Anticoagulant rodenticides in predatory birds 2011: a Predatory Bird Monitoring Scheme (PBMS) report

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Executive Summary

The Predatory Bird Monitoring Scheme (PBMS; http://pbms.ceh.ac.uk/) is the umbrella project that encompasses the Centre for Ecology & Hydrology's National Capability contaminant monitoring and surveillance work on avian predators. By monitoring sentinel vertebrate species, the PBMS aims to detect and quantify current and emerging chemical threats to the environment and in particular to vertebrate wildlife.

Anticoagulant rodenticides, and in particular second generation anticoagulant rodenticides (SGARs), can be toxic to all mammals and birds. Predators that feed upon rodents are particularly likely to be exposed to these compounds. The PBMS, together with other studies, have shown that there is widespread exposure to SGARs of a diverse range of predators in Britain and that some mortalities occur as a result. This report summarises the PBMS monitoring for anticoagulant rodenticides in barn owls (*Tyto alba*), kestrel (*Falco tinnunculus*) and red kites (*Milvus milvus*) that were found dead in 2011 and presents long term trend analysis for barn owls.

During this year's analysis, a change to the analytical methods used by the PBMS was trialed where the use of matrix-matched standards was compared to solvent-matched standards. Matrix matched standards gave higher % recoveries for spiked samples and more repeatable results, and consequently resulted in reporting of higher liver SGAR concentrations in birds with detectable residues. It was concluded that matrix-matched standards provided a better analysis and would be used in this and future years analysis, but that a predicted solvent-matched equivalent SGAR residue would be calculated for use in time and spatial trend analysis that involved comparisons with data from previous years. This would eliminate biases that could otherwise be introduced into the analysis due to changes in analytical methodology.

In birds that died in 2011, SGARs were detected in 84% of 58 barn owls analysed and the most prevalent compounds were difenacoum and bromadiolone. The majority of the residues were low and not diagnosed as directly causing mortality. The livers from 18 red kites were analysed in 2011. Most (94%) had detectable liver SGAR concentrations, again mainly difenacoum and bromadiolone, although brodifacoum was also detected in 78% of the birds. Six of the red kites analysed showed signs of haemorrhaging thought possibly to be associated with rodenticide poisoning. However, only two of these birds had relatively high sum SGAR liver concentrations (> 0.4 µg/g wet weight) and the contribution of SGARs, if any, to the death of the other four birds is uncertain. SGARs were detected in all 20 kestrel analysed. The most prevalent rodenticides detected in kestrel livers were difenacoum and bromadiolone. The co-occurrence of multiple residues was also prevalent with 19 out of 20 kestrels having more than one SGAR present in their liver.

Due to a new collaborative arrangement with the Hawk Conservancy Trust the PBMS received a higher proportion of its barn owls and kestrels from the counties of Berkshire, Hampshire, Oxfordshire & Wilstshire. These counties are within a focus of rodenticide resistance in the Norway rat (*Rattus norvegicus*) and we tested whether there were significant differences for the prevalence and magnitude of SGAR residues in barn owls and kestrels between this area and other counties. There were no significant differences between either barn owls or kestrels from resistance and non-resistance counties in either the proportion of birds with detectable liver SGAR residues or the magnitude of liver SGAR concentrations in those birds with detected residues. However, the sample size examined was relatively small and it would be valuable in the future to conduct an analysis of the potential impact of resistance on residue prevalence and

magnitude for birds collected over a longer time-scale and incorporating all counties where resistance to SGARs in rats has been documented.

SGARs have been monitored in barn owls since 1983. Data on long-term trends have been adjusted to account for changes over time in sensitivity of analytical methods. This has meant that very low residues (<0.025 µg/g wet weight), which are now detectable, are not included in the time trend analysis. Overall, the proportion of barn owls with detectable liver concentrations of one or more SGAR has increased significantly over the course of monitoring. The highest value was recorded in 2008 while the value for 2011 was 25.9%. The proportion of barn owls with detectable SGAR residues over the period 1990-2011 was two-fold higher in England than in Scotland and Wales and also varied significantly between different regions of England. Between 1997 and 2011 there has not been any significant progressive increase or decrease in detectable SGAR residues in kestrels.

1. Introduction

1.1 Background to the PBMS

The Predatory Bird Monitoring Scheme (PBMS; http://pbms.ceh.ac.uk/) is the umbrella project that encompasses the Centre for Ecology & Hydrology's long-term contaminant monitoring and surveillance work on avian predators. The PBMS is a component of CEH's National Capability activities.

By monitoring sentinel vertebrate species, the PBMS aims to detect and quantify current and emerging chemical threats to the environment and in particular to vertebrate wildlife. Our monitoring provides the scientific evidence needed to determine how chemical risk varies over time and space. This may occur due to market-led or regulatory changes in chemical use and may also be associated with larger-scale phenomena, such as global environmental change. Our monitoring also allows us to assess whether detected contaminants are likely to be associated with adverse effects on individuals and their populations.

Overall, the PBMS provides a scientific evidence base to inform regulatory decisions about sustainable use of chemicals (for example, the EU Directive on the Sustainable Use of Pesticides). In addition, the outcomes from the monitoring work are used to assess whether mitigation of exposure is needed and what measures might be effective. Monitoring also provides information by which the success of mitigation measures can be evaluated.

Currently, the PBMS has two key objectives:

(i) to detect temporal and spatial variation in exposure, assimilation and risk for selected pesticides, biocides and pollutants of current concern in sentinel UK predatory bird species and in species of high conservation value

(ii) in conjunction with allied studies, to elucidate the fundamental processes and factors that govern food-chain transfer and assimilation of contaminants by top predators.

Further details about the PBMS, copies of previous reports, and copies of (or links to) published scientific papers based on the work of the PBMS can be found on the PBMS website.

1.2 PBMS monitoring of anticoagulant rodenticides

Second generation anticoagulant rodenticides (SGARs) can be toxic to all mammals and birds. Predators that feed upon rodents are particularly likely to be exposed to these compounds. The PBMS (see previous reports, also Newton et al., 1999, Shore et al., 2006, Walker et al., 2008a,b) together with other studies (Dowding et al., 2010, McDonald et al., 1998, Shore et al., 2003a,b) have shown that there is widespread exposure to SGARs of a diverse range of predators in Britain. Defra's Wildlife Incident Monitoring Scheme (WIIS)[2] and the PBMS have shown that in the UK some mortalities result from this exposure.

In response to conservation concerns over the potential impacts of SGARs on predators, the PBMS has monitored trends in exposure to second generation anticoagulant rodenticides (SGARs) in a sentinel species, the barn owl (*Tyto alba*). This has been done since 1983 and the findings from previous years and analyses of long-term trends are given in previous PBMS reports and by Newton et al., (1990, 1999). The red kite (*Milvus milvus*) is a high conservation priority species that has been reintroduced to England as part of the red kite species recovery programme (Carter and Grice 2002). SGAR-induced deaths of kites have been detected by the Wildlife Incident Investigation Scheme. Up until 2007, only a small number of red kites were received and analysed by the PBMS each year although this showed that a large proportion of reintroduced birds were exposed to SGARs (Walker et al. 2008a). The development of a collaboration with the Institute of Zoology has meant that the number of red kite livers available for analysis has now usually increased. Kestrels (*Falco tinnunculus*) have been monitored since 2000 following a pilot study that demonstrated a relatively high level of exposure compared with barn owls (Shore et al. 2001) and conservation concerns over declines in kestrel populations (http://www.bto.org/birdtrends2009/wcrkestr.shtml).

This report describes the results of PBMS monitoring of barn owls, kestrels and red kites submitted to the PBMS in 2011 (Table 1.1). This involved measuring liver residues in carcasses submitted to the PBMS by members of the public. The birds died from various causes, but mainly from road traffic collisions and from starvation. The provenance of the birds is shown in Figure 1.1.

Table 1.1. Number of barn owls, kestrels and red kites submitted to the PBMS in 2011

Species		Received	Analysed
barn owl	*Tyto alba*	104	66[1]
red kite	*Milvus milvus*	18	18
kestrel	*Falco tinnunculus*	31	20
Total		153	104

[1] Nine of the birds had died in 2012 but were received before analysis commenced. They were included in this analysis run to facilitate a comparison between the Berkshire/Hampshire rodenticide resistance area and other counties; see section 3.2 of this report.

All red kites were autopsied and analysed. All the barn owls received were autopsied but, because of the large number, a sub-sample of just over 50 birds per year (stratified by date found) were analysed. Similarly a sub-sample of 20 kestels has been analysed. Tissues from

[2] Annual WIIS reports are available at www.pesticides.gov.uk/environment.asp?id=58

all birds received were archived in the PBMS tissue and egg archive where they are available for future research purposes.

