Predatory Bird Monitoring Scheme
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Anticoagulant rodenticides in red kites (*Milvus milvus*) in Britain 2010 to 2015: a Predatory Bird Monitoring Scheme (PBMS) report

L.A. Walker, J.S. Chaplow, C. Moeckel, M. G. Pereira, E.D. Potter, A.W. Sainsbury[*], R.F. Shore[1]

Centre for Ecology & Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster, LA1 4AP, UK;
[*]Institute of Zoology, Zoological Society of London, Regents Park, London NW1 4RY, UK

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[1]Corresponding author: RF Shore, Centre for Ecology and Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster, LA1 4AP, UK. E-mail: rfs@ceh.ac.uk.Website: http://www.ceh.ac.uk/

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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, including red kites (*Milvus milvus*) which will scavenge dead rats, a target species for rodent control. Defra's Wildlife Incident Monitoring Scheme (WIIS) and the PBMS have shown that some mortalities result from this secondary exposure. The aims of the current study were to build on our earlier results by analysing liver SGAR residues in a further 24 red kites that had been submitted to the Predatory Bird Monitoring Scheme between 2010 and 2015. We (i) assessed the scale and severity of exposure and, (ii) by combining the data with that from birds collected earlier (since 2006), we determined if age and sex affects the magnitude of liver SGARs residues accumulated in red kites.

All of the 24 red kites contained detectable liver residues of one or more SGAR, and all but one bird (96%) contained residues of more than one SGAR. Difenacoum was detected most frequently (96% of birds) but bromadiolone and brodifacoum were both also detected in a large proportion of birds (83-88%). Most (approximately 75%) of the kites had sum SGAR livers concentrations >100 ng/g wet wt. and SGAR poisoning was likely to have been the cause of death in two birds. Relatively high liver SGAR residues were also detected in four other birds but they had external signs of trauma indicating they may have died from other causes. The monitoring of SGAR residues in red kites remains important contribution to our understanding of SGAR exposure in wildlife, particularly those issues related to scavenging species.

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 measured liver SGAR residues in a range of predatory birds to determine the scale and severity of secondary exposure. The red kite (*Milvus milvus*) is one of the species that we have monitored. It is a conservation priority species that was reintroduced to England [and later Scotland] in the late 20th Century as part of an official species recovery programme (Carter and Grice 2002). Red kites are scavengers and feed on dead rats. This propensity to scavenge species that are the target of anticoagulant rodenticide control may mean that red kites are particularly vulnerable to secondary exposure. 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 the analysis undertaken (Walker et al. 2008a) indicated that it was likely that a large proportion of proportion of reintroduced birds may be exposed to SGARs. Subsequent development of a collaboration with the Institute of Zoology, facilitated by WILDCOMS network, has meant that the number of red kites available to the PBMS for SGAR analysis has increased and residues can be reported more frequently.

2.3 Aims of the current study

Our aim was to provide an update on previous studies (Walker *et al.*, 2010,Walker *et al.*, 2012, Walker *et al.*, 2013) by analysing liver SGAR residues in a further 24 red kites. These birds died between 2010 and 2015. In this report we: (i) assess the current incidence and magnitude of liver SGAR residues, (ii) determine if age and sex affect the magnitude of liver SGARs residues.

3. Methods

Between 2010 and 2015 dead red kite carcasses were collected as part of the Predatory Bird Monitoring Scheme and the Disease Risk Analysis and Health Surveillance Programme operated by the Centre for Ecology & Hydrology and the Institute of Zoology, respectively (Table 1 and Fig. 1). Both projects are partners in the WILDCOMS network and rely on citizen science in that members of the public submit to the scheme dead birds that they find. A post-mortem examination is undertaken on all carcasses and, as part of this, the age class and sex of each bird was determined. Various tissue samples, including liver, were excised and stored at -20ºC prior to analysis. For the purposes of this study, juvenile birds are classed as individuals that hatched in the current or previous year to that in which they were found dead.

Liver SGAR residues were quantified by Liquid Chromatography Mass Spectrometry and a summary of the analytical methods can be in annex 1 to this report. Taking into account the extract volume, dilution of the extract and the sample weight, the maximum tissue Limit of Detection (LoD) for each of the five active ingredients was 1.7 ng/g wet weight (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). Recoveries from spiked chicken livers for different compounds ranged between 69 and 86% and data are not recovery corrected.

Table 1. Summary of number of red kite livers analysed for SGARs.

