Predatory Bird Monitoring Scheme
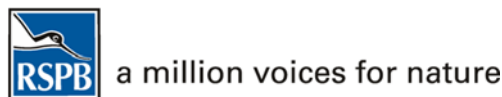http://pbms.ceh.ac.uk/

Anticoagulant rodenticides in sparrowhawks: a Predatory Bird Monitoring Scheme (PBMS) report

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1.   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 activities for contaminant monitoring and surveillance work on avian predators. The PBMS aims to detect and quantify current and emerging chemical threats to the environment and in particular to vertebrate wildlife.

Second generation anticoagulant rodenticides (SGARs) can be toxic to all mammals and birds. The PBMS together with other studies have shown that in Britain, there is widespread exposure to SGARs in a diverse range of predators of small mammals. Defra's Wildlife Incident Monitoring Scheme (WIIS) and the PBMS have shown that some mortalities result from this exposure. The main transfer pathway for SGARs has been thought to be most likely via target and non-target rodents that eat SGAR bait. However, recent studies, including a preliminary analysis by the PBMS on sparrowhawks, *Accipiter nisus*, have suggested that that SGAR transfer through avian transfer pathways may also be important. The aims of the current study were to build on our earlier results by analysing a further 52 sparrowhawks for liver SGAR residues, and using the combined dataset to (i) assess overall levels of exposure in sparrowhawks from across Britain and (ii) determine if age and sex affect the magnitude of liver SGARs residues. A final aim was to compare exposure (assessed from liver residues) in sparrowhawks with that of the barn owl *Tyto alba*, a predator that takes predominantly small mammals

We found one or more SGARs in the livers of 89% of the 94 sparrowhawks we analysed; all birds were collected by the PBMS between 2010 and 2013. A high proportion of these residues were relatively low and none were associated with haemorrhaging unconnected with physical trauma. The proportion of birds with detectable residues and the magnitude of those residues were significantly higher in adults than juveniles, but residues did not vary significantly between males and females. Comparison of liver SGAR residues in barn owls and sparrowhawks that had died over a similar time period indicated that, for both adults and juveniles, sparrowhawks were as likely to have detectable liver SGAR residues as barn owls but the magnitude of the residues was lower in sparrowhawks.

Overall this study has demonstrated that food-chain transfer of SGARs can occur via a predominantly avian trophic pathway and lead to secondary exposure.

2.   Introduction

2.1   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 scientific evidence of 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 and policy 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 general 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.

2.2 Second generation anticoagulant rodenticides in predatory birds

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, Walker *et al.*, 2008b) together with other studies (Dowding *et al.*, 2010, McDonald *et al.*, 1998, Shore *et al.*, 2003a, Shore *et al.*, 2003b) have shown that there is widespread exposure to SGARs in a diverse range of predators in Britain. Defra's Wildlife Incident Monitoring Scheme (WIIS) and the PBMS have shown that 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). In some previous years, the PBMS has also measured SGARs in red kites (*Milvus milvus*), a high priority species that has been reintroduced to England, and kestrels (*Falco tinnunculus*) which can accumulate relatively high liver SGAR concentrations (Walker *et al.*, 2013, Walker *et al.*, 2012) and about which there are concern over population declines (http://www.bto.org/birdtrends2009/wcrkestr.shtml).

These previous PBMS studies focused on avian predators that predominantly feed on target and non-target rodents. This reflected the expectation that the main transfer pathway for SGARs is via target and non-target rodents that eat SGAR bait. However, a recent study demonstrated that, in Scotland, the proportion of sparrowhawks (*Accipiter nisus*) that contained detectable liver SGAR concentrations was comparable to that of avian predators that mainly eat small mammals (Hughes *et al.*, 2013). Sparrowhawks feed largely on birds (Newton, 1986), and the results of Hughes *et al.* (2013) suggests that SGAR transfer through avian transfer pathways may be important and perhaps as significant as that mediated via small mammals. In a recent report (Walker et al 2014), we investigated whether the observations from Scotland were likewise observed over a wider spatial scale in Britain. This involved analyzing liver SGAR residues in 42 sparrowhawk carcasses collected from across Britain. Our observations generally supported those of Hughes *et al.* (2013) in that a high proportion of sparrowhawks had detectable liver SGAR concentrations. However, neither study analysed a sufficiently large number of samples to characterize how exposure in sparrowhawks may vary with such factors as age and sex. Age has been found to affect the magnitude of liver SGAR residues in barn owls (Walker *et al.*, 2014), while diet is likely to be a major factor that influences exposure (Shore *et al.*, *in press*) and the diet of males and female sparrowhawks differs to some extent (Newton, 1986).

