<u>THE EXPOSURE OF NON-TARGET WILDLIFE TO</u> <u>RODENTICIDES, WITH SPECIAL REFERENCE</u> <u>TO THE RED KITE (MILVUS MILVUS)</u>

Thesis submitted for the degree of Doctor of Philosophy at the University of Leicester

by

Craig R. Brakes BSc (Plymouth), MSc (Swansea) Department of Biology University of Leicester

October 2003

UMI Number: U179195

All rights reserved

INFORMATION TO ALL USERS

The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if material had to be removed, a note will indicate the deletion.



UMI U179195 Published by ProQuest LLC 2013. Copyright in the Dissertation held by the Author. Microform Edition © ProQuest LLC. All rights reserved. This work is protected against unauthorized copying under Title 17, United States Code.



ProQuest LLC 789 East Eisenhower Parkway P.O. Box 1346 Ann Arbor, MI 48106-1346 For my Grandmother

.

,

ABSTRACT

The Exposure of Non-Target Wildlife to Rodenticides, With Special Reference to the Red Kite (*Milvus milvus*)

Craig R. Brakes Department of Biology, University of Leicester, Leicester LE1 7RH

Factors affecting exposure of wildlife to rodenticides were studied in the field. Time lapse video monitoring demonstrated changes in rat behaviour during normal routine rat control. Rats suffering pre-lethal anticoagulant toxicosis lost their thigmotactic behaviour, becoming increasingly active in open space. Poisoned rats also sat motionless in open space for relatively long periods of time and exhibited lethargic and uncoordinated movement. The observed behavioural change is likely to increase the exposure and vulnerability of rodenticide contaminated rats to their predators. Rat carcasses disappeared rapidly where local scavengers were active. Video monitoring showed several scavenging mammals and birds taking carcasses. The red kite is potentially the avian species most at risk of secondary poisoning by rodenticides, owing to its scavenging niche and foraging around farms. Observations of captive adult birds highlighted a preference for viscera of rats, which may increase the likelihood of rodenticide exposure if rats were poisoned. Monitoring of parental provisioning of prey items to nestlings showed rats to be a major component of diet. Hatchlings were fed only the viscera, indicating that nestlings of this age may be at greatest risk of secondary poisoning. Study of non-target small mammals as routes of exposure revealed that a large proportion of local woodmouse and vole populations fed from bait stations during normal rat control. Residue analysis demonstrated substantial body burdens that may be transferred to small mammal consumers. Calculations indicated that some predators might be at a high risk of receiving a lethal dose if they foraged in a rodenticide treated site and consumed poisoned non-target small mammals.

ACKNOWLEDGEMENTS

I sincerely thank my supervisor, Robert Smith, for giving me the opportunity to perform this research and for all his advice and guidance throughout. It was a real pleasure to work under his supervision. Many thanks are due to Frank Clark for his reassuring words, advice, and in particular, for training me in small mammal capture and handling. Thank you to all the staff of the biology department, in particular; Sue, Malcolm, Helen, Maurice, Tony, Ted, Andre, Yvonne, Mina, and especially Penny, for all their practical help and kindness. Thanks also to the workshop for supplying tools and equipment when required and for fixing my Longworth traps.

I would like to thank English Nature for providing me with funding to contribute to fieldwork costs and also for funding the work on non-target small mammal rodenticide residue extraction and analysis. In particular, I would like to thank Ian Carter and Alastair Burn for the interest they have shown in my research and for the help and advice they have given.

Special thanks to Alistair Leake, Phil Jarvis and Kate Draycott at the Allerton Trust for allowing me to work unrestricted at Loddington and for making it a pleasant experience. Grateful thanks are also extended to all farmers, farm workers and landowners who allowed me to work on their land.

Many thanks to Jemima Parry-Jones and all the friendly and helpful staff at the National Birds of Prey Centre for permitting me to work there and for making my time there so enjoyable. I would also like to thank Karl Ivens of Forest Enterprise and all the RSPB staff and volunteers at the Red Kites @ Rockingham centre for all their friendly assistance.

Thank you to Ainsley Jones at the Central Science Laboratory (CSL) and Peter Farmer and John Lamb at the University of Leicester's Biocentre for their advice in the analysis of rodenticide residues and for allowing the work to be carried out in their laboratories. In particular, I would like to thank Sheonaidh Charman and Jon Roach for all their help in performing the analyses and for answering all my questions. Thank you also to Richard Shore at the Centre of Ecology and Hydrology for his advice and guidance, Mark Lambert at CSL for instructing me in the black art of making tracking plates, Colin Prescott of Reading University for supplying rats, David Houston at Glasgow University for his helpful comments on feeding preferences in wild birds and Malcolm Brown for some fieldwork assistance.

Finally, sincere thanks to Sandra, my family and friends for all their kind words and support throughout my research.

This research was funded by the Natural Environment Research Council.

CONTENTS

	Page
Dedication	i
Abstract	ii
Acknowledgements	iii
Contents	iv

Chapter 1: Introduction

1.1	The need for rat control	1
1.2	Anticoagulant rodenticides and the potential for secondary	
	poisoning of non-target species	2
1.3	Factors affecting exposure	3
1.4	Population effects	5
1.5	Justification for and scope of the presented research	7

Chapter 2: Video analysis of behavioural change in rats following rodenticide intoxication

2.1	Introduction 8		
2.2	Methods and materials		
	2.2.1	Selection of sites	10
	2.2.2	Site descriptions	11
	2.2.3	Video monitoring	15
	2.2.4	Measurement and interpretation of behavioural video	
		data	19
	2.2.5	Rodent baiting	21
	2.2.6	Population estimation	22
2.3	Result	ts	
	2.3.1	Overall activity of rats	25
	2.3.2	Feeding on bait	27
	2.3.3	Population estimation	28
	2.3.4	Thigmotactic behaviour	29
	2.3.5	Diel rhythm	32
	2.3.6	Abnormal behaviour	33
	2.3.7	Activity of predators and scavengers	35
	2.3.8	Exposure of non-target species to rodenticide	
		2.3.8.1 Primary exposure	38
		2.3.8.2 Secondary exposure	38
2.4	Discu	ssion	
	2.4.1	Rat activity	40
	2.4.2	Foraging behaviour	41
	2.4.3	Exposure of scavengers to poisoned rats	42
	2.4.4	Exposure of predators to poisoned rats	43
2.5	Concl	usions	45

Chapter 3: Rates of rat carcass disappearance and potential scavengers

3.1	Introduction	46
3.2	Methods and materials	47

3.3	Result	Results			
	3.3.1	Carcass disappearance rates	49		
	3.3.2	Carcass fate	52		
	3.3.3	Potential scavengers	52		
	3.3.4	Video monitored carcasses	53		
3.4	Discu				
	3.4.1	General discussion	56		
	3.4.2	The importance of cover	56		
	3.4.3	Blowfly infestation	57		
	3.4.4	Secondary poisoning	58		
3.5	Conclusions				

Chapter 4: Studies of the feeding behaviour of red kites when scavenging rats

4.1	4.1 Introduction		
	4.1.1	The diet of red kites	60
	4.1.2	Red kites and secondary poisoning with rodenticides	61
	4.1.3	Feeding behaviour of captive red kites when scavenging	
		rat carcasses	63
	4.1.4	The importance of rats in the diet of red kite chicks	64
4.2	Metho	ods and materials	
	4.2.1	Behavioural observation of red kites feeding in captivity	66
	4.2.2	Direct observation of wild red kite chick diet at nests in	
		Rockingham Forest, east Midlands	67
4.3	Result	ts	
	4.3.1	The feeding behaviour of captive red kites when	
		scavenging rats	
		4.3.1.1 Behaviour at the carcass	69
		4.3.1.2 Management of the carcass	69
		4.3.1.3 The respective roles of the bill and talons	70
		4.3.1.4 The area of initial attack and point of carcass entry	71
		4.3.1.5 The tissue/organ first consumed and subsequent	
		order of consumption	72
		4.3.1.6 Feeding bouts	74
		4.3.1.7 Carcass remains at end of each feeding bout and	
		final remains	74
		4.3.1.8 Pair dynamics	75
	4.3.2	The importance of rats in the diet of wild red kite chicks	
		4.3.2.1 Wild red kite chick diet	75
		4.3.2.2 Parental provisioning behaviour with regard to rats	77
		4.3.2.3 Parental foraging activity of red kite and the diel	
		rhythm of rodenticide intoxicated rats	79
4.4	Discu	ssion	
	4.4.1	Foraging behaviour	81
	4.4.2	Carcass feeding behaviour	82
	4.4.3	Tissue preferences	83
	4.4.4	Feeding behaviour on rats and secondary poisoning by	
		rodenticides	84
4.5	Concl	usions	86

Chapter 5: Non-target small mammal rodenticide exposure around farms and pheasant feeders

5.1	Introduction 8		
5.2	2 Methods and materials		
	5.2.1	Site descriptions	89
	5.2.2	Rat baiting	89
	5.2.3	Longworth small mammal trapping	89
	5.2.4	Population estimation	89
	5.2.5	Small mammal rodenticide exposure	93
	5.2.6	Control populations to allow for natural fluctuations	94
5.3	Result	ts	
	5.3.1	Evidence of small mammal exposure	96
	5.3.2	Rodenticide exposure	100
	5.3.3	Small mammal population changes	100
	5.3.4	Population recovery	104
5.4	Discu	ssion	
	5.4.1	Exposure of small mammals to rodenticide bait	107
	5.4.2	Small mammal community structure	108
	5.4.3	Small mammal population changes	109
	5.4.4	Exposure of predators and scavengers to rodenticide	112
	5.4.5	Rodenticide toxicity to predators and scavengers	115
	5.4.6	Effects of reduced prey abundance	116
	5.4.7	Sub-lethal exposure	117
	5.4.8	Effects on predator and scavenger populations	117
5.5	Concl	usions	119

Chapter 6: Analysis and quantification of rodenticide residues in non-target small mammals

6.1	Introduction		120
	6.1.1	Physiological handling and dynamics of anticoagulants	
		in mammals	120
	6.1.2	HPLC theory	122
	6.1.3	Aims and objectives	122
6.2	Metho	od and materials	
	6.2.1	Collection and preparation of small mammal carcasses	124
	6.2.2	Extraction and clean up of rodenticide residues	124
	6.2.3	Using HPLC to measure rodenticide residues	126
	6.2.4	Quality assurance	127
6.3 Results		ts	
	6.3.1	Residues measured in small mammals	129
	6.3.2	Statistical analysis: Woodmice and bank voles	135
	6.3.3	Coumatetralyl residues found in other species	137
6.4	Discu	ssion	138
	6.4.1	Residue distribution in tissues	138
	6.4.2	Residue differences between death, species and site	139
	6.4.3	Comparison with other studies	140
	6.4.4	Refining the risk assessment	141
	6.4.5	Factors that may modify risk under field conditions	143

6.5	Conclusions	145
Chapter 7: G	eneral discussion	
7.1	Rat infestations and the population effects of rodenticide	
	control	146
7.2	An ecological approach to rat control	147
7.3	The ecotoxicology of anticoagulant rodenticides	148
Appendix 1		155
Appendix 2		157
References		159

Appendix	1
Appendix	2

.

1	*P	P		IA	
	Re	fer	en	ce	S

CHAPTER 1: INTRODUCTION

1.1 The need for rat control

Many rodent species compete with humans for agricultural resources, spoil consumables, and can carry diseases deleterious to human health. The use of rodenticides to control rodents is practised throughout the world, such is the prevalence of rodents and the Microtines as pest species.

In the UK, the brown or Norway rat (Rattus norvegicus) and the house mouse (Mus domesticus) are the main commensal rodents targeted for rodenticide control. Both species are considered kleptoparasitic pests (MacDonald & Fenn 1994) because of the economic damage they cause and the potential hygiene risk they present. A rodent infestation may inflict serious damage to stored crops, in terms of consumption, fouling (entire harvests may be rejected if contaminated by rodent hairs, urine or faeces (Lund 1994)) and exposure (e.g. destruction of grain and silage packaging results in spillage and ruin, respectively). Rats are also responsible for considerable structural damage due to burrowing and gnawing. Burrows and tunnelling complexes within building foundations may result in the collapse of buildings or subsidence. Materials softer than the enamel of rodent incisors (5.5 on Mohr's hardness scale) can be gnawed, including plaster, wood, door frames, window sills, floors, textiles and electric cables (which may result in electrical faults or even fires) (Lund 1994). Rats and mice are reservoirs and vectors for a wide range and large number of parasitic diseases. Ectoparasites e.g. fleas and ticks, helminths (tapeworms), bacteria e.g. Leptospira sp. and Salmonella sp., protozoa e.g. Toxoplasma gondii, rickettsial and viral diseases, are all diseases dangerous to man and domestic livestock (Gratz 1994, Webster & MacDonald 1995).

Rodenticides, when applied correctly, are specifically targeted to control commensal rodent populations in agricultural environments and urban developments. To date, in most circumstances, the application of anticoagulant rodenticide baits is the only effective, practical and economical means of control.

1.2 Anticoagulant rodenticides and the potential for secondary poisoning of non-target species

Anticoagulant rodenticides are based on a naturally synthesised plant toxin, coumarin (the 4-hydroxy coumarin moiety) (originally isolated from mouldy sweet clover in the USA), which acts as an anticoagulant. The liver is the target organ for anticoagulant rodenticides where they act to prevent the reduction of vitamin K epoxide by inhibiting the epoxide reductase enzyme (Mount 1988), thus they interfere with the normal synthesis of vitamin K-dependent clotting factors (Hadler & Shadbolt 1975). Rodenticides are toxic to all organisms with a physiology dependent on such blood clotting mechanisms i.e. vertebrates. Rodenticides take some time to poison animals (Fletcher *et al* 1998). Their mode of action is to delay the onset of symptoms so as to prevent the intended target species, rodents, from becoming bait shy (Fletcher *et al* 1998).

Rodenticides can be categorised either as second-generation anticoagulants, e.g. difenacoum, bromadiolone, brodifacoum and flocoumafen, or as their predecessors the first-generation anticoagulants, e.g. warfarin, pindone, coumatetralyl. Second-generation rodenticides were introduced between 1975 and 1985 to replace warfarin and other long-used rodenticides, to which rats and mice had become resistant (Cowan et al 1995). The second-generation rodenticides are approximately 100-1000 times more toxic than warfarin and the other first generation compounds, a single meal of bait being sufficient to kill a rodent (Newton et al 1999). The greater potency of second-generation rodenticides in comparison to first-generation compounds is likely to be a result of a greater binding affinity to binding sites in the liver and consequently greater accumulation and persistence (Huckle *et al* 1988; Parmar et al 1987). They also have long biological half-lives in tissues such as the liver (Huckle et al 1989a,b; Parmar et al 1987). For example, flocoumafen retained within the liver is eliminated extremely slowly, with a half-life exceeding 100 days (Newton et al 1994). It is these attributes and the fact that death as a result of anticoagulant poisoning occurs after a time lag of four to nine days following ingestion (Meehan 1984), which enhance the potential hazard of secondary poisoning in scavengers and predators of poisoned rodents. Rodenticide victims typically show faint subcutaneous bleeding along the keel (of birds) and on the skull, and external bleeding around the leg joints, mouth and nostrils (Newton et al 1990), as well as internal organ haemorrhaging. However, rodenticide victims do not always show such

obvious signs of haemorrhaging and post mortems should also investigate other lines of analysis, including chemical analysis (Newton *et al* 1999).

Studies on secondary poisoning with rodenticides have shown markedly different results concerning the toxicity of both first and second generation anticoagulants in bird species, but to a small degree only for mammal species (Joermann 1998). Further, species-specific differences may also occur for each individual anticoagulant. However, it has been suggested that the risk to non-target species depends more on the application regime practised and on the feeding behaviour of non-target species rather than on the toxicity of specific rodenticides to individual species (Eason & Spurr 1995). Non-target species susceptible to consuming contaminated prey are those predatory and scavenging birds and mammals that forage in and around agricultural premises and other sites where rodent control is practised, e.g. red kite (*Milvus milvus*), barn owl (*Tyto alba*), buzzard (*Buteo buteo*), polecat (*Mustela putorius*), weasel (*Mustela nivalis*), stoat (*Mustela erminea*) and fox (*Vulpes vulpes*).

1.3 Factors affecting exposure

Following consumption of a lethal dose of bait, a rodent may not die for several days, during which time it is still active and available to predators as rodenticide contaminated prey. On death, a rodent carcass will still contain a substantial amount of anticoagulant, which may then be available to scavengers. As with the targeted primary consumers, residues in the predators/scavengers do not match the actual body burden of the prey because an essential part of the ingested anticoagulant is excreted in faeces and, in the special case of the owls, another substantial proportion in regurgitated pellets (Myllymäki et al 1999). Exposure is likely to be chronic with possible consequences of sub-lethal effects to behaviour and fitness. However, the long biological half-lives of second-generation rodenticides and their common mode of toxicity means that repeated exposure may result in both accumulation of residues and additive toxicity, thereby enhancing the potential for secondary poisoning (Newton et al 1999). Exposure may be exacerbated in areas where rodents exhibit physiological resistance to rodenticides. Increasing quantities of bait may be applied in a misguided effort to control populations in areas with high levels of resistance. Resistant rodents that gorge themselves on the bait are able to move around with few

or no ill effects and each resistant rat potentially provides a substantial parcel of poison to any predators that might attack them (Smith 1999).

The hazard of predation on contaminated rodents is likely to be enhanced following a change in behaviour of rodents as a result of anticoagulant ingestion. Cox and Smith (1992) suggest that rat foraging behaviour will shift from that which is perceived normal (i.e. thigmotaxis, the maximal use of available cover, and preference for nocturnal activity), to a pre-lethal anticoagulant toxicosis induced behaviour, which increases exposure and vulnerability to predation. The ability of predators to kill prey depends on the ease of capture and handling and therefore the issue of prey vulnerability becomes paramount (Cicero 1993).

In order to assess the risk of exposure to scavengers it is necessary to quantify the potential availability of contaminated rodent carcasses. Of the few studies attempting to examine whether moribund rodents retreat to their burrows or if they die out in the open, the results are variable and inconclusive. A laboratory-based enclosure study by Cox and Smith (1992) found that 50% of rats did not die under cover and appeared to move deliberately out from the shelter of the nest box to the open area of the enclosure. If such a behaviour is common to rats in the field, this represents a major route of rodenticide exposure to scavengers. Concerning this point, as part of a rat control campaign on a seabird colony on Langara Island, British Columbia, a radio-tracking study of 19 rats found that 13 of the 15 rats recovered died underground in their burrows (Howald et al 1999). Routine carcass searches found only 35 individuals of the estimated pre-eradication rat population of 3000 above ground, which represents 1.2% of the rat population. Other studies in rat control report similar findings (Fenn et al 1987, Taylor & Thomas 1993). If poisoned moribund rodents do retreat under cover, then the secondary poisoning hazard to avian and other larger scavengers may be substantially reduced, though they still may be accessible to smaller mammalian scavengers that can access burrows and other rodent harbourage. The apparent low numbers of rats which die above ground may be an underestimation as carcass searching studies typically have low success rates, due to the efficiency with which scavengers and predators remove the carcasses, as well as the low efficiency of human searchers (Balcomb 1986, Brown et al 1988, Mineau & Collins 1988, Linz et al 1991; cited in Howald et al 1999). In a study of songbird carcass loss rates from agricultural fields, Balcomb (1986) reported that scavenger pressure was greatest during the first 24 hours of exposure, and that the majority of

birds were scavenged without leaving readily observable remains. Thus, although evidence suggests the availability of contaminated carcasses above ground to be minimal, this may be a significant underestimation of true numbers. Research is required to investigate the fate of poisoned rats; the rate of exploitation and the consumers involved.

1.4 Population effects

Various laboratory and pen studies have demonstrated the transfer of rodenticide and secondary poisoning through feeding contaminated rodents to a variety of predatory species (Mendenhall & Pank 1980, Anon. 1982; cited in Newton et al 1999, Poché 1988, Newton et al 1990, Newton et al 1994, Gray et al 1994). It is clear that rodenticide levels in prey can cause mortality in predators when poisoned rodents form a substantial part of the diet. For example, weasels fed poisoned mice that contained average residue levels of 3.33 μ g.g⁻¹ of difenacoum and 2.21 μ g.g⁻¹ of brodifacoum died after 9-33 and 16-52 days respectively (Anon. 1982). Such secondary poisoning can occur under field conditions (Newton et al 1999), but the level at which it becomes significant in terms of population viability may depend on both the specific foraging behaviour of the non-target species and local environmental conditions. For example, a study of the foraging behaviour of barn owls on farmland in the United States (Hegdal & Blaskiewicz 1984) showed that while baiting with brodifacoum in and around farm buildings occurred, owls were not affected as they foraged for rodents in grassland away from the farm buildings. In contrast, a high proportion of barn owl carcasses from the Isle of Man and the Channel Islands were contaminated, as on these islands field voles (Microtus sp.) and other alternative prey are lacking and owls feed much more heavily on commensal Norway rats and house mice (Newton et al 1999). Predatory and scavenging species that rely on rodents as a major constituent of the diet are certainly at risk, and of those it is the opportunistic predators/scavengers foraging in areas where rodent control is practised that are most at risk.

Analysis of carcasses of predatory and scavenging species has provided evidence that a substantial proportion are contaminated with rodenticide (Shore *et al* 1996, McDonald *et al* 1998, Newton *et al* 1999, Shore *et al* 2000, Shore *et al* 2001). For example, of the 717 barn owls analysed by the Centre of Ecology and Hydrology (formerly the Institute of Terrestrial Ecology, ITE) during 1983-96, 187 (26%)

contained detectable residues of second-generation rodenticides in the liver (Newton et al 1999); and, in a study of polecats in western England rodenticide residues were found in 31% of the carcasses analysed (Shore et al 1996). These species are predominantly predators. A similar analysis on a sample of red kite livers reported rodenticide residues in 70% (Shore et al 2000), more than twice that reported for the barn owl and polecat samples, suggesting that as a scavenger, the red kite is more vulnerable to exposure. Rats do form a significant diet component for red kites (Carter & Grice 2002) and polecats (Blandford 1987) and, in some areas, for barn owls (Glue 1974), indicating their implication as the source of exposure for these species. However, surveys of rodenticide contamination in kestrels (Falco tinnunculus) (Shore et al 2001), stoats (Mustela erminea) and weasels (Mustela nivalis) (McDonald et al 1998) have shown that a large proportion of samples, of species that do not normally predate the target species, also contain rodenticide residues. Barn owls, kestrels, stoats and weasels are specialist predators of non-target small mammals (a collective term used here to mean wood mice (Apodemus sylvaticus), bank voles (Clethrionomys glareolus) and field voles (Microtus agrestis)). Research is required to determine whether small mammals are exposed to rodenticide and to quantify their importance as a route of exposure to their consumers.

Only a small percentage of carcasses recovered are diagnosed as having died from rodenticide poisoning, the majority containing only sub-lethal levels. However, it must be noted that a reduction in fitness as a result of sub-lethal rodenticide toxicosis may increase the likelihood of mortality from other causes. Further, there may be an under-estimation of both the level of exposure and the number of deaths attributable to rodenticide. Carcasses that are most likely to be found and sent in for analysis are those killed suddenly by trauma, particularly collision with vehicles (Carter & Burn 2000). Poisoned birds may retreat to concealed roost sites and poisoned animals may die out of sight, where they are not easily discovered by the casual observer (Birks 1998, Newton et al 1999). In the UK, the Wildlife Incident Investigation Scheme (WIIS) investigates deaths of wildlife, including beneficial insects, pets and some livestock, where there is strong evidence that pesticide poisoning may be involved (Fletcher et al 1998). In 1997 there were 27 incidents involving seven rodenticides: Seven involved deliberate abuse, nine misuse, and in eleven the cause was not established (Fletcher et al 1998). Strict criteria are applied to potential incidents prior to acceptance to prevent large numbers of animals being

submitted for analysis and investigation (Fletcher *et al* 1998), and again the scheme depends on members of the public discovering and submitting carcasses (Birks 1998). At present, the numbers of recorded deaths as a result of rodenticide poisoning are relatively low in all non-target species concerned and, currently, there is no evidence of significant effects on populations. Certainly, however, current monitoring does not reflect the true level of contamination (Birks 1998), and dedicated studies are required to determine whether or not secondary rodenticide poisoning is more intensive and extensive than current evidence suggests.

1.5 Justification for and scope of the presented research

The untargeted mode of action and physiological persistence of anticoagulant rodenticides has engendered hazardous exposure to a wide range of non-target wildlife via the food web of species that forage in proximity to treatment areas. A considerable amount of laboratory research has been conducted on the toxicities of rodenticides to various non-target species and on assessments of primary and secondary exposure (Joermann 1998). Monitoring is valuable in providing information on species exposed and the level of exposure (Shore *et al* 1996, McDonald *et al* 1998, Newton *et al* 1999, Shore *et al* 2000, Shore *et al* 2001). It is field research, however, that is crucial in investigating the routes of exposure in order to properly assess the risk to populations of non-target species and also to develop strategies for risk mitigation.

The aim of this research was to perform field studies that would fill important gaps in current knowledge of rodenticide ecotoxicology, providing an enhanced understanding of exposure and contributing to risk assessment.

Chapter 2 investigates the behavioural change in free-living poisoned rats.
Chapter 3 studies the rate of loss and potential scavengers of rat carcasses on farms.
Chapter 4 provides studies in red kite feeding behaviour with regard to rats.
Chapter 5 investigates the role of non-target small mammals as routes of exposure.
Chapter 6 provides data of residue levels in non-target small mammals.

CHAPTER 2: VIDEO ANALYSIS OF BEHAVIOURAL CHANGE IN RATS FOLLOWING RODENTICIDE INTOXICATION

2.1 INTRODUCTION

The home range of a rat (Rattus norvegicus) will comprise harbourage, food and water sources, and access to mates. Its extent may vary depending on the proximity of these resources, ranging from, for example, about 30m in diameter (Davis et al 1948) to several hundred metres (Taylor 1978). Rats develop an intimate knowledge of their home range. Wild rats move in response to an immediate need, and also have a highly developed exploratory instinct; exploration and re-exploration are crucial in order to provide information about the resources and dangers of the environment (Barnett 1963). Movement and orientation within the home range are directed by two principal mechanisms; thigmotaxis and kinaesthesis. Thigmotaxis or 'movement orientated by tactile stimuli' defines a rat's tendency to move in contact with a vertical surface; rats also prefer to eat in a corner rather than an open space (Barnett 1963). It remains unclear whether the behaviour is innate (Crozier 1928) or learned (Patrick & Laughlin 1934). Kinaesthesis or 'muscle sense' defines the ability of an animal to learn all parts of its environment by bodily contact alone, through the subconscious memory of sequences of muscle movements (Meehan 1984). Adaptations such as these evolve because they enhance survival. Thigmotactic behaviour reduces the risk of predation and kinaesthesis improves flight efficiency and predator escape. Predation pressure is also undoubtedly responsible for the nocturnal activity of rats; diurnal predation is normally a greater risk than nocturnal predation.

Anticoagulant rodenticides have been shown to exert a pre-lethal behavioural change in rats, manifested as a loss of thigmotactic behaviour and an alteration in diel rhythm (Cox 1991, Cox & Smith 1992). During their cage and enclosure trials, Cox & Smith (1992) also noted a change in startle response from bolt to freeze, staggering movement and sluggish reactions. Under pre-lethal anticoagulant toxicosis, the reversal of behaviours, which normally act to reduce predation risk, will increase exposure and vulnerability to predation. Predators are therefore increasingly liable to rodenticide exposure and the risk of secondary poisoning through eating poisoned rats.

Cox & Smith (1992) stressed the need to extend their behavioural studies to the field. MacVicker (1998) performed a limited set of observations of farm rats feeding in barns using time-lapse video monitoring. With regard to changes in the diel

rhythm and the exhibition of abnormal behaviours MacVicker (1998) did not confirm the results of Cox & Smith (1992), and a change in thigmotaxis as an effect of anticoagulant rodenticide was not observed.

The new research presented here builds on these studies, but is based on freeliving populations of wild rats, inhabiting (outdoor) agricultural environments where associated predators and scavengers may forage. The research was based on remote video-surveillance as a means of monitoring any changes in rat behaviour during a period of rodenticide treatment. The research tested the hypothesis that, following application of anticoagulant rodenticide, rat foraging behaviour will shift from that which is perceived normal (i.e. thigmotaxis, the maximal use of available cover, and preference for nocturnal activity), to altered behaviour induced pre-lethally by anticoagulant toxicosis, which increases exposure and vulnerability to predation.

2.2 METHODS & MATERIALS

2.2.1 Selection of sites

In order to test the hypotheses, study sites were required to meet a number of criteria:

- A site with a large population of rats (infestation), the nest site and main habitual foraging areas for which are discrete and can be located through survey.
- A site with no current programme of rodent control in operation, but where the site owner required rodent control.
- The permission, cooperation, trust and non-interference of the site owner.
- The facility to set up and position the video camera and infrared lamp trained on a particular area, which will be left undisturbed.
- A reliable and safe electrical supply, which can serve the requirements of the video monitoring equipment.
- Appropriate secure housing for the safe storage of video monitoring equipment.

Within the study site the area chosen for video monitoring should fulfil the following criteria:

- 1) An area of high rat activity;
- composing an open outside area situated far from human and mechanical disturbance.
- accessible to both avian and terrestrial predators.
- adjacent to mediums of cover such as building sides, walls, vegetation etc.
- 2) An area suitable for the placement of several bait stations;
- ideally no other source of alternative food supply will be available to rats in the vicinity of the bait stations.
- bait stations must be left undisturbed and unavailable to domestic animals.

A considerable amount of time and effort was invested in searching for, and identifying, suitable study sites for this research. Indeed, for the duration of this research degree, it has been a constant and relentless exercise. Despite a large number of informed and helpful contacts and visits to a great many and varied farm sites, all but two were found to be unsuitable. The major problem was that few farms seemed to have a severe rat problem in the Leicestershire / Northamptonshire area. Most of the farms visited were relatively clean, tidy and well kept, being free of rat harbourage and available food, and already practicing an established rat control programme. Very often farms that had rat problems were either too risky with respect to equipment safety or did not have a proximate electrical source. In some cases all study site criteria appeared to be satisfied, yet only after introduction of the video equipment and the preliminary observations of rat activity could it be confirmed whether or not the site was completely suitable. A notable failure concerned a farm site that, although it had a sizeable rat population, was found to have no definitive area of high rat activity in which to site the video camera. This particular area looked promising, but led to the waste of two months fieldwork time.

At the time of the foot and mouth disease (FMD) epidemic in 2001, the first farm trial was being carried out. In line with MAFF and English Nature guidelines fieldwork was suspended and the trial had to be terminated following six weeks preparation and data collection. The trial was restarted at a later date on the lifting of fieldwork restrictions, with permission of the landowner and the institution of appropriate disinfectant procedures. FMD delayed the start of this part of the study by four months.

2.2.2 Site descriptions

Two trials produced sufficient data for quantitative analysis. The first trial was carried out at Farm 1, a recently derelict mixed farm operating as an organic produce supplier, during May/June 2001. The second trial was carried out at Farm 2, a working pig farm, during May/June/July 2002. Thorough and accurate site surveys were needed in order to produce site maps. Figures 2.1 and 2.2 illustrate the layouts of the farms, the main habitat features, the positions of rat burrows and the baited areas.

Farm 1 exhibited a history of mixed farming, comprising disused pig units, a cow shed, grain storage units and a feed mill. Only a small number of buildings, including the cow shed and main barn, were still being used, essentially only as

Figure 2.1 Site Map of Farm 1.







storage for imported organic goods prior to dispatch. The vast majority of the farm was not in use and remained undisturbed. The local environment appeared ideal for rats. The site was bounded by 2m high earth banks overgrown with scrub in which large colonies of rats had infested. Further cover and harbourage was available around the farm in the form of large amounts of accumulated 'farm junk' facilitating the free movement of rats around the farm. In addition to the water resource resulting from poor drainage at various points around the farm, freshwater was also available in the form of a lake at the farm's centre. As the rats did not represent any immediate economic threat, no rat control programme was in place. The main focus for rat activity was the derelict mill, situated towards the rear of the farm, completely undisturbed by farming activity. This was clearly evident through the copious amounts of droppings, numerous rat runs linking the mill to adjacent burrows, and the observation of rats diurnally active in the mill on site visits. Spilled grain and milling waste probably represented the main food supply for the rats, and clearly attracted them to the mill. This site was chosen for baiting and video monitoring, as it was the hotspot for rat activity on Farm 1.

Figure 2.3 illustrates the baited area at Farm 1, including a plan of the mill, bait points, rat runs and the video monitoring area. The video camera was set to record the behaviour of rats moving between the main rat burrow site and the mill. Baiting was carried out according to principles of best practice as in a normal rat control operation. The majority of bait points were set within the mill, to reduce the risk of non-target exposure, in addition to routine measures such as covering all bait points. The video monitoring area fulfilled the criteria essential in measuring any behavioural changes in the rats, incorporating a proportion of edge, cover and open space.

Farm 2 was an intensively operated pig farm comprising a number of pig units (separately housing breeding and fattening stock), a feed-formulation barn, a barn containing straw bales and an extensive manure heap. Rats were a serious pest here, causing extensive structural and mechanical damage to pig units, as potential vectors of disease to pigs, and in the consumption of pig feed. Infestations were clearly evident both within and behind the pig-breeding unit, and around the manure heap. Rat control, in the form of shooting, trapping and poison baiting, was ongoing at the pig-breeding unit, where the effects of the infestation were most damaging and visible. Rat control was not practiced around the manure heap. Survey and farm

worker's experience indicated that the two infestations represented separate local populations or clans. The unmanaged population at the manure heap, however, would serve as the source for reinvasion into the pig-breeding unit, ensuring that any control achieved there would only be temporary.

This study focused on the rat population based around the manure heap. No control was practiced here, the site was relatively undisturbed being situated at the rear of the farm, and a suitable area of moderately high rat activity was available for video monitoring. The burrows of this rat population were located in earth banks surrounding the manure heap and in areas of raised ground along the boundary of the wheat field. Overgrown scrub on and around the manure heap provided cover, and rat runs were also present through the wheat crop. It was likely that the rats foraged on the manure heap, feeding on undigested pig feed and invertebrates. Where the rats were active in the wheat crop, much of the grain had been eaten. Water was available as a result of poor drainage in the vicinity and rainwater also collected in the disused pigsty.

Figure 2.4 illustrates the baited area at Farm 2, showing the layout of the main features, bait points, rat runs and the video monitoring area. The area selected for video monitoring included an open patch of grass between the manure heap and the straw barn, which provided proportions of edge and cover. Fresh rat runs were located leading to, and around the edge of, this area, indicating its importance as a main conduit for foraging rats. Covered bait points were set close to all burrows and along rat runs to promote take, but were concentrated around the video monitoring area where there were high levels of activity.

