Anticoagulant rodenticides in red kites (*Milvus milvus)* in Britain 2015

L.A. Walker, J.E. Jaffe*, E.A. Barnett, J.S. Chaplow, S. Charman**, A. Giela***, A. Jones**, M.G. Pereira, E.D. Potter, A.W. Sainsbury*, D.Sleep, N.J. Thompson, C. Senior***, E.A. Sharp***, R.F. Shore[1]**

NERC 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
**Fera Science Ltd, Sand Hutton, York, YO41 1LZ, UK
*** Science and Advice for Scottish Agriculture, Roddinglaw Road, Edinburgh, EH12 9FJ, Scotland

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

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Contents

1. Executive Summary

Second generation anticoagulant rodenticides (SGARs) can be toxic to all mammals and birds. Various studies have shown that, in Britain, there is widespread exposure to SGARs in a diverse range of predatory mammals and birds, including red kites (*Milvus milvus*) which scavenge dead rats, a target species for rodent control. The Wildlife Incident Monitoring Scheme (WIIS) and the Predatory Bird Monitoring Scheme (PBMS) have shown that some mortalities result from this secondary exposure.

The aim of the current study was to assess the scale and severity of exposure to SGARs (as assessed from the presence of liver SGAR residues) in red kites found dead in Britain in 2015. Carcasses, typically found by members of the public, were submitted for examination and analysis either to the Institute of Zoology's Disease Risk Analysis and Health Surveillance programme (DRAHS) or to the Centre for Ecology & Hydrology's Predatory Bird Monitoring Scheme, partners in the WILDCOMS network. We also report the results of SGAR analysis of red kites from England, Wales and Scotland that died in 2015 and analysed by the WIIS, who are also partners of the WILDCOMS network.

Eighteen red kites from England & Wales were necropsied by either the DRAHS or the PBMS and the livers of the birds were analysed for SGARs by the PBMS. All had detectable liver residues of difenacoum and brodifacoum, and most also contained detectable liver bromadiolone residues. Difethialone was less frequently detected and flocoumafen was not detected in any birds. The presence of detectable brodifacoum residues in all birds may partly reflect the predominance of adult birds in the 2015 sample but may also indicate a growing prevalence of exposure to this compound. The percentage of red kites found by the PBMS to contain brodifacoum has increased since 2010 although any influence of age on this trend has not yet been examined.

Sum liver SGAR concentrations in the 18 kites ranged between 50 and 1266 ng/g wet wt. (arithmetic mean: 463 ng/g). Post-mortems indicated that 7 of the kites had internal hemorrhaging that was not associated with detectable trauma; these birds typically had elevated sum SGAR liver concentrations. On the basis of these two factors, it is considered probable that SGARs were a contributory cause of death in these birds.

The exposure pattern observed in 8 red kites from England & Wales analysed by the WIIS was very similar to that observed in birds analysed by the PBMS, with detectable liver residues of difenacoum and brodifacoum in all birds and bromadiolone in most. SGARs were assessed to be a contributory cause of death in two birds. Thus, of the 26 red kites from England & Wales analysed overall, SGARs were considered to be implicated in the deaths of 9 (35%).

Residue data were available through the WIIS for 6 red kites from Scotland that died in 2015. Three kites (50%) had liver residues of at least two SGARs (bromadiolone and difenacoum); brodifacoum was also detected in one of these kites. SGARs were assessed to be a contributory cause of death in the bird that had residues of three SGARs. The data, although sample size is small, suggest that exposure of kites to SGARs may have been less marked in Scotland than in England & Wales in 2015, as has been found for other species.

2. Introduction

2.1 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,b) together with other studies (Dowding *et al.*, 2010, McDonald *et al.*, 1998, Ruiz-Suárez et al., 2016, Shore *et al.*, 2003a,b) have shown that there is widespread exposure to SGARs in a diverse range of predators in Britain. Some mortalities resulting from this exposure have been documented.