		Year collected					
Age	Sex	2010	2012	2013	2014	2015	Total
Adult	Male		2	1	1	1	5
	Female		4	3	3	1	11
	Unknown			1			1
Juvenile	Male		1	1		1	3
	Female	1		2			3
Unknown	Male			1			1
TOTAL		1	7	9	4	3	24

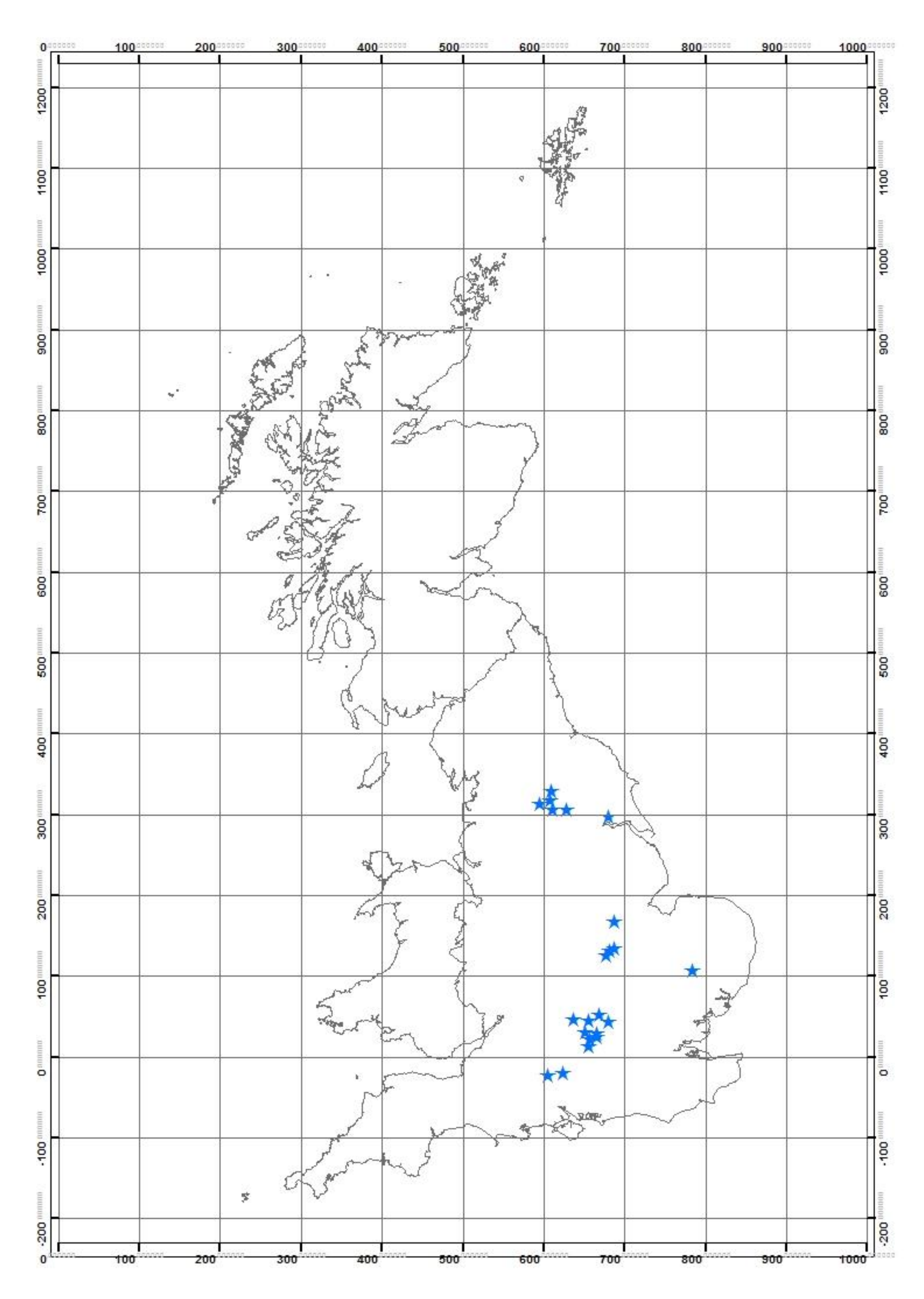

Figure 1. Location of red kites, *Milvus milvus*, found dead in 2010 to 2015 for which livers were analysed for second generation anti-coagulant rodenticides (SGARs). Location not shown for two birds, one from Buckinghamshire and one from Hampshire, as their precise location is unknown.

