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Spatial and temporal analysis of second-generation anticoagulant rodenticide residues in polecats (*Mustela putorius*) from throughout their range in Britain, 1992–1999

R.F. Shore[a,*], J.D.S. Birks[b], A. Afsar[a], C.L. Wienburg[a], A.C. Kitchener[c]

[a]*NERC Centre for Ecology and Hydrology, Monks Wood, Abbots Ripton, Huntingdon, Cambridgeshire PE28 2LS, UK*
[b]*The Vincent Wildlife Trust, 3&4 Bronsil Courtyard, Eastnor, Ledbury, Herefordshire HR8 1EP, UK*
[c]*Department of Geology & Zoology, National Museums of Scotland, Chambers Street, Edinburgh EH1 1JF, Scotland, UK*

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"Capsule": *There is no evidence that extent of exposure of polecats to second-generation anticoagulant rodenticides has changed in Britain during the 1990s nor is it higher in recently recolonised areas in England where usage is higher.*

Abstract

Polecats (*Mustela putorius*) in Britain are currently expanding their range eastwards from Wales to reoccupy central and eastern areas of England. Second-generation anticoagulant rodenticides (SGARs), to which polecats are exposed by eating contaminated prey, are used more extensively in these central and eastern regions, leading to fears of increased exposure, and possible resultant mortality. We measured bromadiolone, difenacoum, flocoumafen and brodifacoum concentrations in the livers of 50 polecats from areas that included newly recolonised habitats and found that at least one SGAR was detected in the livers of 13 out of 37 (35.1%) male and 5 out of 13 (38.5%) female polecats. Difenacoum and bromadiolone were detected most frequently. We then combined these data with measurements on another 50 individuals from earlier studies to create a dataset for 100 polecats collected throughout the 1990s from across the whole of their current range. Using this dataset, we determined if there was any evidence that contamination in polecats had increased during the 1990s and whether animals from England were more contaminated than those from Wales, as might be expected given regional differences in the patterns of SGAR use. Overall, 31 of the 100 polecats analysed to date contained SGAR residues. The incidence was a little higher (40%) in animals that died between January and June and this probably better reflects the overall proportion of animals that are sub-lethally exposed. There was no statistically significant change during the 1990s in the proportion of polecats exposed to SGARs nor any evidence that greater use of SGARs in England resulted in more contamination of polecats. Contrary to expectation, the proportion of animals that contained difenacoum was marginally higher in Wales than elsewhere.

Keywords: Secondary poisoning; Difenacoum; Bromadiolone; Second-generation anticoagulant rodenticides; Mustelids

1. Introduction

Some 80% of the farms growing grass, fodder and arable crops in Britain currently use rodenticides (De'Ath et al., 1999; Garthwaite et al., 1999), as do other types of farms such as pig and poultry units, and most gamekeepers (McDonald and Harris, 2000). Anticoagulants are the most popular of these compounds and are used in a variety of locations, including in and around buildings, along field boundaries and in woodlands. Of the anticoagulants, it is the second-generation compounds that are generally the most toxic and also the most persistent in terms of the longevity of residues in body organs such as the liver (WHO, 1995). Difenacoum and bromadiolone are the most commonly used second-generation anticoagulant rodenticides (SGARs) in Britain and can be deployed in and out of doors; brodifacoum and flocoumafen are more toxic, less widely employed, and restricted to indoor use (De'Ath et al., 1999; Garthwaite et al., 1999).

The potency and persistence of SGARs enhance the potential for secondary exposure and poisoning. Such

* Corresponding author. Tel.: +44-1487-772501; fax: +44-1487-773467.
E-mail address: rfs@ceh.ac.uk (R.F. Shore).

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exposure occurs when predators take contaminated prey or scavenge the carcasses of poisoned rodents. Experimental investigations and risk assessment studies have both indicated that secondary exposure to anticoagulant rodenticides can result in mortality (Joermann, 1998; Luttik et al., 1999). In the last decade, concern has grown about the secondary exposure of vertebrate predators, particularly to SGARs. In Britain, this has been fuelled by mounting evidence that a surprisingly diverse array of predators is exposed, including the weasel (*Mustela nivalis*) and stoat (*Mustela erminea*) (McDonald et al., 1998), which have uncertain population status (McDonald and Harris, 1999), the kestrel (*Falco tinnunculus*) (Shore et al., 2001) which has declined in numbers (Gibbons et al., 1993; Thewlis et al., 2001), and the red kite (*Milvus milvus*), which is currently relatively rare because of past persecution and is subject to a reintroduction programme (Carter and Burn, 2000). Furthermore, long-term studies on the barn owl (*Tyto alba*) in Britain have indicated that the extent of exposure, in this species at least, has increased, the proportion of carcasses containing SGARs rising progressively from 5% in the early 1980s to approximately 40% in the late 1990s (Newton et al., 1999a,b). Although secondary exposure to SGARs has arguably been better documented in Britain than elsewhere, with the exception perhaps of New Zealand, the potential wildlife problems associated with anticoagulant rodenticides are not peculiar to this country. Secondary exposure and poisoning of non-target predators has been documented throughout the world (Mendenhall and Pank, 1980; Duckett, 1984; Hegdal and Colvin, 1988; Poché, 1988; Deisch et al., 1990; Berny et al., 1997; Murphy et al., 1998; Eason et al., 1999; Howald et al., 1999; Mylly-mäki et al., 1999; Stone et al., 1999).