2.3 Aims of the current study

Our aim was to build on the results of Walker *et al.* (2014) by analysing a further 52 sparrowhawks (from the same time period as the 42 analysed previously) for liver SGAR residues, and using the combined larger dataset to (i) assess overall levels of exposure and (ii) determine if age and sex affect the magnitude of liver SGARs residues. We therefore present in this report summary data and statistical analysis for all 94 sparrowhawks combined. A second aim was to compare exposure (assessed from liver residues) in sparrowhawks with that of barn owls that died over the same period.

3.   Methods

Sparrowhawk carcasses were submitted to the PBMS by members of the public. Carcassess were sexed and separated into two age classes, adults and juveniles (where juveniles were defined as birds hatched in the current or previous calendar year relative to when they died). A post-mortem examination investigated putative cause of death and birds were found to have died from various causes, mainly road traffic collisions, other trauma and from starvation. Tissues from all birds were archived in the PBMS tissue and egg archive where they are available for future research purposes.

A subsample of 52 sparrowhawks that had died between 2010 and 2013 was analysed. The data we obtained were combined with those already reported by Walker *et al.* (2014) such that the final dataset consisted of 94 birds with roughly equal numbers of adults and juveniles and males and females (Table 1).

Liver SGAR residues were quantified by Liquid Chromatography Mass Spectrometry and a summary of the analytical methods can be downloaded at http://pbms.ceh.ac.uk/docs/AnnualReports/PBMS_Rodenticides_Methods.pdf. Taking into account the extract volume, dilution of the extract and the sample weight, the mean tissue Limit of Detectuion (LoD) for difenacoum, bromadiolone, brodifacoum and flocoumafen was 2.3 ng/g wet weight (wet wt) and for difethialone was 3.0 ng/g wet wt. Anticoagulant rodenticide concentrations are reported as ng/g wet wt. and have been statistically analysed using Minitab 16.1 (Minitab Ltd., Coventry, U.K.) and illustrated using Graphpad Prism version 5.04 for Windows (GraphPad Software, San Diego, USA).

Table 1. Summary of number of sparrowhawk livers analysed for SGARs.

Age	Sex	Year collected 2010	2011	2012	2013	Total
Adult	Male	0	2	6	10	18
	Female	0	5	10	8	23
Juvenile	Male	2	7	7	9	25
	Female	7	8	8	5	28
TOTAL		9	22	31	32	94

4. Results and Discussion

4.1 Second Generation Anticoagulant Rodenticides (SGARs) in sparrowhawks

Overall, 84 sparrowhawks (89.4% of the sample) contained detectable liver residues of one or more SGAR, and 55 contained residues of more than one SGAR. Difenacoum was detected most frequently (76% of birds) but bromadiolone and brodifacoum were both detected in just under half of all sparrowhawks. Difethialone was only detected in one bird while flocoumafen was not detected in any bird tested.