In response to conservation concerns over the potential impacts of SGARs on predators, the Centre for Ecology & Hydrology's Predatory Bird Monitoring Scheme (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 Scotland in the late 20th Century as part of an official species recovery programme (Carter and Grice 2002). Red kites are scavengers and their diet may include 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 and potential poisoning, and SGAR-induced deaths of kites have been documented.

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 reintroduced birds may be exposed to SGARs. Subsequent development of a collaboration with the Disease Risk Analysis and Health Surveillance (DRAHS) programme, run by the Institute of Zoology, has meant that the number of red kites available for chemical analysis and reporting of SGAR residues has increased. Red kite necropsies are performed predominantly by the DRAHS, occasionally by the PBMS, and analysis of liver SGARs is undertaken by the PBMS.

SGAR residues in red kites from England & Wales that are suspected of being poisoned are analysed and reported by Fera Science as part of the Wildlife Incident Investigation Scheme (WIIS) for England &Wales. The WIIS is a post-registration monitoring scheme designed to inform the pesticide approval process, and investigates the death or illness of wildlife, pets and beneficial invertebrates that may have resulted from pesticide poisoning. Monitoring through the WIIS for England & Wales and PBMS/DRAHS is complimentary in that carcasses/tissues of kites that died in England & Wales are exchanged so that birds suspected of being poisoned are analysed by WIIS while birds that would not qualify for analysis under the WIIS (typically because poisoning is not suspected) are analysed by the PBMS. The WIIS for Scotland is run by SASA (Science & Advice for Scottish Agriculture) and examines SGAR residues in any raptors found dead in Scotland. Kite carcasses from Scotland that are offered to the PBMS are redirected so that they are submitted to the Raptor Health Scotland study for post-mortem investigation and then onto WIIS for Scotland for chemical analysis. WIIS data (for England & Wales and for Scotland) are collated and published quarterly on line[2]. Data for birds that

[2] http://www.hse.gov.uk/pesticides/topics/reducing-environmental-impact/wildlife/wiis-quarterly-reports.htm

died in 2015 and analysed by the WIIS have kindly been made available for the current report so that as full a picture as possible can be presented for SGAR exposure in red kites in Britain that died in 2015. This complex collaboration between five separate organizations/schemes (PBMS, DRAHS, WIIS for England & Wales, Raptor Health Scotland and the WIIS for Scotland) has been facilitated by the WILDCOMS network, in which all are partners.

2.2 Aims of the current study

Our aims were:

(i) to provide an update on previous studies (Walker *et al.*, 2010, 2012, 2013) by analysing liver SGAR residues in 18 red kites (including 3 birds reported in previous PBMS report (Walker *et al.*, 2016) that had been found dead in 2015 and submitted to the DRAHS/PBMS. In this report we assess the current incidence and magnitude of liver SGAR residues in this sample.

(ii) to provide an overview of all the available information for Britain for kites in 2015 by also detailing the findings of SGAR residues in red kites that were examined and analysed by the WIIS for England & Wales, and the WIIS for Scotland.

3. Methods

The carcasses of 18 red kites that died in 2015 were collected as part of the PBMS and the DRAHS programmes (Table 1 and Fig. 1). Both projects rely on citizen science in that members of the public send in dead birds that they find. A post-mortem examination was undertaken on all carcasses during which age class and sex was determined. In all, one juvenile female, eight adult females, seven adult males, and two adults for which a sex was not determined were examined. 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 in kites submitted were quantified by Liquid Chromatography Mass Spectrometry and the analytical methods used by the PBMS are described in Appendix 2. Taking into account the extract volume, dilution of the extract and the sample weight, the maximum tissue Limit of Detection (LoD) for difethialone and the other four of the active ingredients was 3.1 and 1.5 ng/g wet weight (wet wt.), respectively (Table 1). Liver samples were spiked with deuterated bromadiolone and brodifacoum. Mean (± SD) recovery for deuterated bromadiolone and brodifacoum was 70.0±11.3 and 64.2±7.5%, respectively; sample concentrations were not recovery corrected. Anticoagulant rodenticide concentrations are reported as ng/g wet wt. and were 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).