Of the mammalian predators in Britain, the polecat (*Mustela putorius*) may be at particular risk of exposure. This is because of its habit of preying heavily on rats in and around agricultural premises in autumn and early winter (Birks, 1998), when rat numbers are usually at a peak (Taylor et al., 1991) and rodenticide use on farms is also at a maximum (De'Ath et al., 1999; Garthwaite et al., 1999). The potential for secondary exposure of polecats to rodenticides in such circumstances is obvious and, in previous studies on small numbers of individuals (Shore et al., 1996, 1999), we have found residues of SGARs in the livers of animals. Despite or regardless of this exposure, in the last 30–40 years polecats have expanded their range eastwards from Wales, to which they had been largely restricted following widespread persecution in the nineteenth century (Langley and Yalden, 1977). The recent recolonisation of central and eastern Britain has arisen mainly through natural spread, with some translocations (Birks, 1999). As individuals penetrate eastwards, they may increasingly be exposed to SGARs that are used more extensively in central and south-eastern Britain (De'Ath et al., 1999; Garthwaite et al., 1999, also Table 1). There is concern that any rise in the extent of exposure may increase the probability of mortality and hamper, or even prevent, further population and/or range expansion.

Since our initial studies (Shore et al., 1996, 1999), we have analysed the livers of another 50 polecats for SGARs. We report the results of those analyses here. The animals came from newly recolonised areas in the east and south-east, about which there was little information on the extent of exposure to SGARs, and also from more long-established recolonised areas in the Welsh borders and the original core area in Wales. The animals had died between 1993 and 1999, which spanned some of the period in which previously-analysed polecats had died (1992–1997), but most (60%) had died in 1998 or 1999 and so provided a more current picture of contamination. In total (previous and current studies combined), therefore, we have analysed 100 polecats to date and our sample is comprised of animals that died throughout the 1990s along a broadly west-east transect running from the original core area of Wales to newly recolonised areas in eastern and south-east England. The collection of these data has enabled us to examine whether there are patterns of spatial and temporal variation in exposure, the first time, as far as we are aware, that this has been attempted for non-target predatory mammals. Our aims were to determine whether there was evidence of any increase in contamination in polecats over time, as has been observed in barn owls, and whether the extent of contamination varied between different regions of Britain. With regard to spatial variation, we hypothesised that contamination in polecats would reflect regional differences in usage and that polecats from England, and particularly from south-

Table 1
Usage (% of national use) of second-generation anticoagulant rodenticides on farms growing arable crops or grassland and fodder crops in Wales, Midland and Western, Eastern and South-Eastern regions. Areas covered by different regions are shown in Fig 3. Data are from De'Ath et al. (1999) and Garthwaite et al. (1999)

Farm type	Compound[a]	% Of national use		
		Wales	Mid and West	East/SE
Grass and fodder	Bromadiolone	<1	12	12/21
	Difenacoum	3	24	16/10
	Brodifacoum	0	<0.01	15/<1
	Flocoumafen	0	0	11/89
Arable crops	Bromadiolone	0	5	24/11
	Difenacoum	4	23	22/5
	Brodifacoum	0	12	42/9
	Flocoumafen	0	0	71/0

[a] Data do not include use of difenacoum in combination with hypercalcemic rodenticides.

eastern areas, would be more contaminated (greater proportion of animals containing residues and possibly higher residue magnitude) than animals from the original core areas in Wales.

2. Materials and methods

2.1. Collection of polecat carcasses

Corpses of polecats presumed to have died from rodenticide poisoning have been found in farmyards in Britain since the 1960s (Walton, 1970). However, since such animals tend to die out of sight on private land, sampling this source was unlikely to generate the abundant fresh material necessary for analysis. Therefore, a network of volunteers was engaged to collect the carcasses of polecats that had been killed accidentally on roads. Sex, date found and provenance were recorded for each animal. Each carcass was stored in a deep freeze until dissected, when the liver was excised and homogenized before being sub-sampled for residue analysis. The mean ± SE sample weight that was analysed was 1.570 ± 0.023 g ($n = 50$).

2.2. Residue analysis

Second-generation anticoagulant rodenticide residues were determined by high-performance liquid chromatography (HPLC) following extraction methods described by Hunter (1985) and subsequently modified by Jones et al. (1999). The dichloromethane, acetone, methanol (all from Rathburn Chemical Co. Ltd, Walkerburn, Scotland), glacial acetic acid, sodium sulphate and ammonium acetate (all from Merck, Poole, Dorset, UK) used as reagents in the analysis were of a grade suitable for HPLC analysis.

Each liver sample was weighed accurately and ground to a homogenous paste with furnace-cleaned sand and anhydrous sodium sulphate. Approximately 20 ml of extraction solvent (30% v/v acetone in dichloromethane) was mixed thoroughly with the ground tissue, left to stand for 1 h, then decanted and collected through a funnel containing glass wool and sodium sulphate. The ground tissue was subsequently washed with 20 ml aliquots of the extraction solvent and the washings were added to the original extraction aliquot until a total volume of 100 ml was collected. This was mixed and then left to stand for 12 h, after which three-quarters of the extract was reduced to zero volume and the lipid content determined gravimetrically; the remaining 25 ml of the extract were archived. The reduced extract was re-dissolved in 2–3 ml of extract solvent and cleaned up using a SPE LC-alumina-N column (Supelco UK, Poole, Dorset) that had been conditioned with 2–3 ml of extraction solvent. Once the re-dissolved extract had been poured onto the column with washings, the column was washed with 2 ml of acetone:dichloromethane (75:25) and eluted with 3 ml of 5% acetic acid in methanol. The eluate was reduced to dryness by standing in a water bath under a stream of nitrogen and then re-suspended in 0.5 ml methanol.

The eluate was analysed by HPLC (HP Series 1100, Agilent Technologies, Bracknell, Berkshire) using a Hypersil ODS (C18) 250 mm × 4.6 mm 5 μm column (Alltech Associates Applied Science, Carnforth, Lancs) at 27 °C. A 15 μl aliquot was injected onto the column using 76:24 methanol:water (v/v), supplemented with 0.25% (v/v) acetic acid and 40 mM ammonium acetate, as the mobile phase pumped at 1.1 ml min^{-1} isocratically. Bromadiolone, difenacoum, flocoumafen and brodifacoum residues were detected by fluorescence spectrometry (HP 1100 series fluorescence detector) using three excitation wavelengths (310, 320 and 350 nm) and a single emission wavelength (390 nm). The excitation wavelength of 310 nm gave the greatest emission signal at 390 nm and was used for quantification. The ratio the emission response elicited by the 320 nm wavelength to that elicited by 310 nm and the ratio elicited by 350 nm to that elicited by 310 nm were both used to aid identification. A chromatographic peak was identified as a specific SGAR if the ratios of the signals for each excitation wavelength matched the ratios in the standards and if the absolute retention time of the peak fell within the retention time window of the calibration standards.