Sum liver SGAR concentrations in sparrowhawks with detectable SGAR residues ranged between 2 and 157 ng/g wet wt. (equivalent to 0.002 and 0.157 µg/g wet wt.). The majority of birds had relatively low residues with nearly 75% having a sum SGAR liver concentration below 40 ng/g wet wt. (Figure 1). Four sparrowhawks had sum SGAR liver residues greater than 100 ng/g wet wt but, on post-mortem examination, there was no evidence of hemorrhage other than that associated with physical trauma. Therefore the contribution, if any, of rodenticides to the death of these four individuals is equivocal.
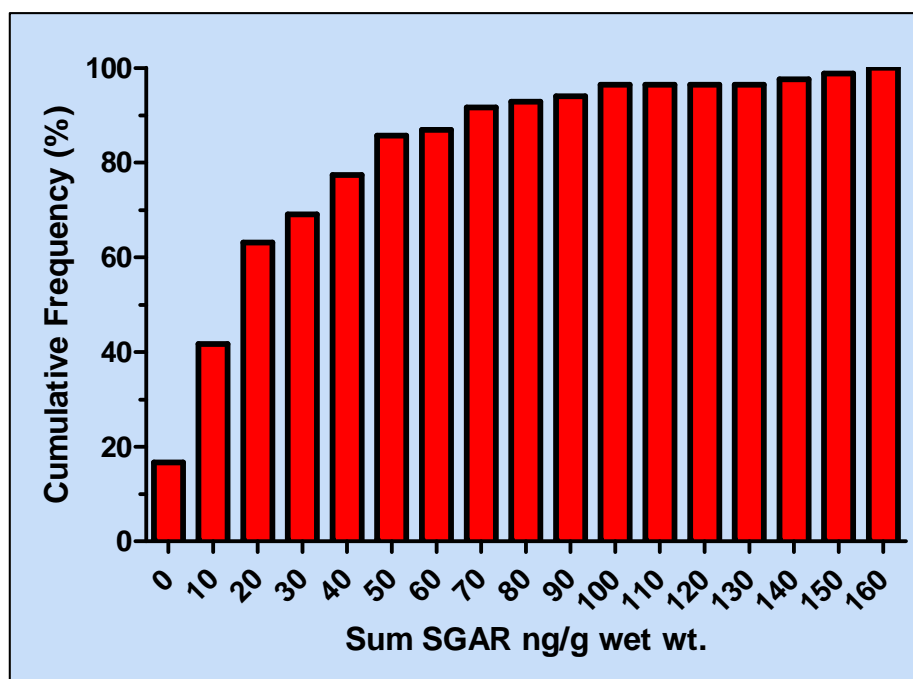

Figure 1. Cumulative frequency for Sum SGAR residues in the livers of sparrowhawks found dead between 2010 and 2013

When birds were separated by age and sex, we found that 19/25 (76%) juvenile males, 24/28 (86%) juvenile females, 18/18 (100%) adult males and 23/23 (100%) adult females had detectable liver concentrations of at least one SGAR. Thus, there was little evidence of any difference in the proportion of birds exposed between males and females, suggesting that exposure was unaffected by any sex-related differences in diet. When data for the sexes were combined, a significantly higher proportion of adult than juvenile sparrowhawks were found

to have one or more SGARs present in their livers (Table 2; Fisher's Exact Test, P=0.004); all adults had detectable residues of at least one SGAR compared with 81% in juvenile birds. Higher exposure rates in adults than juveniles may simply reflect a difference in the average time available to birds to encounter contaminated prey.

Summary statistics for the incidence of detectable concentrations of SGARs in adult and juvenile sparrowhawks are given in Table 2.

Table 2. Percentage (%) of all sparrowhawks analysed with detectable liver SGAR concentrations and the liver concentrations in birds with detected values. Total number of birds analysed was 94.

	Brom	Difen	Flouc	Brod	Difeth	Sum SGARs
Limit of Detection (ng/g) wet weight	2.3	2.3	2.3	2.3	3.0	-
Adults						
N	41	41	41	41	41	41
not detecteds	21	4	41	13	40	0
detecteds	20	37	0	28	1	41
% detected	48.8	90.2	0.0	68.3	2.4	100.0
Conc. of detected compounds						
Geometric mean	7.737	17.26	-	5.535	3.3	22.17
Lower 95% CI	4.933	12.82	-	4.283	-	16.05
Upper 95% CI	12.14	23.24	-	7.152	-	30.62
Juveniles						
N	53	53	53	53	53	53
not detecteds	29	19	53	37	53	10
detecteds	24	34	0	16	0	43
% detected	45.3	64.2	0.0	30.2	0.0	81.1
Conc. of detected compounds						
Geometric mean	10.41	8.329	-	5.343	-	13.39
Lower 95% CI	6.440	5.942	-	3.507	-	9.411
Upper 95% CI	16.82	11.67	-	8.141	-	19.05

Figures are calculated based on methods using matrix matched standards

We used a general linear model on log-transformed data to analyse how liver sum SGAR concentrations varied with age and sex in sparrowhawks. Age was a significant factor ($F_{1,90}=12.92$, P=0.001) but neither sex ($F_{1,90}=0.00$, P=0.962) nor the interaction term between age and sex ($F_{1,90}=0.05$, P=0.832) were statistically significant. Liver concentrations were some 65% greater in adults than juveniles. This difference suggests that the number of exposures increases over time and frequency of exposure exceeds the rate of elimination from the liver.