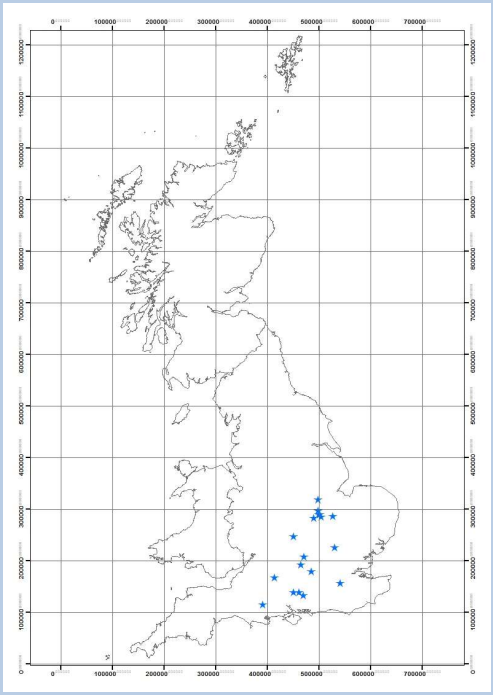

Figure 1. Location of red kites, *Milvus milvus*, found dead in 2015 for which livers were analysed for second generation anticoagulant rodenticides (SGARs) by the PBMS. Locations of kites analysed by the WIIS (Table 2) are not included on the map.

The methods used by Fera and SASA as part of the WIIS are similar in principle to those used by the PBMS but the precise methodology, limits of detection and recoveries will differ to some extent.

4. Results and Discussion

4.1. Red kite carcasses analysed by the PBMS

Summary statistics for the incidence of detectable concentrations of SGARs in the 18 red kites that were analysed are given in Table 1. All contained detectable liver residues of difenacoum and brodifacoum, and 15 (83%) contained detectable bromadiolone residues. Difethialone was detected in five birds (28%) but flocoumafen was not detected in any of the kites. The prevalence of one or more liver SGAR residues was similar to that reported for previous years (95% of kites analysed since 2010 have contained at least one detectable liver SGAR residues (Walker *et al.*, 2010, 2012, 2013, 2016). However, this is the first time that all of the birds tested had detectable residues of brodifacoum. Previously, brodifacoum was found in 16/24 (67%) of kites that died in 2010, in 14/18 (78%) of kites that died in 2011, and in 13/16 (81%) of kites that died in 2012 or 2013 (Walker *et al.*, 2010, 2012, 2013, 2016).

Sum liver SGAR concentrations ranged between 50 and 1266 ng/g wet wt.[3] (equivalent to 0.05 and 1.27 µg/g wet) with an arithmetic mean concentration of 463 ng/g wet wt. Similarly to our previous report (Walker *et al.*, 2016) the majority of birds (83%) had sum SGAR liver concentration > 100 ng/g wet wt., and the median concentration was > 300 ng/g wet wt.

Post mortem examinations by the Institute of Zoology and CEH indicated that seven of the kites (39%) had internal hemorrhaging that was not associated with detectable trauma. These birds had significantly higher sum SGAR liver concentrations than those with no haemorrhaging or with haemorrhaging only associated with physical trauma (student t test on log10 transformed data: t_{14}=2.6, P=0.021; Fig. 2). 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 those seven birds. However, previously we have reported 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 (Walker *et al.*, 2013, 2016). This suggests that although residues may on average be higher in kites thought to have been lethally poisoned by SGARs, deterministic approaches to defining a sum SGAR liver concentration diagnostic of death is difficult. 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.

[3] Liver SGAR residues are sometimes given in units of µg/g wet. Concentrations of 50 and 1266 ng/g wet wt. are equivalent to 0.05 and 1.27 µg/g wet wt.

