Anticoagulant rodenticides in predatory birds 2007 & 2008: a Predatory Bird Monitoring Scheme (PBMS) report

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Contents

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*), kestrels (*Falco tinnunculus*) and red kites (*Milvus milvus*) that were found dead in 2007 and 2008 and presents long term trend analysis for barn owls and kestrels.

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 81% of barn owls and 68% kestrels and the most prevalent compounds were difenacoum and bromadiolone. The majority of the residues were low and not diagnosed as directly causing mortality. Most of the red kites (91%) had detectable liver SGAR concentrations, again mainly difenacoum and bromadiolone although brodifacoum was also detected in over half the birds. A quarter of the 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.

Kestrels have been monitored between 1997 and 2008. Over this period the proportion of birds with detectable SGAR residues was higher in kestrels than in barn owls, although this was not the case the last two years. There has been no progressive increase or decrease over time in the proportion of kestrels with detectable SGAR residues.

Continued monitoring is required to determine whether the high detection rate for SGARs in barn owls is anomalous and perhaps due to random variation in sampling of owls. The high proportion of red kites exposed to SGARs and the relatively large number of birds with signs of haemorrhaging suggests that 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). 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). 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 (WIIS; http://www.pesticides.gov.uk/environment.asp?id=58). 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 increased to approximately 15-20 per year.

This report describes the results of PBMS monitoring of barn owls, kestrels and red kites submitted to the PBMS in 2007 and 2008 (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.

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

Table 1.1. Summary of barn owls, kestrels and red kites submitted to the PBMS in 2007 & 2008

		Year	
Species		**2007**	**2008**
barn owl	*Tyto alba*	122	114
kestrel	*Falco tinnunculus*	10	15
red kite	*Milvus milvus*	21	11
Total		150	140

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

All the kestrels and red kites were autopsied and analysed. All the barn owls received were autopsied but, because of the large number, only a sub-sample of approximately 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 (indandione derivatives) 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 (see Section 3).

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.

