rats can neither see nor hear that person. Such a problem would mean that the behaviour observed may not be truly natural and the observer may need to go to great lengths to avoid being detected (Macdonald, 1987).

A disadvantage of using videos is their relative immobility. The camera with its recorder and light source needs a reliable power supply, so it is generally confined to indoors or near to a building.

## 3.2 METHODS AND MATERIALS

### 3.2.i The farm sites

The aim of the video photography for this part of the project was to record normal feeding of rats at bait points and then any changes that developed as the effects of poisoning set in. As a result, sites where rats were susceptible to the poison were chosen. Unfortunately, some problems with site choice were encountered; most sites with high rat activity either had no electricity supply for the video equipment or the electricity supply was unsafe. On other sites, the owner was not willing to allow video photography to go ahead. In the end, three sites were selected, but due to constant "tripping" of the electricity at one of these, only two sites (B4 and B5) were used over a satisfactory period.

At both the sites, rats were feeding primarily indoors, even before baiting with brodifacoum began, and so the video work was carried out inside too. It had been the aim to record rat behaviour outside, but with these sites being the only ones available, more importance was placed on covering an adequate amount of the populations' behaviour. Despite being indoors, both barns used in the video work allowed free access to rats from outside as well as inside. In fact, in both cases, the rats simply fed inside, but lived elsewhere. Avian predators were almost certainly excluded from these sites, but passerines were seen in the barns by day when doors had been left open. Predation by cats was evident at both sites and so the rats were still exhibiting fairly normal behaviour, such as wariness while feeding, at the onset of videoing.

Site B5 (Plate 3.1) consisted of a large barn that housed a combine harvester and about 30 large fertiliser bags. There was no substantial food supply for rats in this barn, yet a small population was regularly disturbed by the farm workers when they opened the barn doors to go inside. The previous year's crop had been kept in this barn and it appeared that the rats were feeding on the remnants that had been swept into one corner. In the same corner were some stored plastic drums, which provided some additional cover for the rats. The rats had also been observed among the fertiliser bags on the other side of the barn. The video was set up to monitor the corner of the barn where the grain sweepings were lying. Rat droppings in this corner revealed that this was where the rats were concentrating their activities. Bait trays containing untreated (*i.e.* no poison) wheat

were positioned within view of the video. The bait was switched to brodifacoum after two weeks. Some bait trays were positioned against the walls of the barn and others were positioned about 6-8m from the walls. Bait covers were placed over some of the bait trays by the walls and over some of the trays in the open. The rest of the bait trays were left uncovered. Twelve bait trays were used in total and these formed the nucleus of baiting on the site. Video monitoring was carried out from mid March to mid April.

At B4 (Plate 3.2), the video was again set up in a barn where rat activity was high. In this barn, an underground pit with a mesh wire cover at ground level was frequently visited by rats. The pit contained the remnants of the previous year's grain harvest. The majority of rats entered the barn via holes in the wooden doors, which were positioned about 0.5m from the corner of the pit. A few other rats entered the barn from other directions. The barn was positioned in between storage areas where harbourage was plentiful. The only major food source in the vicinity was within the pit. A grain mill was also positioned in the barn next to the pit and a chute ran from the top of the mill diagonally down to the top of the pit. Grain remnants were also found throughout this mill and beneath it where spillage had occurred. The only other objects in the barn were some pallets that were laid out against the wall between the wooden doors and the mill. A cat frequently visited the barn and a wagtail was also seen feeding on spilled grain when the doors had been left open.

The video was set up to monitor rats entering the barn *via* the wooden doors and entering the pit in the corner nearest to the doors. The grain mill and the pallets were also in view. Bait trays contained brodifacoum from the start of the trial (*i.e.* no pre-baiting) and the trays were placed against the walls and under the pallets and also in the open. Half of these bait points were covered and half were left uncovered. Six points were monitored in total. There were 51 bait points located at this site in total. Videoing started at the beginning of May and continued until the end of May when the trial was finished.

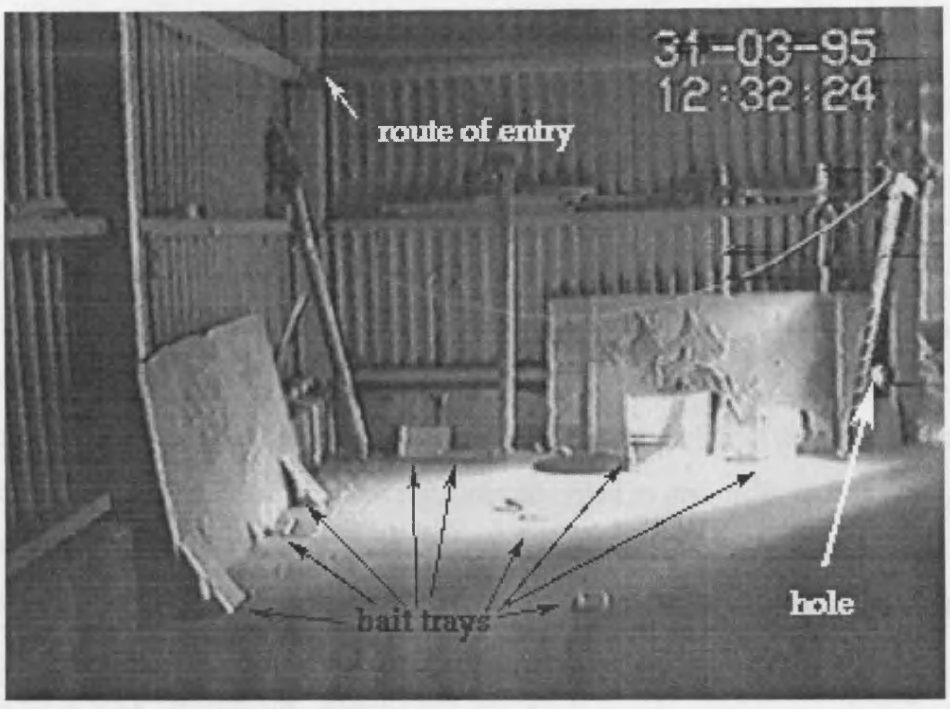



Plate 3.1 The set-up at B5

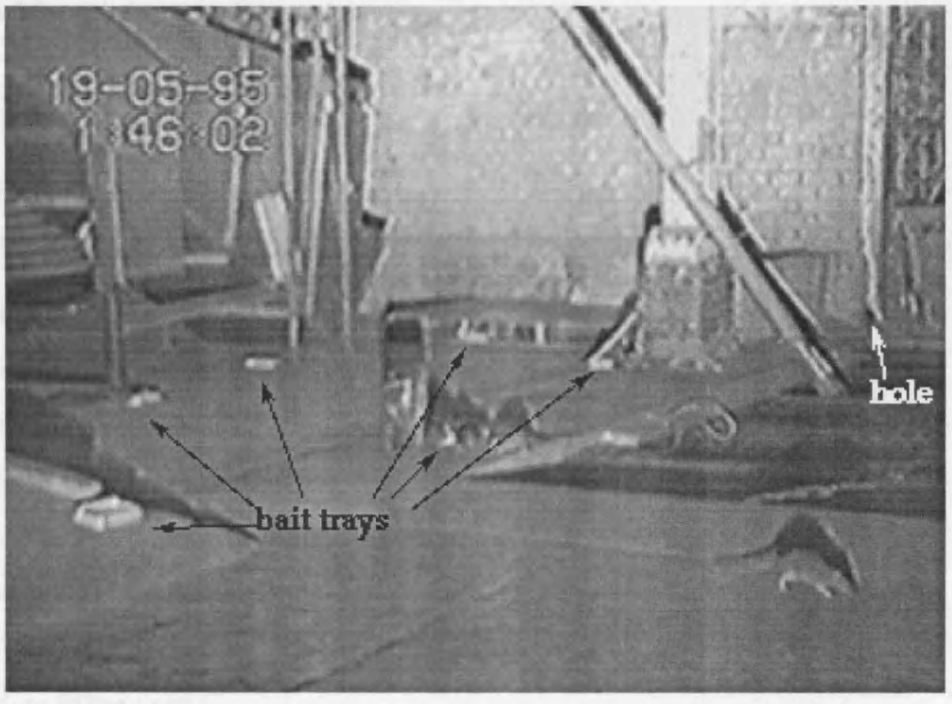



Plate 3.2 The set-up at B4

### 3.2.ii The video and its set-up for monitoring

A black and white ('Ultimate' low light, 0.02 lux) video camera with an auto-iris lens was positioned within a weather-proof cover on a tripod. The video camera was sensitive to near infra-red radiation. The camera was connected to an Hitachi 480 Lr VTL 2000E time-lapse video cassette recorder (vcr) set at 2 frames per second for 72 hours at a time (125 frames per minute). The camera and recorder were also attached to a monitor that was used to adjust the field of vision and the focus on site. "Black light" (near infra-red) was supplied by a Dennard 240V a.c. infra red lamp designed to give infra-red illumination outdoors up to 100m. The video cassettes were later viewed to assess the behaviours shown by the rats. A period of 10 minutes of analysis per hour was selected for the two sites B4 and B5. The video cassettes were played back on an Hitachi VT-5890E (UKN) vcr with a jog-shuttle facility allowing frame-by-frame advance of the recording when required. Activity appeared to be very sporadic at B5 and so a five minute interval every half hour was assessed. Rat activity was far more consistent at site B4 and so a 10 minute interval every hour was assessed.

### 3.2.iii Behaviours assessed by video

The following categories of behaviour were assessed at B5 within each 5 minute interval:

Date and time

Dark or light outside (a gap through the wall could be seen, which was used to monitor daylight)

Approximate number of rats

Movement at the edge or within 20 cm of the walls

Movement in the open

Feeding at the edge

Feeding in the open

Feeding at a covered bait point

Feeding at an uncovered bait point

Feeding alone

Feeding with other rats

Any antagonistic behaviour

Individual rats could not be identified. Behaviour displayed by any individual rat was therefore attributed to the group being observed at the time. Group activities were recorded in the categories listed above.

The behaviour categories assessed at B4 within each 10 minute interval included:

Date and time

Dark or light outside (this could be viewed via the rat hole in the doors)

Approximate number of rats

Movement at the edge or under cover of the miller or pallets

Movement in the open

1 rat feeding alone

More than 1 rat feeding - number feeding

Which bait point(s) used - to show whether rats were feeding at the edge or in the open and under cover or exposed.

Any obvious antagonism

Behaviour of the whole group was monitored as one, but notes were made of anything extraordinary. The use of a "jog shuttle" allowed a few sections of video tape to be monitored frame by frame. A computer (Apple Quadra 840 AV) was used to capture individual frames as digital images and these allowed a much closer investigation of behaviour between individual rats.

### 3.2.iv Behaviours assessed from bait-take and tracking-tiles

As well as assessing the behaviour of farm rats with the video, certain aspects of their behaviour could be ascertained from studying the bait-take measurements and tracking tile records. Particularly of interest was whether feeding was uniform throughout the farm site or whether there were hot spots of activity, and whether any such patterns changed as rodent control progressed. Also of interest was whether outdoor bait points were more or less popular than indoor ones. Anything gleaned from these data would most probably be the result of population dynamics as well as individual behaviour, but understanding

how the population as a whole behaves during control with poisons has important practical implications. These data are referred to in the Discussion.

## 3.3 RESULTS

Data were summarised in figures for overall activity and in tables for individual behaviour categories. Although the tables show frequencies of observed behaviours, chi-squared analysis was considered inappropriate because the observations were in no sense independent (rats clearly respond to the behaviour of other rats). The results are therefore treated descriptively.

### 3.3.i Farm A7

The video was set up to monitor rat activity at one of the sites in the east midlands, A7. The monitoring had to be abandoned after a few days, however, because rats had eaten through the electricity wires in the building and the electricity supply kept tripping. Incomplete daily observations meant that the tape could not be analysed satisfactorily. Two things were noted however. First, a wagtail was frequently observed feeding in the vicinity of the bait trays. Second, the two rats that were captured on film moved in close contact to the walls to reach the bait points. The bait was positioned indoors, so this observation showed that rats maintain thigmotactic behaviour inside as well as outside. Rats have previously been noted to show thigmotaxis in indoor enclosures (Cox and Smith, 1992; Inglis *et al.*, 1996).

### 3.3.ii Group behaviour at Farm B5

General activity patterns were monitored for 21 days at site B5. Bait trays containing non-poison wheat were placed in the barn that was being monitored on 14th March 1995. This wheat was replaced with brodifacoum bait on 31st March. Video-monitored activity for the 24 hour period prior to brodifacoum being placed and for the following five days is shown in Figures 3.1-3.3 and Table 3.1. There was no activity noted after the five day (post-bait) period had ended.

The rats at site B5 were diurnal throughout the pre-baiting period. The rats did not use the non-poison bait at all for the first week of the monitoring period. This was probably because there was plenty of other spilled grain on the floor, but it may also be

the result of neophobic responses to the bait trays. During the three to four days prior to the brodifacoum being used, the rats began to investigate the wheat in the bait trays. They started to feed after about 07:30 each morning. Daybreak was at about 06:30 during this time. There were small peaks in activity, usually about twice each day, when a group of rats would be active within the area being monitored. Single rats or pairs would arrive at the barn at other points during the day. All the rats appeared to arrive at ground level by descending the inside corner of the barn from the roof. Activity generally stopped by about 17:00 hours each afternoon. Individual rats were occasionally seen after this time. Nightfall was at about 19:15.

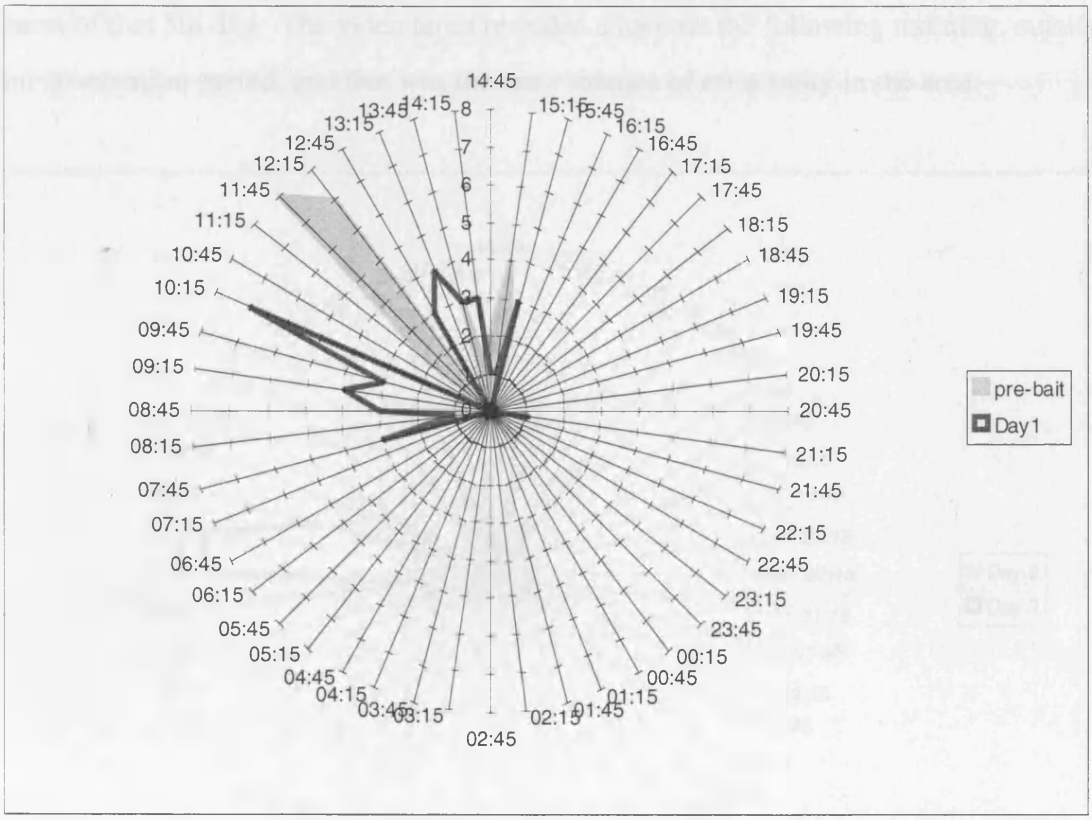



Figure 3.1 Daily activity patterns for rats at B5 prior to baiting (1 typical day) and on the first day after bait was laid. Observations were made every half hour for 5 minutes. Number of rats observed per 5 minute interval is plotted on the axes radiating from the centre of the plot, time (BST) is plotted around the edge. The plot joins observations for clarity, although the rats may not have been active during the 25 minutes between sampled observations. Sunset was at about 19:15 and sunrise was at about 06:30.

There was generally no change to this daily pattern as a result of feeding on anticoagulant bait (Figures 3.2 and 3.3). There was no activity before about 07:15 and very little activity after 17:30. The reason for the diurnal feeding is most probably because the risk of predation within the feeding area was low. The barn was infrequently used by humans and the farm cat was only observed in the area at night; the rats may have become habituated to the behaviour of the cat. General activity in the area increased between the 1st and 3rd days after baiting, but activity levels (measured by the number of rats present and the length of time in view) dropped in the 4th and 5th days after baiting. Only 3 rats in total were observed during the 5th day and there was no activity at all for most of that 5th day. The video tapes revealed a lone rat the following morning, outside an observation period, and that was the last evidence of rat activity in the area.

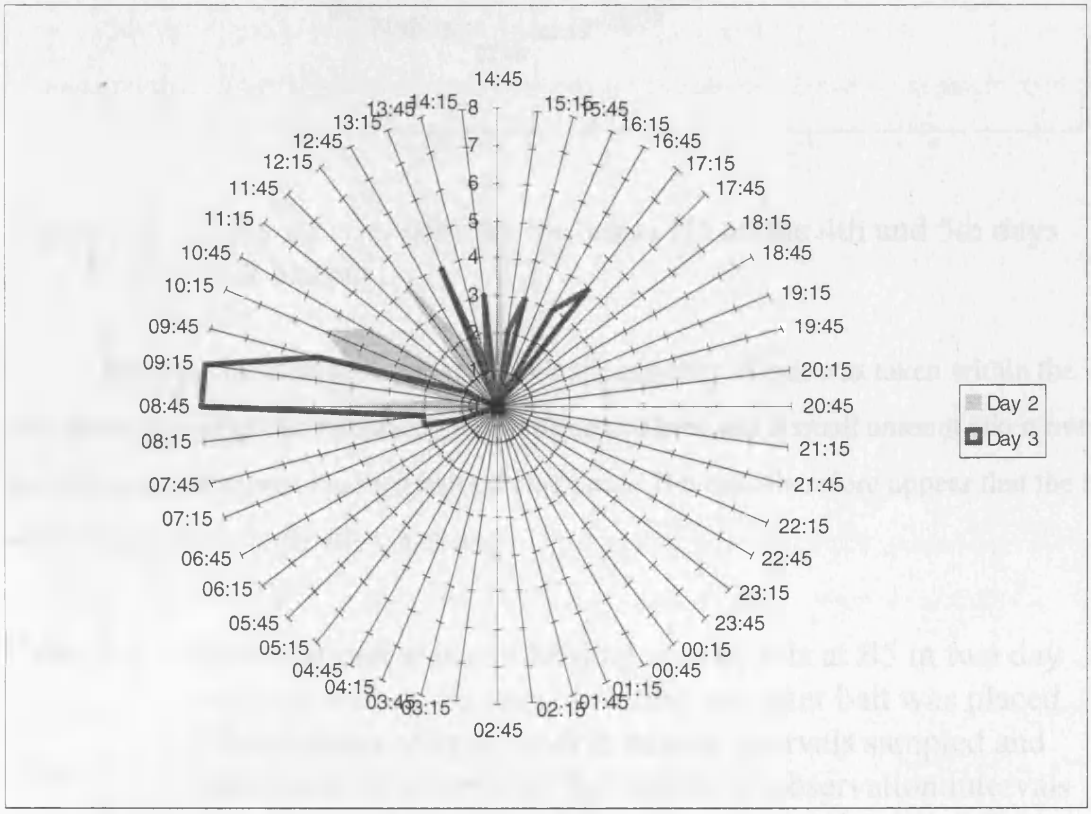



Figure 3.2 Daily activity patterns for rats at B5 on the 2nd and 3rd days after baiting

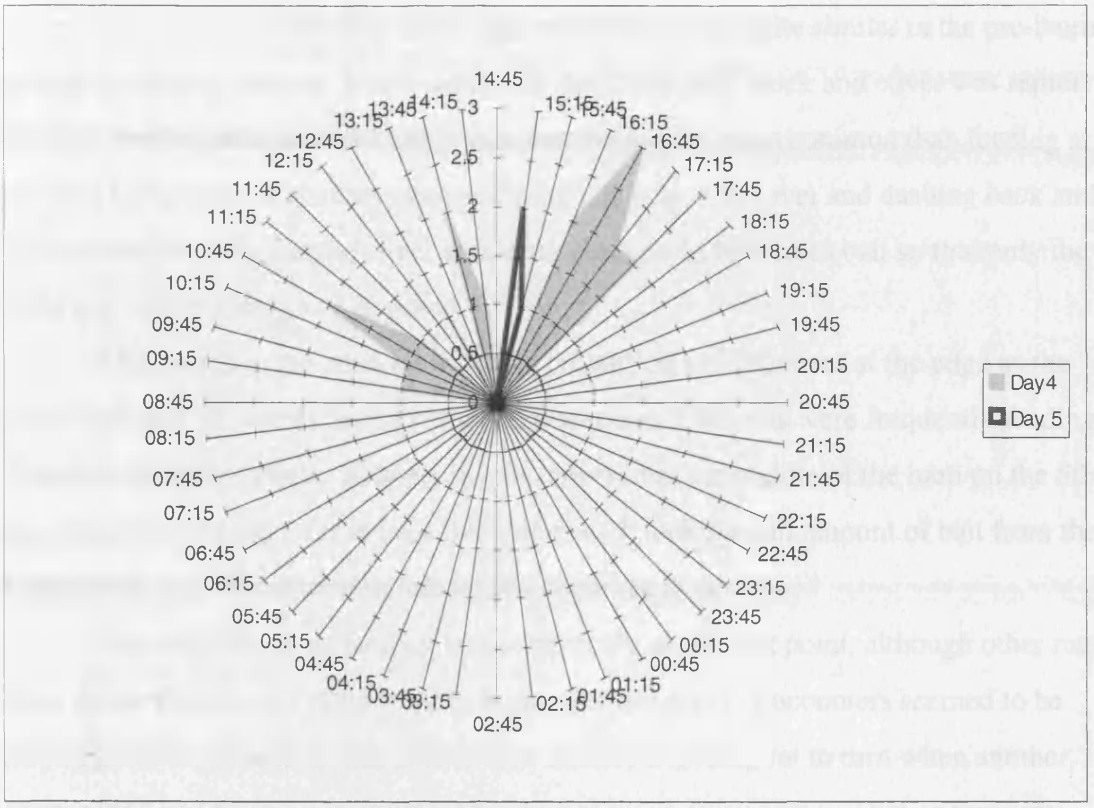



Figure 3.3 Daily activity patterns for rats at B5 on the 4th and 5th days after baiting

Bait-take measurements revealed that the majority of bait was taken within the first three days after the brodifacoum was placed. There was a small amount taken over the following two days and then bait-take stopped. It would therefore appear that the rats at this site were very rapidly controlled.

Table 3.1 Observations of use of feeding area by rats at B5 in two day intervals before the start of baiting and after bait was placed. Observations refer to the five minute intervals sampled and individual values refer to the number of observation intervals that contained the defined activity.

| Site | Dates | Total observations | Counts at edge | Counts in open | Feed under cover | Feed in open |
|---|---|---|---|---|---|---|
| B5 | pre-bait & Day 1 | 96 | 25 | 20 | 14 | 18 |
| | Days 2 & 3 | 96 | 29 | 11 | 15 | 17 |
| | Days 4 & 5 | 96 | 14 | 1 | 3 | 7 |

Movement and feeding at the edge of the barn were quite similar in the pre-baiting period and during baiting. Movement in the open was very quick and cover was rapidly sought. Feeding at uncovered bait points was marginally more common than feeding at covered bait points. Rats were observed remaining close to cover and dashing back and forth to feed or remaining in cover and bending round to feed from bait so that only the front part of their body was exposed.

Movement in the open increased in proportion to movement at the edge as the trial continued. Towards the end of day 3 it was noted that rats were frequently feeding from the open bait points. A large rat was observed in the middle of the barn on the 5th day and remained there for at least five minutes. It took a small amount of bait from the nearest bait tray, but otherwise just sniffed about the floor.

The majority of rat feeding was done singly at any bait point, although other rats were in the vicinity and often feeding from other bait trays. Encounters seemed to be non-aggressive. The most usual behaviour was for a feeding rat to turn when another approached and for the one approaching then to move away again. Two or three rats were seen feeding together on the first three days after baiting. The pairs consisted of two adult rats together, two juveniles together or a mixture. The threes always contained at least one juvenile.

### 3.3.iii Group behaviour at Farm B4

Monitoring of the rats at site B4 began on 5th May 1995 soon after brodifacoum bait had been laid out in bait trays. Rats failed to use the bait until after the pit was cleared of grain on the 16th May. General activity data for the 9th to the 11th May are shown as the equivalent to the pre-baiting period at site B5. Further data for the 17th to the 25th May are shown as this was the period within which the rats took the bait. No activity was observed in the barn after the 25th May.

Rats were observed at site B4 during much of each 24 hour period prior to bait-take starting. Most activity occurred during the late afternoon and evening and lasted for a few hours. Many rats could be seen arriving in the barn together, usually entering the area from outside via the hole at the base of the wooden doors. Virtually all activity involved rats going into and out of the pit, but they also travelled up over the small wall

and along the back wall via the pallets to get to the mill machine. Rats were frequently seen climbing all over the mill or feeding underneath it. Rats would occasionally be seen dashing across the barn from a different direction to reach the pit. Much of the peak activity in the barn occurred before it was dark outside at around 21:30 hours. Further activity, usually involving smaller numbers of rats, occurred at various times throughout the night.

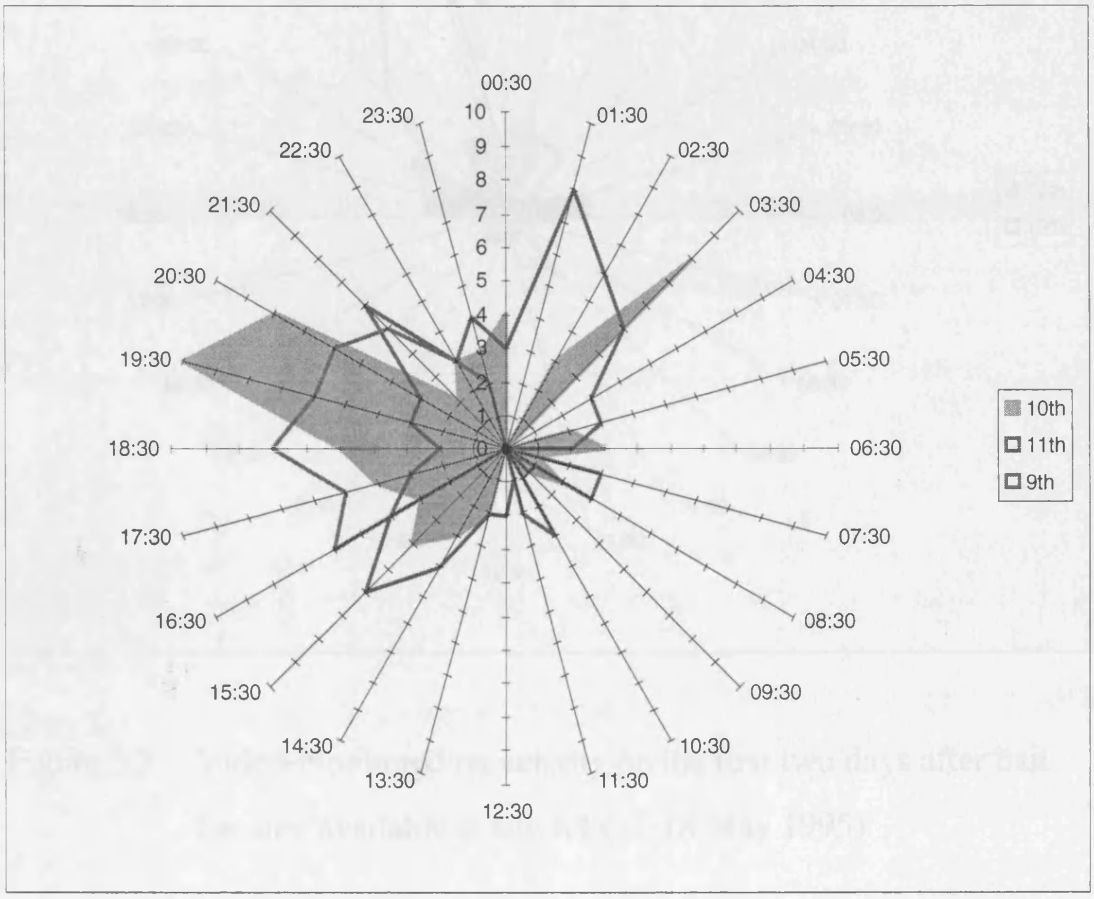



Figure 3.4 Video-monitored rat activity for 9th-11th May 1995 at site B4 prior to bait-take starting. Observations were made every hour for 10 minutes. The times shown are British Summer Time. Sunrise was at about 05:30 and sunset was at about 21:30.

Daybreak was at about 05:30 BST. The least active time of day was from about 06:00 to about 15:00. This may have been because of disturbance from general farm activities in the vicinity or because the rats were naturally less active at these times.

Predation, by the local cat and the farm terriers, may have also acted as a deterrent to the rats.

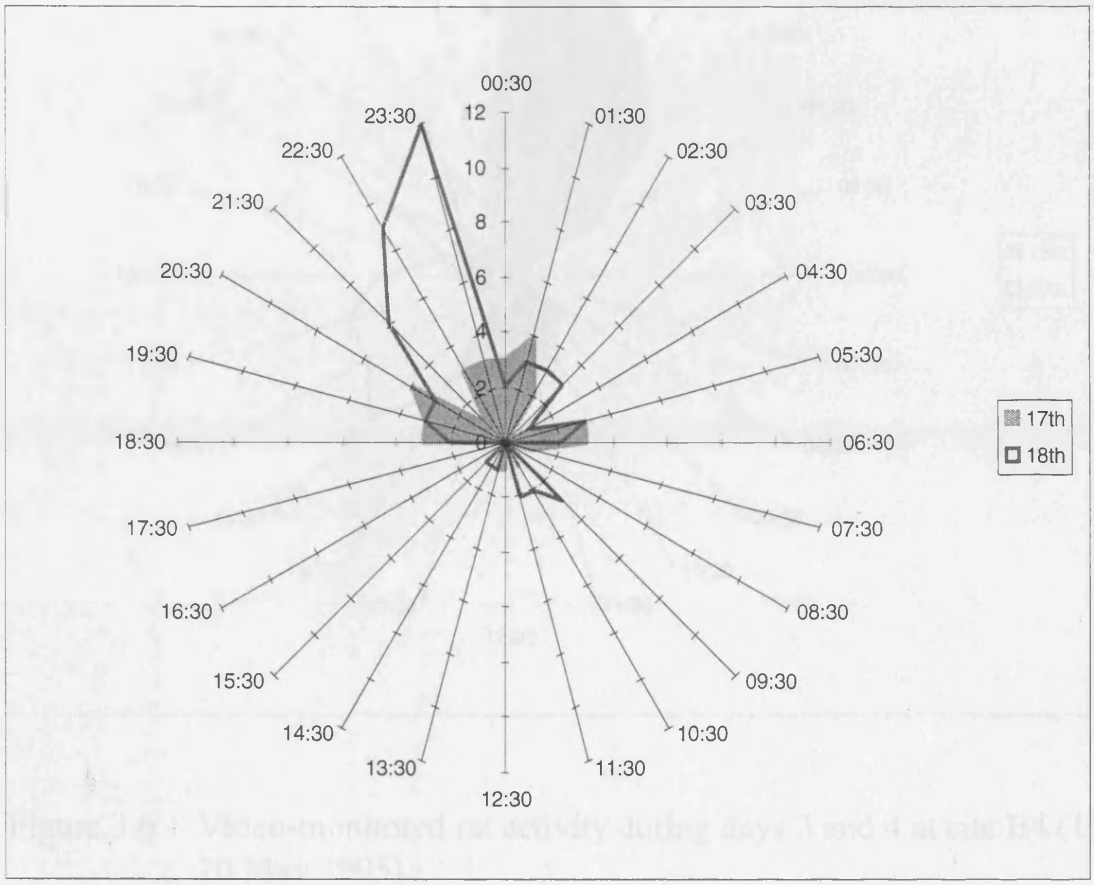



Figure 3.5 Video-monitored rat activity on the first two days after bait became available at site B4 (17-18 May 1995)

The rat activity changed as the trial progressed. There was very little activity at all on the 17th, the day after the pit had been cleared. The whole local habitat of the rats had been drastically changed in the process of the clean-up. Food was no longer available in the pit, and the pallets and some other items of cover had been removed. Activity was back to fairly normal intensity the next day, the 18th, but was much delayed in the day, with peak activity occurring around 23:00.

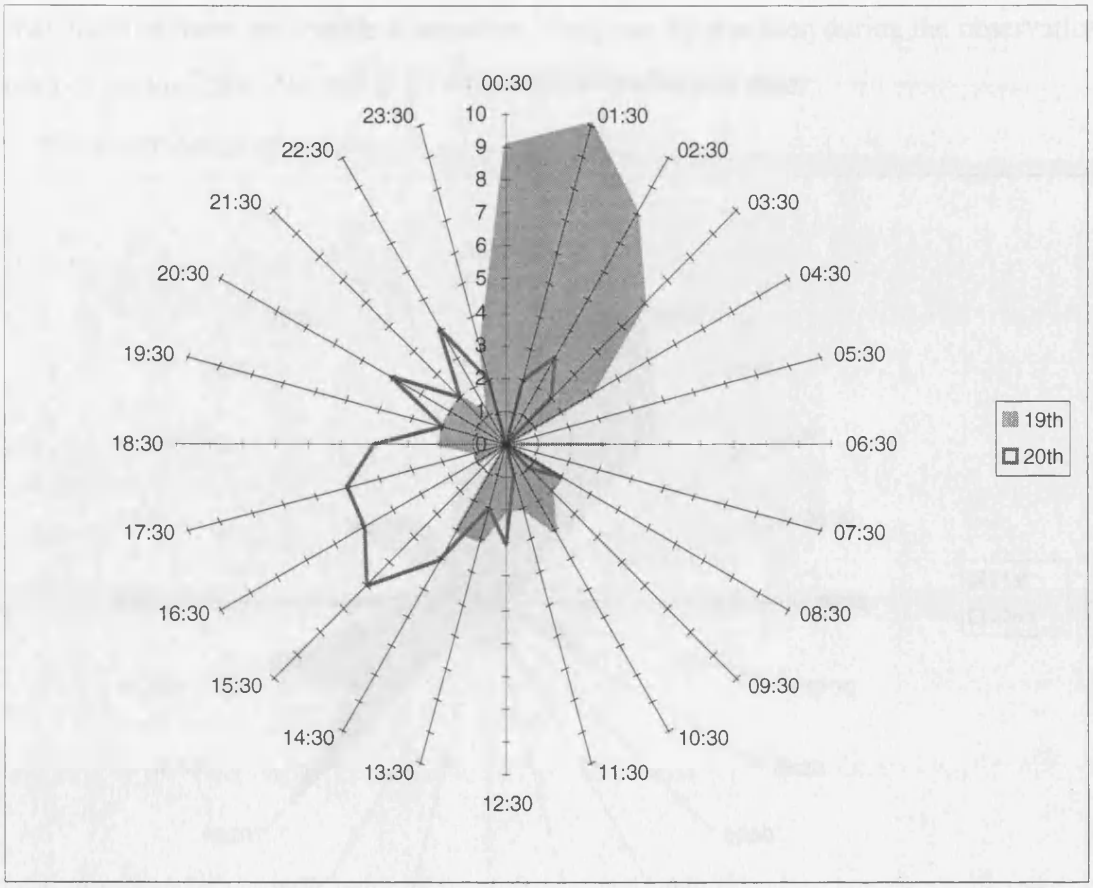



Figure 3.6 Video-monitored rat activity during days 3 and 4 at site B4 (19-20 May 1995)

The peak in rat activity on day 3 was a continuation of the peak on the 18th, extending from midnight until about 04:00. Rats were seen to move progressively around the bait trays in a feeding "frenzy" until all the bait had run out. Only adult rats appeared to be involved in this extended feeding period. Rats were seen across the whole area, including the open spaces, throughout the observations. The rats continued feeding until they had taken all the bait supplied in the bait trays. Smaller peaks involving just a few rats occurred during the late morning and again in the evening. These activity periods seemed to involve rats checking each of the bait points for any left over bait. On the next day (20th), the main activity occurred earlier, in the formerly busy period during the mid afternoon to early evening. Fewer rats were involved.

Activity resumed in the early hours of the 21st and again in the late afternoon that day. A large reduction in activity was noted thereafter from the 22nd (Day 6), with a

maximum of three rats visible at any time. Only one rat was seen during the observation periods on the 25th. No rats at all were observed after this date.

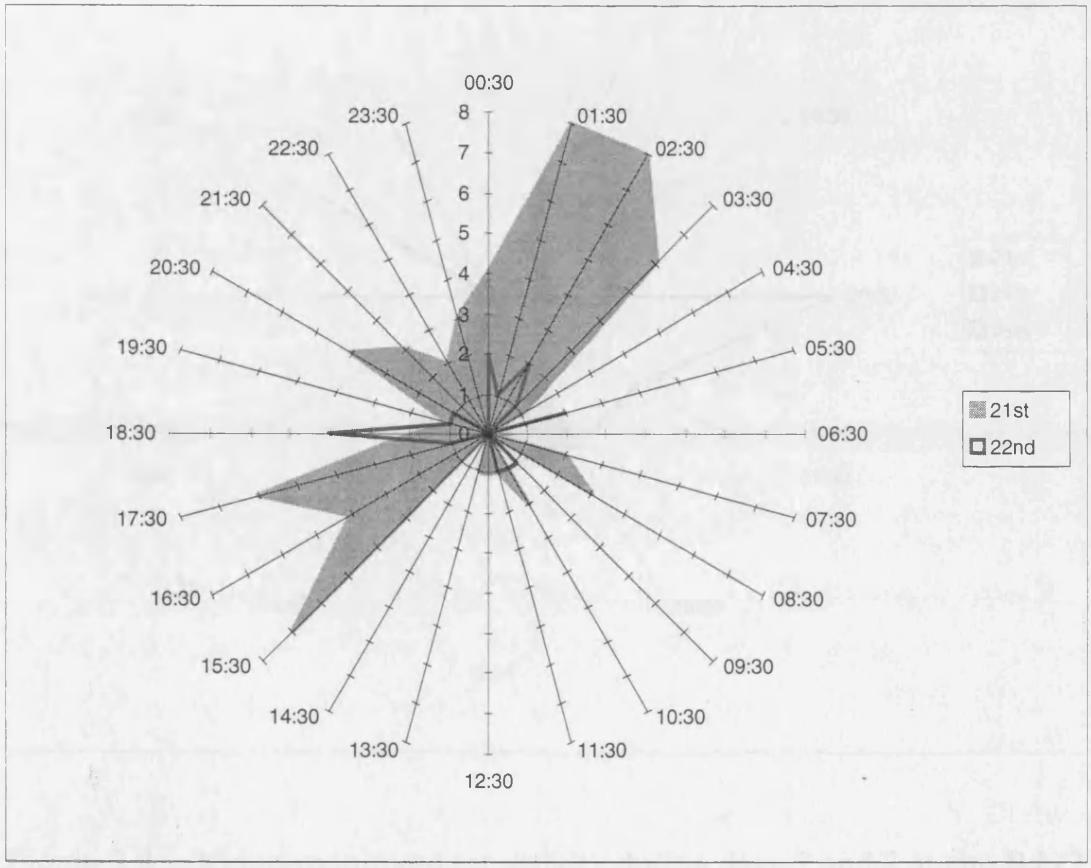



Figure 3.7 Video-monitored rat activity during days 5 and 6 at site B4 (21-22 May 1995)

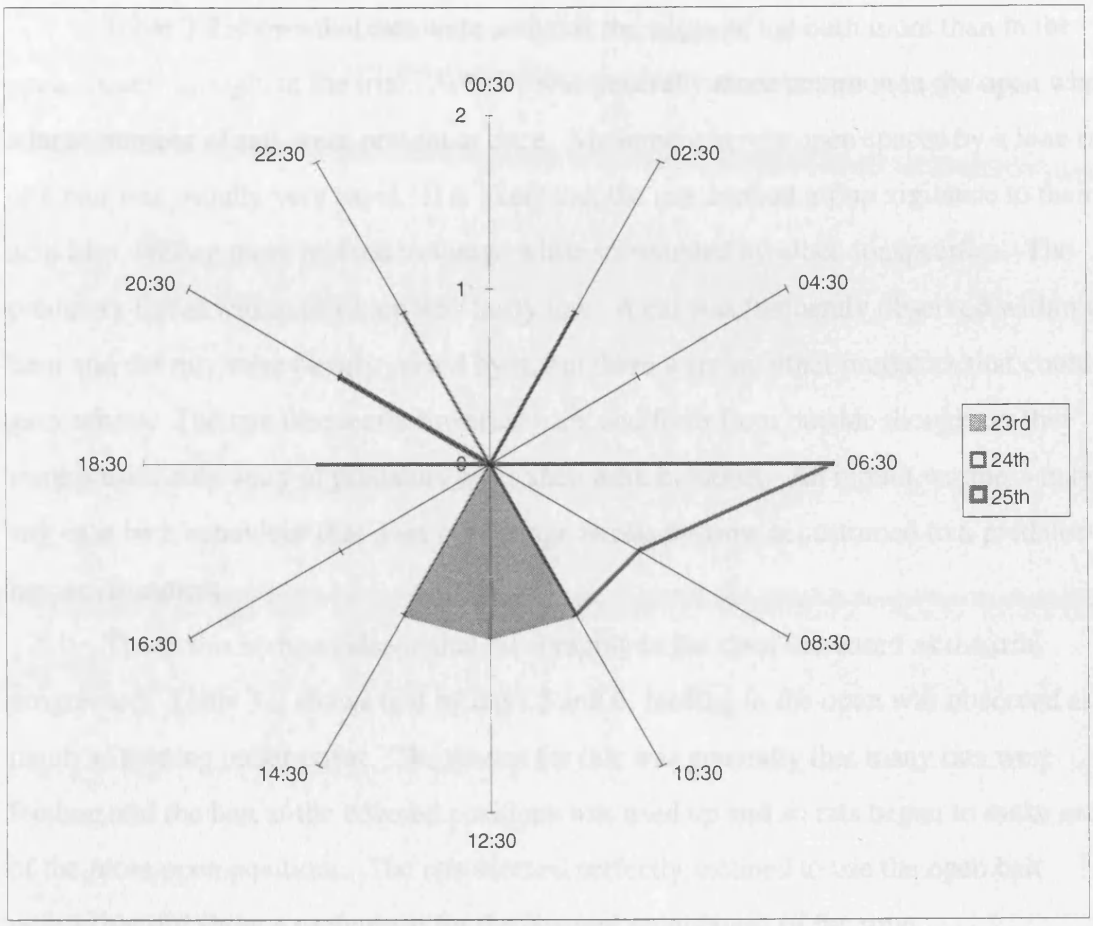



Figure 3.8 Video-monitored rat activity during days 7 and 8 at site B4 (23-24 May 1995)

Table 3.2 Observations of use of feeding area by rats at B4 in two-day intervals before the start of baiting and after bait was placed. Observations refer to the number of 10 minute sample periods and the individual values refer to the number of observation intervals in which the defined behaviour was exhibited.

| Site | Dates | Total observations | Counts at edge | Counts in open | Feed under cover | Feed in open |
|---|---|---|---|---|---|---|
| B4 | pre-bait | 48 | 42 | 27 | 2 | 0 |
| | days 1 & 2 | 48 | 35 | 19 | 8 | 5 |
| | days 3 & 4 | 48 | 38 | 21 | 22 | 14 |
| | days 5 & 6 | 48 | 31 | 23 | 14 | 14 |
| | days 7 & 8 | 24 | 6 | 6 | 1 | 3 |

Table 3.2 shows that rats were active at the edges of the barn more than in the open spaces throughout the trial. Activity was generally more common in the open when a large number of rats were present at once. Movement across open spaces by a lone rat or a pair was usually very rapid. It is likely that the rats applied group vigilance to their activities, feeling more relaxed to forage while surrounded by other conspecifics. The predatory threat within this barn was fairly low. A cat was frequently observed within the barn and the rats were clearly scared by it, but there were no other predators that could gain access. The rats frequently travelled back and forth from outside though, so they were presumably wary of predators when they were outdoors. An inbuilt wariness may in any case be a behaviour that does not change as rats become accustomed to a predator-free environment.