Table 1. Percentage (%) of red kites with detectable liver SGAR residues and liver concentrations (ng/g wet weight) of individual and of summed (Σ) SGARs. Total number of birds analysed was 18.

	Active Ingredient[1]					
	Brom	Difen	Floc	Brod	Difeth[3]	ΣSGARs[4]
Limit of Detection (ng/g) wet weight	1.5	1.5	1.5	1.5	3.1	-
Number of birds with non- detected (ND) concentrations	3	0	18	0	13	0
Number of birds with detected concentrations	15	18	0	18	5	18
% detected	83	100	0	100	28	100
Summary statistics for liver SGAR concentrations (ng/g wet wt.) in all 18 birds[2]						
Arithmetic mean	92.3	124	-	183	65.1	463
Geometric mean	24.9	67.8	-	50.1	4.39	329
Median	30.6	84.1	-	29.4	ND	373
Minimum	ND	4.50	-	1.90	ND	50.2
Maximum	296	378	-	914	987	1266
Summary statistics for liver SGAR concentrations (ng/g wet wt.) in birds with detected concentrations						
Arithmetic mean	111	124	-	183	231	463
Geometric mean	50.2	67.8	-	50.1	65.6	329
Median	47.9	84.1	-	29.4	30.7	373
Minimum	3.90	4.50	-	1.90	19.8	50.2
Maximum	296	378	-	914	987	1266

ND = Non-detected. [1]Brom = bromadiolone, Difen = difenacoum, Floc = flocoumafen, Brod = brodifacoum, and Difeth = difethialone. [2]Non-detected assigned a value of zero for calculating arithmetic means and medians but a value of 0.75 ng/g wet wt. (½ limit of detection, except for difethialone for which a value of 1.55 ng/g wet wt. assigned) when calculating the geometric mean. [3]Difethialone had a higher limit of detection than other SGARs (see methods) but there were no detected liver concentrations for other SGARs below the difethialone (see section 8); comparison between compounds of % occurrence or magnitude of residues is therefore unaffected by the slight variance in LoD; [4]ΣSGARs calculated as the sum concentrations of individual compounds. ND concentrations for individual compounds assigned a value of zero in this calculation

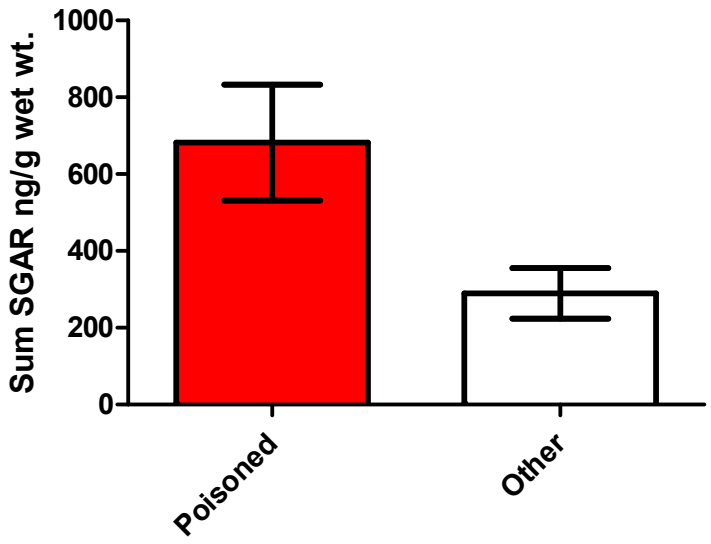

Figure 2. Mean (±SEM) of sum (Σ)SGAR concentrations in red kites with haemorrhaging not associated with physical trauma (poisoned; n=7) and those died from other causes (other; n=10). One of the 18 birds in the current study was excluded from analysis as it was unclear whether observed haemorrhaging was associated with trauma or not.