Since 2006, the concentrations of warfarin and coumatetralyl (first generation hydroxycoumarins) and the presence or absence of diphacinone and chlorophacinone (indandiones) have been quantified in addition to SGARs. A summary of the analytical methods can be downloaded from the PBMS website (http://pbms.ceh.ac.uk/docs/AnnualReports/PBMS_Rodenticides_Methods.pdf). Anticoagulant rodenticide concentrations are reported as µg/g wet weight (wet wt) throughout this report.

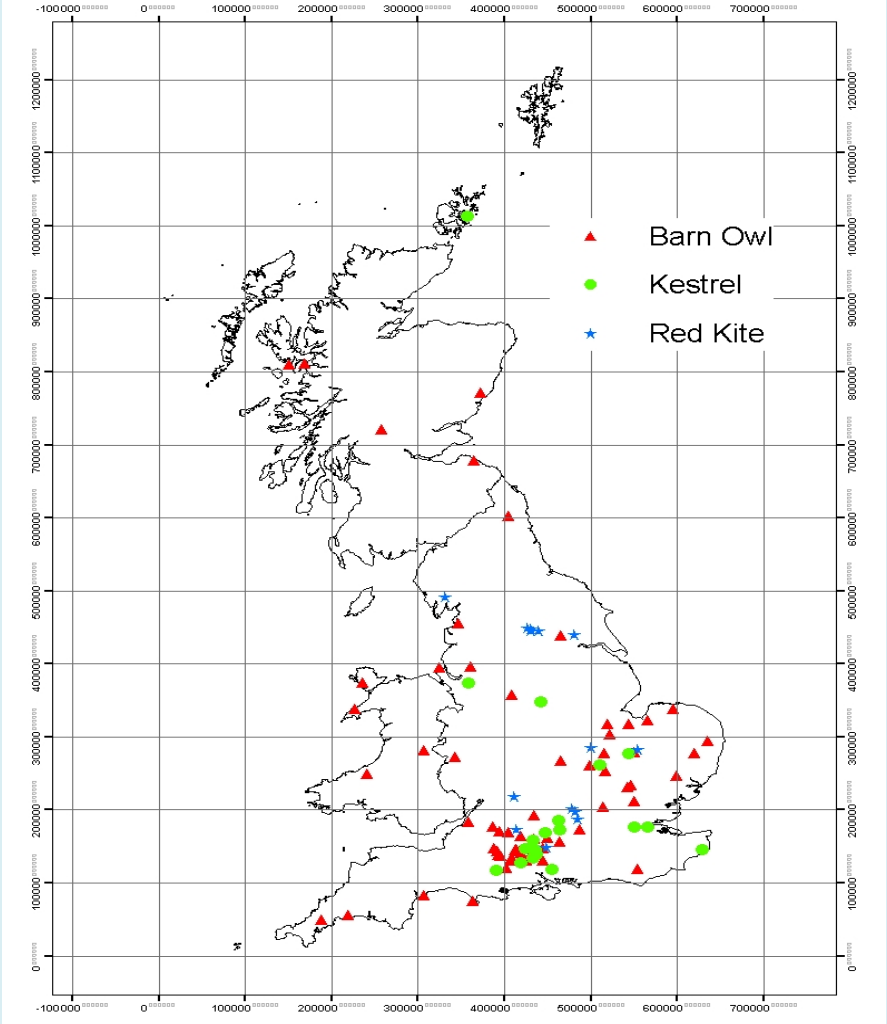

Figure 1.1. Location of birds found dead in 2010 that have been analysed for hepatic anticoagulant rodenticide concentrations. Grid lines are 100km squares.

2. Changes to analytical methods

2.1 Background

Since 2006, the PBMS has used liquid chromatography mass spectrometry (LC-MS) techniques to quantify anticoagulant rodenticide residues in the livers of predatory birds. This method utilised solvent-matched standards for describing calibration curves for each rodenticide. However, an alternative approach is to use matrix-matched calibration standards which are constituted in solvent that has been used previously to extract uncontaminated chicken liver; the aim is to match as closely as possible the matrix of extracts of livers from predatory birds. The advantage of using matrix-matched standards is that it they effectively account for potential variations in accuracy of contaminant quantification caused by interferences associated with naturally occurring organic compounds in the liver extract. In addition, use of matrix matched standards in the PBMS analysis would make our results more directly comparable to those produced by schemes, such as the Wildlife Incident Investigation Scheme (WIIS), which use matrix-matched standards in their analysis. However, introducing the use of matrix-matched standards into the PBMS analysis could potentially result in a step change in the prevalence of detected residues and/or the reported magnitude of detected residues in PBMS samples. This is because use of matrix-matched standards is likely to improve analytical recovery.

We therefore conducted a study to assess whether use of matrix-matched rather than solvent-matched standards would significantly alter the reported prevalence of detected residues or the reported magnitude of residues that were detected. This involved analysing barn owl, kestrel and red kite livers from birds that died in 2011, together with the associated quality control samples, using both solvent-matched and matrix-matched calibration standards. We specifically compared data derived from solvent-matched and matrix-matched calibration curves for three sets of samples:

(i) % recoveries associated with topically spiked but otherwise uncontaminated chicken liver samples that were run with each batch of unknowns
(ii) detected concentrations in sub-samples of the liver of an individual buzzard (*Buteo buteo*) known to contain detectable residues of bromadiolone, difenacoum and brodifacoum, the three most commonly detected second generation anticoagulant rodenticides found in predatory birds in the UK
(iii) the proportion of barn owls, kestrels and red kites that died in 2011 that had detectable residues of one or more second-generation anticoagulant rodenticide and the magnitude of detected residues

2.2 Percentage recoveries from topically spiked chicken livers

Eight chicken liver samples topically spiked with SGARs were analysed, one with each batch of unknowns. The calculated % recoveries using matrix-matched standards were significantly higher than those calculated using solvent-matched standards (Paired t-tests, $t_7 >= 3.27$, $P < 0.05$ in all cases; Table 2.1).

Table 2.1 Summary statistics for % recoveries for eight spiked chicken liver when analysed using solvent-matched and matrix-matched standards

Compound	Standards	Mean	Standard Deviation	S.E.M.[1]	R.S.D.[2]	Lower 95% CI[3]	Upper 95% CI
Bromadiolone	Solvent	62.8	28.5	10.1	45.4	38.9	86.6
	Matrix	89.7	24.7	8.7	27.5	69.1	110.3
Brodifacoum	Solvent	67.1	29.1	10.3	43.3	42.8	91.4
	Matrix	102.3	38.7	13.7	37.8	69.9	134.7
Difenacoum	Solvent	82.4	19.9	7.0	24.2	65.7	99.0
	Matrix	108.0	22.3	7.9	20.6	89.4	126.7
Difethialone	Solvent	51.4	27.7	9.8	53.8	28.3	74.5
	Matrix	79.1	40.6	14.3	51.3	45.2	113.1
Flocoumafen	Solvent	70.9	18.2	6.4	25.7	55.7	86.2
	Matrix	91.0	27.4	9.7	30.2	68.0	113.9

[1]Standard Error of the Mean; [2] Relative standard Deviation; [3]Confidence Interval

For four of the five compounds, the % recoveries calculated using matrix-matched standards had a lower relative standard deviation than those calculated using solvent-matched standards, suggesting that use of matrix-matched standards gave better repeatability.

2.3 Magnitude of residues in sub-samples of buzzard liver

As with the spiked chicken liver, a sub-sample of the buzzard liver was included in each of the eight analytical batches that were run.

The mean (± SEM) bromadiolone concentration, as measured using matrix-matched samples, was 20.2 (± 3.83) ng/g wet wt. but there was no significant difference between concentrations calculated using matrix-matched or solvent-matched standards (Paired t-test; t_7=0.06, P=0.957). The mean concentration for brodifacoum derived using matrix-matched and solvent matched standards was 3.99 (± 1.09) ng/g wet wt. and 2.70 (± 0.74) ng/g wet wt., respectively, and the mean (± SEM) elevation in brodifacoum residues in each sample that was associated with using matrix-matched rather than solvent-matched standards was 1.29 (±0.61) ng/g wet wt., although this was not statistically significant (Paired t-test; t_7=2.105, P=0.073). The mean (± SEM) difenacoum concentration using matrix-matched and solvent matched standards was 10.9 (±1.79) ng/g wet wt. and 9.08 (± 0.79) ng/g wet wt., respectively but, as with brodifacoum, this elevation in reported concentration within each sample as not quite statistically significant (Wilcoxon matched-pairs signed rank test; W=28, P=0.055).

