2.
Effects on Mammals

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METHODS FOR STUDYING POPULATIONS OF WILD MAMMALS

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ABSTRACT

The secretive and often nocturnal habits of mammals dictate that specialised methods of observation or capture are necessary for their study. In situations where mammals are likely to encounter pesticide applications in their own habitat or through their diet a variety of direct and indirect techniques are available. Whether live or dead capture is necessary will be dictated by the aims of the study and particularly the need to detect possible sub-lethal effects of pesticides; radio tracking may be appropriate for the detailed study of movement and/or the observation of places and causes of death. Trapping techniques are suitable for small rodents and insectivores and will vary with the habitat, the behaviour of the study species, the area to be studied, the likely range of population density and the accuracy required of the results. Indirect methods which can be calibrated, such as faecal pellet counts, and presence or absence techniques such as 'hair tubes' and the monitoring of feeding signs may also be considered. Direct observation and counting may be more suitable for larger species.

INTRODUCTION

The interactions of wild mammals and pesticides occur in the agricultural environment in two main ways. Firstly, non-target species can be affected in specific pest control situations, which often involve rodents, such as in the control of rats (Rattus norvegicus) and house mice, (Mus musculus) (Richards, in press) or in the control of wood mice (Apodemus sylvaticus) to protect newly drilled sugar beet (Anon, 1980, Green 1979). Secondly, in the control of diseases and invertebrate pests, where mammals are again non-target organisms, a number of interactions are possible. Such applications may be wide-spread and repeated at least annually. Non-target mammals may ingest seed dressings (Dimond & Sherburne, 1969; Jefferies et al., 1973, Westlake et al., 1982) be sprayed, ingest the spray via contaminated food (Jett, et al., 1986) or ingest the pesticide directly in pelleted form (Greig-Smith, 1987b, Tarrant & Westlake, in press).

Whether immediate or delayed mortality of the non-target species is involved or simply a sub-lethal effect (Holdgate, 1979), knowledge and/or samples of dead or contaminated individuals may be required. Many mammals are secretive and nocturnal (Southern, 1964) and specialised methods are necessary for their study. In this review I discuss methods for the study of wild mammals, concentrating on their capture for demographic analysis; such methods may sometimes be used for the removal of samples for biochemical or histological examination in the laboratory or the removal of blood samples (e.g. Westlake et al., 1982, Leigh Brown, 1977). The discussion is divided into sections on radio-telemetry, trapping, indirect methods and direct counting techniques for monitoring mammal populations. In the space available a comprehensive review is not possible and so the emphasis will be placed on the smaller British species.

RADIO-TELEMETRY

Studies of mammals using radio-telemetry have allowed great advances in the knowledge of their ecology and behaviour. (Macdonald, 1978, Amlaner & Macdonald, 1980, Cheeseman & Mitson, 1982). It may be very labour-intensive and it usually depends on the initial capture of the individual so that a transmitter can be attached; thus trapping biases (see TRAPPING SMALL MAMMALS) may also apply.

Telemetry has been used successfully to answer questions concerning movement, activity, social behaviour and feeding in a wide range of species in agricultural environments, e.g. rats (Hardy & Taylor, 1980; Fenn _et al_, 1987), hares (_Lepus europaeus_) (Tapper & Barnes, 1986) and badgers (_Meles meles_) (Cheeseman & Mallinson, 1980). The technique has potential for detailed studies of survival or comparing individual differences, but there are problems associated with the number of animals which can be followed at one time and the size of the transmitter, particularly with smaller species. The size constraints of transmitters may limit their use to adult individuals (e.g Wolton, 1985) and limited battery life and detection range in small transmitters also cause problems. Significant advances have been made in the miniturisation of transmitters (Kenward, 1987) so it is to be hoped that the full size range of small species will be studied in the future.

TRAPPING SMALL MAMMALS

General considerations

Trapping is often taken at face-value without questioning the behavioural response on which it relies; comparisons between species or even within species may be invalidated by variation in the reaction of the individual to the trap. Thus the following consideration of variables affecting the response of the individual to the trap is an important foundation for the study of trap-revealed results. Detailed points of technique are reviewed by Flowerdew (1976) and Gurnell & Flowerdew (1982).

Technical problems

Traps for small rodents and insectivores in Britain have developed from open ended wooden tunnels, activated by a spring trap mechanism, to the aluminium Longworth trap (Chitty & Kempson, 1949). The latter has an adjustable tripping weight but careful adjustment is necessary (Grant, 1970). Cheaper alternatives are the Davis trap (Davis, 1961, Green, 1979) and the plastic 'Trip-trap'. In North America Sherman traps are a common alternative to Longworths but may also be subject to a setting bias (Bergstrom, 1985). Many other designs are available (e.g. Andrzejewski _et al_., 1966 Hansson, 1967, Karlsson, 1986, Getz, 1987).

Comparisons between various types of live trap are common (e.g Chitty & Kempson, 1949, Hansson, 1967, Mihok, _et al._, 1982) but trap choice is often made on the basis of cost or availability. Comparisons of pitfall traps with Longworth traps are enlightening as they indicate the difficulties of using both types; the tests (Beacham & Krebs, 1980) show that many individuals caught in pitfalls fail to enter live traps and others may take several weeks to be recruited into live traps. Pitfalls tended to capture lower weight voles than live traps. In the repeated capture of voles, however, pitfalls are only about half as effective as live traps (Table 1), implying that voles learn to avoid pitfalls as they grow older. The time-lag in recruitment of young to live traps in the winter led to population estimates from live traps alone which increased into the winter despite the fact that breeding had stopped. However, by spring the live trap estimates were close to those from all the traps.

TABLE 1

Trappability# of Townsend voles (Microtus townsendii) in enclosed and free-living populations sampled by pitfall and livetraps (adapted from Beecham & Krebs, 1980).

Trap type	Season	Trappability (%)	N
Longworth	Summer 1976	61	324
Longworth	Summer 1977	65	1692
Pitfall	Summer 1976	35	727
Pitfall	Summer 1977	28	1726

$$\#\text{Trappability} = \frac{\sum \frac{\{\text{no. of captures of individual}\}}{\{\text{no. of possible captures of that individual}\}}}{N}$$

where N = the number of individuals caught more than twice. In calculating the total number of captures of an individual the first and the last captures are excluded as it is necessarily caught at these times.

The baiting of traps usually increases trap success in small rodents (Chitty & Kempson, 1949, Patric, 1970, Gurnell, 1976) and insectivores (Pernetta, 1977) but the attractiveness of baits may vary seasonally (see 'Environmental influences on trap success'). In studies of carnivores (eg. the weasel, Mustela nivalis), elaborate baiting and odour addition may be necessary to effect capture (King, 1973, 1975).

The technique of 'prebaiting' (Chitty & Kempson, 1949), where the baited trap is placed in position but set so that it will not catch, is common practice in small mammal trapping (Flowerdew, 1976). It was designed to accustom trap-shy individuals to traps and thus provide a larger and more 'random' sample on the first day of trapping of marked (if present) and unmarked animals than would otherwise occur. However, its practice has been questioned by Gurnell (1980) who compared the capture success of bank voles, Clethrionomys glareolus, and wood mice, Apodemus sylvaticus in traps prebaited for one or two days or not prebaited at all. With wood mice the effect of prebaiting for one or two nights on capture success was never large in comparison with no prebaiting (Table 2); the effect of prebaiting for two nights was similar to prebaiting for one night. In bank voles prebaiting for one or two nights led to increased captures on the first night of trapping after which the differences between prebaited and non-prebaited traps were small (Table 2).

Thus it is recommended that unless trapping periods are relatively short, viz. one or two days, when weather factors may seriously affect results, prebaiting is not necessary and the extra days may be better used adding to the total amount of information obtained.

TABLE 2

The percentage of the total catch of individual wood mice or bank voles caught on successive nights of trapping. Data tabulated from graph in Gurnell (1980).

		Night of trapping			
Species	Prebaiting	1	2	3	4
Wood mouse	Yes	55	22	15	8
	No	51	25	15	9
Bank vole	Yes	50	23	14	13
	No	41	23	22	14

Early research on field voles, Microtus agrestis led to the recommendation of prebaiting (Chitty & Kempson, 1949) and many subsequent trapping studies of voles in Britain have prebaited and/or utilized a three- or four-day trapping period (Southern, 1973, Ferns, 1979). Both prebaiting and/or a relatively long trapping period (three or more days) have been used for population studies of British shrews, Sorex araneus and Sorex minutus (Pernetta, 1977, Dickman, 1980). Others, (e.g. Churchfield 1980), used no bait but attended to the traps at frequent intervals in order to minimise trap mortality.

The density of traps in the study area should be related to the density of small mammals and their range of movement (Andrzejewski et al., 1966, Janion & Wierzbowska, 1970, Gurnell, 1976). Kikkawa (1964) observed that woodland trapping grids of 11.5 x 23 m missed individual bank voles known to be present inbetween the trap points (c.f. King (1975) for similar observations of carnivores). If small mammals occupy 70-80% of the traps then the number of traps used should be doubled (Southern, 1973, Gurnell & Flowerdew, 1982). In contrast, a large number of traps at a single point or at relatively distant points (>150 m apart) may be sufficient for residue analysis and long-term studies (Dimond & Sherburne, 1969) and such methods may be calibrated for density estimation (Thomson, 1986).

Environmental influences on trap success

In wood mice activity above ground starts at sunset but the last return to the nest is not correlated with sunrise (Wolton, 1983); activity is reduced by moonlight (Kikkawa, 1964, Wolton, 1983) and by wet and cold conditions (Brown, 1956, Gurnell, 1975, 1976). However, if the temperature suddenly rises or there is a storm activity may increase (Evans, 1942). Seasonal changes in activity are common in small mammals (e.g. Gipps (1985) for bank voles, Churchfield (1982) for common shrews). With harvest mice (Micromys minutus) traps placed on the ground are more successful in the autumn and winter than in the spring and early summer (Corbet & Southern, 1977).

Mast crops also affect the reaction of small mammals to traps so that trappability is reduced (Kikkawa, 1964, Smith & Blessing, 1969) and grey squirrel (Sciurus carolinensis) trappability is low in autumn in deciduous woodland (Gurnell, 1983). The presence of other species may also inhibit capture; bank voles are subordinate to yellow-necked mice (Apodemus flavicollis) (Andrzejewski & Olszewski, 1963) and bank vole activity is influenced by the presence of wood mice (Greenwood, 1978). The odour of short-tailed shrews, (Blarina brevicauda) and other species in the trap inhibits the entry of meadow voles (Microtus pennsylvanicus) (Boonstra, Rodd & Carlton, 1982).