4.2   Comparison of liver SGAR residues in sparrowhawks and barn owls

We compared differences in both the prevalence and magnitude of detectable liver sum SGAR residues between the 94 sparrowhawks and 173 barn owls (41 adults, 132 juveniles) that had been collected between 2010 and 2012 and analysed by the PBMS. Prevalence did not differ between the two species for either adult or juvenile birds (Figure 2). These results are consistent with those of Hughes *et al.* (2013) in that they suggest sparrowhawks may be equally likely to be exposed to SGARs as predators that specialize on small mammals. It was also notable that the percentage of sparrowhawks with detectable residues in the present study was almost twice that in birds that came exclusively from Scotland.

Comparison of the differences between sparrowhawks and barn owls in the magnitude of liver sum SGAR concentrations accumulated Involved analysis of log10 transformed to meet the assumptions of the two-sample student t-test; juvenile and adult birds were considered separately. There were significant differences between the two species for both age classes. Sum SGAR liver concentrations in adult birds with detectable residues were on average 2.5 fold higher in barn owls than sparrowhawks ($t_{(78)}$=4.05, P=0.0001) while for juveniles this difference was 1.7 fold ($t_{(148)}$=2.48, P=0.014; Figure 2). Possible reasons for this species difference is that residues in prey and/or frequency of exposure is lower for sparrowhawks than barn owls, although it is possible that pharmacokinetic also play a factor.
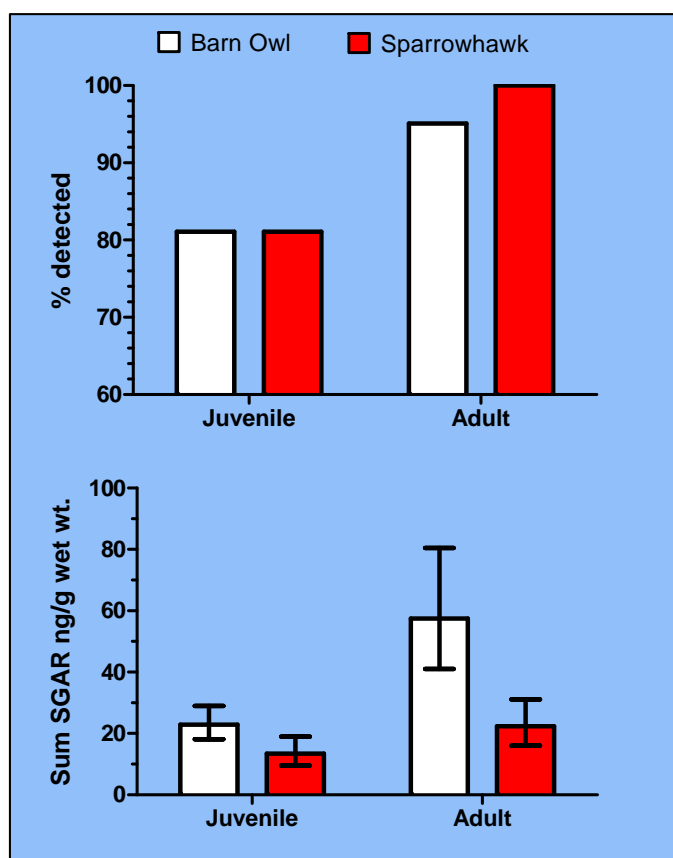

Figure 2. Percentage of barn owls and sparrowhawks that died between 2010 and 2013, with detectable concentrations of SGARs present in their livers and the geometric mean (±95% confidence interval) of sum SGAR residues. Sample numbers are indicated in the figure for each group, livers in which any SGAR was not detected were excluded from the calculation of the sum SGAR concentration.

5.   Conclusions

This study has demonstrated that SGARs are present in a large proportion of sparrowhawks submitted to the Predatory Bird Monitoring Scheme. One or more SGARs were detected in 89% of 94 birds received between 2010 and 2013. A high proportion of these residues were relatively low and none were associated with post-mortem evidence of haemorrhaging that was not linked to physical trauma. Our study has demonstrated clearly that adult sparrowhawks are more likely to accumulate detectable levels of SGARs than juvenile birds and that the magnitude of those residue are higher in adult birds.

Comparisons with SGAR residues in barn owls that had died over a similar time period indicated that, for both adults and juvenile birds, exposure to SGARs is as widespread as in barn owls but accumulated liver SGAR concentrations are on average lower than those in barn owls of comparable age class.

Overall this study demonstrates that a predominantly avian pathway represents a significant route of food-chain transfer of SGARs leading to secondary exposure in a non-mammal feeding predatory bird.

6.   Acknowledgements

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