2.2.3 Video monitoring

Remote video monitoring equipment is an extremely valuable tool in the study of wildlife in the field. The rat is an elusive, highly vigilant and largely nocturnal species. A study of behaviour such as this would not have been possible without the use of time-lapse video equipment.

The advantages of a video monitoring system include; easy habituation by the study animal, observation day and night in all conditions, and an almost continuous and permanent record of activity (Stewart *et al* 1997). Once initial neophobia with regard to site preparation and bait point placement had been overcome, rats did not appear to exhibit any behavioural response to the continued presence of a video

Figure 2.3 Map of baited area on Farm 1.



Figure 2.4 Map of baited area on Farm 2.



Key:



camera. Observation at night was possible using a video camera sensitive to infra red light. Infra red light is outside the range of vision of a rat, allowing coverage of rat activity during its peak without influencing behaviour through the use of visible sources of illumination. The use of a time-lapse video function allows continuous recording over a far greater period of time than conventional recording speed. Some disadvantages of a video monitoring system include; the high initial cost of equipment, observation restricted within a limited arena due to the fixed position of the camera, and, unless properly concealed, the security of attractive electronic appliances (Stewart *et al* 1997).

In both trials, the video monitoring area was situated within reach of a mains 240V a.c. power supply. A 100m length of armoured cable was laid and visibly marked. This powered the entire system and had a residual current device circuit breaker as an added safety precaution. Figure 2.5 illustrates a schematic of the video monitoring system used in the trials. The camera used was a monochrome video camera (Model; NCL 1100 'Ultimate' low light, 0.02 lux), with an auto-iris wide-angle lens sensitive to infra red light. The lens and circuit board were housed in a weatherproof cover and mounted on a tripod. The video camera was connected to a time-lapse video cassette recorder (VCR) (Model; Hitachi 480 Lr VTL 2000E) set at a resolution of 2 frames per second (125 frames per minute), allowing 96 hours of recording time from a 4-hour cassette. Infra red illumination of the arena at night was provided by a weatherproof infra red floodlight (Model; Dennard 300 W, halogen bulb). The lamp incorporated a photocell enabling it to be switched on and off automatically, at dusk and dawn respectively.

To prevent tampering and theft, each piece of equipment was securely attached to adjacent immovable objects using padlocked chains and security cables. Additionally, the video camera and tripod were covered in camouflage netting. Measures taken to protect equipment from the elements included; housing the VCR in a waterproof box, stabilising the camera tripod against the wind and ensuring all electrical connections were sealed and waterproofed. Video monitoring at a site of a rat infestation presents its own unique problem; namely, the probability that rats will chew cables and damage the equipment. As a preventative measure all exposed equipment cables were fed through hosepipe and, where possible, suspended. The mains power cable was steel armoured and so sufficiently protected.

Prior to setting up in the field, all equipment was tested in the laboratory for safety, performance and reliability. During field assembly, great care was taken to minimise site disturbance; for example, equipment was positioned so as not to obstruct rat runs. A period of field testing was then required in order to ensure the desired field of view and optimal picture quality. This included adjustments in positioning, direction and focus of both the video camera and infra red lamp.



Figure 2.5 Diagram of video monitoring set-up

2.2.4 Measurement and interpretation of behavioural video data

Video monitoring generated eight 4-hour video tapes of behavioural data in each trial representing 768 hours of continuous recording. Videos were analysed in playback on a VCR with a jog-shuttle facility (Model; Hitachi VT-5890E) permitting detailed analysis in frame-by-frame slow motion, when necessary. The behaviours chosen for analysis are those pertinent in testing the hypotheses; categorised and defined in Table 2.1. Behaviour was manually digitised under a continuous sampling protocol split into sequential five minute sample intervals, for later analysis.

Measure	Definition
Date & Time	Date & time (BST) function displayed and imprinted on picture
Weather conditions & Temperature	Main weather conditions observable via camera & temperature records
Number of Rats	Maximum number of individual rats observed at any one time during sample interval
Movement or activity at Edge	Activity within < 20cm of vertical surfaces of features in filming arena (e.g. walls, vegetation line, cover)
Movement or activity in Open	Activity beyond > 20cm of vertical surfaces of features in filming arena
Abnormal behaviour	Motionless in open space > 20 seconds (i.e. not active; drinking, eating) or clear exhibition of ill health
Predator incidence	Observation of potential predators & scavengers of rats in filming arena
Notes	Supplementary notes where required on behaviours and other points of interest

Table 2.1 Behavioural measures in video analysis

Although the study would not have been possible without the utilisation of video monitoring, certain aspects of behavioural observation and measurement were limited by the method, and also by the standard of equipment available. It was not possible to identify rats individually so if, for example, a rat moved out of the camera's field of view and then re-entered, it could not be confirmed as the same rat. As a result, all activity recordings are representative of the population as a whole. Although desired, the measurement of particular behaviours in high detail and the observation of more subtle behaviours were not possible. Using a monochrome camera to record in time lapse (playback of time-lapse video is 'jerky' and can be distorted) significantly reduces picture quality and information. Where a specific image was required or a particularly interesting event occurred, the VCR could be connected to a computer and a video 'frame-grabber' used to capture and produce hard copies of individual frames.

During video analysis, a behavioural measurement was only recorded if it was completely certain. The behaviours measured here, although crude, satisfy the hypotheses and confidence can be placed in the results. Any changes in behaviour during rodenticide treatments were defined by reference to 'normal' behaviour during the pre-bait periods. The period of normal behaviour therefore represented an internal control.

Determination of whether a population has changed after a perturbation to the environment (in this case, the effects of rodenticide on the rat population) ideally requires a number of replications and appropriate statistical analyses (Stewart-Oaten *et al* 1992). In this study, the low number of replicates and the inherent site differences between replicates prevented conventional statistical analyses. Ecosystem-level and other large-scale experiments, such as these, often result in unreplicated perturbations, the statistical assessment of which is problematic (Stewart-Oaten *et al* 1992).

2.2.5 Rodent baiting

Initially, sites were surveyed for rat activity in order to define the boundaries of the study area and to indicate the best areas for bait placement. Bait points were set close to burrows and in areas of concentrated rat activity, determined by the presence of runs and fresh droppings. Specifically, points were set alongside runs in spots sheltered by vegetation, natural objects or undisturbed farmyard junk. Between 15 and 30 bait points were set, with number and spacing dependent on the extent and density of rat populations at specific sites. Bait points consisted of a plastic bait tray placed inside a wooden box $(40 \text{cm} \times 15 \text{cm} \times 15 \text{cm})$ with openings at both ends. Rectangles of hardboard were set against the ends of bait boxes at an angle and weighted down, in order to prevent feeding by birds, notably pheasants. The active ingredient in the rodenticide bait used in all trials was coumatetralyl (375 ppm) (trade name; Racumin), a first-generation, multiple-dose rodenticide. Coumatetralyl was chosen because of the low toxicity to birds associated with first-generation compounds. Avoidance of potential secondary poisoning was especially important since the trials were carried out within the foraging range of a recently reintroduced red kite (*Milvus milvus*) population.

Bait points were pre-baited with non-poisoned grain at the start of the study and the video monitoring of normal rat behaviour. It was deemed necessary to position bait boxes at this time to allow the rats to become familiar with their presence, and to pre-bait, as rats are notoriously neophobic about exploiting a novel food source (Barnett 1963). Following a week of pre-baiting, the pre-bait was replaced with rodenticide bait. Initially, 100g were laid at each bait point, and if, on daily inspection, all 100g were consumed (complete take), the quantity of bait was doubled to 200g. Where takes were partial, containers were topped up to 100g or 200g every four days to maintain a surplus. This surplus-baiting strategy is a standard approach to rodent control using first-generation anticoagulants (Buckle 1994) and is specified on rodenticide labels. A record of bait take was kept using digital scales.

2.2.6 Population estimation

A census technique was required in order to measure the efficacy of rodent baiting and to assess the population change over the baiting period. Video monitoring provided some measure of the changes in rat activity, but this would be unreliable as a census method had rats, for example, altered their activity patterns. Changes in bait take provide an indication of population change, but this method is flawed as a census technique as it assumes that every rat is attracted to and feeds from bait. A census based on bait take alone would be complicated further if some individuals were resistant to rodenticides. The census method on which population estimation was ultimately based was tracking. The use of tracking tiles to estimate population size represents an independent census technique based on the population size and its activity. Weather-resistant tracking plates have been designed and tested by Shepherd & Greaves (1984). The method is applied and the population size estimation

Tracking plates, measuring 100 x 200mm, were cut from vinyl floor tiles (B&Q Ltd) and covered with adhesive book binding film (Staples Ltd). A 5% solution of activated carbon powder (Fisher Scientific) suspended in methanol was then lightly brushed onto the surface of the tiles, which had been lightly roughened with sand paper to prevent crazing. As the methanol evaporates, a thin, evenly coated film of carbon powder remains, which is removed on contact, leaving, in this case, a footprint record (Plate 2.1). The area of rat activity defined for each farm was divided into 10 x 10m squares. Using the site maps (rat burrows, runs and areas of activity) and

supplementary detailed information on rat signs, up to four tracking plates per square were placed individually along perceived segregated runs. It was essential that only one plate was placed per run, in an attempt to ensure that all rats were accounted for and to reduce the chances of the same rat marking several plates. Two activity indices were recorded, one based on whether a plate was marked or not (binary index) and the other on a 4-point scoring system (referred to as a 4-point index):

.

0	=	no prints
1	=	1-25% of plate covered by prints
2	=	26-95% covered
3	=	96-100% covered

(Quy et al 1993)

Once *in situ*, the plates were inspected, scored and recoated every 24 hours for four consecutive days. Summation of plate scores provided a daily measure of rat activity on each of the four days, from which the mean was taken. Quy et al (1993) provide a linear regression model based on the results of 14 farm trials testing their methodology. The equation (y = 1.77x - 30.21) can be used to estimate the size of rat populations before and after rodenticide treatments, based on the 4-point scoring system (y=track score index, and x=population size). Although the farms used in their study were located in southern England, the farm sites used in this study are broadly similar. This method was used to provide population estimates at the beginning and end of the control periods on both sites. Any change in rat activity and population size could then be calculated.

Plate 2.1 Rat footprints on a tracking tile



2.3 RESULTS

2.3.1 Overall activity of rats

Each and every registration of a rat entering the video-monitoring area was measured as a count of rat activity. Figures 2.6 and 2.7 illustrate the changes in rat activity over the study period at Farms 1 and 2 respectively.

Figure 2.6 Rat activity on Farm 1; measured as the count of the number of times any rat entered the video monitoring area during a 24h period.







At Farm 1 an initial sharp decline in activity was followed by a comparatively sharp increase in activity to prior levels. The decline in activity is interpreted as a neophobic response to the introduction of bait boxes. Four days after the introduction of rodenticide bait there was a sharp decline in activity (3/6-4/6). This decline was then followed by two smaller momentary increases then declines in activity. These changes in activity are interpreted with the application of concurrent observations on rat size, corresponding to social dominance.

Initially, it was observed that the majority of feeding bouts at visible bait points involved relatively large, apparently dominant, individuals. Smaller, apparently subordinate, rats were seen, but they either fed minimally at bait points outside periods of peak activity or, as was observed on numerous occasions, they were aggressively excluded from bait points by larger rats. Thus, the first decline in activity following rodenticide application may have been the result of mortality of the larger dominant rats. The removal of the dominant individuals would then permit the subordinate rats unconfined access to bait stations. Following further periods of about four days, the two smaller declines in activity may have been a result of mortality events involving the subordinate rats (12/6-13/6 and 16/6-19/6). Following the mortality of the subordinate rats and observed minimal site activity, it was assumed that the population was close to being controlled. However, a rapid increase in counts of apparently 'new' larger rats indicated a clear case of reinvasion (20/6-22/6). The subsequent drop in activity was likely to be a result of their mortality.

Activity at Farm 2 followed an apparently less complicated profile. There was initially a lower level of rat activity in the video-monitoring area than at Farm 1. A similar decline and subsequent rise in activity during the pre-baiting period again indicated a neophobic reaction. The introduction of rodenticide bait was followed by a decline in activity for three days. Activity then sharply increased for five days and then fell. This peak and fall in activity (20/6-21/6) represents the only clear mortality event at Farm 2, which infers the absence of the effects of social dominance, in contrast to Farm 1. Rats of all sizes were indeed observed during peak times of activity throughout the study period. However, social dominance was again apparent at bait points (aggressive exclusion of smaller rats by larger rats), and there is an indication of a second mortality event five days later (26/6-27/6).

2.3.2 Feeding on bait

Figures 2.8 and 2.9 illustrate bait consumption at Farm 1 and Farm 2 respectively. Bait-take profiles reflect the changing status of both activity and population size; an increase in bait-take was concurrent with an increase in rat activity, and bait-take declined as rats died.





Figure 2.9 Bait-take (g/measured every four days) at Farm 2.


Only a few counts of rats in the video monitoring showed them moving through the video monitoring area; most counts involved individuals feeding at bait points. During a feeding bout, rats would either remain within the bait box or make frequent short trips between the bait box and an adjacent place of cover. No pattern was apparent between which of these behaviours was exhibited and, for example, numbers of rats present, rat status or time of day. Generally, only one rat at a time fed at a bait point, though occasionally pairs of rats fed together, tolerating each other, exhibiting no apparent aggression. Investigation of bait stations over the treatment period highlighted frequent spillage within the immediate vicinity, blue (bait marker) dyed rat droppings, and sometimes, blood trails.

2.3.3 Population estimation

Tracking tile scores (4-point index) and calculated population estimations (calibration of Quy *et al* 1993) for Farm 1 and Farm 2 are presented in Tables 2.2 and 2.3 respectively. For Farm 1, the population reduction over the period of baiting was calculated to be 48%. Due to reduced bait take and activity counts, rat control was presumed a success after three weeks, and the second tracking tile session was carried out. Unfortunately, the post-rodenticide score covers the period of reinvasion, thus inflating the population estimate. Had the session been carried out after four weeks, a predicted lower score would show control had been achieved. For Farm 2 the population was reduced by 98% (* a post-bait population of 1 was given as the index score was too low to be entered into the calibration equation). Bait-take data and activity counts also indicated this level of control. A paired t-test indicates that there was a significant effect of control on the pre-rodenticide rat populations, when compared to the post-rodenticide rat populations, at Farms 1 and 2 (P<0.05).

Table 2.2 Tracking tile data, Farm 1 (40 tiles).

Date	4-point index	Date	4-point index
30/05/01	58	19/06/01	47
31/05/01	67	20/06/01	44
01/06/01	66	21/06/01	37
02/06/01	68	22/06/01	39
Initial activity (mean)	64.75	Post-bait activity (mean)	41.75
Initial population	85	Post-bait population	44

Date	4-point index	Date	4-point index
11/06/02	47	29/06/02	14
12/06/02	56	30/06/02	11
13/06/02	36	01/07/02	8
14/06/02	40	02/07/02	9
Initial activity (mean)	44.75	Post-bait activity (mean)	10.5
Initial population	49	Post-bait population	1*

Table 2.3 Tracking tile data, Farm 2 (24 tiles).

2.3.4 Thigmotactic behaviour

Rats normally exhibit thigmotactic behaviour (movement/activity at the edge). The hypothesis to be tested is that rodenticide intoxication leads to the loss of thigmotaxis. Thigmotactic behaviour was quantified by measuring activity within 20cm (edge) or >20cm (open) from a vertical surface. The video monitoring area of both sites comprised a much higher proportion of 'open' area to 'edge' at a ratio of approximately 5:1. Figures 2.10 and 2.11 illustrate counts of rat activity at the edge and in the open over the study periods at both sites. At both sites, during the whole study period, the proportion of activity at the edge was almost always greater than activity in the open, indicating that thigmotaxis was indeed the preferred behaviour, especially considering the comparatively smaller edge area available. However, it is the change in the use of open space that indicates an effect on thigmotactic behaviour. Normal behaviour involves some activity in the open, but rodenticide intoxication appears to increase the proportion of use of open space. On Farm 1 as highlighted in Figure 2.10, during the 24 hours prior to a mortality event the proportion of open space use increases. In other words, during the 24 hours before death rats are making greater use of open space than at other times. On Farm 2 (Figure 2.11), the effect is even more pronounced. Figures 2.12 and 2.13 show the effect in a different way, displaying open space use as a percentage of total activity.

Figure 2.10 Rat activity at Edge/Open (Log scale) on Farm 1; the areas marked are where the proportion of activity in the open approaches activity at the edge. These corresponded to the mortality events (\pm 1d) described in Figure 2.6.



Figure 2.11 Rat activity at Edge/Open (Log scale) on Farm 2; in this case, the proportion of activity in the open approaches activity at the edge from 15/6 onwards, five days before the first mortality event.





Figure 2.12 Rat activity in open (% activity with 3-point moving average) on Farm 1. • denotes no data

Figure 2.13 Rat activity in open (% activity with 3-point moving average) on Farm 2. • • denotes no data



2.3.5 Diel rhythm

To examine any changes in diel rhythm, rat activity was plotted over a 24-hour scale for both the pre-bait and post-bait periods. The data is expressed as average counts of rat activity for each hour during the pre-bait (Farm 1, 6 days; Farm 2, 8 days) and post-bait (Farm 1, 28 days; Farm 2, 20 days) periods. During the pre-bait period, the rats at Farm 1 (Figure 2.14) showed peaks of activity at 18:00, 01:00 and 09:00 BST. This activity pattern did not appear to change following rodenticide application, not supporting the hypothesis that rats, normally nocturnal, become more diurnal as a result of rodenticide intoxication. However, the interpretation here was complicated by the fact that the rat's normal activity was already diurnal to a degree. These data are discussed further in section 2.3.7 in relation to predation pressure. At Farm 2 (Figure 2.15) the rats showed a more typical activity pattern; activity through the night with peaks at 19:00 and 07:00 BST. Again, there was no change in diel rhythm and rat activity remained largely nocturnal following rodenticide intoxication.









2.3.6 Abnormal behaviour

Abnormal behaviour (Table 2.1) was measured following the observations of Cox (1991), who described the appearance of certain behaviours with the onset of rodenticide toxicosis. Although arbitrary, the definition of abnormal behaviour used in this study is objective. On Farm 1, there was an increased incidence of abnormal behaviour (Figure 2.16) associated with the periods surrounding mortality events, also related to increased use of open space. Abnormal behaviour, as defined, was also apparent at the start of the pre-bait period, when in fact, the largest count was observed. On Farm 2, there was a clear contrast between pre-bait and post-bait periods (Figure 2.17); abnormal behaviour occurred with the onset of rodenticide toxicosis. Again, however, abnormal behaviour occurred at the start of the pre-bait period following initial site disturbance. The counts of abnormal behaviour correspond with counts in rat activity, indicating that all rats were affected.





Figure 2.17 Incidence of abnormal behaviour (defined as motionless in open space for >20s). Farm 2. • • denotes no data



2.3.7 Activity of predators and scavengers

Observations of potential predators and scavengers of rats entering the video monitoring area were recorded at both sites. Figure 2.18 displays the incidence of predators and scavengers at Farm 1; the species concerned were foxes (*Vulpes vulpes*) and a farm cat (*Felis catus*). There was an observed increase in fox and cat activity around the times of the mortality events. This may indicate an increase in the attractiveness of the site to predators or the availability of carcasses for scavengers. The incidence of predators and scavengers at Farm 2 is shown in Figure 2.19, where the main species concerned were foxes and badgers. At this site, the activity of predators and scavengers was less clearly related to the potential availability of intoxicated rats and carcasses, around the period of the mortality event. It was only towards the end of the study that there was an increase in observations, most likely in response to carcass availability.







Figure 2.19 Incidence of activity of predators and scavengers on Farm 2. denotes no data

On Farm 1 foxes (nocturnal only) were active prior to the application of rodenticide, but their activity increased at the time of the first mortality event. Concurrently, the nocturnal activity of rats diminished to virtually zero, and all rat activity became solely diurnal. Figure 2.20 illustrates the apparent relationship. These rats may have altered their activity pattern in response to the perceived predation risk carried by foxes. Video monitoring of the same site four months earlier showed the rats to be solely nocturnal, in the absence of foxes (Figure 2.21).

Figure 2.20 Diurnal (~04:30 to 21:45 BST) and nocturnal (~21:45 to 04:30 BST) activity pattern of rats & fox incidence on Farm 1.



Figure 2.21 Diurnal (~07:30 to 17:30 GMT) and nocturnal (~17:30 to 07:30 GMT) activity pattern of rats on Farm 1 in February 2001.



2.3.8 Exposure of non-target species to rodenticide

2.3.8.1 Primary exposure

Every morning at dawn (04:30 BST approx.), during both trials, flocks of chaffinch (*Fringilla coelebs*) and house sparrow (*Passer domesticus*) would be observed to forage around bait stations, presumably feeding on grains of bait, spilled by rats the previous night. Occasionally, small birds entered bait stations, although took flight with the approach of a foraging rat. Other species also took bait, e.g. jackdaw (*Corvus monedula*). Pheasants (*Phasianus colchicus*), at both sites, and badgers (*Meles meles*), at Farm 2, demonstrated a clear partiality for rodenticide bait. Despite the preventative measures of boards, weighed down by bricks, covering bait box entrances, these species still managed to gain access. Pheasants were observed to manipulate their necks in order to squeeze their heads in and feed (Plate 2.2), while badgers would merely push boards aside, even when weighted with breezeblocks (Plates 2.3 and 2.4).

2.3.8.2 Secondary exposure

Several incidences of rat predation were observed during the treatment period. Plate 2.5 shows a fox stalking a rat; on two occasions foxes were observed to be successful in their predation attempts. At Farm 1 the farm cat would frequently stalk rats during the treatment period; Plate 2.6 shows a picture of the result of one such predation attempt. Rat predation by a barn owl (*Tyto alba*) was also observed at Farm 1 during the treatment period.

Only one rat carcass was found (in nettles, under a corrugated iron sheet on Farm 1), despite extensive and exhaustive carcass searches, every four days on Farm 1 and every day on Farm 2.

Plate 2.2 Pheasant feeding from bait station Plate 2.3 Badger foraging around bait station



Pheasant entering bait station

Plate 2.4 Badger feeding from bait station



Badger attempting to access bait station





Badger entering bait station



Fox hunting rats

Plate 2.6 Farm cat in successful predation attempt



2.4 DISCUSSION

2.4.1 Rat activity

It must be emphasised that rat activity is not a true measure of rat abundance, as rats were not individually identifiable, but a measure of activity from the population as a whole. The measure is likely to overestimate population size as individual rats may leave and re-enter the arena repeatedly.

Neophobia, or new object reaction, describes avoidance when there is a change in an otherwise familiar situation (Barnett 1963). This innate response confers survival value as it serves to protect rats from the potential dangers of new objects or food. Neophobia is, however, temporary, and may be overcome by the exploratory nature of rats, resulting in habituation (Barnett 1963, Berdoy & MacDonald 1991). A neophobic response and succeeding habituation, after approximately 3-4 days, was evident during the pre-bait period on both farms as activity declined then subsequently increased. This was probably a response to unavoidable disturbance in setting up video equipment and the placement of bait points. The initial neophobic response was overcome as the rat's initial avoidance was superseded by their exploratory instinct and the capitalisation of a new food source. On Farm 2, the application of rodenticide, replacing the pre-bait, also caused a neophobic reaction, though substantially reduced compared with the pre-bait period. These findings support the work of Shepherd & Inglis (1987) who found that rats exhibited different degrees of neophobia towards new food containers and new foods. The neophobic response to the rodenticide was also supported by the data on bait take, which initially rose at both sites as rats became more familiar and took more food (Buckle et al 1987, Berdoy & Macdonald 1991).

Several observations of larger, apparently dominant rats exhibiting aggressive behaviour to smaller, apparently subordinate individuals may have restricted the feeding activity of subordinates. This may have led to a situation where dominant rats may have consumed a lethal dose before subordinates. These interpretations are based on limited observations of social interactions between rats and the assumption that body size positively correlates with social rank. Cox & Smith (1992) and Cox (1991) collected data in farm trials that supported Dubock's (1982) hypothesis that dominant rats exclude subordinates from bait points; in their studies, the body masses of

carcasses collected declined through time, suggesting that larger (more dominant) animals fed and died first.

The activity profile at Farm 1 indicates mortality events at regular intervals of four days, and at Farm 2 after seven days, in accordance with the mode of action of anticoagulant rodenticides. The effects of anticoagulant poisoning were apparent 2-3 days after the introduction of bait, physiologically in the form of blood trails on bait containers, and behaviourally in terms of altered behaviour.

2.4.2 Foraging behaviour

Movement of rats is characterised by the formation of easily defined, habitually used runs, connecting burrows with foraging sites. The run network is established and maintained by the deposition of residual cues (e.g. rat urine) by conspecifics (Galef & Buckley 1996), resulting in communal utilisation. Run layout is directed by thigmotaxis and the exploitation of protective cover, although rats may occasionally move quickly across open ground (Taylor 1978). Cox (1991) and Cox & Smith (1992) demonstrated that anticoagulant toxicosis induces behavioural changes in enclosed rats, which lost their thigmotactic nature and preference for cover. The results described here confirm and extend that observation to free-living farm rats. Further, it appears that the neophobic response may also be affected. The rat activity profiles of both farms show a significant increase in activity just before a mortality event, which may reflect loss of neophobic restraint. Behavioural alteration was overtly apparent in the 24 hours prior to mortality events, though could be observed as little as two days after first application of bait.

Behavioural measurements may have been influenced by the effect of group foraging on individual behaviours of rats. Individuals were more active in open space and appeared more 'relaxed' when in the presence of several other rats, probably as a result of group vigilance (Krebs 1994). However, this group effect occurred both before and during rodenticide treatment so should not have biased the results.

At least one other pathological condition causes behavioural changes in brown rats. *Toxoplasma gondii* infection has been shown to cause an increase in activity (Webster 1994b), a decrease in neophobic behaviour (Webster *et al* 1994), and manipulation of a rat's perception of cat predation risk, reversing aversion into attraction (Berdoy *et al* 2000). Such manipulations of the intermediate host would

enhance the chances of predation by cats, the definitive host, permitting completion of the parasite's life cycle (Berdoy *et al* 2000).

Rats were also observed to sit motionless for long periods in areas of open space, apparently dazed and unresponsive, in accordance with the observations of Cox (1991). In addition, rats were observed to move in a fashion that could only be interpreted as uncoordinated, for example, moving in circles, haphazardly, or in directions for no perceivable purpose. This behavioural occurrence was clear when compared to normal movement behaviour. Several studies on anticoagulant poisoned rats have described similar behaviours (Farag 1982, Desheesh 1983, Cox & Smith 1992). Without laboratory investigation, the possible pre-lethal effects on rat physiology that induce such behaviours can only be hypothesised. Certainly, the discomfort caused by haemorrhage at the joints, skeletal muscle, lungs and other organs could account for some abnormal behaviour. But such extreme changes in behaviour as the loss of thigmotaxis, neophobia and kinaesthesis, and the observations of apparent torpor, could really only be explained by brain damage. Indeed, Cox & Smith (1992) suggest that such aberrant behaviour could be accounted for by sublethal cerebral haemorrhage, often revealed in post mortem examinations of anticoagulant poisoned rats. Whatever the causes, these behavioural changes have clear implications for predation risk and consequentially, the risk of secondary poisoning.

The occurrence of abnormal behaviour, as defined, at the start of the pre-bait periods on both sites may have been due to a temporary change in rat activity due to site disturbance in placing bait boxes and setting up video equipment.

2.4.3 Exposure of scavengers to poisoned rats

It is possible that, despite intensive searching effort, carcasses may still have been overlooked, or scavenger pressure was such that rapid exploitation prevented their detection. Alternatively, moribund rats may well retreat to burrows, thereby reducing availability and rodenticide exposure to scavengers. This hypothesis is supported by the results of a number of studies that have reported observations on the frequency and proportion of poisoned rats which are found dead above ground. Only 2/15 radio-tagged rats died above ground during rodenticide poisoning on Langara Island, British Columbia (Howald *et al* 1999). In poisoning operations on New Zealand Islands, all 16 radio-tagged rats were found to die in their burrows on Ulva Island (Taylor 1993),

most rats died underground on Breaksea Island (endorsed by the strong smell of decomposition) (Taylor & Thomas 1993), and no rats were found on the surface of Hawea Island, New Zealand (Taylor & Thomas 1989). Following rodenticide trials on UK farms, Harrison *et al* (1988) estimated that only 4% of the original rat population died on the surface and during poisoning at an Oxfordshire farm, Fenn *et al* (1987) reported that the majority of rats died under cover. In contrast, Cox & Smith (1992) reported that 12/18 rats died in the open rather than nest boxes, and similarly, Gemmeke (1990) found anticoagulant poisoned caged rodents to die as often above ground as below. Considering the evidence available and the likelihood that captive animals may behave differently to wild individuals, it is possible that only a small number of poisoned rats would have died above ground and been available to scavengers in the studies reported here.

2.4.4 Exposure of predators to poisoned rats

The ability of predators to kill prey depends on the ease of capture and handling and therefore prey vulnerability (Cicero 1993) is an important issue in secondary poisoning. Rats deficient in self or environmental awareness represent easy prey. Further, there is a wide variety of observational and experimental evidence to show that prey behaving oddly, exhibiting abnormal tendencies, or are clearly unfit, are preferentially selected by predators (Popham 1943, Rudebeck 1950-51, Kenward 1978, Cicero 1993). For example, in a study by Hunt et al (1992), house sparrows exposed to the contact avicide fenthion were selectively predated by American kestrels (Falco sparverius). Kestrels appeared to recognise aberrant behaviour resulting from fenthion intoxication e.g. decreased response to external stimuli, reduced stamina and impaired anti-predator behaviour, and targeted these individuals in preference to unexposed birds. The movement of rats away from cover and into the open increases predatory exposure. Kotler et al (1988) showed that rates of predation were higher on rodents foraging in the open than on rodents foraging with cover. Development by a predator of a specific searching image (a tendency to continue to select a given type of prey (Tinbergen 1960)) for prey exhibiting rodenticide intoxicated behaviours, would also increase the proportion of contaminated individuals in the diet and therefore, the likelihood that a lethal dose be achieved. The increased incidence of foxes at both sites may indeed indicate predatory success aided by the exposure and reduced escape response of rats.

With regard to a significant change in diel rhythm, in terms of rats switching activity from nocturnal to diurnal, there was no evidence in this study to support the results of Cox (1991). What was apparent however, was a cessation of nocturnal activity in response to the increased incidence of foxes on Farm 1. Initially, these rats were active both during the night and, prominently, during the day; the diurnal activity possibly facilitated by minimal human disturbance on a disused farm (Taylor 1975), or forced by the nocturnal foraging activity of foxes (Fenn & MacDonald 1995). These observations are supported by the work of Fenn & MacDonald (1995) who observed a similar relationship at a farm midden in Wytham, Oxfordshire. It appears that, at a particular level of fox activity, the predation risk becomes greater than that presented by diurnal predators leading rats to become solely diurnally active. Certainly, the fear and risk of fox predation in rats is significant. Laboratory experiments have shown rats to avoid foraging in the presence of fox odour (Berdoy & MacDonald 1991), and stress-inducing odorant molecules have been isolated in fox faeces (Vernet-Maury et al 1984). The observed seasonal changes in fox activity and the resulting changes in the activity patterns of rats on Farm 1 were also observed by Fenn & MacDonald (1995).

2.5 CONCLUSIONS

- 1) The coumatetralyl-based rodenticide 'Racumin' was effective in controlling populations of rats on two farms in Northamptonshire.
- Rats exhibited abnormal behaviours and a loss of normal thigmotactic behaviour during the pre-lethal period of anticoagulant toxicosis, which may increase their exposure and vulnerability to predators.
- Rats did not exhibit a change in diel rhythm as a result of anticoagulant toxicosis, contrary to the results of Cox (1991) who studied rats in enclosures.
- Video monitoring provided evidence of primary and secondary rodenticide exposure to a number of non-target species.

CHAPTER 3: RATES OF RAT CARCASS DISAPPEARANCE AND POTENTIAL SCAVENGERS

3.1 INTRODUCTION

One aspect in the assessment of the potential secondary poisoning hazard represented by rodenticide controlled rats (Rattus norvegicus) is the rate of carcass scavenging and the identification of scavengers that feed on poisoned rats. Current UK label guidelines for the safe use of rodenticide specify 'to search for and burn or bury all rodent bodies', to reduce the risk of secondary poisoning incidents. Carcass recovery is, however, limited by a number of variables. Poisoned or moribund individuals may retreat to the relative safety of home sites or other forms of cover (Mineau & Peakall 1987), reducing the likelihood that a carcass will be discovered during a search. The extent to which cover-seeking behaviour inhibits carcass discovery will depend on the nature of the cover, e.g. vegetation structure and density (Tolbin & Dolbeer 1990) and the manoeuvrability of farmyard junk. In addition, relatively small, cryptically coloured animals (such as brown rats) are less conspicuous than large brightly coloured animals (Vyas 1999). Carcass discovery is heavily reliant on searcher effort and experience (Linz et al 1991). On a rodenticide treated farm site, farmers may not spend much time on thorough daily searches, although site-specific experience may define the most likely sites of carcass recovery, increasing searcher efficiency. Searches for poisoned carcasses are essential, yet even the most responsive and thorough are unlikely to account for all bodies (Mineau & Collins 1988, Balcomb 1986), leaving a proportion available to scavengers. For example, in a searcher efficiency trial conducted by Stutzenbaker et al (1986), 0/50 waterfowl placed in cover and 6/50 waterfowl placed on top of vegetation were recovered. Scavengers and predators, in contrast, are highly adept in rapid and comprehensive capitalisation of a new food source (Kostecke et al 2001, Peterson et al 2001, Wobeser & Wobeser 1992, Hiraldo et al 1991, Balcomb 1986, Houston 1986), although rates of carcass discovery will depend on habitat (Mineau & Collins 1988).

The objectives of this study were to investigate rates of rat carcass removal on farm premises and to identify their scavengers, i.e. those species most at risk from secondary rodenticide poisoning. Specifically, testing the hypotheses that the removal of rat carcasses around farm sites is rapid, and that the degree of cover affects the rate of disappearance.

3.2 METHODS AND MATERIALS

The study was carried out on two farms, and three trials were performed on each. Farm 2 was a medium scale intensive pig farm in Northamptonshire (Farm 2 as used in Chapter 2), and Farm 3, a mixed arable and sheep farm, and game estate, in Leicestershire. Farm 2 had a recent history of severe rat infestations, both within pig units and along field boundaries. Rat control was practiced in and around the pig units, and shot or trapped rats were, on at least one occasion, left in situ or discarded on a manure heap. A large number of carrion crows were attracted to the site. Both crows (Corvus corone) and magpies (Pica pica) were controlled and their carcasses were discarded on the manure heap. Rat infestations were rare on Farm 3, and rats were pre-emptively controlled using rodenticide and traps at a low intensity. Scavengers, such as crows, were not attracted to the site in numbers, as any food was largely made unavailable. In addition, a resident gamekeeper had practiced predator control on the entire estate until early 2002 in order to promote pheasant (Phasianus colchicus) rearing. Both farms were situated in a rural arable and pasture environment of a variable topography interspersed with small copses, and adjacent to small villages. The suite of potential scavengers included both mammals (fox Vulpes vulpes, badger Meles meles, weasel Mustela nivalis, stoat Mustela erminea, hedgehog Erinaceus europaeus) and birds (crow, magpie, buzzard Buteo buteo, and at Farm 2 red kite Milvus milvus). All trials were carried out in the summer of 2002, during June on Farm 2 and during August on Farm 3.