There was some evidence that rat-foraging in the open increased as the trial progressed. Table 3.2 shows that by days 5 and 6, feeding in the open was observed as much as feeding under cover. The reason for this was generally that many rats were feeding and the bait at the covered positions was used up and so rats began to make use of the more open positions. The rats seemed perfectly inclined to use the open bait points, but did show a preference for the covered points most of the time.

Agonistic behaviour was evident within the groups feeding, especially at peak feeding times when many rats were in the barn together. Some rats were displaced from bait stations by other rats, but more usually, rats met away from a bait point to contest their dominance; this could be seen in individual video frames as classic postures (e.g. hunched back, sideways approach) were adopted (see next section). Much of the feeding in the early stages of the trial occurred singly or occasionally in pairs. These pairs consisted of juveniles or adults in any combination. During the peak periods, juveniles were notable by their absence. At less favoured times, groups could be seen feeding together. These may have been family groups, presumably feeding at one point to make use of group vigilance. An alternative may be that the juveniles were copying (or being taught by) the adults (Galef and Whiskin, 1995a; 1995b).

On two occasions a small bird (unidentified) was seen within the barn, feeding near the bait points but not from them. A wagtail was also seen on one occasion. The cat that visited the barn also sniffed from a bait tray on one occasion, although it was probably sniffing the rat odour rather than contemplating taking the bait.

### 3.3.iv Individual and interactive rat behaviour at Farm B4

The frame by frame analyses of video footage from site B4 allowed individual rats to be observed as they interacted with other rats. Some of the behaviours noted are shown in the frame sequences in Plates 3.3-3.7. Agonistic behaviour was observed both in apparent defence of a bait point (Plate 3.6) and as a territorial type behaviour in the whole arena within view (Plates 3.5 and 3.7). Adult rats were clearly very aware of the presence of others in the vicinity. Some rats were tolerated, while others were deterred immediately or avoided. Rats within a family group are likely to tolerate each other (Macdonald and Fenn, 1994; Shepherd and Inglis, 1987), whereas intruders will be attacked (Adams and Boice, 1983). It is impossible to say whether the rats observed feeding in the barn at B4 were an extended family group or whether they were members of different families. Macdonald and Fenn (1994) described rats as forming colonies that are probably loose conglomerations of small family units or "clans" with a greater degree of tolerance within rather than between groups. It is likely therefore that the tolerated rats were members of the same group whereas those that were attacked were members of an alien group. Within large infestations, the smaller groups that make up the colony are likely to defend a particular area (Macdonald and Fenn, 1994). This may have been happening in the pair attack seen in Plate 3.6.

Submissive or cautious behaviour was also observed (Plate 3.4). Such behaviour may have been displayed by what Adams and Boice (1983) referred to as the beta males. These animals are subordinate to the dominant alpha animals and can only use resources when the alpha animals have finished. Female rats are also noted to be less aggressive when they encounter each other and rely on posture rather than attack to decide dominance (Adams and Boice, 1983; Ziporyn and McClintock, 1991). Ziporyn and McClintock also described a behaviour, termed "passing", which females use when they meet to display social dominance. An example of this behaviour is possibly exhibited in Plate 3.3 where the two rats meet. Juvenile rats were not observed interacting with any other rats. In some cases, they were seen feeding throughout an agonistic encounter in the vicinity of the bait point. Such an observation would imply that juveniles are tolerated and are not involved in inter-group contests.

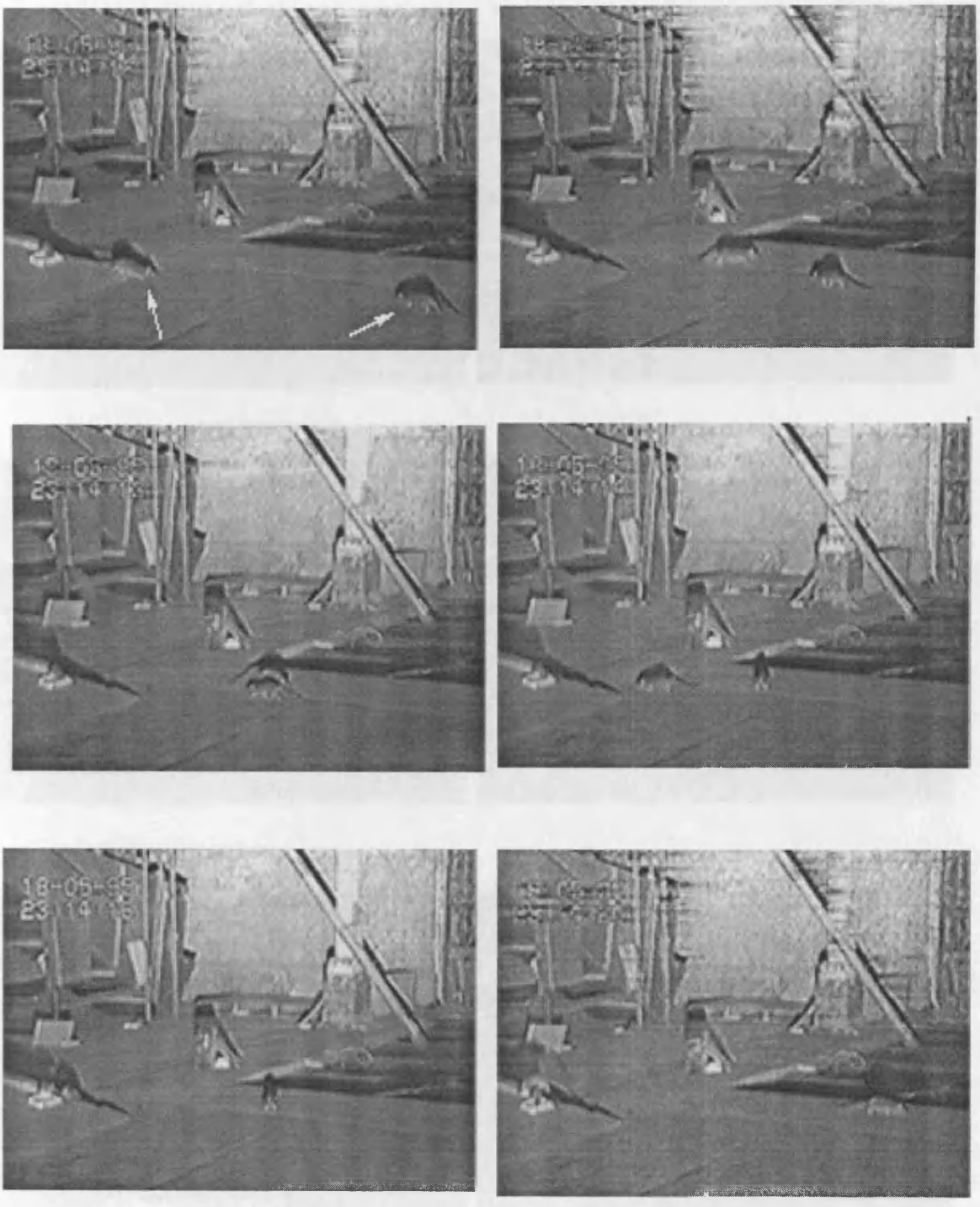


**Plate 3.3 Putative "Passing" behaviour**

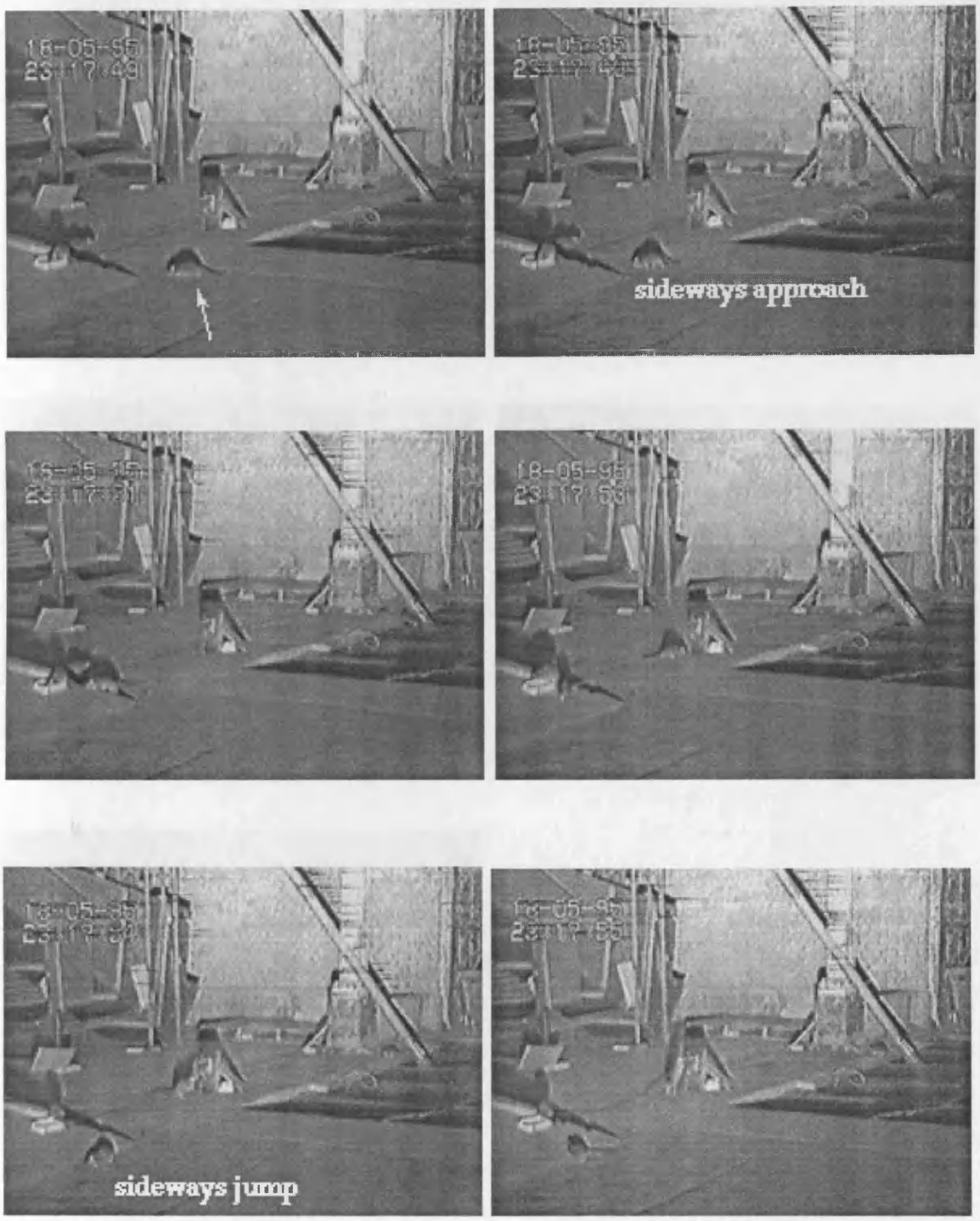



Plate 3.4 Timid approach

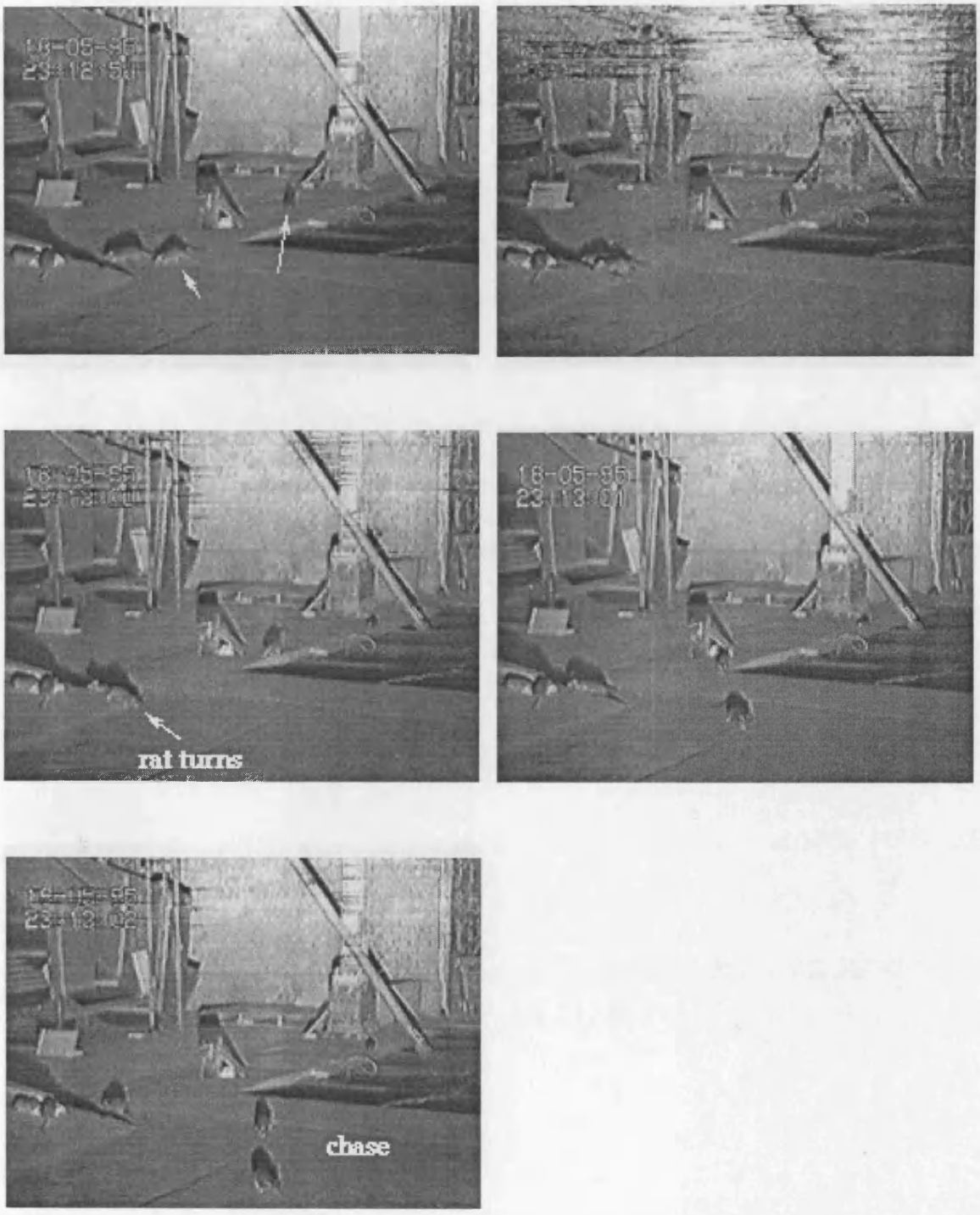



Plate 3.5 Attack/Territorial behaviour

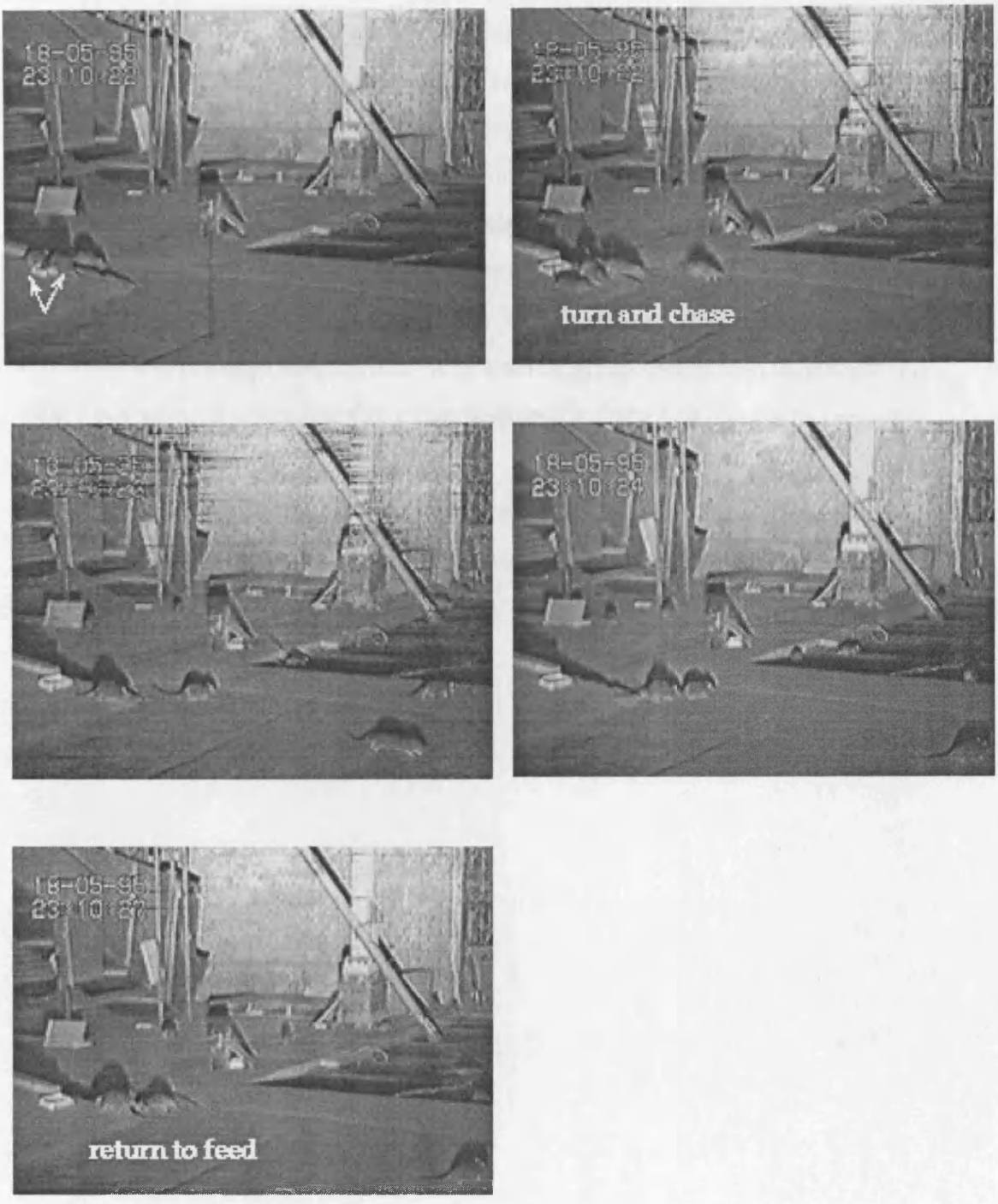



Plate 3.6 Double attack

Inglis *et al.* (1996) noted that adult rats within an enclosure would frequently feed with juveniles from the family group but would never feed with other adults at the same bait points. Plate 3.9 shows a group of five rats feeding together on two separate occasions. This may have been a family group. There is more than one adult present, however, which contradicts the observation made by Inglis *et al.* (1996). The group can be seen feeding from the same bait point while all the other bait points are unused. This suggests copying behaviour, and may also be a mechanism to increase group vigilance against predators or aggressive conspecifics. The absence of other rats also implies that this was a less favoured time to feed. Why a whole group should feed together at this time is not clear. It is possible that group feeding at a single bait point is incompatible with holding a position of dominance.

Plate 3.10 shows the arrival of the farm cat at the observation site and a rat feeding nearby. The rat was apparently unaware of the cat at first, but then made a very speedy escape.

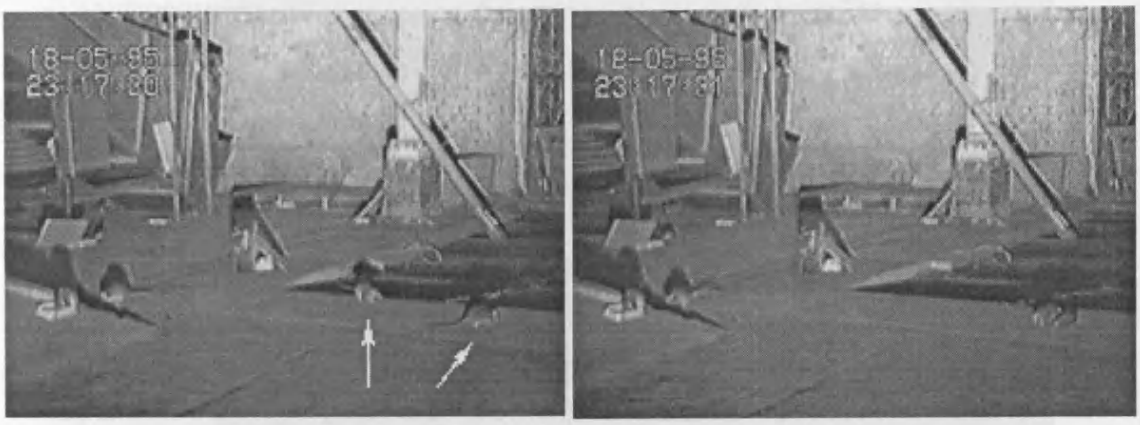


Plate 3.7 Aggressive contact

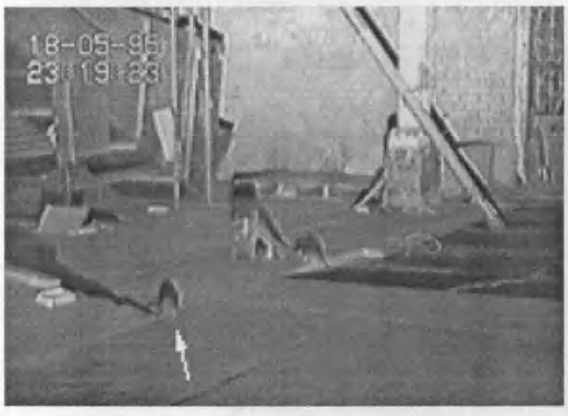


Plate 3.8 Hunchback posture

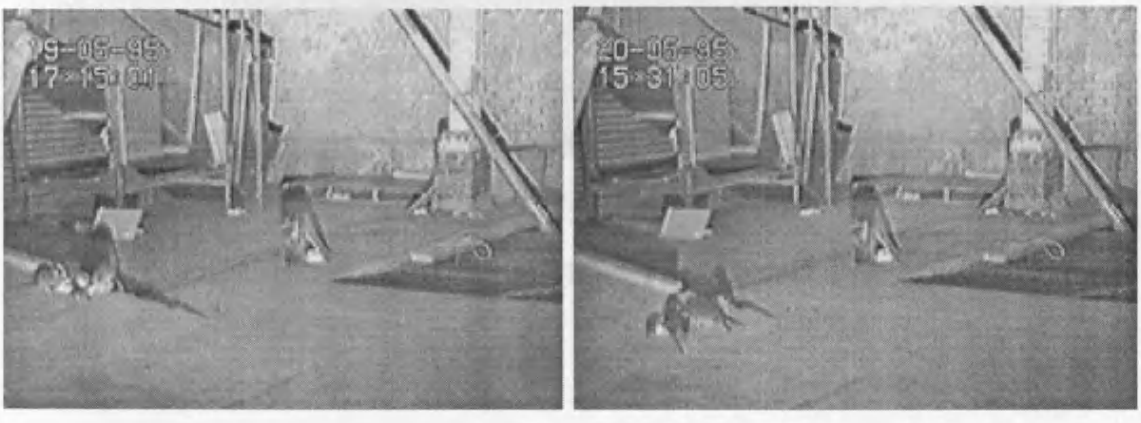


Plate 3.9 Group feeding on 2 occasions

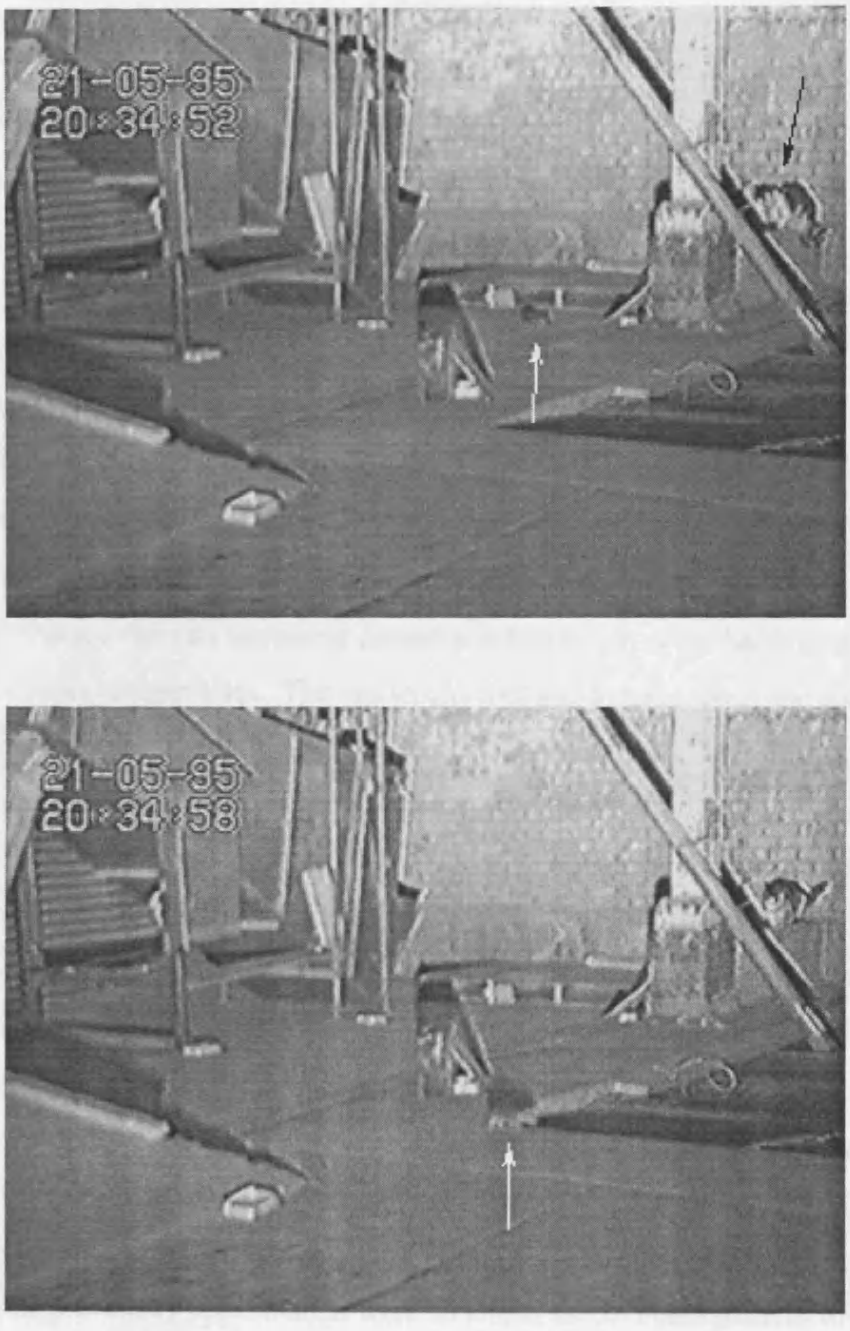



**Plate 3.10 Escape from predation**

### 3.3.v Other observations noted from the farm trials

Many observations were made during the baiting trials on the 18 farm sites that gave a clue to behaviour exhibited by wild rats during the period before death. Blood stains were observed on bait trays, which implied that rats were continuing to feed (or at least visit bait points) even at the stage of haemorrhage.

Rats were clearly having to contest bait point access; bait trays were found dragged into rat holes on many occasions, especially at sites where the rat density was perceived to be high. It would seem therefore that the rats were attempting to monopolise the resource and were only able to do this within their own home site. Taylor and Thomas (1989) noted that rats invariably carried wax blocks out of the baiting tunnels and removed the blocks to their holes. The rats would continue to travel back and forth between bait tunnel and their hole until all the blocks had been removed. This hoarding behaviour has been noted with rats elsewhere (Nakatsuyama and Fujita, 1995; Whishaw and Whishaw, 1996). Removing wax blocks was noted to leave no bait residue above ground (Taylor and Thomas, 1989), whereas dragging around bait trays that contain granular bait may leave a trail of bait residue (Quy *et al.*, 1995). This spillage of bait was noted during these trials.

Rat activity and bait point use on many of the farm sites used during the trials seemed to occur in patches. Some bait points were very popular, whereas others were not used or were used very little, despite efforts to position all bait trays in suitable rat terrain. It was also noted that some parts of a farm site only became used by rats later on in a treatment, while previously popular areas were no longer used. These patterns of activity infer that a farm rat population may indeed be made up of a number of subgroups or clans (Macdonald and Fenn, 1994).

Cox (1991) noted that outdoor bait points were used more than indoor points. No such definite pattern was observed at the sites used during this trial and each site was very different. The notable factor was that rats accessed mostly the bait points that were close to the rat harbourage. Thus at sites where rats were living largely indoors, most bait-take was observed from indoor bait points, and outdoor bait trays were used mostly where rat harbourage was outside.

Cox (1991) noted that rats and wood mice were feeding from the same bait trays. There was evidence of mice and rats feeding from the same trays during these trials, although the mice involved were mostly house mice. At one of the sites, A1, house mice apparently also lived within the main straw rick used as harbourage by the rats. This was a surprising observation, as it is commonly believed that rats deter, or even predate, house mice.

Rats were noted behaving abnormally during the trials. "Dozy" rats were observed at a few sites; usually they were crouched in an exposed location such as on hay bales or on the floor and once in an exposed drain pipe. All the rats were observed in this state during broad daylight. The rats were clearly alive but seemed unable to move away. This "freezing" behaviour was observed among females by Cox and Smith (1992). Cox and Smith also described male rats staggering during the pre-lethal phase of anticoagulant treatment. Two rats were observed showing this behaviour during the trials. One rat staggered out through a doorway into the open yard during broad daylight.

Finally, an example of just how adaptable rats can be was shown at site B3 where there was a fire that destroyed the whole of a barn during the second night of the trial. Rats had been using the barn for harbourage (in hay) and they had been feeding on bagged pig feed that was stored in the barn. The morning after the fire, the earth floor was still smouldering and yet there were fresh rat excavations in the earth.

## 3.4 DISCUSSION

The rats at B4 were largely nocturnal throughout the trial whereas those at B5 were active only by day. The reason for this difference between the two sites is likely to be related to the relative disturbances at each site. Farm workers were always active in the vicinity of the feeding area at site B4 between 07:30 and 16:30 and the rats were mostly active outside these times. The barn at B5 was rarely visited and so the rats remained undisturbed through the day. Nieder (1985) noted that more than 95% of rat activity was around sunset and Taylor (1978) noted activity peaks around sunset and continuing for a few hours and then a smaller peak before sunrise. This approximate pattern occurred at B4, but not at B5. It is possible that either night time predation was high at B5 making the day time relatively safer, or that in the absence of day time risks, the day is actually a preferred time for feeding. It has been noted previously that outdoor rats usually use the relatively safer night time for feeding, mating and play (Robitaille and Bovet, 1976; Hardy and Taylor, 1979; Berdoy and Macdonald, 1991), but it has also been noted that if there is little disturbance, rats will be active during the day (Shepherd and Inglis, 1987; Shekarova *et al.*, 1995).

The video monitoring of activity at the two sites B4 and B5 revealed no changes in circadian activity as a result of baiting. This is in contrast to the observations made by Cox and Smith (1992) who found that rats changed their preferences after ingesting bait from being active at night to being more active by day. It may be that the indoor location of feeding at the two sites video-monitored during this trial was the reason for no change; because effectively it was dark 24 hours a day. The rats observed by Cox and Smith (1992) were either in cages or in an enclosure, *i.e.* those rats were in far more of an artificial setting, with predators absent, few other rats and so on. The set-up during those trials did, however, include artificial light that was used to mimic daylight. The reason for the contrasting data from this study and the one reported by Cox and Smith (1992) may therefore be simply due to the absence of a light period at the feeding areas observed at sites B4 and B5. It would obviously be preferable to repeat these types of video-observation studies on more farm sites, and viewing outdoor activity of rats as well as indoor activity in order to discover whether activity preferences between day and night do occur in wild free rats.

Although abnormal behaviour in the pre-lethal period was noted on a few of the farm sites during the baiting trials, no such behaviour was observed on video. This again is in contrast to the data produced by Cox and Smith (1992). It may be that the video footage was played back relatively too fast (playing time-lapse at normal speed effectively speeds up the film) to notice such behaviour or perhaps such behaviour is only found among rats during daylight (the direct, personal observations made on "staggering" rats and "frozen" rats all occurred during daylight and those observations made by Cox and Smith (1992) were in artificial daylight). Such abnormal behaviour is likely to alter the risks of predation, and any circadian changes in activity will determine whether it is the night-time predators (and scavengers) or the day-time ones that are potentially exposed to a rodenticide-affected rat. Erlinge (1975) noted that weasels avoid prey that "freeze", although badgers are often observed to take unhealthy prey as they are relatively easy to catch (Neal, 1986). Owls have been noted to take rodents in the open more than from cover (Kotler *et al.*, 1988). The data produced by Cox and Smith (1992) and the personal observations made infer that daytime hunters, such as kestrels, buzzards, kites and weasels are likely to gain increased exposure to poisoned rats compared to largely nocturnal animals such as badgers, foxes and owls. Scavengers are likely to be unaffected by any activity change because the carcasses may be found day or night. Scavengers will obviously be exposed to any rats that die above ground. Carcasses were infrequently discovered away from cover during these trials, an observation also noted during previous similar trials (Fenn *et al.*, 1987; Harrison *et al.*, 1988). Whether the rats died under cover or whether they were quickly scavenged before researchers found them is not sure. Kenward (1988) noted that 43% of grey squirrels died in their dreys after a poison treatment. This result would imply that scavengers could be exposed to about half the poisoned population. The relative exposure is then determined by what percentage of the prey item, or perhaps the hunting territory, is affected. Hegdal and Colvin (1988) discovered that the proportion of poisoning incidents and mortality among Eastern screech owls was greatly affected by the percentage of the owl's home range that was treated with anticoagulant to control meadow voles.

It is apparent that replicated trials including video-monitoring of rat activity outdoors would be desirable. Only by such replications can any patterns of circadian activity, or the reasons for deviation from normal patterns, be discovered and understood.

Pre-lethal period changes in behaviour that relate specifically to wild free rats can then also be determined and the repercussions on predators and scavengers can be more accurately assessed.

Rats during this trial apparently preferred to move close to cover while rats were few in number, but use of open spaces increased as more rats were present. Covered bait points were initially more preferred, but open trays were used later, especially by rats feeding together. Cox and Smith (1992) found that covered bait points were used more than open ones and this pattern did not change during the pre-lethal period. It may be that, with a large number of rats together, group vigilance is used to reduce risks of individual predation, thus allowing use of open spaces and uncovered bait points more freely. Rats generally fed in groups. These were not normally tight groups (as in Plate 3.9), but individuals feeding within the peak times of activity. During these times there would be many rats in the barns simultaneously. It would seem that there must be some advantage to feeding as a group over feeding alone because lone rats were rarely observed. Group vigilance is probably the advantage gained. Rats were certainly observed more often in the open (although within the confines of the barn) when many rats were present; lone rats almost always stayed near cover.

Agonistic behaviour at peak times was frequently observed. Dominant animals that can monopolise feeding points and that win dominance contests (even if only displayed by posture) will gain most from feeding at peak times because they are protected from predators while they feed. It is probably also worthwhile for juveniles and beta animals to feed with a large group, especially as juveniles were noted to be excluded from agonistic encounters in these trials. The antagonism received by omega animals is likely, however, to be detrimental. Omega rats will be unable to access bait points at all or may be confined to feeding from more risky bait points on the edge of the territory or away from cover. Omega rats may thus choose to feed on their own at less good times to avoid antagonism or they may be confined to feeding at off-peak times through exclusion by conspecifics. On their own, they will get more food but will also be more prone to predation (Taylor, 1978; Hardy and Taylor, 1979; Shepherd and Inglis, 1987; Berdoy and Macdonald, 1991). This may actually mean that the rats that are predated are the least likely to have eaten a large dose of poison. Colvin (1984) argued that barn owls were not

at risk of poisoning by anticoagulants because the subordinate (juvenile) rats that were selected were likely to be excluded from bait points.

The family groups observed in Plate 3.9 were feeding at an isolated bait point with no other rats around; it was not a peak time. Family groups may be confined to feeding at less good times in order to protect their young from antagonism or because the adults are themselves subordinate animals. A possible alternative may be that some form of teaching occurs between adult and juvenile rats and the group is unable to monopolise a single bait point for group feeding when many rats are present.

Rats infected with *Toxoplasma gondii* have been shown to be less neophobic and more prone to predation (Webster *et al.*, 1994; Berdoy *et al.*, 1995). This may mean that these rats are more likely to consume novel baits and so any predator that consumes the rats will be exposed to both the poison and to *Toxoplasma gondii.*

*The future:* Activity of wild rats has been studied by radio-telemetry (Hardy and Taylor, 1979; Fenn *et al.*, 1987) and within enclosures (Shepherd and Inglis, 1987; Cox, 1991; Inglis *et al.*, 1996). There is still, however, a lack of behavioural studies on rats following dosing with anticoagulant rodenticides, especially in the wild. Such work is vital because then a real pattern of activity, abnormal behaviour and exposure to non-target predators and scavengers can be assessed and further predicted. Behaviour is a key part of ecotoxicology and others have realised the importance of including behavioural studies for post-regulation monitoring of pesticide exposure to vertebrates (Hart, 1990).

Video-monitoring is likely to be the best way forward with the study of farm rat behaviour after an anticoagulant treatment; there is negligible interference and continuous and permanent observation can be achieved. Difficulties of site access will hopefully be overcome in the future so that outdoor sites can be monitored. Farms in areas of physiological resistance can then also be studied. This may give an insight into reasons why control failures occur (Quy *et al.*, 1992b; Brunton *et al.*, 1993) and provide proof or contention for putative behavioural causes of treatment failure such as the Poisoned Partner Effect, increased neophobia and conditioned aversion (Berdoy and Macdonald, 1991; Brunton *et al.*, 1993) in free-living wild rats.

## 3.5 CONCLUSIONS

Field observations at all the farm sites and detailed analysis of video-monitored rat activity at two of the farms provided evidence of the following rat behaviours:

1. Rats may be primarily active by day or by night, depending on local disturbances and the relative safety of their food source location.
2. Where large numbers of rats are active at one time, other (possibly subordinate) rats also feed away from peak times.
3. Rats showed a preference for moving and feeding under cover. During peak times of activity, however, when many rats were present together, rats readily used the open spaces to feed and move about. It is proposed that group vigilance allowed this apparent drop in individual wariness.
4. There were no real changes in circadian patterns of activity as a result of poison bait application. This result contadicts the findings of Cox and Smith (1992) from their study of rats in cages and enclosures. Instead, there was less activity in general as a result of the poison application.
5. "Freezing" and "staggering" behaviours were exhibited by some rats prior to death. These rats were found in the open during daylight as well as indoors.
6. Interactions between individual rats were frequently observed. Interactions between pairs of rats were also noted. There was evidence of direct aggression (attacks and chases). There was also evidence of passive displays of dominance (hunched back posture and "passing"). All interactions appeared to relate to access to bait points, although some contests were over a larger area (*e.g.* Plate 3.5).
7. Juveniles were apparently immune to aggressive interactions between two or more rats. Juveniles were noted to continue feeding at bait points located at the heart of an interaction.
8. Some rats were timid and appeared to have subordinate status in their approach to a bait point (*e.g.* Plate 3.4). These rats, nevertheless, were able to feed often from the bait trays during peak activity periods.
9. Groups of up to five rats were observed feeding from a single bait tray, despite there being other bait trays available in the vicinity. These groups consisted of adults and juveniles and were possibly family groups.

10. This study of wild, free, farm rat populations was the first of its kind. Additional studies of farm rats would enable confirmation or contention of putative behaviours such as the Poisoned Partner Effect, increased neophobia and conditioned aversion.

# CHAPTER 4: ANALYSING RESIDUES IN RODENT TISSUE

## 4.1 INTRODUCTION

### 4.1.i The aims of this study

Various field studies have included the use of labelled or marked food to follow the path of a chemical into primary feeders and even on through the food chain (Crier, 1970; Cox, 1991; Sanchez-Hernandez, 1994). More specifically, certain polychlorinated biphenyls (PCBs) have been used previously for studies of rodent feeding (Buckle *et al.*, 1986; Quy *et al.*, 1992; Sanchez-Hernandez, 1994; Townsend *et. al*, 1995; Cowan *et al.*, 1995) because they are relatively stable metabolically and so can be detected in the rodent some weeks after it has consumed the marked bait. A method developed by MAFF, Central Science Laboratory (unpublished), and referred to by Cowan *et al.* (1995) and Quy *et al.* (1992), used the relationship between known amount of ingested PCB marker versus amount of PCB marker retrieved from carcasses and applied this to field studies where only the amount of PCB retrieved could be measured.

During this study, Hexachlorobiphenyl (HCBP) was incorporated with the brodifacoum and coumatetralyl baits at 98 $\mu g\ g^{-1}$ (and in a later batch at 89 $\mu g\ g^{-1}$). These levels were chosen to fall within the maximum limit allowable of 100 $\mu g\ g^{-1}$ set by the UK Department of the Environment.

There were two aims for this part of the project. The main aim was to measure the residues of HCBP in the rat carcasses (following the method used by MAFF, Central Science Laboratory) and thereby test the hypothesis that there are differences in individual bait-consumption levels of coumatetralyl versus brodifacoum, and that there are differences in bait consumption in an area of physiological resistance versus an area where resistance is absent. The second aim, if possible, was to determine levels of rodenticide residues in the carcasses and trapped bodies and thereby measure the actual rodenticide load carried by rats at any time. This would test the hypothesis that resistant rats carry a higher load of rodenticide than susceptible rats.

## 4.1.ii Storage and metabolism of rodenticides

Anticoagulants are insoluble in water but are very lipophilic, so they accumulate in fat deposits around the body (Meehan, 1984). They are also known to accumulate in the liver, their site of action (Newton *et al.*, 1990; Gray *et al.*, 1994b; Jones, 1996). Thijssen and Janssen (1994) noted that the half-life of coumatetralyl in rat livers was 7-10 days. Second-generation anticoagulants are little metabolised and may be stored in a very stable form in the body for months. Thijssen (1995) reported that the half-life of brodifacoum in the rat liver is more than 100 days and Masterd and Thijssen (1991) noted that rat liver microsomes were saturated with brodifacoum for at least 30 days. Newton *et al.* (1994) found that flocoumafen was slowly eliminated from the livers of barn owls and had a terminal elimination half-life of more than 100 days. The body half-life may, however, be less than this. Brown *et al.* (1988) noted that the mean residue of brodifacoum in rats after pulsed baiting dropped from 0.45 mg $Kg^{-1}$ to around zero in 35 days, with an estimated half-life of around 14 days.