4. Results and Discussion

Summary statistics for the incidence of detectable concentrations of SGARs in red kites are given in Table 2. All of the 24 red kites contained detectable liver residues of one or more SGAR, and all but one bird (96%) contained residues of more than one SGAR. Difenacoum was detected most frequently (96% of birds) but bromadiolone and brodifacoum were both also detected in a large proportion of birds (Table 2). Difethialone and flocoumafen were each only detected in two (8%) of the birds tested.

Table 2. Percentage (%) of all red kites with detectable liver SGAR concentrations (ng/g wet weight) and the liver concentrations in birds with detected values. Total number of birds analysed was 24.

	Active Ingredient[2]					
	Brom	Difen	Floc	Brod	Difeth	Sum SGARs
Limit of Detection (ng/g) wet weight	1.7	1.7	1.7	1.7	1.7	-
Number of birds with non- detected (ND) concentrations	4	1	22	3	22	0
Number of birds with detected concentrations[1]	20	23	2	21	2	24
% detected	83	96	8.3	88	8	100
Summary statistics for liver SGAR concentrations (ng/g wet wt.) in all 24 birds						
Arithmetic mean	59.2	91.5	2.0	111	3.8	266
Geometric mean	21.9	28.6	1.0	24.8	1.1	169
Median	35.6	27.4	0.8	24.0	0.8	243
Minimum	0.8	0.8	0.8	0.8	0.8	2.0
Maximum	255	595	26.8	593	61.9	663
Summary statistics for liver SGAR concentrations (ng/g wet wt.) in birds with detected concentrations						
Arithmetic mean	70.8	95.5	14.6	127	35.3	266
Geometric mean	42.3	33.5	8..0	40.4	23.2	169
Median	40.8	33.9	14.6	29.5	35.3	243
Minimum	2.3	2.0	2.4	1.90	8.70	2.0
Maximum	255	595	26.8	593	61.9	663

[1] Non-detected assigned a value of 0.8 ng/g wet wt. equivalent to ½ limit of detection
[2] Brom = bromadiolone, Difen = difenacoum, Floc = flocoumafen, Brod = brodifacoum, and Difeth = difethialone.

Sum liver SGAR concentrations ranged between 2 and 663 ng/g wet wt. (equivalent to 0.002 and 0.663 µg/g wet wt.) with a geometric mean concentration of 169 ng/g wet wt. The majority of birds (almost 75%) had sum SGAR liver concentration > 100 ng/g wet wt. and median concentrations were > 200 ng/g wet wt. The incidence and magnitude of SGAR residues were similar to those reported in red kites previously (Walker *et al.*, 2010, Walker *et al.*, 2012, Walker *et al.*, 2013).

Post mortem examinations by the Institute of Zoology indicated that two of the kites had internal hemorrhaging that was not associated with detectable trauma. The birds had sum SGAR liver concentrations of 491 and 594 ng/g wet wt. Given the lack of evidence for trauma and relatively high liver residues, it is probable that SGARS were a contributory factor in the deaths of these birds. A further four birds had sum SGAR liver concentrations greater than 400 ng/g wet wt., including the bird with the highest residues of 663 ng/g wet wt., but all had suffered physical trauma of some kind. It is unclear whether exposure to SGARs contributed to the death of these birds. As observed by Walker *et al*., (2013), there is a great deal of overlap in residue magnitude between birds that are thought to have died of SGAR poisoning and those thought to have died of other causes and deterministic approaches to defining a sum SGAR liver concentration diagnostic of death is problematic. Probabilistic approaches to interpreting the significance of liver residues, as proposed by (Thomas *et al.*, 2012), may be a better means of understanding the likely impact of SGARs on mortality in this species and other species.

Sum SGAR concentrations detected in red kites submitted to the PBMS between 2006 and 2015 (data from the current and previous PBMS reports) were analysed to determine whether residue magnitude varied with age and sex. Residue data could not be transformed so that the assumptions of generalized liner models were met and so the data were analysed using a non-parametric Kruskal-Wallis (and *post-hoc* Dunn's Multiple Comparison tests). This analysis indicated that adult female birds had significantly higher median concentrations than juvenile females (P<0.005; Figure 2). The difference in median liver SGAR magnitude between adult and juveniles in males was similar to that in females but was not statistically significant. (Figure 2). Median liver concentrations were similar in males and females of the same age class.