The rats used in the study were uncontaminated white laboratory rats of medium (100-200g) to large (>200g) size (Rep-Tech Ltd). Carcasses were shipped and stored frozen then thawed prior to field placement. At the beginning of each trial, 15 rats were placed randomly around the farm premises. Carcasses were handled only with disposable latex gloves and every care was taken to reduce site disturbance and contamination with human scent. All rats were placed during the afternoon, within about an hour. To assess the effect of cover on disappearance rates, carcasses were placed in three different levels of cover; cover (100% concealment overhead), partial cover (~50% concealment overhead), or open (0% concealment overhead). In order to reduce the possibility of habituation to carcass location by potential scavengers over the three trials on each site, carcasses were positioned as follows:

Position	Trial 1	Trial 2	Trial 3
1	Cover	Partial Cover	Open
2	Cover	Partial Cover	Open
3	Cover	Partial Cover	Open
4	Cover	Partial Cover	Open
5	Cover	Partial Cover	Open
6	Partial Cover	Open	Cover
7	Partial Cover	Open	Cover
8	Partial Cover	Open	Cover
9	Partial Cover	Open	Cover
10	Partial Cover	Open	Cover
11	Open	Cover	Partial Cover
12	Open	Cover	Partial Cover
13	Open	Cover	Partial Cover
14	Open	Cover	Partial Cover
15	Open	Cover	Partial Cover

Carcasses were placed realistically, in positions perceived to be likely places where a rodenticide-poisoned rat might die. Sites were not disturbed in order to manipulate the level of cover required. Farmyard junk and natural vegetation were fully utilised to provide cover and only existing open spaces were used.

Carcasses were placed on a patch of silver sand (50cm×50cm) for the purpose of gaining evidence of prints left by scavengers. Carcasses were checked daily for five days or until disappearance. Separate measurement of nocturnal and diurnal scavenging pressure, although desired, was not possible, as access to Farm 2 was prohibited outside the hours of 08.00 and 18.00 BST, preventing the inspection of carcasses at dusk (21:30 approx.) and dawn (04:30 approx.). On inspection, carcass remains were characterised as 1) removed with no observable trace, 2) some body part or tissue remaining, 3) some degree of internal evisceration, 4) carcass intact/untouched. Detailed notes on carcass state were also taken.

As a video-monitoring study was being concurrently performed on Farm 2 (Farm 2 as used in Chapter 2) the opportunity to observe the fate of carcasses (within the field of view) was taken. The video camera set up is described in detail in section 2.2.3.

3.3 RESULTS

3.3.1 Carcass disappearance rates

At Farm 2 over the three trials a mean of 73.3% (SD = 29, n=45) of rat carcasses were scavenged and removed (Table 3.1). Carcass disappearance was rapid with 37.8% removed within the first 24 hours. The rate of carcass disappearance was greatest within the first 24 hours than in the days that followed ($\chi^2_{(3)} = 8.8$, P<0.05). In contrast, at Farm 3 a mean of only 19.9% (SD = 6.7, n=45) of rat carcasses were ultimately removed over the 3 trials. A Chi-Square test was not possible for Farm 3 as all expected counts were too small. Carcass disappearance rates for both farms are illustrated in Figure 3.1.

Trial no.	No. carcasses remaining at daily checks after placement							
Farm 2	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5		
1	15	10	8	4	3	2		
2	15	7	7	4	2	1		
3	15	11	10	9	9	9		
Total	45	28	25	17	14	12		
Farm 3								
1	15	15	13	13	12	12		
2	15	14	14	14	13	13		
3	15	14	13	11	11	11		
Total	45	43	40	38	36	36		

Table 3.1 Results of carcass disappearance trials.

Figure 3.1 disappearance of rat carcasses from Farms 2 and 3 (3 trials each)



Regression analysis of the disappearance of carcasses over the trial periods showed a good fit to an exponential disappearance and a significant overall difference between farms (Farm 2; slope=-0.259, r^2 =96.7%, Farm 3; slope=-0.057, r^2 =99.6).

Figures 3.2 and 3.3 show disappearance rates at the different levels of cover, for Farm 2 and Farm 3 respectively. Graphically, the level of cover does appear to influence rates of carcass removal (carcasses disappear faster in the order open>partial>complete). In a comparison of the rates of disappearance in relation to cover on both farms, an analysis of covariance of log numbers remaining, using a general linear model (GLM), showed that there was a significant decrease in the number of carcasses with time (Day effect) and that this decrease was significantly affected by the amount of cover (Cover *Day effect) (Farm 2, Table 3.2a; Farm 3, Table 3.2b). Note that some of the regressions eliminated the final day when there was no further loss of carcasses from the previous day. Regression coefficients (rates of disappearance of carcasses) for each farm are given in Table 3.3.









Table 3.2a Analysis of Covariance (GLM) fitted to log N transformed data of carcass disappearance in relation to cover (Farm 2).

Source	DF	Seq SS	Adj SS	Adj MS	F	Р
Day	1	2.88442	3.92786	3.92786	432.10	0.000
Cover	2	1.44237	0.01209	0.00605	0.67	0.534
Cover*Day	2	0.58072	0.58072	0.29036	31.94	0.000
Error	11	0.09999	0.09999	0.00909		
Total	16	5.00750		·		

Table 3.2b Analysis of Covariance (GLM)) fitted to Log N transformed data of
carcass disappearance in relation to cover	r (Farm 3).

Source	DF	Seq SS	Adj SS	Adj MS	F	Р
Day	1	0.064846	0.079541	0.079541	67.10	0.000
Cover	2	0.034919	0.002588	0.001294	1.09	0.381
Cover*Day	2	0.023699	0.023699	0.011849	10.00	0.007
Error	8	0.009483	0.009483	0.001185		
Total	13	0.132947				

Table 3.3 Exponential regression	coefficients	(slopes)	with	standard	error	for
each level of cover on both farms						

Cover		Farm 2	Farm 3		
	Coefficient	Standard Error	Coefficient	Standard Error	
Open	-0.488	0.037	-0.084	0.015	
Partial	-0.222	0.022	-0.112	0.018	
Complete	-0.208	0.018	-0.033	0.005	

3.3.2 Carcass fate

Of the carcasses scavenged, means of 72% (SD=13.6, n=3) and 47.3% (SD=20.9, n=3) were removed without trace, on Farm 2 and 3 respectively. In such cases carcasses would have either been entirely consumed in situ or carried away for consumption elsewhere. Prior to complete removal, or by the final day of each trial, means of 19.9% (SD = 13.3, n=3) and 24.4% (SD = 10.2, n=3) of carcasses had been eviscerated or only slightly scavenged, at Farm 2 and 3 respectively. Closer inspection of carcasses revealed signs indicative of feeding by particular species. For example, evisceration using the technique of pulling the carcass inside out would only be possible for species with sufficient manual dexterity, size and strength (e.g. raptor, fox). Scavenging by small rodents was apparent in the development of small wounds at a variety of points over a carcass. At Farm 2 a mean of 13.3% (SD = 9.4, n=3) showed remains of body parts. In all cases, the body parts remaining were intestines or the digestive tract in its entirety. Some of the potential scavengers in this study are known to discard the guts of prey; for example, foxes and badgers (Hewson & Kolb 1976). Over the trial periods, means of 31.1% (SD = 25.3, n=3) and 82.2% (SD = 7.7, n=3) of rat carcasses became colonised and infested by blowfly maggots, at Farm 2 and 3 respectively. Maggots were observed on some carcasses as soon as 48 hours after placement. Carcasses that became infested with maggots were almost always entirely consumed by those maggots; the infestation apparently deterred subsequent scavenging by mammals and birds. By the end of the 5-day trial period, infested carcasses were almost entirely consumed (only bones and some hide remaining), before maggots left as prepupae. In all trials, those carcasses that were not removed within 48-72 hours of placement were colonised by blowfly maggots.

3.3.3 Potential scavengers

It was rare that a perfect set of tracks would be found in the sand following carcass utilisation by a scavenger. Footprints masked one another and sand was disturbed as a result of carcass handling. In a number of cases tracks were not legible. Very often identification was made solely on the basis of one clear footprint. Where tracks of more than one species were found, all species would be recorded as potential scavengers. Scavenging species were only confirmed if identification was absolutely certain. Identification texts were used as aids (Sargent & Morris 1999, Bang & Dahlstrom 1974) and observation of scavengers through video monitoring

complemented identification of species. Figure 3.4 shows the incidence of scavengers at both farms. Nine vertebrate species, six mammals and three birds, were identified. The most common scavengers were rats, foxes and crows, accounting for 35%, 24% and 20% of tracks respectively on Farm 2, and 67%, 17% and 17% of tracks respectively on Farm 3.





3.3.4 Video monitored carcasses

As part of the carcass disappearance trials on Farm 2 twelve carcasses were placed within the field of view of a video camera, set up for use in another study. Video monitoring provided data and information on scavenging species and possible interactions, carcass feeding/handling behaviour, discovery time, length of feeding bouts, and carcass fate.

Rat carcasses were discovered by scavengers between 85 and 525 min (mean = 242min, SD=162.8, n=12) after placement. In 8/12 of cases, crows were first to discover and scavenge carcasses, foxes 3/12, and red kite 1/12. Crows appeared to be the least cautious in scavenging carcasses, feeding immediately with no apparent prior investigation. Crows exhibited a common feeding behaviour where carcasses were moved/rearranged a short distance then pinned in order to pluck an entry point, before feeding (Plate 3.1). Crows were proficient in carcass entry. Crows would normally eat

for about 2 minutes, scanning intermittently, then leave the field of view for about 2 minutes, before returning. This pattern of feeding would cycle until the crow finally left, and when a carcass was shared, involved both birds. Crow feeding bouts lasted between 20 and 85 min (mean = 38.75min, SD=20.8, n=8), before the crow left a carcass or removed a carcass from the field of view. Crows removed carcasses by both dragging them along the ground and in taking flight whilst holding a carcass in the claws.

Foxes clearly behaved cautiously, approaching carcasses slowly whilst sniffing the surrounding ground. In two cases, a fox visited a carcass, sniffed, scanned for about 2 minutes, then left without it, before returning several hours later (appearing to be the same individual) finally to remove it (Plate 3.2). Foxes removed carcasses quickly and cleanly, never eating them *in situ*. A badger was observed only once, investigating the remainder of a carcass before removing it (Plate 3.3).

The scavenging incident involving the red kite was personally observed, as fieldwork was being conducted at the time. Initially, the kite circled the carcass at a significant height (~25m) before descending. The kite never alighted on the ground and only made closer inspections during 3 flypasts at a height of about 3-4 metres. Eventually, an attempt at retrieving the carcass was made on the fourth flypast, although it was unsuccessful as the rat was dropped (Plate 3.4). The kite then perched in a nearby tree for five minutes before leaving the site as a result of human disturbance.

Carcasses were never entirely consumed *in situ* by any species; being removed from the field of view immediately on discovery (fox and red kite) or at different stages of consumption (crow). Following placement, time to removal varied between 100 and 1560 min (mean = 517min, SD=533.1, n=12).

Plate 3.1 Crow feeding on rat carcass

29-05-02 5:55.00

Crow pinning and feeding on rat carcass

Plate 3.3 Badger scavenging rat carcass

Plate 3.2 Fox scavenging rat carcass



Fox taking rat carcass



Badger taking rat carcass

Plate 3.4 Red kite scavenging rat carcass



Red kite attempting to take rat carcass

3.4 **DISCUSSION**

3.4.1 General discussion

Despite the successful identification of scavengers with the use of silver sand, the method is limited. In an appraisal of tracking methods used to monitor primary non-target rodenticide exposure Edwards *et al* (1988) list 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; and rainfall washing out tracks. In performing the study there is likely to be an unavoidable bias with regard to the species of scavenger attracted to the carcasses. Attraction to, or avoidance of, carcasses or areas tainted with human scent will determine species occurrence (Kostecke *et al* 2001).

Carcass scavenging rates may be variable due to a number of factors, e.g. temperature (DeVault & Rhodes 2002), carcass condition (Bumann & Stauffer 2002), ground cover (Tobin & Dolbeer 1990), and carcass density and visibility (Linz *et al* 1991). Scavenging rates were clearly greater on Farm 2 than on Farm 3, yet, with regard to the factors given above, there was no difference between sites. A difference in the number of scavenger species and their abundance between the sites appears to have been the prime variable. Rats were a reliable and common food resource on Farm 2 that was already utilised by an established suite of scavengers. Thus, scavenger pressure remained high with the introduction of a common prey type. At Farm 3 a combination of low food availability and estate-wide predator control was not conducive to a significant level of scavenger pressure and rates were consequently low.

3.4.2 The importance of cover

The proportion of cover in influencing rates of carcass disappearance was significant. The importance of cover in carcass discovery is dependent on the importance of vision in the repertoire of scavenger senses, specific to each species. Many scavengers are predominantly olfactory, reliant on chemical cues, rendering cover an insignificant variable. Several studies have found the level of cover to be inconsequential in carcass disappearance rates (Bumann & Stauffer 2002, DeVault & Rhodes 2002, Houston 1986). In this study the proportion of cover was significant, highlighting the importance of visual cues in foraging. The use of white rats in this study may have resulted in visual scavenger bias, considering their conspicuousness relative to brown rats. However, the use of white rats was deemed acceptable, as there was very little colour contrast between the rats and the underlying silver sand used for recording tracks. Any bias would only have resulted from an attraction to patches of silver sand. The importance of olfaction in mammal behaviour and foraging is well established. Although birds are still considered primarily visual scavengers, many bird species have been shown to possess a functioning olfactory system (Roper 1999, Rowe & Guilford 1996, Papi 1991, Houston 1986). In this study a crow was observed to land and walk directly towards a slate tile, then reach under with its beak and pull out a completely hidden carcass, which it proceeded to scavenge. The crow arrived at the carcass 95 minutes after placement and it is unlikely it would have waited so long to investigate had it observed placement. Indeed, carrion-eating corvids that cache food are likely to possess a highly developed olfactory ability (Buitron & Nuechterlein 1985).

3.4.3 Blowfly infestation

All trials were carried out during the summer, when warm temperatures increase the competition between vertebrate scavengers, invertebrates and bacteria, for carrion (Janzen 1977). Diptera and Coleoptera are the major components of the invertebrate necrophage community, playing crucial roles in the decomposition of carrion (Kocarek 2003). Apparently, the presence of blowfly maggots may deter vertebrate scavengers, signifying a carrion resource to be unpalatable and toxic. Janzen (1977) hypothesised that bacteria release toxins in order to deter larger scavengers. DeVault & Rhodes (2002) contend that the odours released by bacteria actually attract insects and vertebrates to carcasses for the purpose of opening and burrowing through them, thereby creating previously inaccessible habitats for bacteria to occupy. The chemical cues released by bacteria may change according to resource availability, appearing to compel and manipulate larger scavengers in order to maximise resource utilisation.

When maggots eat almost all of a rat carcass, it is likely that the rodenticide loading of a poisoned rat is also consumed. The mode of action of rodenticides is unlikely to affect the condition of a fly or normal functioning of its physiology. Although the utilisation of poisoned rat carcasses by blowflies may indeed reduce rodenticide exposure to vertebrate scavengers, a new exposure pathway may be initiated, contaminating those species that predate flies. Individually, concentrations

of the active ingredient may be minute, but considering the persistent nature of rodenticides (certainly 2nd generation), there may be potential for bioaccumulation in insectivorous species that forage on farm premises, e.g. swallow (*Hirundo rustica*).

The rate of carcass decomposition is correlated with temperature (Kocarek 2003). A reduction in the degree of blowfly carcass colonisation and infestation during winter will increase the availability of poisoned rats for vertebrate scavengers, at a time when food may be scarce. Rat populations in farm buildings are at their highest in winter as rats move in from the fields seeking food and shelter (Huson & Rennison 1981). As rodenticide application increases so will the provision of poisoned carcasses, just at the time when rats become a readily utilised predator and scavenger resource, e.g. polecats (Birks 1998).

3.4.4 Secondary poisoning

All of the species identified as scavengers must be considered at risk of secondary poisoning. Clearly, the secondary poisoning of rats through cannibalism may inadvertently aid control. However, the other species attracted to forage in farm premises will scavenge carcasses of poisoned rats if they are available. The degree of risk to non-target species is dependent on adherence to the guidelines for best practice with regard to carcass disposal. Evidently, it is not enough just to promote the necessity for carcass searches. The rapid disappearance of carcasses, regardless of cover, requires search effort that is responsive, frequent and extensive. For those scavengers that cannot access burrows, carcass availability is dependent on the number of rats dying above ground (discussed in section 2.4.3). Even a small number of carcasses may represent a significant hazard. For example, it would only be necessary for a fox to eat one poisoned rat carcass per day for 5 days for a lethal dose to be achieved (based on coumatetralyl LD50 for a rat; 0.3 mg.kg⁻¹×5 days (Eason *et al* 2002), and stored residue in a rat; 6.8 mg.kg⁻¹ (MacVicker 1998)).

3.5 CONCLUSIONS

- 1) A wide range of non-target mammals and birds scavenged rat carcasses at farms.
- On Farm 2 rates of carcass disappearance were rapid. Results suggest that the rate of disappearance depends on the abundance and activity of local scavengers.
- 3) The level of cover affected the rate of carcass disappearance.
- 4) Of the carcasses scavenged, most were removed without trace, with the remainder only partially scavenged.
- Invertebrate scavengers, i.e. blowfly maggots, colonised a large proportion of carcasses, apparently deterring vertebrate scavengers. This observation suggests an alternative route of rodenticide exposure, to non-target insectivores.
- 6) On the farms studied, the most common scavengers of rat carcasses were rats themselves, foxes and crows.

CHAPTER 4: STUDIES OF THE FEEDING BEHAVIOUR OF RED KITES WHEN SCAVENGING RATS

4.1 INTRODUCTION

4.1.1 The diet of Red Kites

The red kite (Milvus milvus) has a wide food spectrum, tending to take the most readily available items (Walters Davies & Davis 1973); it actively varies its diet in line with the abundance of prey. The red kite is both a predator and scavenger. It hunts by soaring and circling, ranging widely over open ground at considerable height and also by gliding low (3-5 m above ground (Wildman et al 1998)) (Shaw & Perrins 1998). Despite its predation on a variety of prey, the kite is better suited to a scavenging role. There is no advantage in killing prey if the animal could easily obtain good quality meat by scavenging (Houston 1979). The kite's ability to cover vast distances on minimal energy, often ranging up to 5-8 km from the nest and 10-12 km from the roost, must give to each individual a wide awareness of the presence of carrion and a greater choice of potential food sources than is enjoyed by most scavengers (Davis & Davis 1981). On sighting a carcass it will descend in tighter circles, settling within the vicinity and approach cautiously (Walter Davies & Davis 1973). If the carcass is occupied by other species the kite may wait at a distance before pursuing any bird which flies off carrying food and oblige it to drop the morsel, which the kite then retrieves from the ground (Davis & Davis 1981, Walters Davies & Davis 1973). The kite seems quite adept in such kleptoparasitism; it has been observed robbing a kestrel (Falco tinnunculus), on the ground, of a rat, and a sparrowhawk (Accipter nisus), in flight, of a blackbird (Turdus merula) (Wildman et al 1998). Indeed, it is a food-pirate on a host of raptors and corvids, ranging in size, both smaller and larger than itself (Shaw & Perrins 1998). If predating live prey, a steep dive with talons outstretched will attempt to catch prey by surprise, rather than using speed and pursuit (Shaw & Perrins 1998). Many small mammals such as voles, rats and mice are caught in this way. In flight, red kites are highly manoeuvrable and prove successful in hawking large insects, and birds (Wildman et al 1998, Davis & Davis 1981, Walters Davies & Davis 1973). Other foraging techniques include: A sit and wait strategy, occasionally adopted, where the bird will perch in a tree scanning an area frequented by rodents or other small mammals (Zawadzka 1999, Wildman et al 1998, Walters Davies & Davis 1973). Red kites in Scotland have been observed

foraging in a harvested pea field and on freshly ploughed land, feeding on worms and, presumably, other invertebrates (Wildman *et al* 1998). They may also visit rookeries in search of young rooks (Wildman *et al* 1998) and scavenge carcass remains around peregrine falcon (*Falco peregrinus*) eyries (Davis & Davis 1981).

In contrast with most other raptors the red kite is strongly attracted to anthropogenic sources of food, for example: abattoirs, rubbish dumps, farm middens, village streets and gardens, wildlife killed on roads, and wildlife shot and left in fields by farmers and gamekeepers. It is clearly evident from diet analyses (Zawadzka 1999, Larraz 1999, Garcia et al 1998, Wildman et al 1998, Davis & Davis 1981) that human waste is an important source of food for red kites, especially during autumn and winter when natural prey may be scarce. In an evaluation of the importance of livestock carcass disposal sites for wintering red kites, Larraz (1999) concluded that such sites supported a higher number of wintering kites than other localities where only live prey was available, and that the use of predictable localised carrion in dumps resulted in greater winter survival of kites (Donazar 1992; cited in Larraz 1999). A study of red kite diet in the English east Midlands, using direct observations, the recording of food remains at nest sites, and the analysis of regurgitated pellets, showed that the majority of the diet was made up of carrion (Carter & Grice 2000). Studies were conducted year round and it was found that lagomorphs were the principal food throughout, and especially important during the breeding season (82% occurrence in pellets, by number). The common rat (Rattus norvegicus) and other small mammals were the next most important food sources, especially during winter (27% and 33% occurrence in pellets, by number, respectively). Medium-sized/large birds, particularly gamebirds, pigeons and crows, formed a major part of the diet during the breeding season (Carter & Grice 2000). The majority of crows and pigeons were juveniles, vulnerable to predation both at the nest and as a result of inexperience.

4.1.2 Red Kites and secondary poisoning with rodenticides

Red kites are predominantly scavengers but are also known to predate live prey on occasion where the effort required for capture is relatively minimal. Such a mode of foraging make the species especially vulnerable to consumption of both rodent carcasses and live, but lethargic, rodents suffering sub-lethal anticoagulant toxicosis. Also, red kites are not wary of people and are likely to forage around farm buildings where rodent control is practised. Additionally, the gregarious nature of the species, evident in communal roosting and social foraging behaviours, may provoke the risk of mass poisoning incidents where rodent carcasses are available in numbers following a baiting campaign. In Switzerland, for example, the use of bromadiolone to control water voles (*Arvicola terrestris*) caused a mass mortality involving no fewer than 25 red kites among other raptor species (Petroli 1983, Beguin 1983; cited in Carter & Burn 2000).

The Red Kite Reintroduction Programme in England and Scotland has proved successful in establishing viable breeding populations, and subsequent detailed monitoring, including diet analysis, radio-tracking and wing-tagging, has provided valuable data and information on their ecology and progress (Carter & Burn 2000). Both radio-tracking and wing-tagging have provided information on the welfare and the fate of individual birds, and greatly increase the chances that dead birds will be located, either by project staff or through reports by members of the public (Carter & Burn 2000). Eight kites found dead in 1998 and 1999 were found to contain the residues of second generation anticoagulant rodenticides and post-mortems revealed that, in at least three cases, these had caused the death of the bird (English Nature 2000). Further, there may be a significant under-representation of deaths attributable to rodenticide as it is estimated that only one in five of the red kites that die are found in good enough condition to establish the likely cause of death (Holmes et al. in press; cited in Carter & Burn 2000). Estimates of red kite mortality in English populations in 1998-99 may mean that as many as 40 rodenticide-poisoning incidents occurred, a worryingly high figure given the currently small populations (Carter & Burn 2000). The problem of secondary poisoning with rodenticide is also affecting the Scottish red kite population. Analysis of liver tissue from red kites received during 1997 and 1998 identified rodenticide residues in five out of nine samples, although in four of these cases the immediate cause of death was attributed to other pesticide types (Sharp & Hunter 1999). Since this report several more kites have died in Scotland as a result of ingesting poisoned rats including three nestlings, three juveniles and an egg-laying adult female (B. Etheridge; pers. comm.). Pellet analysis of the English population in the east Midlands has shown that rats form a major part of the diet both in winter and during the breeding season (Carter & Grice 2000). Pellet analysis of red kites in northern Scotland, however, has indicated that rats are a minor prey item in both summer and winter (Wildman et al 1998), although more recent information from

nest visits in northern Scotland has indicated that rats can form an important part of the diet, at least in some years (Carter & Burn 2000).

In a recent analysis of 20 red kite livers, bromadiolone, difenacoum, flocoumafen and brodifacoum were detected in nine, ten, one and six livers respectively (Shore et al 2000). In total, 14 (70%) livers contained at least one rodenticide and nine kites (45%) contained more than one rodenticide (Shore et al 2000). The median (Inter Quartile Range) wet weight (ww) concentrations in those kites with detectable residues were 0.051 (0.027-0.134) $\mu g.g^{-1}$ for bromadiolone (n=9), 0.044 (0.024-0.132) µg.g⁻¹ for difenacoum (n=10) and 0.202 (0.040-0.575) $\mu g.g^{-1}$ for brodifacoum (n=6) (Shore *et al* 2000). The threshold level of rodenticide contamination that causes mortality is not known in red kites. Laboratory toxicity tests on barn owls (Tyto alba), however, have measured lethal concentrations of 0.33-1.72 μ g.g⁻¹ for bromadiolone, 0.11-0.17 μ g.g⁻¹ for difenacoum, and 0.29-1.25 μ g.g⁻¹ for brodifacoum (Newton et al 1999). Although the lethal concentrations are liable to be different between the two species it is interesting to note that three kite livers measured concentrations within the barn owls' lethal range for difenacoum and two for brodifacoum. It can be inferred that on the basis of this sample a substantial proportion of English red kites are ingesting potentially lethal concentrations of rodenticide.

In response to the number of poisoned birds and the vulnerability of the red kite to rodenticide contamination, WIIS (Wildlife Incident Investigation Scheme) now monitor levels of rodenticides in all red kites found dead, irrespective of the known cause of death.

4.1.3 Feeding behaviour of captive Red Kites when scavenging rat carcasses

Research on the exposure of red kites to rodenticides requires detailed observations on their feeding behaviour when scavenging rodent carcasses. It is pertinent to establish whether or not there are clear preferences for different tissues/organs/parts of the carcass since this could affect exposure of kites to rodenticides; anticoagulant rodenticides are stored mostly in the liver, while the stomach and intestines could contain substantial quantities of bait if the rat had recently fed on poisoned bait. It is hypothesised that red kites consume particular organs/parts of the carcass in preference to others and that they might consume organs of the carcass in a particular order of preference. Detailed observations of feeding are only feasible in captive birds
where it is possible to achieve experimental control, data significance and to establish feeding preferences with greatest sensitivity. In captive birds, however, feeding preferences may be exaggerated compared with the natural environment where food supply is not guaranteed.

4.1.4 The importance of rats in the diet of Red Kite chicks

The majority of lowland Britain is suitable for red kite with its undemanding habitat requirements and generalist diet (Carter 2001). The reintroduction of 70 birds (from Spain and the Chilterns, England) in the east Midlands between 1995 and 1998 has led to the establishment of 16 breeding pairs by 2001. The population is centred on Rockingham Forest, Northamptonshire, an area of mixed farmland and mixed deciduous/coniferous woodland, providing ideal foraging and breeding habitat for red kite.

In 2001 the nesting of a breeding pair (\mathcal{O} untagged, \mathcal{Q} red 1) close to the Forest Enterprise office at Top Lodge, Fineshade, Northamptonshire, in May 2001, offered the scope to install a nest camera and to observe the activities of parents and chicks throughout the breeding season. An information centre run by RSPB staff was set up providing live footage, educational visits and evening slide shows (English Nature 2002). The 'Red Kites @ Rockingham' initiative, a collaboration of English Nature, Forest Enterprise and RSPB, is now in its third year, following successful camera coverage of the same red kite pair, which again nested and bred close to the centre in 2002 and 2003.

Live footage of red kites rearing young from hatching to fledging provided the opportunity to study the provisioning behaviour of parents and chick diet. The main aim of this part of the study was to assess the importance of rats in the diet of nestlings. It was hypothesised that rats may be important as prey items delivered to the nest as they represent a readily available source of carrion of an optimal size (a significant meal, which can be carried to the nest in its entirety without requiring division of a larger carcass). It was also hypothesised that red kite parents may feed chicks the most easily digestible and nutritious parts of a carcass such as the liver and intestines, in preference to less easily assimilated parts. This provisioning behaviour with regard to rodenticide-poisoned rats could potentially provide a maximal dose of rodenticide. Additionally, data on times of prey delivery, indicating parental foraging

times, were compared with data on the diel rhythm of rodenticide-intoxicated rats (section 2.3.5) to see if there was any significant overlap, providing an indication of risk.

•

4.2 METHODS AND MATERIALS

4.2.1 Behavioural observation of Red Kites feeding in captivity

With the kind permission of Jemima Parry-Jones MBE, feeding experiments were carried out in avian enclosures at The National Birds of Prey Centre, Newent, Glos. Four red kite were available for study; one enclosure housed a wild injured male from Germany (Kw) and a captive bred male (Kc), and in the other enclosure there was a pair consisting of a wild injured male from the UK (K1) and a wild injured female from Germany (K2). The results of this study should be treated with caution if extrapolated to free-living individuals, although three of the four were originally wild.

Initially it was expected that kites that were used to daily public exposure would feed in the presence of an observer. Following a day of patient observation, none of the birds would even approach the carcasses let alone feed. After trials with hides and other attempts at concealment of the observer, it was realised that the kites would not feed in the known presence of an observer. Fortunately, small holes cut in the rear wall of each aviary conferred excellent points of observation. From these points, successful, undisturbed observer. The kites only fed at times during the day when human activity was minimal, for example, early in the morning before the centre was opened to the public and during flying displays when the public were gathered in the demonstration field.

Carcasses fed to the birds were uncontaminated, whole and undamaged, dark welsh Norway rats, provided by the Vertebrate Pests Unit, Reading University. Carcasses were completely thawed from frozen storage before being offered to birds at their usual feeding time (around 08:30 GMT). Carcasses were weighed both before being offered to birds and again on cessation of each feeding bout to provide measurements of amounts consumed. On cessation of each feeding bout carcasses were photographed using a 35mm SLR camera both *in situ* and following examination.

During observations of feeding the following was noted:

- Behaviour at the carcass (e.g. immediate attack, neophobic behaviour)
- Management of the carcass (e.g. repositioning/movement)
- The respective roles of the bill and talons

- The area of initial attack and point of carcass entry
- The tissue/organ first consumed and the subsequent order of consumption
- The carcass remains following each feeding bout and at the perceived end of carcass utilisation

A stopwatch was started on introduction of the carcass and measurements were made of time elapsed between placement of carcass and a) arrival b) commencement of feeding bout c) carcass opening d) first consumption e) cessation of feeding bout and departure (duration of feeding bout included time spent standing on or near carcass). Carcasses were placed on the floor of the enclosure in order to simulate the situation of kites locating rat carcasses in a natural environment. The placement of the carcass and the position it was laid in was varied between cases. In total 24 rat carcasses were offered to birds on two days per week over a period of three months, providing 24 sets of feeding reports.

4.2.2 Direct observation of wild Red Kite chick diet at nests in Rockingham Forest, east Midlands

Camera pictures (camera mounted within 1m of nest), relayed to the information centre at Fineshade, were recorded on a time-lapse video recorder for later analysis. An appraisal of the use of time-lapse video monitoring in wildlife observation is given in section 2.2.3.

Red kite nests were video-monitored during the nestling periods in the breeding seasons of 2001, 2002 and 2003. In 2001 and 2003, the first weeks of the hatchling periods could not be monitored owing to logistical problems and equipment failure. Nests were monitored until young had fledged and left the nest. The 2002 nesting period was monitored entirely, from hatching to fledging. Prey delivery and chick diet were only recorded during the hatchling period, until young fledged and could forage for themselves. Nestling information and the periods of video monitoring for each breeding season are given in Table 4.1.

Nest Year	Numbers of chicks & their hatching dates	Fledging dates	Video-monitored period	
2001	2 (01/05/01, 02/05/01)	~28/06/01	15/06/01-28/06/01	
2002	2 (09/05/02, 13/05/02)	20/06/02, 29/06/02	12/05/02-29/06/02	
2003	3 (~13/05/03)	~26/06/03	29/05/03-26/06/03	

Table 4.1

During the video-monitoring period coverage was continual, except in brief periods of equipment failure. Video monitoring covered 22%, 94% and 64% of the nestling periods in 2001, 2002 and 2003 respectively. Results have been combined and not compared between years because of the differences in monitoring period.

The use of time-lapse video monitoring allows continuous recording over a far greater period of time than conventional recording speed. Picture quality is compromised, however, resulting in grainy, flickering monochrome footage. As a result, the resolution of identification of prey was reduced. Prey were identified to the lowest taxonomic level possible and, when available, identification was confirmed using RSPB records at the 'Red Kites @ Rockingham' information centre. In many cases identification was only possible to order, and prey was placed in generic categories. Many prey items comprised dismembered parts and were unidentifiable; these were placed in a separate category. In some instances, video analysis clearly showed the female parent removing prey from the nest following a feeding bout, then returning later with the same prey item. Where identified, this behaviour was accounted for to minimise the potential for repeat counts of the same item. Video observations of prey delivery provided counts of a particular prey over the three breeding seasons. Percentage frequencies for each prey type were then calculated.

4.3 **RESULTS**

4.3.1 The feeding behaviour of captive Red Kites when scavenging rats4.3.1.1 Behaviour at the carcass

Following placement of the carcass kites, would remain perched for, on average, 51 mins \pm 26 SD (N=24), before approaching and commencing feeding. The time elapsed varied with the birds' hunger, the level of local disturbance, the birds awareness of wider disturbance, and time of day. However, the time spent perched appeared also to have a behavioural function as the birds would intermittently turn their heads and intently scrutinise the carcass.