Some rodenticide is broken down and removed from the body; Meehan (1984), in a review of rodenticide metabolism, stated that the rate of metabolism depends on the genetics of the rat. Excretion rates of warfarin in laboratory rats ranged from 14% after 48 hours to 50% after 6 hours, depending on the strain of rat tested. Rodenticides remained in females for longer than in males. About 66% of metabolites occurred in urine and the rest occurred in the faeces.

Huckle *et al.* (1989a) used a sub-lethal dose of $^{14}C$-labelled flocoumafen to measure absorption and elimination rates from caged rats. The flocoumafen was rapidly absorbed, with a maximum level occurring in the blood at four hours after dosing. It was very slowly eliminated from the body, with 74-76 % being retained at day 7. Half of the dose was found in the liver as unchanged flocoumafen and from here, elimination was incredibly slow (elimination half life was 220 days). In separate studies, Huckle *et al.* measured elimination rates of $^{14}C$-labelled flocoumafen from rats after repeated oral dosing (1988) and after percutaneous injections (1989b). Huckle *et al.* found that elimination from the body via the urine was minimal, but faecal elimination accounted for the loss of between 18 and 59% of the dose, with more being eliminated when repeated doses were administered. Much of the flocoumafen was stored within the liver, which appeared to become saturated after a certain level of dosing so that no further

accumulation occurred. Huckle *et al.* (1988) suggested that lethal anticoagulant action only occurs after the binding sites in the liver are fully saturated. Eadsforth *et al.* (1993) also found evidence for what they believed to be saturable binding-sites in the livers of hens that were fed with flocoumafen.

Metabolism of warfarin (breakdown and excretion) is apparently the same in resistant and non-resistant rats (Meehan, 1984), although Thijsen (1995) noted that in the Scottish-type of physiological resistance there is little or no persistence of anticoagulants in the body.

## 4.1.iii Storage and metabolism of PCBs

Polychlorinated biphenyls are lipophilic and persistent (O'Neill, 1993). They have made history in environmental biology for their adverse effects on marine mammals following bioaccumulation through the food chain (Begon *et al.*, 1990). Townsend *et al.* (1995) noted that HCBP is one of the more benign PCBs. HCBP could be released into the environment at concentrations up to 100 $\mu g\ g^{-1}$ (though the level allowed without a licence from the Department of the Environment has since been reduced to 50 $\mu g\ g^{-1}$). The particular isomer of HCBP used in this study is shown in Figure 4.1, along with the TCBP isomer used as an internal standard in the laboratory analyses.

Mizutani *et al.* (1980) described the properties of different HCBP isomers and noted their respective elimination rates from mice. Mizutani *et al.* found that 2,4,5,2',4',5'-HCBP was more rapidly eliminated than most other HCBP isomers and it was comparable to TCBP isomers. TCBP was used as an internal standard during the analyses of HCBP for this study. The internal standard is supposed to mimic the action of the compound under investigation, so the similarity of their elimination rates is a good thing. The biological half-life of 2,4,5,2',4',5' HCBP was found to be about 94 days in mice, but Mizutani *et al.* stated that elimination rates, and thus the biological half-life, were likely to depend more on release from fatty tissues in the body than on rates of metabolism. Cowan *et al.* (1995) found that residues of HCBP remained stable for at least six weeks in rats. Some metabolism of PCBs was noted by Sanchez-Hernandez (1994) in wood mice and grey squirrels following oral consumption of bait formulated with dichloro- and decachloro-biphenyls. Accumulation occurred in the body until dosing

stopped and then a small amount of metabolism was noted. Sanchez-Hernandez (1994) found a good correlation between the level of marker in the blood of both wood mice and squirrels and the amount of marked bait ingested. This type of relationship has also been reported by Quy *et al.* (1995), Townsend *et al.* (1995) and Cowan *et al.* (1995) for PCB markers within bodies of wood mice and rats.

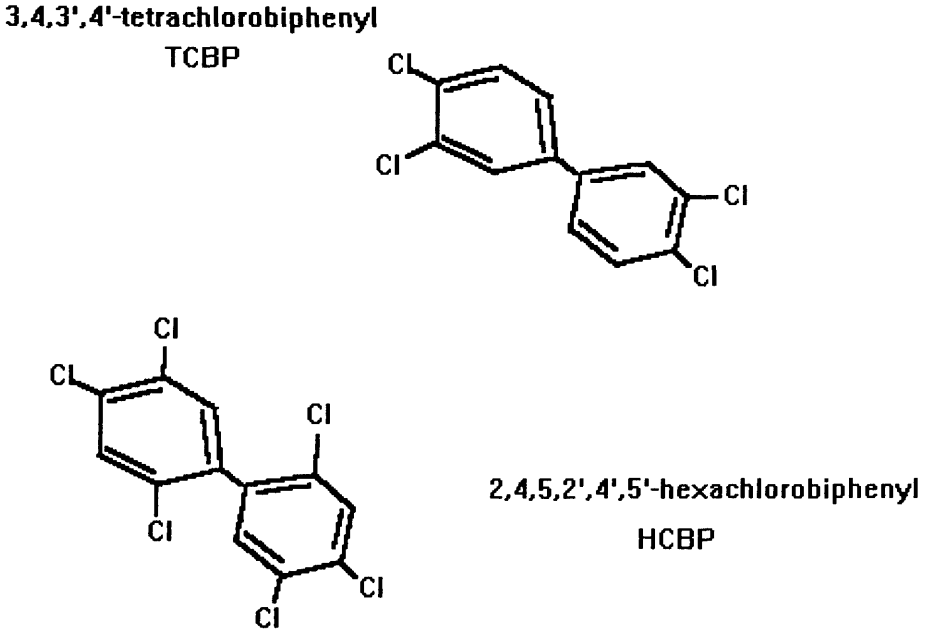



Figure 4.1 The chemical structures of the internal standard, TCBP and the marker incorporated in the rodenticide bait, HCBP.

For the purposes of this study, the HCBP and the TCBP internal standard were assumed to be metabolically stable and therefore able to provide a true indication of consumption levels. Metabolism, if it occurred, would mean that the levels of HCBP residue measured were minimum levels and the real consumption of bait was greater than the values indicated.

## 4.1.iv Liquid chromatography

The low volatility of anticoagulant rodenticides makes liquid chromatography the method of choice for their determination (Jones, 1996). Substances suitable for Liquid Chromatography will be soluble and miscible in a mobile phase or solvent. This covers a very wide range of substances.

**TLC**: Thin Layer Chromatography (TLC) relies upon capillary action to draw the components under investigation through the solid phase with the solvent. The component is then deposited behind the solvent front and the distance the component has travelled indicates its strength and polarity. Using an ultraviolet light source, fluorescence detection of compounds such as hydroxycoumarin anticoagulants can be made because they glow under the u.v. light. TLC provides a non-quantitative measure of the presence or absence of a residue in the body extract. TLC has been used for determining anticoagulant rodenticide residues in the past (Yuen, 1978; Stahr *et al.*, 1991).

**HPLC:** High Performance Liquid Chromatography (HPLC) also uses a mobile phase that flows through a stationary phase or column. The column consists of tightly packed particles of silica. A mobile phase of up to four different solvents in a mixture is pumped through the column. The ratio of different solvents and the flow rate can be varied throughout the run. An injection of the substance under investigation is made and the solvents continue to pump through the column for a set period of time to wash the column through. If a component has a strong affinity for the mobile phase then its retention time in the column will be short. In reverse phase HPLC (used during this study), the most polar substances will elute before the less polar compounds. Either ultra-violet or fluorescence detection can be used with HPLC. The detector reacts to the presence of a component and produces an electric signal that is recorded.

A number of studies have used HPLC to measure or determine rodenticide in animal tissue (Reynolds, 1980; Mundy and Machin, 1982; Hunter, 1983; Merson *et al.*, 1984; Hunter, 1985; Hunter *et al.*, 1988; review by McGarvey, 1994; Kuijpers *et al.*, 1995). HPLC with fluorescence detection has been used to determine a range of anticoagulant rodenticides in animal tissue (Kelly *et al.*, 1993; Panadero *et al.*, 1993; Jones, 1996; Coly and Aaron, 1996).

## 4.1.v The basics of GCMS

Gas Chromatography with Mass Spectrometry (GCMS) was used for the PCB marker-residue analyses. This combined technique can easily detect polychlorinated biphenyls as they are particularly volatile. It is also highly sensitive and can detect very small amounts of compound.

In the GC, a gaseous mobile phase travels through a stationary phase or column. The stationary phase is a high molecular weight polymer that is coated on the inner walls of the capillary column. The mixture of compounds under investigation is injected into the mobile phase and is then carried in the mobile phase to the column. The components are retarded by the stationary phase on the column to differing degrees depending on their volatility. The components reach the end of the column and enter the mass spectrometer. They are immediately ionised by electrons in the source chamber before being filtered off to the mass analyser. Here, the analyser either scans for the ions, or selective ion recording (SIR) can search for specified ions. GCMS usually employs SIR as the method of detection. The ions finally reach the detector multiplier (DM) where an electrical signal is multiplied (for easier detection) and then detected.

The GCMS used in this study made use of select ion recording (SIR). The electric field of any compound can be scanned and a mass spectrum will then be produced. This will reveal the strong ions within the compound. An intense ion, representative of the compound, is usually selected and the mass spectrometer is then set specifically to detect that ion. If an internal standard that is closely related to the sample compound is used (as TCBP is related to HCBP), then the mass spectrometer can switch rapidly between detection of the ion representing the compound being analysed and detection of the ion representing the internal standard. In this way, accurate, sensitive and selective monitoring and quantifying of samples can be made.

Braselton *et al.* (1992) used MS to determine the rodenticide diphacinone from chlorophacinone. Gas chromatography has been used to determine residues of organochlorine insecticides and their relatives in the past (Telling *et al.*, 1977; Buckle *et al.*, 1986; Quy *et al.*, 1992; Sanchez-Hernandez, 1994; Townsend *et. al*, 1995; Cowan *et al.*, 1995).

## 4.2 METHODS AND MATERIALS

Bait used during the farm trials (Chapter 2) had been formulated with brodifacoum at 50 $\mu g\ g^{-1}$ and HCBP at 98 $\mu g\ g^{-1}$, coumatetralyl at 375 $\mu g\ g^{-1}$ and HCBP at 98 $\mu g\ g^{-1}$ (east midlands), or coumatetralyl at 375 $\mu g\ g^{-1}$ and HCBP at 89 $\mu g\ g^{-1}$ (central southern England). The cut wheat baits were bought already formulated with anticoagulant, and the HCBP was added at Zeneca, Jealott's Hill Research Station, using heated corn oil to emulsify the marker and coat the grain evenly with it.

### 4.2.i Preparing the carcasses for analyses

A protocol supplied by MAFF, Central Science Laboratory (unpublished) was followed, as it had successfully enabled good detection of PCB marker residues from rat carcasses previously. As soon as a rodent body had been found dead or trapped, it was weighed and then the tail, feet and guts, including the stomach, were removed. The tail and feet were removed because they do not homogenise easily and the assumption was made that rodenticide and marker compound would not have accumulated in those body parts. The stomach and guts were removed so that the carcass residue load would not include any bait still in the gut, but just that absorbed into the rest of the body. As soon as possible after these preliminary preparations, the bodies were double-wrapped inside two plastic bags and deep frozen.

It is very difficult to homogenise a whole rat carcass because of its mechanical properties, especially those of the skin. A conventional blender will take out most of the body contents but leaves the skin wrapped around the blade. The skin on frozen carcasses is easier to cut, but the ice (and to some extent, the bones) blunts the cutting blades very rapidly. The Central Science Laboratory protocol used an electric bacon slicer and electric mincers, but the manual method described below was found to be faster, safer and cheaper to implement.

Each body was in turn partially defrosted at room temperature for approximately 20-30 minutes. A butcher's meat cleaver was then used to chop the whole body in a fume cupboard into thick sections approximately 4cm wide. The sections were fed through a domestic hand mincer and the resulting mince was then fed through again in order to

produce a truly homogenous sample. The sample was then labelled and refrozen until it was needed for residue extraction.

Old-fashioned, metal, hand mincers were found to give more torque than an electric mincer and a bank of 6 mincers could be set up along a bench to make the most efficient use of time for processing and washing up. Mincers were dismantled for washing and the tissue residue within was removed. The mincer parts were then scrubbed clean using teepol detergent in hot water. *Leptospirosis* spirochaetes are destroyed by freezing, though other potential pathogens (*e.g. Salmonella*) are not. Great care was therefore taken in order not to place anyone in the laboratory at risk. Protective clothing, eye protection and a face mask were worn at all times while handling the rats. Materials and bench tops were cleaned and then sterilised with an antibacterial detergent at the end of each processing session.

## 4.2.ii Extraction of Hexachlorobiphenyl (HCBP)

This method was based on one supplied by MAFF, Central Science Laboratory (unpublished). The sample of mince was defrosted and a 3g sample was taken. At this stage, 100µl of a 1 mg/ml solution of tetrachlorobiphenyl (TCBP) was added to the mince as an internal standard for the analyses. The mince was then ground with 17g of anhydrous sodium sulphate and left for one hour until the sample was completely dry. Meanwhile, a soxhlet condenser was set up on a heating mantle in the fume cupboard (see Figure 4.2). About 80ml of hexane (HPLC grade) was placed with a few anti-bumping granules in a 150ml round-bottomed flask and this was attached to the bottom end of the soxhlet. A cellulose extraction thimble (26 mm x 60 mm) was placed in the column of the soxhlet and was rinsed with the condensing solvent for an hour. After this time, the mince mixture was placed into the pre-rinsed cellulose thimble in the soxhlet column with 80ml of fresh hexane in the round-bottomed flask beneath. The soxhlet was set on a medium heat and left for 3.5 hours. After this time, the soxhlet was allowed to cool and then the solvent was collected in the round-bottomed flask. This sample extract was evaporated to about 5 ml by gently heating it on the heating mantle in the fume cupboard. (Rotary evaporation could be used at this stage as an alternative).

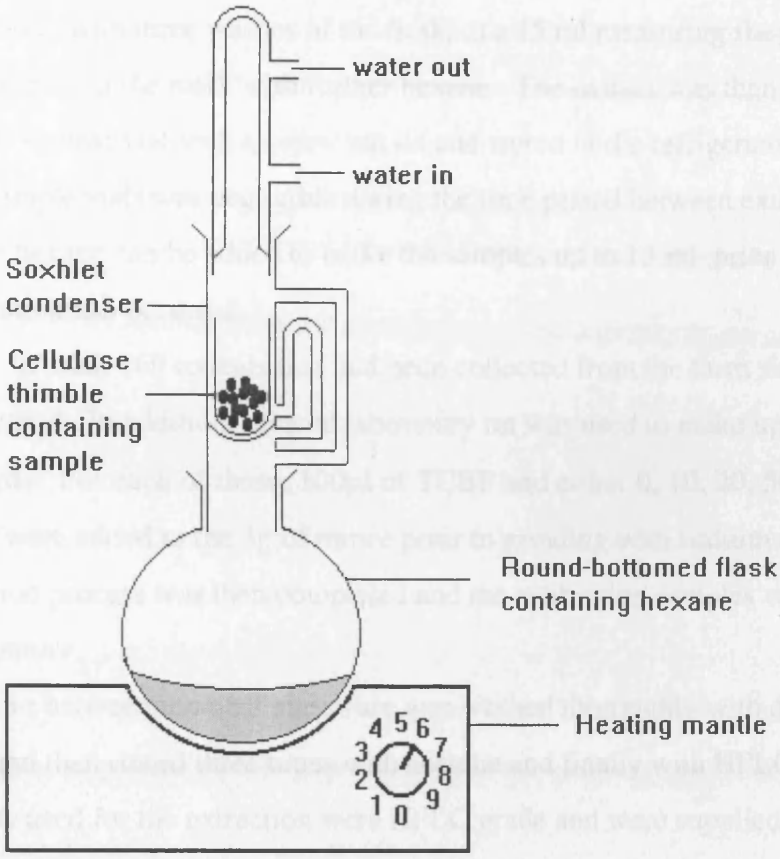



Figure 4.2 Diagram to show a soxhlet condenser set up on a heating mantle and containing a cellulose thimble filled with a rat mince and sodium sulphate mixture.

For the next stage, a 20 mm diameter chromatography column (40-50 cm long) was clamped by a retort stand in a fume cupboard. A small amount of glass wool was placed in the base and then about 90 ml of hexane was added to the column. Using a filter funnel, 20g of Grade IV alumina powder was added to the top of the column. The alumina was allowed to settle and then the hexane was drained from the column until the meniscus level sat at the top of the powder. The 5 ml of sample extract was transferred by a long glass pipette onto the top of the alumina along with three hexane rinses of the round-bottomed flask. A 150 ml conical flask was placed under the column and the tap was opened to allow the sample to gravitate into the alumina. Hexane (90 ml) was then added to the column to wash the sample through. When the column had drained, the extract that had collected in the conical flask was evaporated to about 2-3 ml. It was then

transferred, with three washes of the flask, to a 15 ml measuring flask using a glass pipette and made up to the mark with further hexane. The extract was then transferred to a labelled sample vial with a screw top lid and stored in the refrigerator. Evaporation from these sample vials was negligible during the time period between extraction and analyses. Further hexane can be added to make the samples up to 15 ml prior to analyses if evaporation has occurred.

In total, 169 rodents that had been collected from the farm sites were processed by this method. In addition, minced laboratory rat was used to make up 6 calibration standards. For each of these, 100µl of TCBP and either 0, 10, 20, 50, 100 or 200µl of HCBP were added to the 3g of mince prior to grinding with sodium sulphate. The extraction process was then completed and the calibration samples were stored in the refrigerator.

In between uses, all glassware was washed thoroughly with detergent, rinsed with water and then rinsed three times with acetone and finally with HPLC grade hexane. All solvents used for the extraction were HPLC grade and were supplied by Fisher Scientific, Loughborough. Alumina powder, supplied by Sigma Aldrich, Poole, was Grade Super I but was down-graded to IV by mixing with 10% (*i.e.* 2g) of HPLC grade water. HCBP (2,2'4,4'5,5') was supplied by MAFF, originally from Palmer Research Laboratory. TCBP (2,4,2',4') was provided by the MRC Toxicology unit at Leicester University, originally supplied by Analabs Inc., Connecticut. Later calibration standards and samples used 3,4,3',4' TCBP as the internal standard as the previous batch had run out.

## 4.2.iii Using GCMS to measure HCBP

Tetrachlorobiphenyl (TCBP) was used as the internal standard for these analyses as it is a very similar compound to HCBP and so the two should be affected equally by the extraction and analytical processes. Ionisation of HCBP produced a spectrum with major peaks at 360, 362, and 290 m/z (mass to charge ratio; the charge is +1, so the m/z values given are equivalent to the mass of the ions described). A major ion of TCBP was shown at 292 m/z. After initial testing of the analytical procedure to check that the samples were showing clean peaks of TCBP and HCBP, it was decided that the mass spectrometer would be set on select ion recording (SIR) to detect ions of TCBP at 292 m/z and HCBP

at 360 m/z. It was also decided that a dilute version of each sample should be injected onto the GCMS for the actual analytical runs as the samples that were used in the initial test runs caused massive overloading.

A Hewlett Packard GC (model 5890) was used with a J & W Scientific DB-5 column No.1 (length 15 m, id 0.25 mm and a film thickness of 0.25 μm). Helium was used as the mobile phase gas at a flow rate of 4 p.s.i. and split injections (1:60) of 0.5μl were made with the purge on to prevent overloading. The injector temperature was 280 °C. The oven temperature started at 80 °C and remained at that temperature for one minute before being raised by 30 °C per minute to reach 290 °C by 8 minutes. The oven temperature was then held at 290 °C for 5 minutes. The interface temperature was 270 °C and the source was 250 °C. The MS model was a VG Trio-1 (VG, Manchester) and the detector multiplier was set at 500.

For the actual runs, a Gilson pipette was used to take 10μl of each sample or calibration standard and 190 μl of hexane and these were then placed together in a 3 ml vial. This was equivalent to a 20 x dilution of the sample. A Pasteur pipette was then used to fill a volume reduction insert vial within a crimp-top vial for each sample that was loaded on the autosampler. Each run consisted of 54 injections. (The format for the injections is shown by an example in the Appendices). An injection of the strongest standard was made to start with (*i.e.* calibration standard containing 200μl of HCBP) to condition the column. After this, two injections of ethyl acetate were made to wash the column. Next, two injections of each of four of the calibration standards were made, going in order of increasing HCBP concentration. After this two further ethyl acetate injections followed. The samples were then injected in turn (the samples had been placed in random order on the autosampler), with two injections from each sample vial and with one ethyl acetate injection after every 8 sample injections. The last two calibration standards were injected at the end of the run. The autosampler automatically washed the needle thoroughly with ethyl acetate between every injection. The autosampler and GCMS were left to run overnight. Each sample run took 15 minutes and the whole batch of 54 injections lasted for approximately 14 hours.

Quantitation of peaks was made by manually setting the baseline for the peaks with the aid of a computer mouse on a Lab Base Program. The computer then calculated the peak area. The percentage peak area ratio of TCBP over HCBP for the calibration

standards was used to plot a calibration line for each run. The equation of this line was then used to convert the percentage peak area ratio values for the samples to a HCBP concentration. The HCBP concentration was a value found in 3g of minced rat and a conversion factor was used to calculate the total body load of HCBP. The total body load calculated was the value of HCBP in the whole body excluding the tail and feet and guts. For this, a corrected mass value was used as the body weights of carcasses had been taken before, rather than after, the removal of the guts, feet and tails. (See Appendix 2 for a conversion graph of body mass before to body mass after removal of guts, feet and tail; mass after ≈ 85% of mass before.) A value for equivalent bait-take (in g) was then calculated by dividing the body load (in μg) by 98 or 89 for the relevant $\mu g\ g^{-1}$ value of the HCBP in the bait formulation for that site. Values of mg of marker per Kg of body mass were also calculated for display in the figures.

## 4.2.iv Extraction of rodenticide residues

This method was based on the method used by Jones (1996). A small sample of mince (0.5-1.0g) was taken and ground with 10x as much (by mass) of anhydrous sodium sulphate. This mixture was left for about 30 minutes and was then transferred to a 25 ml screw-top conical flask with 15 ml of extraction solvent (30% HPLC grade acetone in HPLC grade dichloromethane). The flask was placed on an oscillator at 220 revs/min for 1.5 hours. After this time, the extract was decanted into a centrifuge tube and spun at 10,000 rpm for 10 minutes. Meanwhile, a further 10 ml of extraction solvent was added to the conical flask which was then placed back on the oscillator for another 30 minutes. The extract of this was similarly centrifuged for 10 minutes on the same setting. The two lots of supernatant were transferred to a 25 ml volumetric flask and made up to the mark with extraction solvent.

Next, a 'Sep-Pak' cartridge was conditioned with 10 ml of dichloromethane (DCM) at a rate of 5-10 ml per minute. 10 ml of the sample extract was added to the Sep-Pak cartridge at 3-5 ml per minute. The cartridge was washed with 10 ml of extraction solvent and then 2 ml of DCM:acetone (25:75). Finally, the anticoagulants were eluted by adding 5 ml of 5% methanolic acetic acid to the cartridge and the anticoagulants were

collected in a glass sample vial. The sample was evaporated to dryness and then re-dissolved in 0.5 ml methanol and stored in a screw-top glass vial until use.

All solvents were HPLC grade and were supplied by Fisher Scientific, Loughborough. Anhydrous sodium sulphate was supplied by Fisher Scientific, Loughborough. Alumina N Sep-Pak cartridges (1850 mg) were supplied by Waters Ltd, Watford.

## 4.2.v Using TLC and HPLC to measure the rodenticide residues

The method of HPLC determination of anticoagulant rodenticides described by Hunter (1985) was used to detect pure solutions of brodifacoum, coumatetralyl and HCBP by HPLC at Zeneca's Jealott's Hill research station during a four week training period at the start of this project. The method was not, however, used for testing animal tissue and so the clean-up technique described by Hunter was not tested.

No HPLC machine was available for use at Leicester during the period of analyses of rodent tissue and Thin Layer Chromatography (TLC) was chosen as the next most suitable technique. It was intended that TLC results would give qualitative results of the presence or absence of anticoagulant and that a Mass Spectrometer with a fluorescence detector would then be used to quantify the residues. Coumarin anticoagulants fluoresce in ultraviolet light and TLC and Mass or fluorescence Spectrometry have been used for such analyses in the past (Yuen, 1978; Stahr *et al.*, 1991; Panadero *et al.*, 1993). Brodifacoum and coumatetralyl were positioned in 10µl spots at the base of the reverse phase silica TLC plate. A solution of 1% methanol in dichloromethane was used as the solvent. The rodenticides were successfully deposited behind the solvent front, but an ultra violet light was unable reliably to pick out the fluorescing rodenticides from other contaminants on the TLC plate. Many days were spent trying to optimise the conditions in order to detect the rodenticides consistently, but to no avail. This meant that TLC, using these conditions, was not a reliable method to use for detection of the coumatetralyl and brodifacoum.

Eventually, a HPLC machine with fluorescence detection became available for just a day and a half. It was decided to use this time to measure a small number of samples from coumatetralyl sites. A method already provided by Jones (1996) was followed as

exactly as possible and some solutions of pure anticoagulant and some spiked laboratory rat extracts were made up as described. A Shandon HPLC machine with fluorescence detection was used with a hypersil BDS reverse phase column with measurements 250 mm x 4.6 mm and with a 5μm guard column. The excitation wavelength was 310 nm and the emission wavelength was 390 nm. Solvent A was 0.25% (v/v) acetic acid in water and solvent B was 0.25% (v/v) acetic acid in methanol. Solvent B was linearly increased from 25% to 80% over the first 1.5 minutes, then to 100% over the next 7.5 minutes, maintained at 100% for 8 minutes and then decreased to 25% over the next 1 minute. This was then maintained for 5 minutes. The whole run lasted for 23 minutes. The flow rate was kept constant at 0.8 ml per minute. Injections of 50μl were made of the 10 samples from Coumatetralyl sites (five from the east midlands, five from central southern England; two sites in each region). Pure coumatetralyl in acetone in a 1 mg/ml solution and diluted by 20 was also injected as used as an external standard for the samples. A laboratory rat extract that had 100μl of a 1 mg/ml solution added at the first stage of extraction was also used as a comparison. Quantitation of sample peaks were made by comparing the peak areas of standards and samples.

## 4.3 RESULTS

### 4.3.i HCBP marker residues

The clean-up method for the extraction of the HCBP and TCBP appeared to work very well. Clean traces were produced that revealed just the two compounds without any other background contaminants (see Figure 4.3). The TCBP ion eluted at about 5.5 minutes and the HCBP ion eluted at about 6.5 minutes. Quy *et al.* (1992) noted a recovery rate of 55% for HCBP residue in rats fed on known quantities of HCBP bait and Cowan *et al.* (1995) noted a 61% recovery rate for HCBP. The losses reported by Quy *et al.* (1992) and Cowan *et al.* (1995) were likely to have occurred mostly during the extraction procedure rather than as a result of metabolism in the rat (Cowan *et al.* (1995) noted that residue levels of HCBP remained stable in rat carcasses for at least six weeks). The analytical procedures used by Quy *et al.* (1992) and Cowan *et al.* (1995) made use of an external standard for calibrating the residues. This study used an internal standard (the TCBP) so that losses through extraction could be accounted for, as the loss would involve both the HCBP and the TCBP in equal ratio. It was therefore felt that the HCBP residues measured in this study were true representatives of the body loads found in the whole rats. In the event that metabolism of HCBP did occur, the residue values calculated here would be the minimum residues likely to be found in the rat bodies. In summary, the values given in this chapter assume:

1. No loss through metabolism and excretion
2. No storage of HCBP in the feet, tail or intestines
3. That loss of HCBP via extraction processes was the same for the internal standard and was therefore cancelled out.

The data from analyses of the carcasses from all the trial sites were put together so that the marker residue levels and equivalent bait-take values for the two regions and the two poisons could be compared. The data for 26 mouse carcasses were then removed from the analyses so that comparisons could be made that related specifically to rats. This left data for 143 rats in total. Figures 4.4 and 4.5 relate to values of HCBP marker in rats that were found dead and presumed poisoned. Figures 4.6 and 4.7 refer to rats that were killed by trapping or by unmarked brodifacoum after the poison treatment had failed.

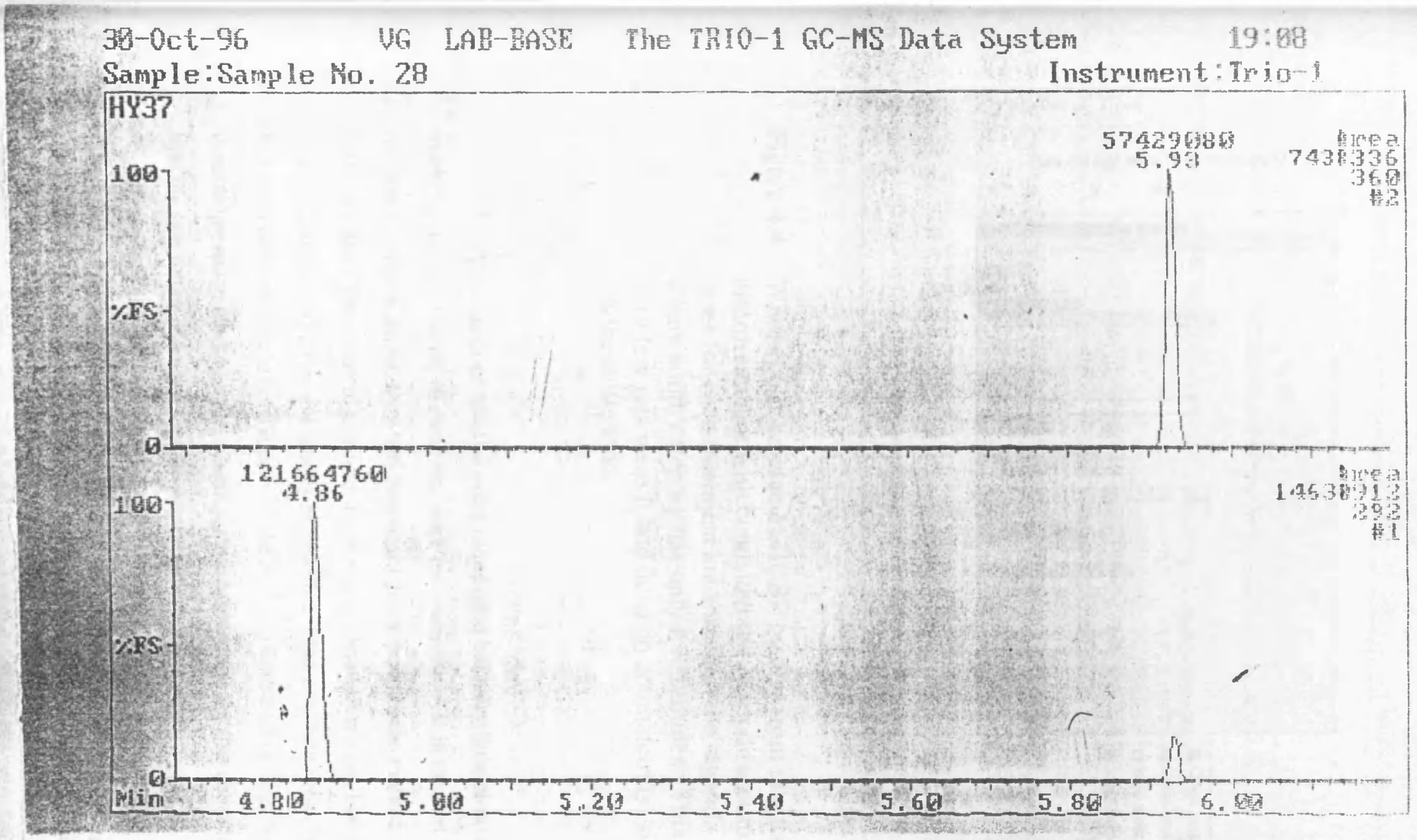



Figure 4.3 A typical trace produced from the GCMS. The HCBP ion peak is shown in the top section, the TCBP ion peak is shown underneath. A small peak of a HCBP ion also appears on the bottom right of the trace. This peak was ignored as the selected ion was the one shown above.

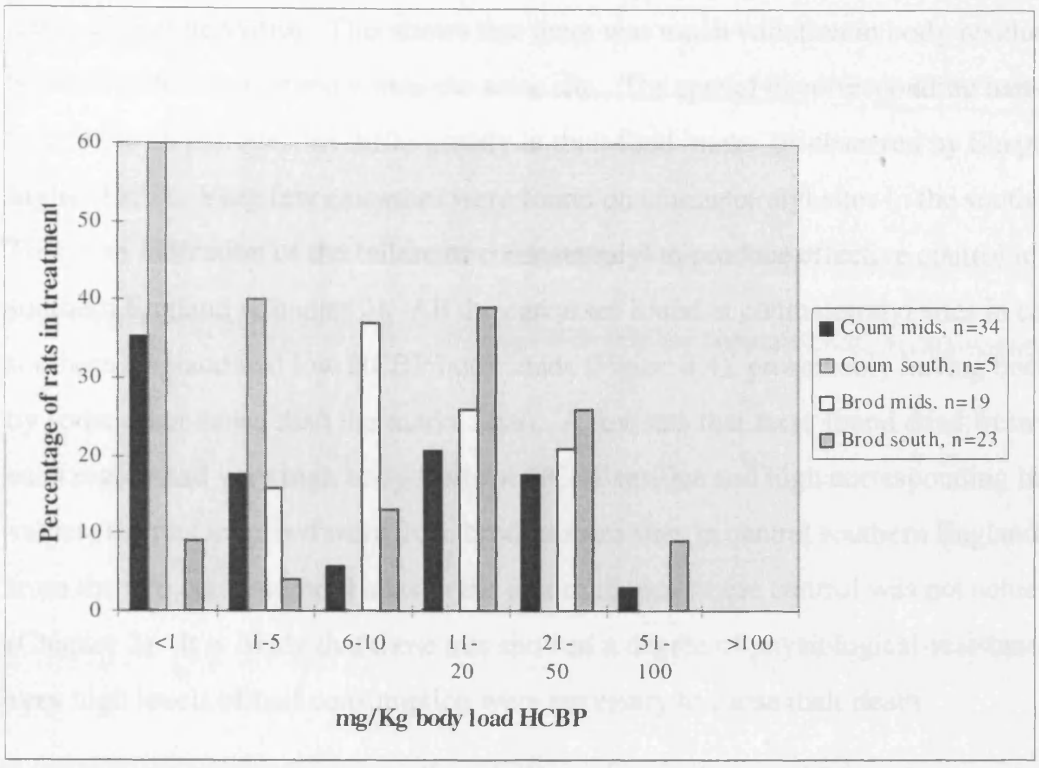



Figure 4.4 Whole body residue of HCBP marker from rats found dead after treatment with coumatetralyl or brodifacoum. Sample sizes for each treatment are shown in the legend. Note that the Coum south category has only five samples. This is because very few rats were found dead on coumatetralyl sites in central southern England.

A large number of rats that were found dead had very little or no residue of marker in them. Twelve of these rats were from one site, A1, in the east midlands. The rats were found dead, yet they had clearly not eaten much of the marked coumatetralyl bait provided. The cause of death for these rats is therefore unsure, but it would appear that the coumatetralyl was not wholly responsible. One possibility may be that the rats had been targeted with a poison prior to the trial and then died during the trial without consuming much marked bait. An alternative explanation for this and other sites may be that the rats found dead had died of natural causes.

Most of the rats had HCBP body residues lower than 50 mg $Kg^{-1}$ and most body residues were spread, within each treatment category, in a normal distribution over a

range of residue values. This shows that there was much variation in body residue between individuals, even within the same site. The spread in corresponding bait-take values shows that rats can differ greatly in their food intake, as observed by Shepherd and Inglis (1987). Very few carcasses were found on coumatetralyl sites in the south (n=5). This is an indication of the failure of coumatetralyl to produce effective control in central southern England (Chapter 2). All the carcasses found at coumatetralyl sites in central southern England had low HCBP body loads (Figure 4.4), presumably having been killed by some other cause than the marked bait. A few rats that were found dead from sites in each region had very high body loads of HCBP residue and high corresponding bait-take values; the rats involved were from brodifacoum sites in central southern England and from the two coumatetralyl sites in the east midlands where control was not achieved (Chapter 2). It is likely that these rats showed a degree of physiological-resistance so that very high levels of bait consumption were necessary to cause their death.

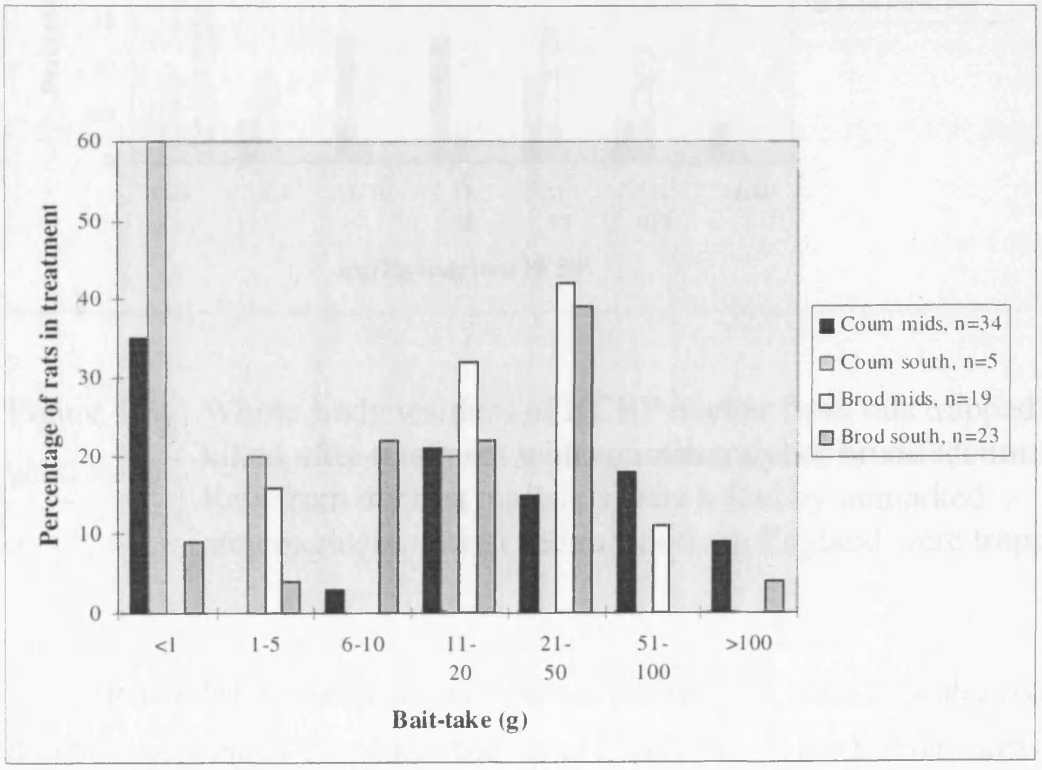



Figure 4.5 Individual minimum bait-take values calculated from whole body residues of HCBP marker in rats found dead after treatment with coumatetralyl or brodifacoum.

No rats were trapped at brodifacoum sites in the east midlands. Some rats were killed following use of unmarked brodifacoum at sites A7 and A8 (where physiological resistance was suspected) and were counted as "trapped". This is because the marker found in them came from just the coumatetralyl, not the brodifacoum. The coumatetralyl had failed to kill them, which is why the brodifacoum was then used.

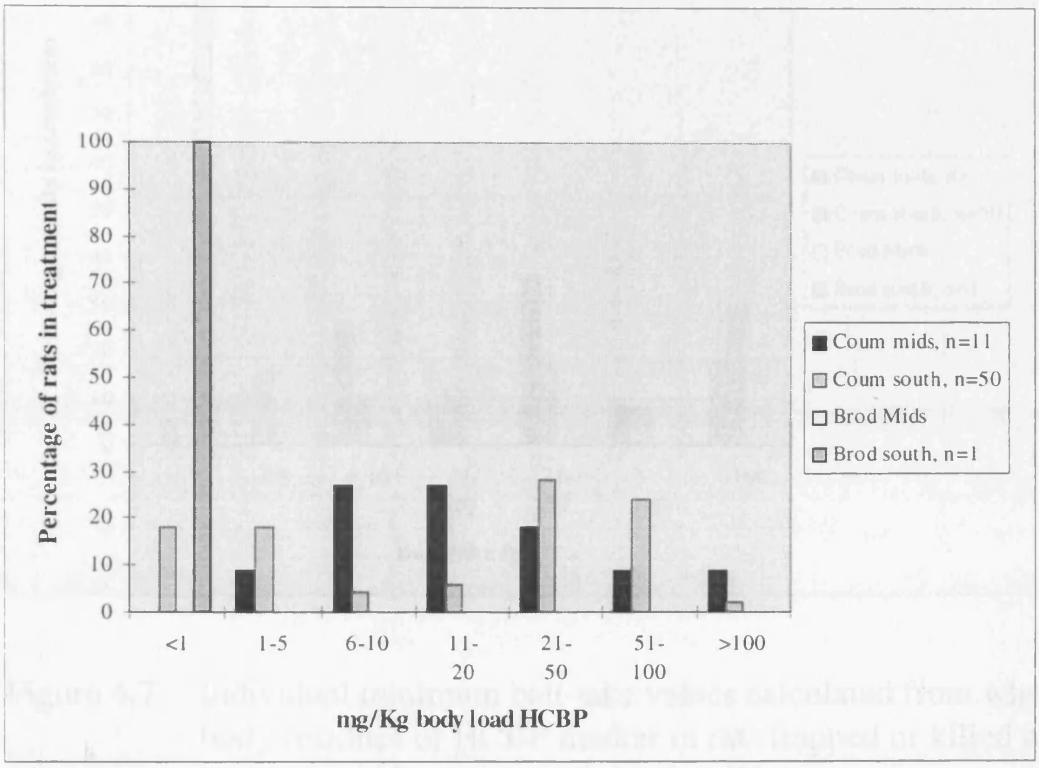



Figure 4.6 Whole-body residues of HCBP marker from rats trapped or killed after treatment with coumatetralyl or brodifacoum. Rats from the east midlands were killed by unmarked anticoagulant, rats in central southern England were trapped.

Rats killed in the east midlands showed residue levels of HCBP with a normal distribution around 11-20 mg $Kg^{-1}$ body mass (Figure 4.6). A few had very high residues, indicating the likelihood of physiological resistance at the sites involved. A few rats had surprisingly low residues, indicating that they had eaten the unmarked brodifacoum (hence their death) but had not eaten much of the coumatetralyl bait before. These rats may have

been new immigrants at the site or may have been previously excluded from bait points so that they were unable to feed until late into the trial (Chapter 3).