2.4 Prevalence and magnitude of residues in barn owls, red kites and kestrels

We pooled data for barn owls, red kites and kestrels from this year's monitoring programme and found that the proportion of birds with detectable residues did not vary significantly depending on whether residues were quantified using matrix-matched or solvent-matched standards (Table 2.2),

Table 2.2 Comparison of percentage of barn owls, red kites and kestrels (n= 95 in total) with detectable SGAR residues measured using solvent-matched and matrix-matched calibration standards

Compound	Solvent	Matrix	Fisher's exact test P-value
Bromadiolone	78.8	76.9	0.868
Difenacoum	63.5	68.3	0.559
Flocoumafen	6.7	7.7	1
Brodifacoum	48.1	49.0	1
Difethialone	3.8	5.8	0.748

In contrast, the magnitude of the bromadiolone, difenacoum and brodifacoum residues that were detected were significantly higher when matrix matched standards were used to quantify these compounds (Table 2.3).

Table 2.3 Comparison of log10 transformed concentrations of SGARs in predatory bird livers quantified using either matrix matched or solvent matched standards. Methods were compared by paired T-test where the compound was detected by both methods.

Compound	N	T-value	P-value	Pairing significant?
Bromadiolone	73	6.86	<0.0001	Yes
Difenacoum	62	5.69	<0.0001	Yes
Brodifacoum	47	10.7	<0.0001	Yes

We subsequently analysed the relationship between concentrations quantified using solvent-matched and matrix-matched standards for the different SGARs (Figure 2.1). This analysis demonstrated that detected concentrations were generally higher when matrix-matched rather than solvent-matched calibration standards were used. However, this difference was most marked for larger residues and concentrations of residues close to the limit of detection were similar when they were reported using the different calibration standards; the calculated regression lines tended to converge with the line of equality at low concentrations (Figure 2.1). This explains why varying the type of calibration standard had no significant effect on the proportion of birds that had detected residues, and also accounts for the somewhat equivocal effects of varying calibration standard on the quantification of residues in the buzzard liver (Section 2.3); the magnitude of the effect of changing the type of calibration standard will depend upon the magnitude of the residue of each SGAR in the liver.

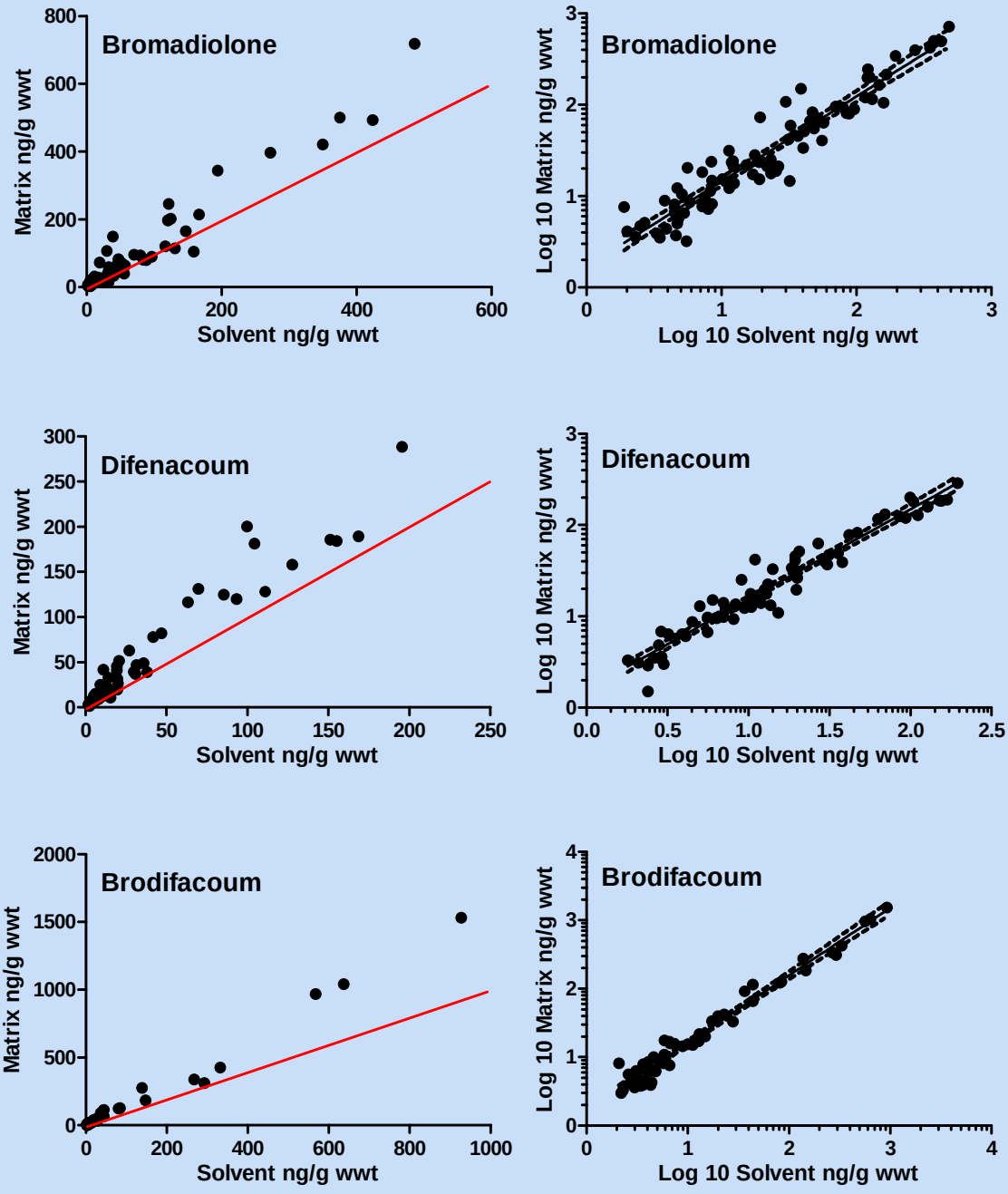

Figure 2.1 Relationships between concentrations of SGARs in liver samples as calculated using matrix-matched and solvent- matched standards. Solid line indicates fitted linear regression and dotted line the 95% confidence limits around that regression. Red line indicates line of equality. Correlations are shown for untransformed data and regression models for log 10 transformed data, as log transformation was necessary to meet the assumptions of the regression models.

2.5 Conclusions and implications for future PBMS reporting

Our results indicate that the introduction of matrix-matched standards for the quantification of SGAR residues in PBMS monitoring is unlikely to affect the proportion of birds found to have detectable residues. However, it is likely to lead to the reporting of higher liver SGAR concentrations in birds that have detectable residues and this will be most marked in birds with the highest residues. In fact, our results indicate that the magnitude of liver residues in birds given in previous PBMS annual reports is likely to be underestimated.

Given these results, it is proposed to analyse future samples using matrix-matched samples. This has the advantage of better % recoveries and repeatability and is likely to provide a better representation of the true liver concentrations accumulated by birds.

This change, however, presents a challenge for the maintenance of data on long-term trends. Long term data on the % of birds with detected residues are already "adjusted" by using an elevated limit of quantification (0.025 µg/g wet wt) to take account for the effects of earlier methodological changes when analysis was switched from fluorescence HPLC to LCMS (Section 4 of this report). All previous analyses have been based on analysis using solvent-matched calibration standards. To eliminate future potential biases due to a switch in use from solvent-matched to matrix-matched standards, we will convert matrix-matched concentrations for bromadiolone, difenacoum and brodifacoum to values that we predict would have been reported if solvent-matched standards had been used. This conversion will be done using the regression equations described in Table 2.4, which are effectively the inverse of the relationships depicted in Figure 2.1. The elevated limit of quantification of 0.025 µg/g wet wt used for time trend analysis will then be applied to these predicted concentrations to determine which birds are scored as having detected liver residues for the purposes of analysing long-term trend data.

Any long-term comparison of residue magnitude (in birds with detected residues) across years will also require normalisation of data to account for the effect of the change in type of calibration standard used. We will again convert matrix-matched concentrations for bromadiolone, difenacoum and brodifacoum to values that we predict would have been reported if solvent-matched standards had been used. Thus, any time trend analysis on residue magnitude would underestimate actual liver concentrations but would be appropriate for detecting changes between years.

We do not have comparative data on flocoumafen and difethialone concentrations quantified using solvent-only and matrix-matched standards. However, very few flocoumafen residues have been reported in birds previously sampled by the PBMS and no difethiolone concentrations have been detected in samples analysed only using solvent-matched standards. Thus, it is not anticipated that the change to matrix-matched standards will have any significant effect on our ability to examine time trends in residue prevalence or magnitude for these two compounds.