The limit of detection (LOD) for peaks identified as SGARs was determined from the linear regression of the multilevel calibration using the equation $Y = Y_0 + 3S_{y/x}$ where Y is the LOD response, Y_0 is the Y intercept and $S_{y/x}$ is the standard error of the regression line. The LODs for bromadiolone, difenacoum, flocoumafen and brodifacoum were 0.027 0.010 0.011 and 0.005 μg, respectively which were very similar to those determined previously in analyses of polecat livers (Shore et al., 1996).

Detectable residues were quantified by comparing the peak area of samples with those of standards. The standards had been made up from technical grade material that had originally been supplied by rodenticide manufacturers and has been used for long-term monitoring of residues in wildlife (Newton et al., 1999b). Comparison of these Monks Wood standards with those that have, in more recent years, become commercially available (Greyhound Chromatography and Allied Chemicals, Birkenhead, Merseyside, UK) indicated that there was no significant difference between standards in the response obtained for either bromadiolone or brodifacoum but that the Monks Wood standard gave a significantly higher response for difenacoum. As a result, the liver difenacoum concentrations in polecats reported in the present study

would have been quantified as 1.24-fold higher if the commercially available standard had been used. Difenacoum data presented here are for residues as detected using Monks Wood standards.

Procedural blanks were analysed to detect possible contamination during the sample preparation. Duplicate liver samples were spiked with known concentrations of SGARs and analysed to determine sample matrix interference and % recovery data. The mean ±SE% recovery, determined from analysis of eight spiked samples, was 83.7±9.8%, 62.7±8.5%, 74.5±9.5% and 53.9±7.6% for bromadiolone, difenacoum, flocoumafen and brodifacoum, respectively; the difference between compounds was not significant ($F_{(3, 28)}=2.17$, $P>0.05$). Concentration data in tissue samples were not recovery-corrected.

2.3. Categorisation of provenance data for all polecats

When the data for the 50 polecats analysed in the present study were combined with those for the 50 animals analysed previously, individuals were categorised by the geographical regions used in the UK Department for Environment, Food and Rural Affairs surveys of pesticide usage in England and Wales (Garthwaite et al., 1999). Overall, 26 polecats came from Wales, 35 from the Midlands and Western region, four from Gloucestershire (just on the border of the Midlands and Western region), eight from the Eastern region and 27 from the South-Eastern region. For the purposes of the present study, the animals from Gloucestershire were included in the Midlands and Western group and polecats from the Eastern and South-Eastern regions were pooled. Thus, there were three general areas from which polecats were collected. The use of SGARs in these regions broadly increased along a transect from west to east and there was a particularly marked difference in usage between Wales and the English regions (Table 1).

3. Results

3.1. Second-generation anticoagulant rodenticide residues in polecats analysed in the present study

At least one SGAR was detected in the livers of 13 out of 37 (35%) male and five out of 13 (38%) female polecats. Difenacoum and bromadiolone were found most frequently (28 and 10% of animals, respectively) whereas brodifacoum was only detected in two polecats and flocoumafen was not detected in any individuals (Table 2). Residues of more than one SGAR were found in two polecats; bromadiolone and difenacoum co-occurred in both animals and brodifacoum was also detected in one of these individuals.

The magnitude of bromadiolone and brodifacoum residues was generally low but difenacoum residues were somewhat greater. One polecat in particular contained a high difenacoum residue (0.917 μg g^{-1}) and it was this individual that was also contaminated with bromadiolone and brodifacoum.

3.2. Temporal and spatial variation in second-generation anticoagulant rodenticide contamination in all polecats examined to date

In the 100 polecats analysed so far (this study and Shore et al., 1999), 31 contained liver residues of one or more SGAR (Table 3). It is predominantly males that are killed on roads (Birks and Kitchener, 1999), and hence males have made up almost three-quarters of the individuals that have been examined for SGARs. However, the proportion of those females that have been analysed and that contained SGARs was identical to that in males (Table 3). Overall, difenacoum and, to lesser extent, bromadiolone, have been detected most frequently in polecats, occurring in just over 1 in 5 and 1 in 10 animals, respectively. Brodifacoum has been detected only rarely in polecats and flocoumafen not at all (Table 3). Multiple exposures, as indicated by the presence of more than one compound in the liver, occurred but with low frequency; out of all 100 polecats analysed, two individuals contained residues of bromadiolone and difenacoum and two others contained both compounds and brodifacoum.

Previous analysis of the pattern of contamination in polecats that died between 1992 and 1997 indicated that there was a seasonal bias in contamination, the proportion of animals that contained residues being higher for individuals that died in the first half rather than the second half of the year (Shore et al., 1999). When the whole sample of 100 individuals was considered, the proportion of animals from each region that was contaminated was always higher (by between 1.6 and 4.5 fold) in the first half than the second half of the year (Fig. 1a). Overall, 40% of the 63 animals killed between January and June contained SGAR residues as opposed to 16% of the 37 polecats that died between July and December (Fisher's Exact test, $P=0.015$). The relatively small size of the dataset precluded using generalised linear models to analyse how the extent of contamination might be affected by all possible interactions between region, year and season. Therefore, it was necessary to determine whether the observed seasonal difference in contamination might bias analysis of temporal (inter-year) and regional differences. This was likely if the relative numbers of animals sampled in the first and second half of the year varied significantly between regions or between years. There was no evidence of a significant difference between regions in the proportion of polecats sampled in each half of the year