Behavioural variation affecting reactions to traps

Within species, dominance leads to early capture (e.g Brown, 1969 for wood mice) and subordinance may lead to continued trap-shyness, even after first capture, as in bank voles (Kikkawa, 1964). Reproductive individuals of yellow-necked mice had higher trappability than non-breeders and subadults (Jensen, 1975). Individuals which have been marked are more trappable than unmarked bank voles and wood mice and trap-revealed sex-ratios may be biased for any of the above behavioural reasons (Gurnell, 1982).

The previous occupant of a trap may affect subsequent captures to such an extent that results are biased. In studies of North American Microtus species individuals entered dirty traps (left with urine, faeces and debris from the previous occupant) more frequently than clean traps (Boonstra & Krebs, 1976); similar results are shown for bank voles, wood mice and yellow-necked mice, with the wood mouse preferring the odour of the same sex and yellow-necked mouse the odour of the opposite sex (Montgomery, 1979). Stoddart (1982) found that in the field vole previously occupied traps deterred subsequent captures and recommended that traps should be cleaned between captures to avoid bias. However, the difference in capture rate of bank voles and wood mice in a further test of clean compared with dirty traps was found to be insignificant, although too few captures of field voles prevented further study of the effect in this species (Tew, 1987).

Trapping small mammals in agricultural habitats

Studies of small mammals in farmland (Pollard & Relton, 1970, Jefferies et al., 1973, Green, 1979) usually allow only a relatively small study area for logistic reasons, and comparisons between fields are not replicated (Green, 1979). These restrictions have been overcome for the study of the effects of pesticides on wildlife in cereal ecosystems in the Boxworth Project (Hardy, 1986, Greig-Smith, 1987a,b). Trap lines are used in conjunction with 'intensive study area' grids and comparisons may be made between individual fields or between larger areas of similar treatments on up to three or four fields. The method (Hare, in prep.) is based on the use of index trap lines (c.f. Hansson, 1967) which are randomly assigned to tractor wheelings. Each trap line has ten points 20m apart with two Longworth traps at each. The number of trap lines is related to the field area so that there is one trap line to approximately 2 ha. The traps are set for two days and a total of 59 lines over 120 ha have been operated by one person over two weeks with 300 traps. Total captures of individuals over the two days for all lines on each field are divided by its area to provide a density index. The intensive study grids (8 x 8 points, 20m apart, 2 traps per point trapped for 4 days) in three fields are trapped at a similar time to the index lines to provide more accurate population data and to allow the calibration of the density index. After 11 grid and line studies on each of the three intensive study fields over four years the calibration of the density index is possible; the regression ($y = 9.46x + 0.67$, $p < 0.001$, 31 d.f., where y = total individuals on the 2 ha grid and x = density index (number per ha)) and the correlation coefficient ($r = 0.80$) seem satisfactory.

Trapping studies in hedgerows have utilized many different techniques (Southern, 1965, Eldridge, 1971). In attempting to provide a standard trapping scheme for hedgerows to cover a wide variation in population density as well as wide-ranging or relatively sedentary small mammal species, a dual approach has been adopted by ADAS (A. Swash & S. Poulton, pers. comm.). This utilizes a high density of traps, 3 per point at 33 points at 5 m intervals for the capture of voles and shrews at high and low density and a further line of 4 traps per point at 25 points placed 20 m apart for the capture of wood mice at high and low densities. It is hoped that these lines will sample the hedgerow species satisfactorily at all times but their efficiency has still to be evaluated.

INDIRECT METHODS

Faecal pellet counts, individually in lagmorphs or as groups in ungulates, along transects provide population estimates suitable for calibration or data on distribution (Putman, 1984, Krebs *et al.*, 1987). Transect counts of a number of signs of rabbits (*Oryctolagus cuniculus*) have been combined with direct counts of individuals to provide a population index (Trout, Tapper & Harradine, 1986) and quadrat searches for faeces and feeding signs of field voles gave an index which has been calibrated in a weasel/prey study in farmland (Tapper, 1979): however, caution is urged in that such a method is only suitable if large fluctuations in relative magnitude are being investigated and the index is shown to be related to vole population density. Putman (1984) reviews the problems involved in the techniques involving faecal pellet counts.

Hair tubes (Dickman, 1986) provide samples of small mammal hair for identification which are collected on double-sided sticky tape in lengths of PVC pipe. The tubes are placed or pegged firmly to the ground and left for 7-10 days and then the guard hares stuck to the tube are removed. In the study of small mammals tubes of 10cm x 3cm were used but the method could be adapted for larger species. The tubes efficiently detect species' presence and calibration of the percentage of tubes visited by a species may provide an index of abundance.

A specialised presence/absence technique dependent on the detection of feeding signs has beed devised for harvest mice (Warner & Batt, 1976). Tennis balls with a 15mm hole in the side are supported on stakes above the ground and baited with budgerigar seed. The balls are left for a few days, as long as 5-7 days if the mice are at low density, and evidence of feeding on the seed indicates the presence of the species. Observation of nests and/or feeding at the balls should confirm that harvest mice are present.

DIRECT COUNTING

Counting and direct observation is often possible with larger species of mammals. In studies of rabbits spotlight counting is possible at night, and combined with live trapping provides adequate population data (Henderson, 1979). Barnes & Tapper (1985) describe a spotlight method for hares. It is possible with fallow deer (Dama dama) for a large team of people to drive and count all the individuals leaving or entering a particular habitat (Chapman & Chapman, 1975) and for many ungulate species aerial photography may be possible, although not perhaps in many British habitats (Flowerdew, 1976).

CONCLUSION

Agricultural habitats, although often a mosaic of hedgerow and woodland as well as fields, provide large scale, relatively open areas for the study of mammals. The methods discussed above are likely to be at least as easy to operate in fields and hedgerows as they might be in woodland and with many species of mammals, especially the rodents, insectivores and small carnivores, farmland is still an area where knowledge of their ecology is in its infancy. The continued use of pesticides provides a practical and pressing reason for their study in the long- and short-term.

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FIELD METHODS FOR STUDYING THE NON-TARGET HAZARD OF RODENTICIDES

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ABSTRACT

During the safety evaluation of brodifacoum, ICI has developed a programme of field methods in a range of environments throughout the world. These methods have been used to estimate risk, measure exposure and hazard. Risk indices for primary exposure to palatable baits and secondary exposure to poisoned rodents are dependent on the quality of baiting and prey/scavenger interaction respectively. The low palatability of wax block baits to non-targets has been demonstrated by "exposed bait placement". Species exposed and survival have been assessed by direct counts, interviews and by using animal signs, while hazard has been measured directly by corpse searching. Radiotracking has been used for monitoring individual species and can allow accurate measurement of exposure and hazard. Residue methods have been used to confirm exposure, establish liver concentrations associated with death and measure residues in target rodents.

INTRODUCTION

Rodents adversely affect the production and storage of many of the world's food commodities (Meehan 1984); often soiling with faeces, urine and hairs much that they do not consume (Dubock 1978). Many species of rodent also serve as either vectors of diseases passed to man and his domesticated stock or as disease reservoirs (Gratz 1984). As a result of these depredations, acute and chronic rodenticides have become widely used in many environments. Because of their phylogenic and physiological similarities, some non-target vertebrates share a similar level of susceptibility to rodenticides with target animals. Thus, in practice, safe use often depends upon reducing the exposure to non-target animals by careful bait placement and the use of formulations that are more attractive to target than to non-target animals. However, there are cases where secondary exposure and hazard from rodenticides have been underestimated and incidents have followed the widespread use of rodenticide baits in the field (Mendelssohn 1972, GELAC 1983). Although the anticoagulant rodenticides have, in general, a good safety record (Hardy et al. 1986), the increasing use of the more potent second generation anticoagulant rodenticides, such as bromadiolone, brodifacoum and, most recently, flocoumafen has made it necessary to evaluate certain use patterns for their safety to non-target animals, both for responsible product stewardship and as a requirement in registration procedures.

ICI has developed field methods for a diversity of environments throughout the world, including urban, village, farm and field. Many of the methods have been adapted from well established techniques and integrated into a programme to estimate risk and measure exposure and hazard to non-target wildlife and domestic animals. Hazard is defined as the product of toxicity and exposure (Johnson 1982). Risk is a prediction of hazard or one of its components. In some circumstances exposure may be easier to measure than hazard. Both measurements are essential in field trials. Exposure may take two forms: primary, if baits are eaten by non-targets; and secondary, if residues persist in rodents after they have eaten bait.

Primary exposure measurements have combined assessment of bait palatability; baiting quality (concealment); abundance of non-target species and confirmation by the presence of residues in non-targets. While for secondary exposure, measurements have combined assessment of baiting quality (size of placements and frequency of baiting); size of the target infestation; location of baiting (e.g. around farm buildings or in woodland), the interaction between predators and scavengers with intoxicated and poisoned animals respectively; rodenticide concentration and its duration in target rodents; and confirmation of exposure by the presence of residues in predators and scavengers. Hazard may be measured directly in terms of survival (i.e. census before and after treatment) and directly as mortality (i.e. corpse location). In addition, indirect methods such as tracking can produce population indices for survival or mortality. Many of the methods overlap in their application but are described separately below. Examples used are from ICI field trials in South East Asia, USA and Europe, evaluating the second generation anticoagulant rodenticide, brodifacoum.

It is essential that the methods and the study design are tailored specifically to the environment in which the rodenticide is applied. For example, if there is likely to be high variability in the quality of bait placement (i.e. level of concealment by different farmers) and if this may greatly influence exposure and hazard, relatively large numbers of sites (up to 16) have been used in a single study. This compares with far fewer sites when intensive field trials are combined with machinery application of insecticide granules, dressed seed and molluscicide baits (Bunyan et al. 1981, Greig-Smith and Westlake 1988) where exposure via application is less variable.

METHODS

Baiting Practice and Risk Indices

Baiting practice has an impact on non-target exposure. Variables such as bait concealment (C), quantity of bait placed (Q), location of baiting (L) area baited (A) and size of the infestation (I) can be monitored during trials. Baiting practice is numerically classified, albeit sometimes subjectively. For example, bait concealment at bait points is scored 1, 2 and 3 where concealment is good, intermediate and poor. When combined with non-target animals numbers (N) this can provide a measure of risk. The risk index $\sqrt[3]{CxQxN}$ gives a good indication of primary exposure and hazard to birds when baits are palatable and demonstrates the need for a high standard of bait placement, Fig. 1 and 2, respectively. Risk indices for secondary exposure have not been fully evaluated and have been used to assess secondary exposure risks when farmers have been instructed in "pulsed baiting" (Dubock 1984). Hazard from secondary exposure is indicated by risk indices ($RI= 3\sqrt{LxIxA}$; Fig 3). Location (L) has been scored 1 (restricted to around farm buildings), 2 (around farm buildings and neighbouring uncultivated areas), 3 (fields and hedgerows) and 4 (in woods). Thus the development of indices based on location, infestation and area of baiting, together with some knowledge of the interaction between predators, scavengers and exposed rodents gives a good indication of ultimate hazard.