4.3.1.2 Management of the carcass

On approaching the carcass, birds would first alight on the ground in the vicinity of the carcass, and then walk towards it. On no occasion did a kite relocate, or attempt to relocate, a carcass to the feeding tray or a favoured feeding perch. Carcass management depended on the individual kite's method and skill in carcass entry and feeding. The two wild-caught kites from Germany were the most proficient in carcass entry (K2, Kw) and would, on most occasions, quickly manoeuvre the carcass to expose the area they would enter at and purposefully make the attempt. The two UK kites exhibited inferior abilities (K1, Kc) and would, on occasion, explore the entire carcass with the bill, searching for weak points or natural openings for access, often leaving the carcass without success. Both carcass placement on the aviary floor and the position the carcass was laid in (on ventral or dorsal surface, or side) were varied throughout the study. All individuals were capable of picking up a carcass in the bill and turning it over, or dragging it along the ground. There were individual differences in feeding ability but all kites, either immediately or following some exploration, turned the carcass onto its back for attempted entry at points along the ventral side. In all cases carcass entry was successful only in the thoracic and abdominal regions or at the anus. The birds fed from the carcass where it was placed or, following management, within the immediate vicinity. Altering the placement appeared to have no consequence.

4.3.1.3 The respective roles of the bill and talons

On arrival at the carcass the bill was initially employed in repositioning the carcass to expose the desired part of the body. The bird would then stand on the carcass with both feet before attempting to make entry with the bill. The weight of the bird pinning the carcass down was clearly necessary in order to provide the resistance against which the bill could work. The bill would pinch the hide, the neck would strain, and the head would jerk backwards as grip was lost, in attempts made to rip a wound. The bill would rip at the same spot, in quick succession until either a small wound was made or the bird gave up and tried another area. Only the bill was utilised for carcass entry and on no occasion were the talons used to puncture the carcass in order to aid entry. Once a wound was successfully made feeding and consumption would commence, utilising the bill to tear organs and flesh from the carcass. Only when a bird experienced difficulty in entering the carcass would it spend a period of time plucking tufts of fur from the area of the hide it desired to gain entry to. It was clear from all observations of feeding that the kites were loath to place anything more than the tip of the bill into the carcass. In order to eviscerate the carcass (section 4.3.1.5), the kite employs a method of turning the carcass inside out, 'skinning out'. The kite consumes organs attainable from the initial small wound, then enlarges the wound somewhat through tearing the hide and consuming localised muscle and flesh before using the bill to pull the carcass inside out in order to attain all the organs desired. Plate 4.1 shows an eviscerated, skinned out carcass. The talons played an important role in this method of feeding by clasping the hide at the edges of the wound and thus allowing the hide to be pulled inside out. In common with most birds, the kite is fastidious in keeping itself unsoiled and its feathers in good condition; following every feeding bout the bird would return to perch, wipe the bill energetically against the perch and pick the feet clean.

Plate 4.1 A 'skinned out' rat carcass



4.3.1.4 The area of initial attack and point of carcass entry

Differences in the abilities of the kites in carcass entry were clearly seen, although the methods used were essentially the same. One bird of each pair (Kw, K2) demonstrated a greater efficiency and adeptness in carcass entry than its companion (Kc, K1) and, as a result, was the first to feed in the majority of cases. In cases where Kc and K1 approached and attempted the carcass first, on every occasion but one the attempts would end in failure. The bird would return to perch and its companion would go to feed soon after. Typically, Kc and K1 would pluck and rip ineffectively at the carcass, appearing hesitant in deciding which part of the carcass to attempt. However, despite a lack of success in gaining entry in initial attempts at one or two areas, these individuals then made a systematic examination of the whole carcass, no doubt searching for a wound or natural opening which they could exploit. The eyes, ears, nose and mouth of the rat would be attacked, and periods of time would be spent plucking fur from areas of the hide to aid in entry. In contrast, Kw and K2 exhibited a purposeful and determined behaviour in carcass entry. These birds indicated a predetermined preferred point of entry and would, in most cases, immediately expose such areas before making sustained and effective attacks. In the sole incidences of carcass entry success, Kc and K1 took 240s and 170s, respectively, to open the carcass. Kw and K2 took 169 \pm 186s (means, SD) (n=10) and 48 \pm 31s (n=12), respectively, to open carcasses. For all birds, the preferred areas of attack were concentrated in thoracic-abdominal regions and in all cases successful carcass entry

was made only in these areas: Abdomen 46% (n=11); Thorax 33% (n=8); Anus 21% (n=5). In attacks on the abdominal region, birds would generally place one foot on the hindquarters of the carcass and one foot on the thorax, pinning the carcass, before ripping at the abdomen. In attacks on the thoracic cavity, either a similar position would be adopted to an attack on the abdomen, though with feet being placed further up the carcass or, both feet would be placed on the abdomen. On occasion, in both attacks on the abdomen and the thorax, use would be made of the limbs to aid entry; birds would rip both at the limb in order to tear the hide and attack the slightly looser hide under-limb, which would provide a better grip. In attacks on the anus, birds would either place both feet on the hind limbs or approach from the opposite side, placing both feet on the abdomen. Plate 4.2 shows a carcass where initial entry was made at the abdomen.

Plate 4.2 Rat carcass with initial entry wound at abdomen



4.3.1.5 The tissue/organ first consumed and the subsequent order of consumption

Feeding observations were made at a distance of 2-3 metres, allowing detailed and accurate notes to be made on feeding behaviour and any tissue preferences. It was possible to identify the consumption of all major organs and body parts. Cases were omitted from the data analysis where observer view of feeding was obstructed by the bird or where excessive disturbance altered the feeding behaviour of the bird to a large extent. In order to avoid unnecessary complexity in data analysis, organs and associated tissues were appropriately grouped for χ^2 analysis: Sub-mandibular and

ventral cervical soft tissues (including tongue, trachea and oesophagus to thoracic inlet) and thoracic organs (heart and lungs), urinogenital organs (including kidneys, bladder, glands and genitalia), small intestines, and liver. The importance of the intestines and liver in this study warranted consideration separately and thus were not grouped as 'abdominal organs'. Table 4.2 provides the observed and expected counts of organ consumption in a χ^2 table.

Table 4.2 χ^2 table showing observed and expected counts of organ consumption during feeding observations of red kites. 24 rat carcasses were consumed by two pairs of birds. The sharing of carcasses between a pair means that totals may exceed 24 where both birds consumed (partially) a particular organ of the same carcass at the same level of order.

Viscera⇒	Small Intestines	Liver	Thoracic / sub- mandibular	Urinogenital organs	Totals
Order ↓			organs		
	O = 10	O = 6	O = 7	O = 1	
1					24
	E = 6.70	E = 6.70	E = 5.02	E = 5.58	
	O = 4	O = 14	O = 4	O = 4	
2					26
	E = 7.26	E = 7.26	E = 5.44	E = 6.05	
	O = 10	O = 4	O = 7	O = 15	
3					36
(& later)	E = 10.05	E = 10.05	E = 7.53	E = 8.37	
Totals	24	24	18	20	86

Analysis of data indicated that there was an order of preference in viscera consumption ($\chi^2_{(6)}$ = 23.97, P<0.001, n=24). Further, examination of individual components of χ^2 highlighted the following order of preference: small intestines > liver > urinogenital organs. The most preferred organs, small intestine and liver, were consumed in every case in the first feeding bout. The small intestines are specifically noted as the kites exhibited a high degree of selectivity in consumption of the digestive tract. When feeding on the digestive tract, in 71% (n=17) of cases the caecum was deliberately separated and discarded, in 63% (n=15) of cases the large intestine was ignored and in 33% (n=8) of cases the stomach was discarded. It appears then that there is a preference not to consume these parts of the digestive tract, but this behaviour was certainly dependent, in part at least, on the hunger of a bird and, if feeding second, what was left of the carcass. Records of feeding show that when an individual fed from a carcass on successive days it was likely to be more selective in consumption of the digestive tract than when it had not fed for one or two days. Further, as the first bird to feed consumed the majority of the viscera, where parts of the digestive tract had been discarded, there were several records of the second bird of the pair taking the rejected organs whilst the first bird was still feeding.

4.3.1.6 Feeding bouts

The average duration of initial feeding bouts for all birds was 9.8 ± 6.7 min, n=29 (mean, SD). Only undisturbed bouts, where the kite left the carcass to return to perch, apparently satiated, were included. Bout lengths include the amount of time taken to enter the carcass. The average fresh weight of carcass consumed in initial feeding bouts for all birds was 62.73 ± 21.56 g, n=16, and as a percentage of the whole carcass, 25.33 ± 8.51 %, n=16. Only those feeding bouts where a single kite fed were considered. Normally the carcass could be retrieved for weighing following feeding by the first bird. Retrieval was sometimes not possible as the second bird would either immediately begin feeding from the carcass on cessation of feeding by the first bird or, on rare occasions 'steal' the carcass from the first bird.

4.3.1.7 Carcass remains at end of each feeding bout and final remains

Examination of carcasses following feeding allowed confirmation of observations and a closer inspection of feeding methods. Although evisceration was evidently the initially preferred feeding method, kites were very capable in managing and consuming all parts of the carcass. It was not unusual to observe birds swallowing whole limbs, large pieces of hide and sections of the spine, and on one occasion, even an intact head and tail. Ultimately, in most cases, the entire carcass was eventually consumed. Following the study of initial feeding bouts carcasses were returned to the aviaries and by the following day they would be gone, often without readily observable trace. Only in four cases were remains found; three intact hides with tails, and on one occasion a skull, picked clean.

4.3.1.8 Pair dynamics

The housing of the kites as pairs enabled some study of behaviour and interactions in relation to carcass utilisation. Further, the observation of any intraspecific competition was promoted as only one carcass was offered between a pair of birds, in contrast to normal feeding practice at the centre which provided birds with multiple portions. The kites always fed singly at the carcass and in most cases, only when the first bird had finished feeding and went to perch did the other approach the carcass. As discussed in section 4.3.4 Kw and K2 were more proficient in carcass utilisation than Kc and K1 and accordingly were normally the first to attempt to feed. There were a few occasions where the birds did interact at the carcass although the behaviours were variable between pairs. On one occasion while K2 was feeding, K1 approached and gave a relatively quiet call, at which point K2 stopped feeding and retreated from the carcass allowing K1 to feed. This behaviour was in contrast to similar interactions between Kw and Kc. Several times, soon after Kw had opened the carcass and had begun feeding, Kc arrived and attempted to steal the carcass by dragging it away. Kw would normally permit this without challenge and leave to perch, having consumed some viscera, but if Kc attempted to steal the carcass too soon Kw would defend the carcass; excited calling and aggressive posturing, even physical contact, often ensued.

4.3.2 The importance of rats in the diet of wild Red Kite chicks

4.3.2.1 Red Kite chick diet

Table 4.3 provides a complete listing of identified prey items delivered to nests during the video monitoring periods in all three years. The wide diversity of prey is indicative of the kites' scavenging niche. Many of the prey species are common road casualties e.g. lagomorphs, others may be available following lethal control e.g. rat, and some bird species may be taken as vulnerable juveniles or nestlings e.g. corvids. Video monitoring over the available periods indicated that lagomorphs and rats were the main identifiable prey by observed frequency.

Species	2001	2002	2003	Total	
Mammals					
Rabbit (Oryctolagus cuniculus)	7	15	14	36	
Hare (Lepus capensis)	. 1	4	1	6	
Rat (Rattus norvegicus)	1	16	7	24	
Stoat (Mustela erminea)	1	0	0	1	
Weasel (Mustela nivalis)	1	1	2	4	
Grey Squirrel (Sciurus carolinensis)	0	1	0	1	
Woodmouse (Apodemus sylvaticus)	0	5	3	8	
Vole (Clethrionomys glareolus or	0	3	0	3	
Microtus agrestis)					
Birds					
Pigeon (Columba sp.)	1	4	2	7	
Crow (Corvus sp.)	4	1	4	9	
Magpie (Pica pica)	0	1	0	1	
Pheasant (Phasianus colchicus)	0	1	0	1	
Red-legged Partridge (Alectoris rufa)	0	1	0	1	
Moorhen (Gallinula chloropus)	1	0	0	1	
Chicken (Gallus domesticus)	1	2	1	4	

Table 4.3 Identified prey spectrum delivered to red kite nestlings by parents during the 2001, 2002 and 2003 breeding seasons.

Figure 4.1 provides percentage frequencies of video monitored identified prey items, with additional data on prey remains at nests and occurrence in pellets during the breeding season (English Nature 2001) for comparison. Of the video monitoring data thirty five percent of prey remains delivered to nestlings were unidentified, mainly constituting dismembered parts. Eight percent of prey were unidentified mammals and eleven percent were unidentified birds. Prey remains and pellets were collected from red kites of the east Midlands population during the breeding seasons prior to 2001 (English Nature 2001). Clearly there are differences in prey frequencies between methods. Prey remains and pellets tend to bias diet estimates in favour of different prey types (Redpath *et al* 2001).

Figure 4.1 Comparison of percentage prey in diet identified by three different methods: video monitored prey delivered to red kite nestlings in 2001, 2002 and 2003 (pooled data), food remains found at kite nests and occurrence in pellets during the breeding season (source; English Nature 2001). Prey are categorised and unidentified prey items are not included.



4.3.2.2 Parental provisioning behaviour with regard to rats

The female mainly performed feeding of young. Although the male delivered prey to the nest, only in a few instances were attempts made to feed young, usually with little success (the male was observed to offer food to several areas of chicks, including their tails). During the first few weeks, chicks were unable to feed themselves and relied solely on the female for provision. In many cases it was not possible to observe the female parent feeding nestlings, as she would be facing away from the camera. However, several feeding bouts involving rats were witnessed over the three breeding seasons. Plate 4.3 shows a picture of the female parent (red 1) delivering a rat to chicks (4 weeks old) before feeding them. The female parents feeding behaviour, with regard to rats, was the same as described in captive kites (sections 4.3.1.3 to 4.3.1.5). The female would enter the carcass along the ventral side and proceed to eviscerate the rat before consumption of the rest of the carcass. As hypothesised, young chicks were fed the viscera (small pieces of easily digestible tissue), although it was not possible to identify specific organs. Plate 4.4 shows the female parent about to open and eviscerate a rat to feed to her three-day-old chick. After about four weeks, the chicks began to take an interest in food delivered to the nest and in the absence of the

female, would attempt to manipulate carcasses and feed themselves. During the following weeks the female would continue to feed them, providing the majority of their intake, yet as they became stronger and more experienced, she increasingly left them to feed themselves. When left alone with an opened rat carcass, older chicks (> four weeks) were observed to feed, though only on viscera via the existing wound. When fed by the female, older chicks were offered and consumed all parts of the rat, including, in one observed instance, a complete hind quarters and tail.



Female parent

Plate 4.4 Female parent about to feed a rat to chick



4.3.2.3 Parental foraging activity of Red Kite and the diel rhythm of rodenticide intoxicated rats

Is there a pattern between the foraging activity of the red kite parents and the diel rhythm of rodenticide intoxicated rats? Figures 4.2a-d show data on red kite parents prey delivery times to nestlings (a), delivery times of rat carcasses (b), daily activity of rats on Farm 1 during rodenticide treatment (c), and daily activity of rats on Farm 2 during rodenticide treatment (d). Over the three breeding seasons both parents delivered rats to the nest. Rats were delivered throughout the day from 05.00 to 17.00 BST (for all years; pooled data). Studies of the daily activity patterns in farm rats both before and after rodenticide treatment showed no change in diel rhythm (section 2.3.5). At Farm 1 rats were active during the day prior to rodenticide application and remained so during the treatment period, and at Farm 2 rats showed very little activity during the day both before and after rodentice to the nest throughout the day, from the first foraging flight at dawn to the evening. There are no peaks in the number of rats delivered at nests that correspond to times of high rat activity on farms.

Figure 4.2a Red kite parental delivery times of all prey to nestlings (2001,2002,2003; pooled data).



Figure 4.2b Red kite parental delivery times of rat carcasses to nestlings (2001,2002,2003; pooled data).



Figure 4.2c Daily rat activity at Farm 1 during rodenticide treatment period (May/June 2001).



Figure 4.2d Daily rat activity at Farm 2 during rodenticide treatment period (May/June/July 2002).



4.4 **DISCUSSION**

4.4.1 Foraging behaviour

As a scavenger the kite is often seen foraging close to human settlement, indeed many populations frequently exploit human waste and refuse in their diet and those prev that are available as a result of human activities. As a result, the kite has a reputation for being fairly tolerant of people. This may well be true of a kite in flight, but a kite on the ground is highly vulnerable and very wary. Certainly, observations of captive birds highlighted a tentative nature and precautionary behaviours when approaching the carcass on the ground. Despite the relative safety of the aviary, much time elapsed between carcass placement and arrival of the bird; time that was spent scanning the area and scrutinising the carcass. As the method of observation describes, observation was only possible if the kites were completely unaware of human presence. Otherwise the birds would remain perched and on no account would they even approach the carcass. As captive birds, on view to the public everyday, some desensitisation may be expected. If sensitivity was reduced, considering their behaviour, wild kites must certainly be very wary when feeding. Similar behaviour is expressed in wild birds; on location of a carcass, they circle slowly down to a nearby perch, where they wait and observe, ensuring a measure of safety, until a final approach is made.

In the study of captive birds, none of the kites attempted to move the carcass to the feeding tray or a favoured feeding perch. It is likely that the relative safety afforded by the enclosure was conducive to feeding on the ground as prey, or parts of prey, would normally (in wild kites) be picked up and carried to a feeding perch, or even be eaten on the wing. Certainly, in wild kites during the breeding season food is carried to the nest site or plucking station. Despite its size and impressive wingspan, the red kite is not a particularly heavy (0.7-1 kg) or powerful bird. It has a large wing area in relation to body weight enabling its characteristic gliding and soaring flight over large distances, but the lifting power, in terms of prey, is not known. Video monitoring evidence showed that rats are an important prey item for nestlings and rats of various sizes were delivered to nests. Wild Norway rats can weigh anything up to 550g (Meehan 1984). It is likely that an average sized adult rat is close to the limit of what an adult kite can carry for any significant distance.

4.4.2 Carcass feeding behaviour

Regarding the captive birds, differences in carcass opening abilities were probably dependent on factors such as experience in utilising whole rat carcasses and individual physical abilities. The poor abilities of Kc and K1 in carcass opening may be explained as follows: Kc was a captive bred bird probably inexperienced in scavenging whole carcasses of large rats which it may never have been offered; when attempting to gain entry to a carcass K1 only ever made what could be described as 'weak' rips with the bill, a possible result of the injuries sustained as a wild bird. In general, difficulties could be attributed to the fact that the kite is considered to be a relatively weak-billed raptor, and certainly with larger carcasses, e.g. sheep, the kite depends on other scavengers, e.g. foxes, dogs, buzzards and ravens, to open the carcass (Walters Davies & Davis 1973). Otherwise, unless a carcass has putrefied sufficiently, softening the hide, access is limited to the anus, mouth, eyes and ears. Taking advantage of natural openings and any wounds obviously aids carcass entry, although this may not provide access to preferred tissues and organs. In 79% of cases the point of initial attack and successful carcass entry was concentrated in the abdominal and thoracic regions indicating a predetermined attack for the attainment of preferred tissues. Video footage of kite parents opening rat carcasses in order to feed nestlings confirmed this behaviour. However, it must be noted that the abdominal and thoracic regions may just represent the weakest points in the hide where the kite has most chance of success. Obviously, methods of carcass entry and utilisation, and the application of bill and talons will vary according to the prey in question, size of prey being an important factor. Concerning the pair dynamics in carcass feeding observed in captive kites, it is possible that those kites which were less proficient in carcass entry may actually benefit from waiting until a carcass is opened, be it by another kite or another species, before feeding itself. Time spent on the ground (where highly vulnerable) and the energy required in gaining entry to a carcass may be saved if a kite waits for others to open it. Further, allowing others to feed from a carcass indicates its suitability for ingestion (Knight & Knight 1983). The disadvantage is that unless the initial scavenger(s) at the carcass can be displaced, the waiting kite may only be left with carcass remnants.

The red kite has a relatively large bill, typical of scavenging raptors. This adaptation enables proficient feeding from carcasses, and aids in reducing the extent to which the head is immersed in the carcass. Indeed, in all observations of feeding

behaviour, only the tip of the bill was entered into the carcass. Two important reasons may explain this restrictive trait: although kites do feed in groups if the food source permits, when feeding alone on the ground, the kite must remain keenly vigilant, scanning frequently; the feathers around the head are kept clean and in good condition, and bacterial spread and the risk of infection are reduced.

4.4.3 Tissue preferences

Observations of feeding by captive birds clearly highlighted evisceration as the initial objective before the consumption of other parts of the carcass. In view of this behaviour any consumption of flesh and major muscle groups in the early stages of feeding was interpreted as a method essential in the attainment of further viscera and not the preferred tissue for consumption. Certainly, in the common practice of 'skinning out', the initial wound must be widened through the consumption of localised flesh and muscle in order to pull the carcass inside out. The clear order of tissue/organ preference found may reflect the higher nutritional content and digestive efficiency of viscera in comparison to other tissues and such differences between viscera. Ingestion of the small intestines of herbivorous prey may provide a carnivore, such as the kite, with a source of vegetation nutrition not directly obtainable, in addition to the protein and fat content. The liver is a large, easily digested organ of high nutritional content. A similar order of preference has been documented in avian scavengers when utilising waterfowl carcasses (Peterson et al 2001). When feeding on larger carcasses (e.g.> than a small mammal), evidence both in these studies and in observations of wild birds suggests that the kite does not habitually gorge itself in one long session, but rather returns periodically for small feeds (Walters Davies & Davis 1973). It is likely that the carcass may be utilised by other scavengers between feeding bouts. It is therefore prudent for the kite to exhibit tissue preferences, consuming the most nutritious parts when given the choice, before competitors locate a carcass and feed.

Rejection of the contents of the stomach and caecum is common in many predators and scavengers. Aversion to such organs probably arises from the risks to health in ingesting possibly toxic or unpalatable matter. However, when faced with a choice of starvation or the consumption of distasteful and low quality tissues, kites feeding on carcass remnants were indiscriminate in tissue ingestion, consuming discarded digestive organs, hide and the skeleton.

4.4.4 Feeding behaviour on rats and secondary poisoning by rodenticides

The importance of these studies is clear in understanding the potential exposure of rodenticides to red kites. An evident preference for the small intestine and liver is a crucial finding because it will increase exposure. The digestive tract of a rat, which has been feeding on rodenticide bait, is likely to contain a quantity of undigested and partially digested bait, which may then be ingested by a scavenger. The liver, in comparison to all other organs, will contain the highest concentration of anticoagulant (chapter 6). Ingestion of the gut and liver presents the highest secondary poisoning hazard (Mineau *et al* 1999). The rejection of the stomach is an equally important finding, possibly removing a substantial proportion of potential rodenticide intoxication in the form of undigested bait. However, despite it being selectively removed in preference to other viscera, it was eventually consumed in the majority of cases. Certainly, at times of food scarcity, wild birds would be expected to be non-discriminate in organ consumption.

The observed eventual consumption of carcasses in their entirety in the studies of both captive and wild birds was not only surprising, but highlights the potential bias in diet studies that use carcass remains at nest sites and plucking stations in data collection. As a result, the importance of some prey, including the rat, in the diet of the red kite may be underestimated. Consequently, the level of exposure to rodenticides may also be underestimated. Conversely, it is conceivable that at times when prey is abundant, the kite may scavenge only lightly at each carcass, consuming only the most preferred parts. Where rodent carcasses are available in numbers following a baiting campaign, selective feeding behaviour is likely to enhance exposure.

Studies of feeding here have shown that red kites may be especially vulnerable to secondary poisoning by exhibiting a preference for eating potentially the most contaminated parts of a carcass. Red kite nestling diet monitoring showed that the rat is an important resource for chicks, second only to lagomorphs. Organs such as the liver and intestines represent the most easily consumed, nutritious and digestible parts of a carcass; thus the organs that potentially contain the highest concentrations of rodenticide (chapter 6) are those offered to chicks. Red kite chicks may well be most at risk from secondary poisoning as newborn hatchlings when they are fed the viscera of rats.

Red kite diet studies have shown that the rat is an important food resource (Carter& Grice 2000, Carter & Burn 2000). Additionally, red kites do forage around farm buildings where rat control may be undertaken (Plate 3.4). As a result of its foraging and feeding behaviour the red kite is clearly one of the species most at risk of secondary poisoning by rodenticides.

4.5 **CONCLUSIONS**

- 1) In the majority of cases, red kites fed on rat carcasses by entering at the abdominal and thoracic regions to expose viscera.
- 2) Red kites exhibited a clear tissue/organ preference in the order small intestines>liver>urinogenital organs, when feeding on rat carcasses. The evident preference for the small intestines and liver may increase the exposure of red kites to rodenticide when feeding on poisoned rats.
- Red kites were able to consume rat carcasses in their entirety, suggesting that rats may be underestimated in studies of diet that are based on feeding remains.
- 4) Rats were a main prey item in the diets of red kite nestlings.
- 5) When feeding on rats, hatchling red kites were fed only the internal viscera by the female parent. Red kite chicks may well be most at risk from secondary poisoning with rodenticides as newborn hatchlings.

CHAPTER 5: NON-TARGET SMALL MAMMAL RODENTICIDE EXPOSURE AROUND FARMS AND PHEASANT FEEDERS

5.1 INTRODUCTION

Recent studies from around the world have demonstrated extensive exposure of a wide range of non-target species to anticoagulant rodenticides (Berny *et al* 1997, Stone *et al* 1999, Howald *et al* 1999, Shore *et al* 1999, Burn *et al* 2002). Exposure may be direct or primary (through the consumption of bait by non-target species), secondary (through the consumption of contaminated prey), or even tertiary. The persistent, bio-accumulative nature of several of the chemicals involved provides scope for contamination beyond direct exposure, and their mode of action offers a lethal hazard to exposed individuals of many vertebrate species.

Rats (*Rattus norvegicus*), house mice (*Mus musculus*), and grey squirrels (*Sciurus carolinensis*) are the main target species for rodenticide control in Britain. The predators and scavengers that eat the target species are likely to be most at risk from secondary poisoning. However, surveys of rodenticide contamination in kestrels (*Falco tinnunculus*), stoats (*Mustela erminea*) and weasels (*Mustela nivalis*) (McDonald *et al* 1998, Shore *et al* 2001) have shown that a large proportion of samples, of species that do not normally predate the target species, also contain rodenticide residues. Kestrels, stoats and weasels are specialist predators of non-target small mammals (a collective term used here to mean wood mice (*Apodemus sylvaticus*), bank voles (*Clethrionomys glareolus*) and field voles (*Microtus agrestis*)), and this study attempts to determine whether small mammals could be an important route of exposure for rodenticide to predators and scavengers.

Small mammals have been shown to be attracted to rodenticide bait in other studies (Harradine 1976, Wood & Phillipson 1977, Cox 1991, Townsend *et al* 1995). The main aims of this study were to estimate the proportion of small mammals exposed to rodenticide bait, to estimate any population changes following exposure to rodenticides, and to quantify rodenticide transfer from exposed animals, both alive and dead, to their predators and scavengers. Exposure studies were carried out as part of a routine rat control programme, in order for results to be relevant to normal, routine rat-control and not considered just as experimental trials. Two sorts of rat control were examined: around agricultural premises, and around pheasant feeders

(feed hoppers on game estates can also attract a rodent infestation). This study details the results of replicate trials on two farms and three pheasant feeder sites on a game estate, where rat infestations were present. The specific hypothesis tested was that small mammal populations at rat-control sites would decline compared with populations at untreated control sites.

5.2 METHODS & MATERIALS

5.2.1 Site descriptions

Farms 3 and 2 are described in sections 3.2 and 2.2.2 respectively. Pheasant feeder sites were situated adjacent to the arable fields of Farm 3, as part of a game estate. Site maps are provided for Farms 3 (190×100m) and 2 (rat control area; $100\times115m$) in Figures 5.1a,b respectively, and for Pheasant feeder sites 1 ($10\times150m$), 2 ($15\times160m$) and 3 ($50\times80m$) in Figures 5.1c-e respectively. Studies were conducted during the following months:

Site Farm 3 Pheasant feeder 1 Farm 2 Pheasant feeder 2 Pheasant feeder 3 Date February 2002 March/April 2002 June 2002 July/August 2002 September/October 2002

5.2.2 Rat baiting

The protocol for rat baiting is described in section 2.2.5.

5.2.3 Longworth small mammal trapping

Live trapping, using Longworth small mammals traps, was used in order to monitor small mammal bait exposure and to estimate small mammal populations. Traps were placed within and around the baited area, yet independent of bait points. Fifty traps were placed in pairs in a grid system, spaced according to habitat, as recommended by Gurnell & Flowerdew (1994), and marked with a numbered cane. Specifically, traps were positioned in sheltered areas next to grass tussocks, logs, under bushes, and adjacent to any small mammal burrows and runs. Traps were placed on solid ground and at a slight angle, to prevent rainwater entering. Traps were filled with hay for bedding and warmth, and small handfuls of rolled oats to sustain the catch. Fly castors were also provided in case shrews (*Sorex* sp.) were accidentally captured. Site maps (Figures 5.1a-e) define small mammal trapping areas and the positions of trapping points.

5.2.4 Population estimation

In order to quantify the effects of rodenticide treatment on non-target small mammals, population estimates are required both before (Trap session 1) and after (Trap session



Figure 5.1a Site Map of Farm 3, showing small mammal trapping area and trap points

Figure 5.1b Site Map of Farm 2, showing small mammal trapping area and trap points





Figure 5.1c Site Map of Pheasant feeder 1, showing small mammal trapping area and trap points

Figure 5.1d Site Map of Pheasant feeder 2, showing small mammal trapping area and trap points



Figure 5.1e Site Map of Pheasant feeder 3, showing small mammal trapping area and trap points



KEY	
-----	--

木口	Trees	¥.	Hedge		Pheasant feeder
<u>sulu</u>	Grass (scrub)		Rat burrows	¢	Trapping point (2 Longworth traps)

2) application. It is important to have an operational definition of a population. In the context of this study, the population is defined as 'the animals that move and feed within the area enclosed by the traps, or whose home range encompasses the traps'. Following a trap pre-bait period of 2-3 nights, small mammal trapping was carried out for five nights and population size was estimated using mark-release-recapture (MRR). Two measures of population size estimation were applied to MRR data; Jolly-Seber (JS) and minimum-number-alive (MNA). The JS method of mark-releaserecapture was chosen as this analysis accommodates open populations (Greenwood 1996). The JS method is a fully stochastic model that allows for births, deaths, immigration and emigration. The extent of rat infestations and hence the area of small mammal trapping defined the study areas and none of the sites was isolated from surrounding small mammal habitat. Additionally, the provision of a grain-based bait would undoubtedly lead to the immigration of animals. Marking was batch-specific, as required for a JS estimate, with each mark on a different part of the body corresponding to a specific trap night. Populations could then be estimated with data on the number of animals with each possible capture history. For the purposes of this study it was not necessary to identify animals individually.

JS estimates are very imprecise unless the number of marked animals in each sample is more than ten (Greenwood 1996). In some of the studies not enough animals were marked in order to perform a species-specific JS estimate, owing to the number of traps used and the potential reduction in animal abundance following rodenticide application. For this reason, JS estimates were made only for the small mammal community as a whole. MNA was used to provide a measure of any changes in species-specific abundance. MNA is the sum of all individuals known to be alive during a trapping session. The use here of MNA as a population index assumes that animals do not die from being first trapped to the end of the trapping period. MNA is criticised as a measure of population size for being negatively biased as it assumes that the entire population has been marked, an assumption that cannot be validated and is unlikely to be true (Pollock *et al* 1990). MNA has the advantage that it is precise; it is measured without error rather than being estimated. Because of the inherent bias, however, it should be thought of only as an index of population size.

Population estimation was repeated after three months in order to estimate population recovery. This was not done at Farm 2 because the farmer would not allow further access to the site.

Traps were set at dusk and checked at dawn (times dependant on time of year). Animals were identified, sexed, weighed, and marked by clipping guard hairs to reveal undercoat of a different colour. The marking scheme that was used in all JS population estimations is detailed in Figure 5.2.

Figure 5.2 Marking scheme for Jolly-Seber small mammal population estimation



Day	Trap session 1	Trap session 2
Day 1	RH (Right Hind)	T (Tail)
Day 2	LH (Left Hind)	MR (Middle Right)
Day 3	RF (Right Front)	ML (Middle Left)
Day 4	LF (Left Front)	MM (Middle Middle)

5.2.5 Small mammal rodenticide exposure

Evidence of small mammal rodenticide exposure was based upon the presence of a bait marker dye, pre-mixed with bait, which showed in faeces. Small mammals invariably defecate within the trap tunnel during the period prior to release providing a reliable means of obtaining and examining faeces. The basis of the bait was composed of a mixture of coarsely cut cereal and wholegrain wheat. Although the commercial bait was already dyed blue (as a deterrent to birds (Pank 1976)), Chicago sky blue 6B dye was also added (700 mg.kg⁻¹) to ensure exposure was identified.

During the period of initial small mammal population estimation (Trap session 1), bait points were pre-baited with non-poisoned grain in order to overcome any neophobia in the rats (section 2.2.5). Following this period of pre-baiting, the pre-bait was replaced with rodenticide bait. Daily inspections of bait points allowed monitoring for evidence of small mammal feeding: grain remains and faeces. Rats and mice have been shown to differ in their feeding technique (Calhoun 1962). Rats will normally eat the whole grain, whereas wood mice will nibble the cheeks of the grain and discard the husks (Cox 1991). The use of tracking tiles to record the footprints of animals entering bait boxes also proved very informative (method described in section 2.2.6). During the first five days of rodenticide application, small mammal trapping continued in order to record bait exposure. Trapping also provided carcasses of individuals that succumbed to poisoning within the traps. It was assumed that, since the mode of action of anticoagulant rodenticide is delayed, resulting in death 3-4 days after consumption of a lethal dose, exposed individuals would have died or be close to death after 5 days. Small mammal rodenticide victims were collected, dissected and stored in deep-freeze in the laboratory until required for residue analysis (chapter 6). On dissection, the carcass was examined for internal haemorrhaging symptomatic of anticoagulant poisoning and the presence of dyed bait in the gut. The liver and digestive tract were then removed and stored separately in a freezer with the remainder of the carcass.

During the second 5 days of rodenticide application, another, 'postrodenticide' small mammal population estimation was carried out (Trap session 2).

5.2.6 Control populations to allow for natural fluctuations

Small mammal population densities fluctuate annually and numbers can be influenced by short-term climatic extremes. It is therefore necessary to monitor control populations simultaneously in order to allow for such natural effects and to separate them from the possible effects of rodenticide treatment. In each trial, control sites were chosen to be as similar in habitat structure as possible to treatment sites. Control sites were 300-1000m from treated sites. Fifty traps were placed at control sites in the same manner as in treatment sites and trapping was performed in tandem.

The rodenticide bait used in this study was grain based, attractive to rodents as a source of food. A similar grain was provided at bait points in control sites to allow for the effects of supplementary feeding influencing population estimates through

immigration from surrounding habitat. In order to test for any difference in palatability between the rodenticide and the untreated grain, the pre-bait was dyed with rhodamine B (500 mg.kg⁻¹) to monitor feeding during the first two trials. Rhodamine B and Chicago sky blue 6B dyes were chosen as bait markers as both have been shown to have no effect on food acceptability in either rats or wood mice in laboratory trials (Cox 1991).

