Anticoagulant rodenticides in predatory birds 2009: 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*), and red kites (*Milvus milvus*) that were found dead in 2009 and presents long term trend analysis for barn owls.

Since 2006, anticoagulant rodenticide concentrations have been quantified using the more sensitive Liquid Chromatography – Mass Spectrometry (LC-MS) method. This has resulted in lower concentrations of these compounds being detected than was previously possible. Consequently, for samples from 2006 onwards, the proportion of birds in which anticoagulant rodenticides have been detected has increased compared to previous years.

SGARs were detected in 89% of barn owls and the most prevalent compounds were difenacoum and bromadiolone. The majority of the residues were low and not diagnosed as directly causing mortality. Only five red kites were received by the scheme in 2009. Most of the red kites (4 o/5 birds) had detectable liver SGAR concentrations, again mainly difenacoum and bromadiolone although brodifacoum was also detected in over half the birds. Two of the five red kites analysed showed signs of haemorrhaging thought possibly to be associated with rodenticide poisoning.

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 (<0.025µg/g wet weight), which are now easily detectable, are not included in the time trend analysis. The proportion of owls with detectable SGAR residues was found to be two-fold higher in England than in either Scotland or Wales. 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 but this was approximately twice that for the previous three years. The value for 2009 was lower than 2008 but remains one of the highest recorded since monitoring began.

Continued monitoring is required to determine whether the high detection rate for SGARs in barn owls in 2008 and 2009 will change. Although our data for red kites in 2009 is limited, it is consistent with a high proportion of red kites being exposed to SGARs and some dying as a result. This species remains at particular risk from anticoagulant rodenticides.

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 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 Natural England's reintroduction programme (Carter and Grice, 2002). SGAR-induced deaths of kites have been detected by the Wildlife Incident Investigation Scheme. 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 recent collaboration with the Institute of Zoology has meant that the number of liver samples available for analysis has now usually increased.

This report describes the results of PBMS monitoring of barn owls and red kites submitted to the PBMS in 2009 (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. Summary of barn owls and red kites submitted to the PBMS in 2009

		Year	
Species		**Received**	**Analysed**
barn owl	*Tyto alba*	100	52
red kite	*Milvus milvus*	6	5
Total		106	57

It is not always possible to take a liver sample from a bird and so the number of samples analysed may differ from the values in this table

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

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. Tissues from 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. This is because the analytical method used by the PBMS changed to a Liquid Chromatography-Mass Spectrometry (LC-MS) approach that facilitated the simultaneous measurement of all the compounds. It is also a more sensitive analytical method and so can detect lower concentrations of these compounds than was possible previously. This has implications for interpretation of long-term monitoring data (Walker et al., 2010).

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.