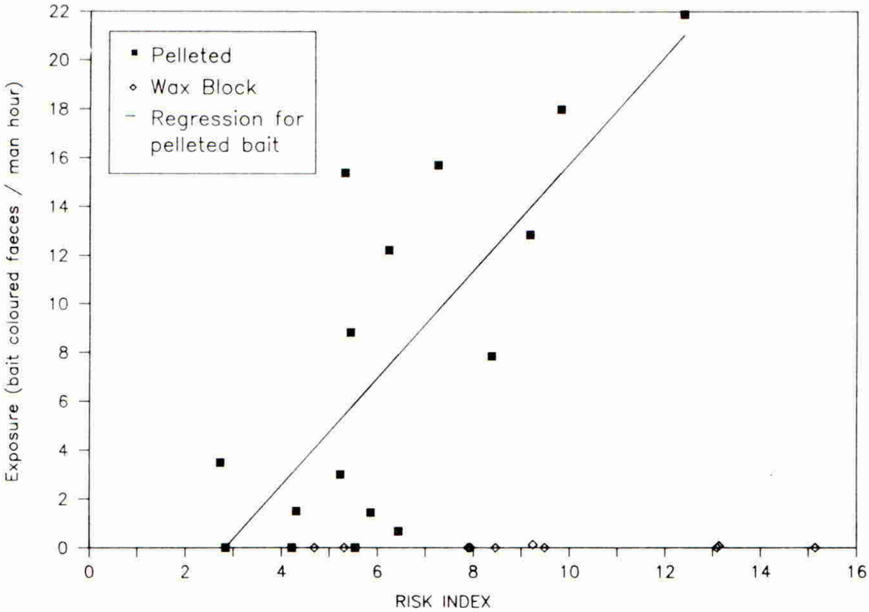

Fig. 1. The relationship between a risk index for birds through primary exposure (concealment x quantity of bait x bird numbers) and measured exposure (bait coloured bird faeces/man hour) for pelleted and wax block baits on U.K. farms, ($r=0.77$, $P<0.001$).

Exposed Bait Placements

This technique measures the attractiveness and palatability of baits to wildlife and domestic animals. Baits are placed where they are conspicuous, in areas regularly frequented by domestic animals or wildlife. These are then observed from a distance for a period of time. Animals approaching, investigating and eating baits are recorded. Alternatively baits may be placed on patches of fine dry sand or other suitable surfaces for tracking. Distinction is made between animals approaching (tracks pass bait) and eating bait (tracks round bait or crumbs of bait on sand). The time over which tracking is measured is recorded and the identity of the tracking animals estimated (i.e. large or small bird). This technique has only been attempted for diurnal feeders because the activity of target rodent species during the night normally obliterates any non-target tracks.

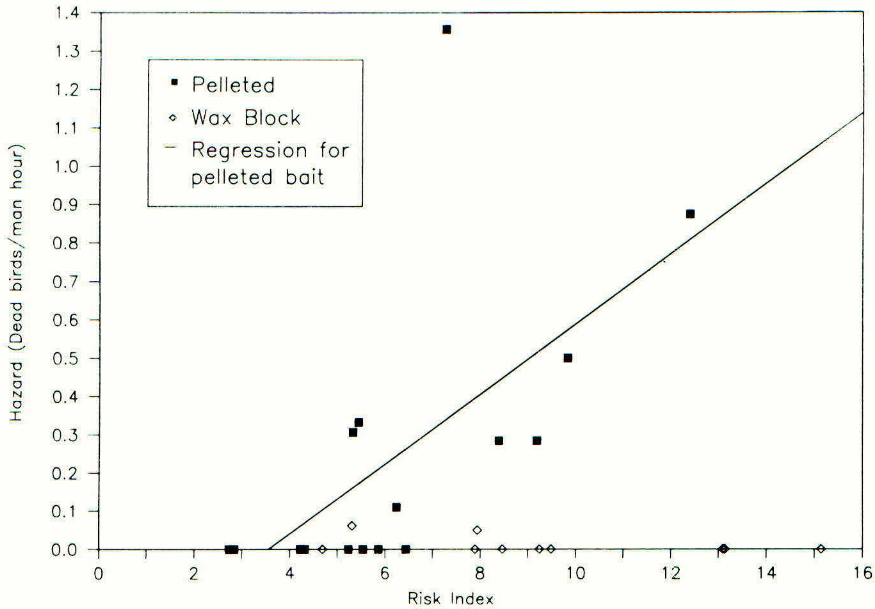

Fig. 2. The relationship between a risk index for birds through primary exposure (concealment x quantity of bait x bird numbers) and hazard (dead birds/man hour) for pelleted and wax block baits on U.K. farms, (r=0.61, P<0.05).

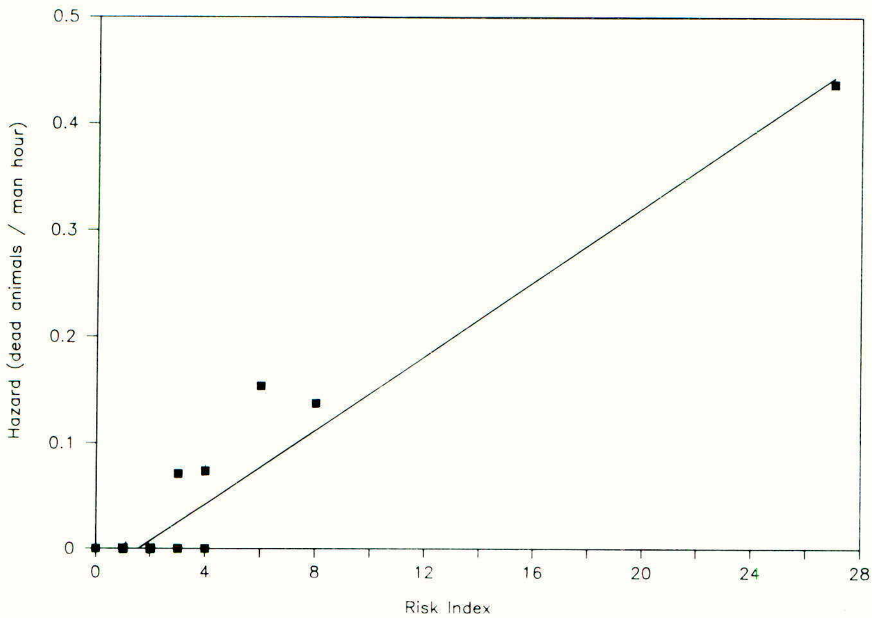

Fig. 3. The relationship between a risk index for secondary exposure (location of baiting x size of infestation x area baited) and hazard to predatory and scavenging animals (dead animals/man hour) on U.K. farms.

Direct observation and tracking methods are complementary. While tracking can be done with low inputs of manpower there are four disadvantages; difficulty in species identification; overestimation of exposure because it does not indicate how long animals were in the vicinity of the bait; removal of baits without leaving tracks by dogs etc; and rainfall washing out tracks. Tables 1 and 2 present the results for 56 hours of observations of wax blocks in Tubuan, Philippines (Hoque et al. 1988) and 1194 hours tracking at wax blocks around farm buildings, respectively. The identification of species most at risk allows exposure of these animals to be minimised during bait applications.

TABLE 1

Results of exposed wax block bait placements in Tubuan, Philippines.

Animal	Total No's	No's Approaching	No's Investigating	No's Eating
Children	6	0	0	0
Doves	10	0	0	0
Cats	4	0	0	0
Goats	5	2	0	0
Ducks	21	8	2	0
Chickens	33	20	5	0
Dogs	10	9	4	3*

* Animals trying to eat bait were stopped from doing so by the experimenter.

TABLE 2

Results of small bird tracking at exposed wax block baits around U.K farm buildings.

Farm	Tracking Hours	Approaching % of Total	Eating % of Total	No's Dead Small Birds
A	332	9.4	0	0
B	24	0	0	0
C	35	0	0	0
D	144	7.4	0	1
E	79	0	0	0

Use of Tracks and Signs

Signs can be used to identify species exposed and to measure changes in activity before and after treatment. Their use for estimating relative changes in populations of mammals is well established (Davis 1983). These under used methods have much potential but they can be misleading; for example, rodenticide use may remove a predator's source of food or provide an abundant but temporary source of food for scavengers and thus affect their activity.

Species exposed to baits and poisoned rodents may be identified from their footprints on sand or other tracking surfaces. The presence of dye in faeces, from the rodenticide bait is further evidence of exposure. The importance of rodents in the diet of predators and scavengers may be determined from indigestible remains in faeces e.g. fur; bones and fur in bird pellets and from the condition of rodent carcass remains. For example, Barn Owl Tyto alba pellets were collected at owl roosts and examined to identify prey in a coastal part of New Jersey, USA where rodenticides were used to control commensal rodents (Hegdal & Blaskiewicz 1984). Only 6% of pellets contained commensal species (Rattus norvegicus and Mus musculus). Radio-tracking of the owls revealed that these rodents had probably been taken in the field and not from around farm buildings.

Activity indices may be developed by methods based on tracking. Phillips (1982) describes a tracking method for the fox Vulpes vulpes using a scent as an attractant. Interpretation of the results requires untreated control areas and an understanding of the speed of immigration and emigration in the population.

Non-Target Animal Counts

Direct counts can be used to establish wildlife potentially exposed to rodenticide baits and to measure changes in local populations of sedentary species. Some census methods which can be adapted include transect (Ferry and Frochot 1958), point (Braae and Nohr 1983), roost and call counts, territory mapping (IBCC 1970) and, for domestic animals only, by interviewing owners (Liberg 1984).

Counts of wildlife and free-ranging domestic animals around buildings on farms and in villages have been done by transect counts and interview respectively. Transects involve counting non-targets while walking a fixed route around buildings. The value of these data for assessing changes in the local population depends on how sedentary they are and the size of the study area. For example, farmyard populations of European Robins Erithacus rubecula may remain relatively constant while Chaffinches Fringilla coelebs may be much less so. This is shown in Fig. 4 for a situation in which the rodenticide had been shown by other methods not to have effected these birds. The speed at which the population of a sedentary species can recover from mortality in winter is unclear. In more open environments, such as rice fields, a method combining permanent transects and 5 minute point counts has been used (Tongtavee et al. 1987). In the same study Barn Owls and Spotted Owlets Athene brama were monitored by regular counts of them leaving communal roosts at dusk. Tawny Owls Strix aluco have been monitored on farmland and neighbouring woods by "territory mapping" during the autumn using a tape lure (Fig. 5). Again interpretation of the results requires untreated control areas and an understanding of immigration and emigration in the population.