Table 2.4 Descriptive parameters of linear regression analysis of log10 transformed SGAR concentrations calculated using matrix matched vs solvent matched standards with units expressed in ng/g wet weight

Compound	df*	F-value	R^2	P-value	Linear?	b**	c**
Bromadiolone	1, 79	773	0.907	<0.0001	Yes	0.974	-0.098
Difenacoum	1, 68	1093	0.941	<0.0001	Yes	0.955	-0.125
Brodifacoum	1, 49	1538	0.969	<0.0001	Yes	0.982	-0.182

* df indicates degrees of freedom on statistical test.

** The equation of the line can be calculated as: log10[Solvent] = b*log10[Matrix] + c.

3. Anticoagulant rodenticide concentrations in birds submitted to the PBMS in 2011

Summary statistics for the incidence of detectable concentrations of anticoagulant rodenticides in the barn owls and red kites that were analysed are given in Table 3.1. Results for individual birds are given in a downloadable addendum to this report (https://wiki.ceh.ac.uk/display/pbms/Home).

The data reported here (Section 3) are for concentrations quantified using matrix-matched standards.

Table 3.1. Number of birds (No/) with detectable liver concentrations of anticoagulant rodenticides and the percentage this comprised of all birds analysed (%). Total number of barn owls, red kites and kestrels analysed was 58, 18 and 20, respectively.

	Limit of	barn owls		red kites		kestrels	
	Detection[1]	No/	%	No/	%	No/	%
2nd Generation (SGAR)							
bromadiolone	1.4	40	69	15	83	20	100
difenacoum	1.2	31	53	15	83	18	90
flocoumafen	1.1	4	7	2	11	0	0
brodifacoum	1.4	19	33	14	78	11	55
difethialone	1.0	4	7	1	5.6	1	5
Any SGAR	-	49	84	17	94	20	100
Multiple SGARs	-	30	53	16	89	19	95

[1] Method LoDs reported in ng/g wet wt.

NB. These figures are calculated based on methods using matrix matched standards and should not be directly compared to previous year's results, which used solvent matched standards (see Section 2)

3.1 Difethialone

Difethialone has recently been licensed for indoor use only in Britain. This compound (CAS Number 104653-34-1), has now been added to the analytical suite for the PBMS. It was detected in the livers of six birds; four barn owls, one kestrel and one red kite. However all of these detected concentrations occurred in only one of the eight analytical batches that were run. Re-examination of the analytical data and the data from the analytical controls gave no reason to doubt the veracity of the analytical data but the clustering of detected residues within a single batch is suspicious. Thus, the data are not reported further here. Fresh sub-samples of the livers of these birds (and some that tested negative for difethialone) will be re-analysed as part of the monitoring programme in 2013 (analysis for 2012 samples) to confirm that difethialone was present in these samples.

3.2 Use of samples from the Berkshire/Hampshire rodenticide resistance area

In the last year the PBMS developed a collaborative arrangement with the Hawk Conservancy Trust (HCT) by which the HCT submits to the PBMS the carcasses of birds that its rehabilitation hospital is unable to treat successfully. This has resulted in a larger proportion of the barn owls and kestrels analysed by the PBMS originating from an area of known rodenticide resistance in the Norway rat (*Rattus norvegicus*). Hampshire/Berkshire resistance in the Norway rat has been described by Buckle and Prescott (2012) and confers resistance to all first generation anticoagulant rodenticides (warfarin-type ARs), bromadiolone, and probably difenacoum. The focus of this resistant strain is Berkshire and Hampshire but also extends to Wiltshire and south Oxfordshire. Rodenticide resistance in Norway rats may lead to increased contamination of SGARs in non-target predatory birds through one of two routes. The first stems from the increased probability that the bodies of resistant target species are likely to contain larger amounts of SGARs than non-resistant animals and that resistant animals survive their exposure, thus remaining active and available as prey to predators. Consequently predatory birds that prey upon these target species or that may scavenge their bodies may be exposed to higher concentrations of SGARs. The second route is that the large-scale and prolonged use of SGARs that tends to occur when trying to control resistant populations may lead to increased primary exposure in non-target species, such as the wood mouse (*Apodemus sylvaticus*), and this may increase exposure in predators that primarily feed upon non-target species.

To determine whether the recent greater availability of carcasses from the HCT is likely to significantly increase the overall detection of SGAR residues by the PBMS, we compared the prevalence of detectable SGAR residues, and the magnitude of those residues, in barn owls and kestrels from the resistance area (Berkshire, Hampshire, Oxfordshire and Wiltshire inclusive) with that in birds from other counties. This analysis was conducted on the 2011 birds that were analysed for the current report (see Section 3.3 and 3.4) but a further eight barn owls that had died in early 2012 were included in the analysis to increase sample size for this particular analysis.

The proportion of barn owls with detectable residues of one or more SGARs in their liver was not significantly different between owls from the Berkshire/Hampshire resistance area and those found in other counties within the UK (22 out of 27 vs 32 out of 39; Fisher's exact test; P=1.000). Neither sum SGAR liver concentrations nor concentrations of individual SGARs differed significantly between barn owls found dead in the resistance area and those found in other counties within the UK (Figure 3.1), although the difference between areas for difenacoum residues was close to significance (Figure 3.1).

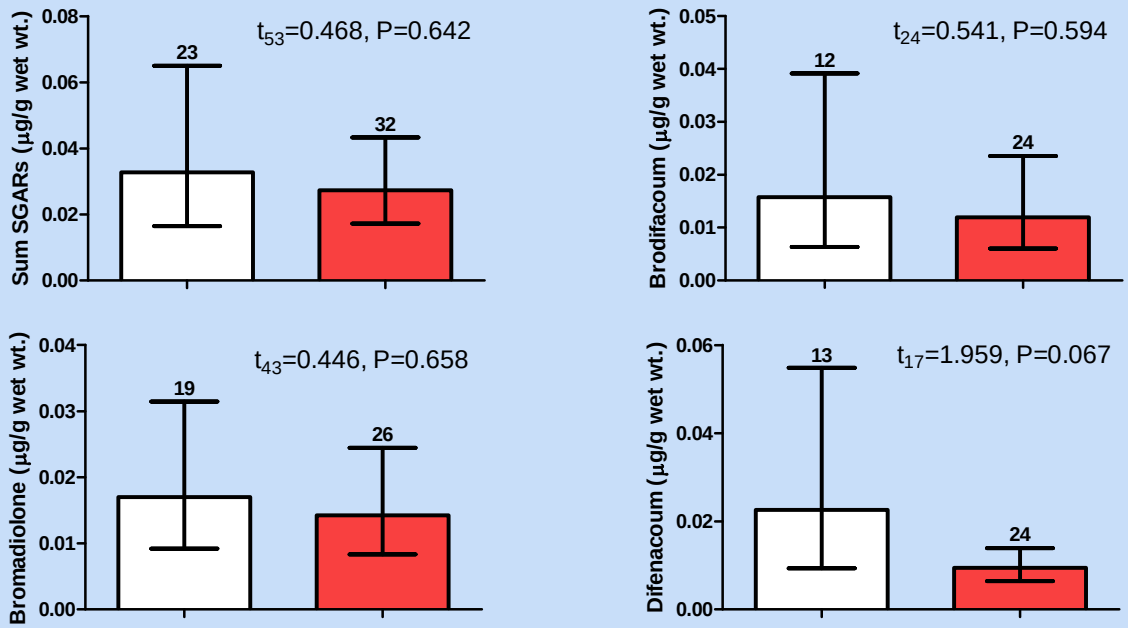

Figure 3.1 Geometric mean (+95% CI) for sum SGAR, brodifacoum, bromadiolone and difenacoum concentrations in livers barn owls found dead in the Berkshire/Hampshire resistance area (white bars) and other areas (red bars). Non-detected values were not included in the analysis. Sample size indicated above each column, and students t-test staistic and P-value indicated for each compound. Difenacoum tested using Welch's correction.

There were detectable residues of one or more SGARs in all of the kestrels livers analysed and so only residue magnitude was compared for this species. None of sum SGAR, brodifacoum, bromadiolone or difenacoum concentrations differed significantly between birds from the Berkshire/Hampshire resistance area and other counties (Figure 3.2).

Overall, we found no evidence from samples analysed as part of the 2011 monitoring programme to indicate that the provision of extra samples by the HCT from resistance areas would significantly increase our overall detection of SGARs in barn owls and kestrels. However, the sample size examined was relatively small and it would be valuable in the future to conduct an analysis of the potential impact of resistance on residue prevalence and magnitude for birds collected over a longer time-scale and incorporating all counties where resistance to SGARs in rats has been documented.