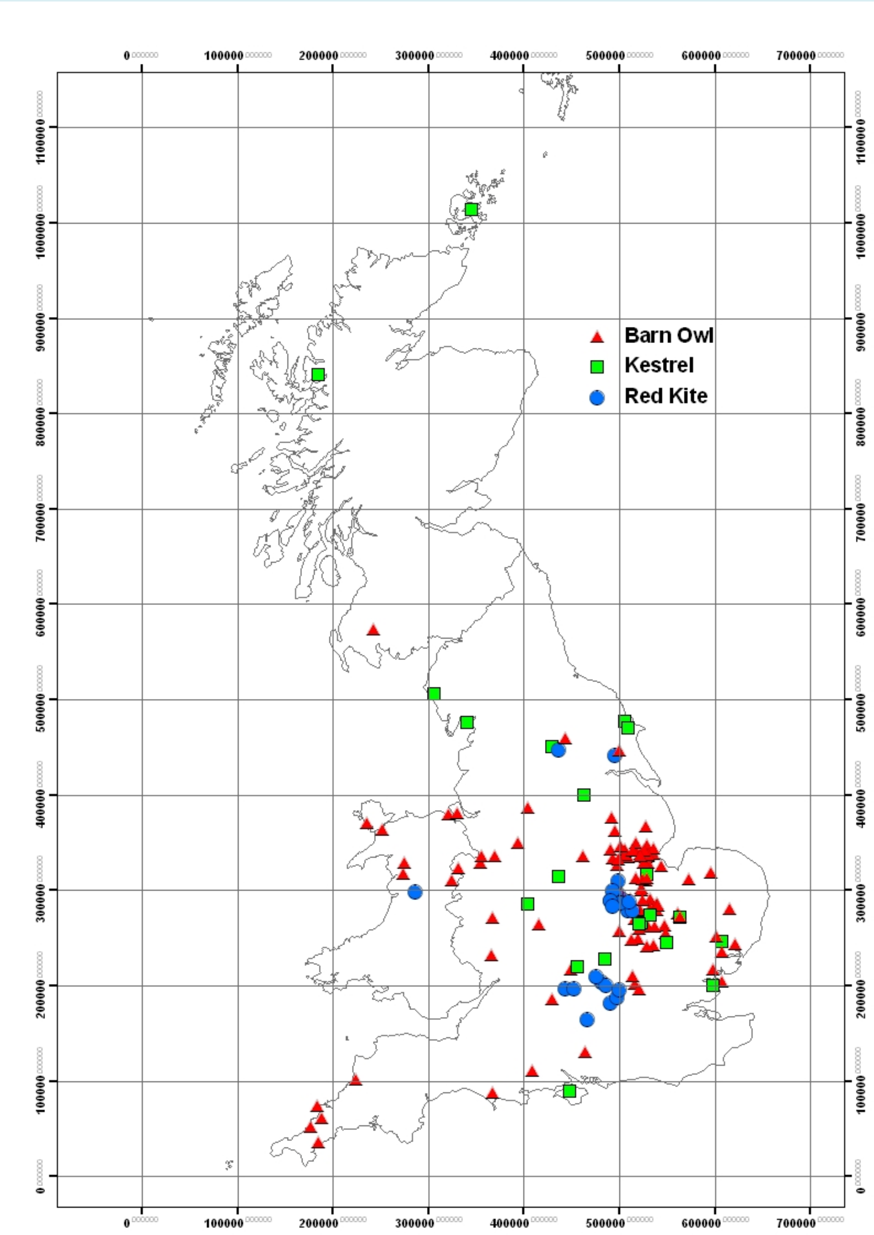

Figure 1.1 Location of birds found dead in 2007 and 2008 that have been analysed for hepatic anticoagulant rodenticide concentrations. Grid lines are 100km squares.

2. Anticoagulant rodenticide concentrations in birds submitted to the PBMS in 2007 & 2008

Summary statistics for incidence of detectable concentrations of anticoagulant rodenticides in the three species studied are presented below (Table 2.1). Results for individual birds are given in an addendum to this report which can be downloaded from the PBMS website (http://pbms.ceh.ac.uk/docs/AnnualReports/PBMS_Rodenticides_2007-8_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, kestrels and red kites analysed was 98, 22 and 32, respectively

	barn owls		kestrels		red kites	
	No/	%	No/	%	No/	%
2nd Generation (SGAR)						
bromadiolone	55	56	12	55	19	59
difenacoum	61	62	12	55	28	88
flocoumafen	2	2	1	5	1	3
brodifacoum	28	29	6	27	17	53
Any SGAR	79	81	15	68	29	91
Multiple SGARs	53	54	11	50	26	81
1st Generation (FGAR)						
warfarin	0	0	0	0	1	3
coumatetralyl	1	1	0	0	1	3
chlorophacinone	4	4	0	0	1	3
diphacinone	0	0	0	0	1	3
Any FGAR	5	5	0	0	4	13
Multiple FGARs	0	0	0	0	0	0
				0		
Any rodenticide	79	81	15	68	29	91
Multiple rodenticides	53	54	11	50	26	81

2.1 Barn Owls collected in 2007 &2008

Ninety eight barn owls were submitted to the PBMS in 2007 and 2008 collectively; all had died in those years. Seventy (80.6% of the sample) contained detectable liver concentrations of one or more SGAR (Table 2.1). This was the highest proportion reported since monitoring began in 1983, although this largely reflects the new lower limits of detection achieved by the LC-MS analysis.

As in previous years, the majority of exposure was to bromadiolone and difenacoum (77% of barn owls analysed). Brodifacoum was detected less frequently (Table 2.1) and flocoumafen was found in only two owls. Of the other anticoagulant rodenticides, coumatetralyl was detected in one barn owl and chlorophacinone in four (4%); residues were all below 0.03 µg/g wet wt. Warfarin and diphacinone, which has not been approved for use in the EU since September2006, were not detected in any of the barn owls tested. Overall, multiple SGAR residues were detected in approximately half 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).

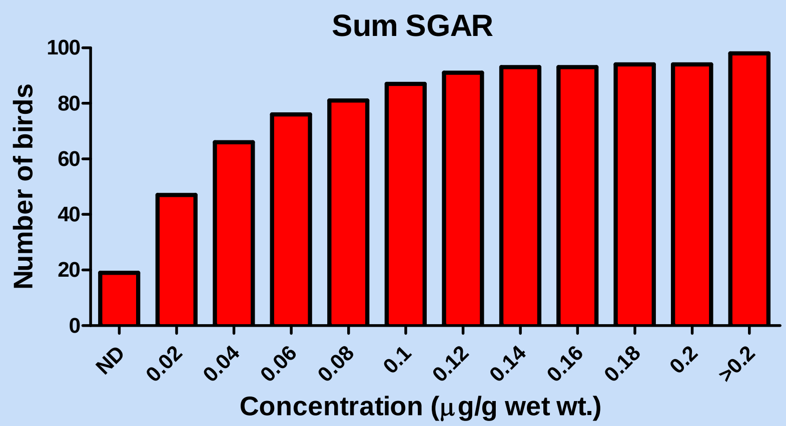

Fig. 2.1. Cumulative frequency for sum SGAR residues in the livers of barn owls found dead in 2007 and 2008 combined.