In Thailand, interviews have been conducted before and after organised house baiting of entire villages (Fig. 6). The data again need to be interpreted carefully; the apparent decline in chickens was due to their sale and death from other causes.

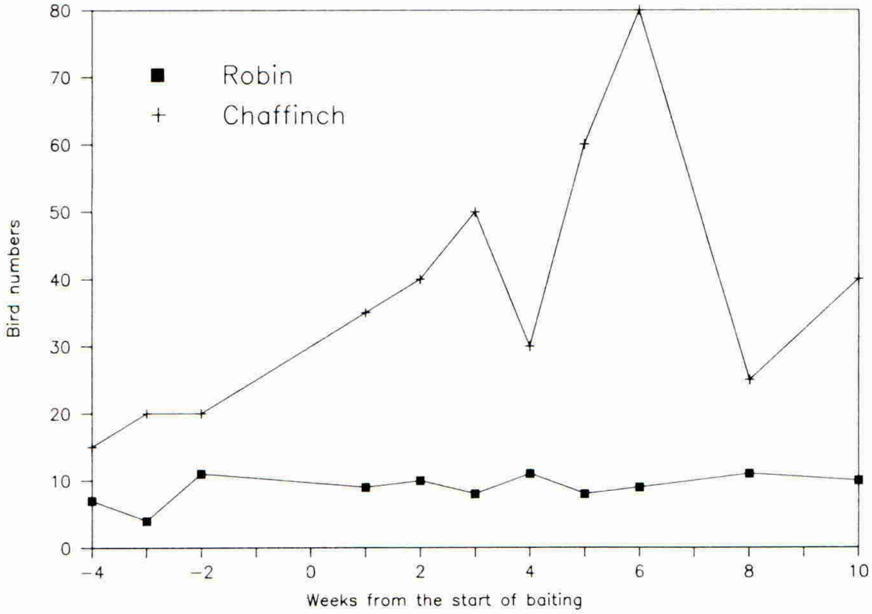

Fig. 4. Farmyard counts of small birds while baiting with wax blocks on U.K. farms.

Corpse Searches

Searches for rodenticide casualties need to be made around the baited area and in any accessible places visited by exposed animals, identified by direct observations or location of signs. For example, small flocking birds may regularly spend time in a particular stretch of hedgerow when not feeding on spilt grain at the entrance to a grain store, or Barn Owls may roost daily in the roof of a barn or village temple. Farm questionnaires have been used concurrently with field trials. Reporting of dead animals by farm staff and gamekeepers as a percentage of those found by study personnel for small/ medium sized mammals and small birds was low (0 and 16% respectively) higher for large wild birds (43%) and high for domestic free range animals (100%). For comparative purposes, when a series of trials are being conducted, e.g. with different bait formulations, it is important to record man-hours spent corpse searching to allow estimated hazard to be adjusted for searching effort. A substantial proportion of corpses may remain undetected (Balcomb 1986) or will have died in locations that are not searched or may be scavenged by other animals. This is particularly so during the early stages of rat control when infestations are heavy. In these circumstances there may be evidence for non-target corpses being taken by rats and dragged down their burrows. The use of dogs to find and recover corpses has potential for improving efficiency, as demonstrated by their use by the police, but so far they have not been used in field trials. Corpse recovery experiments have been used to establish search efficiency for deliberately placed carcasses (Balcomb 1986). This is a useful technique for training personnel and to measure scavenging rates. Accurate quantification of the search efficiency is probably not possible owing to unrealistic concealment of test carcasses and animals dying outside the searched area. Thus, corpse searching provides a qualitative and not a quantitative assessment of hazard.

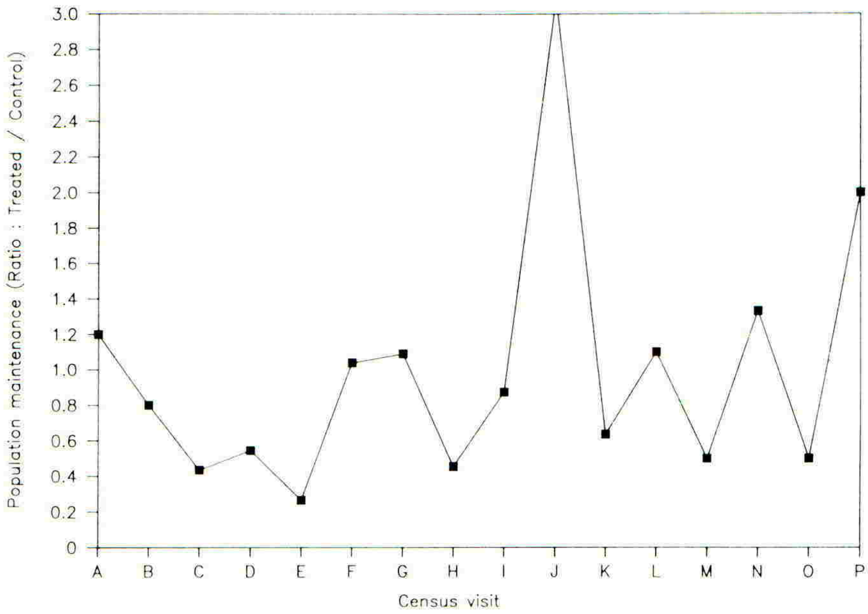

Fig. 5. A nocturnal census of Tawny Owls by territory mapping using a tape lure.

Radio-tracking

Radio-tracking is potentially the most powerful technique available, allowing both assessment of exposure and mortality. A number of animals, representing the population, are fitted with a radio-transmitter and followed to establish their interaction with baits or poisoned rodents and their subsequent fate during the study. Normally, studies have been conducted because there are no suitable alternative methods for the species concerned. Studies need to be intensive to ensure a good assessment of exposure and a high recovery rate of animals which die. Development of mortality circuits and automatic recording systems are making this technique more efficient. It is essential that the tracked population is representative of the population as a whole and that there is a low risk of losing radio-contact with individuals, through emigration, movement within a large home range, transmitter detachment and premature battery drain. Examples include the radio-tracking of Barn Owls over farmland in New Jersey (Hegdal & Blaskiewicz 1984) and Screech Owls Otus asio over orchards in Virginia (Hegdal & Colvin 1988). These studies have confirmed the importance of measuring the interaction between the target rodent and the predator.

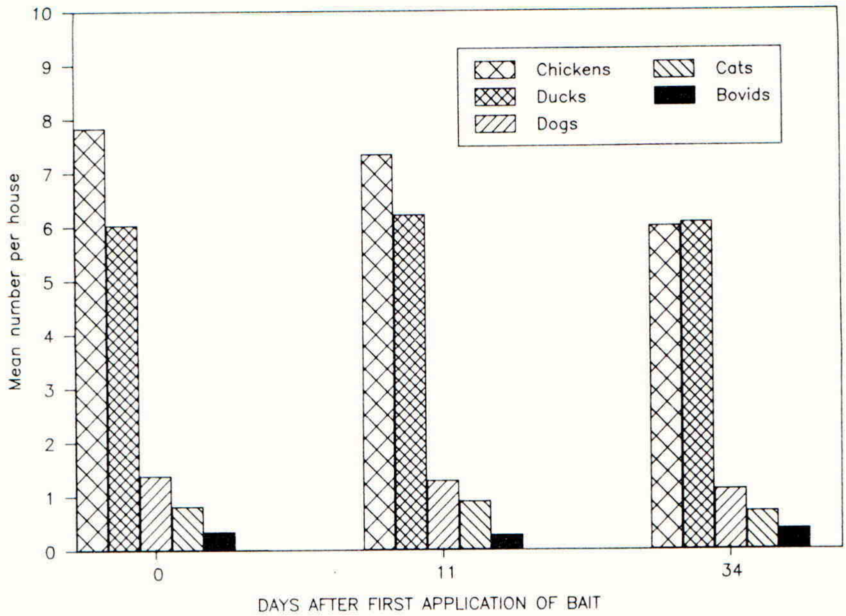

Fig. 6. A domestic animal census conducted by interviewing householders in a Thailand Village.

<u>Residues</u>

Residue methods give confirmation of exposure and an indication of the likely cause of death when lethal thresholds have been established. These thresholds can only be determined following many analyses of animals suspected of being poisoned on the evidence of autopsy findings, which in the case of anticoagulants includes haemorrhage. Liver residues of brodifacoum in wildlife suspected of being poisoned are presented in Table 3.

TABLE 3

Residues of brodifacoum in the livers of wildlife poisoned during field trials.

Type	Mean Residue ($mg\ kg^{-1}$)	Range ($mg\ kg^{-1}$)
<u>Primary Exposure</u>		
Bird	1.2	0.6-2.4 (n=9)
<u>Secondary Exposure</u>		
Mammal	1.8	0.8-2.8 (n=5)
Bird	1.4	0.4-3.8 (n=13)

Measurement of the concentration and decline of rodenticide residues in the rodent population is important for an assessment of risk. The sample population should be representative of that being taken by predators and scavengers. Residues can be measured in live-trapped and dead rodents and be expressed as a mean or median. The latter is arguably better as residues in the population are typically skewed (Brown et al. 1988) with a few very high residues associated with undigested bait. Regular sampling of live rodents may provide data on the persistence of exposure to predators. This has been done in the Philippines during a typical rat control programme in rice fields (Hoque et al. 1988). Residue analysis may be used to estimate potential risks via secondary exposure in resistant and susceptible populations of rats and from different baiting strategies, i.e. "pulsed" and "saturation" baiting (Dubock 1984).

Long Term Population Monitoring

Long term studies may be necessary when a rodenticide or group of rodenticides with similar risks are in commercial use. Monitoring should be conducted over extensive areas of representative usage. In the UK two organisations can monitor the risks from the commercial use of pesticides on wildlife. The Ministry of Agriculture, Fisheries and Food run a Wildlife Incident Investigation Scheme and the British Trust for Ornithology (B.T.O.) run several surveys which may be valuable in this context. Establishing the methods and determining a baseline population level in a specified area should be the first objective, followed by regular repeat censuses using the same method. Such a study on owls is being conducted by the B.T.O. and funded by four rodenticide manufacturers (Ciba-Geigy, ICI, Shell and Sorex).

DISCUSSION

Many of the methods described have been adapted from well established techniques and integrated into a programme to measure exposure and hazard to non-target wildlife and domestic animals and to estimate risk. Not all the methods are established and some speculative ideas are introduced, with problems highlighted. There is considerable scope for further development and validation of methods.