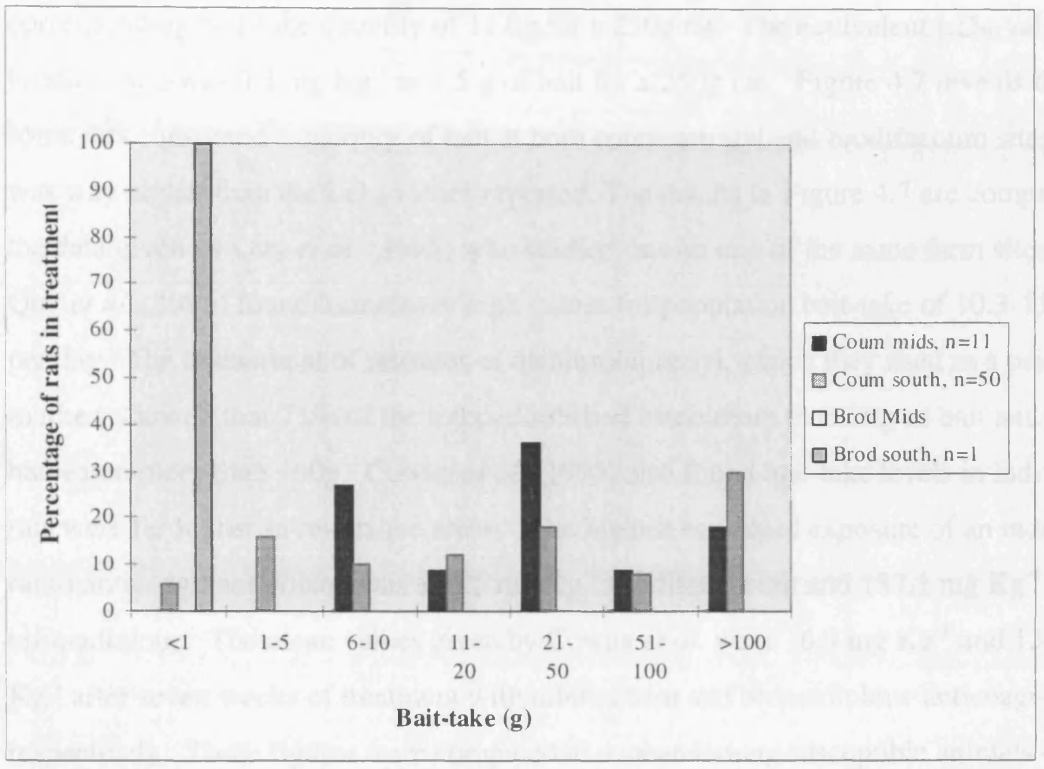



Figure 4.7 Individual minimum bait-take values calculated from whole body residues of HCBP marker in rats trapped or killed after treatment with coumatetralyl or brodifacoum.

There were many rats trapped from coumatetralyl sites in southern central England (n=50). Some of these showed very little or even no marker residue. This result indicates that some rats found on the farms and trapped within the baiting areas were not feeding from the bait points. Buckle *et. al* (1987) found that 4.1% of rats failed to feed from bait points during poison treatments. The rats may have chosen not to feed (Brunton *et al.*, 1993, showed some physiologically resistant rats ate very little bait) or they may have been excluded from the bait by other, perhaps dominant rats. The majority of rats trapped in central southern England contained 21-100 mg of HCBP per Kg of body mass and one rat contained more than 100 mg of HCBP.

Greaves and Cullen-Ayres (1988) reported $LD_{50}$ values in susceptible rats of 1.1 mg $Kg^{-1}$ and 0.45 mg $Kg^{-1}$ for coumatetralyl and brodifacoum respectively. Johnson and Prescott (1994) reported a higher $LD_{50}$ value of 16.5 mg $Kg^{-1}$ for coumatetralyl, with a corresponding bait-take quantity of 11.0g for a 250g rat. The equivalent $LD_{50}$ value for brodifacoum was 0.3 mg $Kg^{-1}$ or 1.5 g of bait for a 250g rat. Figure 4.7 reveals that some rats consumed a quantity of bait at both coumatetralyl and brodifacoum sites that was way higher than the $LD_{50}$ values reported. The results in Figure 4.7 are comparable to the data given by Quy *et al.* (1995) who studied rats on one of the same farm sites, B2. Quy *et al.* (1995) found immensely high values for population bait-take of 10.3-11.8 Kg per day. The assessment of residues of dichlorobiphenyl, which they used as a bait marker, showed that 71% of the trapped rats had eaten more than 20g of bait and 51% had eaten more than 100g. Cowan *et al.* (1995) also found bait-take levels in individual rats were far higher in resistance areas. The highest estimated exposure of an individual rat to anticoagulant poison was 110.5 mg $Kg^{-1}$ for difenacoum and 187.1 mg $Kg^{-1}$ for bromadiolone. The mean values given by Cowan *et al.* were 16.9 mg $Kg^{-1}$ and 13.2 mg $Kg^{-1}$ after seven weeks of treatment with difenacoum and bromadiolone anticoagulants respectively. These figures were compared to a mean among susceptible animals of 9.6 mg $Kg^{-1}$. Interestingly, just four weeks of treatment with bromadiolone produced a mean residue of 9.1 mg $Kg^{-1}$, which is a similar figure. It therefore seems that the phenomenon of over-consumption of bait by resistant rats is comparatively more noticeable as the treatment time is extended.

A summary of the data for the highest residue levels and corresponding bait-take values for this trial are shown in Tables 4.1 and 4.2.

Table 4.1 Percentage of rats that showed high levels of marker residue and corresponding bait-take in each of the four treatment categories. Total sample size was 143. Treatment sample sizes were as follows: cm=45, cs=55, bm=19, bs=24

| Totals | mg $Kg^{-1}$ >20 | >100 | bait-take (g) >20 | >100 |
|---|---|---|---|---|
| **coum mids** | 24 | 2 | 47 | 11 |
| **coum south** | 49 | 2 | 51 | 27 |
| **brod mids** | 21 | 0 | 53 | 0 |
| **brod south** | 33 | 0 | 42 | 4 |

Table 4.2 Worst incidents of body residues (>50 mg $Kg^{-1}$) and/or bait-take values (>100g) taken from HCBP marker data for rats found dead or trapped in the east midlands and in central southern England

| mg $Kg^{-1}$ body wt. | bait-take (g) | site | region | poison | trap/carcass |
|---|---|---|---|---|---|
| 90.95 | 102.55 | A8 | e. midlands | coumatetralyl | C |
| 39.42 | 144.28 | A5 | e. midlands | coumatetralyl | C |
| 49.73 | 106.97 | A7 | e. midlands | coumatetralyl | T |
| 76.12 | 158.45 | A7 | e. midlands | coumatetralyl | T |
| 102.25 | 70.95 | A8 | e. midlands | coumatetralyl | T |
| 88.9 | 33.93 | B4 | c.s.England | brodifacoum | C |
| 58.16 | 133.18 | B4 | c.s.England | brodifacoum | C |
| 51.93 | 201.37 | B2 | c.s.England | coumatetralyl | T |
| 22.56 | 108.79 | B2 | c.s.England | coumatetralyl | T |
| 88.62 | 406.27 | B2 | c.s.England | coumatetralyl | T |
| 96.21 | 417.17 | B2 | c.s.England | coumatetralyl | T |
| 83.29 | 372.29 | B2 | c.s.England | coumatetralyl | T |
| 99.55 | 489.62 | B2 | c.s.England | coumatetralyl | T |
| 47.94 | 204.21 | B2 | c.s.England | coumatetralyl | T |
| 39.86 | 197.95 | B2 | c.s.England | coumatetralyl | T |
| 39.05 | 176.03 | B2 | c.s.England | coumatetralyl | T |
| 40.51 | 156.31 | B2 | c.s.England | coumatetralyl | T |
| 51.5 | 211.51 | B2 | c.s.England | coumatetralyl | T |
| 67.47 | 50.26 | B3 | c.s.England | coumatetralyl | T |
| 51.12 | 94.72 | B3 | c.s.England | coumatetralyl | T |
| 106.67 | 199.68 | B3 | c.s.England | coumatetralyl | T |
| 60.12 | 42.49 | B3 | c.s.England | coumatetralyl | T |
| 61.51 | 97.52 | B3 | c.s.England | coumatetralyl | T |
| 90.21 | 220.55 | B1 | c.s.England | coumatetralyl | T |
| 61.89 | 119.4 | B1 | c.s.England | coumatetralyl | T |
| 42.05 | 105.21 | B1 | c.s.England | coumatetralyl | T |

There were clearly more cases of high bait-take and body residues of marker (worst incidents) in rats from coumatetralyl sites. There were, however, a few rats from brodifacoum sites that had high HCBP residues or bait-take values also.

Table 4.3a Analysis of Variance (GLM) fitted to square-root transformed data of bait take over mass, taken from HCBP residue results. Sums of Squares are adjusted to take account of unbalanced numbers.

| Source | DF | Seq SS | Adj SS | Adj MS | F | p |
|---|---|---|---|---|---|---|
| **Mass** | 1 | 0.17914 | 0.33603 | 0.33603 | 6.45 | 0.012 |
| **Region** | 1 | 0.38056 | 0.23272 | 0.23272 | 4.47 | 0.036 |
| **cou/brod** | 1 | 0.19696 | 0.18376 | 0.18376 | 3.53 | 0.062 |
| **Region*coum/brod** | 1 | 0.05186 | 0.05186 | 0.05186 | 1.00 | 0.320 |
| **Error** | 138 | 7.18673 | 7.18673 | 0.05208 | | |
| **Total** | 142 | 7.99525 | | | | |

Table 4.3b Adjusted means (g bait/g body mass) for the corresponding data of bait take over mass for each treatment category. Adjusted (rather than simple) means take account of unbalanced numbers. Back-transformed ("real") means are also shown for ease of interpretation. These means are bait take(g) expressed as a percentage of the body mass (g).

| | Treatment | Mean | StDev | "Real" mean |
|---|---|---|---|---|
| **Region** | e. mids | 0.1381 | 0.03125 | 1.91 |
| | c. s. eng | 0.2267 | 0.02805 | 5.14 |
| **coum/brod** | coum | 0.2229 | 0.02313 | 4.97 |
| | brod | 0.1419 | 0.03578 | 2.01 |
| **Region*coum/brod** | coum, mids | 0.1573 | 0.03402 | 2.47 |
| | brod, mids | 0.1188 | 0.05242 | 1.41 |
| | coum, south | 0.2884 | 0.0314 | 8.32 |
| | brod, south | 0.1649 | 0.04806 | 2.56 |

An analysis of variance (general linear model) was carried out on the data for individual minimum bait take (calculated from the residue data) divided by body mass (Tables 4.3a and b). The analysis was applied to square-root transformed data, as this transformation made the residuals more even. There was found to be a significant difference in bait take between rats of different masses (p=0.012), with smaller rats taking proportionately more bait. Rats from central southern England had consumed

significantly (on average 64%) more bait per unit body mass than rats from the east midlands (p=0.036). Rats from coumatetralyl-treated sites had consumed on average 57% more bait per unit body mass than rats on brodifacoum sites, though the difference was not quite significant (p=0.062). This last result is surprising, but is likely to be related to the fact that saturation baiting was not maintained on some of the coumatetralyl sites in central southern England because of the problems discussed in Chapter 2. The rats on brodifacoum sites ate the quantity of bait that they chose, whereas access to bait at coumatetralyl sites was limited for the reasons given in Chapter 2. Thus the rats at some of the sites in central southern England, B2 and B3 in particular, could have consumed bait far in excess of what was provided. This would have undoubtedly altered the residue levels of HCBP seen.

An alternative analysis of variance (general linear model) that included the comparison between poisoned rats and trapped or killed rats produced a different result (Tables 4.4a and b). The differences between bait take levels of individual rats in any of the treatment categories were no longer significant, but there was a highly significant difference between values for trapped rats compared to those found dead (p=0.007). This result indicates that where trapping (or further poisoning) was necessary to control the rat population at any site, the individual rats within those populations were consuming significantly greater quantities of bait than rats at other sites (*i.e.* the population pattern reflected the pattern shown by individual rats).

Table 4.4a Analysis of Variance (GLM) fitted to square-root transformed data of bait take over mass, taken from HCBP residue results and including the category trap v. poison. Note that the adjusted Sums of Squares are altered by the inclusion of poison v. trap and the error Mean Square is also reduced.

| Source | DF | Seq SS | Adj SS | Adj MS | F | p |
|---|---|---|---|---|---|---|
| **Mass** | 1 | 0.17914 | 0.29027 | 0.29027 | 5.84 | 0.017 |
| **Region** | 1 | 0.38056 | 0.02147 | 0.02147 | 0.43 | 0.512 |
| **coum/brod** | 1 | 0.19696 | 0.00283 | 0.00283 | 0.06 | 0.812 |
| **Region*coum/brod** | 1 | 0.05186 | 0.00259 | 0.00259 | 0.05 | 0.82 |
| **Poi/Trap** | 1 | 0.37782 | 0.37782 | 0.37782 | 7.60 | 0.007 |
| **Error** | 137 | 6.80891 | 6.80891 | 0.0497 | | |
| **Total** | 142 | 7.99525 | | | | |

Table 4.4b Adjusted means (g bait/g body mass) for the corresponding data of bait-take over mass for each treatment category. Back-transformed ("real") means are also shown for ease of interpretation. These means are bait take(g) expressed as a percentage of the body mass (g).

| | Treatment | Mean | StDev | "Real Mean" |
|---|---|---|---|---|
| **Region** | e. mids | 0.2009 | 0.03125 | 4.04 |
| | c. s. Eng | 0.2312 | 0.02805 | 5.35 |
| **coum/brod** | coum | 0.2096 | 0.02313 | 4.39 |
| | brod | 0.2225 | 0.03578 | 4.95 |
| **Region*cou/brod** | coum, mids | 0.1997 | 0.03402 | 3.99 |
| | brod, mids | 0.2022 | 0.05242 | 4.09 |
| | coum, south | 0.2195 | 0.0314 | 4.82 |
| | brod, south | 0.2429 | 0.04806 | 5.9 |
| **Poi*Trap** | poison | 0.1332 | 0.02721 | 1.77 |
| | trap | 0.2989 | 0.04701 | 8.93 |

## 4.3.ii Marker residues in mice

Out of a total of 24 mice found as carcasses or killed in traps, 15 had residues of HCBP marker in them. The body load of marker (µg) along with the equivalent µg/g body concentration and calculated bait take value (g) for each mouse is shown in Table 4.5. Townsend *et al.* (1995) found that the limit of detection of HCBP in small rodent carcasses was 0.05 µg/g. Residues of HCBP in wood mice were 0.05-241 µg/g and the median was 3.0 µg/g. Townsend *et al.* (1995) assumed a storage ratio of 0.5 to calculate bait-take values for wood mice of 0.01-35g (about 0.05-135µg/g difenacoum). These values, Townsend *et al.* stated, were often higher than the reported $LD_{50}$ value for wood mice of 0.35 µg/g difenacoum. The data in Table 4.5 show that on sites used during this study, house mice ate between 0 and 22.14 g of rodenticide bait each. In some cases, the bait consumption far exceeded the $LD_{50}$ value for brodifacoum of 0.45µg/g (Meehan, 1984).

Table 4.5 Values of HCBP residues in mice from brodifacoum (B) and coumatetralyl (C) sites in the east midlands (sites A7, A8 and A9) and in central southern England (B7 and B9). Cause of death was either poison (P) or trap (T).

| site | Corrected body mass | μg $g^{-1}$ | bait take (g) | c.o.d. | treatment | body load (μg) |
|---|---|---|---|---|---|---|
| A7 | 14 | 23.58 | 3.27 | P | C | 320.75 |
| A8 | 14 | 4.19 | 0.58 | P | C | 56.96 |
| A9 | 15 | 1.58 | 0.25 | P | B | 24.16 |
| A9 | 3 | 46.87 | 1.63 | P | B | 159.35 |
| A9 | 15 | 29.26 | 4.57 | P | B | 447.75 |
| A9 | 15 | 41.72 | 6.51 | P | B | 638.28 |
| B7 | 7 | 20.93 | 1.45 | P | B | 142.33 |
| B9 | 14 | 29.37 | 4.49 | P | C | 399.48 |
| B9 | 15 | 0.90 | 0.15 | T | C | 13.72 |
| B9 | 8 | 24.33 | 2.09 | T | C | 186.12 |
| B9 | 15 | 16.71 | 2.87 | T | C | 255.73 |
| B9 | 14 | 26.12 | 4.24 | T | C | 377.46 |
| B9 | 17 | 63.63 | 12.15 | T | C | 1081.72 |
| B9 | 15 | 74.71 | 12.84 | T | C | 1143.03 |
| B9 | 16 | 122.02 | 22.14 | T | C | 1970.59 |

## 4.3.iii Rodenticide residues

It was only possible to analyse 10 carcasses in the limited time that was available to use the HPLC. The method used for determination of the rodenticide residues was not as satisfactory as the clean-up and detection of HCBP residues. Furthermore, it was not possible within the short time available using the HPLC to produce totally clean and reproducible traces. A solution of pure coumatetralyl in acetone produced a relatively tidy trace with a peak easily detectable at 17 minutes (see Figure 4.8). A sample of laboratory rat spiked with pure solution and extracted by the method described in section 4.2.iv produced a far more complex trace with a number of peaks and a variable baseline. A coumatetralyl peak nevertheless eluted at 17 minutes. Thereafter, the samples produced multi-peak traces despite frequently washing the column with solvent to remove contaminants.

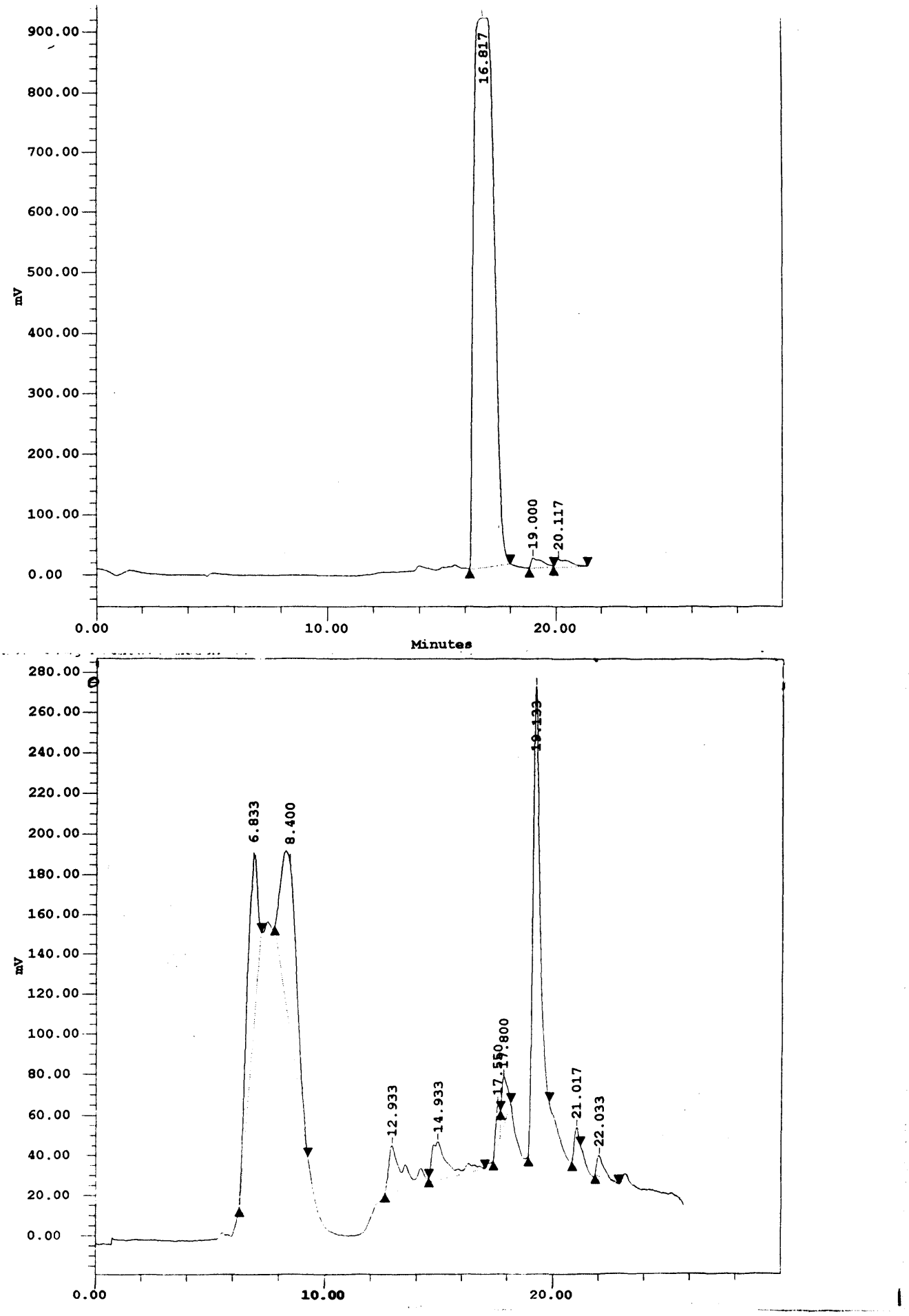



Figure 4.8 Traces of Coumatetralyl produced on the HPLC

a. Pure solution of coumatetralyl (external standard)

b. Sample No. 63 from site A8 (a typical trace)

All sample traces produced significant peaks, but they were not all eluted at 17 minutes. Bearing in mind that rodenticides break down fairly rapidly in the body system, it was deduced that some of these extra peaks were metabolites of the coumatetralyl, similar in nature to the original form, but having slightly different properties and therefore eluting at different times. Rather than ignoring the results altogether, an attempt was made to quantify all the significant peaks on each trace and to compare them with the standard coumatetralyl traces. This was done using the peak area values produced by the computer. Where more than one peak was observed, each peak area was added together. The peak area totals for each trace were converted to coumatetralyl quantity by comparison with the peak area of the external standard (pure solution of coumatetralyl). These values gave the quantity of coumatetralyl in just 0.75g rat, so the total body load was calculated using the corrected mass values (mass after feet, guts and tail had been removed). From this, a value of mg coumatetralyl per Kg body weight and an equivalent value of bait-take (g) were calculated. These residue values were believed to be a minimum figure. The samples were compared to a pure solution of coumatetralyl as an external standard, and no allowance was made for loss through extraction of the samples. The real levels may have been higher, although not a great deal more. Hunter (1985) recovered 90% of anticoagulant rodenticides from spiked liver tissue at levels from 0.05 to 1 mg $Kg^{-1}$. Kelly *et al.* (1993), found 100% recovery from plasma and 95% from liver tissue for difenacoum.

The calculated residue results are shown in Figures 4.9 and 4.10. It is immediately clear that the body residues of three of the five rats from central southern England were in excess of the residues in the other rats sampled. These rats were taken from sites that showed physiological resistance; the rats were trapped. The other two rats from central southern England that were used for these residue analyses (B2c and B3b) were also trapped and they do not show such high body loads of coumatetralyl. All the rats shown here from the east midlands had been found dead rather than killed after the treatment.

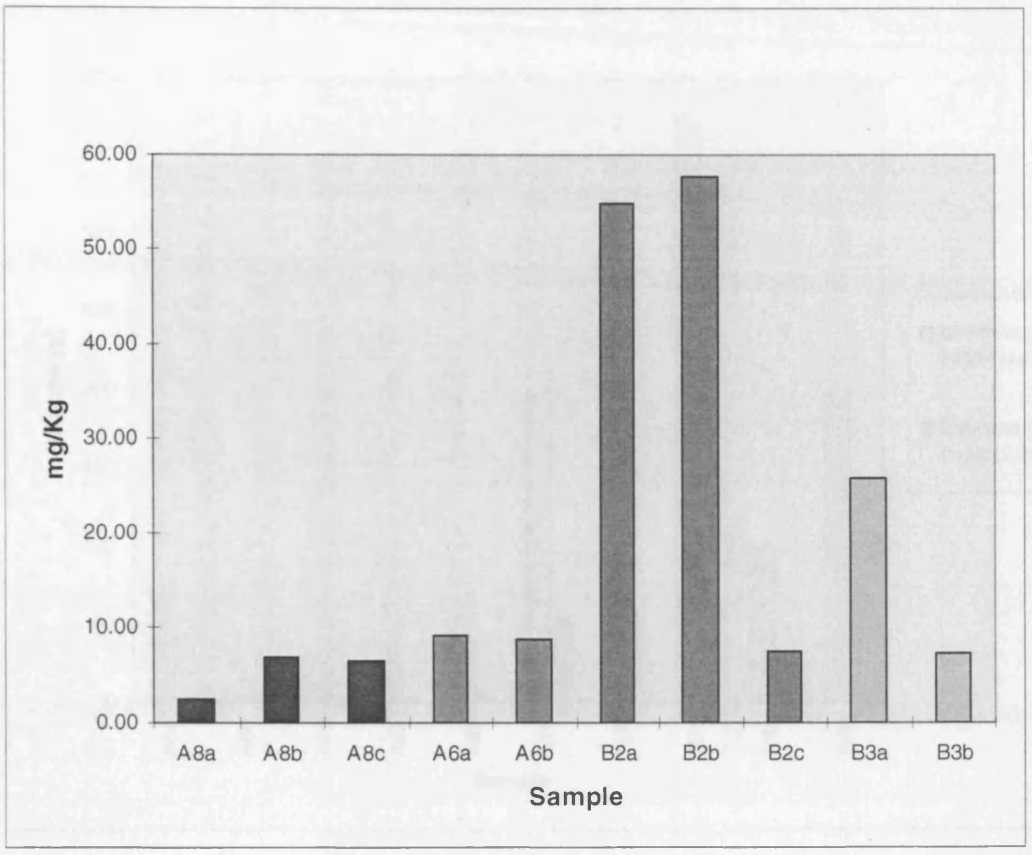



Figure 4.9 Body residues of coumatetralyl in 10 rats, expressed as mg of residue per Kg of body mass. Samples prefixed with A were from the east midlands, those with a B were from central southern England. Rats were taken from two sites within each region; A6, A8, B2, B3.

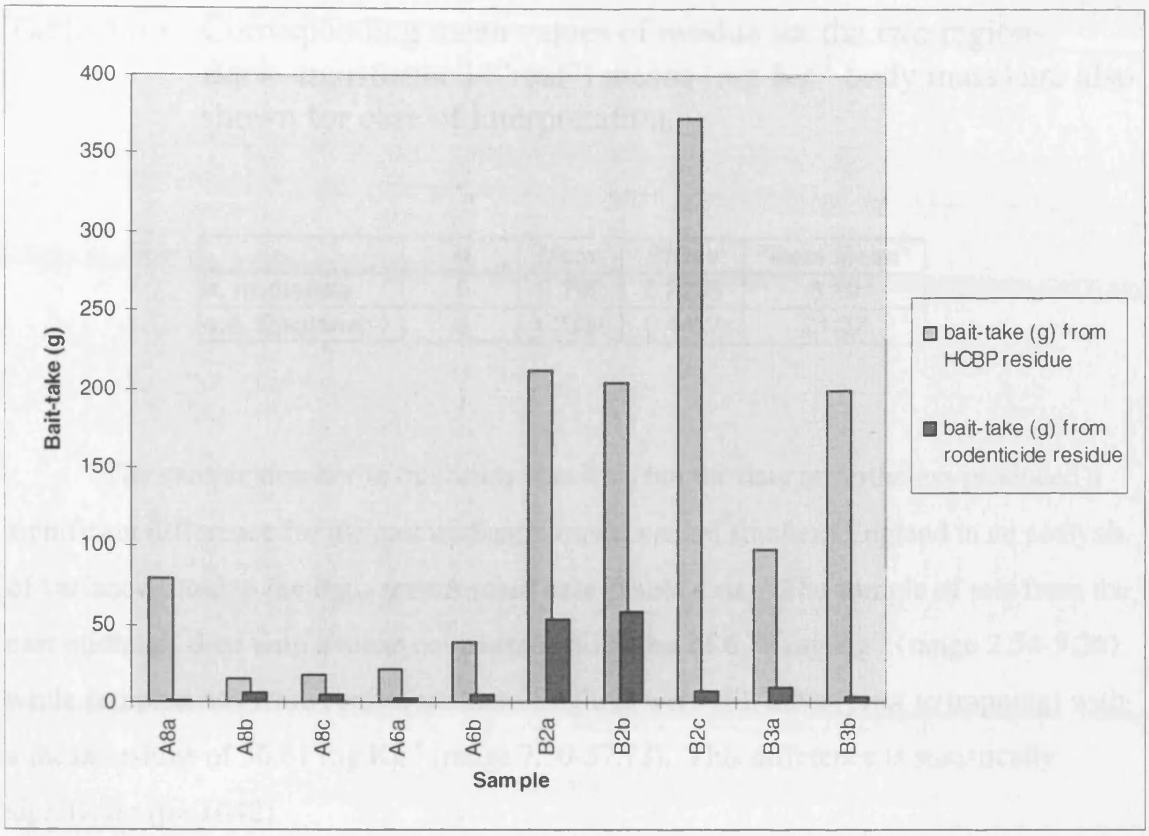



Figure 4.10 Graph to show a comparison of the calculated bait-take values from residues of HCBP and residues of coumatetralyl.

Table 4.6a Analysis of Variance (one-way) fitted to the data for $\log_{10}$ transformed coumatetralyl residue data from 10 sample rats, five from each of the two regions.

| Source | DF | SS | MS | F | p |
|---|---|---|---|---|---|
| Region | 1 | 0.723 | 0.723 | 5.85 | 0.042 |
| Error | 8 | 0.989 | 0.124 | | |
| Total | 9 | 1.712 | | | |

Table 4.6b Corresponding mean values of residue for the two regions. Back- transformed ("real") means (mg $Kg^{-1}$ body mass) are also shown for ease of interpretation.

| | N | Mean | StDev | "Real Mean" |
|---|---|---|---|---|
| **e. midlands** | 5 | 0.792 | 0.2265 | 6.19 |
| **c.s. England** | 5 | 1.3298 | 0.4427 | 21.37 |

The sample number in this study was low, but the data nevertheless produced a significant difference for the east midlands versus central southern England in an analysis of variance fitted to the $\log_{10}$ transformed data (Table 4.6a). The sample of rats from the east midlands died with a mean coumatetralyl residue of 6.78 mg $Kg^{-1}$ (range 2.54-9.26) while sampled rats from central southern England were still alive (prior to trapping) with a mean residue of 30.61 mg $Kg^{-1}$ (range 7.30-57.73). This difference is statistically significant ($p=0.042$).

Greaves and Cullen-Ayres (1988) reported a $LD_{50}$ value for susceptible rats of 1.1 mg $Kg^{-1}$ for coumatetralyl. All the rats sampled here carried a body load in excess of the reported $LD_{50}$ value and some of the rats carried more than 50 times more coumatetralyl than the $LD_{50}$ dose.

Figure 4.11 shows that there is not a clear relationship between the amount of bait ingested (as measured by the HCBP residue) and the level of poison remaining as a residue. This is not surprising as coumatetralyl metabolism is known to be faster than HCBP metabolism (section 4.1). Much metabolism of the coumatetralyl appears to have occurred, but the rate of metabolism seems to be different between the rats sampled. An alternative may be that the residues shown are representative of the last few days prior to death. In this case, the difference shown between the regions may also represent a difference between the trapped rats, which may feed up until death, with those found dead, which presumably stopped eating some time prior to death (Cox and Smith, 1992; Chapter 3). Anticoagulants such as coumatetralyl have quite a short half-life of seven to ten days (Thijssen and Janssen, 1994) and so bait eaten two or three weeks before trapping would not be expected to be present unless great quantities had been consumed.

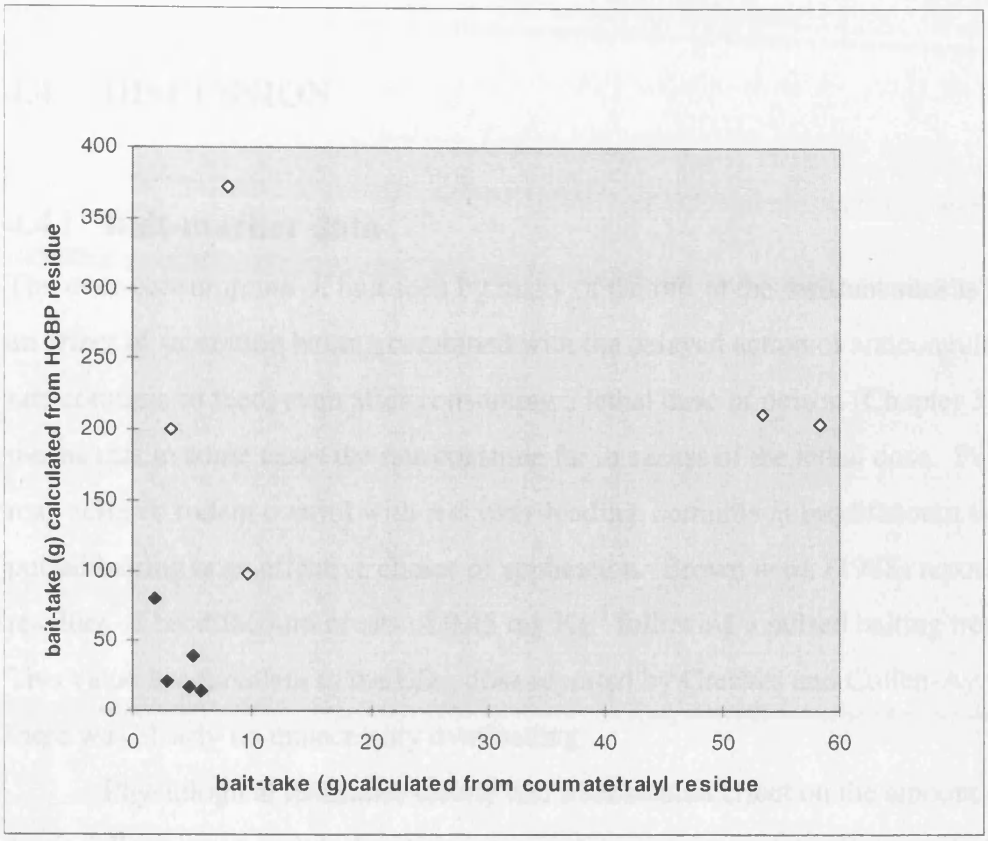



Figure 4.11 Graph to show the relationship between bait-take values calculated from the coumatetralyl and the HCBP residues (pale points are from central southern England, dark points are from the east midlands).

## 4.4 DISCUSSION

### 4.4.i Bait-marker data

The over-consumption of bait seen by many of the rats at the resistant sites is likely to be an effect of saturation baiting combined with the delayed action of anticoagulants. The rats continue to feed, even after consuming a lethal dose of poison (Chapter 3). This means that in some cases the rats consume far in excess of the lethal dose. Pulsed baiting may achieve rodent control with less over-loading, certainly at brodifacoum sites where pulsed baiting is an effective choice of application. Brown *et al.* (1988) reported mean residues of brodifacoum in rats of 0.45 mg $Kg^{-1}$ following a pulsed baiting treatment. This value is equivalent to the $LD_{50}$ dose reported by Greaves and Cullen-Ayres (1988) so there was clearly no unnecessary overloading.

Physiological resistance clearly had a substantial effect on the amount of bait (especially coumatetralyl) that was consumed during these trials. Cowan *et al.* (1995) concluded, however, that resistance only caused 10% of treatment failure in an area of strong physiological-resistance. Alternative foods and other problems were considered to cause a far greater lack of efficacy. Quy *et al.* (1992a) used marker residue levels to infer that the majority of farm rats had eaten insufficient bait to kill them even if they had been susceptible; Quy *et al.* (1992a) suggested that insufficient feeding on bait rather than physiological resistance was responsible for treatment failure, though they may have underestimated bait consumption because they did not use an internal standard in their analyses.

The residue results for individual rats shown in this chapter match the values given for bait consumption by the entire populations shown in Chapter 2. Clearly some rats in central southern England ate high levels of coumatetralyl bait without showing adverse effects. This means that the provision of bait in such cases may be both costly and ineffective. It could also mean that rats are carrying a very high dose of coumatetralyl, with potential repercussions for predatory and scavenging species in the ecosystem.

There were also large differences between individuals in the amount of bait consumed. Some rats ate a huge amount of bait, others had eaten no detectable quantity.

These differences are consistent with some sort of bait point monopolising at farm sites, with some rats managing to exclude others. Behavioural exclusion was apparent with the work done in Chapter 3, and the results in this chapter imply that effective control may be hampered as a result. This conclusion was also reached by Nott and Sibly (1993). The residues of poison in these dominant rats will govern how much of a risk is imposed on other wildlife species in the farm rat's ecosystem.

Finally, the values of marker residue as measured here may underestimate potential effects on scavengers or predators that eat a whole rat, including its guts. Many of the rats, particularly in the resistance areas, had guts filled with bait when they were dissected. The guts were removed prior to residue analyses, but a scavenger or a predator could be exposed to this added load of poison.

## 4.4.ii Rodenticide-residue results

Rats from brodifacoum sites were not analysed, but if they had consumed similar quantities of bait to rats from coumatetralyl sites their residues could be higher because brodifacoum is metabolised more slowly than coumatetralyl (Masterd and Thijssen, 1991; Gray *et al.*, 1994a; Thijssen, 1995). The bait-marker data show that some of the rats during this trial certainly ate large quantities of brodifacoum, so the possibility of high residues exists. Brodifacoum consumption was, however, generally lower than coumatetralyl consumption. Moreover, brodifacoum consumption reached an end when the rats died (there was no evidence of resistance to brodifacoum). Coumatetralyl bait may, conversely, continue to be consumed indefinitely if there is resistance. The body residue of coumatetralyl may therefore remain constantly high, as metabolism and excretion may not exceed consumption. Where second-generation anticoagulants are used excessively and ineffectively, such as the bromadiolone baiting observed prior to the trials at site B4, then the body load may be even greater than found for coumatetralyl because the resistance (excessive consumption) is combined with slow metabolism (second-generation compound).

With so few rats sampled during this study, these explanations are speculative but provide an obvious area for further research. The fact remains, however, that resistant rats that were fed coumatetralyl are capable of carrying very high loads of poison residue.

This factor must surely be taken into account when rodenticides are screened for non-target wildlife hazards in the future. These resistant rats could represent a serious risk of secondary poisoning to predatory animals and this risk must be way in excess of the risk assessed during screening and post-regulation monitoring of the coumatetralyl bait.

Gray *et al.* (1994a) found that mice dosed with 50 $\mu g\ g^{-1}$ second-generation compounds such as brodifacoum and fed to barn owls contained 0.95-5.2 mg $Kg^{-1}$ of residue. The owl livers were later found to contain just 0.5-0.7 mg $Kg^{-1}$ of brodifacoum, whereas the pellets contained 29% of the amount of brodifacoum ingested. Gray *et al.* concluded that the risk of poisoning barn owls is low, because much of the rodenticide is taken out of the body in the pellets. Townsend *et al.* (1981) similarly concluded that tawny owls fed on mice dosed with warfarin (1.6 mg $Kg^{-1}$, with a few up to 17 mg $Kg^{-1}$) were not at risk of secondary poisoning. The owls were able to metabolise, rather than accumulate, the warfarin. The mice used in these two toxicity experiments contained body residues that were way below the coumatetralyl levels found in some of the rats in the field during this study. It is therefore likely that different results would be encountered if highly-dosed rodents, such as the resistant rats in these trials, were used to feed the owls. Merson *et al.* (1984) found that meadow voles containing residues between 0 and 9.47 mg $Kg^{-1}$ (mean around 3 mg $Kg^{-1}$) did cause adverse effects on wild screech owls. It may be that wild animals are under very different stresses to caged animals of the same species and the levels of toxin tolerance are likely to be similarly altered. Gray *et al.* (1994a) noted that all the barn owls that had a dose of >1.9 mg $Kg^{-1}$ showed behavioural effects. Hart (1990) pointed out that low-dose behavioural changes may mean that an otherwise environmentally benign compound can cause damage to non-target wildlife by disrupting normal patterns of behaviour and survival. It therefore seems sensible to take caged toxicity trial conclusions as a higher estimate of tolerance and to continue the vital post-regulation monitoring of environmental toxicants that is part of the regulation process in this country.

## 4.5 EVALUATION OF APPROACH

In summary, the use of bait markers such as benign PCBs provides an opportunity to quantify bait take of individual rats in the field. These data may then be compared with analysis of rodenticide residues to make inferences about metabolism and excretion of rodenticides consumed by wild animals. Execution of the field experiment here was complicated by two factors: (i) the unexpected appearance of resistance in the east midlands, (ii) prevention by some farmers of full saturation baiting at some sites in central southern England. Despite this, the data revealed:

1. Individual rats in central southern England (area of resistance) ate significantly more poison bait than rats in the east midlands ($p<0.05$).
2. Individual rats from both areas ate more coumatetralyl than brodifacoum ($p=0.06$).
3. More than 60 % of the rats from coumatetralyl sites and more than 80 % of the rats from brodifacoum sites in each region had consumed bait quantities in excess of the $LD_{50}$ values given for susceptible rats (up to 16.5 mg $Kg^{-1}$ and 0.3 mg $kg^{-1}$ for coumatetralyl and brodifacoum respectively).
4. The majority of the "worst cases" of excessive bait consumption occurred at farms in central southern England. One rat had consumed at least 490 g of coumatetralyl before it was trapped and another rat had eaten at least 133 g of brodifacoum.
6. The marker residue data from trapped rats showed that some individuals had eaten very little or no poison bait. This result is consistent with the theory of bait point exclusion. An alternative explanation may be that some rats chose not to access bait points because other food supplies were preferred or due to "increased neophobia".
7. The body loads of coumatetralyl actually found in rats from central southern England were significantly greater than the residues in rats from the east midlands ($p<0.05$). Some rats carried body loads of coumatetralyl at least 50 times more than the reported $LD_{50}$ dose.
8. These results indicate that predators and scavengers potentially are exposed to rodenticide levels that far exceed the quantities against which they are tested during pesticide regulation. This is particularly the case in areas where physiological resistance is common.

# CHAPTER 5: GENERAL DISCUSSION

## 5.1 POPULATION EFFECTS OF ANTICOAGULANT RODENTICIDE USE

Rat populations that are targeted during rodenticide control treatments may be discrete and confined to a small area of the farm, or they may be extensively spread. The size of population is likely to depend largely on the availability of the rats' basic requirements: food, water and shelter. If any one of these three ingredients is absent, then rats are unlikely to colonise a farm, although they may travel from some distance to visit it daily (Hardy and Taylor, 1979; Fenn *et al.*, 1987). Where water, food and shelter are present, there is little to stop colonisation, but the size of the population will still depend on the relative availability of each resource. If any resource is in short supply, then it is likely to become a limiting factor on population growth. Begon and Mortimer (1986) described the "scramble and contest" strategies employed by plants and animals in populations that are limited by any resource. The scramble strategy places all individuals as equivalents; they compete equally for resources and if the population carrying capacity with regard to that limiting resource is reached, all the individuals suffer. This may be as drastic as the death of the whole population or may take effect on the growth or fecundity of the individuals, so that reproduction is inhibited. An example of the scramble strategy of intraspecific competition is shown by blowfly pupae that are pollutant-stressed (Forrest, 1996). The contest strategy places some individuals above others in their ability to monopolise a resource, so that if the resource becomes a limiting factor on the population growth, the advantaged individuals will still be able to survive and reproduce as normal, whereas the disadvantaged individuals may die or stop breeding. An example of this strategy is shown by a strain of the southern cowpea weevil, *Callosobruchus maculatus* (Broadhurst, 1997).

Previous studies of rat populations have implied that rats adopt the "contest" strategy to deal with intraspecific competition. It has been reported that dominance hierarchies form within loose family groups and the dominant individuals are able to monopolise food and perhaps mates (Adams and Boice, 1983; Macdonald and Fenn, 1994). Dominant individuals also aggressively exclude rats that attempt to immigrate to the population (Robitaille and Bovet, 1976; Adams and Boice, 1983). This social

structure may therefore actively regulate the size of populations, both by preventing immigration and by minimising the reproductive status of subordinate individuals (Smith and Greaves, 1987). Butler and Whelan (1994) discovered that the population density of wild rats in County Kildare, Ireland, was maintained by a complex social structure. The limiting resource was the number of reproducing females.

This study provided evidence in support of the theory of bait point exclusion, but not complete monopoly. Rats were observed interacting at feeding locations and some rats were actively deterred by others (Chapter 3). Marker residue data (Chapter 4) indicated that some rats never actually access bait points, although this may be through choice rather than exclusion. Rodent control failure should not, however, be attributed to bait point exclusion if sufficient bait points are supplied over a suitable area to target the entire population. Rat mass data (Chapter 2) indicated that rats of all sizes were feeding and dying throughout the trials and so bait point monopoly could not have been achieved on the larger scale of the whole farm site. Behavioural observations also implied that all rats eventually accessed bait points if they chose to and there was no obvious monopoly to the point of total exclusion of subordinate rats. It is important that farmers are aware of the need to bait frequently and extensively in order to avoid bait point exclusion at the expense of a failed control programme. Too many farms visited during these trials maintained just a very few permanent bait stations at which they heaped out the bait, often uncovered. This practice not only fails to target the whole rat population, but it adds to the risk of poisoning non-target bait feeders.