The median summed rodenticide concentration in barn owls with detected residues was 0.031 µg/g wet wt. Most owls had concentrations below the potentially lethal range (Figure 2.1) but eleven (11% of the sample) had residues (summed values for all four SGARs) greater than 0.1 µg/g wet wt; four of these exceeded 0.2 µg/g wet wt. The maximum liver concentration amongst these 11 owls was 0.727 µg/g wet wt (0.723 µg/g wet wt. brodifacoum and 0.004 µg/g wet wt. difenacoum) but there were no signs of haemorrhaging and the bird was diagnosed as having died from starvation. The second highest liver residue was 0.694 ug/g wet wt (0.663 µg/g wet wt. brodifacoum, 0.017 µg/g wet wt. difenacoum and 0.014 µg/g wet wt. flocoumafen) in a bird diagnosed of dying from unknown trauma. There was hemorrhaging on one side of the body consistent with a severe blow and the role of rodenticides is uncertain. In the other nine birds, summed SGAR liver residues were below 0.35 µg/g wet wt. and, at post mortem examination, there were either no signs of hemorrhage or the circumstances in which the bird was found suggested physical trauma had caused any hemorrhaging.

2.2 Kestrels collected in 2007 & 2008

Twenty-two kestrels were received in 2007 and 2008 collectively. Seven had died in 2007 and 15 in 2008. Fifteen kestrels (68% of sample) contained detectable levels of one or more SGAR in their livers. Twelve kestrels (55% of sample) each had detectable concentrations of difenacoum and bromadiolone; brodifacoum was detected in 6 birds and flocoumafen was detected in one bird. Warfarin, coumatetralyl, chlorophacinone and diphacinone were not detected in any of the kestrels. In all, half of the kestrels had multiple SGAR residues (Table 2.1).

The median summed rodenticide concentration was relatively low (0.056 µg/g wet wt). No "potentially lethal range" has been suggested for SGAR concentrations in kestrels but most birds had relatively low liver concentrations (Figure 2.2) and any observed hemorrhaging was associated with physical trauma.

Unlike in previous years, the proportion of individuals with detectable residues was not significantly greater than in barn owls (Fishers exact test; P=0.251).

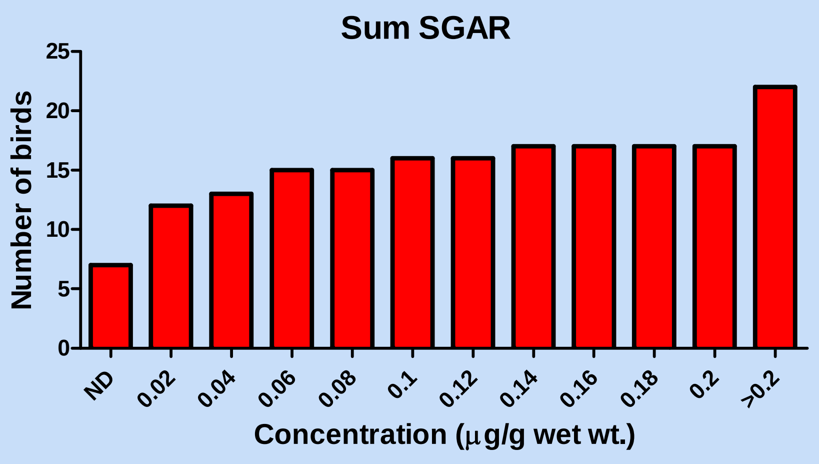

Figure 2.2. Cumulative frequency for sum SGAR residues in the livers of kestrels found dead in 2007 and 2008 combined.

2.3 Red kites collected in 2007 & 2008

Liver samples from 32 red kites that had died in 2007 and 2008 were analysed. Most (91%) contained detectable concentrations of anticoagulant rodenticides (Table 2.1).