5.3 **RESULTS**

5.3.1 Evidence of small mammal exposure

Small mammal visits to bait boxes were evident by the presence of small footprints and tracks on the tracking plates. In every trial, both rat and small mammal footprints were found on individual plates, indicating commensal feeding at common sites, although they may have fed at different times. The activity patterns of rats and small mammals do overlap and, as rats are known to defend a food source, the incidence of small mammals at bait boxes demonstrated an attraction to bait, rather than mere chance visitation. Small mammal faeces were found scattered both in the bait boxes and within the actual bait, showing that mammals fed while sitting in the bait as well as from the edge of the tray. This behaviour would contribute to exposure; not only would an animal be exposed in feeding, but also through the ingestion of bait powder and residue covering the limbs and body, via grooming. Small mammal feeding was also evident in the observation of feeding remains.

The primary indicator of small mammal rodenticide exposure was the presence of bait marker in faeces deposited in the trap. The presence or absence of blue dye in faeces was generally clear. Where there was uncertainty, closer investigation, involving squashing or breaking open droppings, confirmed the presence of dye. Over all the trials, an average of 48.6 % of the small mammal community trapped had fed on rodenticide bait from covered bait boxes. The proportions ranged from 32 % at Pheasant feeder 1 to 67 % at Pheasant feeder 3 (Table 5.1). All four species that were trapped were exposed, though apparently to different degrees. Wood mice were most attracted to bait, with an average of 57.4 % (\pm 14.5 %) (\pm SD) of animals trapped eating bait, bank voles; 30.6 % (\pm 12.6 %), field voles; 19.5 % (\pm 2.1 %), and of the house mice trapped at Farm 2, 30 % had dyed faeces (Figures 5.3a-e).

In the trials at Farm 3 and Pheasant feeder 1 a similar proportion of small mammals feeding on rodenticide and pre-bait, dyed with Rhodamine B, showed that there was no difference in exposure rates ($\chi^2_{(1)} = 0.107$ NS), and hence palatability (Figure 5.4). This shows that there was no significant difference in the palatability of grain treated with rodenticide (and Chicago sky blue) compared with untreated grain (with Rhodamine B).

 Table 5.1 The proportion of small mammals trapped at each site eating rodenticide bait.

Site	% Small mammals eating bait
Farm 3	59 %
Farm 2	43 %
Pheasant feeder 1	32 %
Pheasant feeder 2	42 %
Pheasant feeder 3	67 %

Figure 5.3a-e The percentage of small mammal species trapped that were known to have eaten rodenticide bait in each study.





Figure 5.4 Test to show whether coumatetralyl rodenticide affects palatability. Proportion of small mammals trapped eating untreated pre-bait and rodenticide bait at Farm 3 and Pheasant feeder 1. Rodenticide bait was dyed with Chicago sky blue. Prebait was dyed with Rhodamine B.


5.3.2 Rodenticide exposure

On introduction of the rodenticide bait (replacing the pre-bait), small mammals began feeding immediately, with a high proportion of traps containing dyed faeces the next day. The first signs of rodenticide poisoning in small mammals were observed only 2-3 days after bait introduction. Bleeding from the orifices (nose, ears anus and genitals) was noted in live wood mice and bank voles, which were subsequently released. If animals were found dead in traps, only those carcasses accompanied by dyed faeces were considered rodenticide victims. On the final day of the trial, all animals trapped with dyed faeces were humanely sacrificed by carbon dioxide asphyxiation, to represent those rodenticide-contaminated individuals potentially available to predators. All bodies were collected and taken back to the laboratory for analysis (chapter 6), as with the carcasses of rodenticide victims.

Exposed animals exhibited various stages of rodenticide intoxication through their behaviour, in contrast with the behaviour of an unexposed and healthy animal. Both wood mice and bank voles showed reduced escape responses, and in some cases, uncoordinated movement and a staggering gait, indicating clear discomfort. Such behavioural symptoms have previously been noted in observations of rodenticide intoxicated rats (Cox & Smith 1992).

5.3.3 Small mammal population changes

Table 5.2 provides Jolly-Seber estimates of small mammal populations before and after rodenticide treatment, the percentage population change, and a population estimate following a three-month recovery period, on all rat control sites and their untreated controls. The small mammal populations declined at all sites following rat control treatments. Of the corresponding control populations, three increased, one showed no change and one declined, over the same period. The control population for Farm 3 declined, probably because of torrential rain during February 2002, but this decline (44%) was less than at the corresponding rat treatment site (79%).

The specific hypothesis, that rat control reduced non-target small mammal populations, was tested by a one-tailed sign test. The one-tailed probability of a decline relative to control populations at all five rat-control sites occurring by chance is $p=0.5^5 = 0.03125$, i.e. significant at the 5% level. Thus it can be concluded that there was a significant decrease in small mammal populations as a result of rodenticide poisoning.

Table 5.2 Small mammal population changes following rodenticide exposure at rodenticide treated and control sites using the Jolly-Seber estimate. Small mammals included the following species: wood mice, bank voles, field voles and house mice. Not all species were present at every site (Table 5.3).

Site	Initial population	Population post- rodenticide exp.	Population change (%)	Population after three months
Farm 3	39	8	- 79	0
Control	41	23	- 44	28
Farm 2	19	11	- 42	No site access
Control	25	25	0	No site access
Pheasant feeder 1	35	7	- 80	34
Control	17	25	+ 32	38
Pheasant feeder 2	28	25	- 11	30
Control	26	31	+ 16	45
Pheasant feeder 3	80	38	- 53	25
Control	73	96	+ 24	38
Total: Rodenticide treated sites	201	89	- 56	
Total: Control sites	182	200	+ 9	

Table 5.3 provides MNA estimates of species-specific populations and population estimates when species are combined, before and after rodenticide treatment. Although the JS and MNA estimations are comparable, there are differences that affect the interpretation of results. MNA population estimations are consistently higher than JS estimations because the MNA estimator does not account for the immigration of small mammals into study areas; because of this bias, only JS data is considered in the interpretation of results.

Site	Initial population index	Population index post-rodenticide exposure	Population changes (%)	Population index after three months
	WM 37	11	-70	0
D	BV 3	0	-100	0
Farm 3	FV 3	3	0	0
	Total 43	14	-67	0
	WM 23	27	+15	21
Control	BV 4	2	-50	0
	FV 20	5	-75	22
	Total 47	34	-28	43
	WM 16	7	-56	No site access
Farm 2	BV 7	5	-29	No site access
	HM 7	0	-100	No site access
	Total 30	12	-60	No site access
	WM 20	16	-20	No site access
Control	BV 12	9	-25	No site access
	Total 32	25	-22	No site access
	WM 15	14	-7	11
Pheasant	BV 19	3	-84	32
feeder 1	FV 6	5	-17	0
	Total 40	22	-45	43
	WM 9	8	-11	15
Control	BV 10	15	+33	23
	FV 1	1	0	0
	Total 20	24	+17	38
	WM 5	10	+50	30
Pheasant	BV 25	25	0	5
feeder 2	FV 0	0	0	6
	Total 30	35	+14	41
	WM 5	13	+62	34
Control	BV 24	33	+27	24
	FV 0	0	0	1
	Total 29	46	+37	59
Pheasant	WM 59	55	-7	18
feeder 3	BV 42	5	-88	7
	Total 101	60	-41	25
	WM 45	46	+2	28
Control	BV 45	52	+13	26
	Total 90	98	+8	54

 Table 5.3 Small mammal population changes following rodenticide exposure at rodenticide treated and control sites using the MNA estimate as an index.

Figures 5.5a-e illustrate species-specific trapping trends at each of the rat control sites.







5.3.4 Population recovery

Population estimates made three months after each trial gave an indication of the potential longer-term effects of rodenticide treatments. At each of the pheasant feeders small mammal populations had recovered, and/or were at a level that may be expected for the time of year, suggesting density-dependent migration from adjacent populations. At Farm 3 no small mammals were found after three months. This may have been because small mammal populations are normally at their lowest in April/May.

Population recovery data are analysed in Table 5.4. The proportional rate of change is measured as N_3/N_0 , the small mammal population size (JS) after three months (N₃) compared with the initial population size (N₀) prior to rodenticide

treatment. In all four cases, the rate of change was higher in the untreated control populations than in the corresponding rodenticide treated populations, even when control populations declined outside the breeding period. Thus, effects of rat control were only partly offset by summer breeding, and outside the breeding period the two rat control sites (Farm 3 and Pheasant feeder 3) declined more than the untreated control sites. A paired *t*-test (based on N₃/N₀) indicates that the effect of rat control on small mammal populations was significant, when compared to control sites (P=0.047).

Table 5.4 Population recovery and proportional rate of change three months
after rodenticide treatment. Data considered in relation to the time of year and
small mammal breeding/non-breeding periods.

Site	Population after three months (N ₃)	Initial population (N ₀)	Proportional rate of change (N ₃ /N ₀)	Time of year
Farm 3	0	39	0	February-May
Control	28	41	0.6829	
Pheasant feeder 1	34	35	0.9714	March-June
Control	38	17	2.2353	
Pheasant feeder 2	30	28	1.0714	July-October
Control	45	26	1.7308	
Pheasant feeder 3	25	80	0.3125	Sept-December
Control	38	73	0.5205	
Total: Rodenticide treatment sites	89	182	0.4890	
Total: Control sites	149	157	0.9490	
Breeding: Rodenticide treatment sites	64	63	1.0159	
Breeding: Control sites	83	43	1.9302	
Non-breeding: Rodenticide treatment sites	25	119	0.2101	
Non-breeding: Control sites	66	114	0.5789	

Figures 5.6a-e illustrate both the initial proportions of small mammal species in communities at each trial site, based on trapping frequency, and the proportions of small mammal species in communities post-rodenticide exposure. There was very little change in relative species abundance following rodenticide exposure and community structure remained the same.

Figure 5.6a-e Initial and Post-rodenticide proportions of species in small mammal communities at study sites



5.4 **DISCUSSION**

5.4.1 Exposure of small mammals to rodenticide bait

The differences in the proportions of each species attracted to and feeding from bait stations may be a reflection of species differences in foraging ecology and typical diets. The wood mouse is a generalist seed and insect eater, the bank vole a generalist herb, leaf and seed eater and the field vole specializes in eating grass (Flowerdew 1993). It seems unlikely that the respective species interactions with rats at the bait boxes are responsible for the differences in take. Rats would be expected to defend a food source from small mammals. They are normally only active nocturnally, when the greatest degree of potential interaction would be with the wood mouse, another nocturnal species, yet it shows the highest take of all the small mammals. Interaction at bait boxes may be largely avoided with different peaks of activity during the night. Both vole species may be active nocturnally and diurnally and would be expected to have unhindered access to bait throughout the day. In monitoring poison hoppers, used in the control of grey squirrels, Wood & Phillipson (1977) discovered a wide range of both mammal and bird species to be attracted to and to consume warfarin bait. They estimated that between 55 and 65 % of bait was consumed by non-target animals, including the wood mouse, and that the majority of bait was removed at night, when squirrels do not feed.

As a preliminary investigation, not as part of the experimental design, trapping continued following the removal of bait at Farm 3. Here, there was clear evidence of caching in wood mice and bank voles (data not recorded). Both pre-bait and rodenticide bait were cached, evident with the presence of dyed faeces up to three days after the removal of bait boxes from the site. Food stores and caches are common in the burrows of wood mice and bank voles, particularly in the autumn and winter (Flowerdew 1993). Caching of bait would extend the length of exposure to an individual and may even lead to the exposure of individuals that do not visit bait boxes, especially during the winter when wood mice and bank voles are known to share nests with others of their own species to help keep warm (Flowerdew 1993).

Shrews are insectivores and would not be expected to be attracted to and feed on grain-based rodenticide bait, yet surprisingly, several were found in traps with dyed faeces (Data was not recorded as a licence to trap shrews was not possessed). Residues of bait have also been found in shrews in other studies (Colvin 1984,

Townsend *et al* 1995). The ability to adapt diet according to the availability of particular food items is one reason why many small mammals are so successful (Flowerdew 1993). This opportunistic feeding behaviour may explain why both shrews and field voles consumed grain-based bait. Further, all species may become more attracted to bait where there is shortage of natural food supplies, for example, during winter or in habitats of poor quality. An alternative explanation for bait exposure in shrews is through secondary poisoning, via the consumption of contaminated invertebrates. Insectivorous birds died from eating ants and cockroaches that had fed on brodifacoum baits (Godfrey 1985). Snails found near brodifacoum bait during rodent control were found to contain 0.91 mg.kg⁻¹ (Howald *et al* 1999). Residues of brodifacoum have been found in beetles feeding from bait stations (Eason & Spurr 1995). Indeed, both snails and beetles have been found in bait trays in this study. Shrews are very susceptible to poisoning on low doses of warfarin and they are reported to have a 28 times lower tolerance than wood mice (Churchfield 1990).

The use of dye to assess exposure may underestimate the numbers of animals feeding on bait in some circumstances. During the study at Pheasant feeder 1, the incidence of dyed faeces seemed lower than suggested by the evidence of bait feeding and the reduction in estimated population size. A possible explanation for this may be the high rates of metabolism and digestion in small mammals. Small mammals may not have entered the traps until several hours after feeding on bait, by which point dyed faeces may already have been excreted. Indeed, on investigation of faeces in traps, both dyed and light brown faeces could be found, resulting from bait feeding and excretion of food eaten prior to bait or from feeding on the rolled oats provided in the trap, illustrating the high rate of digestion and elimination.

5.4.2 Small mammal community structure

Small mammal communities at each trial site were typical of the habitat mosaic present. Wood mice are not demanding in their habitat requirements, being found in almost all agricultural habitats as well as more undisturbed sites. Bank voles require good ground cover, preferably with a diversity of dense shrubs and grasses. Field voles are grassland dwellers, requiring a grass sward substantial enough to meet their feeding and living needs (Flowerdew 1993). At Farm 3, a mixed sheep rearing and arable farm, the community was dominated by wood mice, with few voles. The study area covered the whole farm, comprising established hedgerows, grassy and wooded banks, and grassy patches around farm buildings. The low numbers of field voles can be explained by the lack of suitable habitat, but more bank voles may have been expected at this site. Farm 2 was a large pig farm, with rat populations within farm buildings and notably, a substantial infestation in scrub banks around an extensive manure heap at the periphery, where the study was carried out. The small mammal community comprised wood mice, bank voles and house mice, in descending order of abundance. Habitats at Pheasant feeder 1 and 2 comprised established banked hedgerows and dense scrub grassland. The community at Pheasant feeder 1 consisted of wood mice and bank voles, in equal dominance, and a small number of field voles. Only bank voles and wood mice were present at Pheasant feeder 2, but with a greater abundance of bank voles. Pheasant feeder 3 was sited in a small mixed woodland copse with poor ground cover. Again only wood mice and bank voles were found, though wood mice dominated here, as may be expected in such a habitat.

The low number of field voles from any of the study sites, including control sites, was surprising. In northern Britain the field vole shows cyclical fluctuations with periodic peaks at 3-5 year intervals and times of decline, relative scarcity and increase in the years in between (Flowerdew 1993). A possible explanation for the low numbers of field voles may be that the species was currently experiencing a cyclical trough in population status. However, it is more likely that the lack of field voles in the study areas is due to a paucity of good habitat.

The proportions of the species found within the communities and the occurrence of other small mammal species may be different at similar sites where rats are not present.

5.4.3 Small mammal population changes

The largest population declines were observed at Farm 3 and Pheasant feeder 1 trials, carried out during February and late March/early April, respectively. Wood mice and bank vole populations are typically at their lowest at this time of the year; it is the post-winter/pre-breeding season, and individuals that die are unlikely to be replaced. A large population decline was observed in the trial at Farm 2, carried out in June, during the breeding season when numbers may be expected to begin to rise. The smallest population decline was observed at Pheasant feeder 2, where trapping rates actually increased post-rodenticide exposure. This trial was carried out during late July/early August when both wood mice and bank vole populations are increasing

during their respective breeding seasons. The trial at Pheasant feeder 3 was carried out during late September/early October, when both wood mice and bank vole populations are reaching, or at, their peak. The population decline in the small mammal community here was mainly borne by the bank vole population. Trapping rates of wood mice remained high at Pheasant feeder 3, despite a high proportion being exposed to bait. A possible explanation is that individuals that succumbed may have been rapidly replaced by dispersing wood mice from adjacent unaffected populations. Bank voles are unlikely to disperse as rapidly (Wolton & Flowerdew 1985).

It is clear that rat-control treatments had a significant effect on local populations of the small mammal community. The actual effect, however, may be influenced by the time of year and the corresponding typical population densities. For example, the population effects of rodenticide-induced mortalities in the autumn may be tempered by the high dispersal rates of juveniles following the breeding season (Table 5.4). In late winter, when small mammal numbers are at their lowest, rodenticide application may all but wipe out the remainder.

Following the population losses as a result of rodenticide treatment, the immigration of new individuals will depend on both the productivity of adjacent unaffected populations and the quality of wildlife corridors. Certainly, all pheasant feeder sites had a greater degree of habitat connectivity than Farm 3, enabling populations to recover more quickly. The removal of some individuals sacrificed at the end of each trial for rodenticide residue analysis in the laboratory, would have artificially reduced populations. Although this would not affect population estimations at the time of the studies, it could have influenced the degree to which populations could recover. It seems likely, however, that these rodenticide-exposed individuals would have died anyway.

In terms of community status, the proportions of each small mammal species did not change significantly as a result of rodenticide treatment. The dominant species in each community prior to rodenticide application remained dominant post treatment. Any changes in community structure between initial populations and recovered populations, three months after treatment, matched those in control populations, clearly the effects of the natural annual changes in specific species abundances and not as a result of rodenticide induced effects. Data on the changes in rat populations were not collected. If rat populations did not recover as quickly as the non-target

small mammals, then the community structures at each site were altered. Rat control treatments may have aided the recovery of non-target small mammal populations through the removal of a competitor.

In view of the high proportions of small mammal populations exposed to bait and the effects on populations, the duration of rodenticide treatment is likely to be critical for long-term population status and the potential secondary exposure of predators and scavengers of small mammals. The objective of this study was to investigate small mammal rodenticide exposure during rat-control treatment. Treatment periods at the five sites lasted for only 10 days, and the population effects may have been less than in more extended rat control treatments. Rodenticide treatments often take up to four or five weeks, undoubtedly resulting in greater, long term and probably wider negative effects on small mammal population levels. Indeed, in studying the non-target small mammal population effects of permanent warfarin bait stations in Scottish shelter belts, Harradine (1976) found that wood mouse and bank vole populations were reduced to the extent that none of the breeding cohort remained to repopulate the site the following year. Analysis of bait markers in wood mice showed that individuals trapped as far as 80m from a treatment area had fed from bait points (Townsend et al 1995). Further, immigration rates of wood mice have been shown to increase with the provision of supplementary food (Flowerdew 1972). It appears likely then that the attraction to rodenticide bait, essentially a supplementary food source, could extend exposure beyond the treated site. Indeed, rodenticide treated sites, especially where baiting is permanent, could act as local population sinks for small mammals, resulting in the continual supply of intoxicated prey and contaminated carcasses to predators and scavengers. Where rodenticide treatment is temporary, it would be expected that populations could recover. In studying the resilience of small mammal populations, Sullivan (1986) found that the repopulation of experimentally depopulated sites, by five species of small mammal, showed that poison did not effectively suppress populations for long, at least when baiting is carried out over limited areas. Table 5.4 demonstrates, however, that the proportional rate of recovery of rodenticide treated sites was less than in control sites, i.e. the effect of rodenticide treatment persisted at least three months.

5.4.4 Exposure of predators & scavengers to rodenticide

Small mammals are an important component in the diet spectra of many predatory and scavenging species in Britain. Specialist predators for which small mammals are the principal prey item, include the kestrel, weasel, barn owl (*Tyto alba*), long-eared owl (*Asio otus*), short-eared owl (*Asio flammeus*), and tawny owl (*Strix aluco*). Generalists such as the buzzard (*Buteo buteo*), fox (*Vulpes vulpes*), red kite, polecat (*Mustela putorius*) and stoat, do not rely on small mammals and can alter feeding habits depending on the available prey. Non-target species that are susceptible to consuming contaminated rats and small mammal prey include predatory and scavenging birds and mammals that forage in and around agricultural premises, and also other sites where rodent control is practised e.g., around the feed hoppers used in pheasant rearing. The species for which rats and small mammals are predominant prey are clearly most at risk of secondary poisoning.

Secondary poisoning of weasels by warfarin has been shown (Townsend et al 1984), and coumatetralyl dosed mice caused the death of 4/4 weasels over an exposure period of 11-68 days (Anon 1981). In an effort to assess the incidence of rodenticide exposure in stoats and weasels, carcasses (40 stoats and 10 weasels) were collected from estate gamekeepers and analysed for six anticoagulant compounds (McDonald et al 1998). Residues of rodenticide were detected in 30% of weasels and 23% of stoats. A survey of polecats revealed rodenticide residues in 31% of the sample (Shore et al 1996). Studies by Birks (1998) highlighted heavy utilisation of agricultural premises by polecats during the winter when rat populations are high and consequently bait application is likely to be at its highest. A large proportion of polecats may certainly be at risk from eating poisoned rats as analysis of faeces confirmed rats as the principal prey item, although wood mice and voles were also taken. Evidence of rodenticide exposure in kestrels through the Centre for Ecology and Hydrology's (CEH) predatory bird monitoring scheme and the Wildlife Incident Investigation Scheme (WIIS) prompted an analysis of kestrel livers, specifically for second generation anticoagulants (Shore et al 2001). Of the 36 kestrels analysed (collected between 1997 and 2000), 67% contained rodenticide residues, indicating that there must be an important route of exposure in the foraging habits of this species. For comparison, 40% of barn owls analysed in 1997-98 by CEH contained detectable residues of second-generation rodenticides in the liver (Shore et al 2001).

The rodenticide-induced behavioural changes exhibited as symptoms of haemorrhage in rats i.e., reduced escape response and staggering gait (Cox & Smith 1992) were also observed in contaminated wood mice and voles in this study. Internal haemorrhage greatly affects limb joints, which is likely to account for decreased mobility (Wood & Phillipson 1977). Cox and Smith (1992) also suggest that rat foraging behaviour will shift from that which is perceived normal (i.e. thigmotaxis, the maximal use of available cover), to a pre-lethal anticoagulant toxicosis induced behaviour (movement in the open and away from cover), which increases exposure and vulnerability to predation (chapter 2). It is entirely likely that the foraging behaviour of intoxicated small mammals will similarly change (discussed in section 2.4.4). Results in this study have shown that a high proportion of the small mammal population, where rat control is practiced, are exposed to rodenticide (as much as 67%). A large number prey exhibiting rodenticide-intoxicated behaviours could increase the proportion of contaminated individuals in the diet of predators and therefore, the likelihood of ingesting a lethal dose.

While the risk may be highest for small mammal specialists, generalist species, which do not normally contain a large proportion of small mammals in their diets, may also be at risk. The adaptive and opportunistic nature of generalist scavengers and predators may lead some individuals to capitalise on a large number of conspicuous and lethargic prey. Short-term rodenticide treatments resulting in a temporary glut of carcasses could lead to the almost certain death of species that exhibit caching behaviour (e.g. mustelids (King 1989) and foxes (Macdonald 1987)). Additionally, if a species is naturally gregarious, social foraging behaviours may provoke the risk of mass poisoning incidents. In Switzerland, for example, the use of bromadiolone to control water voles (*Arvicola terrestris*) caused a mass mortality involving no fewer than 25 red kites among other raptor species (Petroli 1983, Beguin 1983; cited in Carter & Burn 2000).

Most species are not exclusively predatory or scavenging, and both modes of foraging may be employed; thus a species may be vulnerable to consumption of both rodent carcasses and live, but lethargic, rodents suffering sub-lethal anticoagulant toxicosis. The behaviour of poisoned moribund animals, regarding whether they die out of sight or in the open, is not known in the wild. Yet, it is an important question in quantifying the potential availability of contaminated rodent carcasses to scavengers. It is thought likely that most poisoned animals will retreat to their burrows or nests

(Birks 1998, Newton et al 1999) and this may equally apply to small mammals. Of the few studies attempting to examine whether moribund rodents retreat to their burrows or if they die out in the open, the results are variable and inconclusive. A laboratorybased enclosure study by Cox & Smith (1992) found that 50% of rats did not die under cover and appeared to move deliberately out from the shelter of the nest box to the open area of the enclosure. If such behaviour was common to rats and small mammals in the field, this could represent a major route of rodenticide exposure to scavengers. As part of a rat control campaign on a seabird colony on Langara Island, British Columbia, however, a radio-tracking study of 19 rats found that 13 of the 15 rats recovered died underground in their burrows (Howald et al 1999). Routine carcass searches found only 35 individuals of the estimated pre-eradication rat population of 3000 above ground, which represents 1.2% of the rat population. Other studies in rat control report similar findings (Fenn et al 1987, Taylor & Thomas 1993). Carcass searching is, however, notoriously unreliable (Stutzenbaker et al 1986). If poisoned moribund rodents do retreat under cover, then the secondary poisoning hazard to avian and other larger scavengers may be substantially reduced, though they may still be accessible to smaller mammalian scavengers that can access burrows and other rodent harbourage. This may increase the relative risk to weasels and stoats hunting in the burrows of small mammals, and especially weasels, since being smaller than stoats allows easier and greater access. Rodenticide residues have been found to be more prevalent in female than male stoats (Murphy et al 1998, McDonald *et al* 1998). This may be because female stoats eat more small mammals than male stoats (King 1989). However, both female stoats and weasels may be more exposed to poisoned moribund small mammals and their carcasses than males, due to their greater propensity for accessing small mammal tunnels and hunting underground, as observed in weasels (Erlinge 1975). Owls, hawks and larger predators occasionally predate stoats and weasels, leading to the possibility of tertiary poisoning.

Feeding behaviour at the carcass will also affect exposure. Anticoagulant rodenticides are stored mostly in the liver, while the stomach and intestines could contain substantial quantities of bait if a rodent had recently fed on rodenticide. If a consumer shows a preference for these organs, or of course, if the whole carcass is consumed, then it will receive maximum exposure (chapter 4). The dose contributed by the digestive tract may account for a large percentage of the total body burden. As

some consumers are known to reject this organ, this may have a significant influence on exposure. For example, kestrels and weasels survived eating prey contaminated with zinc phosphide, as they did not eat the guts (Tkadlec & Rychnovski 1990). Such feeding preferences will, however, be influenced by prey availability and requirements, and in times of food scarcity, wild animals could be expected to be less discriminating in organ consumption.

5.4.5 Rodenticide toxicity to predators and scavengers

Various laboratory and pen feeding trials have been conducted over recent decades with a view to quantifying the toxicity hazard that rodenticide contaminated prey may represent to consumers. Variation in procedure and conditions makes comparison of data difficult, but in a review of secondary poisoning studies (Joermann 1998), certain conclusions have been drawn. First generation compounds have rarely been implicated in secondary poisoning incidents involving birds, yet can cause mortality in mammalian predators. The second-generation compounds, however, are highly toxic to both birds and mammals.

In terms of secondary poisoning, the differences in toxicity and exposure hazard between the first and second-generation rodenticides mainly reflect differences in physiological toxicokinetics. Anticoagulants share a common binding site in the liver, but the second generation anticoagulants have a greater binding affinity than the first generation compounds (Parmar et al 1987), providing greater potential for accumulation and persistence (Huckle et al 1988). Hence, the risk of secondary poisoning is greater with second-generation compounds because, relative to first generation compounds, they are not substantially metabolised and excreted before death (Laas et al 1985). Rodenticides differ in their toxicities; 2-3 orders of magnitude between the first and second generation compounds, but also to a large degree within these groups. The inter and intra-specific sensitivities of both target and non-target species to rodenticides are variable, providing no basis for the extrapolation of toxicity hazard from tested species to non-tested species. The hazard potential for non-target species depends more on the application regime practised and on the feeding behaviour of non-target species rather than on the toxicity of specific rodenticides to individual species (Eason & Spurr 1995).

Measuring toxicity under laboratory conditions in order to estimate the risk to non-target species holds a degree of uncertainty. Laboratory bred animals may not be

as resilient as wild individuals and conversely laboratory conditions cannot mimic natural conditions and inherent stress, a factor considered crucial in promoting haemorrhage from anticoagulant ingestion (Jacques & Hiebert 1972). Further, assessing the actual hazard under field conditions is difficult because pharmacological susceptibility is not necessarily an indicator of ecological susceptibility (Moore 1966). Rodenticide treated sites may only represent a fraction of the foraging area for some species and irregular consumption of contaminated animals may only result in sublethal residues. However, some rodenticide compounds are bioaccumulative and a lethal dose may still be achieved if the exposure rate exceeds the rate of metabolism and excretion.

5.4.6 Effects of reduced prey abundance

The distribution, density and reproduction of specialist small mammal predators are intimately linked to the population dynamics of their prey (Flowerdew 1993). Reductions in the numbers of wood mice and voles have been shown to cause local declines and to affect reproductive success in weasels, stoats and tawny owls. In years of low numbers of small mammals, breeding failure is high and juvenile survival low in weasels and stoats (Tapper 1979, Erlinge 1981, 1983, King 1983). Studies of tawny owls and barn owls have shown that when prey availability is low, undernourished birds may not attain breeding fitness. Even if reproduction is viable, clutch size, hatching success, hatchling survival and fledging success are all greatly affected by prey numbers (Southern 1970, Shawyer 1987). The population numbers and breeding of kestrels, long-eared owls, short-eared owls and hen harriers (Circus cyaneus) are all affected by field vole numbers (Flowerdew 1993), where voles comprise a large proportion of the diet. When field vole numbers are low, and where there are differences in hunting habitats, other small mammal species will increase in importance, or may even replace voles as the principal prey in the diet. All small mammal species studied have been shown to be exposed to rodenticide contamination, resulting in local population declines, though perhaps to differing degrees. It is possible that, in contrast with typical diets, the easy capture of intoxicated animals, may increase the proportion of a particular species in the diet. For example, predatory species, for which the principal prey item is normally field voles, may switch to wood mice if capture effort is lower because of rodenticide intoxication.

The rodenticide induced reduction of small mammal populations, and even rats, if predated, in an area may cause declines in local populations of predators, as a result of secondary poisoning and a reduction in food supply.

5.4.7 Sub-lethal exposure

In the majority of cases of secondary poisoning, exposure is likely to be chronic with possible consequences of sub-lethal effects to behaviour and fitness. A reduction in fitness as a result of sub-lethal rodenticide toxicosis may increase the likelihood of mortality from other causes. For example, the impairment of hazard awareness or speed of reaction may result in collision with traffic or power lines. Anticoagulants do not appear to have any subtle sub-chronic effect on laboratory animals, though nonspecific signs such as anorexia and depression may be observed shortly before clinical signs (Berny et al 1997). Sub-lethal effects are very difficult to measure and it is very difficult to differentiate the effect from a wide range of potential causes in the wild. Sub-lethal doses of brodifacoum have caused abortions and reduced lambing rates in sheep (Godfrey 1985) and abortions in rats (WHO 1995). In studying the incidence of rodenticide residues in stoats and weasels, McDonald et al (1998) were unable to detect differences in body condition between contaminated and uncontaminated animals. Townsend et al (1981) concluded that it was unlikely that tawny owls would obtain a lethal dose of warfarin from the consumption of contaminated mice in treated woodlands, but they expressed concern about sub-lethal effects of measured reductions in plasma prothrombin. A study of secondary poisoning of golden eagles (Aquila chrysaetos) (Savarie et al 1979) found prothrombin clotting time had significantly increased, and although clotting times eventually returned to normal (two weeks later), eagles had appeared weaker with evidence of external bleeding. Such clotting disorders may prove hazardous if, for example, a predator is wounded or stressed.

5.4.8 Effects on predator and scavenger populations

While there is no evidence that rodenticide induced mortality in non-target predators and scavengers is causing populations to decline, there is evidence of extensive exposure, with the potential to cause additional mortality that may not be sustained by populations already experiencing critical limitations. For example, kestrel numbers have shown a decline of 29 % in the UK over the period 1994-2000 (Noble *et al* 2001) although this may be linked with overall declines in farmland biodiversity in recent decades (Burn *et al* 2002). Many populations can withstand a certain amount of extra mortality (or reduced reproduction) without declining in the long term, because of density dependent processes that enable remaining individuals to survive better or to reproduce more prolifically (Newton 1988).

Small mammals and other prey species have relatively short developmental periods (time to first breeding) and high reproductive rates. They are therefore capable of recovering rapidly from perturbations. Predators and scavengers, in contrast, are larger, mature more slowly and have a lower reproductive rate. Predatory birds typically forage over large areas encompassing several locations where rodenticide treatments may take place. History shows that recovery from perturbations may take many years (e.g. the sparrowhawk (*Accipter nisus*); Newton 1988), even after the cause of the population decline has been removed. Thus it is a cause for concern that predators and scavengers may be exposed to rodenticide-contaminated animals of non-target as well as target species. In the next chapter, this route of exposure will be quantified by estimation of rodenticide residues, and factors leading to higher or lower rodenticide loads will be assessed.

5.5 CONCLUSIONS

- Non-target small mammals (wood mice, bank voles and field voles) fed on rodenticide bait from bait boxes during normal routine rat control treatments.
- A large proportion (48.6%, n=5) of local small mammal populations were exposed to rodenticide bait. Wood mice were most attracted to bait, followed by bank voles, then field voles.
- 3) Non-target small mammals exhibited similar abnormal behaviours to rats (section 2.3.6) during the period of pre-lethal anticoagulant toxicosis. The abnormal behaviours observed in live animals might increase their vulnerability to predators.
- 4) Local populations of non-target small mammals significantly declined as a result of rat control using rodenticide treatments.
- 5) Population estimations made three months after the cessation of rodenticide treatment indicated that, generally, local populations of small mammals had recovered. The extent of recovery was dependent on the time of year and specifically, the stage of the breeding cycle.
- Small mammal community structure and relative species abundance did not significantly change as a result of the effects of rodenticide treatment.

CHAPTER 6: ANALYSIS AND QUANTIFICATION OF RODENTICIDE RESIDUES IN NON-TARGET SMALL MAMMALS

6.1 INTRODUCTION

Small mammals (wood mice *Apodemus sylvaticus*, bank voles *Clethrionomys glareolus* and field voles *Microtus agrestis*) are an important prey resource in the diets of a large number and wide range of predators and scavengers. Field evidence of extensive exposure to rodenticide of local populations of small mammals around farms and pheasant feeders was demonstrated in chapter 5. It is subsequently highly important to gain information and data on body burdens of rodenticides in order to assess the secondary poisoning risk to predators and scavengers. The anticoagulant rodenticide used in the small mammal exposure studies was coumatetralyl, a first generation compound, formulated as a grain based bait, 'Racumin', at a concentration of 375ppm. Study sites, bait placement and application rates are detailed in Chapter 5.