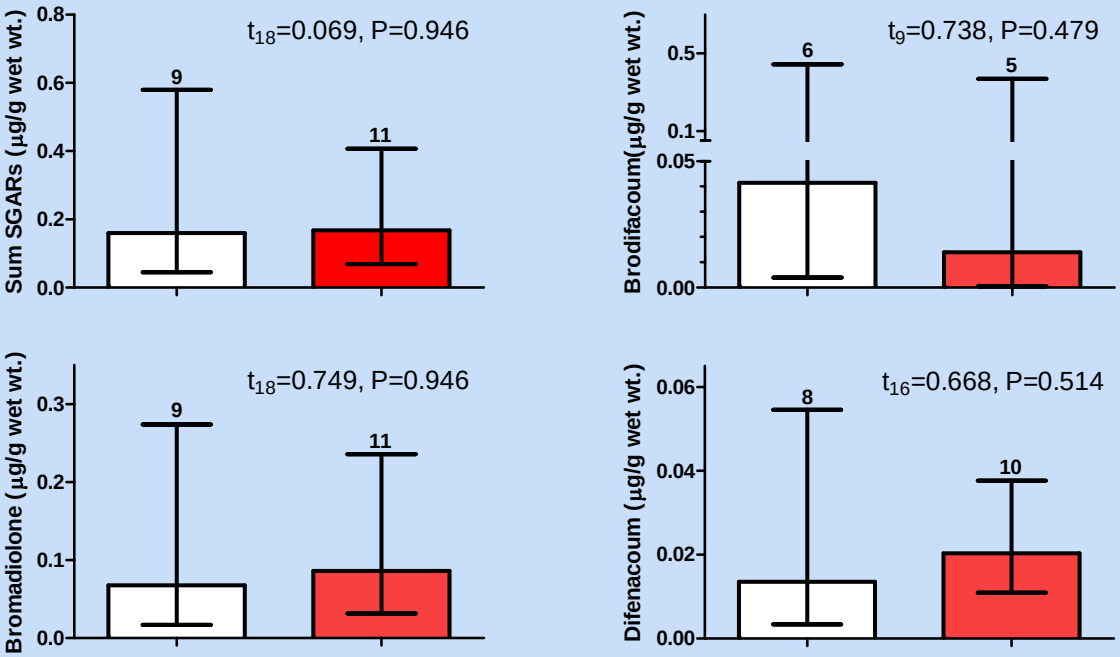

Figure 3.2 Geometric mean (+95% CI) for sum SGAR, brodifacoum, bromadiolone and difenacoum concentrations in livers kestrels found dead in the Berkshire/Hampshire resistance area (white bars) and other areas (red bars). Non-detected values were not included in the analysis. Sample size indicated above each column, and Students t-test staistic and P-value indicated for each compound.

3.3 Barn Owls collected in 2011

Fifty-eight barn owls that had died in 2011 were analysed. Forty-nine (84% of the sample) contained detectable liver concentrations of one or more SGAR (Table 3.1).

As in previous years, the majority of exposure was to bromadiolone and difenacoum (79% of barn owls analysed). Brodifacoum was detected less frequently (33% of birds analysed; Table 3.1). Flocoumafen was detected in four of the 58 owls analysed. Overall, multiple SGAR residues were detected in 52% of the livers analysed.

The potentially lethal range for SGAR residues in barn owls has variously been described as > 0.1 µg/g wet wt (Newton et al. 1998) and > 0.2 µg/g wet wt (Newton et al. 1999) and is so classed on the basis of two sets of observations. The first was that owls diagnosed at post-mortem of having died from rodenticide poisoning (because they had characteristic signs of haemorrhaging from such organs as the heart, lungs, liver, brain and/or subcutaneous areas) almost all had liver residues >0.1 µg/g wet wt. The second was that owls that had been experimentally poisoned had residues of the range 0.2-1.72 µg/g wet wt (Newton et al. 1999). This range has been used in this report as an indicator of concern that SGARs may have an adverse effect on individuals although recent analysis (Thomas et al. 2011) suggests that effects on some individuals may be

associated with residues <0.1 µg/g wet wt.

Most owls had concentrations below the potentially lethal range but ten (17.2% of the sample) had residues (summed values for all four SGARs) greater than 0.1 µg/g wet wt; six of these exceeded 0.2 µg/g wet wt. The maximum sum SGAR liver concentration among these ten owls was 0.337 µg/g wet wt (which consisted entirely of brodifacoum) that was detected in a bird that had been diagnosed as having died due to starvation and in which there was no macroscopic evidence of haemorrhaging characteristic of anticoagulant poisoning. Similarly the remaining nine birds with liver residues in excess of 0.1 µg/g wet wt. did not show any signs of haemorrhaging other than those likely to have been caused by physical trauma. One bird showed signs of haemorrhaging without associated fractures nor any circumstantial evidence to suggest physical trauma. However, the sum SGAR concentration in this bird was 0.060 µg/g wet wt. and so evidence for rodenticide poisoning was inconclusive in this case.

3.4 Red kites collected in 2011

Liver samples from 18 red kites that had died in 2011 were analysed. Seventeen (94%) of the birds contained detectable concentrations of anticoagulant rodenticides (Table 3.1) with 16 birds having been exposed to more than one SGAR.

Interpretation based on such a small sample has to be limited. However, as with barn owls and kestrels, the most prevalent rodenticide detected in red kite livers was difenacoum and bromadiolone (Table 3.1). As in birds that died between 2007 and to 2010, a large proportion (78%) of red kite livers also contained brodifacoum. This was significantly higher than the proportion of owls in which brodifacoum was detected (Fisher's exact test, P=0.001).

The sum SGAR liver concentrations in red kites were generally higher than those observed in barn owls. Concentrations in livers with detectable SGAR residues ranged between 0.008 and 1.02 µg/g wet wt with a median concentration of 0.239 µg/g wet, 8-fold higher than in barn owls. Post mortem examinations by the Institute of Zoology indicated that six of the kites had internal hemorrhaging that was not associated with detectable trauma and so may have been due to anticoagulant poisoning. These six birds included the individual in the whole sample that had the highest sum SGAR liver concentration (sum SGAR of 1.02 µg/g wet wt., consisting of 0.967 µg/g wet wt. of brodifacoum and trace amounts of difenacoum) and another bird with liver residues of 0.200 and 0.310 µg/g wet wt. of difenacoum and brodifacoum, respectively. Thus it seems likely that SGAR poisoning was the cause of death in both birds. The sum SGAR liver concentrations in the remaining four kites ranged between 0.015 and 0.278 µg/g wet wt. Similar residues (up to 0.434 µg/g wet wt.) were detected in birds thought to have died due to other causes. Therefore the contribution, if any, of rodenticides to the death of these four individuals is equivocal.

3.5 Kestrels collected in 2011

All of the 20 kestrels analysed had detectable liver concentrations of one or more SGAR. The most prevalent compounds detected were bromadiolone and difenacoum (100% of birds analysed, Table 3.1). Bromadiolone was found in every bird while difenacoum co-occurred with bromadiolone in 18 of the birds. Flocoumafen was not detected in any of the kestrels tested while brodifacoum was found in 11 birds. As these figures suggest, the co-occurrence of multiple residues was high with more than one rodenticide detected in all but one of the birds.

Concentrations in livers with detectable SGAR residues ranged between 0.010 and 1.77 µg/g wet wt with a median concentration of 0.215 µg/g wet wt., which was significantly higher and than that measured in barn owls (student t-test, T_{32}=4.579, P<0.0001).

There was no macroscopic evidence at post-mortem examination of haemorrhaging characteristic of anticoagulant poisoning in any of the kestrels examined. Thus, despite the presence of large liver SGAR concentrations in some individuals, there was no clear evidence that SGARs were the cause of death.

4. Long term trends in liver SGAR concentrations in barn owls & kestrels

4.1 Long term time trends in the prevalence of liver SGAR residues in barn owls

A common limit of quantification (LoQ) was applied to the long-term dataset for SGARs. This was 0.025 µg/g wet wt. and was applied to each of the four compounds as described in Walker et al. (2010). Any detected values below this 0.025 µg/g LoQ were re-assigned as non-detected values for the purposes of time trend analysis and the percentage occurrence of SGARs were then recalculated for each year - these are termed "adjusted % detected" values. The use of adjusted % detected values under-estimates the true occurrence of liver SGAR residues for compounds and years where the limit of quantification was substantially lower, but it eliminates biases in the long-term data due to improvement in the sensitivity of analysis over time. The adjusted % detected values therefore provide a measure of temporal changes but do not necessarily indicate the actual scale of exposure. Adoption of a common limit of detection for different SGARs eliminates detection biases when comparing % detection values for different rodenticides.