Table 2
Second-generation anticoagulant rodenticide residues in contaminated polecats. Residues were not detected in the livers of 32 other polecats that were analysed and the data are only for the 50 polecats newly analysed for this study

Date located	County	Sex	Sample weight (g)	% Lipid (w/w)	Rodenticide concentration (μg g^{-1} wet weight)			
					Bromadiolone	Difenacoum	Flocoumafen	Brodifacoum
March 1993	Dyfed	M	1.382	4.81	ND[a]	ND	ND	0.070
May 1993	Dyfed	M	1.802	1.99	ND	0.581	ND	ND
June 1993	Dyfed	F	1.540	4.14	ND	0.579	ND	ND
March 1995	Warks	M	1.511	5.66	0.095	ND	ND	ND
March 1997	Powys	M	1.318	4.32	ND	0.184	ND	ND
June 1997	Hampshire	F	1.465	5.22	ND	0.319	ND	ND
July 1997	Dyfed	F	1.414	6.32	0.178	0.019	ND	ND
September 1997	Oxon	F	1.627	3.47	ND	0.319	ND	ND
November 1997	Cardigan	M	1.528	4.11	ND	0.067	ND	ND
February 1998	Warks	M	1.741	4.98	0.034	ND	ND	ND
March 1998	Cardigan	M	1.826	5.71	ND	0.397	ND	ND
April 1998	Herts	M	1.852	5.22	ND	0.064	ND	ND
April 1998	Gwynedd	M	1.869	3.52	ND	0.030	ND	ND
June 1998	Oxon	M	1.643	7.96	ND	0.033	ND	ND
June 1998	Brecknock	M	1.706	4.04	0.094	ND	ND	ND
July 1998	Gwynedd	F	1.642	3.55	ND	0.369	ND	ND
April 1999	Powys	M	1.588	3.97	0.186	0.917	ND	0.052
April 1999	Gwynedd	M	1.613	5.08	ND	0.381	ND	ND

[a] ND = not detected

Table 3
Total number of all polecats with and without detectable residues of second-generation, anticoagulant rodenticides. Data are combined for the 50 polecats analysed in this study and the 50 animals analysed previously (Shore et al., 1996, 1999)

	Bromadiolone	Difenacoum	Flocoumafen	Brodifacoum	All compounds		
					Male	Female	All
Residues	12	22	0	3	22	9	31
No residues	88	78	100	97	50	19	69
% with residues	12	22	0	3	31	32	31

($\chi^2 = 1.85$, $P > 0.05$, df = 2; Fig. 1b) but there was between years (data grouped into pairs of years for analysis: $\chi^2 = 18.1$, $P < 0.001$, df = 3; Fig. 1c).

Analysis for time-trends in contamination in polecats thus had to take account of when animals had died and was done separately for animals that died in the first and second half of the year. There was no apparent progressive increase or decrease over time in the frequency with which SGAR residues were detected in polecats from any one region, although the data for each separate region were sparse (Fig. 2). Pooling data for the different regions improved sample sizes, particularly for polecats that died between January and June, but there was no evidence of a significant progressive change between 1992 and 1999 in the proportion of polecats that were contaminated (weighted linear regression for polecats that died between January and June: $F_{(1,6)} = 0.34$, $P > 0.05$, Fig. 2).

As there were no significant between-year trends in the dataset, nor any significant difference between regions in the proportion of animals sampled in the first and second half of the year, animals that had died in different seasons and years were pooled when regional variability in contamination was investigated. Initial inspection of the geographical distribution of contaminated and uncontaminated carcasses (Fig. 3) did not appear to support our hypothesis that frequency of contamination of polecats would be higher in England (particularly the south-east) than Wales. Indeed, when carcasses were categorised by provenance (Fig. 4), there was no difference between regions in the proportion of animals that contained bromadiolone ($\chi^2 = 0.82$, $P > 0.05$, df = 2). There was, albeit weak ($\chi^2 = 5.69$, P = 0.058, df = 2), evidence of regional differences in difenacoum contamination but, surprisingly, the proportion of polecats containing difenacoum was highest in Wales, more than twice that in polecats from the other two regions (Fig. 4). Of the three animals that contained brodifacoum, two were also from Wales, the other from Herefordshire (Midlands and Western region). Because difenacoum was the most prevalent of the SGARs in polecats, the proportion of animals that