It is inevitable that for rodenticides, some non-target vertebrates will share a similar level of susceptibility to rodents. As a consequence exposure can result in hazard. Development of baiting methods and baits of low palatability to non-targets and their testing in the field have minimised exposure. Ultimately, environmental susceptibility of rodenticides depends on the often difficult risk/benefit analysis in each environment (Brown et al 1988).

Using the field studies described, ICI has developed bait formulations e.g. wax block baits, which present minimal risk to wildlife and domestic animals from primary exposure. The promotion of "pulsed" baiting has lowered residues in target rodents. Hazards through secondary exposure from second generation anticoagulants like bromadiolone, brodifacoum and flocoumafen can be minimised by avoiding use in environments where there is a strong interaction between contaminated rodents and carrion feeding/ predatory wildlife. Where secondary exposure may occur the data from field studies can allow governments and users to determine the risks and the benefits from using a particular product.

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DEVELOPMENT OF METHODS TO ASSESS THE HAZARDS OF A RODENTICIDE TO NON-TARGET VERTEBRATES

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ABSTRACT

The environmental hazard posed by a rodenticide is determined not only by the toxicity to non-target wildlife of the active ingredient but also their exposure to it. Toxicity can be determined in laboratory tests but exposure can best be assessed during trials simulating real use. In trials with STORM* (flocoumafen; a new anticoagulant rodenticide) on UK farms the primary hazard from the consumption of bait was estimated by visual observations of non-target activity around bait points, records of footprints on tracking boards, and carcase searching. Secondary exposure arising from the consumption of poisoned rodents was assessed using censuses and behaviour monitoring. The number and location of rodent carcases was recorded and the concentration of rodenticide in their tissues determined. The possible risk to predators was estimated from the rodenticide concentration in live-trapped rodents. Wildlife species abundance pre- and post-treatment was monitored. The cause of death in non-targets was assessed using post-mortem examinations and residue analysis.

INTRODUCTION

Rodents are important pests in and around farms in the U.K. Many different methods have been devised to control rodent pests but, in practice, effective control largely relies on the use of anticoagulant rodenticide baits. These are chronic poisons that interfere with the blood coagulation mechanism. Second generation anticoagulants such as flocoumafen are highly active rodenticides, distinguished from the first generation poisons by their effectiveness against resistant rodent strains. (Johnson and Scott, 1986).

Rodenticide baits need to have a high palatability and toxicity to effectively control rodents. They may also have a high toxicity to some non-target vertebrates. To assess the hazard to non-target species, knowledge of their exposure to the rodenticide, which is a function of the baiting technique, is just as important as knowledge of its intrinsic toxicity. The attractiveness of a rodenticide bait, and its availability to non-target species must therefore be determined. The only way of doing this is to conduct wildlife trials using the rodenticide in the same way as the proposed 'directions for use' on the commercial pack.

This paper describes some of the methods that can be used to assess the hazards to non-target vertebrates from the use of anticoagulant rodenticides on farms in the U.K. Examples are taken from field trials conducted with flocoumafen (STORM*), a new second generation anticoagulant rodenticide.

*Shell registered trade mark Site selection.

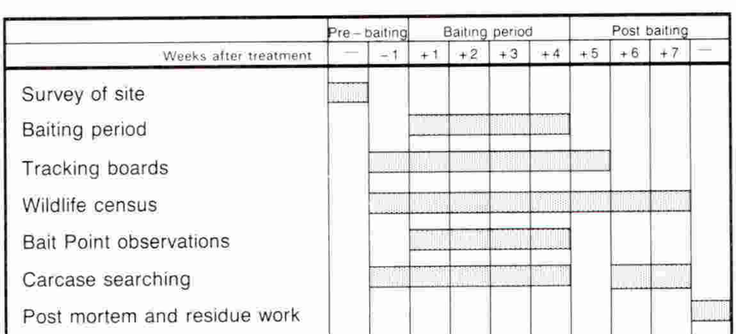

FIGURE 1

Typical trial schedule for a farm wildlife study

Rodenticides are commonly used in and around farms, and since farms and farm buildings are usually areas of wildlife activity, these provide good sites on which trials can be conducted. If possible, farms to be used for trials should combine a localised rat infestation that can be monitored, and sufficient wildlife to assess potential hazards. The farmers must be prepared to co-operate in the trial, and must not have used any other rodenticides in the previous few weeks. Trials should be carried out on a sufficient number of farms to monitor the use of the rodenticide under a range of possible conditions and situations, and if possible, control sites should also be selected representing a similar degree of attractiveness to wildlife as the sites to be baited, but these will not be subjected to rodent control.

Survey of site

Accurate maps of the trial sites are required. These maps should show the layout of buildings, roads, fields, main habitat features and positions of the bait points and can be used throughout the trial to record wildlife activity. They can also be used to mark the positions of any target and non-target carcases recovered. Aerial photographs are useful aids in producing these maps. Figure 2 shows maps of a typical trial site, illustrating the level of detail required.

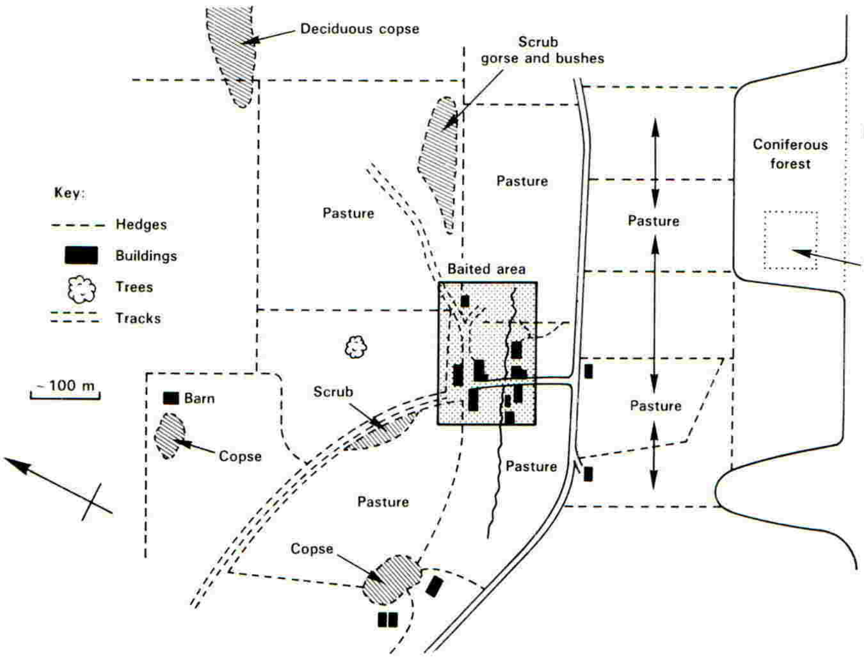

FIGURE 2a

Typical farm trial site map to show the whole site i.e. to limits of carcase searching activities

For all rodenticides the positions chosen for the bait points are critical in ensuring the effective control of a rat infestation. Marking the positions of rat runs, nest holes and foraging areas on to the maps can make this easier.

During pre-baiting visits data on rodent numbers and the abundance of wildlife are collected. This data is useful for determiming the efficacy of the rodenticide, and the possible hazards of its use.

In our trials with flocoumafen pre-baiting censuses of the rodent population, domestic animals and vertebrate wildlife were carried out to give an overview of the farm before baiting started. Normally the rodent activity is assessed by recording the weight of a non-poisonous loose grain bait eaten during a set time, and by scoring the rat footprints on tracking boards placed near bait points. These boards are usually covered with gypsum powder or other material (eg. lampblack) that shows up footprints.

Numbers, location and behaviour of vertebrate wildlife species present on each trial site are recorded. To ensure that pre-treatment deaths are not confused with those occurring during the baiting period, searches are carried out and any carcases present on the sites before baiting starts are removed.

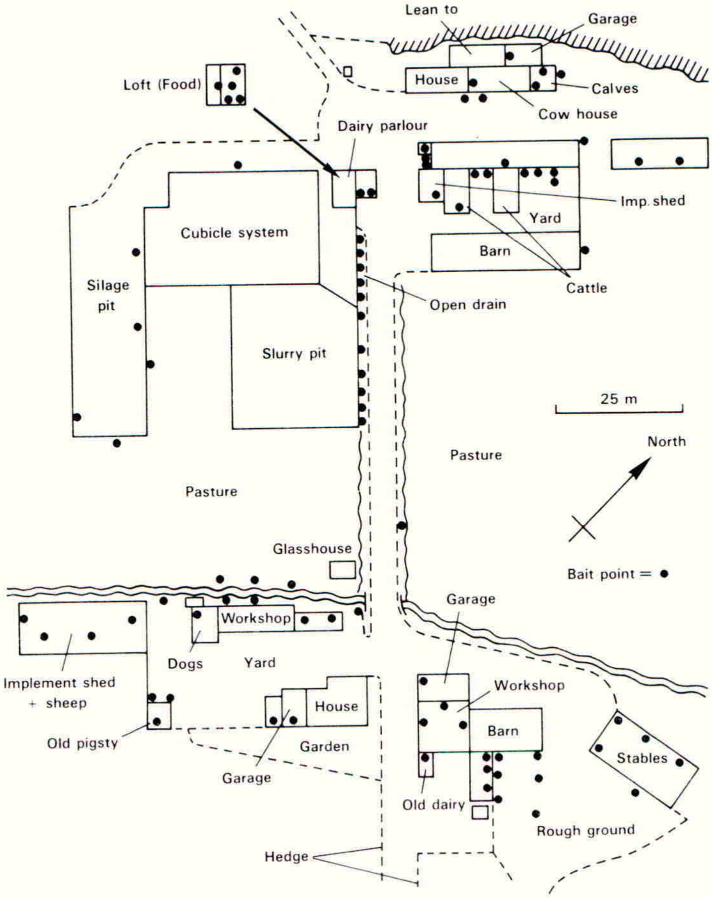

FIGURE 2b

Detailed trial site map to show positions of individual bait points in the baited area

Baiting and Tracking boards:

There are a number of different formulations of rodenticide baits that can be used, and a variety of ways in which the bait can be applied on the farms. Trials with flocoumafen have used both a loose grain bait and a wax block bait formulation, both containing 50 ppm active ingredient. The bait has been applied using a surplus baiting strategy, where large amounts of bait are in place continuously, and a pulse baiting technique where the bait is replaced only once per week. Footprint scores from tracking boards and the weight of rodenticide bait eaten are used to follow the change in rodent numbers during the trial. Figure 3 illustrates the bait consumption and tracking board scores during a typical trial, using the wax block bait formulation and a surplus baiting strategy.