All residues reported in this section have been determined using solvent-matched standards.

The adjusted % detected values for one or more SGAR in barn owl livers has increased from zero in 1983 (based on a small sample size of 4 livers), when monitoring began, to a maximum of 49% in 2008 (Figure 4.1). This long-term change primarily reflects an increase over time in the proportion of birds with detectable residues of difenacoum and/or bromadiolone; the proportion of birds that have multiple compounds in their livers has also increased (Figure 4.1). Brodifacoum, and to a lesser extent flocoumafen, have been detected in barn owls during the course of the monitoring period but there is no evidence of any significant progressive change in exposure over time (Figure 4.1).

The adjusted % detected value for birds in 2011 was 26% and is the second lowest value reported since 2005 (Figure 4.1). However there is considerable inter-year variation over the last 10 years (Coefficient of Variation: 27%) and further monitoring is required to determine if there is any long-term decline in exposure.

In terms of potential adverse effects, the 2011 results are consistent with those previously reported (Walker et al., 2010) in that the proportion of barn owls with liver concentrations above 0.1 µg/g wet wt. has risen during the course of monitoring over time (r_s=0.431, P<0.05) but there has been no significant change in the proportion of birds with liver residues > 0.2 µg/g wet wt. (Figure 4.1). Overall, the average proportion of owls analysed that had SGAR residues > 0.2 µg/g wet wt is 4.3%, but the cause of death in many of these birds has not been attributed to anticoagulant rodenticides.

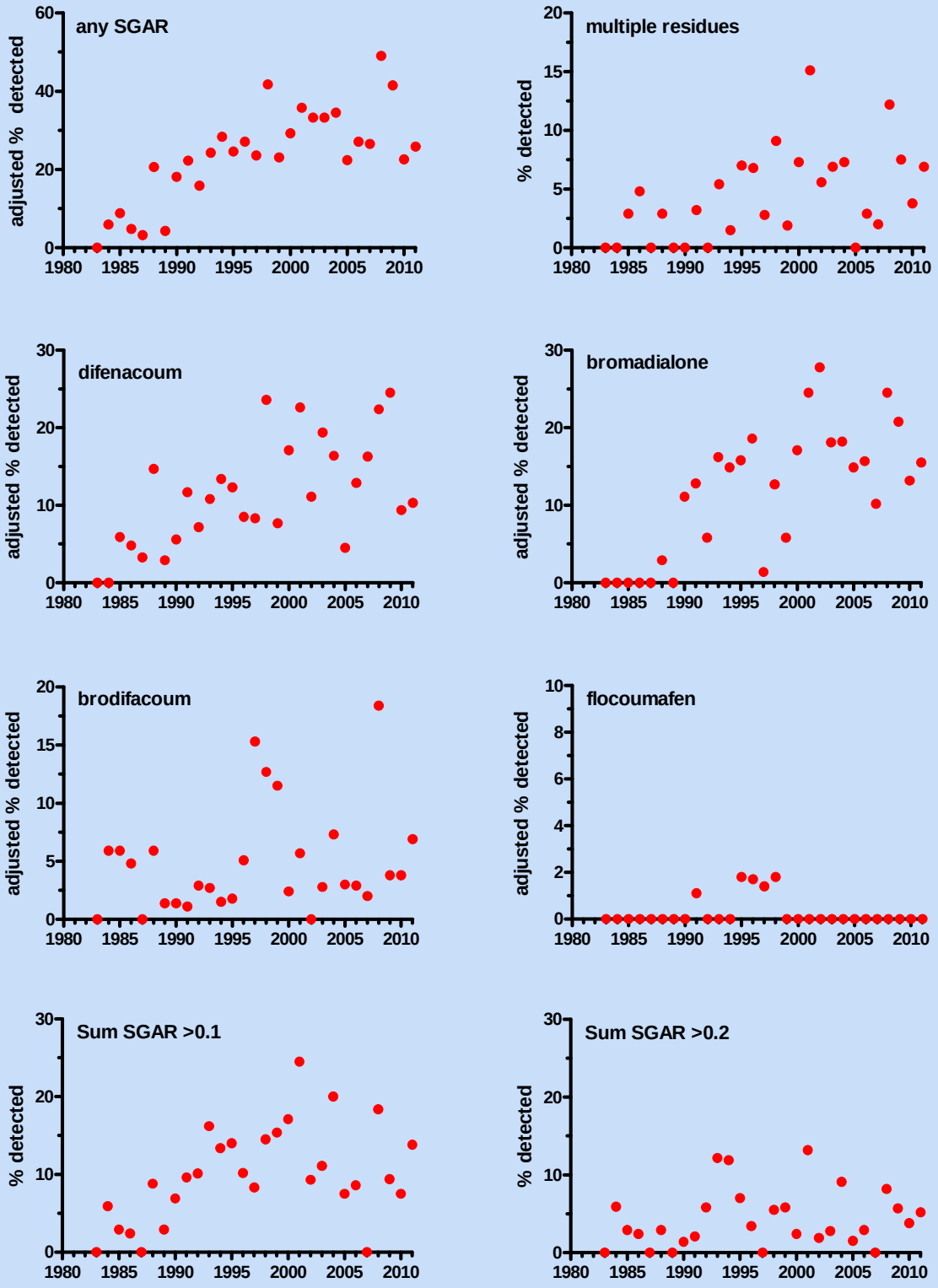

Figure 4.1. Variation over time in adjusted % of barn owls with: detectable (≥0.025 μg/g wet wt.) concentrations of individual or summed SGARs, multiple residues in the liver, or liver concentrations >0.1 μg/g wet wt. or 0.2 μg/g wet wt.

4.2 Long term regional analysis of the prevalence of liver SGAR residues in barn owls

The scale of exposure of barns owls in England, Scotland and Wales has been compared using the data available pooled for the years 1990-2011 to provide sufficient sample size for analysis. The adjusted % of owls with detected residues of any SGAR was approximately two-fold higher in England than in either Scotland or Wales and the difference between the countries was significantly different (Table 4.1). At a smaller scale, there were also significant differences among regional areas of Great Britain as defined by Defra (χ^2=33.6, P<0.0001; Figure 4.2). Similarly to last year's report (Walker *et al.*, 2012), if Scotland and Wales were excluded from the analysis then there was still a significant difference between the English regions (χ^2=13.0, P=0.011).

Table 4.1. Number (n) of owls and the number as a percentage of all birds tested (%) from England, Scotland and Wales between 1990 and 2011 that had detectable liver SGAR concentrations ≥ 0.025 µg/g wet wt. (common limit of quantification applied to all compounds and samples).

	number (% of whole sample tested) of owls with detected residues						
	England (n=1114)		**Scotland (n=116)**		**Wales (n=112)**		**Chi Squared statistic[1]**
Bromadiolone	183	(16%)	13	(11%)	6	(5.4%)	11.2 (**)
Difenacoum	157	(14%)	6	(5.2%)	10	(8.9%)	9.15 (*)
Flocoumafen	2	(0.2%)	1	(0.9%)	0	(0%)	-
Brodifacoum	62	(5.6%)	4	(3.4%)	1	(0.9%)	5.32 (ns)
Any SGAR	335	(30%)	20	(17%)	16	(14%)	19.6 (***)
Multiple SGAR	64	(5.7%)	4	(3.4%)	1	(0.9%)	5.66 (ns)