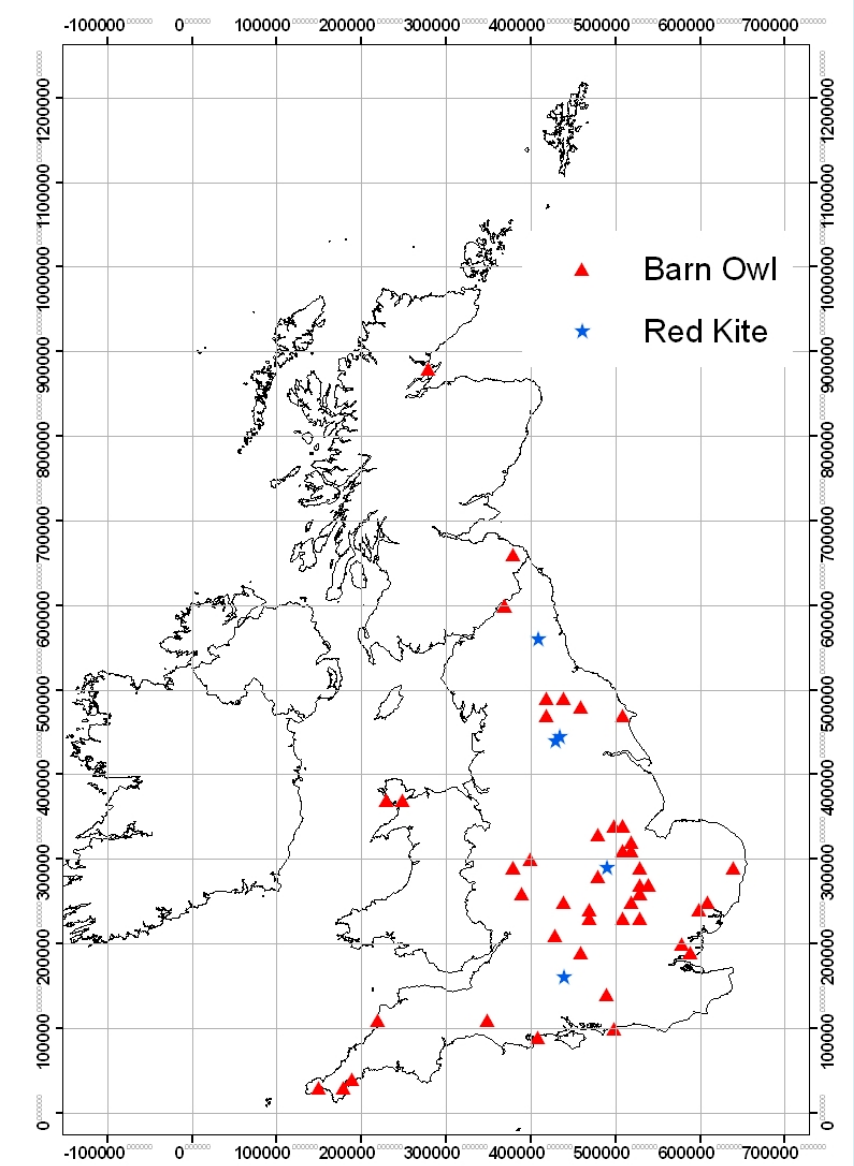

Figure 1.1 Location of birds found dead in 2009 that have been analysed for hepatic anticoagulant rodenticide concentrations. Grid lines are 100km squares.

2. Anticoagulant rodenticide concentrations in birds submitted to the PBMS in 2009

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 2.1. Results for individual birds are given in a downloadable addendum to this report (http://pbms.ceh.ac.uk/docs/AnnualReports/PBMS_Rodenticides_2009_Addendum.pdf).

Table 2.1. Number of birds with detectable liver concentrations of anticoagulant rodenticides (No/) and the percentage this comprised of all birds analysed (%). Total number of barn owls and red kites analysed was 52 and 5, respectively

	barn owls		red kites[1]
	No/	%	No/
2nd Generation (SGAR)			
bromadiolone	34	65.4	1
difenacoum	27	51.9	4
flocoumafen	1	2	1
brodifacoum	23	44.2	3
Any SGAR	46	89	4
Multiple SGARs	28	54	4
1st Generation (FGAR)			
warfarin	0	0	0
coumatetralyl	0	0	0
chlorophacinone	0	0	0
diphacinone	0	0	0
Any FGAR	0	0	0
Multiple FGARs	0	0	0
Any rodenticide	46	89	4
Multiple rodenticides	28	54	4

[1] sample number too low to present % data for red kites

2.1 Barn Owls collected in 2009

Fifty-two barn owls were analysed by PBMS in 2009; all had died in those years. Forty-six (89% of the sample) contained detectable liver concentrations of one or more SGAR (Table 2.1) which is similar to those values reported for 2007 and 2008.

As in previous years, the majority of exposure was to bromadiolone and difenacoum (79% of barn owls analysed). Brodifacoum was detected less frequently (Table 2.1) and flocoumafen was found in only one owl. None of the other anticoagulant rodenticides were detected any of the 52 owls analysed. Overall, multiple SGAR residues were detected in approximately a quarter 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).

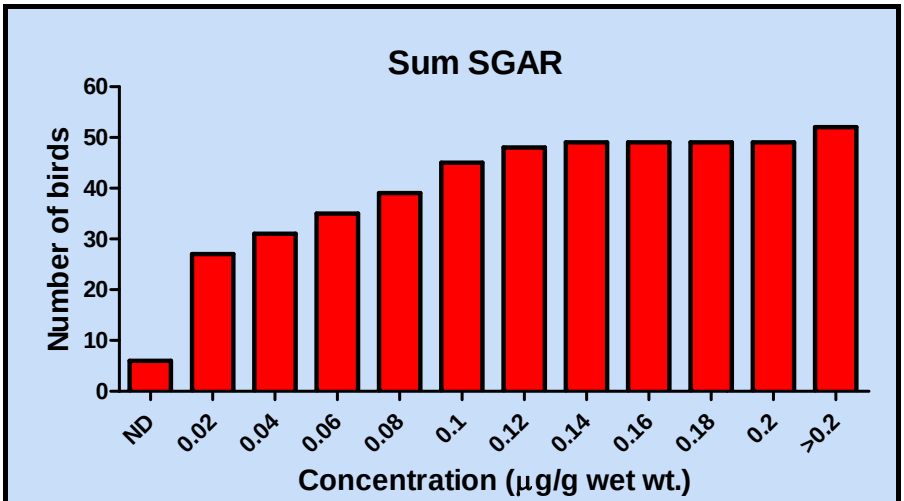

Fig. 2.1 Cumulative frequency for Sum SGAR residues in the livers of barn owls found dead in 2009.