6.1.1 Physiological handling & dynamics of anticoagulants in mammals

Coumatetralyl is the agreed name for 4-hydroxy-3-(1,2,3,4-tetrahydro-1-naphthyl) coumarin [5836-29-3], $C_{19}H_{16}O_3$ (Figure 6.1). It was first introduced in 1956 and is now one of the most widely used of the first generation anticoagulants (Buckle 1994). It accounts for 8% of all rodenticides used on arable farms (Thomas & Wild 1996) and 4% of rodenticides used on game estates (McDonald & Harris 2000). The acute LD_{50} for Norway rats (*Rattus norvegicus*) has been recorded as 16.6 mg.kg⁻¹ (Dubock & Kaukeinen 1978), although the LD_{50} has also been estimated at a considerably lower level of 1.08 mg.kg⁻¹ (mean of male and female LD_{50}) (Greaves & Cullen-Ayres 1988). Coumatetralyl is more toxic when ingested chronically over a number of days; 0.3 mg.kg⁻¹ each day for five days (Buckle 1994).

Figure 6.1 The chemical structure of coumatetralyl



Anticoagulant rodenticides have a common mode of action, blocking the vitamin K cycle in the liver; different compounds differ only in binding affinity and persistence in the target organ. In examining the hepatic binding, metabolism and toxicity of the second-generation rodenticide flocoumafen, Huckle *et al* (1988) showed the anticoagulant to accumulate in the liver and to become lethal only when binding sites were saturated. Although, the liver is the target organ, distribution of anticoagulant in the blood stream may lead to accumulation in other parts of the body. Huckle *et al* (1988) found tissue concentrations of flocoumafen in the rank order; liver>>kidney>>skin>muscle>fat>blood. Anticoagulants are lipophilic and accumulate in fat (Huckle *et al* 1989a, WHO 1995). Following brodifacoum field trials, Rammel *et al* (1984) found residues in rabbit liver, muscle and fatty tissue at levels of 4.4, 0.26 and 0.86 mg.kg⁻¹, respectively.

The proportion of anticoagulant consumed that is metabolised and/or excreted is dependent both on the species and compound concerned. Following a single sublethal dose of flocoumafen to rats, 38% accumulated in the liver and exhibited a halflife of elimination of 220 days (Huckle *et al* 1989a). Metabolism was shown to be limited as the excreted rodenticide was largely unchanged parent compound. Warfarin (a first generation anticoagulant), however, is extensively metabolised hepatically and extra-hepatically in the rat (Barker *et al* 1970), and has a half-life of elimination of 7-10 days (Thijssen 1995). In terms of potency, coumatetralyl may be considered intermediate between flocoumafen and warfarin, being subject to little hepatic metabolisation and with a half-life of approximately 55 days (Parmar *et al* 1987). Anticoagulants have the greatest binding affinity for microsomal sites in the liver and the half-life of anticoagulants stored in other parts of the body is likely to be smaller (Eason *et al* 2002, Myllymaki *et al* 1999). Elimination is therefore typically biphasic because elimination from liver binding sites takes much longer than elimination from other sites.

Typically, only the liver is analysed in post mortem investigations of wildlife poisoning incidents and in monitoring for anticoagulant exposure (e.g. McDonald *et al* 1998, Shore *et al* 2000, Shore *et al* 2001). This protocol is useful for evaluating effects on exposed animals. For non-target prey, however, it cannot provide sufficient information on the potential secondary poisoning hazard for a risk assessment unless the relationship between residues in liver and other organs is known. Predators and scavengers do not only consume the liver, and in an assessment of secondary exposure the whole carcass must be considered. The liver constitutes only a small part of the body mass and the accumulation of anticoagulant at lower concentrations in other tissues may, by mass, contribute significantly to the total body burden. If an animal had been consuming rodenticide bait close to death, a large amount may remain unassimilated along the gastrointestinal tract (GIT). Although consumption of the GIT is species specific and may also depend on prey availability and seasonal requirements; its potential importance must be considered. For these reasons, the liver, GIT and body remainders were individually analysed for coumatetralyl. HPLC with fluorescence detection was used to determine this anticoagulant (Jones 1996).

6.1.2 HPLC theory

Chromatography is a technique of separating a sample into individual analytes, which can be characterised and quantified using a coupled detection system. High performance liquid chromatography (HPLC) involves passing a sample through a column (stationary phase) in a solvent medium under high pressure (mobile phase). Analytes are separated on the basis of their specific polarity. The polarity will determine an analyte's interaction between the mobile and stationary phases and the length of time it remains in the column. Altering the polarity of the mobile phase along a solvent gradient can enhance analyte separation. Analytes will leave the column at a specific time (retention time). Analytes are then qualified and quantified using direct fluorescence detection, a sensitive and selective detection system. The detector reacts to the presence of an analyte, producing an electric signal, the intensity of which depends on the amount of analyte present. Prior measurement of the retention time of a known analyte (calibration standard) allows identification and confirmation of that analyte in a sample. Measurement of the signal intensity of a known concentration of a known analyte allows quantification through calibration.

6.1.3 Aims & objectives

The overall aim of this chapter is to determine whether the body loads of rodenticide in non-target small mammals exposed through primary poisoning presents a risk to their predators and scavengers. The specific objectives are:

.

- To compare body loads between species, between different organs within a species, and between rodenticide victims compared with live but contaminated animals.
- To test whether there is any difference in body loads between small mammals exposed by rat control in different locations.
- To refine the risk assessment using these data.

6.2 METHODS & MATERIALS

6.2.1 Collection & preparation of small mammal carcasses

Rodenticide-exposed small mammals were taken for analysis in conjunction with the rodenticide-exposure study detailed in chapter 5. Rodenticide victims found dead in Longworth traps would represent some of the animals normally available to scavengers. On the final day of exposure studies, rodenticide contaminated, but live, animals were euthanased (by carbon dioxide asphyxiation), representing animals available to predators. Collection of both categories of animals allows an assessment of potential differences in levels of exposure between predators and scavengers.

Animals collected in the field were taken to the laboratory and prepared for analysis. Animals were weighed whole then dissected. Animals were investigated for the presence of rodenticide dye both on the skin/fur and in the GIT, and for clinical signs of anticoagulant toxicity. Following examination, the GIT was dissected from the oesophagus to the rectum and the liver excised. These viscera and their body remainders were then weighed, placed in individual containers and stored in a freezer at -20°C. Details of all rodenticide contaminated small mammals recovered and submitted to the laboratory for residue analysis are provided in appendix 1. Once all carcasses had been examined and dissected, individual body remainders were chopped into small pieces (approx. 1cm wide) and homogenised in a Waring blender (Model; 8011). On occasion, carcass hide would become wrapped around the blades, preventing cutting; however, repositioning material and re-blending overcame this problem. Additionally, blades were prone to blunting as a result of cutting bone. To maintain efficient and true homogenisation, blades were sharpened regularly. In order to prevent cross contamination, all exposed equipment was thoroughly washed and scrubbed between samples. Once homogenised, samples were returned to deep freeze storage.

6.2.2 Extraction & clean up of rodenticide residues

Rodenticide analysis and quantification were based on the method of Jones (1996), a modification of the original method of Hunter (1983a).

In order to measure the rodenticide residue it must be separated from the tissue matrix. This was achieved using a solvent extraction method. Samples were removed from storage and allowed to thaw at room temperature for 45 minutes prior to

extraction. Excised GITs and livers required a degree of homogenisation (fine slicing and mixing), as residue is likely to be non-uniformly distributed in tissues; body remainders were already suitably homogenised. Sample tissue (up to 1g, weighed to two decimal places) was then transferred to a pestle and mortar and ground with 10x the sample weight of anhydrous sodium sulphate, removing the water and exposing the maximum surface area to the solvent extractant. The sample was ground until a dry, free-flowing powder was obtained, then left for 30 minutes. The mixture was then transferred to a 100ml screw-top conical flask with 15ml of extraction solvent; dichloromethane: acetone (70:30 v/v). The flask was placed on an oscillating platform (model; HT Infors AG CH4103) at 300 oscillations/min for 1 hour to aid the extraction process. Following this the liquid extract was decanted into a 25ml centrifuge tube and spun at 3000 revs/min (model; MSE Centaur 2) for 10 minutes. To ensure complete extraction a further 10ml of extraction solvent was added to the conical flask, which was returned to the oscillation platform for another 30 minutes. This extract was similarly centrifuged and the two lots of resulting supernatant were combined in a 25ml volumetric flask, made to volume with extraction solvent.

The extraction process is not specific in isolating the rodenticide, and many naturally occurring unwanted co-extractives are included, e.g. lipids and fatty acids. To purify the sample extract and for determination at low levels (sub-ppm), a clean up process is necessary. Column adsorption chromatography using disposable neutral alumina Sep-Pak (solid phase extraction) cartridges provides chemical filtration, separating the analyte from the interferences. The cartridge was first conditioned with 10ml of dichloromethane using a 10ml glass syringe at a rate of 5-10ml per minute. Next, 10ml of the sample extract was loaded onto the cartridge and eluted at a rate of 3-5ml per minute. To remove the interferences, whilst retaining the analyte, the cartridge was then washed with 10ml of extraction solvent and then with 2ml of dichloromethane:acetone (25:75 v/v). Finally, the rodenticide was eluted with 5ml of methanol:acetic acid (95:5 v/v), a suitable stronger solvent, into a 7ml screw-top glass vial. The rodenticide eluate was then reduced to dryness by standing in a water bath at 70-80°C for approximately 1 hour, and the residuum reconstituted in 0.5ml methanol for HPLC analysis.

Prior to HPLC analysis samples were required to be stored until enough were prepared to justify a run. To prevent sample loss and potential degradation, sample

vials were tightly wrapped in laboratory sealing film (Whatman) and stored in a sealed container at 4°C. The complete sample preparation took approximately 4 hours. Three samples could be prepared at a time, allowing nine samples to be prepared in a 12-hour working day.

All solvents used were HPLC grade and were supplied by Fisher Chemicals, Loughborough, except the dichloromethane, which was supplied by Sigma Aldrich, Gillingham. The anhydrous sodium sulphate was specially formulated for pesticide residue analysis and supplied by Fisher Chemicals, Loughborough. Alumina N classic Sep-Pak cartridges (1850mg) were supplied by Waters, Watford.

6.2.3 Using HPLC to measure rodenticide residues

The first batch of samples was analysed (over two runs) in the pesticides section of the Central Science Laboratory (CSL), Sand Hutton, Yorks. Suspected wildlife poisoning incidents are investigated here and analysis for anticoagulant rodenticides is routinely performed. Samples were analysed according to CSL standard operating procedure (SOP) *PGD/001; determination of anticoagulant rodenticide residues in samples*. The second batch of samples was analysed (over two runs) in the Biocentre at the University of Leicester. Samples were analysed according to SOP PGD/001 of CSL. Ten samples were randomly selected and analysed in both laboratories for the purpose of calibration.

The HPLC system at CSL consisted of an autosampler (model; Waters 717 Plus) and a fluorescence detector (model; Waters 474). A PC running Waters Millennium software controlled the system; the autosampler, solvent pressure, flow rate and gradient, and the measurement of detector signals. The HPLC system at the Biocentre consisted of an autosampler (model; Varian Pro-Star 410) and fluorescence detector (model; Waters 470), controlled by Varian Pro-Star chromatography (v6.20) software. The HPLC analysis conditions suitable for anticoagulant rodenticides are listed in Table 6.1.

Parameter	Conditions				
Column	Spherisorb ODS2, 5µm, 250x4.6mm with				
	guard column (guard column (reverse-phase)			
Solvent A	0.25% (v/v) acetic acid in water				
Solvent B	0.25% (v/v) acetic acid in methanol				
Flow rate	0.8 ml/minute				
Gradient	Time (min)	% A	% B		
	0	75	25		
	5	5	95		
	20	0	100		
	21	75	25		
	25	75	25		
Post-column reagent	6% (v/v) ammo	nia solution			
Flow rate	0.6 ml/minute				
Detector: excitation wavelength	310 nm				
emission wavelength	390 nm				

Table 6.1 HPLC parameters for the analysis of tissue	e extracts.
--	-------------

Anticoagulant rodenticides exhibit an intrinsic fluorescence strong enough to be detected directly (Coly & Aaron 1998). However, the native fluorescence of these compounds is quenched in the acidic mobile phase, necessitating a post-column pH-switching reagent, which enhances the fluorimetric response (Hunter 1983a). In some analyses the separation and resolution of *cis* and *trans* isomers may be desired, e.g. flocoumafen. Coumatetralyl, however, does not have isomers. Over the course of each sample the signals produced by the detector form a chromatogram. Each peak along the chromatogram indicates the presence of an analyte in a sample. A peak was identified as coumatetralyl if it fell within the retention time window of the external calibration standards run at the beginning and end of each batch of samples.

Coumatetralyl residues were quantified using linear equations based on calibration ranges (0.004 to 0.4 μ g/ml) run with each batch of samples. The minimum limit of detection was defined as the estimated peak height that was three times noise levels. Where sample concentrations exceeded the highest concentration calibration standard of the range (maximum limit of detection), they were suitably diluted and rerun.

6.2.4 Quality assurance

Confirmation of coumatetralyl was obtained by re-chromatographing the samples in the absence of the post-column reagent. Under these conditions the fluorimetric responses of coumatetralyl and other anticoagulants reduce by a characteristic amount (90-95%). This response distinguishes the analyte from any co-extractives, which may potentially interfere chromatographically, thus preventing identification of a false positive.

Procedural accuracy and precision were evaluated by measuring standard recoveries. Uncontaminated mouse liver (1 g) was spiked with 0.1 ml of a 1µg/ml solution of pure coumatetralyl in methanol, i.e. 0.1 µg coumatetralyl. A procedural recovery was performed for each batch of samples. These samples were extracted, cleaned up and analysed as all samples. The mean recovery was estimated as 102% (SD= \pm 39, n=4). Residue data were not corrected for recoveries because this result suggests that there was effectively no loss of rodenticide in the extraction and clean up.

6.3 **RESULTS**

6.3.1 Residues measured in small mammals

A total of 41 wood mice, 17 bank vole, 1 field vole, 4 common shrew (*Sorex araneus*), 1 pygmy shrew (*Sorex minutus*) and a woodmouse foetal sample were analysed. The GITs, livers and body remainders of wood mice and vole bodies were dissected out and analysed separately. The bodies of shrews and the foetal sample were too small to separate and were analysed wholly. Of a grand total of 183 samples, 96 were analysed at CSL and 87 were analysed at the Biocentre. Calibration of samples between the two laboratories was tested by fitting a trendline to the data, giving the linear equation y=1.4554x (y=CSL data, x=Biocentre data) (r²=0.9446) (Figure 6.2). Thus, CSL sample values were 1.4554x higher than Biocentre sample values were converted accordingly in order for all of the results to be combined and analysed together. A complete data set from the analyses is provided in appendix 2. Sample chromatograms of a mixed rodenticide standard and body remainder sample are shown in Figures 6.3a,b.

Figure 6.2 Calibration plot – Pesticides department, CSL vs. Biocentre, University of Leicester. Ten samples were randomly selected and analysed in both laboratories.





Figure 6.3a High Performance Liquid Chromatogram; mixed rodenticide standard

Figure 6.3b High Performance Liquid Chromatogram; sample 13 - body remainder



Concentrations of coumatetralyl in the GITs, livers, body remainders, and expressed as whole animal concentrations, of wood mice and bank voles are shown in Table 6.2. Figures 6.4 and 6.5 show histograms of the range of concentrations (mg.kg⁻¹) in the livers and whole bodies of both rodenticide victims and live but contaminated small mammals, respectively.

Table 6.2 Mean tissue concentrations of coumatetralyl in non-target wood mice and bank voles following rat control on farms and around pheasant feeders. Data are displayed as back-transformed mean concentrations averaged across site (95% confidence limits in brackets).

Status	Species	GIT (mg.kg ⁻¹)	Liver (mg.kg ⁻¹)	Body remainder (mg.kg ⁻¹)	Whole body (mg.kg ⁻¹)
Victim	Wood mice (10)	0.97 (0.234-2.21)	0.84 (0.405-1.431)	0.27 (0.108-0.504)	0.445 (0.156-0.882)
Victim	Bank Vole (14)	0.531 (0.282-0.858)	2.927 (1.834-4.275)	0.245 (0.132-0.392)	0.42 (0.259-0.618)
Alive	Wood mice (31)	0.112 (0.029-0.248)	0.195 (0.103-0.318)	0.035 (0.022-0.051)	0.062 (0.029-0.108)
Alive	Bank Vole (3)	0.207 (0-3.336)	0.377 (0-6.113)	0.051 (0-0.463)	0.095 (0-1.256)

Figure 6.4 Histogram showing the distribution of untransformed coumatetralyl residue levels (mg.kg⁻¹) in the livers of non-target small mammals (victims & alive). Victims are animals that died in traps. Animals that were exposed to rodenticide but had not died were euthanased and are referred to as alive. Note that intervals on the x-axis are not even.



mg.kg⁻¹

Figure 6.5 Histogram showing the distribution of untransformed coumatetralyl residue levels (mg.kg⁻¹) in the whole bodies of non-target small mammals (victims & alive). Victims are animals that died in traps. Animals that were exposed to rodenticide but had not died were euthanased and are referred to as alive. Note that intervals on the x-axis are not even.



Since the masses of body tissues were recorded, it was possible to calculate actual residue amounts in each tissue, which were then summed to provide whole body burdens of rodenticide residue (Table 6.3). Figure 6.6 shows a histogram of the range of body burdens (µg) found in rodenticide victims and live animals.

Table 6.3 Mean tissue residues of coumatetralyl in non-target wood mice and
bank voles following rat control on farms and around pheasant feeders. Data are
displayed as back-transformed mean concentrations averaged across site (95%
confidence limits in brackets).

Status	Species	GIT (µg)	Liver (µg)	Body remainder (µg)	Body burden (total µg)
Victim	Wood mice (10)	3.174 (0.571-7.882)	0.652 (0.31-1.121)	3.32 (1.329-6.207)	7.48 (2.423-15.315)
Victim	Bank Vole (14)	1.23 (0.659-1.977)	2.342 (1.409-3.511)	2.644 (1.453-4.19)	6.004 (3.739-8.802)
Alive	Wood mice (31)	0.335 (0.075-0.781)	0.186 (0.089-0.32)	0.433 (0.257-0.655)	1.025 (0.436-1.861)
Alive	Bank Vole (3)	0.577 (0-10.165)	0.379 (0-6.59)	0.635 (0-6.933)	1.639 (0-23.405)

Figure 6.6 Histogram showing the distribution of untransformed coumatetralyl body burdens (μ g) in non-target small mammals (victims & alive). Victims are animals that died in traps. Animals that were exposed to rodenticide but had not died were euthanased and are referred to as alive. Note that intervals on the x-axis are not even.



The livers generally contained the greatest concentrations of coumatetralyl compared with the other tissues. The exception was a high mean concentration in the GITs of woodmouse victims. In comparison with the other tissues, concentrations in body remainders were lowest. Figure 6.7 illustrates the differences in concentrations $(mg.kg^{-1})$ between the GIT, liver and body remainder for both rodenticide victims and live animals. Conversion of concentrations to residue amounts changed the rank importance of tissues. Because of their greater mass, the body remainders contained the greatest absolute amount of rodenticide. Comparatively, the livers of animals contained the least amount of residue, except in the case of bank vole rodenticide victims. Figure 6.8 shows the differences in amounts (μ g) of rodenticide between the tissues for all animals.

Figure 6.7 Mean concentrations (mg.kg⁻¹) of coumatetralyl residue distributed in non-target small mammals (victims & alive). Victims are animals that died in traps. Animals that were exposed to rodenticide but had not died were euthanased and are referred to as alive. Data are presented as backtransformed mean concentrations.



Figure 6.8 Mean amounts (µg) of coumatetralyl residue distributed in non-target small mammals (victims & alive). Victims are animals that died in traps. Animals that were exposed to rodenticide but had not died were euthanased and are referred to as alive. Data are presented as backtransformed mean concentrations.



6.3.2 Statistical analysis: Wood mice & bank voles

Woodmouse and bank vole data were analysed statistically using the analysis of variance (GLM) option in Minitab (v13.30 Minitab Inc.). The most appropriate transformation, square-root (SQRT), was chosen to help satisfy the assumptions required for analysis of variance, primarily homogeneity of variances. Assumptions for the interaction terms species*site and death*species*site could not be met and were not computed. ANOVA results for all tissues are tabulated in Tables 6.4a-e.

The factors referred to are defined as follows:

Factor	Туре	Levels	Categories
Death	fixed	2	Alive, Victims
Species	fixed	2	Bank voles, Wood mice
Site	fixed	2	Farms, Pheasant feeders

Analysis of variance between species highlighted that liver concentrations in bank voles were significantly greater (approx. 3x) than in wood mice. No other species differences in tissue concentrations were statistically significant.

The factor 'death' was especially conclusive; both wood mice and bank vole rodenticide victims had significantly higher concentrations in all tissues than animals trapped alive.

Investigation of the factor 'site' (farm or pheasant feeder) revealed that those animals (both victims and alive) trapped around farms had significantly greater GIT and body remainder concentrations than those trapped around pheasant feeders. Additionally, a significant interaction between site and death was shown as victims that died around farms had higher GIT and body remainder concentrations than victims that died around pheasant feeders. Site had no effect on liver concentrations.

In terms of body burden (actual residue amounts), there was no significant difference in levels of coumatetralyl between wood mice and bank voles. Body burdens were, though, significantly higher in rodenticide victims than those contaminated animals trapped alive. Body burdens were significantly higher for animals (both victims and alive) around farms (rather than pheasant feeders), and rodenticide levels were greatest in rodenticide victims.
Source	DF	Seq SS	Adj SS	Adj MS	F	P
Death	1	3.1801	2.2201	2.2201	11.04	0.002
Species	1	0.2017	0.0065	0.0065	0.03	0.858
Death*Species	1	0.3004	0.0586	0.0586	0.29	0.592
Site	1	0.9353	1.0877	1.0877	5.41	0.024
Death*Site	1	1.0187	1.0187	1.0187	5.06	0.029
Error	52	10.4612	10.4612	0.2012		
Total	57	16.0974			_	

Table 6.4a Analysis of Variance (GLM) of coumatetralyl concentration (mg.kg⁻¹) in GITs (SQRT), using adjusted SS for tests.

Table 6.4b Analysis of Variance (GLM) of coumatetralyl concentration (mg.kg⁻¹) in livers (SQRT), using adjusted SS for tests.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Death	1	10.6408	3.2760	3.2760	18.93	0.001
Species	1	2.8182	2.0229	2.0229	11.69	0.001
Death*Species	1	0.7068	0.9766	0.9766	5.64	0.021
Site	1	0.1653	0.2087	0.2087	1.21	0.277
Death*Site	1	0.4349	0.4349	0.4349	2.51	0.119
Error	50	8.6532	8.6532	0.1731		
Total	55	23.4191			_	

Table 6.4c Analysis of Variance (GLM) of coumatetralyl concentration (mg.kg⁻¹) in body remainders (SORT), using adjusted SS for tests.

Source	DF	Seq SS	Adj SS	Adj MS	F	P		
Death	1	1.33517	0.72290	0.72290	33.09	0.001		
Species	1	0.00010	0.02444	0.02444	1.12	0.295		
Death*Species	1	0.00759	0.00263	0.00263	0.12	0.730		
Site	1	0.20358	0.23720	0.23720	10.86	0.002		
Death*Site	1	0.22729	0.22729	0.22729	10.40	0.002		
Error	50	1.09221	1.09221	0.02184				
Total	55	2.86593			—			

Table 6.4d Analysis of Variance (GLM) of coumatetralyl concentration (mg.kg⁻¹) in whole bodies (SQRT), using adjusted SS for tests.

In others searces	(22	//				
Source	DF	Seq SS	Adj SS	Adj MS	F	P
Death	1	2.26889	1.31386	1.31386	24.22	0.001
Species	1	0.00032	0.06223	0.06223	1.15	0.289
Death*Species	1	0.01167	0.00844	0.00844	0.16	0.695
Site	1	0.38345	0.45118	0.45118	8.32	0.006
Death*Site	1	0.48489	0.48489	0.48489	8.94	0.004
Error	52	2.82104	2.82104	0.05425		····
Total	57	5.97026				

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Death	1	33.0649	21.4323	21.4323	22.03	0.001
Species	1	0.1003	0.6614	0.6614	0.68	0.413
Death*Species	1	0.5693	0.0124	0.0124	0.01	0.911
Site	1	6.9828	8.2293	8.2293	8.46	0.005
Death*Site	1	9.0031	9.0031	9.0031	9.26	0.004
Error	52	50.5813	50.5813	0.9727		
Total	57	100.3018				

Table 6.4e Analysis of Variance (GLM) of total coumatetralyl burden (µg) in bodies (SQRT), using adjusted SS for tests.

Pearson product-moment correlations (r) of concentrations/amounts of coumatetralyl in different tissues are tabulated in Table 6.5. All values of r indicate a significant positive association (P<0.001) between the levels of coumatetralyl in different tissues.

Tissue concentration/amount	GIT (mg.kg ⁻¹)	Liver (mg.kg ⁻¹)	Body remainder (mg.kg ⁻¹)	Whole body (mg.kg ⁻¹)
Liver (mg.kg ⁻¹)	0.703			
	(0.001)			
Body remainder	0.821	0.842		
$(mg.kg^{-1})$	(0.001)	(0.001)		
Whole body (mg.kg ⁻¹)	0.933	0.868	0.955	
	(0.001)	(0.001)	(0.001)	
Body burden (µg)	0.954	0.823	0.922	0.987
	(0.001)	(0.001)	(0.001)	(0.001)

Table 6.5 Correlation coefficients (Pearson) between coumatetralyl concentrations/amounts (SQRT) in different tissues. P-Values given in brackets.

6.3.3 Coumatetralyl residues found in other species

A mean (back-transformed) whole body concentration of 0.009 mg.kg⁻¹ (95% confidence limits; 0.002-0.024) and a mean body burden of 0.063 μ g (0.004-0.189) were found in shrews (4 common and 1 pygmy). Coumatetralyl residues in the sole field vole analysed were 0.020, 0.123, 0.022, 0.028 mg.kg⁻¹ and 0.458 μ g, in the GIT, liver, body remainder, whole body and body burden, respectively. A concentration of 0.117 mg.kg⁻¹ was found in the woodmouse foetal sample (residue amount; 0.249 μ g).

6.4 **DISCUSSION**

The small mammals analysed in this study were retrieved from the non-target rodenticide-exposure studies of chapter 5. The presence of dyed faeces showed that animals had been exposed. Post mortem examination had confirmed exposure, revealing symptoms consistent with anticoagulant poisoning, e.g. pale liver and kidneys, subcutaneous haemorrhage, and the presence of free blood. Laboratory analysis detected coumatetralyl residues in all animals. The presence of coumatetralyl in the bodies of rodenticide victims and live, but contaminated, individuals poses a secondary poisoning hazard to scavengers and predators respectively. But are the levels detected sufficiently high to cause concern?

6.4.1 Residue distribution in tissues

As may be expected, the highest concentrations of coumatetraly were generally found in the liver, a result consistent with anticoagulant tissue distribution studies in rats (Huckle et al 1988) and Japanese quail (Coturnix japonica) (Huckle et al 1989b). Rodenticides act by interrupting the vitamin K cycle in liver microsomes (MacNicoll 1986). Evidence suggests that once a saturating concentration for the binding sites within the liver has been reached, only then do anticoagulants exert their toxic effect (Parmar & Batten 1987, Huckle et al 1988, 1989b). Hence, a high affinity for binding sites within the liver leads to its preferential storage there, at a high concentration relative to other parts of the body. Circulating unbound anticoagulant may remain in the blood stream or accumulate in a variety of tissues around the body (Eason et al 1996, Huckle et al 1989b, Yu et al 1982). Concentrations of rodenticide were found in body remainders of both rodenticide victims and live animals. The relatively high concentrations in the GIT (gastrointestinal tract) were likely to be due to the presence of undigested bait, present if an individual had recently fed prior to death, and/or as anticoagulant accumulated in the intestinal epithelium. Certainly, on dissection, a large number of stomachs and intestines were stained blue, the colour of the dye in the bait. When the concentrations were converted to actual residue amounts, the relative importance of each tissue changed. The contribution of rodenticide from the body remainder becomes most important as it represents most of the body mass (76.83%, n=59) and the liver becomes less important as it constitutes only 5.59% (n=59) of body mass. The GIT remains important, carrying relatively high concentrations of

anticoagulant and representing 17.58% (n=59) of body mass. Thus, when consuming a rodenticide contaminated small mammal, the level of secondary exposure a predator or scavenger receives will crucially depend on the order of tissue preference and extent of carcass consumption (chapter 4).

6.4.2 Residue differences between death, species and site

As expected, rodenticide victims carried greater residues in all body parts than those in live, but contaminated, animals. Residues in victims were likely to be higher as a result of hepatic binding saturation and storage in extra-hepatic sites. This result suggests that it is scavenger species that would receive greater rodenticide exposure and are likely to be more at risk of secondary poisoning than predator species. It must be noted, however, that moribund individuals are likely to contain similar levels of rodenticide to dead animals. Hence, in some cases, predators may be at risk of similar levels of exposure to scavengers.

The significantly greater rodenticide concentration in the livers of bank voles than in the livers of wood mice indicates a difference in handling between the two species. A possible explanation is that wood mice are more proficient in metabolising rodenticide than bank voles, although studies on rats have indicated that coumatetralyl is subject to little hepatic metabolisation (Parmar et al 1987). Theoretically, this species difference may be a corollary of an evolved physiological resistance to rodenticide as a result of typical diet. Bank voles are typically herb, leaf and seed eaters, whereas wood mice predominantly consume seeds and insects (Flowerdew 1993). Rodenticides are based on a naturally synthesised plant toxin, coumarin (the 4hydroxy coumarin moiety), originally isolated from mouldy sweet clover (Melilotus officinalis and M. alba), but present in many plant species. It is possible that a bank vole can physiologically tolerate coumarin to a greater extent than a woodmouse as clover may naturally form part of its diet. Alternatively, bank voles may gain some resistance to rodenticides through a greater intake of vitamin K (which acts as an antidote to the effects of anticoagulant) as part of a green leaf diet, compared to wood mice.

The finding that GIT and body remainder concentrations in animals (both victims and alive) from farms were greater than concentrations found in animals from pheasant feeders suggests that animals around farms consumed more bait than those around pheasant feeders. Certainly, animals inhabiting farms would normally exploit

139

anthropogenic food sources such as spilled grain. Indeed, they may rely on such resources if farms comprise little natural vegetation. Animals inhabiting pheasant feeder sites, largely comprised of natural habitat, may normally feed on natural resources, and in contrast to animals inhabiting farms, spilled grain may only represent an alternative resource. No difference was found between the liver concentrations of these animals, probably because the accumulation in the liver is fixed, relative to the amounts of anticoagulant residue in the GIT and body remainder that are subject to greater variability. Higher body burdens in animals (both victims and alive) found on farms than in animals found around pheasant feeders indicates that predators and scavengers that forage around farms are at risk of a higher level of exposure than those that forage around pheasant feeder sites.

6.4.3 Comparison with other studies

The results presented here are similar to other small mammal residue studies. Brodifacoum rodenticide (10 mg.kg⁻¹ active ingredient) treatment (10.5 kg/ha, aerial application) targeted at meadow voles (Microtus pennsylvanicus) resulted in whole body concentrations of 2.07 ± 0.17 mg.kg⁻¹ (mean \pm SE) in live animals (Merson *et al* 1984). In a similar study of Microtus sp. exposure, Myllymaki et al (1999) found mean brodifacoum residues of 0.38 mg.kg^{-1} in rodenticide victims and 0.03 mg.kg^{-1} in live animals from plots treated with 4 and 5 kg/ha rodenticide. During efficacy trials of chlorophacinone against rangeland rodents, Primus et al (2001) found whole body residues ranging between 0.26 and 4.1 mg.kg⁻¹ (n=3) in carcasses of *Microtus* sp. Residue concentrations in no-choice laboratory trials are in excess of field results (Myllymaki et al 1999, Grolleau et al 1989, Kaukeinen 1982). Clearly, there is some variation in small mammal residue concentrations, and results may not be directly comparable. Principally, differences in residue concentrations will be dependent on species (attraction and accessibility to, and intake rates of, bait), rodenticide type (specifically first-generation or second-generation anticoagulant rodenticides) and concentration of active ingredient, and the mode and rate of application.

In comparison to concentrations of coumatetralyl found in the target species, the rat, the small mammal residues measured here are relatively lower. Whole body concentrations of 6.8 mg.kg⁻¹ (n=5) were measured in rats found dead following rat control on farms in the east Midlands (MacVicker 1998). Higher concentrations in the

target species should be expected, although it is also possible that small mammals are more susceptible to rodenticide poisoning than rats.

6.4.4 Refining the risk assessment

It is not feasible to test the toxicity of a pesticide or to measure tissue residues in all non-target species following lethal and sub-lethal exposure (Brown *et al* 1996). An assessment of the secondary poisoning hazard to non-target species is, in most cases, theoretical, based on extrapolation from toxicity and residue data of other species and other chemicals (Brown *et al* 1996). Risk assessment of pesticides uses worst-case scenarios in order to determine whether the risk is acceptable (Crocker *et al* 2002).

Table 6.6 calculates the theoretical secondary poisoning hazards to weasels and kestrels of consuming small mammal rodenticide victims, based on rat and hen (*Gallus domesticus*) toxicity data, respectively, assuming some physiological similarity in sensitivity. Using published data on both acute and chronic LD₅₀ values, the residue data collected in this chapter can be used to calculate how many wood mice or voles might deliver a lethal dose to a weasel (*Mustela nivalis*) or kestrel (*Falco tinnunculus*). Weasels and kestrels were chosen as model species for their role as small mammal specialists and in light of recent evidence revealing extensive rodenticide exposure in liver samples (McDonald *et al* 1998, Shore *et al* 2001). For each predator the lethal dose was calculated by weight and the number of contaminated small mammals required to achieve that dose was calculated using the mean body burden of animals in this study. For example, for a female weasel of average body weight (0.063 kg) the number of small mammals required to achieve a lethal dose would be: 0.063 kg × 1.08 mg.kg⁻¹ = 0.068 mg = 10 wood mice/bank voles (10 × 0.00674 mg).

Table 6.6 Calculated number of small mammals in diet required to deliver a lethal dose according to published LD₅₀ (coumatetralyl) data and mean body burdens found in this chapter.

Species	Chronic (Five day) LD ₅₀	Small mammals required ^g	Acute LD ₅₀	Small mammals required ^g	
Weasel ^c			l l b		
(Female)	$0.3 \text{ mg.kg}^{-1} \times 5d^{-1}$	$2.8 \times 5d = 14$	1.08 mg.kg ⁻¹	10	
Weasel ^d			i control h		
(Male)	$0.3 \text{ mg.kg}^{-1} \times 5d^{-1}$	$5.3 \times 5d = 26.5$	1.08 mg.kg ⁻¹ °	19	
Kestrel ^e			$50 \text{ mg kg}^{-1 \text{ f}}$	1550	
			JU mg.kg	1330	

^a Coumatetralyl chronic LD₅₀ for rat (Eason *et al* 2002).