[1] ns = not significant, * = P<0.05, *** = P<0.001; unable to test flocoumafen

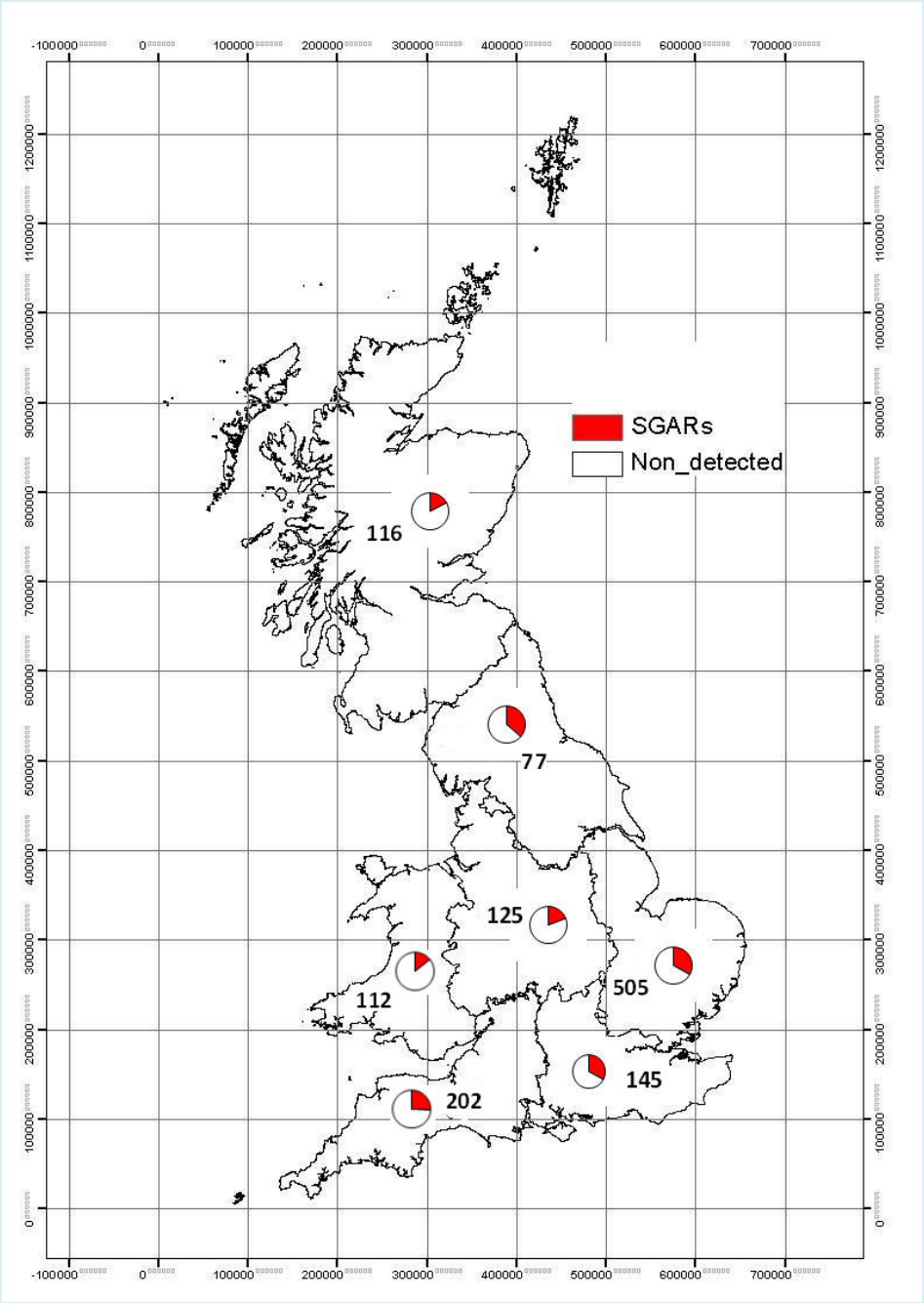

Figure 4.2 Proportion of barn owls (*Tyto alba*) that have detectable residues greater than 0.025µg/g wet wt. in their liver for each Defra region (Dawson and Garthwaite 2004). Number in next to each pie chart indicates number of samples. Grid lines are 100km squares.

4.3 Long term analysis of variation in the prevalence and magnitude of liver SGAR residues with age class and sex

Persistent bioaccumulative contaminants tend to occur at higher residues in older birds and concentrations can also differ between the sexes due to either differing diets and/or contaminant transfer into eggs by females. As part of the post mortem examination of the birds received by the PBMS, the age and sex of the bird is determined. Therefore, to test whether liver SGAR residues vary with age and/or sex in barn owls, we conducted a logistical regression analysis and a general linear model analysis to compare likely occurrence of detectable SGAR residues and residue magnitude, respectively, in barn owls that died between 2000 and 2011.

Juveniles (defined as birds that hatched in the current or previous calendar year) were generally less likely to have detectable SGAR residues or to have multiple liver SGAR residues than adults (Table 4.2). There was no significant difference in the likelihood of detectable SGAR residues occurring in males or females (Table 4.2). The magnitude of liver SGAR residues did not significantly differ with sex or age class for sum SGAR concentrations (Figure 4.3 and Table 4.3) or for individual SGARs (brodifacoum: $F_{1,28}<0.25$; bromadiolone $F_{1,109}<2.43$; difenacoum $F_{1,86}<0.12$; $P>0.05$ in all cases).

Table 4.2 Summary of logistical regression analysis to determine predictive factors in relation to likelihood that a barn owls that died between 2000 and 2011 had detectable residues of one or more SGARs in their liver ≥ 0.025 µg/g wet wt. (common limit of quantification applied to all compounds and samples).

Compound	Predictor	Z	P-value	Odds ratio	lower 95% CI	upper 95% CI
Any	Juvenile	-3.38	0.001	0.53	0.37	0.77
	Male	0.81	0.416	1.15	0.82	1.62
Brodifacoum	Juvenile	-3.88	<0.001	0.23	0.11	0.48
	Male	0.11	0.911	1.04	0.5	2.18
Difenacoum	Juvenile	-2.56	0.010	0.55	0.35	0.87
	Male	-0.4	0.689	0.91	0.59	1.42
Bromadiolone	Juvenile	-1.1	0.271	0.78	0.51	1.21
	Male	0.7	0.486	1.15	0.77	1.73
Multiple Residues	Juvenile	-3.34	0.001	0.32	0.16	0.62
	Male	-0.81	0.421	0.76	0.38	1.49

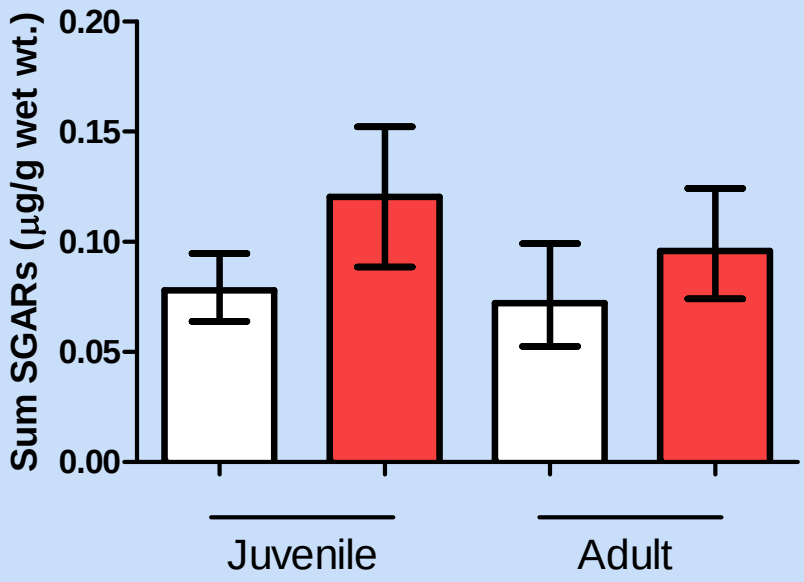

Figure 4.3 Geometric mean (±95%CI) sum SGAR residues in livers of male (white bars) and female (red bars) barn owls found dead in the UK between 2000 and 2011. A common limit of detection of 0.025 µg/g wet wt. was applied to all compounds abnd samples. Non-detected samples are excluded from this analysis.

Table 4.3 Summary of general linear model analysis of sum SGAR residue magnitude in barn owl livers. Only livers with residues present are included in analysis and a common limit of quantification of 0.025 µg/g wet wt. applied to all compounds and samples.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Age	1	0.0092	0.0006	0.0006	0.01	0.943
Sex	1	0.3004	0.3004	0.3004	2.4	0.123
Error	186	23.24	23.24	0.1249		
Total	188	23.55				

These results suggest that male and female barn owls are equally likely to accumulate SGAR residues. However, juvenile birds are less likely to have detectable liver SGAR residues than adults. This may reflect age-related differences in foraging and/or be the result of younger birds having had a shorter period than adults in which to encounter and feed on contaminated rodents. However, there was no age-related difference in the magnitude of liver concentrations in birds with detected residues. This may indicate that a key determinant of residue magnitude is likely to be how recently and frequently birds have fed on contaminated rodents. Our data are not consistent with the idea that residues may be progressively accumulated over the lifetime of birds; if this were the case, it would be expected that, on average, concentrations would be significantly greater in adults than juveniles.

4.2 Long term trends in kestrels

The same common limit of quantification used for barn owls was applied to the whole dataset for kestrels to facilitate inter-year comparisons. SGARs have been monitored in kestrels since 2001, with additional data available for a further 36 birds that had died between 1997 and 2000. However fewer kestrels are received each year than barn owls and so data have been collated into two-three year blocks.