The median summed rodenticide concentration in barn owls with detected residues was 0.027 µg/g wet wt. Most owls had concentrations below the potentially lethal range (Figure 2.1) but seven (13% of the sample) had residues (summed values for all four SGARs) greater than 0.1 µg/g wet wt; three of these exceeded 0.2 µg/g wet wt. The maximum liver concentration amongst these seven owls was 0.585 µg/g wet wt (all of which was brodifacoum) which was

detected in a bird that had been diagnosed as a possible rodenticide poisoning as the post mortem examination indicated haemorrhaging at one or more sites apparently unrelated to trauma. The second highest liver residue was 0.518 ug/g wet wt (0.511 µg/g wet wt. brodifacoum, 0.007 µg/g wet wt. difenacoum) in a bird diagnosed of dying from unknown causes; there was evidence of blood on the breast feathers of this bird but no internal haemorrhaging was apparent. Another bird had sum SGAR liver residues of 0.431 µg/g wet wt (0.330 µg/g wet wt. bromadiolone, 0.094 µg/g wet wt. difenacoum & 0.007 µg/g wet wt. brodifacoum) but haemorrhaging in its skull and lungs were consistent with a collision. Although all the remaining four owls had liver residues between 1 and 1.27 µg/g wet wt. any hemorrhaging present was associated with physical trauma.

2.3 Red kites collected in 2009

Liver samples from five red kites that had died in 2009 were analysed. Four of the five birds contained detectable concentrations of anticoagulant rodenticides (Table 2.1) with all four 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 (Table 2.1). Similarly to 2007 & 2008, a large proportion (3 out of 5 samples) of red kite livers also contained brodifacoum, although unlike in the previous two years this was not significantly higher than the proportion of owls in which brodifacoum was detected.

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 0.267 and 0.659 µg/g wet wt with a median concentration of 0.433 µg/g wet, which was 10-fold higher than in barn owls. The maximum liver concentration was 0.659 µg/g wet wt. (0.487 µg/g wet wt. brodifacoum and 0.174 µg/g wet wt. difenacoum).

Post mortem examinations by the Institute of Zoology indicated that two of the five red kites received showed internal haemorrhages not associated with detectable trauma and therefore consistent with anticoagulant poisoning. The sum SGAR liver concentration in these two birds were 0.270 (including 0.220 µg/g wet wt. brodifacoum) and 0.355 (including 0.290 µg/g wet wt. difenacoum) µg/g wet wt., although similar residues (up to 0.659 µg/g wet wt.) were detected in birds thought to have died due to other causes.

3. Long term trends in liver SGAR concentrations 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 wet wt. LoQ were re-assigned as non-detected values 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.

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 3.1). 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 3.1). 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 3.1).

The adjusted % detected value for birds in 2009 was 40% (Figure 3.1). This was lower than that reported in the previous year (49%) although still higher than any other year in the last ten years of monitoring . Further monitoring is required to determine whether the high value for barn owls in 2008 & 2009 reflects a renewed increased exposure compared to the previous three years.

In terms of potential adverse effects, the 2009 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 but there has been no significant change in the proportion of birds with liver residues > 0.2 µg/g wet wt. (Figure 3.1). Overall, the average proportion of owls analysed that had SGAR residues > 0.2 µg/g wet

wt is 4.5%, but the cause of death in many of these birds has not been attributed to anticoagulant rodenticides.

Table 3.1. Number (n) of owls and the number as a percentage of all birds tested (%) from England, Scotland and Wales between 1990 and 2009 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=1172)		**Scotland (n=122)**		**Wales (n=120)**		**Chi Squared statistic[1]**
Bromadiolone	164	(14%)	11	(9.0%)	6	(5.0%)	9.60 (**)
Difenacoum	150	(13%)	6	(4.9%)	11	(9.2%)	7.47 (*)
Flocoumafen	2	(0.2%)	1	(0.8%)	0	(0%)	-
Brodifacoum	61	(5.2%)	4	(3.3%)	2	(1.7%)	3.65 (ns)
Any SGAR	315	(27%)	18	(15%)	17	(14%)	16.6 (***)
Multiple SGAR	57	(4.9%)	4	(3.3%)	2	(1.7%)	3.05 (ns)

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

The scale of exposure of barns owls in England, Scotland and Wales has also been compared using the data available pooled for the years 1983-2009 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 3.1). At a smaller scale there were also significant differences among regional areas of Great Britain as defined by Defra (χ^2=25.3,P<0.001; Figure 3.2). However, if Scotland and Wales were excluded from the analysis then there was no significant difference between the English regions (χ^2=6.51, P=0.164).