As with barn owls and kestrels, the most prevalent rodenticides detected in red kite livers were difenacoum and bromadiolone (Table 2.1). However, 53% of red kite livers also contained brodifacoum, significantly more than in barn owls (Fisher's Exact test, P=0.018) but not significantly greater than in kestrels (Fisher's Exact test, P=0.093).

The sum SGAR liver concentrations in red kites were generally higher than those observed in the other species (Figure 2.3). The median concentration was 0.208 µg/g wet wt, which was 4-fold and > 6-fold higher than in kestrels and barn owls, respectively. The maximum liver concentration was 1.171 µg/g wet wt. (1.154 µg/g wet wt. brodifacoum and 0.018 µg/g wet wt. difenacoum).

Post mortem examinations by the Institute of Zoology indicated that eight of the red kites received showed internal haemorrhages not associated with detectable trauma and therefore consistent with anticoagulant poisoning. The sum SGAR liver concentration in these eight birds ranged between 0.112 and 1.171 µg/g wet wt., although similar residues (up to 0.533 µg/g wet wt.) were detected in birds thought to have died due to other causes.

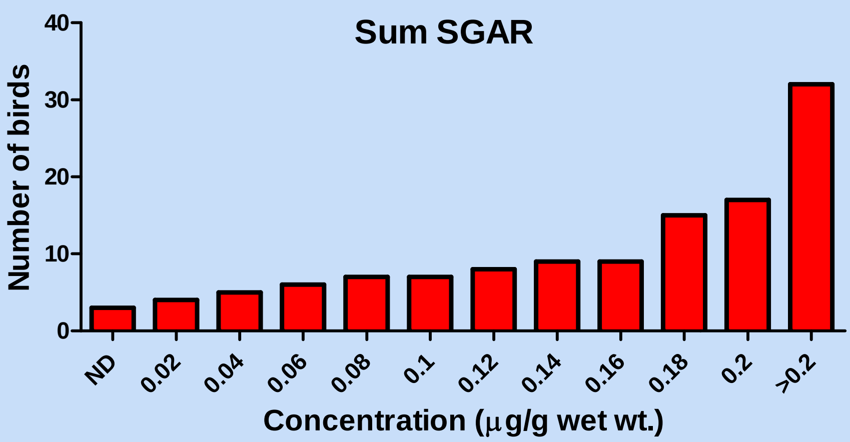

Figure 2.3 Cumulative frequency for Sum SGAR residues in the livers of red kites found dead in 2007 and 2008.

3. Long term trends in liver SGAR concentrations in barn owls and kestrels

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. In our previous report (Walker et al., 2010), the LoQ was based on the least sensitive analytical capability in the earliest years of monitoring which was for difenacoum. However, collection of a further two years of LC-MS based data allowed a more comprehensive analysis of the consequences of switching from HPLC to a LC-MS based quantification. The data indicate that the greater sensitivity of the LC-MS method has resulted in an increase in the proportion of owls that have liver SGAR concentrations up to 0.025 µg/g wet wt. (Table 3.1). Therefore a LoQ of 0.025 µg/g wet wt. has been adopted for the analysis of long-term temporal trends in SGAR concentrations in barn owls and kestrels.

Table 3.1 Comparison of percentage of barn owl livers within different concentration ranges for HPLC and LC-MS analysis methods. HPLC data are from years 2001-2005 (N=301) and LC-MS data are from years 2006-2008 (N=168). Within concentration class comparisons of analytical method were tested by Fisher's Exact test.

		Concentration Class (µg/g wet wt)			
Compound	**Detection Method**	**ND**	**<0.010**	**0.01-0.025**	**>0.025**
Brodifacoum	HPLC	93.4%	1.0%	2.0%	3.7%
	LC-MS	76.8%	10.1%	6.0%	7.1%
	P-value[1]	***	***	*	ns
Bromadiolone	HPLC	77.1%	0.0%	2.7%	20.3%
	LC-MS	50.0%	20.2%	13.1%	16.7%
	P-value	***	***	***	ns
Difenacoum	HPLC	73.1%	3.3%	14.3%	9.3%
	LC-MS	48.2%	23.8%	16.7%	11.3%
	P-value	***	***	ns	ns

[1] ns - Not significant; * - P<0.05; *** - P<0.001

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 underestimates 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.

Long term analysis has not been carried out on the red kite data set because 2007 and 2008 were the first years in which significant numbers of samples have been analysed.