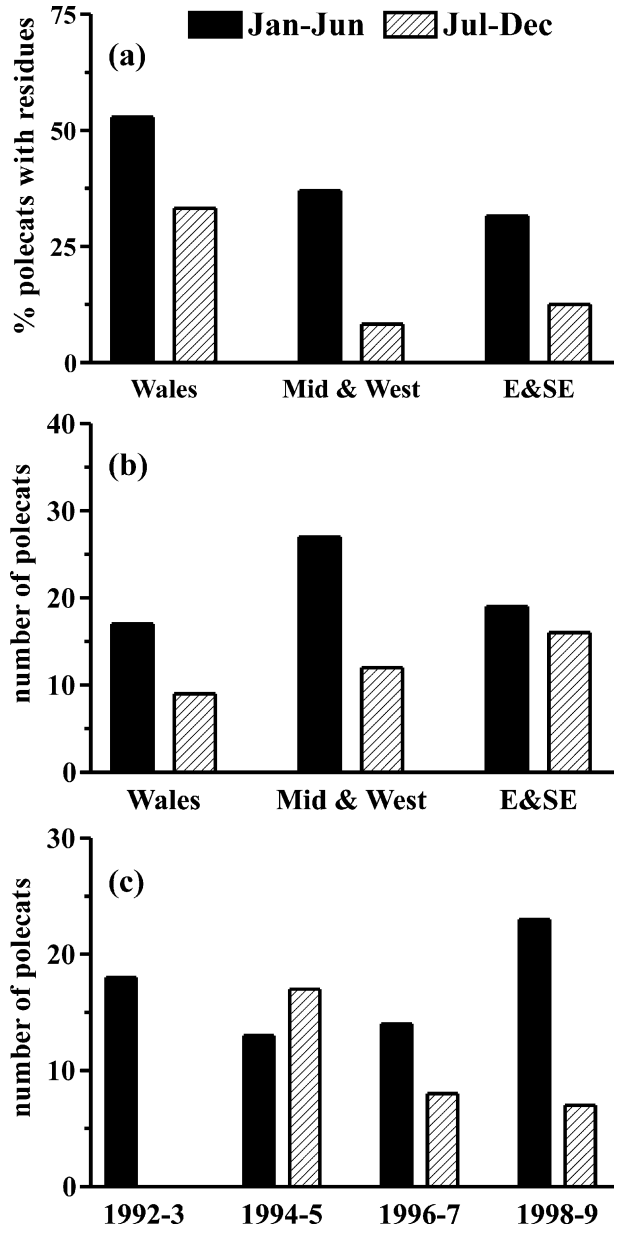

Fig. 1. Data for polecats dying in the periods January–June and July–December: (a) percentage that contained detectable liver residues of one or more second-generation, anticoagulant rodenticide, (b) number of animals sampled from each region, (c) number of polecats sampled in each pair of years during the 1990s. Data for different years are combined in graphs (a) and (b) and data for different regions are combined in graph (c).

contained any SGAR was also somewhat higher in Wales compared with elsewhere (Fig. 4), although the regional differences were not significant ($\chi^2 = 4.02$, $P > 0.05$, df = 2).

Comparison of residue magnitude in contaminated polecats from different regions was necessarily restricted to bromadiolone and difenacoum, the compounds that were detected most frequently. Differences between regions for each compound were evaluated separately using a General Linear Model that included region, the analytical run in which residues had been determined (to check for any possible analytical biases in determining residue magnitude) and time of death (January–June or July–December) as factors. There were no significant differences in residue magnitude between analytical runs (difenacoum: $F_{(1,\ 17)} = 0.63$, bromadiolone: $F_{(1,\ 7)} = 0.31$, both $P > 0.05$), indicating that there was no evidence of analytical bias in the data. There was also no evidence that residue magnitude declined significantly from the first to the second half of the year (difenacoum: $F_{(1,\ 17)} = 1.16$, bromadiolone: $F_{(1,\ 7)} = 1.48$, both $P > 0.05$ for one-tailed test), as might be expected if exposure occurred predominantly in the autumn and winter and residues were metabolised subsequently. Although the mean levels of both bromadiolone and difenacoum were slightly elevated in polecats from Wales than in those from other regions (Fig. 5), these regional differences were not statistically significant for either compound (difenacoum: $F_{(1,17)} = 1.01$, bromadiolone: $F_{(1,\ 7)} = 1.31$, both $P > 0.05$).

4. Discussion

The results from the chemical analyses carried out on the 50 polecats in this study were broadly consistent with those from previous analyses (Shore et al., 1999) in that just over a third of polecats were exposed to SGARs, the proportions of males and females that were contaminated were the same, and the SGARs detected most frequently were difenacoum and bromadiolone. The contamination in many of the polecats was not at trace levels; just over half of contaminated individuals had liver concentrations in the 0.2–1 μg g^{-1} range. The relationship between residue magnitude and mortality, or sub-lethal effects, is poorly defined for SGARs in all species, and so it is difficult to draw conclusions about the possible effects on polecats of their exposure. The 50 animals analysed here had been killed on the roads and so SGARs clearly were not the direct cause of death. However, the liver residues in some animals were close to either the liver concentration of bromadiolone (0.23 μg g^{-1}) measured in a stoat that died after being fed contaminated voles (Grolleau et al., 1989) or the liver concentration of difenacoum (1.4 μg g^{-1}) measured in a polecat that was diagnosed (on the basis of circumstances in which the body was found—dead in a barn, post-mortem haemorrhaging and residue magnitude) to have died from SGAR poisoning (Birks, 1998). Thus, it is possible that some individuals may have succumbed to SGARs if they had not been run over first.

The analysis of all 100 polecats confirmed the conclusion drawn from earlier analysis of a subset of these animals (Shore et al., 1999) that the likelihood of a polecat containing detectable SGAR residues is affected by when the animal died. The observed higher frequency of