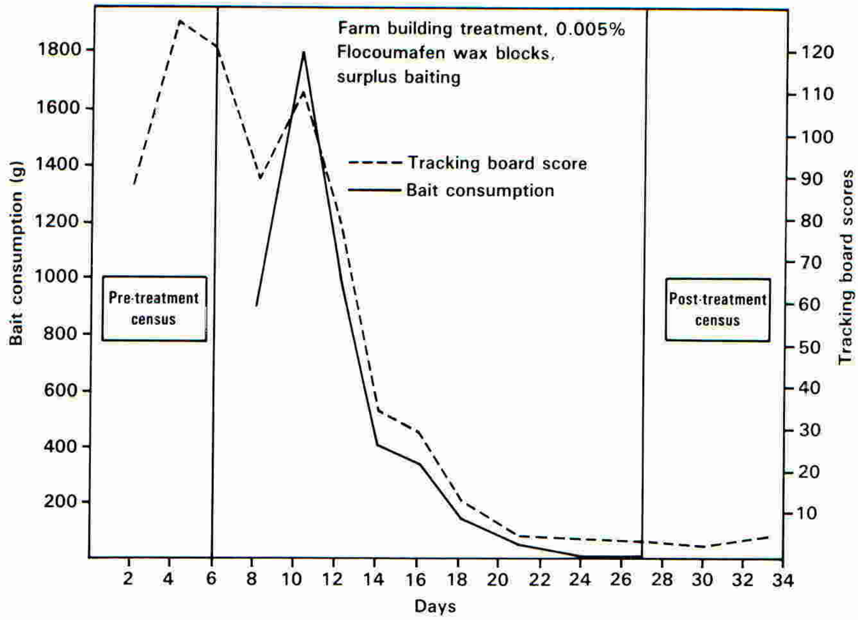

FIGURE 3

An example of bait take cd tracking board scores from a farm treated with 0.005% flocoumafen wax blocks using surplus baiting

Wildlife census and Bait point observations:

The potential exposure of non-target vertebrates to the rodenticide bait, and any gross effects of the treatment can be assessed by monitoring wildlife activity during the baiting and for a suitable post treatment period. Normally, the species are identified, numbers counted, and their behaviour in relation to the bait recorded. Wildlife censuses can be difficult and the strategy employed will be dependent on the location of the site, the season of the year and the prevailing weather conditions on the census day.

In trials with flocoumafen the farm site and buildings were explored and the adjacent land up to about 250m from the baited area was covered by walking slowly around places of wildlife activity and known bird resting and roosting sites, making observations.

The rodenticide bait used, may in some circumstances, be attractive to certain non-target species and, if available to these species during a trial might be consumed. In early trials with flocoumafen a loose grain bait was used and some bait points were observed in detail. Tracking boards were used to record bird footprints and the activity of birds close to the bait points. Any approaches to the bait itself were recorded. To provide control data non-baited points were observed and monitored by recording bird footprints on tracking boards.

Overall the data indicated birds were not attracted to the bait points or to the bait. Their proximity to bait points (and their occasional movement through bait points as shown by the tracking boards) seemed to be solely a result of their normal foraging behaviour. However a few birds were seen feeding on the loose grain bait. To obtain more information on whether birds do feed on a bait it is possible to include in it a blue marking dye which will be detectable in the droppings of birds (or other animals) that have fed on it. In trials with flocoumafen we found this approach particularly useful when plastic sheeting was placed under established roosting sites, allowing the differentiation of fresh and old droppings as well as an estimation of the proportion containing the dye. In trials with the flocoumafen loose grain bait some coloured bird droppings were located and some of the bird carcases found in these early trials showed evidence of rodenticide-related death. Post-mortem examinations revealed haemorrhages, and residue analysis revealed traces of the rodenticide.

Some baits may be more attractive to non-target vertebrates than others. As indicated above, the loose grain bait formulation of flocoumafen was consumed by some seed-eating birds that came into contact with it. To try and overcome this potential environmental hazard a wax block formulation was developed and tested in further trials. This type of formulation was originally developed more than twenty-five years ago for use in damp conditions, such as sewers, and for rodent control in tropical tree crops (Marsh and Plesse 1960, Smith 1967, Wood 1969). Rodents readily accept the flocoumafen block bait in the field, and rodent infestations are rapidly and thoroughly controlled (Garforth and Johnson 1987).

Simple field experiments were used to test the attractiveness of different wheat-based bait formulations to wild birds. Samples of six different formulations were randomly arranged on a bird table and exposed to wild birds for periods ranging from 1 – 12 hours. These formulations contained no rodenticide or added colouring. Bird tables were placed at eight different locations and the results of 35 experiments are summarised in Figure 4. The results clearly indicate that whole wax block bait was less readily taken by wild birds than any of the other formulations. The wildlife trials subsequently carried out using the wax block formulation confirmed that seed eating birds showed no interest in it, nor evidence of, consuming it.

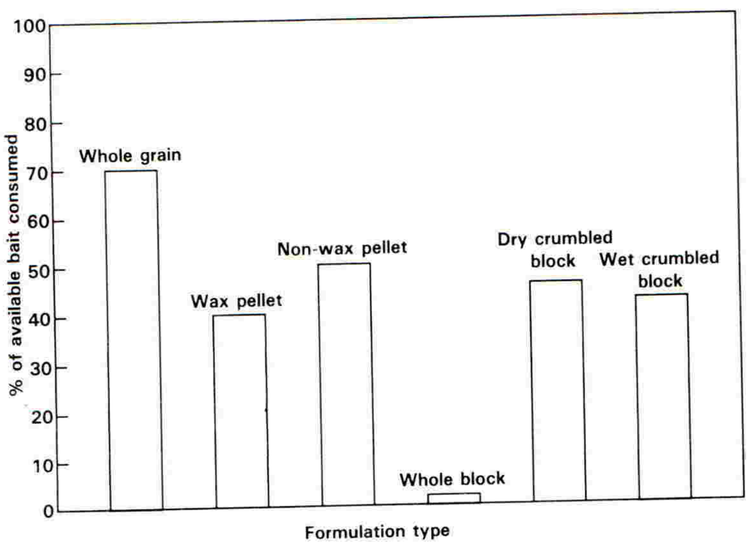

FIGURE 4

Percentages of different formulations consumed from bird table by wild birds

The exposure of wildlife to a rodenticide bait will depend on how well the bait is concealed. Bait trays should be protected from the weather and non-target activity by using lengths of pipe, tiles or slates. Bait can be spilled from the bait trays by rats, or carried to positions where they feel more secure, either to concealed places above ground or back to their nests. Problems may arise if bait is carried and dropped or spilt in exposed positions.

With the loose grain formulation of flocoumafen we found that bait spillage was small and localised, and there was no evidence of it being transported away from the bait points. However, with the wax block bait there is a greater potential for rats to carry and drop the blocks. In trials using this formulation special efforts were made to find and record details of blocks removed from the bait points and left in exposed positions. This was done during our normal farm monitoring work and by questioning farmers and farm employees.

In trials with flocoumafen on seven farms in Kent using a surplus baiting strategy with the wax block bait formulation, generally less than 5 wax blocks per site were found in exposed positions, but on one site up to 10 were found. This represents less than 1% of the blocks laid. Using the pulse baiting technique on six farms in the Welshpool area the amount of bait used to control the rats was considerably reduced. MAFF reported that 'fewer blocks than expected were removed from bait trays and left uneaten. Those lifted were normally deposited immediately beside the tray; these blocks were simply returned to the tray from which they had been taken.' (Lazarus, 1987).

Carcase searching:

The numbers and locations of target and non-target carcases are important in assessing the potential hazards of a rodenticide. Post mortem examinations and residue analysis of non-target carcases can help to determine the cause of death. The location of all carcases and the concentration of rodenticide in them provides an indication of the extent of the hazard to scavengers that may feed on them. The searches for non-targets should concentrate mainly on known bird roosting sites, hedgerows etc. and areas of regular wildlife activity. Carcase searches should be conducted frequently during the baiting period and periodically post-baiting.

In wildlife trials with flocoumafen intensive carcase searches were made at each site before the start of baiting, at weekly intervals during the baiting period and once every 3 weeks for up to 3 months post-baiting. Searches typically involved 2 – 5 people, searching for a total time of about 4 – 8 man-hours per site per occasion. Intensive carcase searches for rodents and non-target animals were conducted in-and-around farm buildings and in the vicinity up to 250m from the baited area.

Post mortem and residue work:

Post mortem examinations can be carried out on non-rodent carcases that are sufficiently well preserved, to identify;

- evidence of physical trauma;
- evidence of disease or poor condition;
- presence/absence of haemorrhage;
- contents of the gut.

Residue analysis can then be carried out on a selection of non-target carcases found at each site. Residue analysis of target and non-target carcases provides data on the concentration of the rodenticide in the animals' tissues and needs to be specific and sensitive. The residue method developed for flocoumafen is based on high performance liquid chromatography with fluorescence detection. This method is highly selective and very sensitive, having a limit of detection of 0.01 mg kg^{-1} (ppm) for each isomer.

Table 1 shows the average flocoumafen residues found in rodents killed on trials with different bait formulations and baiting strategies. The concentration of flocoumafen found in rodent carcases from trials conducted with the wax block formulation and a pulse baiting regime, is less than 50% of that seen in rodent carcases from trials using a loose grain bait and a surplus baiting method.

TABLE 1

Mean flocoumafen residues in rodents found during carcase searches

		Flocoumafen conc. (mg kg^{-1})	
Bait formulation	Baiting strategy	Rats	Mice
Loose grain	Surplus	2.1	2.1
Wax block	Surplus	1.7	2.4
Wax block	Surplus	1.2	2.6
Wax block	Restricted*	0.87	2.3
Wax block	Pulse	0.79	1.2

*bait topped up twice per week.

The positions of carcases in the baited area and immediate vicinity as well as the concentration of rodenticide residues in their tissues are important in assessing the availability of the rodenticide to scavengers. In trials with flocoumafen many of the carcases were found inside buildings or undercover, hidden from view. Using the estimates of rodent numbers from the bait censuses and tracking board scores it is calculated that only about 4% of the original rat population was found as corpses on the surface. It is assumed that the remainder died underground. Thus the amount of rodenticide available to scavengers is only a fraction of that originally laid as bait at the start of the trial. For example in trials in Kent, in which flocoumafen wax block bait and a surplus baiting strategy, were used the total net input of flocoumafen over all seven sites was 4.6 g (91kg bait) of which about 90 mg was recovered in dead rodent carcases on the surface. This represents only 2% of the net input.