3.1 Long term trends in barn owls

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 2008 was approximately twice that detected in the previous three years (22%-28%; Figure 3.1). This apparent rise was not mirrored in the kestrel samples (Section 3.2) which were analysed blind at the same time as the barn owls. Further monitoring is required to determine whether the high value for barn owls in 2008 reflects a renewed increasing trend in exposure or may simply be due to random variation in sampling.

In terms of potential adverse effects, 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 detected 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.

The scale of exposure of barns owls in England, Scotland and Wales has also been compared using the data available pooled for the years 1983-2008 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 3.2).

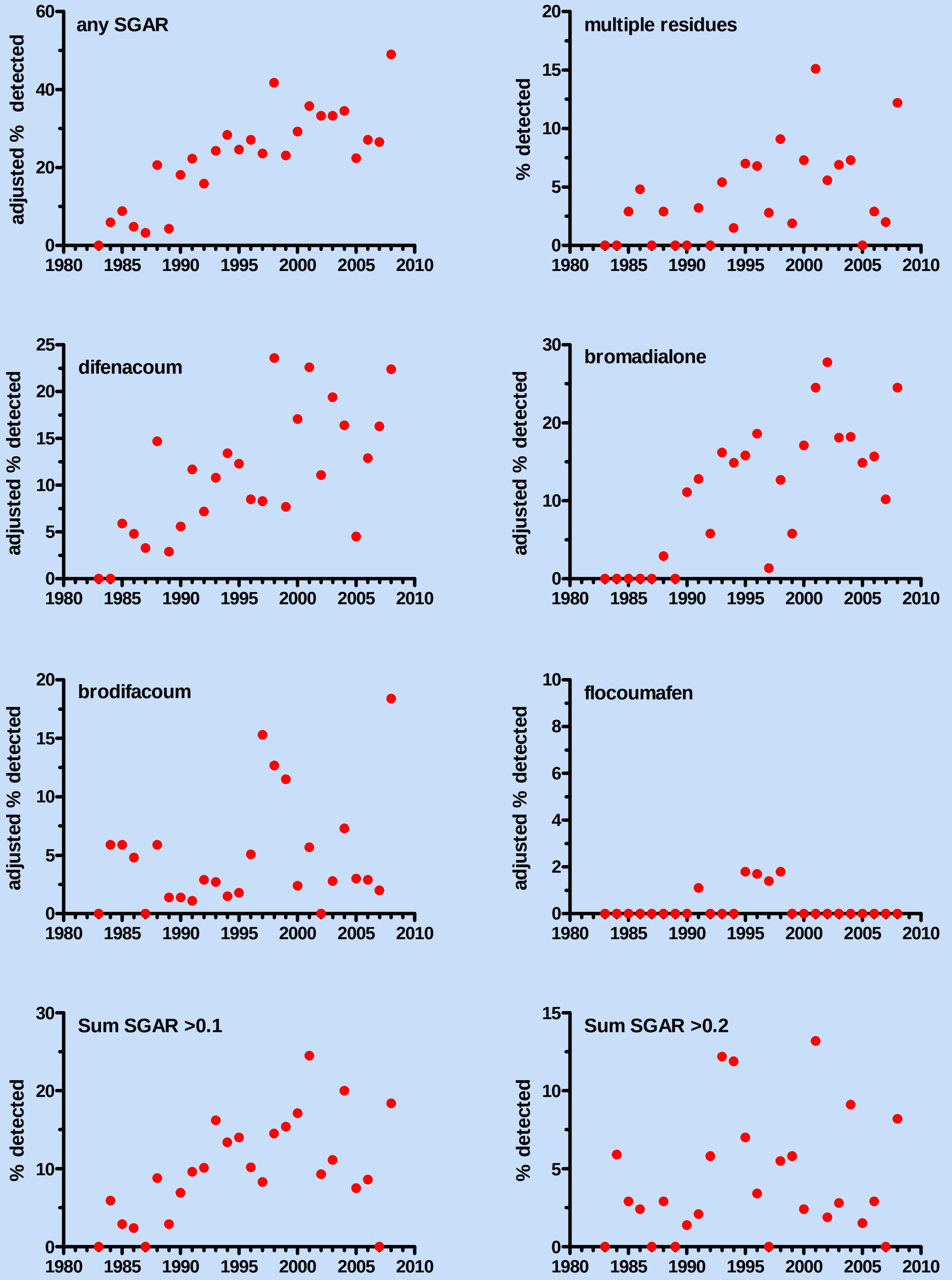

Figure 3.1. Variation over time in adjusted % of barn owls with: detectable (≥0.025 μg/g wet wt.) concentrations of individual or summed SGARs, multiple residues in the liver, or liver concentrations >0.1 μg/g wet wt. or 0.2 μg/g wet wt.