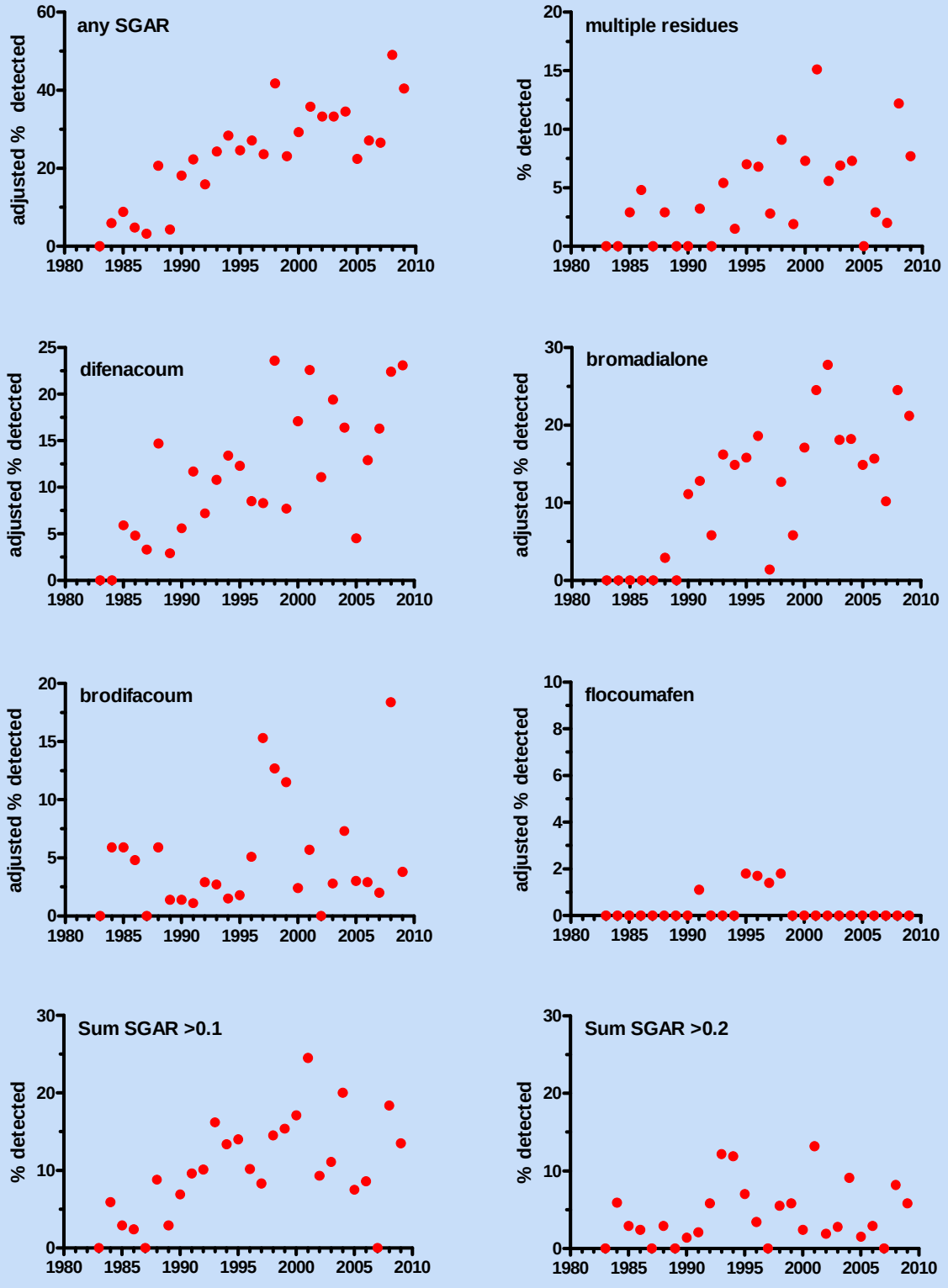

Figure 3.1. Variation over time in adjusted % of barn owls with: detectable (≥0.025 µg/g wet wt.) concentrations of individual or summed SGARs, multiple residues in the liver, or liver concentrations >0.1 µg/g wet wt. or 0.2 µg/g wet wt.