Rat populations are likely also to be regulated to a certain extent by environmental conditions. Bishop and Hartley (1976) noted that a barn population of rats bred all year round whereas a nearby population living in the fields failed to breed through the winter. If environmental conditions are favourable and social interactions with other stable (and thus effectively aggressive) family groups can be avoided, then rats will be able to immigrate on to the site and may even become integrated loosely with the population. Much of the previous failure in rodent control at the sites during these trials was the result of poor farm management. Spilled feed and carcasses were left lying around and these attracted rats. Harbourage, in the form of ricks and fertiliser bags, cannot be removed but other rubbish and debris should be cleared up. Without good farm practice, rats are able to inhabit an area undisturbed and will be able to breed throughout the year.

When poisons are regularly used on a farm, the social structure of the resident rat population is likely to be disrupted. If the subordinate rats are most likely to be poisoned, because they are less neophobic perhaps (Shepherd and Inglis, 1987; Nott, 1988), then the social hierarchy will remain intact. If the dominant rats are targeted, then the social structure may crumble, intruders can immigrate and the site will be recolonised. Where poisoning is repeatedly used, there may be a very unstable and highly changeable population. This effectively means that there is no restraint on population growth (Butler and Whelan, 1994) and the rats' density will be able to increase indefinitely as long as conditions on the farm are favourable. The irony is therefore that unless the rats are eradicated completely and the harbourage and food supply are removed, control may eventually lead to there being more rats than there were to start with.

## 5.2 METAPOPULATION EFFECTS

A metapopulation is the "population of populations" and has developed as an important concept in recent years, especially for animals that are concentrated in discrete patches of resource (Smith, 1995).

In a very heterogeneous environment with a plentiful supply of resources, different groups of rats can avoid interaction and will therefore remain intact. There may therefore be a number of family groups or clans that inhabit such a farm site. Equally, there are likely to be populations of rats living outside the farmstead. Rats are very opportunistic and are therefore good colonisers; they are generalist feeders, they have a high fecundity and they are highly mobile. A poison treatment may clear the farmstead of rats, but it is unlikely that the surrounding populations or metapopulation (Smith, 1994, 1995) as a whole, will be affected. Sullivan (1986) noted that small rodent populations are very resilient and rapidly recolonise areas depleted by use of poisons. Immigrants will rapidly recolonise as long as they remain unaffected by the treatment and as long as the attractive resource remains. Farmsteads are likely to be attractive to rats because they provide all three of the necessary conditions for rat survival; food, water and shelter. The populations that result are likely to be extensive and lacking a rigid or stable social structure. Prolonged rodenticide treatment may result in immigrants being poisoned too. On a local scale there may be an eradication of rats (although eradication efforts in the past have been grossly ineffective; Greaves, 1994). The high mobility and reproductive capacity of rats means that on a metapopulation scale, there will be little adverse effect on a metapopulation of rats from uncoordinated anticoagulant treatment on individual farms (Smith, 1995).

This study indicated that there may indeed be discrete populations of rats living within a farmstead; behavioural observations supported the possibility of "clan" behaviour and field trials revealed that activity occurred within discrete patches of a farm. There was also much evidence of reinfestation, particularly into peripheral areas of a site. These results are therefore conducive to the hypothesis that, given suitable conditions on a farm site, there may be a constant flux of rats onto a site. Data from Chapters 2 and 4 showed that some rats fail to eat the poison bait at all, and this fact supports the theory that, on a metapopulation scale, there will be little effect from uncoordinated rodenticide treatments.

## 5.3 ECOSYSTEM EFFECTS

A compartment model to describe the potential routes of rodenticide transfer through the ecosystem of the UK farm rat was developed by Smith *et al.* (1990) and is shown, in a modified version, below.

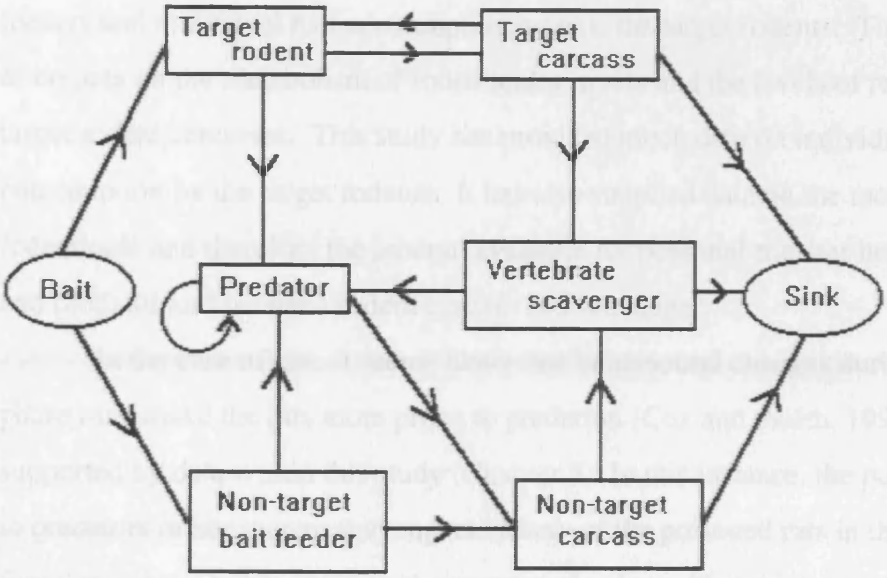



Figure 5.1 The "Rodenticide System", adapted from Smith *et al.* (1990). The arrows represent potential routes of rodenticide transfer between different compartments in the ecosystem. The tertiary-poisoning route of rodenticide transfer (from predator to predator) has been added and the transfer between target carcass and target rodent (cannibalism) has also been included.

The target rodent (rat or house mouse) lives within a fairly complex community. The possible routes of non-target exposure to rodenticides were identified within a compartment model by Smith *et al.* (1990). Non-target bait feeders potentially include wood mice, voles and grain-eating birds as well as some opportunistic feeders such as rabbits, badgers and deer. Any of these animals (non-target or target) may die as a result

of consuming anticoagulant and the carcass may then become available to vertebrate scavengers including other rats. Alternatively, the non-target or target feeder may be killed by a predator and the rodenticide will then be transferred, via the contaminated prey item, to the predatory animal.

Smith *et al.* (1990), whilst developing the compartment model of rodenticide transfer (Figure 5.1), revealed a paucity of data in some areas; scavengers, non-target bait feeders and individual bait consumption by rats, the target rodents. There were also few or no data on the metabolism of rodenticides in rats and the levels of residue found in target rodent carcasses. This study has provided much data on individual bait consumption by the target rodents. It has also supplied data on the metabolism of rodenticide and therefore the amount available for potential transfer between live rodent and predator and between rodent carcass and scavenger.

In the case of rats, it seems likely that behavioural changes during the pre-lethal phase may make the rats more prone to predation (Cox and Smith, 1992), an idea supported by data within this study (Chapter 3). In this instance, the poison is transferred to predators rather than to scavengers. Many of the poisoned rats in this study were found as carcasses however, so the transfer of rodenticide to scavengers is certain.

Many predators and scavengers are themselves exposed to predation and so the possibility of tertiary poisoning exists for predators as well as for scavengers that may consume poisoned predator carcasses. Rats may receive poison by two routes also; directly *via* the bait or by consuming other poisoned rats. Field observations made during this study often noted evidence of rat cannibalism.

These transfers of poison are direct effects of rodenticide on the ecosystem. Other direct, yet less obvious effects, include chronic poisoning or accumulation of poison over a period of time. These would not cause mortality, but may affect reproduction and survival of the species involved due to inability to form mating pairs, inability to raise young, or inability to feed adequately to survive harsh climatic conditions. Some of these effects may be very subtle and not at all easy to detect during routine observation. They may only show up as a result of an unexplained population decrease that is investigated (*e.g.* the Peregrine Falcon: poisoning with dieldrin and DDE in the 1960s; Prestt and Ratcliffe, 1972).

There are also indirect effects that are likely to cause changes to the normal food webs of the ecosystem. The removal of the target rodents may mean that prey items are scarce for some predators. Rats are not a main food item for many species in the UK (Polecats are the one main example), but they are an important secondary food for many more species, particularly when a favoured food, such as field voles are low. Removal of favoured prey by accidental poisoning could have a much more serious effect on predatory species, particularly if they were relatively immobile and therefore unable to hunt elsewhere instead. (Tawny owls are particularly territorial and will only hunt within their own patch: Southern, 1954). There was some evidence from the small mammal investigation in this study (Chapter 2) that voles and wood mice, both of which are favoured prey for many predators, are adversely affected by rodenticide treatments. This view was stated also by Cox (1990). Predators would therefore suffer as an indirect effect of the control programme because their food supply is depleted.

Interspecific competition may be altered as a result of anticoagulant treatments; a competitive species may be depleted or removed, allowing the opportunity for the usually out-competed species to relocate or expand. Tattersall (1992) noted that wood mice competitively exclude house mice. Rats are also generally believed to deter house mice. The removal of either wood mice (by accidental poisoning, Cox (1991)) or rats (by target baiting) may thus allow house mice to spread across a farm. This was seen at one of the sites, A1 in the east midlands (Chapter 2).

Some animal species are very tolerant of short term "catastrophes" and would be able to recover from a poisoning event quite rapidly. Such species are termed r-selected and are capable of high fecundity to recover population numbers (Begon *et al.*, 1990). For example, Colvin (1984) believed that barn owls would quickly recover from any poisoning event involving anticoagulant rodenticides because the barn owl is a r-selected species. Adaptability, such as the ability to switch to a different prey item if the usual one becomes depleted (Erlinge, 1975) and mobility to move away from an area depleted of prey, would assist other predatory species to recover (*e.g.* the barn owl in Malaysia: Smith, 1994). Such species as these may, therefore, remain largely unaffected by rodenticide treatments carried out on a local scale.

Some animal species are very tolerant of toxins and may even thrive in a "polluted" environment. Such species are often used as key indicators to reveal the

presence, or relative concentration, of a pollutant. Key indicator species can sometimes reveal a potential disruption of the ecosystem long before other detrimental effects are noted (Begon *et al.*, 1990).

It is apparent from these complexities that an ecosystem's stability may be affected in a number of ways as a result of pesticide application. Some effects may be easily detected, but others may be so indirect that the cause of the change is not obvious. It is vital, therefore, to take a whole ecosystem approach when considering the environmental fate and possible adverse effects that a pesticide may have on an ecosystem.

## 5.4 THE RESISTANCE FACTOR

In very resistant populations, few rats will be poisoned, and the dominance hierarchy is likely to remain intact. This means that population growth will probably be regulated by the social structure of the colony (Butler and Whelan, 1994). Farmers are rarely inclined, however, to leave a population of rats on the site to its own devices and some form of effective control method will be sought. Baiting with an acute poison or with an anticoagulant to which there is no resistance may be used. Alternative non-chemical methods such as trapping, using ferrets or terriers may be equally successful although such methods are likely to be far less time-efficient. Any control technique may selectively remove a fraction of the population. Trapping has been reported to bias against pregnant females in the past (Kataranovski *et al.*, 1991), the limiting factor for population growth according to Butler and Whelan (1994). Trapping may therefore have little effect on the overall social stability of the colony, although evidence from this study did not find any such bias against trapping pregnant females (Chapter 2).

This study has revealed that resistance is clearly a major concern for rodent control in parts of Britain, such as the borders of Oxfordshire, Hampshire and Berkshire. Farmers, desperate in their attempts to poison the rats, were found often to heap the bait in big piles. This ineffective bait was readily used by the rats as a food source, thus adding to the frustration of the farmers. Farmers need to be informed about the alternatives to ineffective poison use. Much of the resistance problem has occurred because baiting has been carried out ineffectively; with too few bait points and not enough frequency to target all rats. Ineffective poisons are then used *ad. lib.* when in fact this merely targets the susceptible rats within a population and gives the resistant rats added force. Rodenticide resistance in the UK has existed since the 1950s and rats are continuing to evolve further mechanisms (physiological and behavioural) to overcome the use of poisons. It is surely time, therefore, to take the matter of resistance in the UK seriously and implement a systematic monitoring scheme along the lines of the one used in Denmark to combat and manage rodenticide resistance. Resistance monitoring and regulation should be conducted on a region-wide or even nation-wide scale. Farmers should be told which poisons they may or may not use and professional pest control

operators should be made available to assist with application of the more toxic, indoor use poisons, brodifacoum and flocoumafen. Coumatetralyl and other ineffective poisons should be banned from use in resistant areas and the effective poisons should be properly applied, if possible in frequent "pulses" to avoid over-consumption. Much of the excessive consumption witnessed during this study was a result of saturation baiting and the delayed onset to toxicosis. These two factors allow rats to continue eating bait after they have consumed a lethal dose. Saturation baiting is not necessary for brodifacoum and so it should be avoided.

Evidence from this study indicates that physiologically resistant rats may cause anticoagulants to impact on the environment far more than during a "normal" anticoagulant rodenticide treatment. The resistant rats are able, in some cases, to carry an exceptional body load of rodenticide, which means that predators or scavengers that consume the resistant rats could be exposed to excessive levels of anticoagulant; levels against which they have not been tested in toxicity trials for licensing purposes. In the USA, the Environmental Protection Agency (EPA) allows "emergency" use of otherwise strictly regulated chemicals in cases where pesticide resistance cannot be controlled (Matten *et al.,* 1996). UK resistance 'management' in the past has involved merely the development of more toxic poisons to which there is no resistance. These tactics may expose wildlife species to potentially highly damaging chemicals, all because of pesticide resistance. It is, therefore, surely time to seriously address and tackle the resistance issue. Not only is rodent control seriously undermined by the presence of resistance, but non-target wildlife species are being overlooked and endangered in attempts to remove the resistant rats.

## 5.5 BRINGING THE "ECO" INTO ECOTOXICOLOGY

The compartment model produced by Smith *et al.* (1990) went a long way towards assessing the risks of rodenticide use on the environment. The model relied, however, on a great deal of laboratory data. The studies that were used were certainly important for producing data on toxicity levels in different species and the levels of a toxic food that may be consumed, but the studies were unable to mimic the situation in the field. Animals in a natural environment are exposed to entirely different choices, stresses and interactions than animals that are housed artificially. A knowledge of how animals behave and interact in the wild is vital in order to assess accurately the likely transfers of pesticides through the ecosystem. Hart (1994) showed how a knowledge of the field behaviour of wood pigeons was necessary to assess the correct transfers and quantities of insecticide accidentally ingested. This project has investigated aspects of population dynamics, poison bait consumption and individual and group behaviour among wild, free rats. Data produced from these investigations have allowed the transfers of rodenticide through the ecosystem of both susceptible and resistant rats to be detailed more accurately than captivity studies would allow.

Much ecotoxicology work in the UK is used to describe laboratory work on small organisms such as *Daphnia*, particularly in relation to water contamination. Ecotoxicology is used very little to describe the study of toxins within the environment of larger animals and is rarely used to describe work with plants. Ecology is the study of all living things and their interaction with each other and the non-living elements. Toxicology is the study of (man-made) toxins. Ecotoxicology should thus be the study of how toxins affect the natural environment in its whole form. This includes aspects of biology, chemistry and physical processes. All these areas should be investigated during the testing and licensing procedures for pesticides.

Monitoring a pesticide on an ecosystem scale would be very costly (Hart, 1994), yet laboratory studies are not a good enough mimic for natural conditions. The use of models is therefore very important and models could be used to far greater levels than they currently are. Models rely on real data on which to base, and then modify, the factors. Some field experiments, particularly with a replicated and contrasting design, are

therefore vital, whatever the cost. Thereafter, modelling can reduce costs and also reduce the actual exposure of wildlife species to toxins during the course of scientific studies.

Analytical chemistry is now a very useful tool for ecotoxicological studies - limits of detection are very low for many pesticides and highly accurate and reproducable tests can be made, as evidenced in Chapter 4. In this way, transfer of chemicals through the environment can be assessed without lethal doses being issued; this would obviously be far less detrimental to wildlife species.

Pesticides in the UK are currently put through a tiered process of regulation and post-regulation monitoring to assess the environmental risks involved with the chemical's use (Brown, 1994; Smith, awaiting publication). The third tier is the first stage at which field work is conducted and simulated field trials are used to focus on the species at risk. These trials, while carried out in "the field", may still not suitably mimic the natural environment or the conditions under which the pesticide may be used. The post-regulation monitoring involves, realistically, the gathering of data on wildlife poisoning incidents, rather than anything more rigid. This means that many small or less important species may suffer adverse effects without being noticed or reported. The tiered system is popular with regulators and companies, because it seems fairly cost effective. It suffers, however, from a limited ecological perspective, especially in relation to population regulation and the dynamics of species interactions.

The final conclusion from this study is that the scientific community concerned with ecotoxicology should adopt a programme based on use of the following tools to assess more accurately the real routes of transfer and levels of exposure in the environment:

1. Replicated and contrasting field studies (Cox and Smith, 1990) using non-invasive methods (Harrison *et al.*, 1990; Gray *et al.*, 1994b) or environmentally benign, but stable, chemical markers such as HCBP (Sanchez-Hernandez, 1994; Townsend *et al.*, 1995).
2. Studies of changes in behaviour of target and non-target species after exposure to the pesticide (Cox and Smith, 1992), ideally in the field (Hooper *et al.*, 1990).
3. Analytical chemistry - not only for determining wildlife poisoning incidents, but at an earlier stage to detect the transfers of a sub-lethal amount of chemical through the ecosystem. Live sampling is possible using small quantities of blood (Sanchez-

Hernandez, 1994), so this approach could be integrated into post-application monitoring of a substance that can mimic the transfers of the real pesticide.

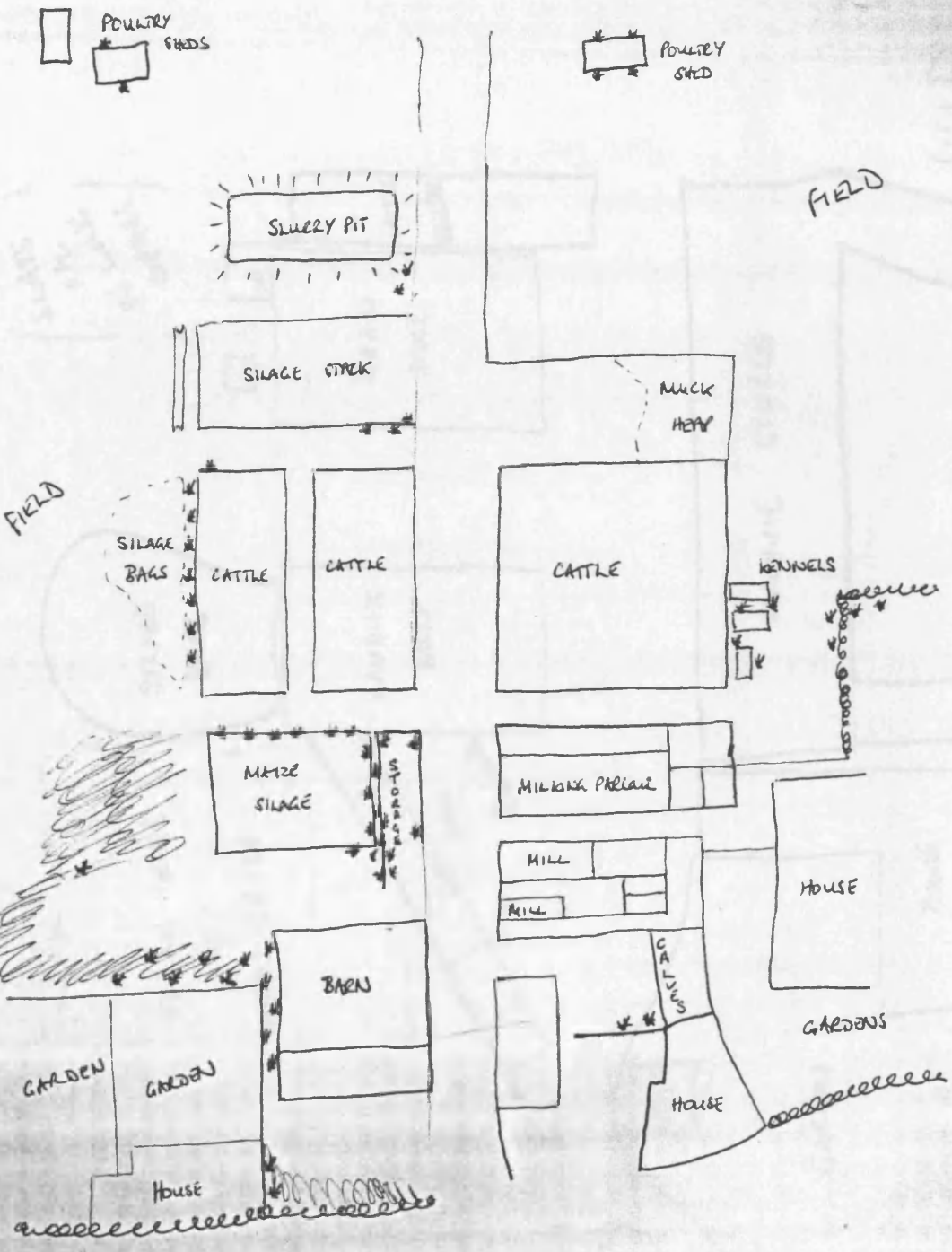
4. When enough basic data are gained, from both the laboratory and the field, models can be used to discover how different environmental stresses *etc.* would impact on the flow and fate of a pesticide (Smith *et al.,* 1990). This would reduce costs and minimise the need to use wildlife species, particularly protected ones such as barn owls, for pesticide testing for every new condition that may arise.

This project was able to assess various aspects of the ecotoxicology of rodenticides using the tools outlined in 1-3 on the previous page. Data were collected that can now be used to further develop the compartment model (Tool 4). A more integrated approach to the study of pesticides in the environment is now achievable, through advanced technology, and is practical, due to the relatively low costs and man power needed. It is also a desirable aim, for as scientists we are largely responsible for the accurate prediction, monitoring and regulation of pesticides in our own environment.

# Appendix 1 Farm site plans

Sites A1-A9 were in the east midlands
Sites B1-B9 were in southern central England

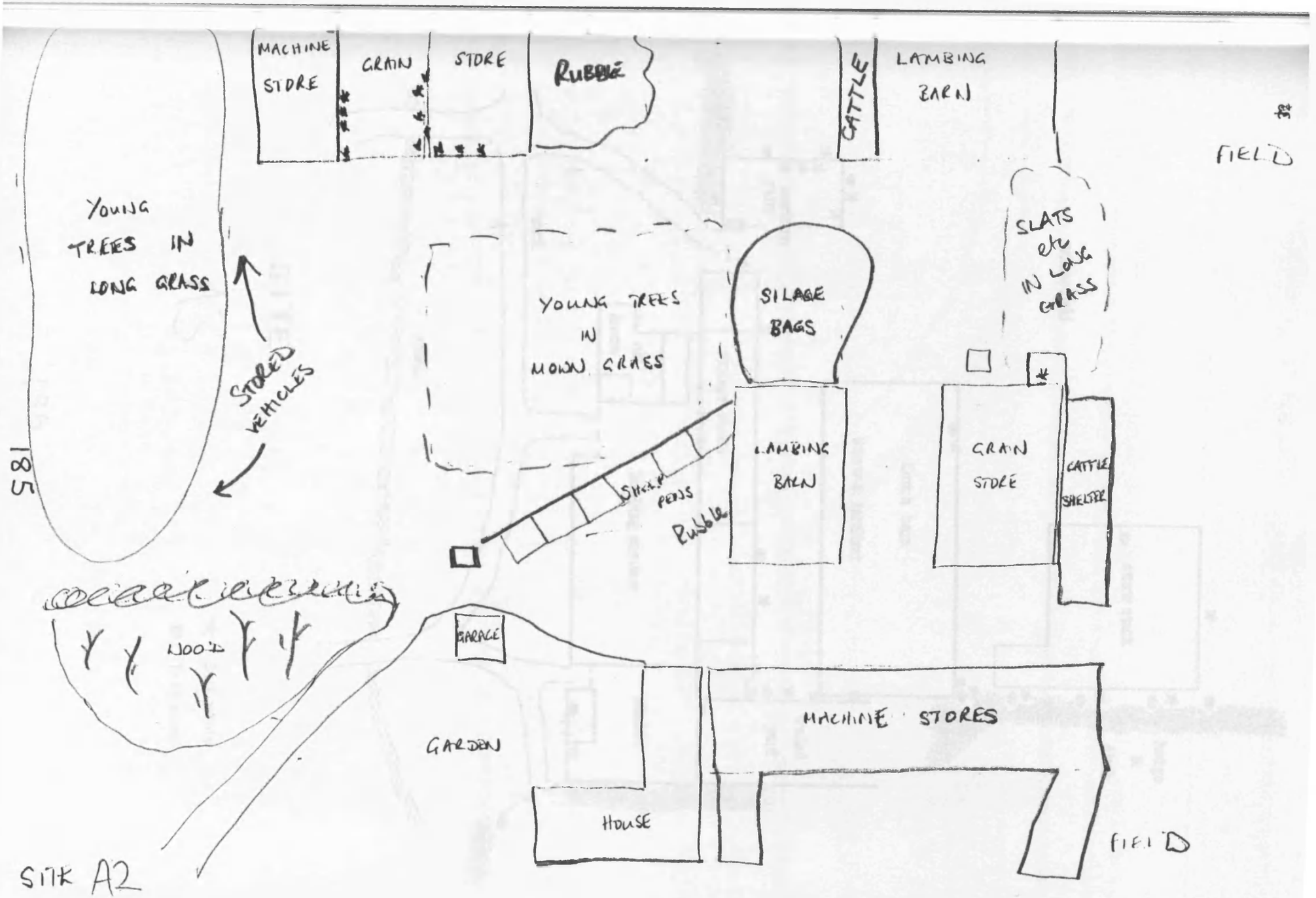
*N. B. Stars indicate bait point locations*

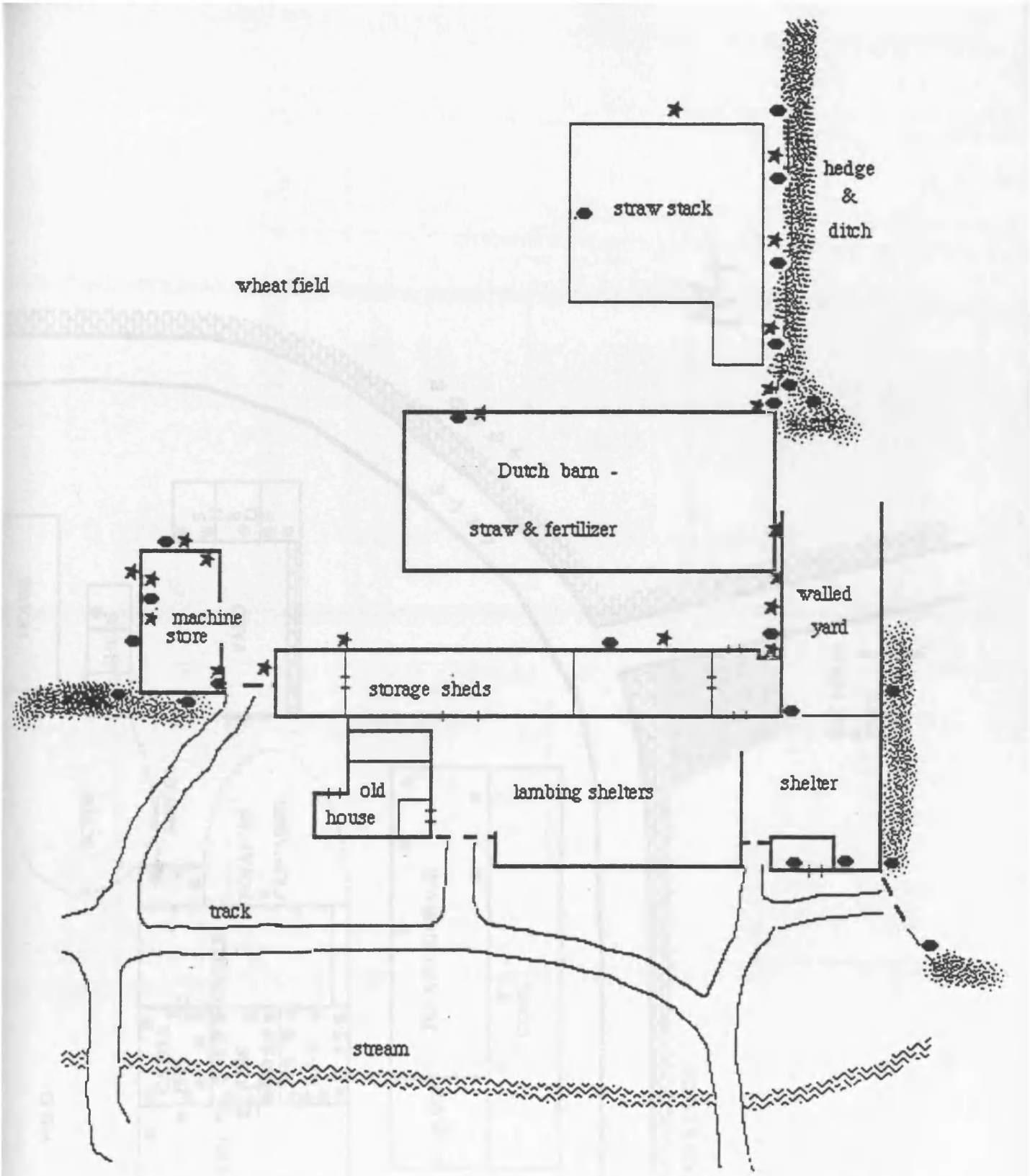



73 BAT POINTS
40 LONGWORTHS
27 T. TILES

A1

184


MACHINE STORE
GRAIN
STORE
RUBBLE
CATTLE
LAMBING BARN
FIELD
YOUNG TREES IN LONG GRASS
STORED VEHICLES
YOUNG TREES IN MOWN GRASS
SILAGE BAGS
SLATS etc IN LONG GRASS
LAMBING BARN
GRAIN STORE
CATTLE SHELTER
PENS
RUBBLE
WOOD
GARAGE
GARDEN
HOUSE
MACHINE STORES
FIELD
SITE A2

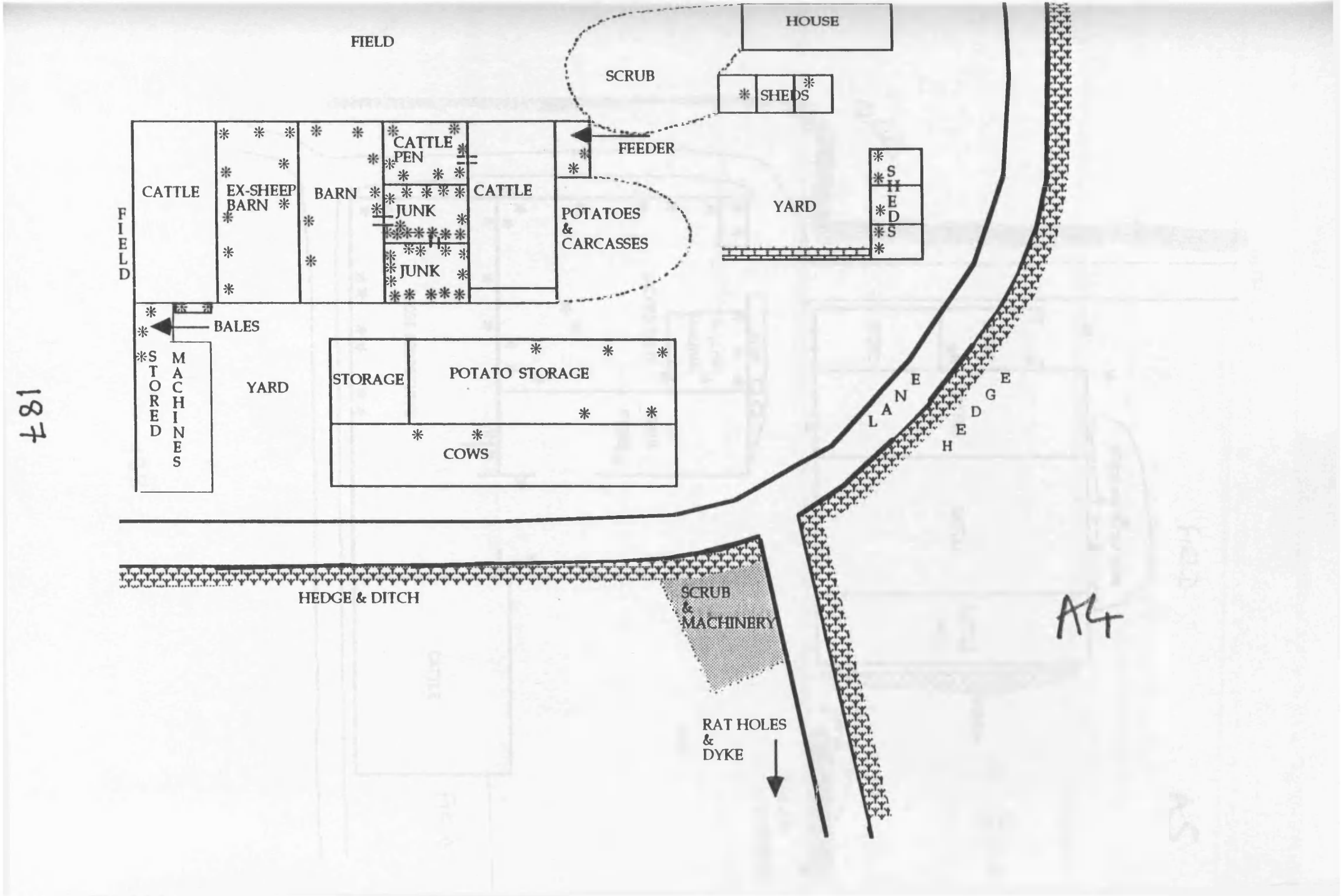


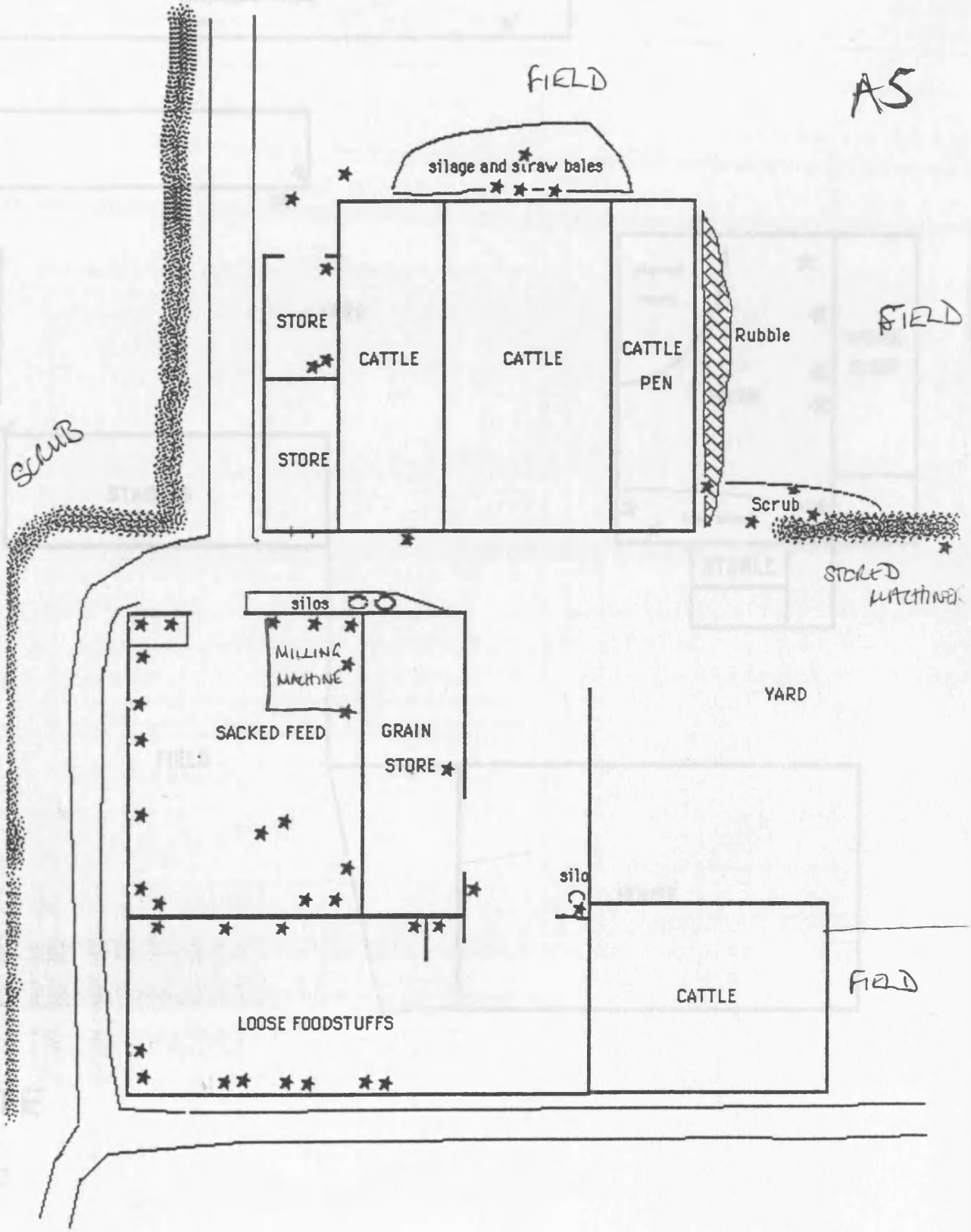

SITE

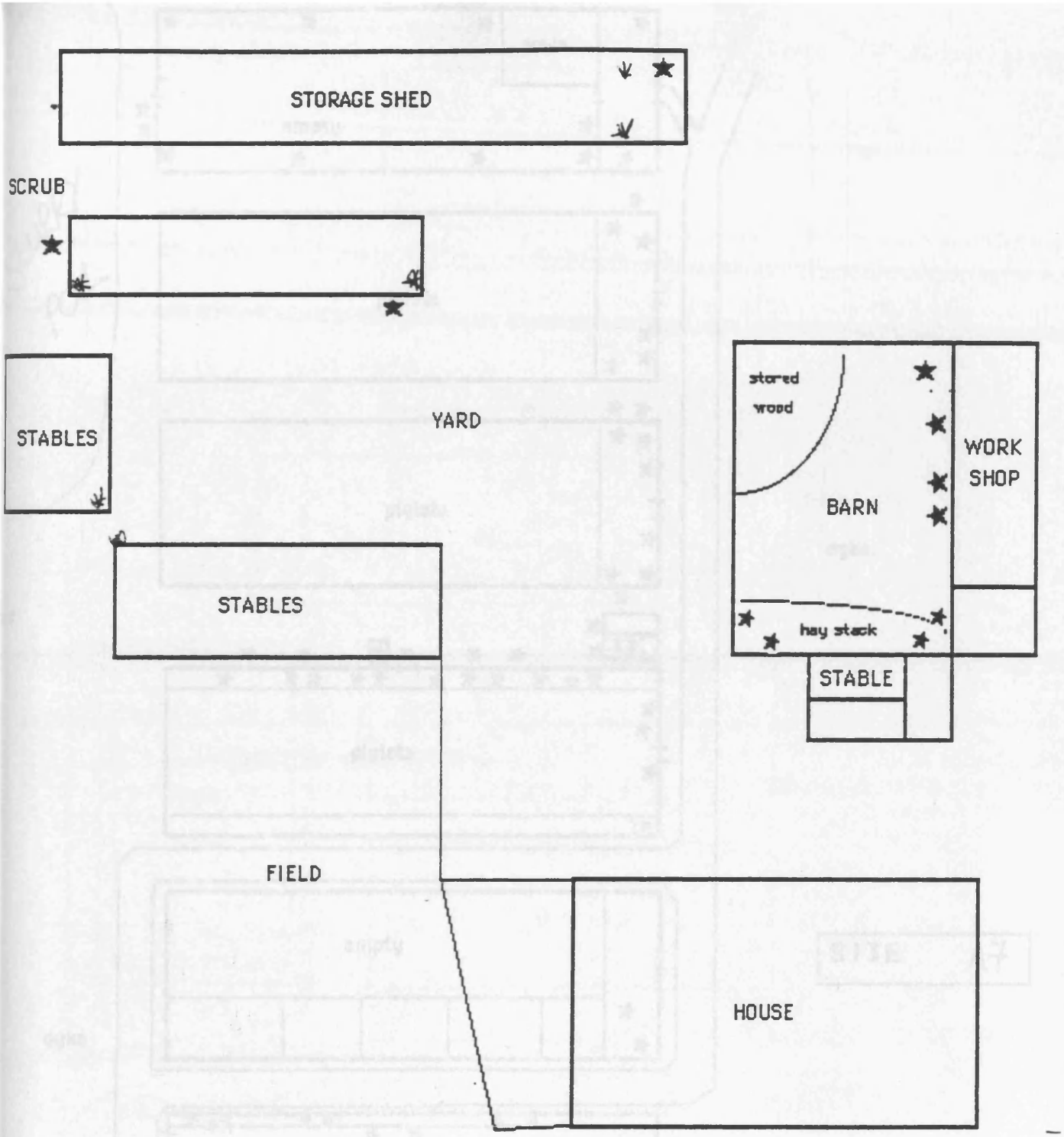
A3

★ Bait points

● Trap sites

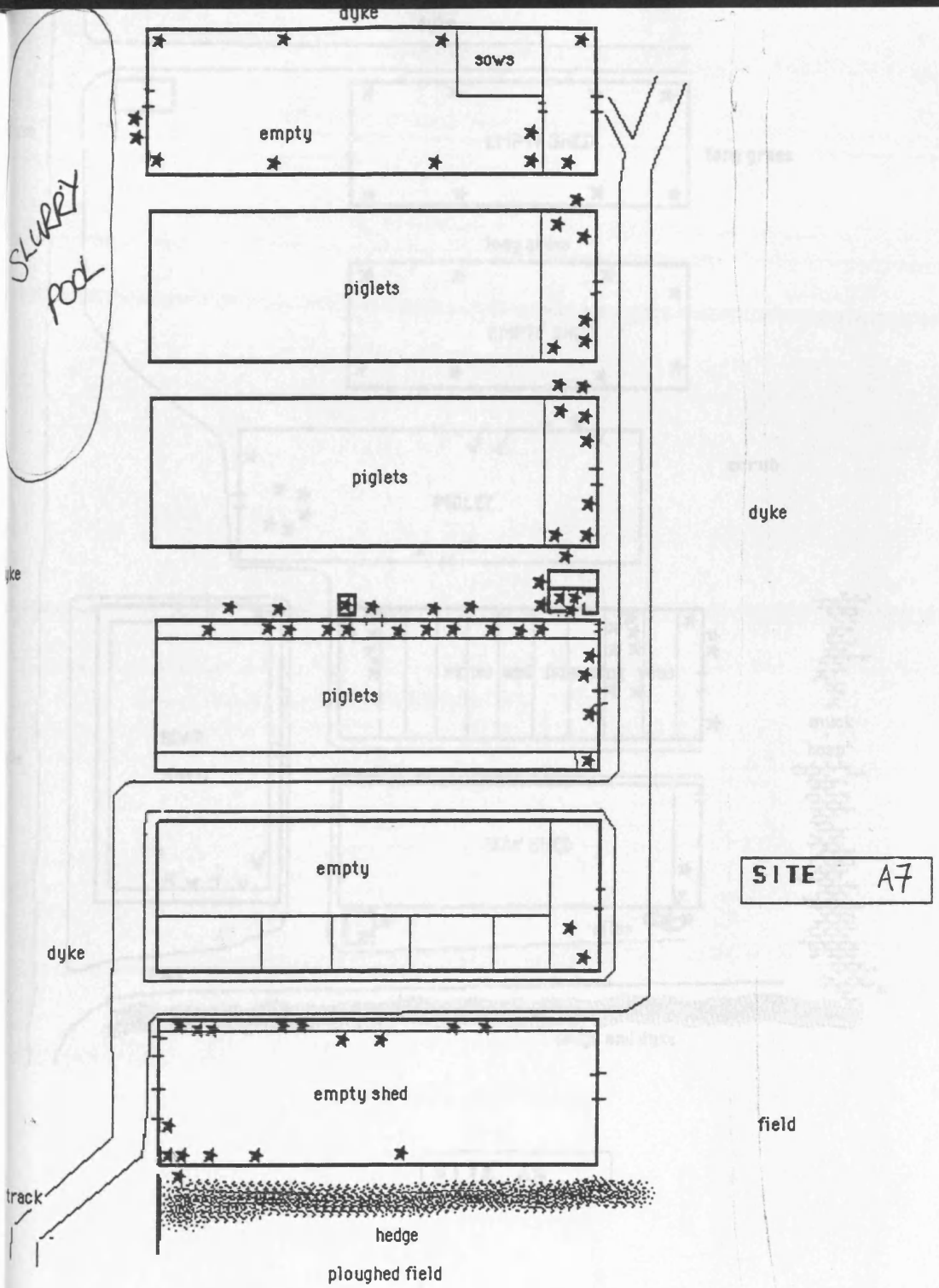

FIELD
HOUSE
SCRUB
SHEDS
FEEDER
CATTLE PEN
CATTLE
EX-SHEEP BARN
BARN
JUNK
JUNK
CATTLE
POTATOES & CARCASSES
YARD
SHEDS
FIELD
BALES
STORED MACHINES
YARD
STORAGE
POTATO STORAGE
COWS
LANE
HEDGE & DITCH
SCRUB & MACHINERY
RAT HOLES & DYKE
A4