^b Coumatetralyl acute LD₅₀ for rat (Greaves & Cullen-Ayres 1988).

^c Body weight 63g (Crocker et al 2002).

^d Body weight 118g (Crocker *et al* 2002).

^e Body weight 209g (Crocker et al 2002).

^f From Luttik *et al* (1999), based on a surrogate bird species; Hen (*Gallus domesticus*) (Hermann & Hombrecher 1962).

^g Assumes mean body weight of woodmouse and bank vole = 15.64g and mean body burden of coumatetralyl = 6.74μ g (rodenticide victims), using this study sample.

These rough calculations suggest that, under field conditions, weasels may be at risk of poisoning by coumatetralyl if contaminated small mammals are consistently eaten over a number of days. Coumatetralyl is not subject to substantial metabolisation within the liver and has a half-life of 55 days (Parmar *et al* 1987), hence there would be some bioaccumulation. Both female and male weasels may be at risk as consumption of contaminated small mammals is only required for periods that are far less than the elimination half-life of coumatetralyl. Male weasels are less susceptible than female weasels owing to their reduced utilisation of small mammals and greater mass. Weasels may be less likely to achieve a lethal dose under a typical diet regime rather than a diet of solely small mammals. McDonald (2002) reports that small mammals typically constitute 66% of the diet of male weasels and 77% of the diet of female weasels: Consumption of a lethal dose, assuming that none is metabolised, could therefore occur in 6.3-8.2 days in females or 8.5-12.9 days in males using the figures in Table 6.6 and intake rates given by Crocker *et al* (2002).

First-generation anticoagulants such as coumatetralyl are known to be of low toxicity to birds. In all experiments with birds, first-generation compounds have caused only a single death (Lee 1994; cited in Joermann 1998). First-generation

compounds have never been implicated in the death of wild birds. It is unlikely that kestrels would be at risk from poisoning by coumatetralyl. In order to achieve a lethal dose, assuming that none is metabolised, a kestrel would have to consume a strict diet of contaminated small mammals for 308 days or, under a typical diet estimate of 78% small mammals (Holliday & Kenington 2001), 395 days, using the figure in Table 6.6 and intake rate given by Crocker *et al* (2002). However, in stark contrast to first-generation compounds, second-generation anticoagulants have caused the mortality of birds after only a few days of feeding on contaminated prey (Joermann 1998) and secondary poisoning has caused the death of wild birds (Barnett *et al* 2002). Using a reported flocoumafen lethal dose for a barn owl (*Tyto alba*) (0.93 mg.kg⁻¹; Newton *et al* 1994) and assuming coumatetralyl body burdens, a kestrel may receive a lethal dose after eating 28.8 contaminated small mammals, attainable after 5.7 days (100% small mammal diet) or 7.3 days (78% small mammal diet).

Even if none of the target animals (rats) are consumed, these calculations show that predators are at risk of secondary poisoning from consuming non-target small mammals around typical farm and pheasant feeder treatments.

6.4.5 Factors that may modify risk under field conditions

Risk calculations assume field situations in which predators forage entirely within a rodenticide treated area. Diets may comprise either 100%, or lower, more typical proportions, of poisoned small mammals. These assumptions are not unreasonable. Predators considered here are those for which a rodenticide treated area comprises a large proportion of an individual's foraging range. In addition, it is possible that predators might preferentially select small mammals exhibiting altered behaviour induced by rodenticide intoxication, over healthy individuals (as discussed in section 5.4.4). Also, if intoxicated prey are easier to catch, a predator might focus it's foraging in a rodenticide treated area regardless of how small a proportion it is of the foraging range. Hegdal & Colvin (1988) reported mortality of screech owls (*Otus asio*) when the area treated with brodifacoum was only 10% of the foraging range of the owl.

If prey are only partially consumed (a possibility when predators are faced with a glut in availability), preferential consumption of viscera that contain disproportionately large amounts of anticoagulant (e.g. livers and GITs) may increase the potential for secondary poisoning. It must be noted that in common with primary consumers, residues in the food of predators will not match the actual body burden of the prey as a portion of the ingested anticoagulant is excreted in faeces (Myllymaki *et al* 1999), either as unchanged parent compound or metabolites. The calculations given do not consider this potential physiological loss and hence predator dose may be overestimated.

Second-generation rodenticides are likely to present the greatest secondary poisoning hazard to predators. Difenacoum and flocoumafen exhibit elimination halflives of 120 and >100 days in the livers of rats (Parmar *et al* 1987) and quail (Huckle *et al* 1989b), respectively. A lethal dose would be achieved more easily as the greater toxicity and, significantly, persistence of second-generation compounds may not require consistent feeding on poisoned small mammals; a lethal dose may accumulate over a longer period of time. One of these compounds, difenacoum, is reported to be the most widely used rodenticide on arable farms (Thomas & Wild 1996) and game estates (McDonald & Harris 1998) in the UK.

Certainly, there is evidence of a secondary poisoning hazard to weasels and other mustelids both in the laboratory and in the field, supporting this theoretical assessment. In New Zealand field trials, secondary poisoning with brodifacoum rodenticide was responsible for poisoning weasels, stoats (*Mustela erminea*) and ferrets (*Mustela furo*) (Alterio 1996, Alterio *et al* 1997, Murphy *et al* 1998a,b). In laboratory studies, stoats and weasels succumbed to secondary poisoning with bromadiolone (Grolleau *et al* 1989) and a range of other anticoagulants (Anon. 1981, Townsend *et al* 1984), respectively.

6.5 CONCLUSIONS

- Analysis of wood mice and bank voles confirmed that these non-target small mammals had consumed substantial amounts of rodenticide aimed at rats.
- 2) The highest concentrations of anticoagulant were generally found in the target organ, the liver. However, significant concentrations were also found in the gastrointestinal tract and body remainder. Indeed, in terms of body burden, most of the anticoagulant was found in tissues other than the liver.
- Levels of anticoagulant residue were significantly higher in rodenticide victims than in live, but contaminated, small mammals, indicating that scavengers could be more at risk of secondary poisoning than predators.
- Levels of anticoagulant were significantly higher in the livers of bank voles than in the liver of wood mice, indicating a difference in physiological handling between the two species.
- 5) Levels of anticoagulant were significantly higher in the gastrointestinal tracts and body remainders of animals trapped around farms than in those trapped around pheasant feeders. Consequently, predators and scavengers that forage around farms could be at a greater risk of secondary poisoning than those that forage around pheasant feeders.
- 6) In assessing the risk to predators of small mammals it was calculated that weasels foraging around rat control sites could be at a high risk of secondary poisoning. Kestrels consuming small mammals contaminated with coumatetralyl are unlikely to be at risk of secondary poisoning. However, if non-target small mammals are exposed to second-generation anticoagulants, the risk to kestrels is likely to be greater.

CHAPTER 7: GENERAL DISCUSSION

7.1 Rat infestations and the population effects of rodenticide control

The availability of food, water and harbourage fulfils the basic requirements of a rat (Rattus norvegicus) and may lead to the establishment of a colony almost anywhere. The size and extent of populations will depend on the amount and concentration of these factors, some of which may be limiting. The common rat is omnivorous, feeding on whatever food may be available, though exhibiting preferences when given the choice (Lund 1994). Rats require a daily source of free-water, normally available in most situations in many exploitable forms, e.g. slurry pits, standing water resulting from poor drainage, and the water supplies of livestock. Rats require harbourage to rear young, provide refuge from predators, and rest (Meehan 1984). Rats are not fastidious in choosing home sites and will burrow in a range of materials, although sloping terrain and loose, well-drained soil are preferred (Meehan 1984). An abundance of resources will allow an infestation of rats to develop in a relatively short space of time. Rats are often described as r-strategists, exhibiting high rates of reproduction and population growth until environmental factors become limiting or environments become unfavourable, at which point emigrants are produced (MacDonald & Fenn 1994).

Colonies of rats are composed of small family units or 'clans' (Fenn & MacDonald 1987). This basic social unit remains constant, but the amount of nest site territory defended by each clan varies inversely with the size of the whole colony (MacDonald & Fenn 1994). In a farm environment rats inevitably interact when foraging, especially if food sources are concentrated. When concentrated, foraging sites may represent neutral ground (Lund 1994) and familiarity between clans may limit the incidence of aggressive interactions. If diffuse, food sources may be defended by individual clans (Buckle *et al* 1987, Fenn & MacDonald 1987). Resident clan members are more likely to be tolerated than transient rats or rats inhabiting the periphery of a colony. The gregarious nature of rats contributes to the proliferation of a colony. However, population density may be regulated by the complex social structure of a rat colony (Adams & Boice 1983, Butler & Whelan 1994). Effective rat control using rodenticide bait requires an understanding of the site-specific social system of rats.

A regional population of rats distributed between patches of favourable environment may absorb the effects of the lethal control of local populations through migration between the metapopulation. The behaviour of dispersing individuals is vital to the establishment of new colonies and thus reinvasion of controlled sites (MacDonald & Fenn 1994). However, little is known regarding the ecology, population dynamics and natural behaviour of rats beyond a site of infestation.

Initial field trials with brodifacoum failed to control rats satisfactorily (Buckle 1994), leading Rennison & Dubock (1978) to suggest that behaviourally subordinate rats may be excluded from bait points by dominants. Observations in this study (chapter 2) support the 'pulsed-baiting' theory of Dubock (1984), based on the concept that aggressive monopolisation of bait points by dominants creates a situation where progressive rat control needs to take account of a social hierarchy. The removal of dominant individuals will disrupt the social structure of a colony, allowing subordinates the opportunity to breed and permitting the invasion of transient rats or rats from peripheral clans. Prolonged baiting is, therefore, likely to be necessary to achieve the desired level of control. However, if the environment remains favourable for rat colonisation, the existence of a metapopulation is likely to ensure that control of a local rat population is short-lived (Smith 1994, 1995). Rat traits of high mobility, opportunism, adaptability and high reproductive capacity predispose the species to colonise, sites if its ecological requirements are met, or continue to be met.

7.2 An ecological approach to rat control

Pest management is basically an ecological problem, not necessarily requiring a chemical solution, and the future solution to rat control should be ecologically based, replacing the current ecologically and economically unsustainable chemical approach (Miller 1992). Although the amount of synthetic pesticides used in the United States has increased 33-fold since the 1940s, U.S. crop losses have increased from about 31% in the 1940s to about 37% today (Pimental 1989; cited in Miller 1992). Poison baiting is only effective in temporarily controlling rat infestations. Rat populations can seldom be eradicated over the long term (Burn *et al* 2002). Indeed, prolonged and excessive rodenticide application may increase the likelihood of the evolution of resistance in local populations of rats. Established resistance to first generation anticoagulants, e.g. warfarin, is widespread in UK rats. Perceived resistance to the

second generation compounds bromadiolone and difenacoum, developed to control warfarin resistant rats, has been reported in the south of England (Gill *et al* 1994, Quy *et al* 1992a).

Since their successful introduction, the emphasis and reliance on anticoagulant rodenticides in rodent control has suppressed the development of ecologically based rodent management (Singleton et al 1999). A rodent management strategy should be based on a sound and thorough knowledge of the animal's biology, behaviour and ecology, which can then be integrated with targeted management practices. The primary aim of pest management should be to reduce damage, rather than to kill a pest, and if possible, efforts made to exclude a pest may negate the requirement for control (Smith 1994). Smith (1994) suggests a variety of non-chemical and non-lethal chemical means that may be adopted in order to manage rodent pests: Immigration reduction and exclusion e.g. rodent-proof buildings, removal of local harbourage, electric fences, diversion feeding; emigration, through the removal of resources; reducing the pest birth rate e.g. the removal of nesting opportunities and the use of reproductive inhibitors or biological sterilants; and, increasing the pest death rate e.g. using parasites, diseases or predators as biological control agents. Research should be directed into investigating the potential of such control methods and developing them into effective and economically viable alternatives to rodenticides.

7.3 The ecotoxicology of anticoagulant rodenticides

The environmental use of toxic chemicals to control a pest may constitute an exposure hazard to non-target wildlife, but it is the level of exposure that determines risk. Rodenticides are highly toxic to vertebrates by design, and therefore have the potential to affect wildlife adversely (Brown 1994). Rodenticides are normally applied to control rat infestations in urban areas, in and around farms, and on game estates. Second generation anticoagulant rodenticides accounted for 74.2% of rodenticide use on arable farms in the UK in 1998, whilst first generation compounds were used on only 17.5% (Bankes & Garthwaite 2001). Brodifacoum and flocoumafen, considered to be the most potent of the anticoagulants (Buckle 1994), are restricted to indoor use only, to limit the risks to non-target wildlife. All rodenticides are subject to conditions of approved use and carry a set of labelled precautionary instructions to prevent misuse. The rodenticide used in this research was the first generation anticoagulant coumatetralyl. The higher toxicity and

148

persistence of second generation compounds represent a greater hazard to non-target wildlife, but the routes of exposure will remain the same. Wildlife may be exposed to rodenticides through primary exposure, the consumption of poison baits, and secondary exposure, the consumption of poisoned prey by predators and scavengers. A third route, tertiary exposure, may potentially exist if predators or scavengers consume carnivores that have been exposed secondarily.

Figure 7.1 Rodenticide ecotoxicology compartment model; adapted from Smith *et al* (1990) and MacVicker (1998). Arrows represent potential routes by which a persistent rodenticide can be transferred from source (bait point) to sink (soil). Species involved are those evident in this research or, in the case of some predators, where evidence of exposure has been reported (Polecats, Shore *et al* 1996; Weasels & Stoats, McDonald *et al* 1998; Red kites, Shore *et al* 2000; and, Kestrels, Shore *et al* 2001).



Figure 7.1 provides a compartment model of rodenticide ecotoxicology, developed from an adaptation of MacVicker (1998), which was based on the initial proposal of Smith *et al* (1990). The model illustrates the routes of rodenticide exposure to a number of non-target species in the UK. The species given are those evident in this research or, in the case of some predators, where evidence of exposure has been reported (Polecats *Mustela putorius*, Shore *et al* 1996; Weasels *Mustela nivalis* & Stoats *Mustela erminea*, McDonald *et al* 1998; Red kites *Milvus milvus*, Shore *et al* 2000; and, Kestrels *Falco tinnunculus*, Shore *et al* 2001). Chapter 2 provides details of non-target species observed to consume bait directly and of predation incidences involving poisoned rats. Chapter 3 documents the suite of scavengers exploiting rat carcasses on farms. Chapter 4 focuses on the exposure of the red kite, potentially the species most at risk of secondary poisoning. Chapters 5 and 6 provide evidence of primary exposure to non-target small mammals.

The research presented in this study provides evidence of rodenticide exposure to a wide range of non-target species. The application of rodenticide bait in chapters 2 and 5 followed guidelines for best practice. The research was realistic in monitoring exposure during normal routine rat control and the results could be indicative of nontarget exposure in rat control elsewhere.

Chapter 2 provided evidence of changes in the behaviour of free-living wild rats suffering pre-lethal rodenticide toxicosis. The increase in open space activity and the incidence of abnormal behaviour (lethargy, uncoordinated movement, and a reduced awareness of surroundings) are likely to increase the proportion of poisoned prey in the diets of predators through the greater conspicuousness and ease of capture of poisoned animals compared with healthy individuals behaving normally. This result is very important as it improves our understanding of predator exposure, yet requires further study to quantify the importance of the route. The route has been identified, but its importance will depend on the foraging behaviour of the predators involved i.e. which predators forage in areas of rat control and to what extent do they exploit the poisoned prey resource. The abnormal behaviours observed in poisoned rats were also observed in non-target small mammals, wood mice (*Apodemus sylvaticus*) and bank voles (*Clethrionomys glareolus*).

Chapter 5 provides an extensive investigation of the importance of non-target small mammals as routes of exposure to their predators and scavengers. The finding that a high proportion of local populations of small mammals are exposed to rodenticide bait provides an indication of the source of exposure to species that do not normally predate rats, e.g. kestrels and weasels, and to species that predate both rats and small mammals, e.g. barn owls (Tyto alba), polecats and stoats. Chapter 6 reveals data of anticoagulant residues in small mammals, refining the risk assessment of exposure to small mammal consumers. Comparison of residues in live contaminated rodents and rodents that had died as a result of poisoning gave an indication of the potential differences in exposure risk to predators and scavengers respectively. Comparison of residues between species provided evidence of a possible difference in handling between wood mice and bank voles, and comparison between sites indicated that predators and scavengers foraging around farms may be at risk of greater exposure than those foraging around pheasant feeder sites. Separate analysis of different parts of the body provided information on the physiological dynamics of anticoagulant in rodents. This knowledge is important in assessing secondary exposure due to the differences in feeding behaviour of predators and scavengers, e.g. weasels and kestrels may not be exposed to anticoagulant residue in the gastrointestinal tract, as they are known to remove and reject this organ (observations in this study and Tkadlec & Rychnovsky (1990)).

Where chapter 2 focussed on the exposure of predators, chapter 3 investigated scavenger exposure. The placement and monitoring of rat carcasses around farms, mimicking the potential availability of poisoned rats during rat control, provided a measure of scavenger removal rates and allowed the identification of scavengers through video monitoring and tracking. Three of the species recorded scavenging rat carcasses were those most frequently recorded by WIIS (Wildlife Incident Investigation Scheme) during the 1990s to contain lethal rodenticide residues; fox (Vulpes vulpes) (32% of 109 examined), badger (Meles meles) (20% of 64 examined) and red kite (18% of 44 examined). At farms frequented by a local scavenger assemblage the disappearance of carcasses may be rapid (Farm 2), whereas, at farms visited rarely by scavengers, carcasses may not be exploited as readily (Farm 3). Knowledge of the abundance and foraging behaviour of scavengers local to a farm where rat control is performed would provide an indication of the potential risk of exposure. The rapid disappearance of carcasses underlines the requirement for carcass searching, recovery and safe disposal, as stipulated on labels of rodenticide baits. Ironically, however, neglecting this obligation may improve the level of rat control, as a result of cannibalism and the secondary poisoning of rats. A significant proportion

151

of rat carcasses were removed and cannibalised by rats at both farms. In addition to rates of loss and scavenger identification, chapter 3 revealed a new and potentially important route of rodenticide exposure via invertebrate scavengers. Carcasses remaining in the field for two days or more were rapidly colonised by blowflies (in summer, winter rates may vary) and, to a lesser extent, necrophagous beetles. The presence of blowfly maggot feeding masses appeared to deter carcass removal by vertebrate scavengers, thus reducing their exposure to rodenticides. However, the ingestion of anticoagulant by invertebrates may lead to the secondary poisoning of insectivores that predate maggots and beetles e.g. shrews (*Sorex* sp.), and flies e.g. swallows (*Hirundo rustica*) (which breed in farm environments during the summer). Incidentally, shrews were also found to be exposed to rodenticide bait, either through the consumption of bait or the consumption of insects that had fed on bait (chapter 5).

As a scavenger and predator that forages around farms, the red kite is considered to be one of the species most at risk of secondary poisoning by rodenticides (Burn *et al* 2002). Indeed, a number of secondary poisoning incidents involving second generation rodenticides have been reported in recent years (Carter & Burn 2000) and in an investigation of residue levels in kites found dead as a result of different causes, 70% of the sample (n=20) contained detectable residues (Shore *et al* 2000). Chapter 3 provides evidence that red kites do forage for rat carcasses around farm buildings (Plate 3.4). Chapter 4 details studies specific to the red kite in an attempt to better understand the routes of exposure and provide an indication of levels of exposure. A study of feeding behaviour when consuming rats indicated that kites preferred the parts of the carcass most likely to contain the greatest concentration of rodenticide. A study of red kite nestling diet showed rats to be an important food item.

The rodenticide ecotoxicology model illustrates the direct effects of rodenticide transfer within the food web. Additionally, rodenticides may also cause a range of sublethal and indirect effects in non-target wildlife, although perhaps not so dramatic as other high profile pesticides e.g. DDE-induced reproductive failure in peregrine falcons (*Falco peregrinus*) (Ratcliffe 1970). Because sublethal effects are difficult to detect and dissociate from other, natural, contributory factors, there is currently no field evidence available that directly relates sublethal rodenticide residues and reduced fitness in non-target wildlife. Possible sublethal effects can currently only be hypothesised. The mode of action of anticoagulant rodenticides involves the inhibition of vitamin-K dependent blood clotting proteins, leading to

152

increased prothrombin times and haemorrhage. If a dose is sublethal, an animal may be vulnerable to death from other causes. For example; with reduced blood coagulation ability a wounded animal may bleed to death; a raptor suffering painful sublethal haemorrhage at the wing joints may not be able to hunt efficiently; and, reduced awareness as a result of minor cerebral haemorrhage may increase the likelihood of foxes and other mammals becoming road traffic casualties. Blood coagulation may not be the only mode of action anticoagulant rodenticides exert. Vitamin-K dependent proteins are involved in many aspects of vertebrate physiology and anticoagulant compounds may act upon these also. For example, bone contains three such proteins, osteocalcin, matrix Gla-protein and protein S, and the anticoagulant antagonism of these may affect bone integrity through calcium loss (WHO 1995). Incidentally, in 2001 The National Birds of Prey Centre, Newent, Glos. reported receiving an injured wild red kite from the Chilterns suffering calcium deficiency (a condition not expected in wild birds) and requiring a leg operation (JP Jones - pers. comm.). It is suggested that post mortem examinations of kite should routinely include looking for bone thinning. Vitamin-K dependent proteins not related to blood coagulation are still discovered regularly (Manfioletti et al 1993; cited in WHO 1995), thus further research is required, which may help to expose the potential sublethal effects of anticoagulant rodenticides.

Despite their pest status, rats are an important component in the diets of a number of predators and scavengers in the UK, the removal of which limits their food supply. The documented effects of rat control on non-target small mammal populations (chapter 5), the prey of many specialist predators, may reduce the local abundance of their sole food supply. The control of rats around farms and in other rural situations removes a significant prey resource that may be crucial to the fitness and reproductive potential of local predator populations. Indeed, prolonged and repeated application of poison baits may permanently hold rodent populations at reduced levels (Harradine 1976), possibly causing the local extinction of some predators in affected areas.

The critical question, which remains unanswered, is to what extent are populations of non-target species affected by rodenticides, both lethally and sublethally? WIIS reports occasional rodenticide poisoning of a wide range of predators and scavengers in the UK. However, this passive monitoring, which relies on the public discovering and submitting carcasses, is likely to underestimate the true level of exposure as poisoned animals are likely to die out of site on private premises (Birks 1998, Newton *et al* 1999). Given the evidence presented in this research, there may be a case for the implementation of an active monitoring programme, e.g. non-lethal blood sampling to measure prothrombin time (Quick 1959).

In the short term, efforts should be made by rodenticide stakeholders to encourage further monitoring and research in qualifying and quantifying routes of exposure, in order to refine the risk assessment for populations of non-target wildlife. As a result, the development of risk management strategies will mitigate the adverse effects in the use of rodenticide baits to control rodent pests.

APPENDIX 1

Sample Details - CSL WM - Wood mouse BV - Bank Vole

T – Death due to rodenticide CO₂ – Sacrificed animals found alive but eating bait

Ν	Species	Trap	Date	Body	GIT	Liver	Sex	Death
				weight	weight	weight		
2	WM	37	12/02/02	18.47	4.74	1.00	F	Т
3	WM	4	13/02/02	16.62	3.72	0.61	F	Т
4	WM	50	17/02/02	17.87	3.23	0.82	Μ	CO ₂
5	WM	77	17/02/02	14.67	2.77	0.73	F	CO ₂
8	BV	31	07/04/02	17.53	2.56	0.95	М	Т
12	BV	2	01/08/02	13.19	2.08	0.74	Μ	Т
13	BV	32	02/08/02	20.16	4.20	1.30	F	Т
15	BV	20	03/08/02	15.17	2.99	0.78	F	Т
16	BV	38	04/08/02	14.25	2.09	0.73	М	Т
18	WM	26	07/08/02	15.68	3.01	0.58	F	Т
19	WM	14	07/08/02	22.20	4.01	1.36	Μ	CO ₂
20	BV	B4	07/08/02	18.30	2.99	1.06	Μ	CO ₂
21	WM	112	07/08/02	19.93	2.86	1.12	М	CO ₂
22	WM	36	07/08/02	21.27	3.37	1.14	Μ	CO ₂
23	BV	10	07/08/02	11.06	1.77	0.57	М	CO ₂
24	WM	15	07/08/02	20.25	2.86	1.07	Μ	CO ₂
25	BV	75	07/08/02	13.29	2.24	0.97	F	CO ₂
26	WM	In open	03/10/02	19.93	2.20	0.74	Μ	Т
27	WM	5	03/10/02	15.07	2.77	0.72	F	Т
28	WM	26	03/10/02	20.85	2.90	1.08	F	Т
33	BV	11	04/10/02	11.09	2.09	0.61	F	Т
34	WM	12	04/10/02	12.31	2.97	0.55	М	Т
35	BV	bait box	04/10/02	10.62	1.80	0.54	М	<u>T</u>
36	BV	50	05/10/02	12.82	2.91	0.85	М	Т
37	WM	5*	10/10/02	15.19	2.66	1.00	M	CO ₂
38	WM	48	10/10/02	15.01	3.00	0.93	F	CO ₂
57	WM	32	10/10/02	17.74	2.57	0.97	M	CO ₂
58	WM	28	10/10/02	19.30	3.15	1.11	F	CO ₂
59	WM	28i	10/10/02	16.36	2.92	0.90	F	CO ₂
60	WM	16	10/10/02	12.31	2.42	0.70	M	CO ₂
61	WM	3	10/10/02	17.42	3.56	0.86	М	Т
62	WM	37	10/10/02	17.11	3.37	0.90	M	CO ₂

Sample Details - Biocentre WM - Wood mouse BV - Bank Vole FV - Field Vole CS - Common Shrew PS - Pygmy Shrew

T – Death due to rodenticide

CO2 - Sacrificed animals found alive but eating bait

Ν	Species	Trap	Date	Body	GIT	Liver	Sex	Death
				weight	weight	weight		
39	WM	8	10/10/02	19.63	3.40	1.18	M	CO ₂
40	WM	102	10/10/02	13.19	2.50	0.82	F	CO ₂
41	WM	83	10/10/02	15.34	2.56	0.78	M	CO ₂
42	WM	A1	10/10/02	12.23	2.05	0.70	F	CO ₂
43	WM	B4	10/10/02	15.00	2.93	0.96	F	CO ₂
44	WM	34	10/10/02	16.27	2.93	1.02	Μ	CO ₂
45	WM	A2	10/10/02	12.27	1.75	0.84	Μ	CO ₂
46	WM	2	10/10/02	14.07	2.17	0.80	Μ	CO ₂
47	WM	50	10/10/02	18.05	3.49	1.15	Μ	CO ₂
48	WM	16	10/10/02	14.12	1.96	0.90	F	CO ₂
49	WM	78	10/10/02	12.61	1.67	0.75	F	CO ₂
50	WM	5	10/10/02	9.96	2.14	0.53	F	CO ₂
51	WM	11	10/10/02	16.34	3.42	1.00	Μ	CO ₂
52	WM	21	10/10/02	19.58	3.40	1.15	F	CO ₂
53	WM	A3	10/10/02	13.70	2.49	0.83	М	CO ₂
54	WM	A4	10/10/02	12.23	2.12	0.74	Μ	CO ₂
55	WM	13	10/10/02	13.99	2.31	0.88	F	CO ₂
56	WM	26	10/10/02	14.18	2.09	0.79	М	CO ₂
6	BV	42	08/04/02	12.95	2.19	0.87	F	Т
11	BV	65	01/08/02	16.77	2.41	0.91	М	Т
14	BV	10	03/08/02	13.94	3.01	0.64	F	Т
29	BV	3	03/10/02	13.42	2.40	0.68	М	Т
31	BV	23	04/10/02	18.17	3.42	NA	М	Т
32	BV	16	04/10/02	12.89	1.81	NA	М	Т
7	FV	25	07/04/02	16.44	3.41	1.03	F	Т
17	WM	32	05/08/02	19.75	3.18	1.17	F(P2.13)	Т
30	WM	28	04/10/02	11.60	2.06	0.70	М	Т
63	CS	94	17/02/02	6.59				Т
64	CS	11	04/08/02	8.49				Т
65	CS	12	04/08/02	7.01				Т
66	CS	18	03/10/02	8.13				Т
67	PS	21	07/04/02	2.89				Т

APPENDIX 2

				GIT	Liver	Body	Whole	Body
Ν	Death	Species	Site	mg.kg ⁻¹	mg.kg ⁻¹	remainder	body	burden
						mg.kg ⁻¹	mg.kg ⁻¹	(µg)
20	CO ₂	BV	PF	1.19	2.18	0.19	0.47	8.58
23	CO ₂	BV	PF	0.01	0.02	0.01	0.01	0.12
25	CO ₂	BV	PF	0.03	0.05	0.02	0.02	0.32
4	CO ₂	WM	F	0.06	0.02	0.01	0.02	0.35
5	CO ₂	WM	F	0.21	0.29	0.08	0.11	1.69
19	CO ₂	WM	PF	0.02	0.08	0.01	0.02	0.36
21	CO ₂	WM	PF	0.05	0.18	0.02	0.03	0.66
22	CO ₂	WM	PF	0.08	0.28	0.04	0.06	1.26
24	CO ₂	ŴM	PF	0.13	0.28	0.04	0.07	1.32
37	CO ₂	WM	PF	0.15	0.29	0.07	0.1	1.5
38	CO ₂	WM	PF	0.18	0.35	0.1	0.13	1.97
39	CO ₂	WM	PF	5.18	4.239	0.312	1.392	27.316
40	CO ₂	WM	PF	0.016	0.065	0.015	0.018	0.241
41	CO ₂	WM	PF	0.012	0.07	0.014	0.017	0.254
42	CO ₂	WM	PF	0.009	0.052	0.008	0.011	0.132
43	CO ₂	WM	PF	0.011	0.143	0.019	0.026	0.386
44	CO ₂	WM	PF	0.015	0.091	0.009	0.015	0.251
45	CO ₂	WM	PF	0.034	0.125	0.011	0.022	0.27
46	CO ₂	WM	PF	0.009	0.086	0.028	0.028	0.397
47	CO ₂	WM	PF	0.019	0.065	0.022	0.024	0.432
48	CO ₂	WM	PF	0.031	0.078	0.02	0.025	0.36
49	CO ₂	WM	PF	0.015	0.051	0.005	0.009	0.114
50	CO ₂	WM	PF	0.009	0.159	0.012	0.019	0.193
51	CO ₂	WM	PF	2.16	0.459	0.125	0.571	9.333
52	CO ₂	WM	PF	0.028	0.072	0.01	0.016	0.321
53	CO ₂	WM	PF	0.014	0.086	0.01	0.015	0.209
54	CO ₂	WM	PF	0.012	0.116	0.017	0.022	0.267
55	CO ₂	WM	PF	0.006	0.083	0.009	0.013	0.181
56	CO ₂	WM	PF	0.033	0.148	0.036	0.042	0.59
57	CO ₂	WM	PF	0.17	0.15	0.05	0.07	1.29
58	CO ₂	WM	PF	0.44	0.39	0.11	0.18	3.47
59	CO ₂	WM	PF	0.18	0.42	0.1	0.13	2.16
60	CO ₂	WM	PF	0.08	0.18	0.04	0.06	0.69
62	CO ₂	WM	PF	0.09	0.25	0.07	0.08	1.43
6	Т	BV	PF	0.142	1.426	0.073	0.176	2.274
8	T	BV	PF	0.69	4	0.26	0.53	9.21
11	Т	BV	PF	0.328	3.436	0.156	0.359	6.013
12	Т	BV	PF	0.75	4.85	0.47	0.76	10.02
13	T	BV	PF	0.53	4.17	0.4	0.67	13.51
14	Т	BV	PF	0.01	0.071	0.012	0.014	0.197
15	T	BV	PF	1.13	3.16	0.33	0.63	9.61
16	Т	BV	PF	0.48	2.24	0.26	0.39	5.61
29	Т	BV	PF	1.193	2.751	0.103	0.432	5.798
31	Т	BV	PF	0.126	0.2	253	0.229	4.165
32	T	BV	PF	0.175	0.	197	0.194	2.504

Coumatetralyl residue concentrations in samples (CSL and Biocentre)

33	Т	BV	PF	0.33	2.68	0.17	0.34	3.75
35	Т	BV	PF	1.63	5.97	0.75	1.16	12.37
36	Т	BV	PF	0.81	3.84	0.42	0.74	9.43
7	Т	FV	PF	0.02	0.123	0.022	0.028	0.458
2	Т	WM	F	4.25	1.71	0.73	1.69	31.15
3	Т	WM	F	2.81	2.21	1.03	1.47	24.45
17	Т	WM	PF	0.256	0.452	0.07	0.127	2.517
18	Т	WM	PF	0.16	0.28	0.05	0.08	1.25
26	Т	WM	PF	0.81	0.66	0.17	0.26	5.16
27	Т	WM	PF	0.56	1.01	0.22	0.32	4.83
28	Т	WM	PF	4.25	1.12	0.32	0.91	18.93
30	Т	WM	PF	0.407	1.113	0.349	0.406	4.708
34	Т	WM	PF	0.62	1.2	0.36	0.46	5.67
61	Т	WM	PF	0.005	0.02	0.04	0.03	0.56
63	Т	CS	PF				0.004	0.028
64	Т	ĊS	PF				0.031	0.262
65	Т	CS	PF				0.005	0.036
66	Т	CS	PF				0.006	0.051
67	Т	PS	PF				0.009	0.025
17 (foetus)	Т	WM	PF		-		0.117	0.249

REFERENCES

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

Alterio, N.C. (1996) Secondary poisoning of stoats (*Mustela erminea*), feral ferrets (*Mustela furo*) and feral house cats (*Felis catus*). New Zealand Journal of Zoology, **23**, 331-338.

Alterio, N., Brown, K. & Moller, H. (1997) Secondary poisoning of mustelids in a New Zealand Nothofagus forest. *Journal of Zoology, London*, **243**, 863-869.

Anon. (1981) Secondary poisoning of weasels by rodenticides. MAFF ADAS. Agricultural Science Service Research and Development Reports, **252**, 37-38.

Anon. (1982) Secondary toxicity hazard to owls from difenacoum. MAFF ADAS. Agricultural Science Service Research and Development Reports, **252**.

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

Bang, P. & Dahlstrom, P. (1974) Animal tracks and signs. Collins.

Bankes, J. & Garthwaite, D. (2001) Pesticide Usage Survey Report 162: Rodenticide use on farms in Great Britain growing arable crops 1998. DEFRA Publications.