The adjusted % of birds with any detectable SGAR liver residue has varied between 45% and 68%, with no significant difference between years ($F_{1,4} \leq 4.920$, $P \geq 0.091$; Figure 4.4) and no. apparent progressive increase or decrease over blocks of years Most exposure is to difenacoum and bromadiolone; between 33% and 64% of kestrels had bromadiolone or difenacoum concentrations > 0.025 µg/g wet wt in their livers. Flocoumafen has not been detected in kestrels during this monitoring period.

None of the birds had macroscopic post-mortem signs of hemorrhaging without accompanying signs of external trauma.

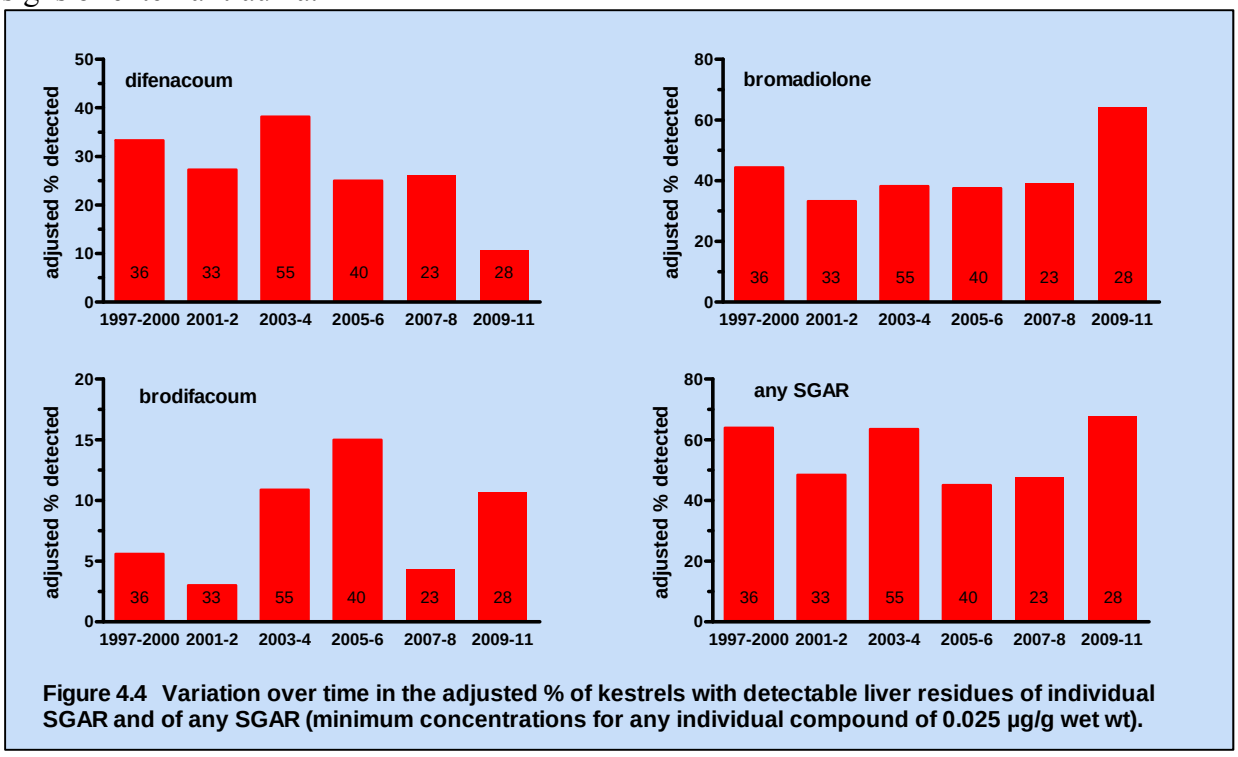

Figure 4.4 Variation over time in the adjusted % of kestrels with detectable liver residues of individual SGAR and of any SGAR (minimum concentrations for any individual compound of 0.025 µg/g wet wt).

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6. References

Buckle, A. & Prescott, C. 2012. The Current Status of Anticoagulant Resistance in Rats and Mice in the UK. Rodenticide Resistance Action Group.

Carter, I. & Grice, P. (2002) Report No. 451: The Red Kite Reintroduction Programme in England. *English Nature Research Reports*, pp. 64. English Nature, Peterborough.

Dawson, A. and D. G. Garthwaite (2004). Pesticide Usage Survey Report 185: Rodenticide Usage by Local Authorities in Great Britain 2001. London, DEFRA Publications.

Dowding, C. V., Shore, R. F., Worgan, A., Baker, P. J. & Harris, S. (2010) Accumulation of anticoagulant rodenticides in a non-target insectivore, the European hedgehog (*Erinaceus europaeus*). *Environmental Pollution,* **158,** 161-166.

McDonald, R. A., Harris, S., Turnbull, G., Brown, P. & Fletcher, M. (1998) Anticoagulant rodenticides in stoats (*Mustela erminea* L.) and weasels (*M. nivalis* L.) in England. *Environmental Pollution,* **103,** 17-23.

Newton, I., Dale, L., Finnie, J. K., Freestone, P., Wright, J., Wyatt, C. & Wyllie, I. (1998) Wildlife and Pollution: 1997/98. Annual Report. JNCC Report No. 285. pp. 61. Joint Nature Conservation Committee, Peterborough, UK.

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. Filander Verlag, Fürth.

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

Shore, R. F., Birks, J. D. S., Afsar, A., Wienburg, C. L. & Kitchener, A. C. (2003a) Spatial and temporal analysis of second-generation rodenticide residues in polecats (*Mustela putorius*) from throughout their range in Britain, 1992-1999. *Environmental Pollution,* **122,** 183-193.

Shore, R. F., Fletcher, M. R. & Walker, L. A. (2003b) Agricultural pesticides and mammals in Britain. *Conservation and conflict: mammals and farming in Britain. Linnean Society Occasional Publication No. 4* (eds F. H. Tattersall & W. J. Manley), pp. 37-50. The Linnean Society, London.

Shore, R. F., Malcolm, H. M., Horne, J. A., Turner, S. & Wienburg, C. L. (2001) Rodenticide residues in the kestrel *Falco tinnunculus*. pp. 17. Centre for Ecology & Hydrology Contract Report to English Nature, English Nature, Peterborough, UK.

Shore, R. F., Malcolm, H. M., McLennan, D., Turk, A., Walker, L. A., Wienburg, C. L. & Burn, A. J. (2006) Did Foot and Mouth Disease control operations affect rodenticide exposure in raptors? *Journal of Wildlife Management,* **70,** 588-593.

Thomas, P. J., Mineau, P., Shore, R. F., Champoux, L., Martin, P. A., Wilson, L. K., Fitzgerald, G. & Elliott, J. E. (2011) Second generation anticoagulant rodenticides in predatory birds: Probabilistic characterisation of toxic liver concentrations and implications for predatory bird populations in Canada. *Environment International,* **37,** 914-920.

Walker, L. A., Lawlor, A. J., Llewellyn, N., Peréira, M. G., Potter, E., Townsend, J., Turk, A. & Shore, R. F. (2010) The Predatory Bird Monitoring Scheme (PBMS) Report 2006-7. A contract report from the Centre for Ecology & Hydrology to Natural England, Centre for Ecology & Hydrology, Lancaster, 65pp.

Walker, L.A., Lawlor, A.J., Llewellyn, N.R., Pereira M.G., Potter, E.D., Molenaar, F.M., Sainsbury, A.W. & Shore, R.F. (2010). Anticoagulant rodenticides in predatory birds 2007 & 2008: a Predatory Bird Monitoring Scheme (PBMS) report. Centre for Ecology & Hydrology, Lancaster, UK. 19pp.

Walker, L. A., Shore, R. F., Turk, A., Pereira, M. G. & Best, J. (2008a) The Predatory Bird Monitoring Scheme: Identifying chemical risks to top predators in Britain. *Ambio,* **37,** 466-471.

Walker, L. A., Turk, A., Long, S. M., Wienburg, C. L., Best, J. & Shore, R. F. (2008b) Second generation anticoagulant rodenticides in tawny owls (*Strix aluco*) from Great Britain. *Science of the Total Environment,* **392,** 93-98.

Walker, L. A., Llewellyn, N. R., Pereira, M. G., Potter, E., Sainsbury, A. W. & Shore, R. F. (2010) Anticoagulant rodenticides in predatory birds 2009: a Predatory Bird Monitoring Scheme (PBMS) report. Centre for Ecology & Hydrology, Lancaster, UK. . pp. 17.

Walker, L. A., Llewellyn, N. R., Pereira, M. G., Potter, E., Sainsbury, A. W. & Shore, R. F. 2012. Anticoagulant rodenticides in predatory birds 2010: a Predatory Bird Monitoring Scheme (PBMS) report. Centre for Ecology & Hydrology, Lancaster, UK. .