Table 3.2. Number (n) of owls and the number as a percentage of all birds tested (%) from England, Scotland and Wales between 1990 and 2008 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=1124)		**Scotland (n=120)**		**Wales (n=118)**		**Chi Squared statistic[1]**
Bromadiolone	153	(14%)	11	(9.2%)	6	(5.1%)	8.43 (*)
Difenacoum	138	(12%)	6	(5.0%)	11	(9.3%)	6.24 (*)
Flocoumafen	2	(0.2%)	1	(0.8%)	0	(0%)	-
Brodifacoum	60	(5.3%)	3	(2.5%)	2	(1.7%)	4.61 (ns)
Any SGAR	295	(26%)	17	(14%)	17	(14%)	15.3 (***)
Multiple SGAR	53	(4.7%)	4	(3.3%)	2	(1.7%)	2.67 (ns)

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

3.2 Long term trends in kestrels

The same common limit of quantification as 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 65%, with no apparent progressive increase or decrease over blocks of years (Figure 3.2). Much of this has been exposure to difenacoum and bromadiolone which have been detected in between 35% and 45% of kestrels. As in barn owls, flocoumafen has not been detected in kestrels during this monitoring period.

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

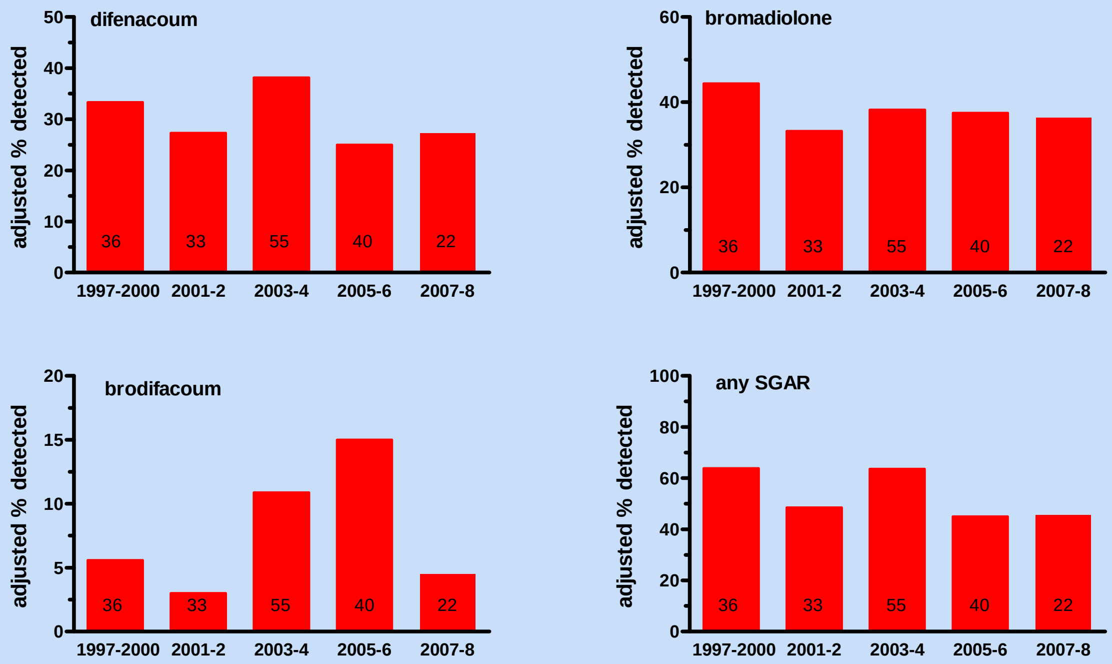

Figure 3.2. Variation over time in the adjusted % of kestrels with detectable liver residues of individual SGAR and of any SGAR (minimum concentrations for any individual compound of 0.025 µg/g wet wt).

4. Acknowledgements

We thank all the members of the public who have submitted birds to the Predatory Bird Monitoring Scheme. Their efforts are key to the success of the scheme. We also thank Andy Bright (www.digiscoped.com) and Gerry Whitlow (www.hyelms.com) for providing photographs of predatory birds and Richard Wadsworth for providing statistical advice and the maps for this report.

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