Barker, W.M., Hermodson, M.A. & Link, K.P. (1970) The metabolism of 4-C14-Warfarin sodium by the rat. *Journal of Pharmacology and Experimental Therapeutics*, **171**, 307-313.

Barnett, E.A., Fletcher, M.R., Hunter, K. & Sharp, E.A. (2002) Pesticide poisoning of animals 2000: Investigations of suspected incidents in the United Kingdom. *A Report* of the Environmental Panel of the Advisory Committee on Pesticides. MAFF, London.

Barnett, S.A. (1963) A Study in Behaviour. Methuen & Co. Ltd.

.

Beguin, J. (1983) *Report on the chemical control of voles*. Neuchatel: The Department of Agriculture of the Republic and Canton of Neuchatel.

Berdoy, M. & MacDonald, D.W. (1991) Factors affecting feeding in wild rats. *Acta Ecologica*, **12**, 261-279.

Berdoy, M., Webster, J.P. & MacDonald, D.W. (2000) Fatal attraction in rats infected with *Toxoplasma gondii*. Proc. R. Soc. Lond. B, **267**, 1591-1594.

Berdoy, M. & MacDonald, D.W. (1991) Factors affecting feeding in wild rats. *Acta Ecologica*, **12**, 261-279.

Berdoy, M., Webster, J.P. & MacDonald, D.W. (2000) Fatal attraction in rats infected with *Toxoplasma gondii*. Proc. R. Soc. Lond. B, **267**, 1591-1594.

Berny, P.J., Buronfosse, T., Buronfosse, F., Lamarque, F. & Lorgue, G. (1997) Field evidence of secondary poisoning of foxes (*Vulpes vulpes*) and buzzards (*Buteo buteo*) by bromadiolone, a 4-year survey. *Chemosphere*, **35**, 1817-1829.

Birks, J.D.S. (1998) Secondary rodenticide poisoning risk arising from winter farmyard use by the European polecat *Mustela putorius*. *Biological Conservation*, **85**, 233-240.

Blandford, P.R.S. (1987) Biology of the polecat *Mustela putorius*: a literature review. *Mammal Review*, **17**, 155-198.

Brown, R.A. (1994) Assessing the environmental impact of rodenticides. *Rodent Pests and Their Control* (eds A.P. Buckle & R.H. Smith), pp.363-380. CAB International. Brown, P., Charlton, A., Cuthbert, M., Barnett, L., Ross, L., Green, M., Gillies, L., Shaw, K. & Fletcher, M., (1996) Identification of pesticide poisoning in wildlife. *Journal of Chromatography A*, **754**, 463-478.

•

Buckle, A.P. (1994) Rodent control methods: chemical. *Rodent Pests and Their Control* (eds A.P. Buckle & R.H. Smith), pp.127-160. CAB International.

Buckle, A.P., Odam, E.M. & Richards, C.G.J. (1987) Chemical bait markers for the study of bait uptake by Norway rats. *Control of Rats and Mice* (eds C.G.J. Richards & T.Y. Ku), pp.199-213. Taylor & Francis, London.

Buitron, D. & Neuchterlein, G.L. (1985) Experiments on olfactory detection of food caches by black-billed magpies. *Condor*, **87**, 92-95.

Bumann, G.B. & Stauffer, D.F. (2002) Scavenging of ruffed grouse in the Appalachians: influences and implications. *Wildlife Society Bulletin*, **30**, 853-860.

Burn, A.J., Carter, I. & Shore, R.F. (2002) The threats to birds of prey in the UK from second-generation rodenticides. *Aspects of Applied Biology*, **67**, 203-212.

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

Calhoun, J.B. (1962) *The ecology and sociology of the Norway rat.* US Department of Health, Education & Welfare.

Carter, I. (2001) The Red Kite. Arlequin Press.

Carter, I. & Burn, A. (2000) Problems with rodenticides: the threat to Red Kites and other wildlife. *British Wildlife*, **11**, 192-197.

Carter, I. & Grice, P. (2000) Studies of re-established Red Kites in England. British Birds, 93, 304-322.

Carter, I. & Grice, P. (2002) The red kite reintroduction programme in England. English Nature Research Reports No. 451. English Nature.

Churchfield, S. (1990) The Natural History of Shrews. Christopher Helm.

Cicero, C. (1993) Vulnerability of prey stimulates attacks by jays and shrikes on adult birds. *Western Birds*, **24**, 101-102.

Colvin, B.A. (1984) Barn owl foraging behaviour and secondary poisoning hazard from rodenticide use on farms. PhD Bowling Green State University.

Coly, A. & Aaron, J-J. (1998) Flourimetric analysis of pesticides: Methods, recent developments and applications. *Talanta*, **46**, 815-843.

Cowan, D., Dunsford, G., Gill, E., Jones, A., Kerins, G., MacNicoll, A. & 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. (1991) Environmental effects of rodenticide use. PhD University of Reading.

Cox, P. & Smith, R.H. (1992) Rodenticide ecotoxicology: Pre-lethal effects of anticoagulants on rat behaviour. *Proceedings of the 15th Vertebrate Pest Conference* (eds J.E. Borrecco & R.E. Marsh), pp.165-170. University of California, Davis.

Crocker, D., Hart, A., Gurney, J. & McCoy, C. (2002) Methods for estimating daily food intake of wild birds and mammals. Unpublished Report. Central Science Laboratory, UK.

Crozier, W.J. (1928) Tropisms. Journal of Genetic Psychology, 1, 213-238.

Davis, D.E., Emlen, J.T. & Stokes, A.W. (1948) Studies on home range in the brown rat. *Journal of Mammalogy*, **29**, 207-255.

Davis, P.E. & Davis, J.E. (1981) The food of the Red Kite in Wales. *Bird Study*, 28, 33-40.

Desheesh, M.A.T. (1983) Effects of anticoagulant poison baits on the behaviour of white rats. *Alexandria Science Exchange*, **4**, 173-180.

DeVault, T.L. & Rhodes, O.E. Jr. (2002) Identification of vertebrate scavengers of small mammal carcasses in a forested landscape. *Acta Theriologica*, **47**, 185-192.

Donazar, J.A. (1992) Muladares y basureros en la biologia y conservacion de las aves en Espana. *Ardeola*, **39**, 29-40.

Dubock, A.C. (1982) Pulsed baiting – a new technique for high potency, slow acting rodenticides. *Proceedings of the* 10^{th} *Vertebrate Pest Conference* (ed R.E. Marsh), pp.123-136. University of California, Davis.

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

Dubock, A.C. & Kaukeinen, D.E. (1978) Brodifacoum (TalonTM rodenticide), a novel concept. *Proceedings 8th Vertebrate Pest Conference* (W.E. Howard & R.E. Marsh), pp.127-137. Sacramento, California.

Eason, C.T., Wright, G.R. & Batcheler, D. (1996) Anticoagulant effects and the persistence of brodifacoum in possums (*Trichosurus vulpecula*). New Zealand Journal of Agricultural Research, **39**, 397-400.

Eason, C.T., Murphy, E.C., Wright, G.R.G. & Spurr, E.B. (2002) Assessment of risks of brodifacoum to non-target birds and mammals in New Zealand. *Ecotoxicology*, **11**, 35-48.

Eason, C.T. & Spurr, E.B. (1995) Review of the toxicity and impacts of brodifacoum on non-target wildlife in New Zealand. *New Zealand Journal of Zoology*, **22**, 371-379.

Edwards, P.J., Brown, R.A., Coulson, J.M. & Buckle, A.P. (1988) Field methods for studying the non-target hazard of rodenticides. *Field methods for the study of environmental effects of pesticides* (eds M.P. Greaves, B.D. Smith, & P.W. Greig-Smith), pp.77-88. BCPC Monograph No.40.

English Nature (2000) *Red Kite Newsletter*. Issue 5. (published leaflet). English Nature).

English Nature (2001) Return of the Red Kite. (published leaflet). English Nature.

English Nature (2002) Red Kites @ Rockingham. (published leaflet). English Nature.

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

Erlinge, S. (1981) Food preference, optimal diet and reproductive output in stoats *Mustela erminea* in Sweden. *Oikos*, **36**, 303-315.

Erlinge, S. (1983) Demography and dynamics of a stoat *Mustela erminea* population in a diverse community of vertebrates. *Journal of Animal Ecology*, **52**, 705-726.

Farag, M.S.H. (1982) Effect of sub-lethal doses of anticoagulants on the reproduction of the rat. *Proceedings of the First Symposium on Recent Advances in Rodent Control* (eds A.H. Helmy Mohammad, T.M. Zaghloul & M. Zakaria), pp.46-52. Ministry of Public Health, Kuwait.

Fenn, M.G.P. & MacDonald, D.W. (1995) Use of middens by red foxes: risk reverses rhythms of rats. *Journal of Mammalogy*, **76**, 130-136.

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

Fletcher, M.R., Hunter, K., Barnett, E.A. & Sharp, E.A. (1998) Pesticide Poisoning of Animals 1997: Investigations of Suspected Incidents in the United Kingdom. MAFF Publications. London.

Flowerdew, J.R. (1972) The effect of supplementary food on a population of woodmice (*Apodemus sylvaticus*). *Journal of Animal Ecology*, **41**, 553-566.

Flowerdew, J.R. (1993) Mice & Voles. Whittet Books.

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

Garcia, J.T., Viňuela, J. & Sunyer, C. (1998) Geographic variation of the winter diet of the Red Kite *Milvus milvus* in the Iberian Peninsula. *Ibis*, **140**, 302-309.

Gemmeke, von. H. (1990) Untersuchungen zur Abschätzung des Sekundärvergiftungsriskos bei Beuregreifern durch Rodentizicide. (Evaluation of the risk of poisoning predators by application of anticoagulant rodenticides). *Nachrichtenbl. Deut. Pflanzenschutzd*, **42**, 22-25.

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

Glue, D.E. (1974) Food of the barn owl in Britain and Ireland. *Bird Study*, **21**, 200-210.

Godfrey, M.E.R. (1985) Non-target and secondary poisoning hazards of 'secondgeneration' anticoagulants. *Acta Zoologica Fennica*, **173**, 209-212. Gratz, N.G. (1994) Rodents as carriers of disease. *Rodent Pests and Their Control* (eds A.P. Buckle & R.H. Smith), pp.85-108. CAB International.

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

Greaves, J.H. & Cullen-Ayres, P.B. (1988) Genetics of difenacoum resistance in the rat. *Current Advances in Vitamin K Research* (ed J.W. Suttie), pp.389-397. Elsevier Science, London.

Greenwood, J.J.D. (1996) Basic techniques. *Ecological Census Techniques; A* Handbook (ed W.J. Sutherland). Cambridge University Press.

Grolleau, G., Lorgue, G. & Nahas, K. (1989) Toxicité secondaire, en laboratoire, d'un rodenticide anticoagulant (bromadiolone) pour des prédateurs de rongeurs champêtres: buse variable (*Buteo buteo*) et hermine (*Mustela nivalis*). *OEPP/EPPO Bulletin*, **19**, 633-648.

Gurnell, J. & Flowerdew, J.R. (1994) *Live Trapping Small Mammals; A Practical Guide*. Occasional Publication No. 3. The Mammal Society.

Hadler, M.R. & Shadbolt, R.S. (1975) Novel 4-hydroxy-coumarin anticoagulants active against resistant rats. *Nature*, **253**, 277-282.

Harradine, J.P. (1976) Anticoagulant rodenticides and non-target wildlife: An ecological evaluation of permanent baiting in rural rat control. PhD University of Edinburgh.

Harrison, E.G., Porter, A.J. & Forbes, S. (1988) Development of methods to assess the hazards of a rodenticide to target vertebrates. *Field methods for the study of environmental effects of pesticides* (eds M.P. Greaves, B.D. Smith & P.W. Greig-Smith), pp.89-96. BCPC Monograph 40. Hegdal, P.L. & 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. & 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.

Hermann, G. & Hombrecher, S. (1962) Ratten und Mäusebekämpfung mit racumin57-Präparaten. *Pflanzenschutz-Nachrischten Bayer*, **15**, 93-112.

Hewson, R. & Kolb, H.H. (1976) Scavenging on sheep carcasses by foxes (*Vulpes vulpes*) and badgers (*Meles meles*). Notes from The Mammal Society No.33. *Journal of Zoology*, **180**, 496-498.

Hiraldo, F., Blanco, J.C. & Bustamante, J. (1991) Unspecialized exploitation of small carcasses by birds. *Bird Study*, **38**, 200-207.

Holliday, S. & Kenington, F. (2001) http://myweb.tiscali.co.uk/hullvalley/hv08017.htm

.

Holmes, J., Walker, D., Davis, P. & Carter, I. (in press) The persecution of raptors in England. English Nature Research Report.

Houston, D.C. (1979) The Adaptations of Scavengers. Serengeti; Dynamics of an Ecosystem, (eds A.R.E. Sinclair & M. Norton-Griffiths). University of Chicago Press.

Houston, D.C. (1986) Scavenging efficiency of turkey vultures in tropical forest. *The Condor*, **88**, 318-323.

Howald, G.R., Mineau, P., Elliott, J.E. & Cheng, K.M. (1999) Brodifacoum poisoning of avian scavengers during rat control on a seabird colony. *Ecotoxicology*, **8**, 431-447.

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

.

Huckle, K.R., Hutson, D.H., Logan, C.J., Morrison, B.J. & 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., Warburton, P.A., Forbes, S. & Logan, C.J. (1989b) Studies on the fate of flocoumafen in the Japanese quail (*Coturnix coturnix japonica*). *Xenobiotica*, **19**, 51-62.

Hunt, K.A., Bird, D.M., Mineau, P. & Shutt, L. (1992) Selective predation of organophosphate-exposed prey by American kestrels. *Animal Behaviour*, **43**, 971-976.

Hunter, K. (1983a) Determination of coumarin anticoagulant rodenticide residues in animal tissue by high-performance liquid chromatography. I. Flourescence detection using post-column techniques. *Journal of Chromatography*, **270**, 267-276.

Huson, L.W. & Rennison, B.D. (1981) Seasonal variability of Norway rat (*Rattus norvegicus*) infestation of agricultural premises. Notes from The Mammal Society No.42. *Journal of Zoology*, **194**, 257-260.

Jacques, L.B. & Hiebert, L.H. (1972) Relation of stress to haemorrhage in laboratory animals. *Proceedings of Animal Care Technology, CALAS*, pp.37-52.

Janzen, D.H. (1977) Why fruits rot, seeds mold, and meat spoils. *The American Naturalist*, **111**, 691-713.

Joermann, G. (1998) A review of secondary poisoning studies with rodenticides. *Bulletin OEPP/EPPO Bulletin*, **28**, 157-176.

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

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

Kenward, R.E. (1978) Hawks and Doves: factors affecting success and selection in goshawk attacks on woodpigeons. *Journal of Animal Ecology*, **47**, 449-460.

King, C. (1989) The Natural History of Weasels and Stoats. Christopher Helm.

King, C.M. (1983) The relationships between beech seedfall and populations of mice, and the demographic and dietary responses of stoats, in three New Zealand forests. *Journal of Animal Ecology*, **52**, 141-166.

Knight, S.K. & Knight, R.L. (1983) Aspects of food finding by wintering Bald Eagles. *The Auk*, **100**, 477-484.

Kocarek, P. (2003) Decomposition and Coleoptera succession on exposed carrier of small mammal in Opava, the Czech Republic. *European Journal of Soil Biology*, **39**, 31-45.

Kostecke, R.M., Linz, G.M. & Bleier, W.J. (2001) Survival of avian carcasses and photographic evidence of predators and scavengers. *Journal of Field Ornithology*, **72**, 439-447.

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

Krebs, C.J. (1994) *Ecology. The experimental analysis of distribution and abundance.* Harper Collins.

Laas, F.Y., Forss, D.A. & Godfrey, M.E.R. (1985) Retention of brodifacoum in sheep and excretion in faeces. *New Zealand Journal of Agricultural Research*, **28**, 357-359. Larraz, D.S. (1999) Dumps for dead livestock and the conservation of wintering Red Kites (*Milvus milvus*). Journal of Raptor Research, **33**, 338-340.

Lee, C.H. (1994) Secondary toxicity of some rodenticides to barn owls. *Proceedings* of the 4th International Conference on Plant Protection in the Tropics, pp.161-163. Kuala Lumpur (MY).

Linz, G.M., Davis, J.E., Engeman, R.M., Otis, D.L. & Avery, M.L. (1991) Estimating survival of bird carcasses in cattail marshes. *Wildlife Society Bulletin*, **19**, 195-199.

Lund, M. (1994) Commensal rodents. *Rodent Pests and Their Control* (eds A.P. Buckle & R.H. Smith), pp.23-43. CAB International.

MacDonald, D. (1987) Running with the Fox. Unwin Hyman.

MacDonald, D.W. & Fenn, M.G.P. (1994) The natural history of rodents. *Rodent Pests and Their Control* (eds A.P. Buckle & R.H. Smith), pp.1-21. CAB International.

MacNicoll, A.D. (1986) Resistance to 4-hydroxycoumarin anticoagulants in rodents. *Pesticide Resistance: Strategies and Tactics for Management*, pp.87-99. National Academic Press, Washington DC.

MacVicker, H.J. (1998) *The ecotoxicology of rodenticide use on farms*. PhD University of Leicester.

Manfioletti, G., Brancolini, C., Avanzi, G. & Schneider, C. (1993) The protein encoded by a growth arrest-specific gene (gas6) is a new member of the vitamin Kdependent proteins related to protein S, a negative coregulator in the blood coagulation cascade. *Molecular Cell Biology*, **13**, 4976-4985.

McDonald, R.A., Harris, S., Turnbull, G., Brown, P. & Fletcher, M.(1998) Anticoagulant rodenticides in stoats (*Mustela erminea*) and weasels (*Mustela nivalis*) in England. *Environmental Pollution*, **103**, 17-23. McDonald, R.A. & Harris, S. (2000) The use of fumigants and anticoagulant rodenticides on game estates in Great Britain. *Mammal Review*, **30**, 57-64.

McDonald, R. (2002) Resource partitioning among British and Irish mustelids. Journal of Animal Ecology, **71**, 185-200.

Meehan, A.P. (1984) Rats and Mice; their biology and control. Rentokil Ltd.

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

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

Miller, G. Tyler Jr. (1992) Living in the environment: an introduction to environmental science, 7^{th} ed. Wadsworth.

Mineau, P. & Collins, B.T. (1988) Avian mortality in agro-ecosystems 2. Methods of detection. *Field methods for the study of environmental effects of pesticides* (eds M.P. Greaves, B.D. Smith & P.W. Greig-Smith), pp.13-27. BCPC Monograph 40.

Mineau, P., Fletcher, M.R., Glaser, L.C., Thomas, N.J., Brassard, C., Wilson, L.K., Elliott, J.E., Lyon, L.A., Henny, C.J., Bollinger, T. & Porter, S.L. (1999) Poisoning of raptors with organophosphorus and carbamate pesticides with emphasis on Canada, U.S. and U.K. *Journal of Raptor Research*, **33**, 1-37.

Mineau, P. & Peakall, D.B. (1987) An evaluation of avian impact assessment techniques following broad-scale forest insecticide sprays. *Environmental Toxicology* and Chemistry, **6**, 781-791.

Moore, N.W. (ed) (1966) Pesticides in the environment and their effect on wildlife. Journal of Applied Ecology (supplement),
Mount, M.E. (1988) Diagnosis and therapy of anticoagulant rodenticide intoxications. Veterinary clinics of North America. *Small Mammal Practice*, **18**, 115-131.

Murphy, E.C., Clapperton, B.K., Bradfield, P.M.F. & Speed, H.J. (1998a) Brodifacoum residues in target and non-target animals following large-scale poison operations in New Zealand podocarp-hardwood forests. *New Zealand Journal of Zoology*, **25**, 307-314.

Murphy, E.C., Clapperton, B.K., Bradfield, P.M.F. & Speed, H.J. (1998b) Effects of rat-poisoning operations on abundance and diet of mustelids in New Zealand forests. *New Zealand Journal of Zoology*, **25**, 315-328.

Myllymäki, A., Pihlava, J-M. & Tuuri, H. (1999) Predicting the exposure and risk to predators and scavengers associated with using single-dose second-generation anticoagulants against field rodents. *Advances in vertebrate pest management* (eds D.P. Cowan & C.J. Feare), pp.387-404. Vilander Verlag. Fürth.

Newton, I. (1988) Determination of critical pollutant levels in wild populations, with examples from organochlorine insecticides in birds of prey. *Environmental Pollution*, **55**, 29-40.

Newton, I., Shore, R.F., Wyllie, I., Birks, J.D.S. & Dale, L. (1999) Empirical evidence of side-effects of rodenticides on some predatory birds and mammals. *Advances in vertebrate pest management* (eds D.P. Cowan & C.J. Feare), pp.347-367. Vilander Verlag. Fürth.

Newton, I., Wyllie, I., Gray, A. & Eadsforth, C.V. (1994) The toxicity of the rodenticide flocoumafen to barn owls and its elimination via pellets. *Pesticide Science*, **41**, 187-193.

Newton, I., Wyllie, I. & Freestone, P. (1990) Rodenticides in British barn owls. *Environmental Pollution*, **68**, 101-117. Noble, D.G., Raven, M.J. & Baillie, S.R. (2001) The Breeding Bird Survey 2000. BTO Research Report, 265. Publ BTO, JNCC, RSPB.

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

Pank, L.F. (1976) Effects of seed and background colours on seed acceptance by birds. *Journal of Wildlife Management*, **40**, 769-774.

Papi, F. (1991) Olfactory navigation. *Orientation in Birds* (ed P. Berthold), pp.52-85. Birkhauser.

Parmar, G., Bratt, H., Moore, R. & Batten, P.L. (1987) Evidence for a common binding site in vivo for the retention of anticoagulants in rat liver. *Human Toxicology*, 6, 431-432.

Parmar, G. & Batten, P.L. (1987) Detection of an anticoagulant binding site in rat liver microsomes and its relevance to the anticoagulant effect. *Human Toxicology*, 6, 432.

Patrick, J.P. & Laughlin, R.M. (1934) Is the wall-seeking tendency in the white rat an instinct? *Journal of Genetic Psychology*, **44**, 378-389.

Peterson, C.A., Lee, S.L. & Elliott, J.E. (2001) Scavenging of waterfowl carcasses by birds in agricultural fields of British Columbia. *Journal of Field Ornithology*, **72**, 150-159.

Petroli, J.C. (1983) *Control of the field vole*. Neuchatel: The Department of Agriculture of the Republic and Canton of Neuchatel.

Poché, R.M. (1988) Rodent tissue residue and secondary hazard studies with bromadiolone. *Bulletin OEPP/EPPO Bulletin*, **18**, 323-330.

Pollock, K.H., Nichols, J.D., Brownie, C. & Hines, J.E. (1990) Statistical inference for capture-recapture experiments. *Wildlife Monographs*, **107**.

Popham, E.J. (1943) Further experimental studies of the selective action of predators. Proceedings of the Zoological Society of London A, **112**, 105-117.

Primus, T.M., Eisemann, J.D., Matschke, G.H., Ramey, C. & Johnston, J.J. (2001) Chlorophacinone residues in rangeland rodents: An assessment of the potential risk of secondary toxicity to scavengers. *Pesticides and Wildlife* (ed J.J. Johnston), pp.164-180. American Chemical Society Symposium 771. OUP.

Quick, A.J. (1959) The development and use of the prothrombin tests. *Circulation*, **19**, 92-96.

Quy, R.J., Cowan, D.P. & 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., Shepherd, D.S. & 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.

Rammel, C.G., Hoogenboom, J.J.L. & Cotter, M. (1984) Brodifacoum residues in target and non-target animals following rabbit poisoning trials. *New Zealand Journal of Experimental Agriculture*, **12**, 107-111.

Ratcliffe, D.A. (1970) Changes attributable to pesticides in egg breaking frequency and eggshell thickness in some British birds. *Journal of Applied Ecology*, **7**, 67-107.

Redpath, S.M., Clarke, R., Madders, M. & Thirgood, S.J. (2001) Assessing raptor diet: comparing pellets, prey remains, and observational data at hen harrier nests. *Condor*, **103**, 184-188.

Rennison, B.D. & Dubock, A.C. (1978) Field trials of WBA 8119 (PP 581, brodifacoum) against warfarin-resistant infestations of *Rattus norvegicus*. *Journal of Hygiene*, **80**, 77-82.

Roper, T.J. (1999) Olfaction in birds. Advances in the study of Behavior, 28, 247-332.

Rowe, C. & Guilford, T. (1996) Hidden colour aversions in domestic chicks triggered by pyrazine odours of insect warning displays. *Nature*, **383**, 520-522.

Rudebeck, G. (1950) The choice of prey and modes of hunting of predatory birds with special reference to their selective effect. *Oikos*, **2**, 65-88.

Rudebeck, G. (1951) The choice of prey and modes of hunting of predatory birds with special reference to their selective effect. *Oikos*, **3**, 200-231.

Sargent, G. & Morris, P. (1999) *How to find and identify mammals*. 2nd Ed. The Mammal Society.

Savarie, P.J., Hayes, D.J., McBride, R.T. & Roberts, J.D. (1979) Efficacy and safety of diphacinone as a predacide. *Avian and Mammalian Wildlife Toxicology* (ed E.E. Kenega), pp.69-79. American Society for Testing and Materials Special Technical Publication No. 693.

Sharp, E.A. & Hunter, K. (1999) The occurrence of second generation anticoagulant rodenticide residues in Red Kites in Scotland. *Scottish Agricultural Science Agency*, unpublished report.

Shaw, D. & Perrins, C. (eds)(1998) The Complete Birds of the Western Palearctic on CD-ROM. OUP.

Shawyer, C.R. (1987) *The Barn Owl in the British Isles – Its past, present and future*. The Hawk Trust, London.

Shepherd, D.S. & Greaves, J.H. (1984) A weather-resistant tracking board. *Proceedings of the 11th Vertebrate Pest Conference* (ed D.O. Clark), pp.112-113. University of California, Davis.

Shepherd, D.S. & Inglis, I.R. (1987) Feeding behaviour, social interactions and poison bait consumption by a family group of wild rats living in semi-natural conditions. *Stored products Pest Control*, pp.97-105. BCPC Monograph 37.

Shore, R.F., Afsar, A., Horne, J.A. & Wright, J. (2000) *Rodenticide and lead* concentrations in red kites Milvus milvus. Unpublished Report. Centre for Ecology and Hydrology.

Shore, R.F., Birks, J.D.S., Freestone, P. & Kitchener, A.C. (1996) Second-generation rodenticides and polecats *Mustela putorius* in Britain. *Environmental Pollution*, **91**, 279-282.

Shore, R.F., Birks, J.D.S. & Freestone, P. (1999) Exposure of non-target vertebrates to second-generation rodenticides in Britain, with particular reference to the polecat *Mustela putorius*. *New Zealand Journal of Ecology*, **23**, 199-206.

Shore, R.F., Malcolm, H.M., Horne, J.A., Turner, S. & Weinberg, C.L. (2001) Rodenticide residues in the kestrel *Falco tinnunculus*. Unpublished Report. Centre for Ecology and Hydrology.

Singleton, G.R., Liers, H., Hinds, L.A. & Zhang, Z. (1999) Ecologically-based management of rodent pests – re-evaluating our approach to an old problem. *Ecologically-based management of rodent pests* (eds G.R. Singleton, L.A. Hinds, H. Leirs & Z. Zhang), pp.17-29. ACIAR Monograph 59.

Smith, R.H. (1994) Rodent control methods: Non-chemical and non-lethal chemical. *Rodent Pests and Their Control* (eds A.P. Buckle & R.H. Smith), pp.109-125. CAB International.

Smith, R.H. (1995) Rodents and birds as invaders of stored-grain ecosystems. *Stored Grain Ecosystems* (eds D.S. Jayas, N.D.G. White & W.E. Muir), pp.289-323. M Dekker Inc., New York.

Smith, R.H. (1999) Population biology and non-target effects of rodenticides: trying to put the eco into ecotoxicology. *Advances in vertebrate pest management* (eds D.P. Cowan & C.J. Feare), pp.331-346. Vilander Verlag. Fürth.

Smith, R.H., Cox, P.R. & Rampaud, M. (1990) Rodenticide ecotoxicology: systems analysis and simulation. *Proceedings of the 14th Vertebrate Pest Conference* (eds L.R. Davis & R.E. Marsh), pp. 47-54. University of California, Davis.

Southern, H.N. (1970) The natural control of a population of tawny owls (*Strix aluco*). *Journal of Zoology*, **162**, 197-285.

Stewart-Oaten, A., Bence, J.R. & Osenberg, C.W. (1992) Assessing effects of unreplicated perturbations: no simple solutions. *Ecology*, **73**, 1396-1404.

Stewart, P.D., Ellwood, S.A. & MacDonald, D.W. (1997) Remote video-surveillance of wildlife – an introduction from experience with the European badger *Meles meles*. *Mammal Review*, **27**, 185-204.

Stone, W.B., Okoniewski, J.C. & Stedelin, J.R. (1999) Poisoning of wildlife with anticoagulant rodenticides in New York. *Journal of Wildlife Diseases*, **35**, 187-193.

Stutzenbaker, C.D., Brown, K. & Lobpries, D. (1986) Special report: an assessment of the accuracy of documenting waterfowl die-offs in a Texas coastal marsh.
Proceedings of Symposium on Lead Poisoning in Wild Waterfowl, 3-4 March 1984, Wichita, Kansas USA (eds J.S. Feierabend & A.B. Russel), pp.88-95. Cooperative Lead Poisoning Control Inf. Program, Washington, D.C.

Sullivan, T.P. (1986) Understanding the resiliency of small mammals to population reduction: poison or population dynamics? *Control of Mammal Pests* (eds C.G.J. Richards & T.Y. Ku), pp.69-82. Taylor & Francis.

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.

Taylor, K.D. (1975) An automatic device for monitoring small mammal traffic on runways. *Journal of Zoology*, **176**, 274-277.

Taylor, K.D. (1978) Range of movement and activity of common rats (*Rattus norvegicus*) on agricultural land. *Journal of Applied Ecology*, **15**, 663-677.

Taylor, R.H. (1993) The Feasibility of Rat Eradication on Langara Island, British Columbia, Canada. Unpublished Report. Environment Canada, Canadian Wildlife Service, Pacific and Yukon Region, Delta, British Columbia.

Taylor, R.H. & Thomas, B.W. (1989) Eradication of Norway rats *Rattus norvegicus* from Hawea Island, Fiordland, using brodifacoum. *New Zealand Journal of Ecology*, **12**, 23-32.

Taylor, R.H. & Thomas, B.W. (1993) Rats eradicated from rugged Breaksea Island (170ha), Fiordland, New Zealand. *Biological Conservation*, **65**, 191-198.

Thijssen, H.H.W. (1995) Warfarin-based rodenticides – mode of action and mechanism of resistance. *Pesticide Science*, **43**, 73-78.

Thomas, M.R. & Wild, S. (1996) Pesticide Usage Survey Report 130: Rodenticide Usage on Farms in Great Britain Growing Arable Crops 1994. MAFF Publications, London.

Tinbergen, L. (1960) The natural control of insects in pine woods. I. Factors influencing the intensity of predation by songbirds. *Arch. Néerl. Zool.*, **13**, 265-343.

Tkadlec, E. & Rychnovsky, B. (1990) Residues of Zn3P2 in the common vole (*Microtus arvalis*) and secondary poisoning hazards to predators. *Folia Zoologica*, **39**, 147-156.

Tobin, M.E. & Dolbeer, R.A. (1990) Disappearance and recoverability of songbird carcasses in fruit orchards. *Journal of Field Ornithology*, **61**, 237-242.

Townsend, M.G., Bunyan, P.J., Odam, E.M. & Stanley, P.I. (1984) Assessment of secondary poisoning hazard of warfarin to least weasels. *Journal of Wildlife Management*, **48**, 628-632.

Townsend, M.G., Entwhistle, P. & Hart, A.D.M. (1995) Use of two halogenated biphenyls as indicators of non-target exposure during rodenticide treatments. *Bulletin* of Environmental Contamination and Toxicology, **54**, 526-533.

Townsend, M.G., Fletcher, M.R., Odam, E.M. & Stanley, P.I. (1981) An assessment of the secondary poisoning hazard of warfarin to tawny owls. *Journal of Wildlife Management*, **41**, 242-248.

Vernet-Maury, E., Polak, E.H. & Demael, A. (1984) Structure-activity relationship of stress-inducing odorants in the rat. *Journal of Chemical Ecology*, **10**, 1007-1017.

Vyas, N.B. (1999) Factors influencing estimation of pesticide-related wildlife mortality. *Toxicology and Industrial Health*, **15**, 186-191.

Walters Davies, P. & Davis, P.E. (1973) The ecology and conservation of the Red Kite in Wales. *British Birds*, **66**, 183-224, 241-270.

Webster, J.P. (1994b) The effect of *Toxoplasma gondii* and other parasites on activity levels in wild and hybrid *Rattus norvegicus*. *Parasitology*, **109**, 583-589.

Webster, J.P., Brunton, C.F.A. & MacDonald, D.W. (1994) Effect of *Toxoplasma* gondii on neophobic behaviour in wild brown rats, *Rattus norvegicus*. *Parasitology*, **109**, 37-43.

Webster, J.P. & MacDonald, D.W. (1995) Parasites of wild brown rats (*Rattus norvegicus*) on UK farms. *Parasitology*, **111**, 247-255.

Wildman, L., O'Toole, L. & Summers, R.W. (1998) The diet and foraging behaviour of the Red Kite in northern Scotland. *Scottish Birds*, **19**, 134-140.

Wobeser, G. & Wobeser, A.G. (1992) Carcass disappearance and estimation of mortality in a simulated die-off of small birds. *Journal of Wildlife Diseases*, **28**, 548-554.

Wolton, R.J. & Flowerdew, J.R. (1985) Spatial distribution and movements of wood mice, yellow-necked mice and bank voles. *The Ecology of Woodland Rodents; Bank Voles and Woodmice* (eds J.R. Flowerdew, J. Gurnell & J.H.W. Gipps), pp. 249-275. Symposia of the Zoological Society of London 55. Clarendon Press, Oxford.

Wood, D.A. & Phillipson, J. (1977) The utilisation of poison hoppers designed for grey squirrel (*Sciurus carolinensis*) control. *Biological Conservation*, **11**, 119-127.

World Health Organisation (1995) Anticoagulant Rodenticides. *Environmental Health Criteria* **175**. WHO.

Yu, C.C., Atallah, Y.H. & Whitacre, D.M. (1982) Metabolism and disposition of diphacinone in rats and mice. *Drug Metabolism and Disposition*, **10**, 645-648.

Zawadzka, D. (1999) Feeding habits of the Black Kite Milvus migrans, Red Kite Milvus milvus, White-tailed Eagle Haliaeetus albicilla and Lesser Spotted Eagle Aquila pomarina in Wigry National Park (NE Poland). Acta Ornithologica, **34**, 65-75.