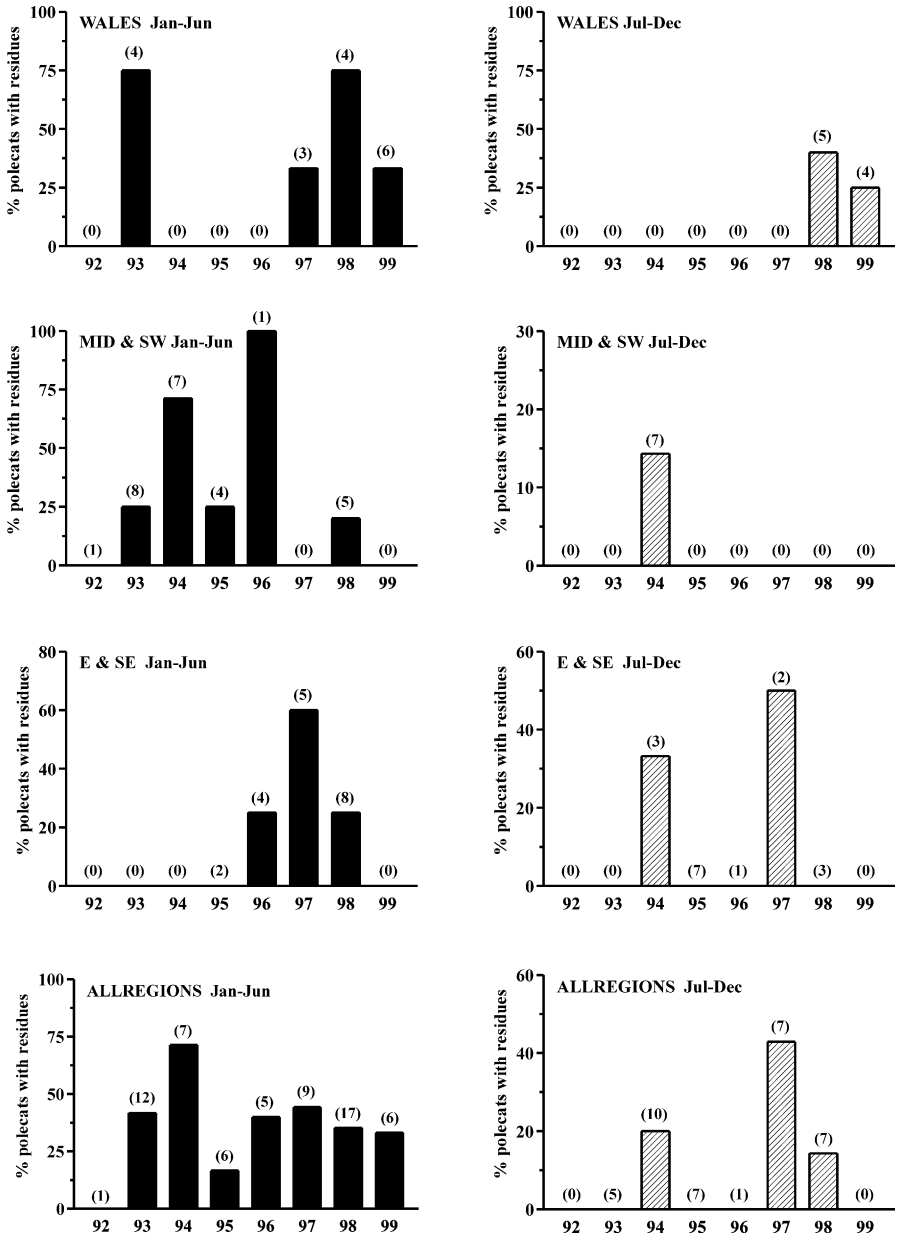

Fig. 2. Percentage of polecats that died between January and June (left hand graphs) and between July and December (right hand graphs) that contained detectable liver residues of one or more second-generation anticoagulant rodenticide between 1992 and 1999. Data are presented for each region and for all regions combined. Numbers in parentheses indicate the number of animals analysed.

residues in animals that died between January and June compared with July–December is consistent with the concept that animals are predominantly exposed in late autumn and early winter, when they feed on rats around farm buildings (Birks, 1998). Polecats that are killed on the roads soon after they leave the farm environs are still likely to contain detectable residues as bromadiolone and difenacoum can persist in the liver for several months (WHO, 1995); in contrast, animals contaminated with small amounts of these rodenticides and that die later in the year may well have metabolised their residues to below detectable levels by the time they are killed. It would be expected that, if polecats do metabolise their liver residues during the year, contaminated animals that died in the first half of the year would have residues of higher magnitude than those that died between July and December. However, we found no significant evidence that this was the case. This may reflect the relatively small sample size for contaminated animals; occasional exposure events in the summer may result in a small number of animals having relatively high residues in early autumn and this could obscure any overall pattern of gradual decline in residue magnitude. Although we cannot rule out the possibility that we were sampling different sub-sets of the population (that differed in their likelihood of

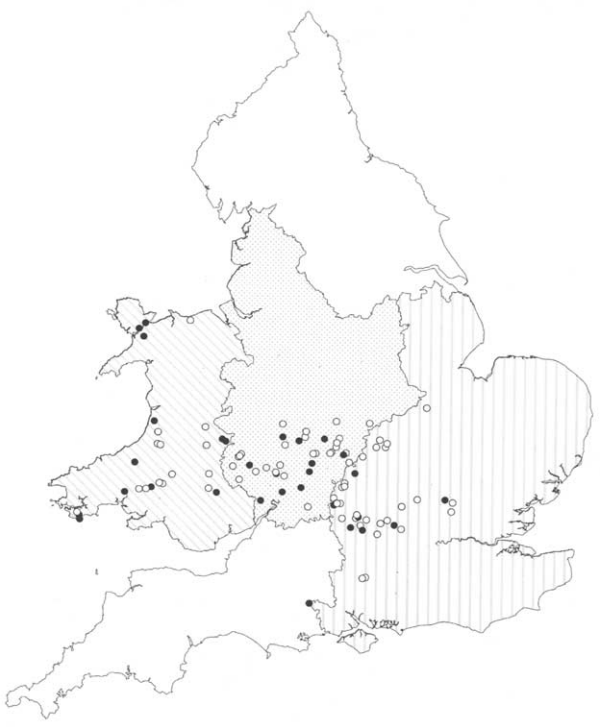

Fig 3. Provenance of polecat carcasses from England and Wales that were analysed and contained detectable (filled circles) or non-detectable (open circles) liver residues of one or more second-generation anticoagulant rodenticide. Data are for carcasses found between 1992 and 1999 inclusive. Heavy borderlines indicate the division of animals into Welsh, Midland and Western and South and South-Eastern (S&SE) regions.

exposure) in the two halves of the year, we have no evidence to think this was so. The low proportion of July to December-killed polecats that contained residues was clearly not a result of sampling bias towards young animals that had not previously fed on farm rats. Analysis of body weights and tooth wear indicated that all of the animals in our sample that were killed between July and December would have been alive in the previous autumn and winter.