4.2. Red kites that died in 2015 and were analysed by the Wildlife Incident Investigation Scheme (WIIS)

The data for red kites from England & Wales that died in 2015 and were investigated by the Wildlife Incident Investigation Scheme (WIIS) (Table 2) were similar to those from the PBMS. All of the kites analysed by the WIIS had detectable concentrations of difenacoum and brodifacoum, and almost all had liver residues of bromadiolone. Difethialone was detected in half of the birds but flocoumafen was not detected in any kites. Summed liver SGAR residues ranged between 50 and 283 ng/g. Two birds (incident codes W/15/16 and W/15/41) were considered to have died from rodenticide poisoning and had summed residues of 283 ng/g and 247 ng/g wet weight. These concentrations were lower than (and outside of the lower 95% CL of) the summed residues in the kites analysed by the PBMS and assessed as likely poisoned by SGARs (Figure 2).

Three of the six red kites (50%) from Scotland that died in 2015 and were analysed by the WIIS for Scotland (Table 2) had liver residues of at least two SGARs (bromadiolone and difenacoum); brodifacoum was also detected in only one of these kites. SGAR poisoning was assessed to be the cause of death in the bird that had residues of three SGARs. These frequencies of residue occurrence of different compounds tended to be lower than those for kites from England & Wales that were analysed by the PBMS or by the WIIS, but overall sample size was small. The highest liver ΣSGAR residue in the Scottish kites was similar to that in the kites from England & Wales that were analysed by WIIS, but the median and mean concentrations for the Scottish birds were 14 and 3 fold lower respectively. We undertook a statistical comparison of kite liver ΣSGAR concentrations by country (pooled PBMS and WIIS data for kites from England & Wales vs data for kites from Scotland); birds from Scotland with no detectable residues were included and assigned a zero liver concentration. The analysis indicated that the median liver ΣSGAR residue was significantly higher in kites from England & Wales than in kites from Scotland (Mann-Whitney U test, U=13, P= 0.0018).

Table 2. Concentrations of second generation anticoagulant rodenticides (SGARs) in the livers of red kites found dead in 2015 and analysed through the Wildlife Incident Investigation Scheme.

Laboratory	Incident code	Month of death	Sex	Age	Location	Concentration of SGAR (ng/g wet wt.)					
						Brom	Difen	Floc	Brod	Difeth	Σ SGARs
WIIS-FERA	103/920	March	M	*	Oxfordshire	10	4.3	ND	5.9	30	50
WIIS-FERA	W/15/08	March	M	Adult	Carmarthenshire	0.3	160	ND	65	ND	225
WIIS-FERA	W/15/12	March	M	Adult	Carmarthenshire	70	120	ND	50	4.9	245
WIIS-FERA	W/15/16	April	F	*	West Glamorgan	110	73	ND	4.8	95	283
WIIS-FERA	103/984	April	M	*	North Yorkshire	ND	26	ND	90	ND	116
WIIS-FERA	W/15/22	May	*	*	Dyfed	7.8	44	ND	1.1	ND	53
WIIS-FERA	104/052	May	F	*	Hertfordshire	69	44	ND	13	0.7	127
WIIS-FERA	W/15/41	Nov	*	*	Glamorgan	9	220	ND	18	ND	247
WIIS SASA	15057/1	March	M	2 year old	Highland	ND	ND	ND	ND	ND	ND
WIIS SASA	15069/1	January	M	juvenile	Tayside	ND	ND	ND	ND	ND	ND
WIIS SASA	15088/1	April	U	Unknown	Highland	10	28	ND	ND	ND	38
WIIS SASA	15098/1	May	U	Unknown	Central	5	20	ND	ND	ND	25
WIIS SASA	15120/1	July	M	8 week old	Grampian	116	39	ND	125	ND	280
WIIS SASA	15194/1	Oct	M	1 year	Highland	ND	ND	ND	ND	ND	ND

Brom = bromadiolone, Difen = difenacoum, Floc = flocoumafen, Brod = brodifacoum, and Difeth = difethialone. ND = Non-detected *not stated

5. Conclusions

The monitoring of SGAR residues in red kites remains an important contribution to our understanding of SGAR exposure in wildlife, particularly in relation to predators and scavengers that feed directly on target prey, such as the brown rat. This is the first time that data for SGAR contamination in red kites has been brought together from the work conducted across five UK organisations/schemes and reflects the benefits of enhanced cooperation and coordination that has been facilitated through the WILDCOMS network.