Most predatory animals feed only on live prey. Since death usually occurrs a number of days after consumption of an anticoagulant bait, rodents that have fed on the bait will be active above ground for a period of time before they die. If a sample of these live rodents can be collected, residue analysis will give an estimate of the potential hazard to predators. Live small rodents (mice, voles and shrews) can be trapped using baited Longworth traps and live rats with unbaited, spring-loaded Fenn traps. Evidence from small rodents trapped during trials with flocoumafen suggest that the residues are generally much lower than those found in carcases. Residues are particularly low (rarely greater than 0.1 mg kg-1) in animals trapped outside the baited area.

CONCLUSIONS

The trials described here are aimed principally at investigating primary hazard caused by the direct consumption of bait. However, useful information can still be obtained about the likely potential for secondary hazard, resulting from the consumption of poisoned rodents by predators and scavengers. For example a knowledge of the residues of rodenticides in carcases or live trapped rodents in the baited area provides information from which the likely exposure levels of pedators and scavengers can be estimated. If this data is then combined with the data on toxicity and feeding habits a reliable assessment of the secondary hazard can be made. In circumstances where there is doubt about whether predators or scavengers are actually feeding on the treated rodents further monitoring studies will be needed. For example, the diet of raptors can be studied by marking their potential prey with chemical bone markers (Crier, 1970) and their foraging behaviour by the use of radiotelemetry techniques (Hegdal and Blaskiewciz, 1984).

The potential primary and secondary hazards will vary considerably with the baiting method and the local environment. These hazards can be minimized:

- by the safe placement of bait, protected from non-target activity;
- by using a formulation that is less attractive to non-target species;
- by using a pulse baiting regime, thereby reducing the total amount of bait placed on a farm and the rodenticide residues in carcases.

The latest trials with flocoumafen formulated as a wax block bait and using a pulse baiting regime confirm a reduction in both the primary hazard and the potential for secondary hazards to non-target wildlife, and will be the commercially recommended formulation and baiting strategy.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the contribution of Sorex Limited and ADAS, Tolworth laboratory in helping to develop the techniques described in this paper, and the assistance of colleagues at Shell Research, Sittingbourne.

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THE POTENTIAL OF RADIO-TAGGING IN TRIALS OF AGROCHEMICALS - A REVIEW

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ABSTRACT

Radio-tagging can be used to aid trials of crop protection chemicals by (i) gathering basic biological data on target and non-target species for planning purposes, (ii) monitoring the mortality rate of target and non-target species during trials, (iii) monitoring exposure and sub-lethal or indirect effects in non-target species, (iv) recovering samples of surviving or freshly dead animals for analysis of chemical loadings, and (v) quantifying the efficiency of corpse-searches which are to be used in more extensive trials. This paper describes the capability of current radio-tagging for trials of crop protection chemicals, drawing examples from its use for planning and testing the control of grey squirrels with Warfarin, and concludes that radio-tagging's potential has been under-exploited both by the agrochemical industry and by conservation interests.

THE DEVELOPMENT OF RADIO-TAGGING

Radio tagging has been available as a wildlife research technique since the early 1960s (Cochran & Lord 1963, Marshall & Kupa 1963, Verts 1963). Although equipment became commercially available in the mid 1960s, the first tags were relatively unreliable and were very often used to make qualitative observations rather than for collecting data from large numbers of animals (Lance & Watson 1980). Although tags were first used in large quantity for mortality studies of deer (Cook *et al.* 1971), pheasants (Dumke & Pils 1973) and lagomorphs (Trent & Rongstad 1974, Brand *et al.* 1975) by the early 1970s, they were still relatively heavy, expensive and unreliable below 0°C.

In the late 1970s, small lithium battery systems became available, giving 3-4 times the energy density of mercury cells (Ko 1980, Kenward 1987) and operating reliably down to -30°C. Competition between tag manufacturers has reduced tag prices (discounting inflation) and further increased tag reliability. Animals weighing more than 250g can be equipped with 12g tags, of which 90-95% will now run for at least 10 months. Tags of 6g with similar cell life are being tested for use on animals with relatively small ranges, and 1g tags transmit for 3-4 weeks on the smallest birds and mammals. Within 5 years there should be reliable 2g tags with up to 6 month life on small rodents.

The use of radio tags for mortality studies has further benefitted from the development of tag designs with minimal adverse impact on the study species, and from improved data collection techniques. Tail-mounts and ponchos or necklaces have become preferable to the original harnesses on birds (Kenward 1978, Small & Rusch 1985, Marcström *et al.* in press), and there have been advances in collar designs for mammals. "Mortality" tags can be built with internal timers, which trigger a change in signal pulse rate if an animal has not moved for a pre-set period (Kolz 1975, Lotimer 1980), and a similar effect can be obtained with light-weight temperature sensors in tags on birds and mammals (Stoddart 1970). The signals from many tag frequencies can be recorded automatically, and it will soon be possible to use low-cost portable microcomputers to monitor tagged populations, triggering an alarm when a tag signal is lost or a death indicated (review in Kenward 1987).

APPLICATIONS IN CROP PROTECTION

There are at least five ways in which radio-tagging can be used in trials of crop protection chemicals.

Recording basic biology

In the control of vertebrate pests, radio-tagging can first be used to gather biological data which indicate how a chemical can best be applied. In Britain, radio tracking has increased knowledge of several pest species which may be amenable to control by biocides, chemo-sterilants or antifeedant chemicals, including rats (*Rattus norvegicus*) and coypu (*Myocastor coypus*) on farmland (Hardy & Taylor 1980, Gosling & Baker in press),, bullfinches (*Pyrrhula pyrrhula*) and starlings (*Sturnus vulgaris*) in orchards (Greig-Smith 1985, Summers 1985), and deer (*Cervus elephas*, *Capreolus capreolus*) in plantations (Staines & Welch in press).

In Bangladesh, Poche *et al.* (1986) showed by radio-tagging that the lesser bandicoot rat (*Bandicota bengalensis*) was more important than its congeneric *B. indica* or *Rattus rattus* as a source of damage in wheat fields. They found that the home ranges of bandicoot rats were very small, so that rodenticide baits needed to be widespread and to be sited within 2m of burrow systems to be effective. In Australia, McElroy *et al.* (1986) recorded the movements of wild dogs (*Canis familiaris*), which are controlled with 1080 poison to reduce predation on livestock, and recommended the use of control zones 12-20 km wide adjacent to grazing land.

Recording mortality

Radio-tagging is ideal for recording mortality during trials of new agrochemicals, both for improving the efficiency of controlling target species, and for minimising the impact on non-target animals. Without radio-tagging, it may be difficult to detect mortality during initial field trials which cover a small area, especially for elusive or wide-ranging predator species which may be sensitive to secondary poisoning. Without the early detection of such "side-effects", there is the risk of investing large sums in further development of a product, which may be banned once it is used on a sufficient scale for the effects to become noticeable. A comparison of mortality in animals visiting treatment and control areas would give early warning of such problems, and perhaps provide the opportunity to devise safer application techniques for the chemical.

In the study of Australian wild dogs (McElroy *et al.* 1986), only two of nine radio-tagged animals were killed by the 1080, partly because the baits rapidly lost toxicity after distribution and partly because they were taken by other animals. The efficiency of the poisoning campaign needed considerable improvement.

Monitoring sub-lethal or indirect effects

Radio-tagging also has great potential for detecting chronic effects of agrochemicals on non-target animals, and for providing information to ameliorate these effects. For example, treatment of cereals with herbicides has greatly reduced populations of the grey partridge (*Perdix perdix*) by removing the food for insects which are vital for chick survival (Potts 1986). Radio-tagging showed that broods of partridges were more sedentary and survived better in fields where a 6 m wide "conservation headland" was left unsprayed (Rands 1986).

There may be risk to non-target species at particular times of year, or in certain types of site. For instance, radio-tagging has been used to determine the wintering grounds of DDE-contaminated night-herons (*Nycticorax nycticorax*) in North America (Henny & Blus 1986). If vulnerable sites or seasons can be identified, restrictions on the use of a product may be limited to those particular circumstances.

Sampling for analysis

With intensive monitoring, radio-tagged animals can be recovered shortly after death, so that tissues can be analysed for toxins. This could lead to changes in concentration of the applied chemical, either to increase efficiency of controlling target species, or to reduce risk to non-target animals. It would also provide evidence for or against the involvement of a chemical in any increased mortality of a non-target species, and enable the sampling of living animals to examine how their loadings were related to their degree of exposure.

A computer search for appropriate keywords revealed no published work in this field. However, the analysis of British woodpigeons (*Columba palumbus*), which had been caught by radio-tagged goshawks in the early 1970s, confirmed that restrictions on organochlorine use were proving effective: organochlorine loadings were too low to be used to study the role of selective predation in contaminating raptors (Kenward & Murton unpublished).

Calibrating other techniques

Although radio-tagging has not been widely used in trials of crop protection chemicals, less direct methods are widely applied. Searching an area for dead animals is a general procedure, and counts of singing birds can also be used as evidence against adverse impact on passerine populations (P. Edwards, personal communication). Radio-tagging in a limited area could be used to check the efficiency of these other methods, and to calibrate them for wider use.

CONTROL OF GREY SQUIRRELS WITH WARFARIN

Grey squirrels (*Sciurus carolinensis*) can cause serious damage by stripping bark from the trunks of young trees (Shorten 1957, Rowe 1984, Rowe & Gill 1985). Although there has been interest in developing chemo-sterilants for male squirrels (Johnson & Taitt 1983), radio tracking shows that their dramatic range expansion during courtship (Kenward 1985) could well render local treatments ineffective. Damage is prevented most effectively by killing squirrels with the anticoagulant Warfarin (Rowe 1983, Kenward *et al.* 1987), which is applied as coated grain in hoppers (Rowe 1980). Nevertheless, there is some concern about the risk of Warfarin as a direct or secondary poison for other wildlife (Marstrand 1974, Wood & Phillipson 1977). Moreover, the cost of Warfarin treatment, at approximately £2 per hectare (Kenward *et al.* 1987), becomes appreciable over the 20-30 years during which a crop may be vulnerable.

Since radio-tagged squirrels frequently foraged at pheasant feed sites in vulnerable woods, experiments were started in 1987 to see whether Warfarin could be used at these sites alone after pheasant feeding ceased, to avoid the cost of siting and pre-baiting hoppers throughout a wood. In March 1987, 40 squirrels were caught and radio-tagged at eight sites in Dorset. Two to six weeks after tagging, hoppers were laid by one or two pheasant feed rides at three sites, whereas hoppers were laid at the recommended density of one per 2 ha throughout the other five sites. The hoppers were prebaited with wheat, and whole maize spread round them for ten days before they were filled with Warfarin-coated wheat.