Given the seasonal bias in the likelihood of exposure to SGARs, analysis of animals collected in the first rather than the second half of the year probably gives a more realistic indication of the proportion of polecats exposed to SGARs. Thus, our data would suggest that some 40% of polecats throughout their range are exposed to SGARs, although this does not account for any fatally poisoned animals (that would not have been present in our sample) but, on the other hand, also assumes that sub-lethal exposure to SGARs does not predispose polecats to being killed on the road. This figure of 40% is somewhat (but not significantly) higher than the 24% of stoats and weasels from eastern England that were found to contain residues (McDonald et al., 1998), is similar to the proportion of barn owls and foxes (*Vulpes vulpes*) in Britain exposed to anticoagulant rodenticides during the 1990s (Newton et al., 1999b; Shore et al., in press), and is significantly lower (Fisher's Exact tests, $P<0.05$) than the proportions of red kites (14 out of 20) and kestrels (24 out of 36) found by recent UK studies to contain SGARs (Shore et al., 2000, 2001). This indicates that, at the current time, polecats in Britain are not particularly any more vulnerable to exposure to SGARs than several other avian and mammalian predators, including some species thought to take far fewer rats. An implication from this is that there may be wider contamination of the prey base by SGARs than previously thought likely.

The lack of evidence of any increase during the 1990s in the proportion of polecats exposed to SGARs appears to contrast with the observation of increasing exposure in barn owls. However, the increase over time in the proportion of barn owls contaminated with SGARs has not been linear (second-order polynomial regression model: $F_{(1,\ 15)}=55.2$, $P<0.001$; Shore, unpublished data), the sharpest rise occurring in the 1980s (Newton et al., 1999a, b). Between 1992 and 1999, the modelled annual increase ranged between 2.2 and 0.8% (Shore, unpublished data), the proportion of birds that were contaminated rising only from 32 to 39%. This small annual rate of increase over the whole of the 1990s was consistent with the apparent broad stability in national usage of SGARs during this period (Olney and Garthwaite, 1994; Olney et al., 1994; Thomas and Wild, 1996; De'Ath et al., 1999; Garthwaite et al., 1999). If the increase in frequency of contamination in barn owls during the 1990s was typical for most non-target predators in Britain, then it would not have been detectable in our dataset because of the relatively small number of polecats that were analysed; the 95% Confidence Limits for the annual rate of change in the proportion of (January–June killed) polecats that were contaminated with SGARs (Fig. 2) were −7.9% and +4.9%. Other factors, such as differences in the dietary preferences of polecats and barn owls, may also mean that temporal trends in contamination vary between species.

Variation in the proportion of polecats contaminated with different SGARs would not be expected to mirror national usage patterns exactly. This is because the likelihood of detecting residues in animals killed on the roads may vary between compounds as a result of differences in their toxicity and biological half-life (Eason et al., 2002), and also because of variation in the analytical sensitivity with which different SGARs can be detected. Despite this, the overall pattern of contamination in polecats did reflect national SGAR usage patterns in that difenacoum and bromadiolone are the most widely used compounds (De'Ath et al., 1999; Garthwaite et al., 1999; Table 1) and were the SGARs most frequently detected in polecats. These compounds are also the SGARs that are most frequently detected in