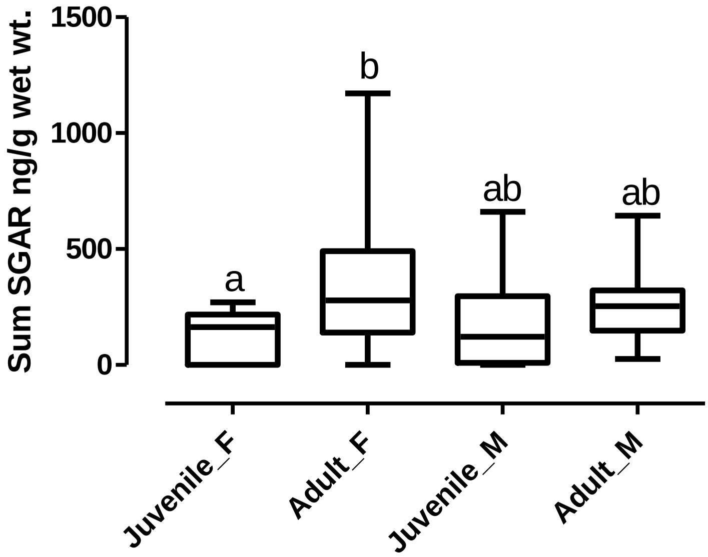

Figure 2. Box and whiskers plot of median (line), interquartile range (box) and minimum and maximum range (whiskers). Sum SGAR concentrations in the livers of juvenile female (N=15), adult female (N=31), juvenile male (N=19) and adult male (N=26) red kites that were submitted to the PBMS between 2006 and 2015. Significant differences (P<0.05) between groups are indicated by different letters.

5. Conclusions

This study has demonstrated that SGARs are present in a large proportion of red kites submitted to the Predatory Bird Monitoring Scheme in recent years (2010 to 2015) with a similar proportion of birds having detectable residues of difenacoum, bromadiolone and brodifacoum in their livers. Two or more SGARs were detected in all but one of the 24 birds examined. On average, adults accumulated higher residues than juvenile birds but residue magnitude did not vary between males and females.

A large proportion of the red kites had sum SGAR livers concentrations greater than 100 ng/g wet wt. with SGAR poisoning likely to have been the cause of death in two birds. However, similarly high residues were also detected in birds that died of other causes. Exposure to rodenticides has not prevented successful reintroduction of kites into parts of Britain. There have been a number of re-introductions in England and Scotland and productive breeding groups established - in some cases, these have benefitted from large-scale provision of food. Breeding at sites distant from release areas has been slow to develop but, overall, the British Trust for Ornithology Breeding Bird Survey (BBS) sightings have increased exponentially since 1994 (https://www.bto.org/birdtrends2010/wcrredki.shtml).

The monitoring of SGAR residues in red kites remains an important contribution to our understanding of SGAR exposure in wildlife, particularly those issues related to scavenging species. Such monitoring will also help to elucidate any effects that exposure may have on further population re-establishment and growth.

6. Acknowledgements

We thank all the members of the public who have submitted predatory bird carcasses to the Predatory Bird Monitoring Scheme. Their efforts are key to the success of the scheme. The Predatory Bird Monitoring Scheme was co-funded in 2015-16 by the NERC Centre for Ecology & Hydrology, the Department for the Environment, Food and Rural Affairs (Defra), Natural England (NE), the Royal Society for the Protection of Birds (RSPB), the Scottish Environment Protection Agency (SEPA) and the Campaign for Responsible Rodenticide Use (CRRU).

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8. Annex 1 - Summary of analysis methods for anticoagulant rodenticides by LC-MSMS

Approximately 0.25 grams of fresh liver was ground with sodium sulphate in a pestle and mortar to form a dry free flowing powder. This was extracted twice into a mixture of acetone and chloroform using a mechanical wrist shaker, the solvent being collected by centrifugation after each extraction. The combined solvent extract was exchanged into a chloroform/acetonitrile mixture and cleaned up by solid phase extraction; the final residue was dissolved in 1 ml of LCMSMS mobile phase. The extract was determined by LCMSMS using negative atmospheric pressure chemical ionization and multiple reaction monitoring. The instrument was calibrated using certified rodenticides standards prepared in mobile phase. The liver samples were run in batches of sixteen which incorporated a blank prepared with chicken liver, and a spiked recovery standard prepared with chicken liver.