All 18 red kites that were analysed by the PBMS died in 2015 and were from England & Wales. All had detectable liver residues of at least two SGARs, one of which was brodifacoum, a SGAR licensed for "indoor" use only in 2015. This exposure pattern was mirrored by the 8 kites from England & Wales that were analysed by the WIIS. This is the first time that all birds sampled by the PBMS in any one year contained brodifacoum. This may partly reflect the predominance of adult birds in the 2015 sample but may also indicate a growing prevalence of exposure to this compound; the percentage of red kites found by the PBMS to contain brodifacoum has increased since 2010 although any influence of age on this trend is yet to be investigated.

Of the 26 red kites from England & Wales analysed overall, 9 (35%) were considered likely to have been poisoned by SGARs. This already high incidence of poisoning does not include any other type of poisoning that may occur because of illegal use of other pesticides and through exposure to lead (Pain et al., 2007; Jaffe-pers. comm) and suggests that poisoning is a significant mortality factor in red kite populations, especially in areas of England & Wales. Our findings clearly indicate a strong need to continue monitoring the exposure and effects of SGARs on red kites in England & Wales and to determine whether they are altered by rodenticide stewardship and concomitant changes in permitted use, such as authorisation of "in and around building" use for brodifacoum, flocoumafen and difethialone.

Data available for kites from Scotland were limited for 2015 but suggests that exposure to SGARs may be lower than for kites from England & Wales. This is consistent with regional differences in SGAR exposure that have been previously observed in barn owls *Tyto alba* (Shore et al., 2015). Variation in exposure of red kites to SGARs at a regional and sub-regional level may be driven by a number of factors. Future analysis of changes in exposure and effects over time should take into account that there may be differences in exposure patterns between non-contiguous populations within the UK.

6. Acknowledgements

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7. References

Carter I, Grice P. 2002. *The red kite reintroduction programme in England*. English Nature Research Reports No. 451. English Nature, Peterborough.

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*) and weasels (*Mustela nivalis*) in England. *Environmental Pollution,* 103**,** 17-23.

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. *In:* Cowan, D. P. & Feare, C. J. (eds.) *Advances in vertebrate pest management.* Fürth: Filander Verlag.

Pain, D.J., Carter, I., Sainsbury, A.W., Shore, R.F., Eden, P., Taggart, M.A., Konstantinos, S., Walker, L.A., Meharg, A.A., Raab, A. 2007. Lead contamination and associated disease in captive and reintroduced red kites *Milvus milvus* in England. *Science of the Total Environment* 376, 116-127.

Ruiz-Suárez, N., Melero, Y., Giela, A., Henríquez-Hernández, L.A., Sharp, E., Boada, L.D., Taylor, M.J., Camacho, M., Lambin, X., Luzardo, O.P., Hartley, G. 2016. Rate of exposure of a sentinel species, invasive American mink (*Neovison vison*) in Scotland, to anticoagulant rodenticides. *Science of the Total Environment*. *569-570 (Supplement C):*1013-1021.

Shore, R. F., Birks, J. D. S., Afsar, A., Wienburg, C. L. & Kitchener, A. C. 2003a. Spatial and temporal analysis of second-generation anticoagulant 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. *In:* Tattersall, F. H. & Manley, W. J. (eds.) *Conservation and conflict: mammals and farming in Britain. Linnean Society Occasional Publication No. 4.* London: The Linnean Society.

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.

Shore, R.F., Pereira M.G., Potter, E.D. and Walker. L.A. 2015. Monitoring rodenticide residues in wildlife. pp. 346-365 in Rodent pests and their Control: 2nd edition. (Eds, A.P. Buckle and R.H. Smith), Wallingford, Oxfordshire, CAB International, pp. 346-365. ISBN: 978 1 84593 817 8.