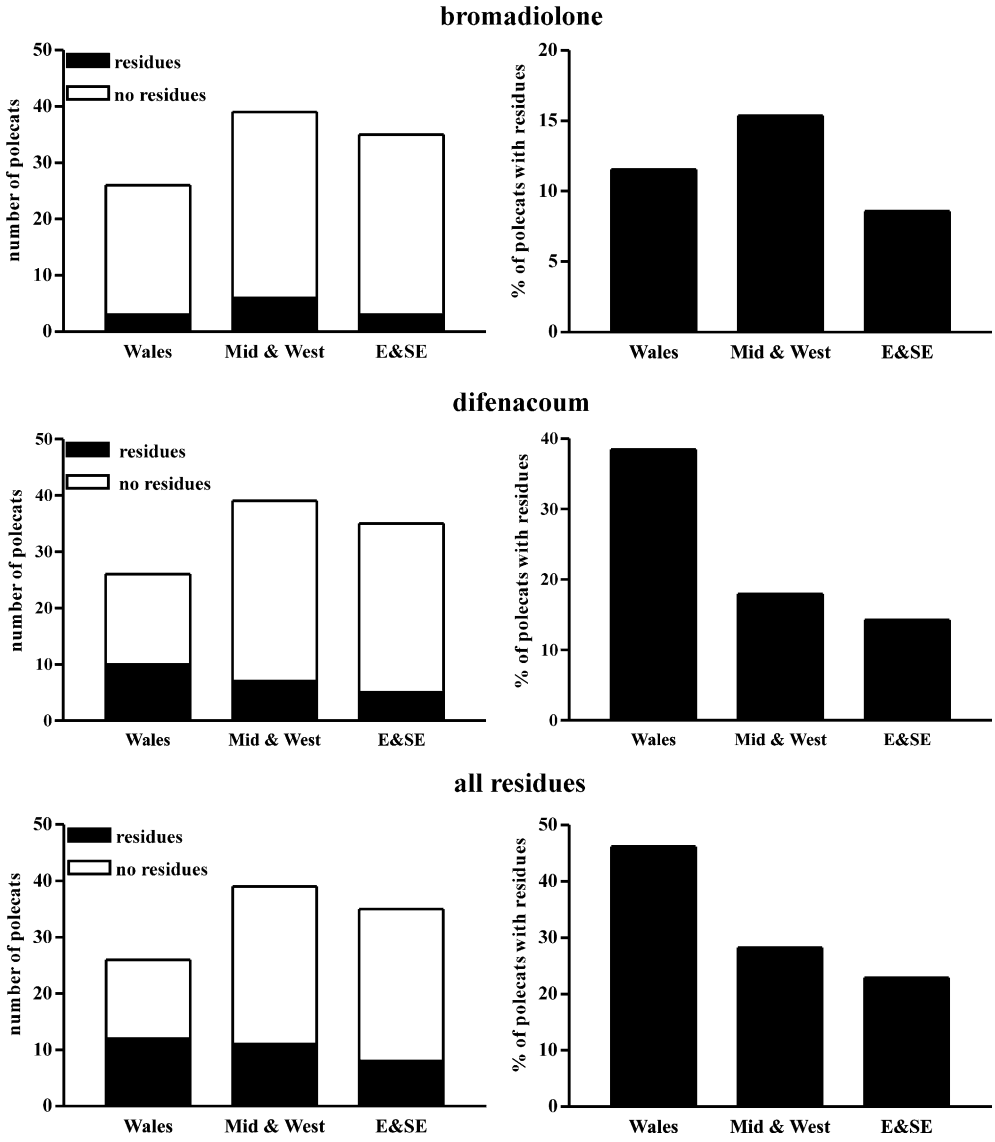

Fig 4. Number (left hand graph) and percentage (right hand graph) of polecats from different regions that contained detectable liver residues of bromadiolone (top graphs), difenacoum (middle graphs) or any second-generation, anticoagulant rodenticide (bottom graphs). Data for different years are combined.

other avian and mammalian predators (Newton et al., 1999b; Shore et al., 2001, in press). Furthermore, residues of brodifacoum and flocoumafen were detected in polecats only rarely or never and this was consistent with the relatively low use of these compounds nationally. However, both brodifacoum and flocoumafen are used relatively extensively in the east and south east regions (Table 1), but were not detected in any of the polecats from those areas. This suggests that the restriction to indoor use for these SGARs may be effective in limiting the exposure of polecats.

In contrast to the national pattern, the variation between regions in the contamination of polecats clearly did not match regional differences in usage. The proportion of polecats from each region that contained difenacoum or bromadiolone was not highest in the central or eastern regions, as we hypothesised would be the case given the greater use of these compounds compared with in Wales. In fact, difenacoum residues were more prevalent and marginally higher in polecats from Wales. Why the extent of contamination in polecats does not match spatial variation in usage is uncertain but could be due to a variety of factors. These may include differences between regions in the distribution of polecats relative to foci of SGAR use and in the reliance of polecats on farmyard rats in autumn and winter. It is also possible that the occurrence of bromadiolone and difenacoum resistance and cross-resistance (between first and second-generation anticoagulant rodenticides) in rats (Greaves et al., 1982; Thijssen, 1995) may enhance secondary exposure in polecats and mask any influence of usage pattern. Studies on the feeding behaviour and

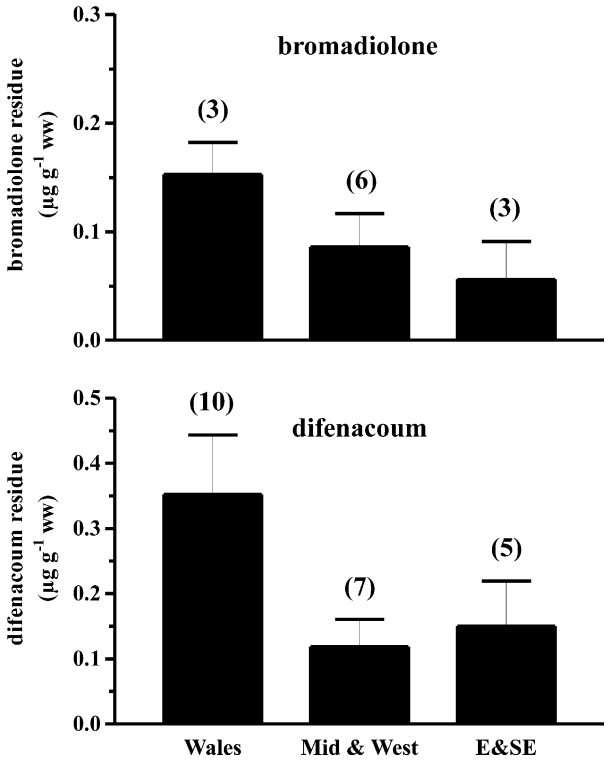

Fig. 5. Mean (±SE) liver bromadiolone and difenacoum concentrations in polecats from different regions that contained detectable residues (number of animals indicated by numbers in parentheses).

general ecology of polecats in their recently-recolonised areas in eastern England are necessary to determine whether there are regional differences between populations that affect their likelihood of exposure. Studies are also needed on the spatial distribution of SGAR residues in rats and other small mammal prey to determine if resistance significantly affects secondary exposure in polecats and other vertebrate predators.

5. Conclusions

Overall, this study demonstrated that some 40% of adult polecats that were killed accidentally (mostly on roads) had been sub-lethally exposed one or more times to SGARs, predominantly difenacoum and bromadiolone. Contrary to our expectations, there was no evidence that the proportion of the polecat population exposed to SGARs increased either during the 1990s generally or as the species expanded its range into more eastern areas. It is evident that exposure of polecats to SGARs in Wales did not prevent the range expansion that occurred particularly in the 1980s and 1990s (Birks, 1999). Given this, we have no reason to suppose that current usage of SGARs will prevent polecats further expanding their range in eastern areas of England. However, changes in the scale or patterns of SGAR use, the extent of resistance in rodents, or dietary shifts by polecats towards greater reliance on farm rats, could all increase the extent of exposure and the associated likelihood of mortalities. Furthermore, our studies have concentrated on SGARs because they are perceived to pose the greatest secondary poisoning hazard. Exposure to first-generation compounds has not been monitored but polecats may be more at risk of exposure in eastern areas to certain of these compounds; 58 and 82% of the estimated national use of chlorophacinone on farms growing arable and fodder crops, respectively, is in the Eastern region (De'Ath et al., 1999; Garthwaite et al., 1999). The potential risk associated with secondary exposure to this and other first-generation rodenticides is largely unquantified for polecats. Periodic studies are needed in the future to evaluate whether the extent of exposure to anticoagulant rodenticides is changing in the polecat population. Such studies should include quantification of both first and second-generation anticoagulant rodenticides and also maximise the number of animals analysed to enhance the power with which temporal and spatial trends in contamination can be detected.

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