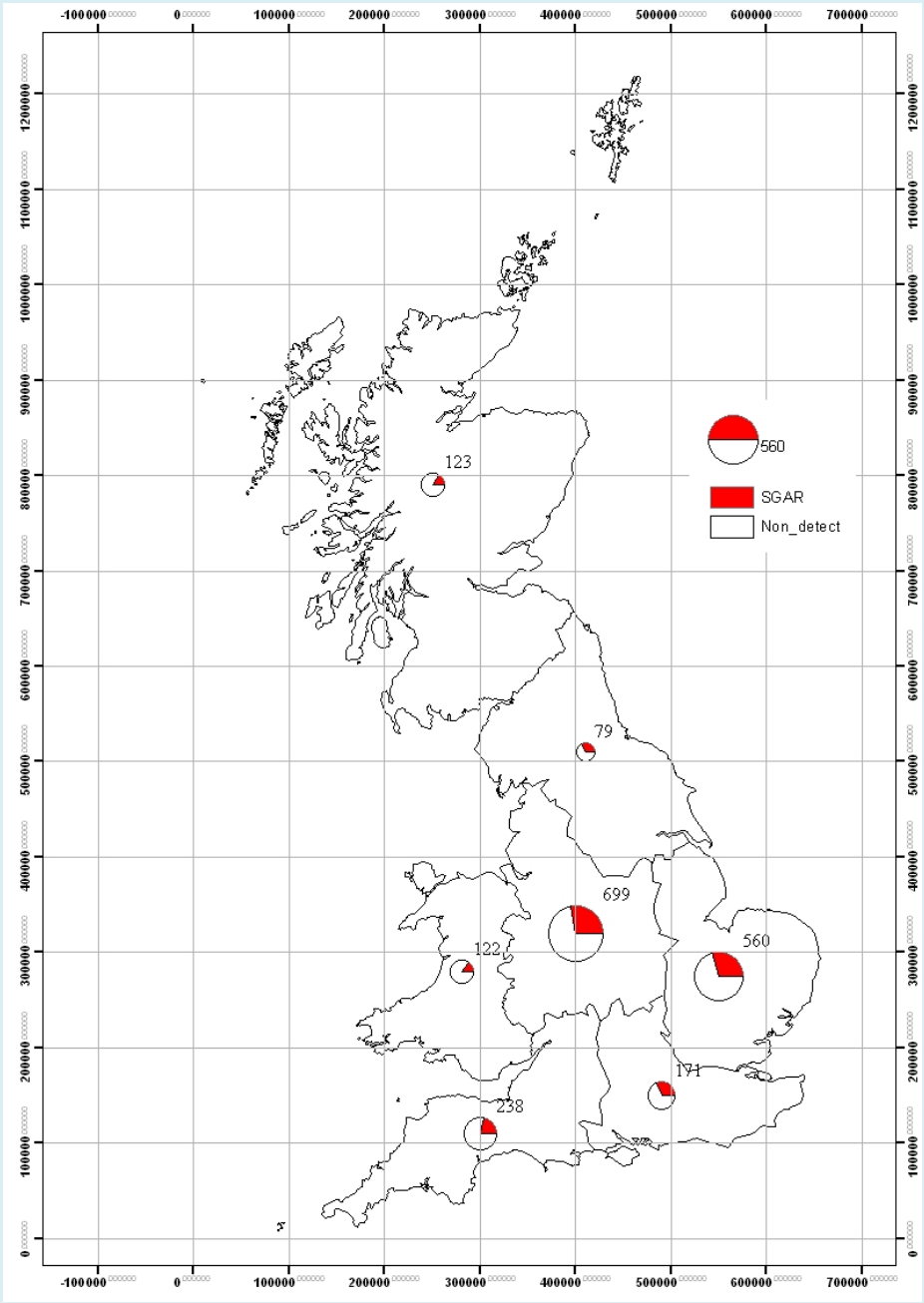

Figure 3.2 Proportion of barn owls (*Tyto alba*) that have detectable residues greater than 0.025µg/g wet wt. in their liver for each Defra region. Number in next to each pie chart indicates number of samples. Grid lines are 100km squares.

4. Acknowledgements

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