Thomas, P. J., Mineau, P., Shore, R. F., Champoux, L., Martin, P. A., Wilson, L. K., Fitzgerald, G. & Elliott, J. E. 2012. Second generation anticoagulant rodenticides in predatory birds: Probabilistic characterisation of toxic liver concentrations and implications for predatory bird populations in Canada (vol 37, pg 914, 2011). *Environment International,* 40**,** 256-256.

Walker, L. A., Chaplow, J. S., Llewellyn, N. R., Pereira, M. G., Potter, E. D., Sainsbury, A. W. & Shore, R. F. 2013. *Anticoagulant rodenticides in predatory birds 2011: a Predatory Bird Monitoring Scheme (PBMS) report*. 17pp.
http://pbms.ceh.ac.uk/sites/pbms.ceh.ac.uk/files/PBMS_Rodenticide_2011.pdf

Walker, L. A., Chaplow, J. S., Moeckel, C., Pereira, M. G., Potter, E., Sainsbury, A. W. & Shore, R. F. 2016. *Anticoagulant rodenticides in red kites (Milvus milvus) in Britain 2010 to 2015: a Predatory Bird Monitoring Scheme (PBMS) report.* Centre for Ecology & Hydrology, Lancaster, UK, 14pp.
http://pbms.ceh.ac.uk/sites/pbms.ceh.ac.uk/files/PBMS_Rodenticide_Red_Kite_2016_FINAL.pdf

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. 17pp.
http://pbms.ceh.ac.uk/sites/pbms.ceh.ac.uk/files/PBMS_Rodenticides_2009.pdf

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. 17pp.
http://pbms.ceh.ac.uk/sites/pbms.ceh.ac.uk/files/PBMS_Rodenticide_2010.pdf

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.

8. Appendix 1 – Results by individual for birds analysed by the PBMS

Table 3. Concentrations of second generation anticoagulant rodenticides (SGARs) in the livers of red kites found dead in 2015 and analysed by the PBMS

PBMS Code	IOZ Code	Month of Death	Sex	Age	Location	Concentration of SGAR (ng/g wet wt.)					
						Brom	Difen	Floc	Brod	Difeth	Σ SGARs
18328		*	F	Adult	N. Hampshire	255	82.5	ND	152	ND	490
18399		*	M	Adult	N. Hampshire	ND	378	ND	25.4	ND	403
18547		Oct	M	Adult	Berkshire	287	13.4	ND	83.8	ND	385
19109	XT 97/16	Apr	F	Adult	N. Wiltshire	235	23.8	ND	30.8	19.8	310*
19110	XT 1050/15	Mar	M	Adult	Hertfordshire	ND	131	ND	414	93.1	638*
19111	XT 719/15	May	F	Adult	Northants	3.9	274	ND	1.9	987	1266*
19112	XT 1257/15	Jul	F	Adult	Northants	6.1	170	ND	26.1	21.7	224
19113	XT 1393/15	Jun	M	Adult	W. Kent	13.2	29.3	ND	15.5	ND	58.0
19114	XT 166/16	Feb	M	Adult	Northants	296	339	ND	53.7	ND	688
19115	XT 785/15	Jul	F	Adult	Dorset	33.4	319	ND	8.6	ND	361*
19118	XT 1274/15	May	F	Adult	N. Hampshire	47.9	41.7	ND	574	ND	664
19121	XT 1114/15	May	U	Adult	Northants	ND	74.3	ND	914	ND	988*
19122	XT 664/15	May	F	Adult	Northants	167	91.2	ND	27	ND	285
19123	XT 125/16	Nov	F	Adult	Oxfordshire	17.8	4.5	ND	27.9	ND	50.2
19124	XT 728/15	Mar	U	Adult	Northants	12	85.7	ND	870	ND	968*
19126	XT 1338/15	Apr	M	Adult	Hunts	66.8	136	ND	22.1	30.7	256
19181	XT 182/15	*	M	1st yr	Leics.	192	16.5	ND	33.5	ND	242*
19191	XT 409/15	*	M	Adult	Bucks	27.8	19.5	ND	10.5	ND	57.8