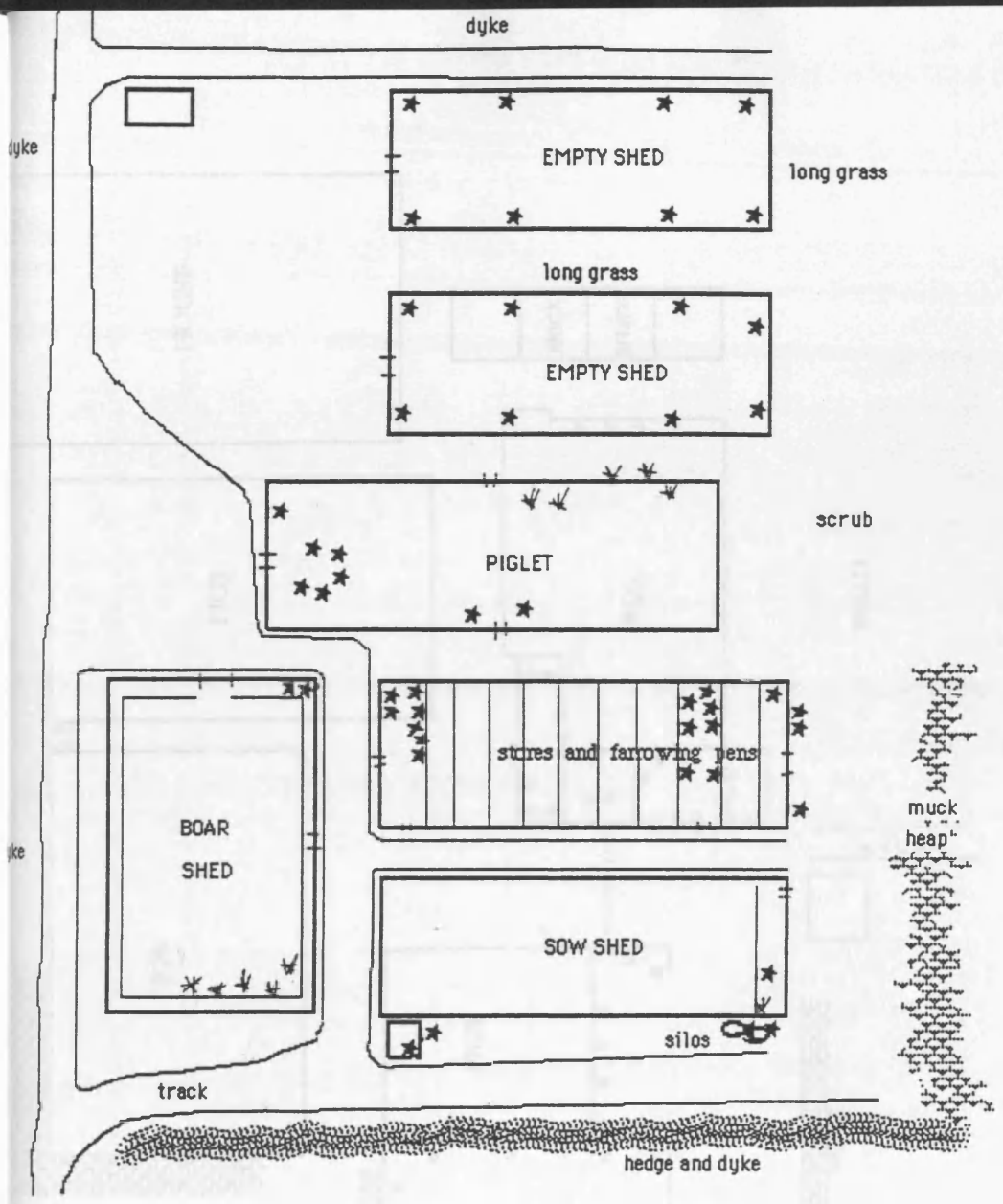

FIELD
A5
silage and straw bales
STORE
CATTLE
CATTLE
CATTLE
PEN
Rubble
FIELD
STORE
SCRUB
Scrub
STORED
MACHINES
silos
MILLING
MACHINE
YARD
SACKED FEED
GRAIN
STORE
silo
CATTLE
FIELD
LOOSE FOODSTUFFS


STORAGE SHED
SCRUB
STABLES
YARD
stored wood
WORK SHOP
BARN
hay stack
STABLE
STABLES
FIELD
HOUSE

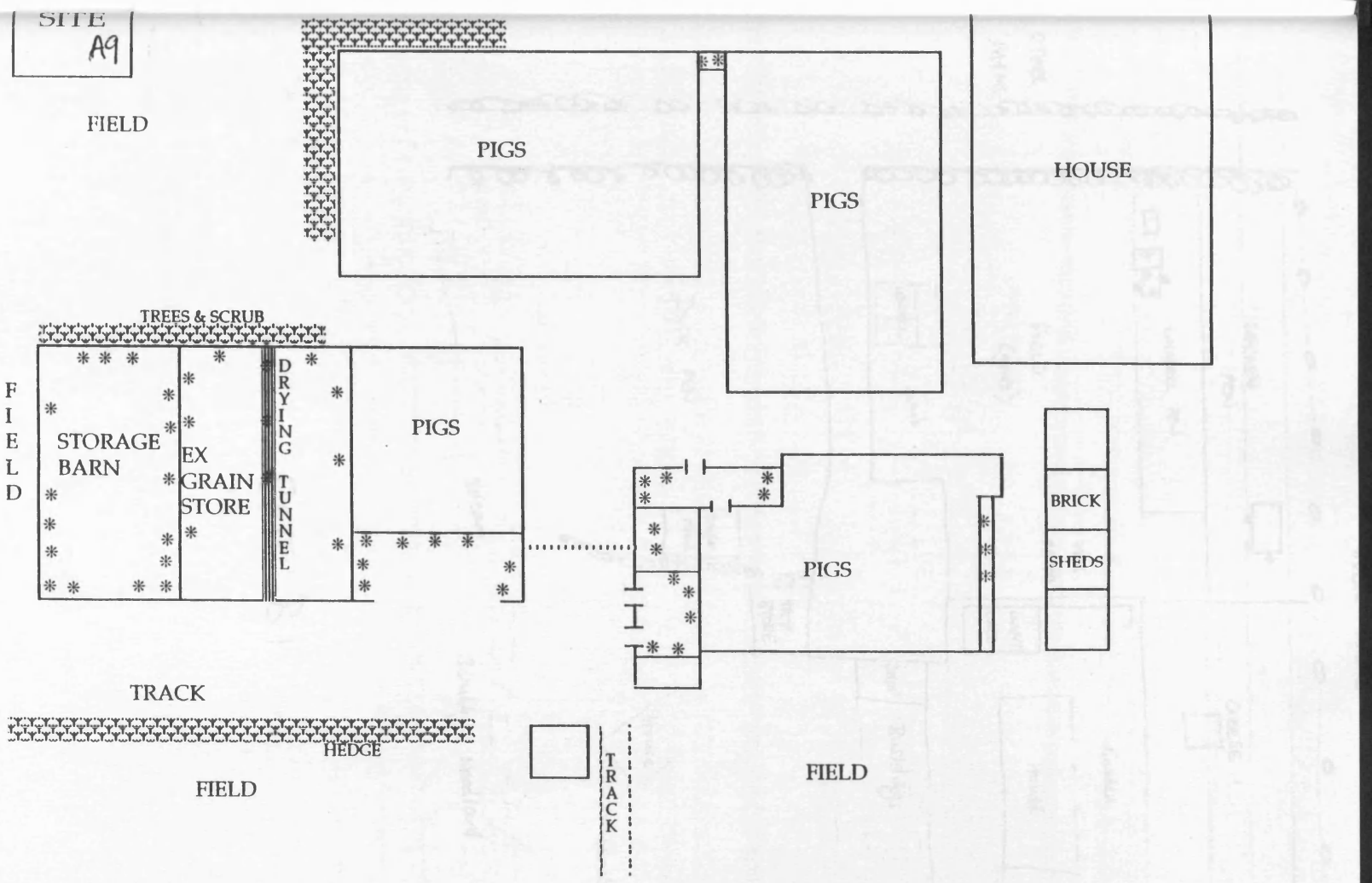
**SITE**

A6

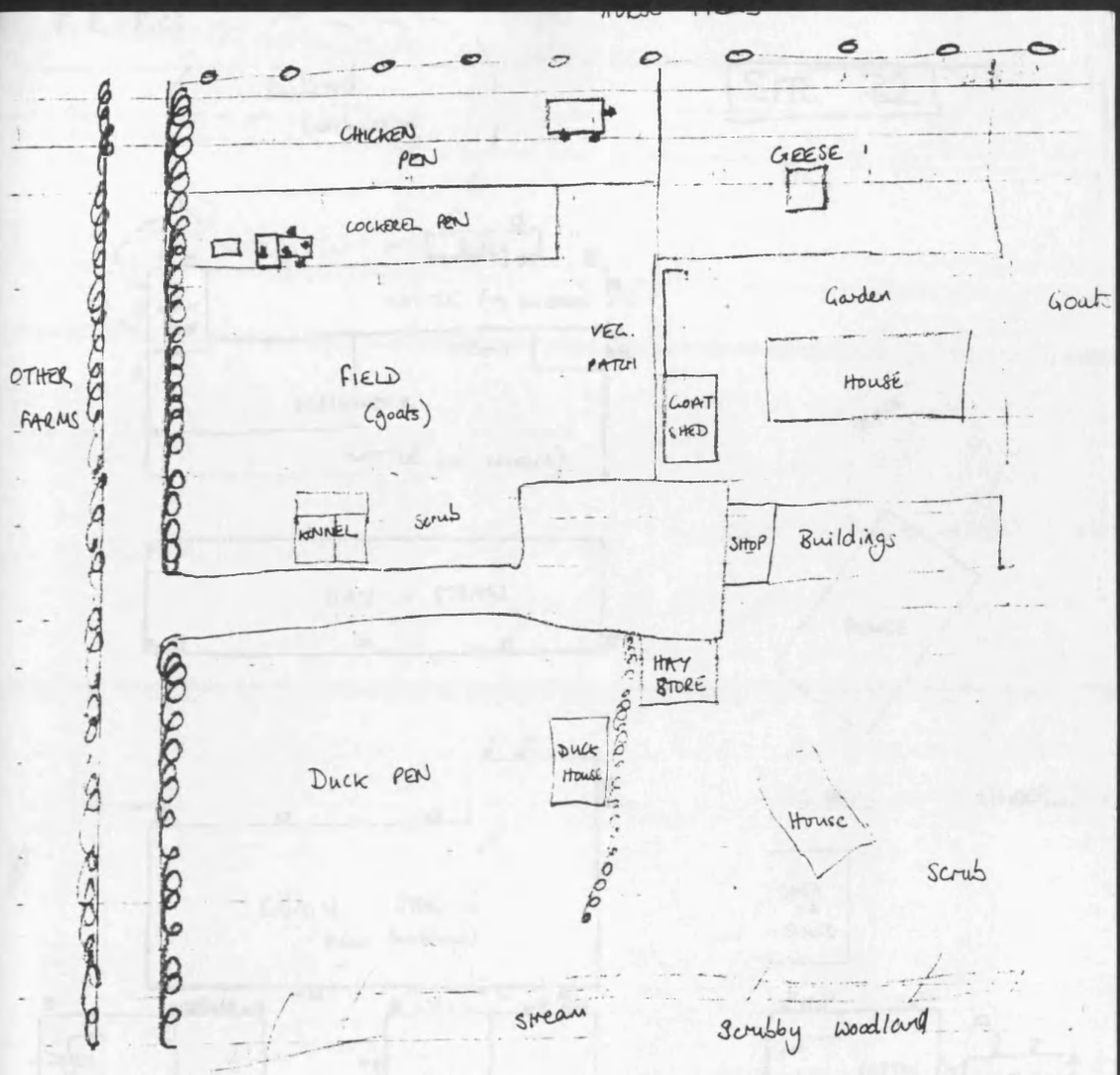

dyke
sows
empty
SLURRY POOL
piglets
piglets
dyke
piglets
empty
SITE A7
dyke
empty shed
field
track
hedge
ploughed field


dyke
dyke
EMPTY SHED
long grass
long grass
EMPTY SHED
PIGLET
scrub
BOAR
SHED
stores and farrowing pens
muck
heap
SOW SHED
silos
track
hedge and dyke

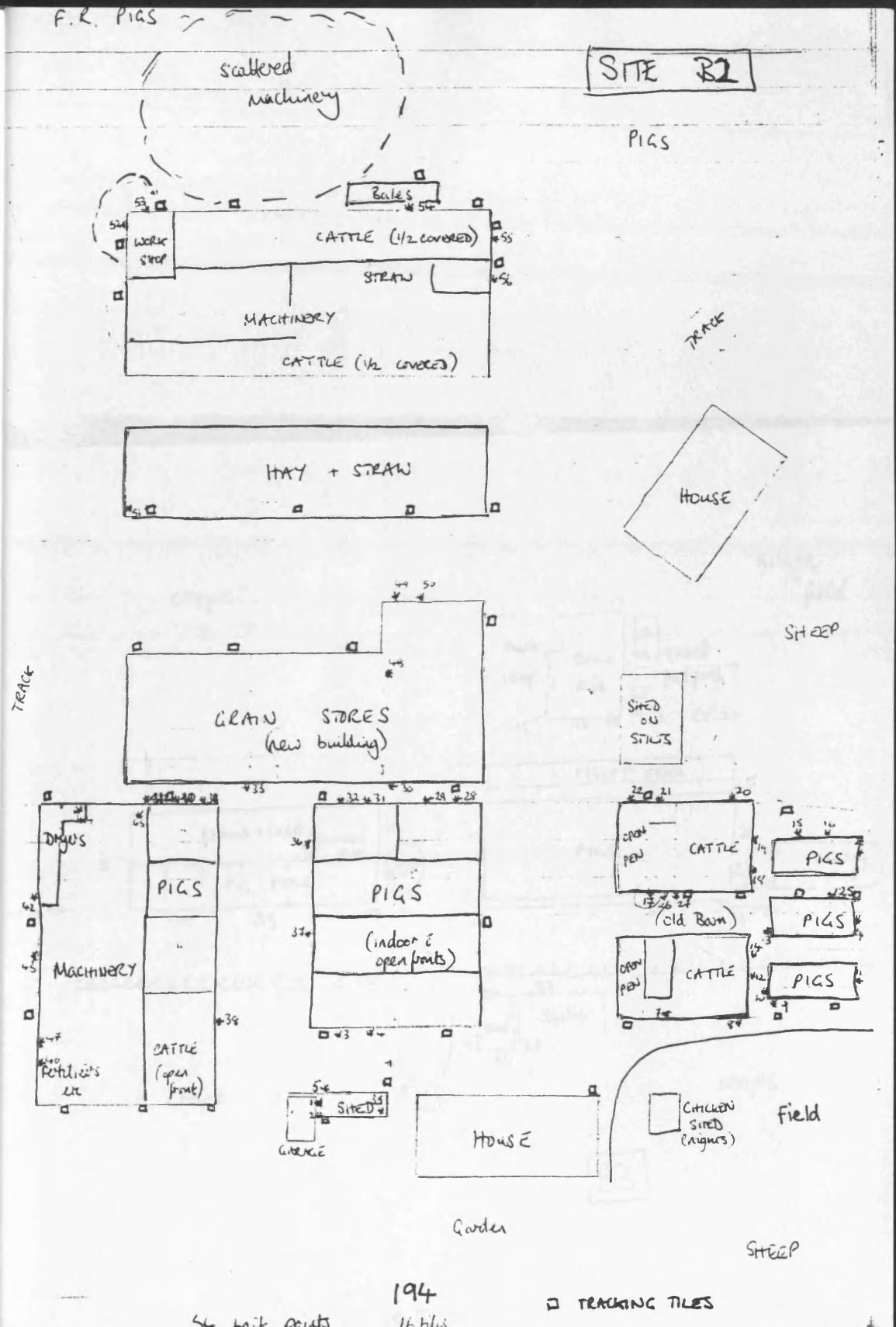
SITE A8


SITE
A9
FIELD
PIGS
PIGS
HOUSE
TREES & SCRUB
FIELD
STORAGE BARN
EX GRAIN STORE
DRYING TUNNEL
PIGS
PIGS
BRICK
SHEDS
TRACK
HEDGE
FIELD
TRACK
FIELD

192


CHICKEN PEN
COCKEREL PEN
GEESE
Garden
Goat
VEG. PATCH
FIELD (goats)
OTHER FARMS
HOUSE
GOAT SHED
KENNEL
Scrub
SHOP
Buildings
HAY STORE
DUCK HOUSE
DUCK PEN
House
Scrub
Stream
Scrubby woodland

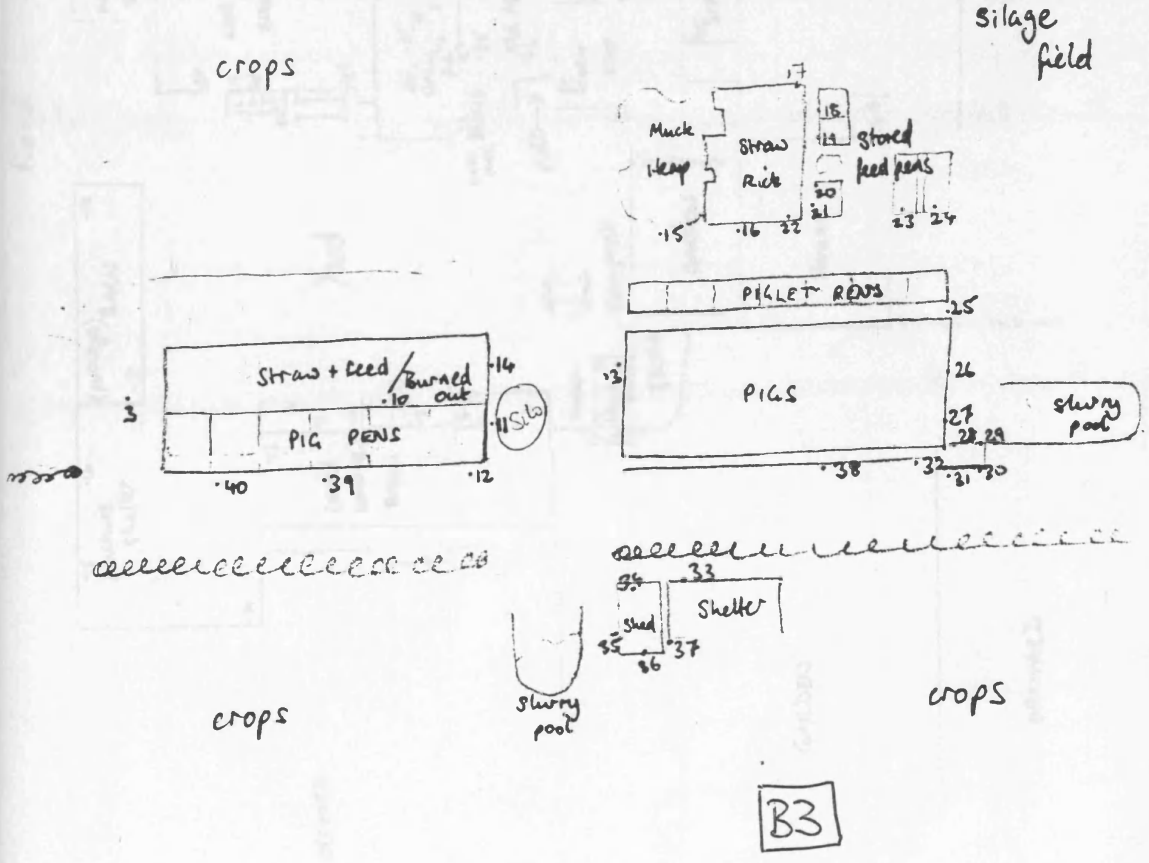
SITE B1



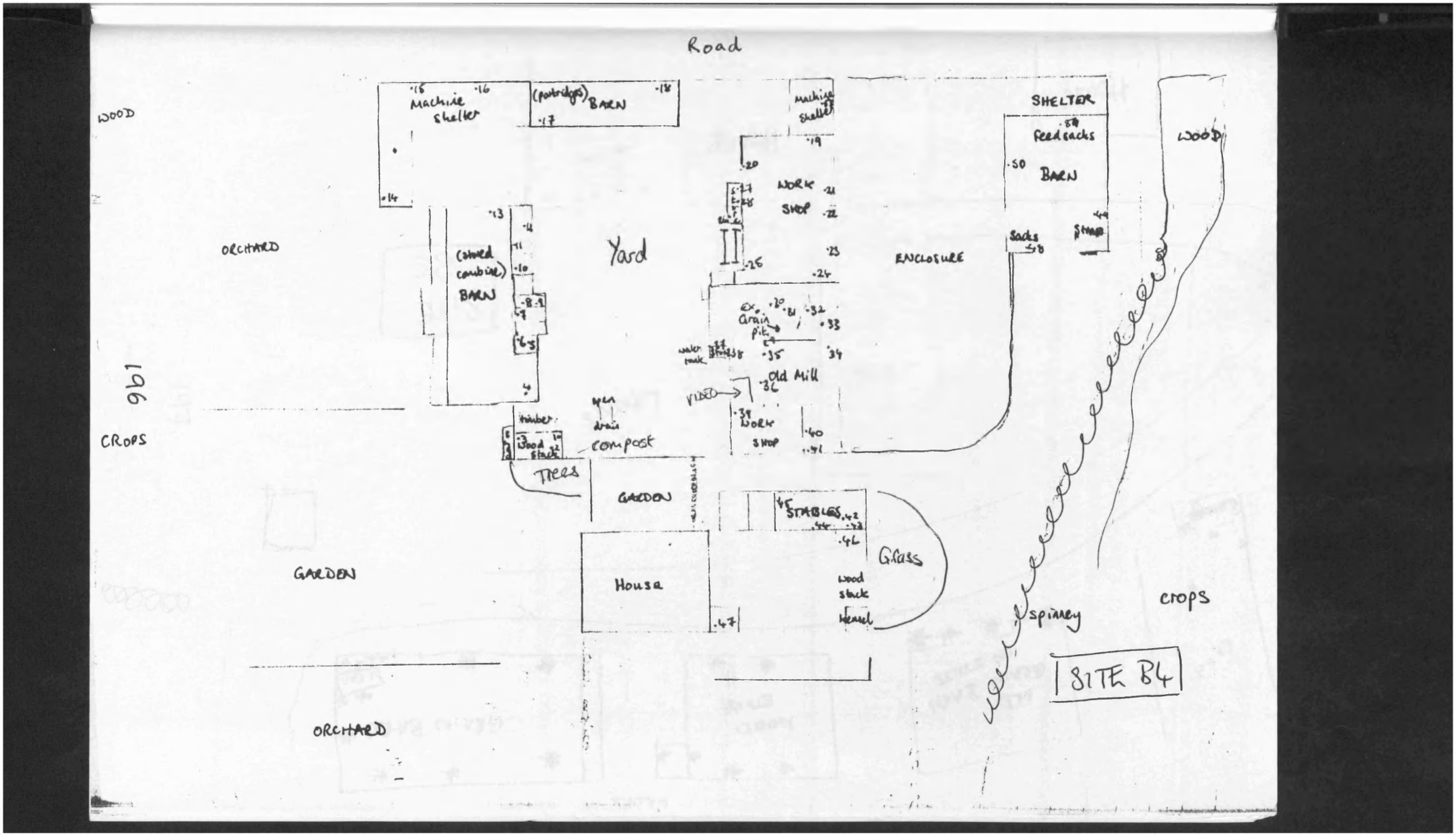

194

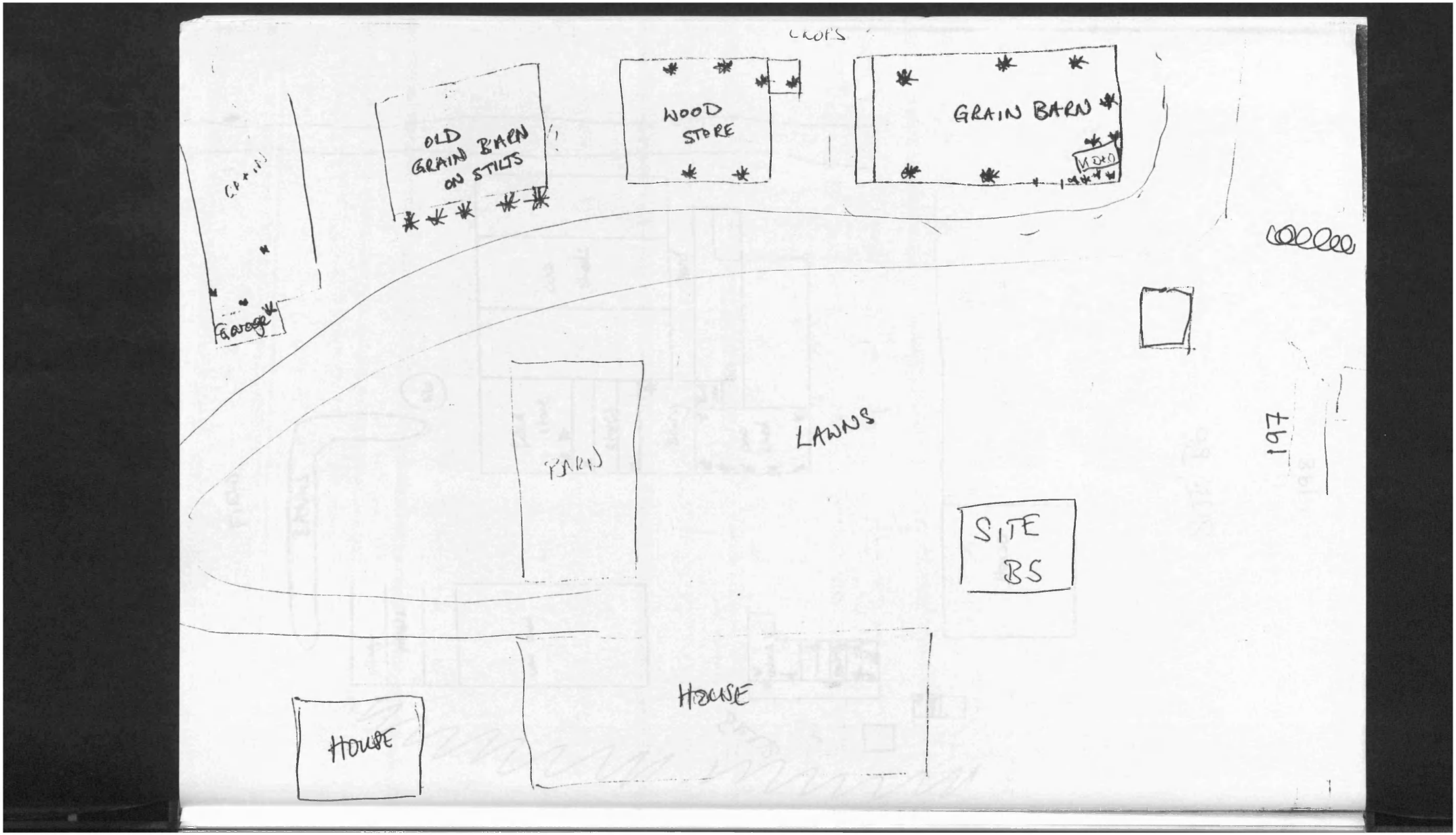
54 bait points 16 kills,

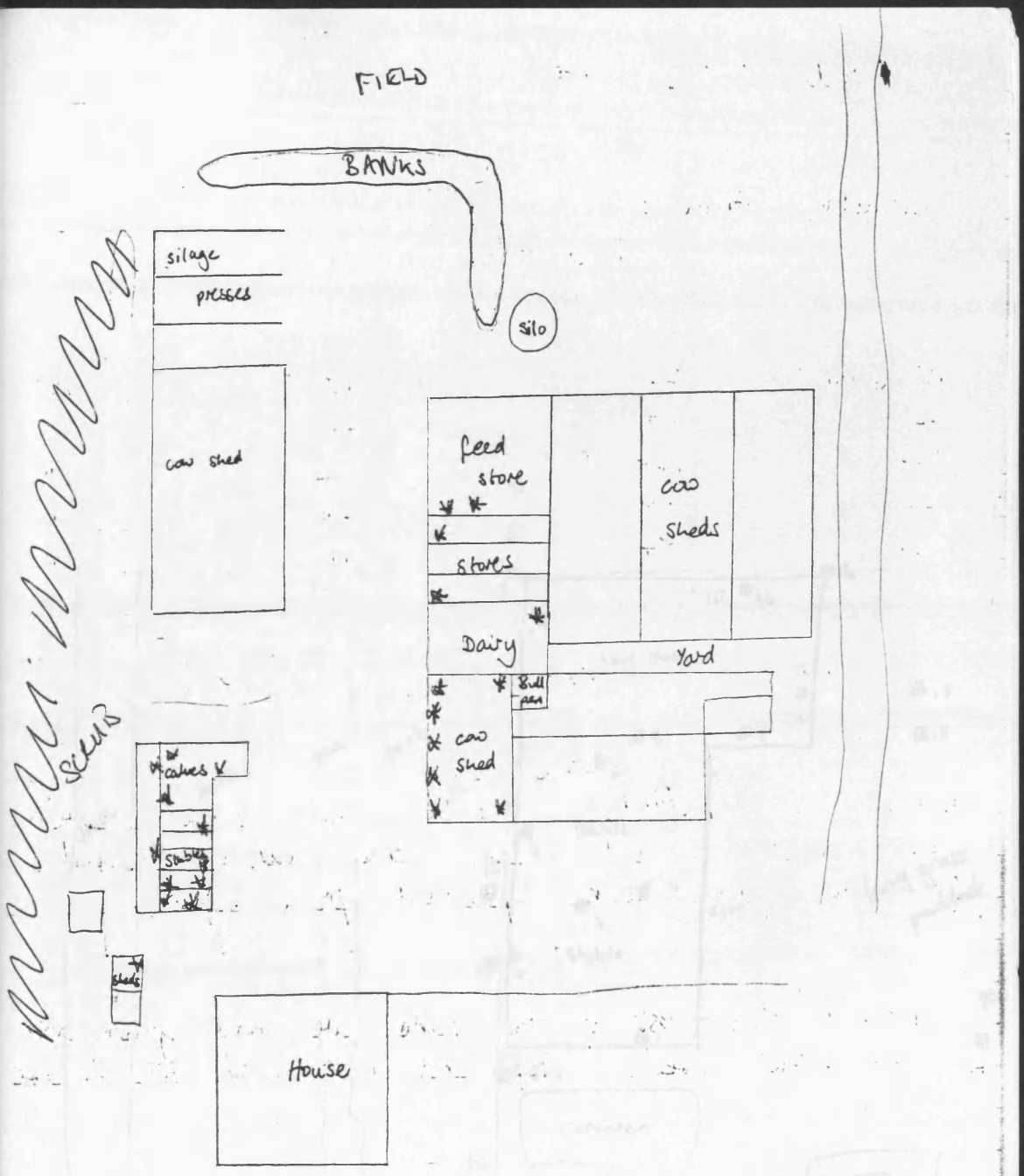
□ TRACKING TILES


crops
silage field
Muck Heap
Straw Rick
Stored feed pens
PIGLET PENS
Straw + feed / burned out
PIG PENS
PIGS
slurry pool
Shed
Shelter
slurry pool
crops
crops

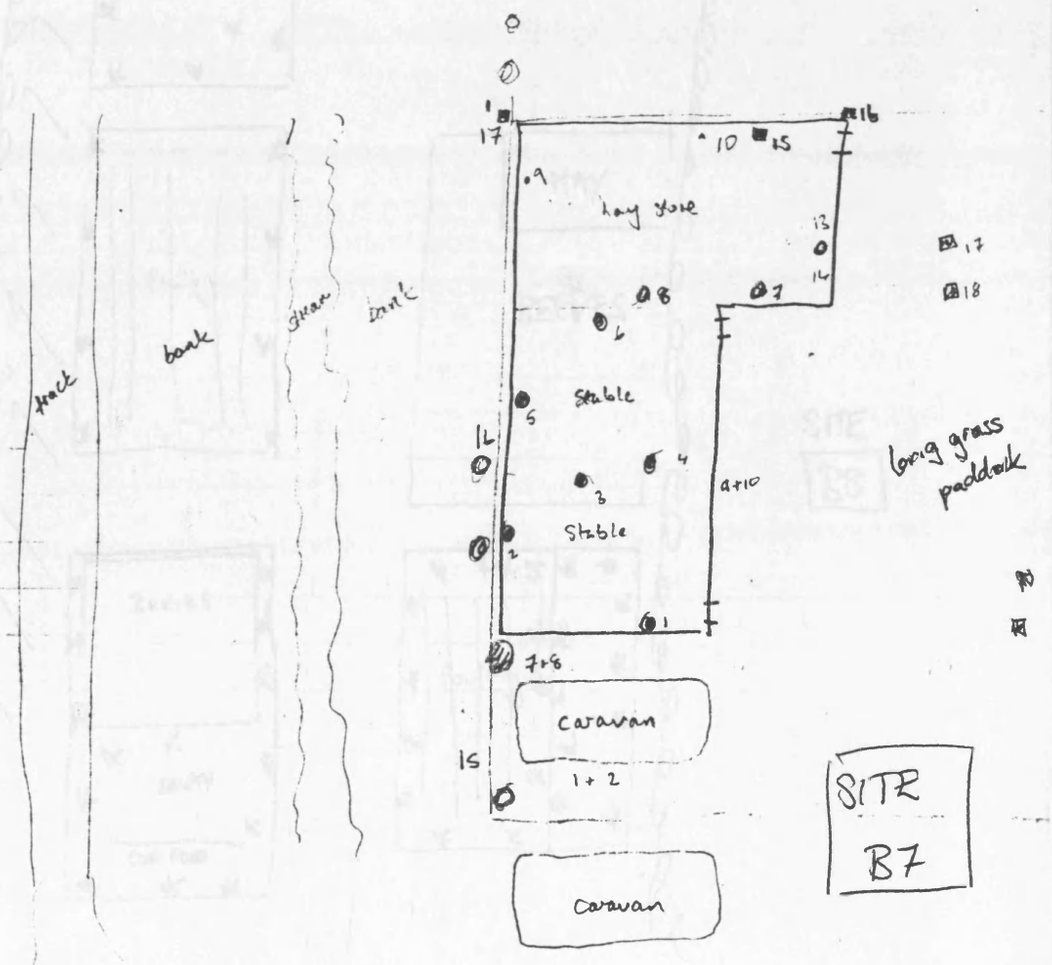
B3

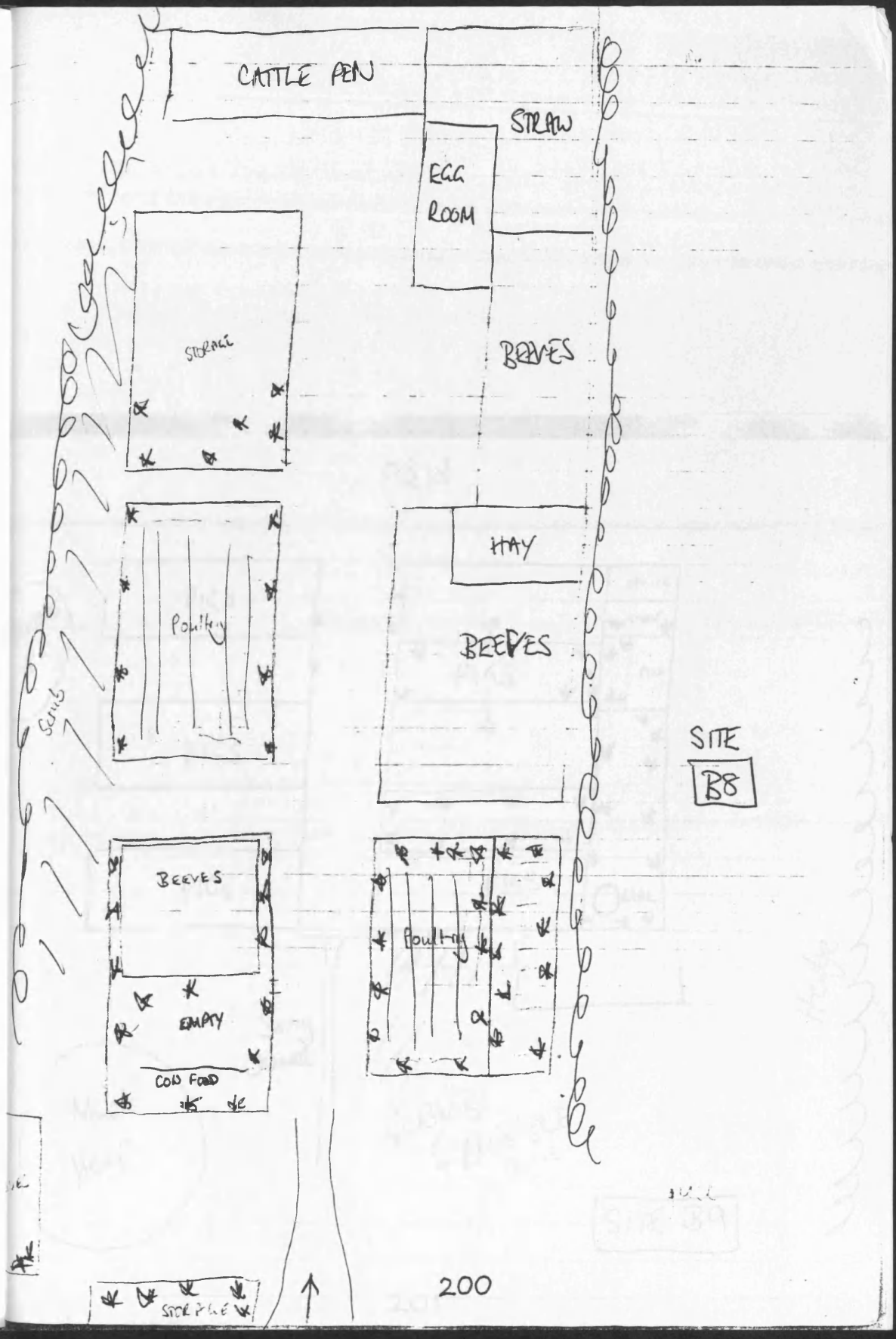

Road
WOOD
Machine shelter
(partridges)
BARN
Machine shelter
SHELTER
Feedsacks
BARN
WOOD
WORK
SHOP
ORCHARD
Yard
(stored combine)
BARN
ENCLOSURE
Sacks
SHOP
Ex. Grain pit
water tank
Old Mill
VIDEO
WORK
SHOP
961
CROPS
timber
wood stacks
open drain
compost
Trees
GARDEN
STABLES
Grass
GARDEN
House
wood stack
Hearth
spinney
crops
ORCHARD
SITE B4


CROPS
OLD GRAIN BARN ON STILTS
WOOD STORE
GRAIN BARN
GRAIN
GARAGE
LAWNS
FARM
SITE BS
HOUSE
HOUSE
191

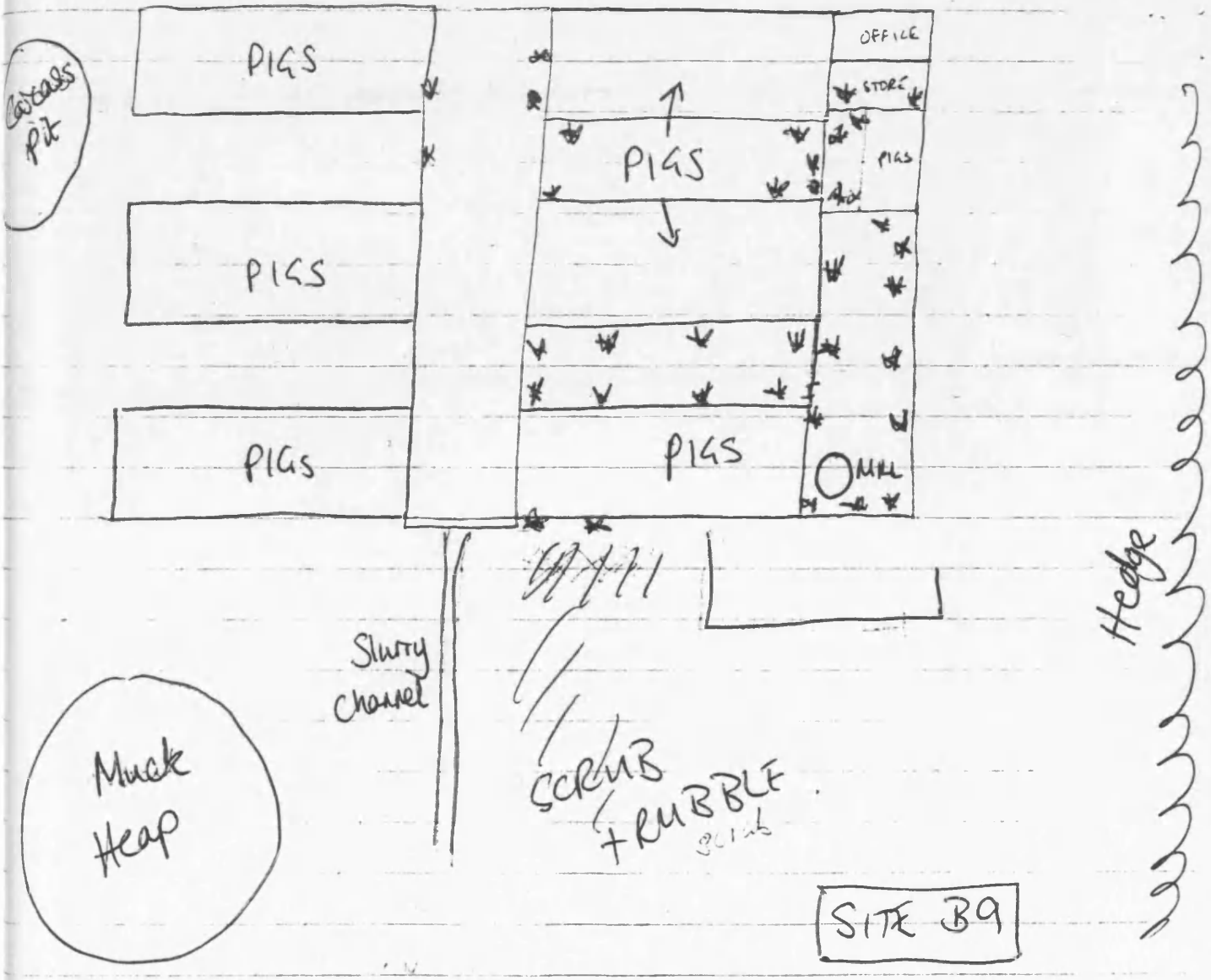

FIELD
BANKS
silage
presses
silo
cow shed
feed store
cow sheds
stores
Dairy
Yard
Bull pen
cow shed
calves
Stable
Sheds
House

SITE B6


track
bank
stream
trail
hay store
Stable
Stable
caravan
caravan
long grass paddock
SITE B7


CATTLE PEN
STRAW
EGG ROOM
BEEVES
STORAGE
HAY
Poultry
BEEVES
SITE
B8
Scrub
BEEVES
EMPTY
COW FOOD
Poultry
STORAGE
200

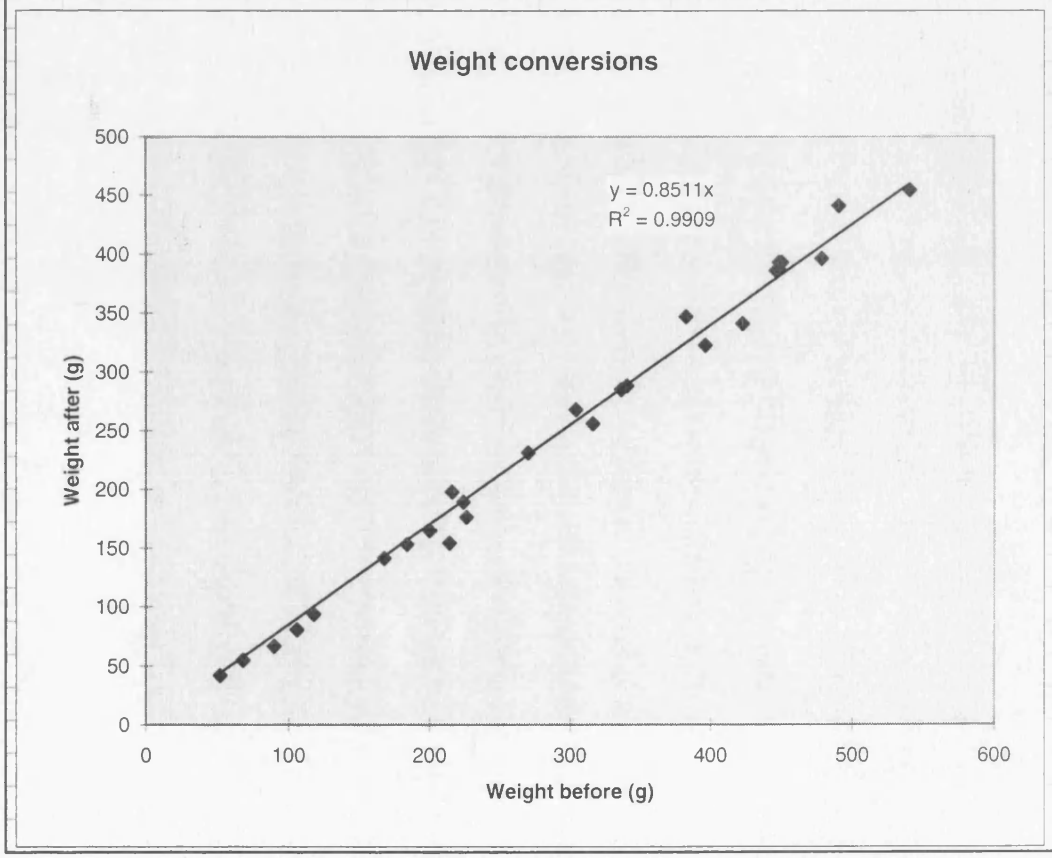
Field


PIGS
PIGS
PIGS
PIGS
PIGS
PIGS
OFFICE
STORE
PIGS
MILL
Carcass pit
Slurry channel
Muck Heap
SCRUB + RUBBLE
Hedge
SITE B9

# Appendix 2 Graph to show conversion from rat body mass before (whole rat) to body mass after the guts, feet and tail had been removed.