The trapped squirrels were equipped with 25g radio collars, which were used both for radio location and also to measure ambient temperature at the collar surface (Kenward 1982). The transmitter circuit contained a thermistor, which increased the rate of the signal pulses when the collar was warmed up in a drey. A slow signal indicated either that the animal was foraging or, if foraging was unlikely (eg at night), that it was dead. The tags therefore facilitated rapid detection of deaths and recovery of the corpses.

Since one squirrel had been killed and two radios failed before the start of poisoning, 37 were available at the start of the treatments. Placing hoppers throughout a wood was the most effective method: it killed 20 of 21 squirrels in 6 to 25 days (average 12 days) after the hoppers were filled with Warfarin (Figure 1), with no noticeable difference between sites in the mortality rate. Placing hoppers at pheasant feed sites alone killed squirrels in 4 to 16 days (average 8 days), significantly more quickly than when hoppers were spread throughout a wood (Mann-Whitney *U*-test, 2-tailed $P = 0.038$). However, only 10 out of 16 squirrels were killed at the pheasant feed sites, a lower proportion than when hoppers were more evenly distributed (Fisher exact test, 2-tailed $P = 0.019$). Radio tracking showed that the remaining 6 squirrels were not visiting feed rides where hoppers were sited. Hoppers were as effective when placed on the ground as when fixed in trees at chest height, which reduced their accessibility to badgers (*Meles meles*) and deer.

There was some difference between individual squirrels in their vulnerability to poisoning, in that breeding females tended to die first. Two died after 4 days at pheasant feed sites, compared with an average of 9 days for the other squirrels, and 5 took an average 10 days to die at the other sites, compared with 13 days for the non-breeders (Figure 1). This result complemented an earlier study, in which only one of four radio-tagged breeding females survived when Warfarin was used, compared with at least nine of fourteen other squirrels. In a one-tailed test (since breeding females would be expected to eat most, and therefore be poisoned first), the combined probability from all these observations (Fisher 1954) is close to significance at the 5% level ($Chi^2 = 12.01$ with 6 d.f.).

Of the 30 radio-tagged squirrels which died, 13 (43%) remained in their dreys. Most of the others were on the ground under their dreys. Twenty squirrels, including 3 poked from dreys, were left where they died to record the incidence of scavenging, which might lead to secondary poisoning. Five of these were eaten or cached by foxes, 3 by rats, 2 by a buzzard and 1 by a sparrowhawk, while 2 were found and removed by the gamekeeper and 7 rotted. In all, 11 of the 20 (55%) were eaten or cached by scavengers. Since squirrels which die in dreys tend to rot there (personal observations), the proportion of poisoned squirrels taken by scavengers can be estimated as 24% (43%x55%).

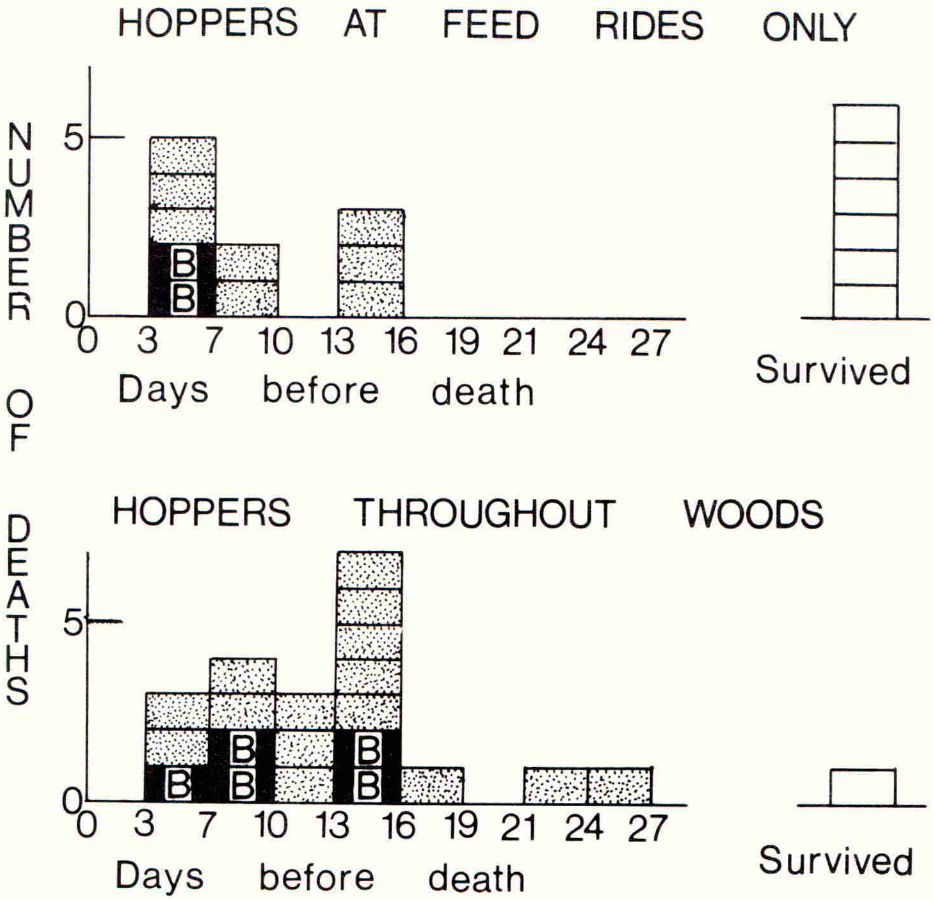

Fig. 1. The numbers of breeding female squirrels (B) and other squirrels ( ) which died during each 3-day period following the start of poisoning with Warfarin, when hoppers were laid at pheasant feed rides only or throughout woods. Numbers of squirrels which survived ( ) are shown on the right.

These data were collected in a year when the acorn crop had failed, and therefore show the impact of Warfarin under optimal conditions. They confirmed that the recommended density of 1 hopper per 2 hectares gave the best results, and also showed that the poison need only be applied for 3-4 weeks to kill most of the squirrels. They indicated that breeding females were more vulnerable than other squirrels: this is an advantage, because damage levels relate more strongly to the density of juvenile squirrels than to any other aspect of squirrel demography (Kenward & Parish 1986). Most importantly, the work cut short a build-up of damage in the areas concerned. A similar experiment in 1988, following an exceptionally good acorn crop, will provide information on the application of Warfarin when there is an abundance of natural food for squirrels.

CONCLUSIONS

In view of the detailed results which can be obtained, it is surprising that radio-tagging has not been used more widely in trials of crop-protection chemicals. The cost of equipment is not great, since £2 000 will provide 30 radio tags, which can usually be re-used, and a simple receiving system. The planning and field-work requires trained personel, but these can be obtained on contract. The entire squirrel project would have cost about £12 000 as a contract from the Institute of Terrestrial Ecology. Radio-tagging may well prove an important tool for the agrochemical industry, both to provide information which increases product efficiency, and to reduce the risks of bad publicity or loss of profit from delayed detection of hazards to wildlife.

ACKNOWLEDGEMENTS

I am very grateful to Morden Estates for providing study sites and other assistance, to the Royal Forestry Society for additional funding, to F.I.B. Doyle, F.C.P. Dutton and A. Carter for help with fieldwork, and to J. Beckett for searching the literature. Comments on the manuscript were kindly provided by Dr M.G. Morris and M.P. Greaves, with the figure drawn by S. Hayward.

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DISCUSSION

A. Hardy: Can we start with questions to Dr Kenward? He has illustrated the use of a sophisticated technique in the sort of trials and methodology we have been talking about this morning with vertebrates.

P. Mineau: What would be your recommended method of attachment of very small transmitters to small songbirds?

R. Kenward: At the moment the only effective means of attachment is with harnesses. There may be mortality associated with this and if you are going to use it in pesticide trials you will also have to use controls in an untreated area. In the future there may be better methods of attaching transmitters.

E. Harrison, Shell Research Limited: How does pheasant feeding result in more damage to trees by squirrels?

R. Kenward: It is partly because young squirrels trigger the damage. Feeding pheasants during the spring encourages squirrel breeding, even if there is not much other food. It also encourages squirrels' over-winter survival.

A. Hardy: Vertebrate pesticides, perhaps, have slightly greater longer term effects than other pesticides. How does this relate to our considerations of what is short-term and what is long-term? A number of the methods that we have been talking about have really been looking at the shorter-term effects and do not really deal with longer term implications. How good are we at tackling those?

R. Brown, ICI Plant Protection Limited: It is very difficult to construct an experiment with any pesticide to measure effects on long-term population dynamics. Any pesticide regime that is set up is, in the long term, going to be affected by environmental variables. Perhaps there is a role for models in looking at what happens in the long term and to try to make predictions. Long term experiments are certainly valuable. Monitoring during these experiments is very important as it allows reptrospective assessment of effects. However, in setting up long-term experiments, it is very difficult to control events. One conclusion that can be drawn from the vertebrate work is that we have got two approaches. One is to take large sites and look at them in great detail and the other is to use many replicates and measure only one or two simple things like carcase numbers. I wonder whether the 2-3 ha plots referred to by Dr Clements are really big enough for vertebrate studies?

A. Hardy: Several speakers have mentioned the need to tailor the design of trials to the characteristics of the compound and the biology of species likely to be at risk. In a number of areas there is no such thing as a standard protocol for maximising the chances of recording whatever impact is likely.

R. Clements: In fact the plots were whole fields and their sizes are typical of grassland fields, contrasting with the 10 to 30 hectares of arable fields.

P. Greig-Smith: With respect to long-term effects, it might be useful to distinguish between the long-term effect that a single event can have and the long-term effects that come from sustained use. These are rather different things. With sustained use, it has been suggested that models might be used to address animal population dynamics. We also need models to relate events on a single site on a single occasion to the frequency of use. This has not been explored so far.

J. Chadwick, Health and Safety Executive, Bootle: I would like to ask those who have used models whether they have achieved as much as they hoped.

P. Mineau: Yes, I think I have seen some successful attempts. One that I was involved in examined the hazards of diazinon sprayed on turf where the usual dietary toxicity perspective was not sufficient. We modelled this on the basis of acute toxicity and approximate time to death based on waterfowl landing at the site and starting to feed. In fact, the model matched very closely the times to death that were observed. I believe that there is room for site- and case-specific modelling. There are now a number of very good examples of studies looking at avian energetics using techniques such as doubly-labelled water. The case of waterfowl grazing on grass is appropriate to model because it deals with continuous food intake.

R. Cousens, Inst. Arable Crops Research, Long Ashton: One of the objections to using models is that predictions usually aren't validated experimentally. Two things do worry me in many of the studies I have heard this morning. One is that they are often done without controls. The other is that they rely on looking for trends after an application but with virtually no survey replication. Perhaps this will be addressed in the discussion workshops later in this meeting?