IOZ=Institute of Zoology; M – male; F- female; U – sex not determined; ND = non-detected;
Brom – bromadiolone; Difen – difenacoum; Floc – flocoumafen; Brod – brodifacoum; Difeth - difethiolone;
*indicates birds with haemorrhaging unassociated with any observed physical trauma. These 7 kites make up the "poisoned" group in Figure 2

9. Appendix 2 - 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.

A sub sample (0.25g) of each liver was thawed, weighed accurately, ground and dried with anhydrous sodium sulfate. Each sample was spiked with labelled standards (d^5- Bromodialone, and d4- Brodifacoum, QMx). Chloroform: acetone (1:1 v/v) was added to each sample and the samples were thoroughly mixed using a vortex.

Samples were extracted on a mechanical shaker (Stuart SF1, Bibby Scientific) for 1h, then centrifuged at 5000 rpm for 5 minutes and the supernatant was transferred to a clean tube. This process was repeated with clean solvent, but the second time, samples were on the mechanical shaker for only 30 minutes. The combined extract was evaporated to dryness using nitrogen, re-dissolved in chloroform : acetone (1:1; v/v) and filtered (O.2 mm PTFE filter). The filtered sample was evaporated to dryness and re – dissolved in acetone: DCM (1:23; v/v).

The sample was re-filtered (0.2mm PFEE filter) and then cleaned using automated size exclusion chromatography (Agilent 1200 HPLC system). The clean extract was evaporated and the residue was re-suspended in chloroform: acetone: acetonitrile (1:1:8; v/v). The extract was further cleaned using solid phase extraction cartridges (ISOLUTE® SI 500mg, 6ml). The cartridges were washed with methanol and activated with acetonitrile. The samples were eluted with acetonitrile and this solvent was then exchanged for the mobile phase.

Analysis was performed using a 'Ultimate 3000' HPLC coupled to a triple quadrupole 'Quantum Ultra TSQ' mass spectrometer (Thermo Fisher Scientific, Hemel Hemsptead; UK) interfaced with an ion max source in Atmospheric Pressure Chemical Ionisation mode (APCI) with negative polarity and operated with Xcalibur software ™ (V.2.0.7.). Analyte separation (10 µL inj. volume) was performed on a Hypersil Gold column (Thermo, 1.9 µm particle size, 50 mm x 2.1mm I.D.) using a H2O : MeOH mobile phase gradient.

The analytes were eluted from the column using a programme which mixed different ratios of mobile phase A: 0.77g/L Ammonium acetate in water and Mobile phase B: 0.77g/L Ammonium acetate in Methanol at a rate of 0.3 ml min-1. Gradient elution started from 70% A and 30% B, increased to 60% B in 2 min and held until 6 min; it was then ramped to 70% B at 8.5 min and finally to 100% B at 12 min, held for 1 min and then returned to starting conditions.

MS/MS was performed in single reaction mode (SRM) using APCI in the negative mode, and characteristic ion fragments were monitored for each compound. Argon was used as the collision gas. Chromatographic peaks were integrated using Xcalibur™ which was also used to generate linear calibration curves with R2>0.99.

For quality control and assurance, in each batch a blank and in house QC were used. The performance of the method was assessed in terms of the limit of detection (LOD), recovery of the internal standards for the analytes and linearity. The rodenticides standards (Dr Ehrenstorfer) were matrix matched. Recovery for the total procedure was calculated using the labelled standards.

Limits of detection (LoD) for each compound are given in Table 1 of the main report. Liver samples were spiked with deuterated bromadiolone and brodifacoum. The mean (± SD) recovery for deuterated bromadiolone and brodifacoum was 70.0±11.3 and 64.2±7.5%, respectively; concentrations in samples were not recovery corrected.