The mass after was used for calculating body residues in rats. The data for this graph were taken from carcasses that had values of mass for both a before- and after- gut, tail and feet removal.

| No. | Weight before | Weight after |
|---|---|---|
| 1 | 216 | 198 |
| 2 | 214 | 154 |
| 3 | 68 | 55 |
| 4 | 226 | 176 |
| 5 | 200 | 165 |
| 6 | 52 | 42 |
| 7 | 270 | 231 |
| 8 | 224 | 189 |
| 9 | 316 | 256 |
| 10 | 478 | 396 |
| 11 | 382 | 347 |
| 12 | 446 | 386 |
| 13 | 106 | 81 |
| 14 | 422 | 341 |
| 15 | 540 | 454 |
| 16 | 340 | 288 |
| 17 | 448 | 393 |
| 18 | 168 | 141 |
| 19 | 184 | 153 |
| 20 | 396 | 323 |
| 21 | 118 | 94 |
| 22 | 448 | 387 |
| 23 | 450 | 393 |
| 24 | 336 | 285 |
| 25 | 90 | 67 |
| 26 | 304 | 268 |
| 27 | 490 | 441 |



# Appendix 3 Data from HCBP analyses using Gas Chromatography with Mass Spectrometry (GCMS)

Mean concentration values refer to the mean of values from the two injections made for each sample
HCBP concentration was amount of HCBP in 3g of tissue. Corrected body mass (85% of original body mass) was used to calculate the amount of HCBP in the whole body. This value was used to calculate bait take and mg $Kg^{-1}$ body load for each rat or mouse analysed.

| Site | Mass (g) | Corrected mass (g) | species r/m | region mid/sth | poison /trap | coum /brod | sample | mean [HCBP] | body load (ug) | bait take (g) | mean value | body load (mg) | mg/Kg body wt. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B1 | 256 | 218 | 1 | 2 | 2 | 1 | 8 | 270.63 | 19629.37 | 220.55 | | 19.63 | 90.21 |
| B1 | 202 | 172 | 1 | 2 | 2 | 1 | 9 | 185.67 | 10626.45 | 119.40 | | 10.63 | 61.89 |
| B1 | 262 | 223 | 1 | 2 | 2 | 1 | 13 | 126.14 | 9364.05 | 105.21 | | 9.36 | 42.05 |
| B1 | 358 | 304 | 1 | 2 | 2 | 1 | 39 | 5.30 | 537.28 | 6.04 | | 0.54 | 1.77 |
| B1 | 482 | 410 | 1 | 2 | 2 | 1 | 42 | 1.02 | 139.88 | 1.57 | 90.56 | 0.14 | 0.34 |
| B7 | 8 | 7 | 2 | 2 | 1 | 2 | 154 | 62.79 | 142.33 | 1.45 | | 0.14 | 20.93 |
| B2 | 170 | 145 | 1 | 2 | 2 | 1 | 1 | 0.22 | 10.51 | 0.12 | | 0.01 | 0.07 |
| B2 | 406 | 345 | 1 | 2 | 2 | 1 | 2 | 155.80 | 17922.26 | 201.37 | | 17.92 | 51.93 |
| B2 | 505 | 429 | 1 | 2 | 2 | 1 | 3 | 67.67 | 9682.35 | 108.79 | | 9.68 | 22.56 |
| B2 | 480 | 408 | 1 | 2 | 2 | 1 | 4 | 265.87 | 36158.01 | 406.27 | | 36.16 | 88.62 |
| B2 | 254 | 216 | 1 | 2 | 2 | 1 | 5 | 20.98 | 1510.17 | 16.97 | | 1.51 | 6.99 |
| B2 | 50 | 43 | 1 | 2 | 2 | 1 | 6 | 139.75 | 1979.76 | 22.24 | | 1.98 | 46.58 |
| B2 | 454 | 386 | 1 | 2 | 2 | 1 | 7 | 288.63 | 37127.95 | 417.17 | | 37.13 | 96.21 |
| B2 | 488 | 415 | 1 | 2 | 2 | 1 | 10 | 16.02 | 2215.64 | 24.89 | | 2.22 | 5.34 |
| B2 | 464 | 394 | 1 | 2 | 2 | 1 | 11 | 13.93 | 1831.36 | 20.58 | | 1.83 | 4.64 |
| B2 | 468 | 398 | 1 | 2 | 2 | 1 | 12 | 249.88 | 33134.04 | 372.29 | | 33.13 | 83.29 |
| B2 | 515 | 438 | 1 | 2 | 2 | 1 | 14 | 298.64 | 43576.00 | 489.62 | | 43.58 | 99.55 |
| B2 | 394 | 335 | 1 | 2 | 2 | 1 | 15 | 0.82 | 91.69 | 1.03 | | 0.09 | 0.27 |
| B2 | 446 | 379 | 1 | 2 | 2 | 1 | 28 | 143.83 | 18174.92 | 204.21 | | 18.17 | 47.94 |
| B2 | 505 | 429 | 1 | 2 | 2 | 1 | 29 | 1.83 | 261.64 | 2.94 | | 0.26 | 0.61 |
| B2 | 388 | 330 | 1 | 2 | 1 | 1 | 34 | 9.58 | 1053.20 | 11.83 | | 1.05 | 3.19 |
| B2 | 448 | 381 | 1 | 2 | 1 | 1 | 35 | 0.00 | 0.00 | 0.00 | | 0.00 | 0.00 |
| B2 | 118 | 100 | 1 | 2 | 1 | 1 | 37 | 5.28 | 176.39 | 1.98 | | 0.18 | 1.76 |
| B2 | 70 | 60 | 1 | 2 | 2 | 1 | 38 | 68.52 | 1358.99 | 15.27 | | 1.36 | 22.84 |
| B2 | 520 | 442 | 1 | 2 | 2 | 1 | 40 | 119.57 | 17617.29 | 197.95 | | 17.62 | 39.86 |
| B2 | 370 | 315 | 1 | 2 | 2 | 1 | 44 | 39.84 | 4177.06 | 46.93 | | 4.18 | 13.28 |
| B2 | 430 | 366 | 1 | 2 | 2 | 1 | 46 | 7.50 | 913.59 | 10.27 | | 0.91 | 2.50 |
| B2 | 80 | 68 | 1 | 2 | 1 | 1 | 79 | 0.00 | 0.00 | 0.00 | | 0.00 | 0.00 |
| B2 | 250 | 213 | 1 | 2 | 2 | 1 | 99 | 102.13 | 7234.50 | 81.29 | | 7.23 | 34.04 |
| B2 | 484 | 411 | 1 | 2 | 2 | 1 | 110 | 52.95 | 7260.89 | 81.58 | | 7.26 | 17.65 |
| B2 | 472 | 401 | 1 | 2 | 2 | 1 | 111 | 117.15 | 15666.23 | 176.03 | | 15.67 | 39.05 |
| B2 | 404 | 343 | 1 | 2 | 2 | 1 | 202 | 121.54 | 13911.87 | 156.31 | | 13.91 | 40.51 |
| B2 | 430 | 366 | 1 | 2 | 2 | 1 | 203 | 154.51 | 18824.28 | 211.51 | | 18.82 | 51.50 |
| B2 | 396 | 337 | 1 | 2 | 2 | 1 | 204 | 24.45 | 2743.82 | 30.83 | 118.22 | 2.74 | 8.15 |
| A9 | 140 | 119 | 1 | 1 | 1 | 2 | 104 | 32.93 | 1306.06 | 13.33 | 13.33 | 1.31 | 10.98 |
| A9 | 18 | 15 | 2 | 1 | 1 | 2 | 146 | 4.74 | 24.16 | 0.25 | | 0.02 | 1.58 |
| A9 | 18 | 15 | 2 | 1 | 1 | 2 | 151 | 125.15 | 638.28 | 6.51 | | 0.64 | 41.72 |
| A9 | 18 | 15 | 2 | 1 | 1 | 2 | 152 | 87.79 | 447.75 | 4.57 | | 0.45 | 29.26 |
| A9 | 4 | 3 | 2 | 1 | 1 | 2 | 156 | 140.60 | 159.35 | 1.63 | | 0.16 | 46.87 |
| A4 | 326 | 277 | 1 | 1 | 1 | 2 | 50 | 36.05 | 3330.07 | 33.98 | | 3.33 | 12.02 |
| A4 | 58 | 49 | 1 | 1 | 1 | 2 | 59 | 25.60 | 420.75 | 4.29 | | 0.42 | 8.53 |
| A4 | 112 | 95 | 1 | 1 | 1 | 2 | 66 | 53.60 | 1700.76 | 17.35 | | 1.70 | 17.87 |
| A4 | 366 | 311 | 1 | 1 | 1 | 2 | 74 | 32.35 | 3354.28 | 34.23 | | 3.35 | 10.78 |
| A4 | 366 | 311 | 1 | 1 | 1 | 2 | 77 | 30.28 | 3139.71 | 32.04 | | 3.14 | 10.09 |
| A4 | 316 | 269 | 1 | 1 | 1 | 2 | 86 | 63.95 | 5726.10 | 58.43 | | 5.73 | 21.32 |
| A4 | 495 | 421 | 1 | 1 | 1 | 2 | 91 | 44.50 | 6241.53 | 63.69 | | 6.24 | 14.83 |
| A4 | 384 | 326 | 1 | 1 | 1 | 2 | 92 | 14.52 | 1579.96 | 16.12 | | 1.58 | 4.84 |
| A4 | 422 | 359 | 1 | 1 | 1 | 2 | 93 | 12.91 | 1544.09 | 15.76 | | 1.54 | 4.30 |
| A4 | 438 | 372 | 1 | 1 | 1 | 2 | 94 | 33.63 | 4173.96 | 42.59 | | 4.17 | 11.21 |
| A4 | 64 | 54 | 1 | 1 | 1 | 2 | 102 | 64.61 | 1171.60 | 11.96 | | 1.17 | 21.54 |
| A4 | 122 | 104 | 1 | 1 | 1 | 2 | 105 | 116.31 | 4020.30 | 41.02 | | 4.02 | 38.77 |
| A4 | 17 | 14 | 1 | 1 | 1 | 2 | 131 | 110.95 | 534.42 | 5.45 | | 0.53 | 36.98 |
| A4 | 100 | 85 | 1 | 1 | 1 | 2 | 205 | 47.35 | 1341.66 | 13.69 | | 1.34 | 15.78 |
| A4 | 300 | 255 | 1 | 1 | 1 | 2 | 206 | 32.70 | 2779.19 | 28.36 | | 2.78 | 10.90 |
| A4 | 324 | 275 | 1 | 1 | 1 | 2 | 208 | 30.72 | 2819.96 | 28.78 | | 2.82 | 10.24 |
| A4 | 138 | 117 | 1 | 1 | 1 | 2 | 209 | 9.81 | 383.40 | 3.91 | | 0.38 | 3.27 |
| A4 | 312 | 265 | 1 | 1 | 1 | 2 | 210 | 32.81 | 2900.56 | 29.60 | 26.74 | 2.90 | 10.94 |
| B9 | 26 | 22 | 2 | 2 | 2 | 1 | 136 | 0.00 | 0.00 | 0.00 | | 0.00 | 0.00 |
| B9 | 18 | 15 | 2 | 2 | 2 | 1 | 137 | 2.69 | 13.72 | 0.15 | | 0.01 | 0.90 |
| B9 | 20 | 17 | 2 | 2 | 2 | 1 | 138 | 190.89 | 1081.72 | 12.15 | | 1.08 | 63.63 |
| B9 | 18 | 15 | 2 | 2 | 2 | 1 | 139 | 50.14 | 255.73 | 2.87 | | 0.26 | 16.71 |
| B9 | 23 | 20 | 2 | 2 | 2 | 1 | 140 | 0.00 | 0.00 | 0.00 | | 0.00 | 0.00 |
| B9 | 9 | 8 | 2 | 2 | 2 | 1 | 141 | 72.99 | 186.12 | 2.09 | | 0.19 | 24.33 |
| B9 | 18 | 15 | 2 | 2 | 2 | 1 | 142 | 0.00 | 0.00 | 0.00 | | 0.00 | 0.00 |
| B9 | 13 | 11 | 2 | 2 | 2 | 1 | 143 | 0.00 | 0.00 | 0.00 | | 0.00 | 0.00 |
| B9 | 19 | 16 | 2 | 2 | 2 | 1 | 144 | 366.05 | 1970.59 | 22.14 | | 1.97 | 122.02 |
| B9 | 17 | 14 | 2 | 2 | 2 | 1 | 148 | 78.36 | 377.46 | 4.24 | | 0.38 | 26.12 |
| B9 | 16 | 14 | 2 | 2 | 1 | 1 | 153 | 0.00 | 0.00 | 0.00 | | 0.00 | 0.00 |
| B9 | 16 | 14 | 2 | 2 | 1 | 1 | 155 | 88.12 | 399.48 | 4.49 | | 0.40 | 29.37 |
| B9 | 18 | 15 | 2 | 2 | 2 | 1 | 200 | 224.12 | 1143.03 | 12.84 | | 1.14 | 74.71 |
| B6 | 292 | 248 | 1 | 2 | 1 | 2 | 41 | 44.84 | 3709.73 | 37.85 | | 3.71 | 14.95 |
| B6 | 80 | 68 | 1 | 2 | 1 | 2 | 75 | 30.80 | 698.15 | 7.12 | | 0.70 | 10.27 |
| B6 | 108 | 92 | 1 | 2 | 1 | 2 | 80 | 47.09 | 1441.00 | 14.70 | | 1.44 | 15.70 |
| B6 | 304 | 258 | 1 | 2 | 1 | 2 | 125 | 46.76 | 4027.95 | 41.10 | 25.20 | 4.03 | 15.59 |
| A1 | 164 | 139 | 1 | 1 | 1 | 1 | 48 | 0.24 | 11.04 | 0.11 | | 0.01 | 0.08 |
| A1 | 635 | 540 | 1 | 1 | 1 | 1 | 51 | 0.00 | 0.00 | 0.00 | | 0.00 | 0.00 |
| A1 | 80 | 68 | 1 | 1 | 1 | 1 | 53 | 0.59 | 13.38 | 0.14 | | 0.01 | 0.20 |
| A1 | 155 | 132 | 1 | 1 | 1 | 1 | 54 | 0.00 | 0.00 | 0.00 | | 0.00 | 0.00 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A1 | 284 | 241 | 1 | 1 | 1 | 1 | 56 | 0.32 | 25.61 | 0.26 | | 0.03 | 0.11 |
| A1 | 336 | 286 | 1 | 1 | 1 | 1 | 57 | 0.32 | 30.06 | 0.31 | | 0.03 | 0.11 |
| A1 | 298 | 253 | 1 | 1 | 1 | 1 | 61 | 0.32 | 27.07 | 0.28 | | 0.03 | 0.11 |
| A1 | 216 | 184 | 1 | 1 | 1 | 1 | 70 | 0.59 | 36.19 | 0.37 | | 0.04 | 0.20 |
| A1 | 486 | 413 | 1 | 1 | 1 | 1 | 87 | 0.00 | 0.00 | 0.00 | | 0.00 | 0.00 |
| A1 | 96 | 82 | 1 | 1 | 1 | 1 | 97 | 0.81 | 21.93 | 0.22 | | 0.02 | 0.27 |
| A1 | 248 | 211 | 1 | 1 | 1 | 1 | 120 | 0.28 | 19.84 | 0.20 | | 0.02 | 0.09 |
| A1 | 414 | 352 | 1 | 1 | 1 | 1 | 133 | 0.35 | 41.04 | 0.42 | 0.19 | 0.04 | 0.12 |
| A1 | 12 | 10 | 2 | 1 | 1 | 1 | 147 | 0.00 | 0.00 | 0.00 | | 0.00 | 0.00 |
| | | | | | | | | | | | | | |
| B8 | 146 | 124 | 1 | 2 | 1 | 2 | 112 | 0.71 | 29.35 | 0.30 | | 0.03 | 0.24 |
| B8 | 308 | 262 | 1 | 2 | 1 | 2 | 129 | 0.30 | 26.37 | 0.27 | | 0.03 | 0.10 |
| B8 | 19 | 16 | 2 | 2 | 2 | 2 | 157 | 0.00 | 0.00 | 0.00 | | 0.00 | 0.00 |
| B8 | 218 | 185 | 1 | 2 | 2 | 2 | 207 | 1.14 | 70.28 | 0.72 | 0.43 | 0.07 | 0.38 |
| | | | | | | | | | | | | | |
| A7 | 248 | 211 | 1 | 1 | 2 | 1 | 43 | 149.19 | 10482.89 | 106.97 | | 10.48 | 49.73 |
| A7 | 240 | 204 | 1 | 1 | 2 | 1 | 64 | 228.37 | 15528.89 | 158.46 | | 15.53 | 76.12 |
| A7 | 336 | 286 | 1 | 1 | 2 | 1 | 67 | 28.60 | 2722.26 | 27.78 | | 2.72 | 9.53 |
| A7 | 274 | 233 | 1 | 1 | 1 | 1 | 69 | 17.37 | 1348.82 | 13.76 | | 1.35 | 5.79 |
| A7 | 118 | 100 | 1 | 1 | 2 | 1 | 72 | 32.27 | 1079.00 | 11.01 | | 1.08 | 10.76 |
| A7 | 182 | 155 | 1 | 1 | 2 | 1 | 76 | 45.71 | 2357.23 | 24.05 | | 2.36 | 15.24 |
| A7 | 476 | 405 | 1 | 1 | 1 | 1 | 88 | 13.73 | 1851.65 | 18.89 | | 1.85 | 4.58 |
| A7 | 84 | 71 | 1 | 1 | 2 | 1 | 103 | 42.30 | 1006.81 | 10.27 | | 1.01 | 14.10 |
| A7 | 126 | 107 | 1 | 1 | 2 | 1 | 122 | 25.60 | 913.82 | 9.32 | | 0.91 | 8.53 |
| A7 | 318 | 270 | 1 | 1 | 1 | 1 | 130 | 10.27 | 925.48 | 9.44 | | 0.93 | 3.42 |
| A7 | 210 | 179 | 1 | 1 | 2 | 1 | 134 | 51.64 | 3072.41 | 31.35 | 38.30 | 3.07 | 17.21 |
| A7 | 16 | 14 | 2 | 1 | 2 | 1 | 145 | 70.75 | 320.75 | 3.27 | | 0.32 | 23.58 |
| A7 | 16 | 14 | 2 | 1 | 1 | 1 | 149 | 0.00 | 0.00 | 0.00 | | 0.00 | 0.00 |
| A7 | 18 | 15 | 2 | 1 | 1 | 1 | 158 | 0.00 | 0.00 | 0.00 | | 0.00 | 0.00 |
| | | | | | | | | | | | | | |
| A8 | 268 | 228 | 1 | 1 | 2 | 1 | 49 | 8.67 | 658.30 | 6.72 | | 0.66 | 2.89 |
| A8 | 326 | 277 | 1 | 1 | 1 | 1 | 58 | 17.84 | 1647.47 | 16.81 | | 1.65 | 5.95 |
| A8 | 484 | 411 | 1 | 1 | 1 | 1 | 60 | 44.95 | 6163.89 | 62.90 | | 6.16 | 14.98 |
| A8 | 368 | 313 | 1 | 1 | 1 | 1 | 63 | 13.65 | 1422.82 | 14.52 | | 1.42 | 4.55 |
| A8 | 308 | 262 | 1 | 1 | 1 | 1 | 65 | 89.63 | 7827.39 | 79.87 | | 7.83 | 29.90 |
| A8 | 130 | 111 | 1 | 1 | 1 | 1 | 68 | 271.62 | 10049.82 | 102.55 | | 10.05 | 90.95 |
| A8 | 268 | 228 | 1 | 1 | 1 | 1 | 73 | 57.52 | 4371.28 | 44.60 | | 4.37 | 19.19 |
| A8 | 80 | 68 | 1 | 1 | 2 | 1 | 82 | 306.75 | 6953.09 | 70.95 | | 6.95 | 102.25 |
| A8 | 458 | 389 | 1 | 1 | 1 | 1 | 90 | 62.84 | 8154.96 | 83.21 | | 8.15 | 20.95 |
| A8 | 202 | 172 | 1 | 1 | 1 | 1 | 106 | 87.48 | 5006.94 | 51.09 | | 5.01 | 29.16 |
| A8 | 300 | 255 | 1 | 1 | 1 | 1 | 113 | 106.05 | 9014.10 | 91.98 | | 9.01 | 35.35 |
| A8 | 280 | 238 | 1 | 1 | 1 | 1 | 117 | 95.18 | 7551.25 | 77.05 | | 7.55 | 31.73 |
| A8 | 120 | 102 | 1 | 1 | 2 | 1 | 118 | 130.11 | 4423.87 | 45.14 | | 4.42 | 43.37 |
| A8 | 280 | 238 | 1 | 1 | 1 | 1 | 121 | 44.89 | 3560.93 | 36.34 | | 3.56 | 14.96 |
| A8 | 280 | 238 | 1 | 1 | 1 | 1 | 123 | 140.60 | 11154.41 | 113.82 | | 11.15 | 46.87 |
| A8 | 422 | 359 | 1 | 1 | 1 | 1 | 128 | 27.42 | 3278.70 | 33.46 | 58.19 | 3.28 | 9.14 |
| A8 | 16 | 14 | 2 | 1 | 1 | 1 | 150 | 12.56 | 56.96 | 0.58 | | 0.06 | 4.19 |
| | | | | | | | | | | | | | |
| A5 | 422 | 359 | 1 | 1 | 1 | 1 | 62 | 118.26 | 14139.53 | 144.28 | | 14.14 | 39.42 |
| A5 | 254 | 216 | 1 | 1 | 1 | 1 | 71 | 26.14 | 1881.24 | 19.20 | | 1.88 | 8.71 |
| A5 | 378 | 321 | 1 | 1 | 1 | 1 | 78 | 12.89 | 1380.21 | 14.08 | 59.19 | 1.38 | 4.30 |
| | | | | | | | | | | | | | |
| A6 | 256 | 218 | 1 | 1 | 1 | 1 | 47 | 52.19 | 3785.25 | 38.62 | | 3.79 | 17.40 |
| A6 | 172 | 146 | 1 | 1 | 1 | 1 | 52 | 39.30 | 1915.08 | 19.54 | | 1.92 | 13.10 |
| A6 | 138 | 117 | 1 | 1 | 1 | 1 | 55 | 53.46 | 2084.81 | 21.27 | 26.48 | 2.08 | 17.77 |
| | | | | | | | | | | | | | |
| B3 | 46 | 39 | 1 | 2 | 2 | 1 | 16 | 99.40 | 1295.50 | 14.56 | | 1.30 | 33.13 |
| B3 | 78 | 66 | 1 | 2 | 2 | 1 | 17 | 202.40 | 4472.97 | 50.26 | | 4.47 | 67.47 |
| B3 | 350 | 298 | 1 | 2 | 2 | 1 | 18 | 0.38 | 38.02 | 0.43 | | 0.04 | 0.13 |
| B3 | 194 | 165 | 1 | 2 | 2 | 1 | 19 | 153.36 | 8429.82 | 94.72 | | 8.43 | 51.12 |
| B3 | 120 | 102 | 1 | 2 | 2 | 1 | 21 | 46.49 | 1580.53 | 17.76 | | 1.58 | 15.50 |
| B3 | 510 | 434 | 1 | 2 | 2 | 1 | 23 | 1.78 | 256.63 | 2.88 | | 0.26 | 0.59 |
| B3 | 196 | 167 | 1 | 2 | 2 | 1 | 24 | 320.01 | 17771.08 | 199.68 | | 17.77 | 106.67 |
| B3 | 118 | 100 | 1 | 2 | 2 | 1 | 25 | 103.94 | 3475.02 | 39.05 | | 3.48 | 34.65 |
| B3 | 464 | 394 | 1 | 2 | 2 | 1 | 26 | 0.40 | 52.32 | 0.59 | | 0.05 | 0.13 |
| B3 | 515 | 438 | 1 | 2 | 2 | 1 | 27 | 14.50 | 2115.41 | 23.77 | | 2.12 | 4.83 |
| B3 | 94 | 80 | 1 | 2 | 2 | 1 | 30 | 134.08 | 3571.00 | 40.12 | | 3.57 | 44.69 |
| B3 | 74 | 63 | 1 | 2 | 2 | 1 | 31 | 180.37 | 3781.66 | 42.49 | | 3.78 | 60.12 |
| B3 | 545 | 463 | 1 | 2 | 2 | 1 | 32 | 5.55 | 857.46 | 9.63 | | 0.86 | 1.85 |
| B3 | 555 | 472 | 1 | 2 | 2 | 1 | 33 | 2.56 | 403.25 | 4.53 | | 0.40 | 0.85 |
| B3 | 134 | 114 | 1 | 2 | 2 | 1 | 81 | 8.15 | 309.33 | 3.48 | | 0.31 | 2.72 |
| B3 | 38 | 32 | 1 | 2 | 2 | 1 | 84 | 82.29 | 886.04 | 9.96 | | 0.89 | 27.43 |
| B3 | 34 | 29 | 1 | 2 | 2 | 1 | 95 | 68.72 | 662.01 | 7.44 | | 0.66 | 22.91 |
| B3 | 342 | 291 | 1 | 2 | 2 | 1 | 100 | 16.87 | 1634.71 | 18.37 | | 1.63 | 5.62 |
| B3 | 530 | 451 | 1 | 2 | 2 | 1 | 101 | 2.70 | 405.89 | 4.56 | | 0.41 | 0.90 |
| B3 | 166 | 141 | 1 | 2 | 2 | 1 | 107 | 184.53 | 8679.29 | 97.52 | | 8.68 | 61.51 |
| B3 | 58 | 49 | 1 | 2 | 1 | 1 | 108 | 0.37 | 6.09 | 0.07 | | 0.01 | 0.12 |
| B3 | 505 | 429 | 1 | 2 | 2 | 1 | 109 | 3.31 | 472.90 | 5.31 | 31.23 | 0.47 | 1.10 |
| | | | | | | | | | | | | | |
| B4 | 44 | 37 | 1 | 2 | 1 | 2 | 20 | 266.70 | 3324.85 | 33.93 | | 3.32 | 88.90 |
| B4 | 336 | 286 | 1 | 2 | 1 | 2 | 22 | 10.80 | 1027.89 | 10.49 | | 1.03 | 3.60 |
| B4 | 264 | 224 | 1 | 2 | 1 | 2 | 45 | 174.48 | 13051.25 | 133.18 | | 13.05 | 58.16 |
| B4 | 32 | 27 | 1 | 2 | 1 | 2 | 83 | 27.22 | 246.77 | 2.52 | | 0.25 | 9.07 |
| B4 | 32 | 27 | 1 | 2 | 1 | 2 | 85 | 144.69 | 1311.82 | 13.39 | | 1.31 | 48.23 |
| B4 | 82 | 70 | 1 | 2 | 1 | 2 | 89 | 66.90 | 1554.34 | 15.86 | | 1.55 | 22.30 |
| B4 | 20 | 17 | 1 | 2 | 1 | 2 | 96 | 122.27 | 692.87 | 7.07 | | 0.69 | 40.76 |
| B4 | 152 | 129 | 1 | 2 | 1 | 2 | 98 | 44.98 | 1937.34 | 19.77 | | 1.94 | 14.99 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B4 | 288 | 245 | 1 | 2 | 1 | 2 | 114 | 60.25 | 4916.60 | 50.17 | | 4.92 | 20.08 |
| B4 | 258 | 219 | 1 | 2 | 1 | 2 | 115 | 58.27 | 4259.56 | 43.46 | | 4.26 | 19.42 |
| B4 | 50 | 43 | 1 | 2 | 1 | 2 | 116 | 69.07 | 978.52 | 9.98 | | 0.98 | 23.02 |
| B4 | 286 | 243 | 1 | 2 | 1 | 2 | 119 | 28.10 | 2277.09 | 23.24 | | 2.28 | 9.37 |
| B4 | 322 | 274 | 1 | 2 | 1 | 2 | 124 | 46.54 | 4245.55 | 43.32 | | 4.25 | 15.51 |
| B4 | 386 | 328 | 1 | 2 | 1 | 2 | 126 | 39.98 | 4372.44 | 44.62 | | 4.37 | 13.33 |
| B4 | 32 | 27 | 1 | 2 | 1 | 2 | 127 | 137.84 | 1249.73 | 12.75 | | 1.25 | 45.95 |
| B4 | 294 | 250 | 1 | 2 | 1 | 2 | 132 | 59.81 | 4982.26 | 50.84 | | 4.98 | 19.94 |
| B4 | 32 | 27 | 1 | 2 | 1 | 2 | 135 | 88.78 | 804.90 | 8.21 | 30.75 | 0.80 | 29.59 |

# Appendix 4 Data taken from the HPLC with fluorescence detection used to measure coumatetralyl residues in 0.75g rat tissue

All the significant peaks on the trace were measured and tallied to give a total value for each sample. This total value was used to calculate the residue of coumatetralyl in the whole body. The pure solution of coumatetralyl was used as an external standard against which the samples were calibrated.

| Sample | Peak area | 2nd pk area | 3rd | 4th | total | no. sig. peaks | amt (ug)/.75g | corrected body mass | tot. body load (ug) | tot. body load (mg) | mg/Kg | site |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| dil coumpure | 63574688 | | | | 63574688 | 1 | | | | | | |
| dil*20 coumpure | 55969504 | | | | 55969504 | 1 | 2.5 | | | | | |
| spiked blank | 41652977 | 32706540 | | | 74359517 | 2 | 3.321430051 | | | | | |
| 203 | 42503973 | 47142210 | 866768 | 1418580 | 91931531 | 4 | 4.106322391 | 0.366 | 20038.85 | 20.04 | 54.75 | B2 |
| 107 | 19542744 | 21747264 | 1456113 | 478581 | 43224702 | 4 | 1.930725614 | 0.141 | 3629.76 | 3.63 | 25.74 | B3 |
| 65 | 671742 | 1524486 | 1123527 | 951372 | 4271127 | 4 | 0.190779205 | 0.262 | 666.46 | 0.67 | 2.54 | A8 |
| coumpure | 82472566 | 13016986 | 12349624 | 9740239 | 117579415 | 4 | 5.251941084 | | | | | |
| 63 | 2627010 | 3768060 | 408967 | 4759210 | 11563247 | 4 | 0.516497654 | 0.313 | 2155.52 | 2.16 | 6.89 | A8 |
| 58 | 10800980 | | | | 10800980 | 1 | 0.482449335 | 0.277 | 1781.85 | 1.78 | 6.43 | A8 |
| 55 | 1904021 | 10958422 | 1998614 | 693108 | 15554165 | 4 | 0.694760713 | 0.117 | 1083.83 | 1.08 | 9.26 | A6 |
| 47 | 2144426 | 6025061 | 6514335 | | 14683822 | 3 | 0.655884944 | 0.218 | 1906.44 | 1.91 | 8.75 | A6 |
| meoh | | | | | | | | | | | | |
| 28 | 86988734 | 9952636 | | | 96941370 | 2 | 4.33009778 | 0.379 | 21881.43 | 21.88 | 57.73 | B2 |
| 12 | 4752134 | 7854952 | | | 12607086 | 2 | 0.563123 | 0.398 | 2988.31 | 2.99 | 7.51 | B2 |
| 24 | 2251071 | 10000083 | | | 12251154 | 2 | 0.547224521 | 0.167 | 1218.49 | 1.22 | 7.30 | B3 |
| dil spike (25ul) | 1267315 | 14127878 | 15153026 | 42787729 | 73335948 | 4 | 3.275710108 | | | | | |

# BIBLIOGRAPHY


Adams, N. & Boice, R. (1983). A longitudinal study of dominance in an outdoor colony of domestic rats. Journal of Comparative Psychology **97**(**1**):24-33.

Anon. (1991). Tawny owls find new home. Reading Evening Post 21st May 1991.

Arnold, H. R. (1993). 'Atlas of Mammals in Britain'. ITE Research Publication No. 6. HMSO, London.

Ashton, A. D., Jackson, W. B. and Peters, H. (1987). Comparative evaluation of $LD_{50}$ values for various anticoagulant rodenticides. In 'Control of Mammal Pests' (Eds. C. G. J. Richards and T. Y. Ku), pp 187-198. Taylor and Francis, London.

Bacon, S. J. and McClintock, M. K. (1994). Multiple factors determine the sex-ratio of postpartum-conceived Norway rat litters. Physiology and Behaviour **56**(**2**):359-366.

Badi, M. J., Iliadis, N. and Sarris, K. (1992). The rat, *Rattus norvegicus*, as a carrier and source of infection for *Salmonella gallinarum*. Berliner und Münchener Tierärztliche Wochenschrift **105**(**8**):264-267.

Balcomb, R. (1986). Songbird carcasses disappear rapidly from agricultural fields. The Auk **103**(**4**):817-820.

Begon, M. (1979). 'Investigating Animal Abundance: Capture-Recapture for Biologists.' Edward Arnold, London.

Begon, M. and Mortimer, M. (1986). 'Population Ecology' 2nd edition. Blackwell Scientific Publications, Oxford.

Begon, M., Harper, J. L. and Townsend, C. R. (1990). 'Ecology. Individuals, Populations and Communities.' Second Edition. Blackwell Scientific Publications, Oxford.

Bendavid, M., Pellis, S. M. and Pellis, V. C. (1991). Feeding habits and predatory behaviour in the marbled polecat (*Vormela peregusna syriaca*) 1. Killing methods in relation to prey size and behaviour. Behaviour **118**:127-143.

Benhamou, S. (1997). Path integration by swimming rats. Animal Behaviour **54** (**2**):321-327.

Berdoy, M. and Macdonald, D. W. (1991). Factors affecting feeding in wild rats. Acta Oecologia **12** (**2**):261-279.

Berdoy, M. and Smith, P. (1993). Arms race and rat race: adaptations against poisoning in the brown rat. Revue d'Ecologie (La Terre et La Vie) **48**:215-228.

Berdoy, M., Smith, P. and Macdonald, D. W. (1995). Stability of social status in wild rats: age and the role of settled dominance. Behaviour **132** (**3-4**):193-212.

Berdoy, M., Webster, J. P. and Macdonald, D. W. (1995). The manipulation of rat behaviour by *Toxoplasma gondii*. Mammalia **59** (**4**):605-613.

Bertram, D. F. and Nagorsen, D. W. (1995). Introduced rats, *Rattus* spp. on the Queen Charlotte Islands - Implications for sea bird conservation. Canadian Field Naturalist **109** (**1**):6-10.

Bishop, J. A. and Hartley, D. J. (1976). Ecology of warfarin resistant rats. Journal of Animal Ecology **45**:623-646.

Bishop, J. A., Hartley, D. J. and Partridge, G. C. (1977). The population dynamics of genetically determined resistance to warfarin in *Rattus norvegicus* from mid Wales. Heredity **39**:389-398.

Boakes, R. A., Westbrook, R. F., Elliott, M. and Swinbourne, A. L. (1997). Context dependency of conditioned aversions to water and sweet tastes. Journal of Experimental Psychology - Animal Behavior Processes **23** (**1**):56-67.

Boyle, C. M. (1960). Case of apparent resistance of *Rattus norvegicus* (Berkenhout) to anticoagulant poisons. Nature **188**:517.

Braselton, W. E., Neiger, R. D. and Poppenga, R. H. (1992). Confirmation of indanedione rodenticide toxicoses by mass-spectrometry mass-spectrometry. Journal of Veterinary Diagnostic Investigation **4** (**4**):441-446.

Broadhurst, L. J. (1997). 'The evolution of larval competition and oviposition strategies in the cowpea weevil (*Callosobruchus maculatus*).' PhD Thesis, University of Leicester.

Brown, P., Charlton, A., Cuthbert, M., Barnett, E., Ross, L., Green, M., Gillies, E., Shaw, K. and Fletcher, M. (1996). Identification of pesticide poisoning in wildlife. Journal of Chromatography A **754**:463-478.

Brown, P.R., Singleton, G.R., Kearns, B. and Griffiths, J. (1997). Evaluation and cost-effectiveness of strychnine for control of populations of wild house mice (*Mus domesticus*) in Victoria. Wildlife Research **24** (**2**):159-172.

Brown, R. A. (1994). Assessing the environmental impact of rodenticides. In 'Rodent Pests and Their Control.' (Eds. A. P. Buckle and R. H. Smith), pp 363-380. CAB International, Wallingford.

Brown, R. A., Hardy, A. R., Greig-Smith, P.W. and Edwards, P. J. (1988). Assessing the impact of rodenticides on the environment. Bulletin OEPP/EPPO Bulletin **18**:283-292.

Brunton, C. F. A. (1995). Neophobia and its effect on the macro- and micro-structure of feeding in wild brown rats (*R. norvegicus*). Journal of Zoology **235** (**2**):223-236.

Brunton, C. F. A., Macdonald, D. W. and Buckle, A. P. (1993). Behavioural resistance towards poison baits in Brown rats, *Rattus norvegicus*. Applied Animal Behaviour Science **38** (**2**):159-174.

Buckle, A. P. (1994). Rodent Control Methods: Chemical. In 'Rodent Pests and Their Control'. (Eds. A. P. Buckle & R. H. Smith), pp 127-160. Cab International, Wallingford.

Buckle, A. P., Odam, E. M. and Richards, C. G. J. (1986). Chemical bait markers for the study of bait uptake by Norway rats. In 'Control of Mammal Pests' (Eds. C. G. J. Richards & T. Y. Ku), pp 199-214. Taylor and Francis, London.

Butcher, A. D. (1965). How easy is it to find the corpses? Oryx **8** (**3**):154-155.

Butler, F.T. and Whelan, J. (1994). Population structure and reproduction in brown rats (*Rattus norvegicus*) from pig farms, Co. Kildare, Ireland. Journal of Zoology, London **233**:277-291.

Chou, L. S. and Richerson, P. J. (1992). Multiple models in social transmission of food selection by Norway rats, *R. norvegicus*. Animal Behaviour **44** (**2**):337-343.

Churchfield, S. (1990). 'The Natural History of Shrews'. Christopher Helm (Publishers) Ltd., London.

Clevedon Brown, J. and Stoddart, D. M. (1977). Killing mammals and general post-mortem methods. Mammal Review **7** (**2**) - 'Techniques in Mammalogy'. (Published for the Mammal Society). Blackwell Scientific Publications, Oxford.

Colvin, B. A. (1984). 'Barn owl foraging behavior and secondary poisoning hazard from rodenticide use on farms.' Phd Thesis, Bowling Green State University, USA.

Coly, A. and Aaron, J. J. (1996) Flow-injection analysis of several aromatic pesticides using fluorescence and photoinduced fluorescence detection. Analysis **24** (**4**):107-112.

Cooke, D., Nagle, A., Smiddy, P, Fairley, J. and Omuircheartaigh, I. (1996). The diet of the barn owl *Tyto alba* in County Cork in relation to land use. Biology and Environment - Proceedings of the Royal Irish Academy **96B** (**2**):97-111.

Cowan, D., Dunsford, G., Gill, E., Jones, A., Kerins, G, MacNicoll, A. and Quy, R. (1995). The impact of resistance on the use of second-generation anticoagulants against rats on farms in southern England. Pesticide Science **43**:83-93.

Cox, P. R. (1991). 'Environmental effects of rodenticide use.' Phd Thesis, University of Reading.

Cox, P. R. and Smith, R. H. (1990). Rodenticide ecotoxicology: assessing non-target population effects. Functional Ecology **4**: 315-320.

Cox, P. R. and Smith, R. H. (1992). Rodenticide ecotoxicology: pre-lethal effects of anticoagulants on rodent behaviour. In 'Proceedings of 15th Vertebrate Pest Conference' (Eds. J. E. Borecca and R. E. Marsh), pp 165-170. Univ. California, Davis.

Crier, J. K. (1970). Tetracyclines as a fluorescent marker in bones and teeth of rodents. Journal of Wildlife Management **34** (**4**):829-834.

Davoust, B., Boui, M., Branquet, D., deLahitte, J. D. and Martet, G. (1997). Research for three parasitic infections within a population of rats trapped in Marseille:

evaluation of the zoonotic risk. Bulletin de l'Academie Nationale de Medecine **181** (**5**):887-897.

Day, M. G. (1968). Food habits of British stoats (*Mustela erminea*) and weasels (*Mustela nivalis*). Journal of Zoology, London **155**:485-497.

Delany, M. J. (1974). 'The Ecology of Small Mammals' Studies in Biology No. 51. Edward Arnold, London.

Dubock, A. C. (1982). Pulsed baiting - a new technique for high potency, slow acting rodenticides. In 'Proceedings 10th Vertebrate Pest Conference' (Ed. R. E. Marsh), pp123-136. Univ. California, Davis.

Dubock, A. C. (1984). Pulsed baiting - a new technique for high potency, slow acting rodenticides. In 'The Organisation and Practice of Vertebrate Pest Control' (Ed. A. C. Dubock), pp 105-142. ICI Plant Protection Division, Fernhurst, UK.

Eadsforth, C. V., Gray, A., Huckle, K. R. and Inglesfield, C. (1993). The dietary toxicity of flocoumafen to hens - elimination and accumulation following repeated oral administration. Pesticide Science **38** (**1**):17-25.

Eadsforth, C. V., Gray, A., Harrison, E.G. (1996). Monitoring the exposure of Barn owls to second-generation rodenticides in southern Eire. Pesticide Science **47** (**3**):225-233.

Erlinge, S. (1975). Feeding habits of the weasel *Mustela nivalis* in relation to prey abundance. Oikos **26**:378-384.

Fenn, M. G. P., Tew, T. E. and Macdonald, D. W. (1987). Rat movements and control on an Oxfordshire farm. Journal of Zoology, London **213**:745-749.

Fitzgerald, B. M., Karl, B. J., Veitch, C. R. (1991). The diet of feral cats (*Felis catus*) on Raoul Island, Kermadec Group. New Zealand Journal of Ecology **15** (**2**):123-129.

Flowerdew, J. R. (1972). The effect of supplementary food on a population of woodmice (*Apodemus sylvaticus*). Journal of Animal Ecology **41**:533-566.

Flowerdew, J. R. (1974). Field and laboratory experiments on the social behaviour and population dynamics of the wood mouse (*Apodemus sylvaticus*). Journal of Animal Ecology **43**:499-511.

Flowerdew, J. R. and Gardner, G. (1978). Small rodent populations and food supply in a Derbyshire ashwood. Journal of Animal Ecology **47**:725-740.

Forrest, M. B. (1996). 'Toxins and blowfly population dynamics.' Phd Thesis, University of Leicester.

Frenkel, J. K., Hassanein, K. M., Hassanein, R. S., Brown, E., Thulliez, P and Quinteronurez, R. (1995). Transmission of *Toxoplasma gondii* in Panama City, Panama - a five year prospective cohort study of children, cats, rodents, birds and soil. American Journal of Tropical Medicine and Hygiene **53** (**5**):458-468.

Fullager, P. J. and Jewell, P. A. (1965). Marking small rodents and the difficulties of using leg rings. Journal of Zoology, London **147**:224-228.

Galef, B. G. and Buckley, L. L. (1996). Use of foraging trails by Norway rats. Animal Behaviour **51** (**4**):765-771.

Galef, B. G. and Whiskin, E.E. (1994). Passage of time reduces effects of familiarity on social learning - functional implications. Animal Behaviour **48** (**5**):1057-1062.

Galef, B. G. and Whiskin, E.E. (1995a). Are socially acquired behaviors irreversible? Behavioral Processes **34** (**3**):279-284.

Galef, B. G. and Whiskin, E. E. (1995b). Learning socially to eat more of one food than of another. Journal of Comparative Psychology **109** (**1**):99-101.

Galef, B. G. and Whiskin, E.E. (1997). Effects of social and asocial learning on longevity of food-preference traditions. Animal Behaviour **53** (**6**):1313-1322.

Galef, B. G. and Wright, T. J. (1995). Groups of naive rats learn to select nutritionally adequate foods faster than do isolated naive rats. Animal Behaviour **49** (**2**):403-409.

Galef, B. G., Beck, M. and Whiskin, E. E. (1991). Protein-deficiency magnifies social influence on the food choices of Norway rats (*Rattus norvegicus*). Journal of Comparative Psychology **105** (**1**):55-59.

Galef, B. G., Iliffe, C. P. and Whiskin, E. E. (1994). Social influences on rats (*Rattus norvegicus*) preferences for flavored foods, scented nest materials and odors

associated with harborage sites - Are flavored foods special? Journal of Comparative Psychology **108** (**3**):266-273.

Gill, J. E. (1992). Laboratory evaluation of the toxicity of flocoumafen as a single-feed rodenticide to 7 rodent species. International Biodeterioration and Biodegradation **30** (**1**):65-75.

Gill, J. E. and MacNicoll, A. D. (1991). Determination of the susceptibility of wild populations of the Norway rat (*Rattus norvegicus*) to the anticoagulant rodenticide brodifacoum. Zeitschrift für angewandte Zoologie **78** (**1**):101-117.

Gill, J. E., Kerins, G. M. and Macnicoll, A. D. (1992). Inheritance of low-grade brodifacoum resistance in the Norway rat. Journal of Wildlife Management **56** (**4**):809-816.

Gill, J. E., Kerins, G. M., Langton, S. D. and Macnicoll, A. D. (1993). The development of a blood clotting response test for discriminating between difenacoum-resistant and susceptible Norway rats. Comparative Biochemistry and Physiology **104** (**1**):29-36.

Gill, J. E., Kerins, G. M., Langton, S. D. and Macnicoll, A. D. (1994). Blood-clotting response test for bromadiolone-resistance in Norway rats. Journal of Wildlife Management **58** (**3**):454-461.

Godfrey, M. E. R. (1984). The toxicology of brodifacoum to target and non-target species in New Zealand. In 'The Organisation and Practice of Vertebrate Pest Control' (Ed. A. C. Dubock), pp 631-638. ICI Plant Protection Division, Fernhurst, UK.

Golani, I., Benjamini, Y. and Eilani, D. (1993). Stopping behavior - constraints on exploration in rats (*Rattus norvegicus*). Behavioral Brain Research **53** (**1-2**):21-33.

Gratz, N. G. (1994). Rodents as carriers of disease. In 'Rodent Pests and their Control' (Eds. A. P. Buckle and R. H. Smith), pp 85-108. CAB International, Wallingford.

Gray, A., Eadsforth, C. V., Dutton, A. J., and Vaughan, J. A. (1994a). The toxicity of three second-generation rodenticides to Barn owls. Pesticide Science **42** (**3**):179-184.

Gray, A., Eadsforth, C. V., Dutton, A. J., and Vaughan, J. A. (1994b). Non-invasive method for monitoring the exposure of Barn owls to second-generation rodenticides. Pesticide Science **41** (**4**):339-343.

Greaves, J. H. (1986). Managing resistance to rodenticides. In 'Pesticide Resistance: Strategies and Tactics for Management' pp 236-244. National Academy Press. Washington D.C.

Greaves, J. H. (1994). Resistance to anticoagulant rodenticides. In 'Rodent Pests and their Control' (Eds. A. P. Buckle and R. H. Smith), pp 197-217. CAB International, Wallingford.

Greaves, J. H. (1995). Managing resistance to anticoagulant rodenticides: an appraisal. Pesticide Science **43**:79-82.

Greaves, J. H. and Ayres, P. B. (1967). Heritable resistance to warfarin in rats. Nature **215**: 877-878.

Greaves, J. H. and Ayres, P. B. (1969). Some rodenticidal properties of coumatetralyl. Journal of Hygiene, Cambridge **67**: 311-315.

Greaves, J. H. and Cullen-Ayres, P. B. (1988). Genetics of difenacoum resistance in the rat. In 'Current Advances in Vitamin K Research' (Ed. J. W. Suttie), pp389-397. Elsevier Science, London.

Greaves, J. H., Redfern, R., Ayres, P. B. and Gill, J. E. (1977). Warfarin resistance: a balanced polymorphism in the Norway rat. Genetics Research Cambridge **30**: 257-263.

Greaves, J. H., Shepherd, D. S. and Quy, R. (1982). Field trials of second-generation anticoagulants versus difenacoum-resistant Norway rat populations. Journal of Hygiene (Cambridge) **89**: 295-301.

Greig-Smith, P. W. (1988). Hazards to wildlife from pesticide seed treatments, applications to seeds and soil. BCPC Monograph **39**:127-134.

Gurnell, J. (1978). Seasonal changes in numbers and male behavioural interaction in a population of wood mice, *Apodemus sylvaticus*. Journal of Animal Ecology **47**: 741-755.

Hammond, N. and Pearson, B. (1983). 'Birds of Prey' Hamlyn Books, London.

Hanney, P. W. (1975). 'Rodents - their Lives and Habits' David and Charles, Newton Abbot.

Hardy, A. R. (1990). Estimating exposure: the identification of species at risk and routes of exposure. In 'Pesticide Effects on Terrestrial Wildlife' (Eds. L. Somerville and C. H. Walker), pp 81-97. Taylor and Francis, London.

Hardy, A. R. and Taylor, K. D. (1979). Radio tracking of *Rattus norvegicus* on farms. In 'A Handbook on Biotelemetry and Radio-Tracking' (Eds. C. J. Amlaner Jnr. and D. W. Macdonald), pp 657-665. Pergamon Press.

Harrison, E. G., Eadsforth, C. V. and Vaughan J. A. (1990). A non-invasive approach for monitoring the exposure of Barn Owls to rodenticides. Brighton Crop Protection Conference - Pests and Diseases (unpublished).

Hart, A. D. M. (1990). Behavioural effects in field tests of pesticides. In 'Pesticide Effects on Terrestrial Wildlife' (Eds. L. Somerville and C. H. Walker), pp165-180. Taylor and Francis, London.

Hart, A. (1994). Ecology and toxicology in the assessment of risks to birds from pesticides. Annual Conference of the Society for Environmental Toxicology and Chemistry, Sheffield (unpublished).

Hegdal, P. L. and Blaskiewicz, R. W. (1984). Evaluation of the potential hazard to Barn Owls of Talon (brodifacoum bait) used to control rats and house mice. Environmental Toxicology and Chemistry **3**:167-179.

Hegdal, P. L. and Colvin, B. A. (1988). Potential hazard to Eastern Screech owls and other raptors of brodifacoum bait used for vole control in orchards. Environmental Toxicology and Chemistry **7**:245-260.

Hegdal, P. L., Colvin, B. A., and Blaskiewicz, R. W. (1984). Field evaluation of secondary hazards to Barn owls (*Tyto alba*) and Screech owls (*Otus asio*) associated with brodifacoum baits used for rodent control. In 'The Organisation

and Practice of Vertebrate Pest Control' (Ed. A. C. Dubock), pp647-672. ICI Plant Protection Division, Fernhurst, UK.

Heyes, C. M., Jaldow, E., Nokes, T. and Dawson, G. R. (1994). Imitation in rats (*Rattus norvegicus*) - the role of demonstrator action. Behavioural Processes **32** (**2**):173-182.

Hole, G. (1991). Proximity measures of social play in the laboratory rat. Developmental Psychobiology **24** (**2**):117-133.

Hooper, M. J., Brewer, L. W., Cobb, G. P. &and Kendall, R. J. (1990). An integrated laboratory and field approach for assessing hazards of pesticide exposure to wildlife. In 'Pesticide Effects on Terrestrial Wildlife' (Eds. L. Somerville and C. H. Walker), pp271-283. Taylor and Francis, London.

Howard, W. E. (1986). Rodent-free using permanent bait stations. In 'Control of Mammal Pests' (Eds. C. G. J. Richards and T. Y. Ku), pp 147-154. Taylor and Francis, London.

Huckle, K. R., Hutson, D. H. and Warburton, P. A. (1988). Elimination and accumulation of the rodenticide flocoumafen in rats following repeated oral administration. Xenobiotica **18** (**2**):1465-1479.

Huckle, K. R., Hutson, D. H., Logan, C. J., Morrison, B. J. and Warburton, P. A. (1989a). The fate of the rodenticide flocoumafen in the rat: retention and elimination of a single oral dose. Pesticide Science **25**: 297-312.

Huckle, K. R., Morrison, B. J. and Warburton, P. A. (1989b). The percutaneous fate of the rodenticide flocoumafen in the rat: role of non-biliary intestinal excretion. Xenobiotica **19** (**1**):63-74.

Hunter, K. (1983). Determination of coumarin anticoagulant rodenticide residues in animal tissue by HPLC **I** Fluorescence detection using post-column techniques **II** Fluorescence detection using ion-pair chromatography. Journal of Chromatography **270**: 267-283.

Hunter, K. (1985). High-performance liquid chromatographic strategies for the determination and confirmation of anticoagulant rodenticide residues in animal tissues. Journal of Chromatography **321**: 255-272.

Hunter, K., Sharp, E. A. and Newton, A. (1988). Determination of diastereoisomers of bromadiolone, an anticoagulant rodenticide, in animal tissues by HPLC. Journal of Chromatography **435** (**1**): 83-95.

Ille, R. (1991). Preference of prey size and profitability in Barn owls *Tyto alba guttata*. Behaviour **116**:180-189.

Inglis, I. R. and Shepherd, D. S. (1994). Rats work for the food they then reject: support for the information-primacy approach to learned industriousness. Ethology **98**: 154-164.

Inglis, I. R., Shepherd, D. S., Smith, P., Haynes, P. J., Bull, D. S., Cowan, D. P. and Whitehead, D. (1996). Foraging behaviour of wild rats (*Rattus norvegicus*) towards new foods and bait containers. Applied Animal Behaviour Science **47** (**3-4**): 175-190.

Inoue, S., Tanikawa, T., Kawaguchi, J., Iida, T. and Morita, C. (1992). Prevalence of *Listeria* (spp.) in wild rats captured in the Kanto area of Japan. Journal of Veterinary Medical Science **54** (**3**): 461-463.

Ivanco, T. L., Pellis, S. M. and Whishaw, I. Q. (1996). Skilled forelimb movements in prey catching and in reaching by rats (*Rattus norvegicus*) and Opossums (*Monodelphis domestica*) - relations to anatomical differences in motor systems. Behavioural Brain Research **79** (**1-2**):163-181.

Jackson, W. B. and Ashton, A. D. (1995). Extended summary of the RRAC Symposium on anticoagulant resistance in North America. Pesticide Science **43**: 95-96.

Johnson, I. P., Flowerdew, J. R. and Hare, R. (1991). Effects of broadcasting and of drilling methiocarb molluscicide pellets on field populations of wood mice, *Apodemus sylvaticus*. Bulletin of Environmental Contamination and Toxicology **46**: 84-91.

Johnson, R. A. and Prescott, C. V. (1994). The laboratory evaluation of rodenticides. In 'Rodent Pests and Their Control' (Eds. A. P. Buckle & R. H. Smith), pp 161-179. Cab International, Wallingford.

Jones, A. (1996). HPLC determination of anticoagulant rodenticide residues in animal livers. Bulletin of Environmental Contamination and Toxicology **56** (**1**): 8-15.

Kahana, A., Rozin, A. and Weller, A. (1997). Social play with an unfamiliar group in weanling rats (*Rattus norvegicus*). Developmental Psychobiology **30** (**2**):165-176.

Kapu, M. M., Akanya, H. O., Ega, R. A., Olofu, E. O., Balarabe, M. L., Chafe, U. M. and Schaeffer, D. J. (1991). Concentrations of trace and other elements in the organs of wild rats and birds from the Northern Guinea Savanna of Nigeria. Bulletin of Environmental Contamination and Toxicology **46**: 79-83.

Kataranovski, D., Milenovic, M., Hrgovic, N. and Dunderski, J. (1991) Age and sex structure of Norway rat (*Rattus norvegicus*) populations in urban and suburban habitats in Belgrade. Acta Veterinaria (Beograd.) **41** (**5-6**):247-254.

Kaukeinen, D. (1982). A review of the secondary poisoning hazard potential to wildlife from the use of anticoagulant rodenticides. In 'Proceedings 10th Vertebrate Pest Conference' (Ed. R. E. Marsh), pp 151-158. Univ. California, Davis.

Kaukeinen, D. E. (1993). Nontarget organism evaluations for rodenticides. American Chemical Society's Symposium Series **522**: 352-363.

Kelly, M. J., Chambers, J. and MacNicoll, A. D. (1993). Simple and rapid method for the determination of the diastereoisomers of difenacoum in blood and liver using HPLC with fluorescence detection. Journal of Chromatography - Biomedical Applications **620** (**1**):105-112.

Kenward, R. E. (1988). The potential of radio-tagging in trials of agrochemicals - a review. BCPC Monograph **40**: 'Environmental Effects of Pesticides.'

Khan, A. A. (1987). Evaluation of four rodenticides against rodents in sugarcane fields. In 'Control of Mammal Pests' (Eds. C. G. J. Tichards and T. Y. Ku), p171 (abs). Taylor and Francis, London.

Kjolholt, J. (1990). Distribution of pesticides and potential exposure of non-target organisms following application. In 'Pesticide Effects on Terrestrial Wildlife' (Eds. L. Somerville and C. H. Walker), pp33-63. Taylor and Francis, London.

Koshimizu, K., Saito, T., Shinozuka, Y., Tsuchiya, K. and Cerda, R. O. (1993). Isolation and identification of *Mycoplasma* strains from various species of wild rodents. Journal of Veterinary Medical Science **55** (**2**):323-324.

Kostrzewa, A. and Kostrzewa, R. (1994). Population limitation in Buzzards *Buteo buteo* and Kestrels *Falco tinnunculus*: the different roles of habitat, food and weather. In 'Raptor Conservation Today' Proceedings of IV World Conference on Birds of Prey and Owls, Berlin, 1992. (Eds. B-U Meyburg and R. D. Chancellor), pp39-48. Pica Press, Helm Info. Ltd., E. Sussex.

Kotler, B. P., Brown, J. S.,. Smith, R. J and Wirtz II, W. O. (1988). The effects of morphology and body size on rates of owl predation on desert rodents. Oikos **53**: 145-152.

Krambias, A. and Hoppe, A. H. (1986). The responses of captive partridges to dosing with anticoagulant rodenticides. In 'Control Of Mammal Pests' (Eds. C. G. J. Richards & T. Y. Ku), pp 181-186. Taylor And Francis, London.

Kruuk, H. and Parish, T. (1977). 'Behaviour of Badgers' ITE Publication, Cambridge.

Kuijpers, E. A. P., den Hartigh, J., Savelkoul, T. J. F. and de Wolff, F. A. (1995). A method for the simultaneous identification and quantitation of 5 superwarfarin rodenticides in human serum. Journal of Analytical Toxicology **19**: 557-562.

Lode, T. (1994). Environmental factors influencing habitat exploitation by the Polecat *Mustela putorius* in W. France. Journal of Zoology **234** (**1**):75-88.

Logan, F. A. and Leavitt, F. (1992). Sexual free behavior in male rats (*Rattus norvegicus*). Journal of Comparative Psychology **106** (**1**):37-42.

Lucion, A. B., Dealmeida, R. M. M. and DaSilva, R. S. M. (1996). Territorial aggression, body-weight, carbohydrate-metabolism and testosterone levels of wild rats maintained in laboratory colonies. Brazilian Journal of Medical and Biological Research **29** (**12**):1657-1662.

Lund, M. (1984). Resistance to the second-generation anticoagulant rodenticides. In 'Proceedings 11th Vertebrate Pest Conference' (Ed. D. O.Clark), pp 89-94. Univ. California, Davis.

Lund, M. (1988). Detection and monitoring of resistance to anticoagulant rodenticides in populations of brown rats (*Rattus norvegicus*) in Denmark. In 'Current Advances in Vitamin K Research' (Ed. J. W. Suttie), pp399-405. Elsevier Science, London.

Macdonald, D. W. (1987). 'Running with the Fox' Unwin Hyman Ltd.

Macdonald, D. W. and Fenn, M. G. P. (1994). The natural history of rodents: preadaptations to pestilence. In 'Rodent Pests and their Control' (Eds. A. P. Buckle and R. H. Smith), pp 1-21. CAB International, Wallingford.

Macdonald, D. W. and Halliwell, E. C. (1994). The rapid spread of red foxes, *Vulpes vulpes*, on the Isle of Man. Global Ecology and Biogeography Letters **4** (**1**):9-16.

MacNicoll, A. D. (1986). Resistance to 4-hydroxycoumarin anticoagulants in rodents. In 'Pesticides Resistance - Strategies and Tactics for Management' pp 87-99. National Academy Press.

MacNicoll, A. D. (1988). The role of altered vitamin K metabolism in anticoagulant resistance in rodents. In 'Current Advances in Vitamin K Research' (Ed. J. W. Suttie), pp 407-417. Elsevier Science, London.

MacNicoll, A. D. (1995). A review of biochemical mechanisms of warfarin resistance in the house mouse. Pesticide Science **43**: 57-59.

MacNicoll, A. D. and Gill, J. E. (1987). The occurrence and significance of rodenticide-resistance in the UK. BCPC Monograph **37** 'Stored Products Pest Control': 89-95.

MacNicoll, A. and Gill, J. E. (1993). Vitamin $K_3$ in feedstuffs: antidotal effects in captive anticoagulant-resistant rats and mice. Journal of Wildlife Management **57** (**4**): 835-841.

MacSwinney, F. J. and Wallace, M. E. (1978). Genetics of warfarin-resistance in house mice of three separate localities. Journal of Hygiene Cambridge **80**: 69.

MacVicker, H. J. and Trout, R. C. (1994). Vegetation management to manipulate vole (*Microtus agrestis*) damage in grassy plantations. In 'Grassland Management and Nature Conservation' (Eds. Hagger and Peel), pp302-305. British Grassland Society Symposium 28, Leeds.

Mallick, S. A. (1992). Urine-marking in three species of *Rattus*. Wildlife Research **19** (**1**):89-93.

Martin, A. D., Steed, L. C., Redfern, R., Gill, J. E. and Huson, L. W. (1979). Warfarin-resistance genotype determination in the Norway rat, *Rattus norvegicus*. Laboratory Animals **13**: 209-214.

Martin, P. and Bateson, P. (1986). 'Measuring Behaviour - an Introductory Guide' Cambridge University Press, Cambridge.

Matten, S. R., Lewis, P. I., Tomimatsu, G., Sutherland, D. W. S., Anderson, N. and Covin-Snyder, T. L. (1996). The US Environmental Protection Agency's role in pesticide resistance management. American Chemical Society's Symposium Series **645**:243-253.

Matuschka, F. R., Endepols, S., Richter, D. and Spielman, A. (1997). Competence of urban rats as reservoir hosts for Lyme Disease spirochetes. Journal of Medical Entomology **34** (**4**): 489-493.

McGarvey, B. D. (1994). Derivatization reactions applicable to pesticide determination by HPLC. Journal of Chromatography B **659**: 243-257.

McInnes, C. (1993). Anticoagulant rodenticide toxicity in a dog. Australian Veterinary Practitioner **23** (**3**):160-162.

Mead, C. (1987). 'Owls' Whittet Books Ltd., London.

Meehan, A. P. (1984). 'Rats and Mice. Their Biology and Control' Rentokil Ltd., E. Grinstead.

Mendenhall, V. M. and Pank, L. F. (1980). Secondary poisoning of owls by anticoagulant rodenticides. Wildlife Society Bulletin **8** (**4**):311-315.

Merson, M. H., Byers, R. E. and Kaukeinen, D. E. (1984). Residues of the rodenticide brodifacoum in voles and raptors after orchard treatment. Journal of Wildlife Management **48** (**1**): 212-216.

Mesmer, M. Z. and Satzger, R. D. (1995). Determination of brodifacoum in commercial rodenticides by using High-Performance-Liquid-Chromatography with fluorescence detection. Analyst **120** (**8**):2195-2197.

Metzgar, L. H. (1967). An experimental comparison of Screech owl predation on resident and transient white-footed mice (*Peromyscus leucopus*). Journal of Mammalogy **48**:387-391.

Meyer, A. N. (1994). Rodent control in practice: food stores. In 'Rodent Pests and their Control' (Eds. A. P. Buckle and R. H. Smith), pp 273-290. CAB International, Wallingford.

Mizutani, T., Hidaka, K., Ohe, T., Matsumoto, M., Yamamoto, K and Tajima, K. (1980). Comparative study on accumulation and elimination of hexachlorobiphenyls and decachlorobiphenyl in mice. Bulletin of Environmental Contamination and Toxicology **25**:181-187.

Morris, P. (1979). Rats in the diet of the Barn Owl. Journal of Zoology, London **189**: 540-545.

Morris, P. (1983). 'Hedgehogs' Whittet Books Ltd., London.

Mosterd, J. J. and Thijssen, H. H. W. (1991). The long-term effects of the rodenticide brodifacoum on blood coagulation and vitamin K metabolism in rats. British Journal of Pharmacology **104**: 531-535.

Mundy, D. E. and Machin, A. F. (1982). The multi-residue determination of coumarin-based anticoagulant rodenticides in animal materials by HPLC. Journal of Chromatography **234** (**2**):427-435.

Mwanjabe, P. S. and Morner, J. (1997). Rodent control using early warning strategy in Tanzania. Bulletin of Pest Management, 8 March 1997.

Myllymaki, A. (1986). Rat control in temperate Europe. In 'Control of Mammal Pests' (Eds.C. G. J. Richards and T. Y. Ku), pp 92-98. Taylor and Francis, London.

Myllymaki, A. (1995). Anticoagulant resistance in Europe: appraisal of the data from the 1992 EPPO Questionnaire. Pesticide Science **43**: 69-72.

Naheed, G. and Khan, J. A. (1989). Poison-shyness and bait-shyness developed by wild rats (*Rattus rattus* L.) **I.** Methods for eliminating shyness caused by Barium Carbonate poisoning. Applied Animal Behaviour Science **24**: 89-99.

Nakatsuyama, E. and Fujita, O. (1995). The influence of the food size, distance and food site on food carrying behaviour in rats (*Rattus norvegicus*). Journal of Ethology **13** (**1**):95-103.

Natynczuk, S. E. and Macdonald, D. W. (1994). Scent, sex and the self-calibrating rat. Journal of Chemical Ecology **20** (**8**):1843-1857.

Navarrette, S. A. and Castilla, J. C. (1993). Predation by Norway rats in the intertidal zone of central Chile. Marine Ecology - Progress Series **92** (**1-2**):187-199.

Neal, E. (1986). 'The Natural History of Badgers' Croom Helm, London.

Newton, I., Wyllie, I. and Freestone, P. (1990). Rodenticides in British Barn Owls. Environmental Pollution **68**:101-117.

Nieder, L. (1985). Daily activity of wild rats. Bollettino di Zoologia **52**: 263-267.

Nott, H. M. R. (1988). 'Dominance and feeding behaviour in the brown rat.' Phd Thesis, University of Reading.

Nott, H. M. R. and Sibly, R. M. (1993). Responses to novel food by rats: the effect of social rank. Crop Protection **12**: 89-94.

Olney, N. J., Davis, R. P., Thomas, M. R. and Garthwaite, D. G. (1991a). Rodenticide usage on farms in England, 1988. Pesticide Usage Survey Report: **89** MAFF, London.

Olney, N. J., Davis, R. P., Thomas, M. R. and Garthwaite, D. G. (1991b). Rodenticide usage on farms in England and Wales, 1989. Pesticide Usage Survey Report: **90** MAFF, London.

O'Neill, P. (1993). 'Environmental Chemistry' 2nd edition. Chapman and Hall, London.

Panadero, S., Gomezhens, A. and Perezbendito, D. (1993). Simultaneous determination of warfarin and bromadiolone by derivative synchronous fluorescence spectrometry. Talanta **40** (**2**):225-230.

Partridge, G. C. (1979). Relative fitness genotypes in a population of *Rattus norvegicus* polymorphic for warfarin resistance. Heredity **43**:239-246.

Pelz, H. J. (1987). Damage by wood mice (*Apodemus sylvaticus*) to sugarbeet seeds in relation to food availability. In 'Control of Mammal Pests' (Eds. C. G. J. Richards and T. Y. Ku), pp 5-14. Taylor and Francis, London.

Pelz, H. J. (1989) Ecological aspects of damage to sugarbeet seeds by *Apodemus sylvaticus*. In 'Mammals as Pests' (Ed. R. J. Putman on behalf of the Mammal Society), pp 34-48. Chapman and Hall, London.

Pelz, H. J., Hanisch, D. and Lauenstein, G. (1995). Resistance to anticoagulant rodenticides in Germany and future strategies to control *Rattus norvegicus*. Pesticide Science **43**: 61-67.

Petty, S. J. (1994). Moult of Tawny owls *Strix aluco* in relation to food supply and reproductive success. In 'Raptor Conservation Today' Proceedings of IV World Conference on Birds of Prey and Owls, Berlin, 1992. (Eds. B-U Meyburg and R. D. Chancellor), pp521-530. Pica Press, Helm Info. Ltd., E. Sussex.

Plesnik, J and Dusik, M. (1994a). Reproductive output of the Kestrel *Falco tinnunculus* in relation to small mammal dynamics in intensively cultivated farmland. In 'Raptor Conservation Today' Proceedings of IV World Conference on Birds of Prey and Owls, Berlin, 1992. (Eds. B-U Meyburg and R. D. Chancellor), pp61-65. Pica Press, Helm Info. Ltd., E. Sussex.

Plesnik, J and Dusik, M. (1994b). Reproductive output of the Tawny owl *Strix aluco* in relation to small mammal dynamics in intensively cultivated farmland. In 'Raptor Conservation Today' Proceedings of IV World Conference on Birds of Prey and Owls, Berlin, 1992. (Eds. B-U Meyburg and R. D. Chancellor), pp531-541. Pica Press, Helm Info. Ltd., E. Sussex.

Prestt, I. R. and Ratcliffe, D. A. (1972). Effects of organochlorine insecticides on European birdlife. Proceedings of the International Ornothological Conference **15**: 486-513.

Quy, R. J., Shepherd, D. S. and Inglis, I. R. (1992a). Bait avoidance and effectiveness of anticoagulant rodenticides against warfarin- and difenacoum-resistant populations of Norway rats (*Rattus Norvegicus*). Crop Protection **11**: 14-20.

Quy, R. J., Cowan, D. P., Haynes, P., Inglis, I. R. and Swinney, T. (1992b). The influence of stored food on the effectiveness of farm rat control. BCPC Monograph **42** 'Pests and Diseases':291-300.

Quy, R. J., Cowan, D. P. and Swinney T. (1993). Tracking as an activity index to measure gross changes in Norway rat populations. Wildlife Society Bulletin **21**: 122-127.

Quy, R. J., Cowan, D. P., Prescott, C. V., Gill, J. E., Kerins, G. M., Dunsford, G., Jones, A. and MacNicoll, A. D. (1995). Control of a population of Norway rats resistant to anticoagulant rodenticides. Pesticide Science **45** (**3**): 247-256.

Redfern, R., Gill, J. E. and Hadler, M. H. (1976). Laboratory evaluation of WBA 8119 as a rodenticide for use against warfarin-resistant and non-resistant rats and mice. Journal of Hygiene, Cambridge **77**: 419-426.

Renner, M. J., Bennett, A. J. and White, J. C. (1992). Age and sex as factors influencing spontaneous exploration and object investigation by preadult rats (*Rattus norvegicus*). Journal of Comparative Psychology **106** (**3**): 217-227.

Reynolds, J. D. (1980). Extraction and identiication of 10 anticoagulant rodenticides from baits and stomach contents by HPLC. Proceedings of 23rd Annual Meeting of the American Association Veterinary Laboratory Diagnostics pp187-193.

Riley, D. (1990). Current testing in the sequence of development of a pesticide. In 'Pesticide Effects on Terrestrial Wildlife' (Eds. L. Somerville and C. H. Walker), pp 11-24. Taylor and Francis, London.

Robitaille, J. A. and Bovet, J. (1976). Field observations on the social behaviour of the Norway rat, *Rattus Norvegicus* (Berkenhout). Biology of Behaviour **1**:289-308.

Roe, N. (1995). Spot the Barn Owl? Alimentary my dear Watsons. The Weekend Telegraph, p3. Saturday January 7 1995.

Rowe, F. P. (1987). The control of house mouse (*Mus musculus* L.) infestations on farms. In 'Control of Mammal Pests' (Eds. C. G. J. Richards and T. Y. Ku), p289 (abs). Taylor and Francis, London.

Sanchez-Hernandez, J. (1994). 'Assessing the risk to non-target species during the control of grey squirrels (*Sciurus carolinensis*).' PhD Thesis, University of Reading.

Shekarova, O. N., Kalinin, A. A. and Suntsov, V. V. (1995). The rythmns of daily activity in synanthropic (*R. rattus and R. norvegicus*) and tropical terrestrial forest rats (*Leopoldamys sabanus, Maxomus surifer* and *Berylmys berdmorei*). Zoologichesky Zhurnal **74** (**6**): 122-128.

Shepherd, D. S. and Greaves J. H. (1984). A weather-resistant tracking board. In 'Proceedings 11th Vertebrate Pest Conference' (Ed. D. O. Clark), pp112-113. Univ. California, Davis.

Shepherd, D. S. and Inglis, I. R. (1987). Feeding behaviour, social interactions and poison bait consumption by a family group of wild rats living in semi-natural conditions. BCPC Monograph **37**: 'Stored Products Pest Control' pp 97-105.

Shepherd, D. S. and Inglis, I. R. (1993). Toxic bait aversions in different rat strains exposed to an acute rodenticide. Journal of Wildlife Management **57** (**3**):640-647.

Shore, R. F., Birks, J. D. S., Freestone, P. and Kitchener, A. C. (1996). Second-generation rodenticides and Polecats (*Mustela putorius*) in Britain. Environmental Pollution **91** (**3**): 279-282.

Sleeman, P. (1989). 'Stoats, Weasels, Polecats and Martens' Whittet Books Ltd, London.

Smith, L. K., Field, E. F., Forgie, M. L. and Pellis, S. M. (1996). Dominance and age-related changes in the play fighting of intact and post-weaning castrated male rats (*Rattus norvegicus*). Aggressive Behaviour **22** (**3**): 215-226.

Smith, P., Townsend, M. G. and Smith, R. H. (1991). A cost of resistance in the brown rat? Reduced growth rate in warfarin-resistant lines. Functional Ecology **5**: 441-447.

Smith, P., Berdoy, M., Smith, R. H. and Macdonald, D. W. (1993). A new aspect of warfarin resistance in wild rats: benefits in the absence of poison. Functional Ecology **7**:190-194.

Smith, P., Inglis, I. R., Cowan, D. P., Kerins, G. M. and Bull, D. S. (1994a). Symptom-dependent taste-aversion induced by an anticoagulant rodenticide in the brown rat (*Rattus norvegicus*). Journal of Comparative Psychology **108** (**3**): 282-290.

Smith, P., Berdoy, M. and Smith, R. H. (1994b). Body weight and social dominance in anticoagulant-resistant rats. Crop Protection **13** (**4**): 311-315.

Smith, R. H. (1993). Terrestrial Mammals In 'Handbook of Ecotoxicology' Volume 1 (Ed. P. Calow), pp 339-352. Blackwell Scientific Publications, Oxford.

Smith, R. H. (1994). Rodent control methods: non-chemical and non-lethal chemical. In 'Rodent Pests and their Control' (Eds. A. P. Buckle and R. H. Smith), pp 109-125. CAB International, Wallingford.

Smith, R. H. (1995). Rodents and birds as invaders of stored-grain ecosystems. In 'Stored-Grain Ecosystems' (Eds. D. S. Jayas, N. D. G. White and W. E. Muir), pp289-323. M Dekker Inc., New York.

Smith, R. H. (awaiting publication) Population biology and non-target effects of rodenticides: trying to put the eco into ecotoxicology.

Smith, R. H. and Greaves, J. H. (1987). Resistance to anticoagulant rodenticides: the problem and its management. In 'Proceedings of 4th International Conference on Stored-Product Protection' (Eds. E. Donahaye and S. Navarro), pp 302-315. Tel Aviv, Israel.

Smith, R. H. and Nott, H. M. R. (1988). Rodent damage to cocoa in Equatorial Guinea. FAO Plant Protection Bulletin **36** (**3**):119-124.

Smith, R. H., Cox, P. R. and Rampaud, M. (1990). Rodenticide ecotoxicology: systems analysis and simulation. In 'Proceedings of 14th Vertebrate Pest Conference' (Eds. L. R. Davis & R. E. Marsh), pp 47-54. Univ. California, Davis.

Sokolov, V. E., Kulikov, V. F. and Tikhonova, G. N. (1992). Use of tactile sense in orientation of *Rattus norvegicus* and *Rattus rattus* in experiments with optical border. Zoologichesky Zhurnal **71** (**9**): 131-139.

Southern, H. N. (1954). Tawny Owls and their prey. The Ibis **96** (**2**): 384-410.

Southern, H. N. and Lowe, V. P. (1968). The pattern and distribution of prey and predation in Tawny Owl territories. Journal of Animal Ecology **37**: 75-97.

Stahr, H. M., Hyde, W., Pfeiffer, D., Wilson, D., Kinker, J. and Moore, R. (1981). Thin layer chromatography (TLC) applied to diagnostic toxicology. Veterinary and Human Toxicology **23** (**6**): 433-435.

Stetter, K. R., McCann, L. I., Leafgren, M. A. and Segar, M. T. (1995). Diet preference in rats (*Rattus norvegicus*) as a function of odor exposure, odor concentration and conspecific presence. Journal of Comparative Psychology **109** (**4**): 384-389.

Sullivan, T. P. (1986). Understanding the resiliency of small mammals to population reduction: poison or population dynamics? In 'Control of Mammal Pests (Eds. C. G. J. Richards & T. Y. Ku), pp 69-82. Taylor and Francis, London.

Tapper, S. (1979). The effect of fluctuating vole numbers (*Microtus agrestis*) on a population of weasels (*Mustela nivalis*) on farmland. Journal of Animal Ecology **48**: 603-617.

Tattersall, F. (1992). 'The ecology of the house mouse *Mus domesticus*, with particular reference to interaction with the wood mouse, *Apodemus sylvaticus*.' PhD Thesis, University of Reading.

Taylor, K. D. (1978). Range of movement and activity of common rats (*R.. norvegicus*) on agricultural land. Journal of Applied Ecology **15**: 663-677.

Taylor, K. D., Quy, R. J. and Gurnell, J. (1981). Comparison of three methods for estimating numbers of common rats (*Rattus norvegicus*). Mammalia **45** (**4**): 403-413.

Taylor, K. D., Turner, L. H. and Everard, J. D. (1991). Leptospires in *Rattus* species on Barbados. Journal of Tropical Medicine and Hygiene **94** (**2**): 102-103.

Taylor, R. H. and Thomas, B. W. (1989). Eradication of Norway rats (*R. norvegicus*) from Hawea Island, Fiordland, using brodifacoum. New Zealand Journal of Ecology **12**: 23-32.

Telling, G. M., Sissons, D. J. and Brinkman, H. W. (1977). Determination of organochlorine insecticide residues in fatty foodstuffs using a clean-up technique based on a single column of activated alumina. Journal of Chromatography **137**: 405-423.

Thijssen, H. H. W. (1988). Warfarin inhibition of Vitamin K epoxide reductase of Scottish resistant rats is reversible. In 'Current Advances in Vitamin K Research' (Ed. J. W. Suttie), pp 429-434. Elsevier Science, London.

Thijssen, H. H. W. (1995). Warfarin-based rodenticides: mode of action and mechanism of resistance. Pesticide Science **43**: 73-78.

Thijssen, H. H. W., and Janssen, Y. P. G. (1994). Target-based warfarin pharmacokinetics in the rat: the link with the anticoagulant effect. Journal of Pharmacological Experimental Theory **270**: 554-558.

Tongtavee, K., Hongnark, S., Artchawakom, T., Hongsbhanich, N., Brown, R. A. and Richards, C. G. J. (1986). The safety and efficacy of brodifacoum (klerat) wax blocks and zinc phosphide for rodent control in Thailand. In 'Control of Mammal Pests' (Eds. C. G. J. Richards & T. Y. Ku), pp 173-180. Taylor and Francis, London.

Townsend, M. G., Fletcher, M. R., Odam, E. M. and Stanley, P. I. (1981). An assessment of the secondary poisoning hazard of warfarin to Tawny owls. Journal of Wildlife Management **45** (**1**): 242-248.

Townsend, M. G., Entwistle, P. and Hart, A. D. M. (1995). Use of 2 halogenated biphenyls as indicators of non-target exposure during rodenticide treatments. Bulletin of Environmental Contamination and Toxicology **54**: 526-533.

Twigg, G. I. (1977). Techniques with captive mammals. Mammal Review **7** (**2**):Ch 6. Published for the Mammal Society by Blackwell Scientific Publications, Oxford.

Urban, D. J. (1990). The use of terrestrial field data in the practical application of ecological risk assessment principles. In 'Pesticide Effects on Terrestrial Wildlife' (Eds. L. Somerville and C. H. Walker), pp 319-333. Taylor and Francis, London.

Vlcek, M. (1991). Small mammals on refuse dumps as sources of pathogens and parasites. Veterinarni Medicina **36** (**9**): 569-575.

Wallace, M. E. and MacSwinney, F. J. (1976). A major gene controlling warfarin-resistance in the house mouse. Journal of Hygiene Cambridge **76**: 173-174.

Webster, J. P. (1994). Prevalence and transmission of *Toxoplasma gondii* in wild brown rats, *Rattus norvegicus*. Parasitology **108** (**4**): 407-411.

Webster, J. P., Brunton, C. F. A. and Macdonald, D. W. (1994). Effect of *Toxoplasma gondii* upon neophobic behaviour in wild brown rats, *Rattus norvegicus*. Parasitology **109**: 37-43.

Webster, J. P. and Macdonald, D. W. (1995a). Cryptosporidiosis reservoir in wild brown rats (*Rattus norvegicus*) in the UK. Epidemiology and Infection **115** (**1**): 207-209.

Webster, J. P. and Macdonald, D. W. (1995b). Parasites of wild brown rats (*Rattus norvegicus*) on UK farms. Parasitology **111**: 247-255.

Webster, J. P., Ellis, W. A. and Macdonald, D. W. (1995). Prevalence of *Leptospira* and other zoonoses in wild brown rats on UK farms. Mammalia **59** (**4**): 615-622.

Whishaw, I. Q. and Dringenberg, H. C. (1991). How does the rat (*R. norvegicus*) adjust food-carrying responses to the influences of distance, effort, predatory odor, food size and food availability? Pyschobiology **19** (**3**): 251-261.

Whishaw, I. Q. and Whishaw, G. E. (1996). Conspecific aggression influences food carrying - studies on a wild population of *Rattus norvegicus*. Aggressive Behavior **22** (**1**): 47-66.

Whishaw, I. Q., Dringenberg, H. C. and Comery, T. A. (1992). Rats (*Rattus norvegicus*) modulate eating speed and vigilance to optimize food-consumption. Effects of cover, circadian rythmn, food deprivation and individual differences. Journal of Comparative Psychology **106** (**4**): 411-419.

Wilmore, S. B. (1977). 'Crows, Jays, Ravens and their Relatives' David and Charles (Publishers) Ltd., Newton Abbot.

Wood, B. J. Rodents in agriculture and forestry. In 'Rodent Pests and their Control' (Eds. A. P. Buckle and R. H. Smith), pp 45-83. CAB International, Wallingford.

Yalden, D. W. (1977). The Identification of Remains in Owl Pellets. An Occasional Publication of the Mammal Society.

Yuen, S. H. (1978). Determination of the rodenticides difenacoum and brodifacoum in finished baits by reverse-phase liquid chromatography. Analyst **103**: 842-850.

Ziporyn, T. and McClintock, M. K. (1991). Passing as an indicator of social dominance among female wild and domestic Norway rats. Behaviour **118**: 26-41.