Second generation anticoagulant rodenticide residues in barn owls 2016

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Contents

1 Executive Summary

A wide range of avian and mammalian predators and scavengers in rural Britain is known to be exposed to Second Generation Anticoagulant Rodenticides (SGARs). The barn owl *Tyto alba* is a sentinel for species that are generalist predators of small mammals in rural areas and monitoring of liver SGAR residues in barn owls has been adopted as an element of the monitoring programme undertaken as part of anticoagulant rodenticide stewardship. Monitoring of liver SGAR residues in some 100 barn owls per year is to be conducted in support of stewardship and annually collected data compared with those from 395 barn owls that died between 2006 and 2012 (hereafter termed baseline years), prior to changes in anticoagulant rodenticide (AR) authorisations and onset of stewardship.

The rationale for using data on SGAR residues in barn owls that died between 2006 and 2012 as a baseline was that all measurements had been made using the same analytical techniques, there had been little clear change in exposure over that time period, and the data were the most recent available. The aim of the current study was to measure SGAR exposure in barn owls in 2016, the year in which changes in restrictions on use and the roll out of stewardship were fully implemented.

As in the baseline years, the compounds detected most frequently in barn owls that died in 2016 were bromadiolone, difenacoum and brodifacoum. Overall, 78% of the owls had detectable liver residues of one or more SGAR.

The metrics to be used for stewardship monitoring are reported below in terms of differences between owls that died in 2016 and in baseline years.

- *Numbers of barn owls containing detectable residues of flocoumafen and difethialone.* There was no significant difference in the proportion of barn owls with detectable liver residues of either flocoumafen or difethialone between the baseline years and 2016.

- *The ratio of birds with "low" (<100 ng/g ww) vs "high" (>100 ng/g wet wt.) concentrations for any single SGAR or for ∑SGARs*. There was no significant difference between barn owls from baseline years and from 2016 for any individual compound or for summed SGARs (∑SGARs)

- *Average concentrations of brodifacoum, difenacoum, bromadiolone and ∑SGARs in the cohort of owls with "low" residues (<100 ng/g ww) and "high" residues (>100 ng/g ww*). There was no significant difference between barn owls from baseline years and from 2016 in the concentrations of either "low" or "high" residues for bromadiolone and brodifacoum, or for all residues summed (∑SGARs). The median "low" difenacoum concentration in birds that died in 2016 was significantly lower than in barn owls from baseline years. This partly reflected a decrease in the proportion of owls with detectable difenacoum residues. There were too few 2016 barn owls with "high" difenacoum residues (two birds) to statistically compare to the baseline values.

Overall, the lack of difference in SGAR accumulation by barn owls in 2016 compared within baseline years suggests that, not surprisingly, full implementation of stewardship in 2016 has yet to be reflected by a detectable general reduction in exposure of barn owls.

2 Introduction

2.1 Exposure of non-target predators and their prey to second generation anticoagulant rodenticides (SGARs) in Britain

A wide range of avian and mammalian predators and scavengers in rural Britain are known to be exposed to Second Generation Anticoagulant Rodenticides (SGARs) (McDonald et al., 1998; Newton et al., 1999; Shore et al., 2003a; Shore et al., 2003b; Shore et al., 2006; Walker et al., 2008a; Walker et al., 2008b; Dowding et al., 2010; Hughes et al., 2013; Walker et al., 2014; Ruiz-Suárez et al., 2016). Defra's Wildlife Incident Monitoring Scheme (WIIS)[1] and the Predatory Bird Monitoring Scheme (PBMS- http://pbms.ceh.ac.uk/) have shown that some mortalities are the result. Exposure is generally thought to be secondary in most predators and scavengers but, as many species rarely feed on commensal rodents, exposure is likely due to feeding on non-target small mammal species (Rattner et al., 2014; Shore et al., 2015; Geduhn et al., 2016). In Britain, such non-target species are primarily wood mice *Apodemus sylvaticus* and bank voles *Myodes glareolus,* which will feed on bait they encounter (Brakes and Smith, 2005; Tosh et al., 2012). It has been argued that this exposure scenario may be most significant where SGARs are used around buildings and in open areas. The predominance of difenacoum and bromadiolone (compounds that until recently have been licensed for in and around building and open area use in Britain) in barn owl livers is consistent with this assumption but they are also the most widely used compounds in Britain and residues in predators may also simply reflect predominant usage (Shore, et al., 2015).

The barn owl *Tyto alba* can be considered as a sentinel for species that are generalist predators of small mammals in rural areas. Monitoring of liver SGAR residues in barn owls has demonstrated increases in exposure largely through the 1980s and 1990s, and an overall widespread prevalence of residues (Walker, et al., 2014).

2.2 Changes in SGAR authorisations and implementation of stewardship

Five SGARs are currently authorised for use in the United Kingdom - difenacoum, bromadiolone, brodifacoum, flocoumafen and difethialone. Until recently, only difenacoum and bromadiolone have been authorised for use both *in and around buildings* and in *open areas* in Britain. The other three compounds were restricted to *indoor* use as a mitigation measure to prevent unintentional primary and secondary exposure and poisoning of non-target species. However, a review of the available ecotoxicological data for the five SGARs concluded that they were indistinguishable in terms of environmental toxicity (risks to non-target species) and should be treated in the same way in terms of authorisation (Health & Safety Executive, 2012). This led to a change in the way authorisations are assessed and all five SGARs are currently eligible for similar authorisations that can include *in and around buildings* and potentially *open area* use.

[1] Quarterly WIIS reports are available at http://www.hse.gov.uk/pesticides/topics/reducing-environmental-impact/wildlife/wiis-quarterly-reports.htm

The changes in authorisations for anticoagulant rodenticide (ARs) have been accompanied by the development and implementation of an industry-led stewardship scheme http://www.thinkwildlife.org/stewardship-regime/. Stewardship is intended to coordinate and deliver best practice in terms of use of ARs and thereby minimize (and reduce from current levels) exposure and risk to non-target species from ARs (Buckle et al., 2017). The stewardship scheme in the UK is being implemented by the Campaign for Responsible Rodenticide Use (CRRU- UK - http://www.thinkwildlife.org/about-crru/)

One element of stewardship is a requirement to monitor outcomes. This will involve five elements:

- A periodic survey on the knowledge, attitudes and practices of all professional rodenticide users in order to observe changes over time. A baseline survey has already been conducted in advance of regime implementation.
- The breeding success at 130 selected barn owl nest sites located across five regions of the UK will be monitored to determine year on year fluctuations in nest productivity. This is to provide insight into the potential impact of SGARs on barn owls at the population level.
- An annual report, compiled by CRRU, of WIIS data concerning vertebrate pesticides used in the UK.
- A review of the current state of knowledge of the distribution, severity and practical implications of anticoagulant resistance in UK rodents.
- SGAR residues in the livers of barn owls from across Britain will be monitored annually to determine whether there has been any change in exposure in this wildlife sentinel.

The current report relates to the last of these elements, the monitoring of SGAR residues in barn owls.

The ways in which monitoring of SGAR residues in barn owls could be used to assess the impacts on non-targets of change in authorisation and associated stewardship were outlined in a report by Shore et al. (2014). That report described an analysis that examined how long it would take to detect change [of 10%, 20% and 50%] in liver SGAR concentrations from average levels of 395 barn owls that died between 2006 and 2012. The dataset of residues for 395 barn owls was considered to be a baseline against which to measure future change

Annual monitoring of liver SGAR residues in barn owls will be conducted in support of stewardship using birds that died in 2016 and in later years—changes in authorisations and implementation of stewardship relate to that year.

2.3 Aims of the current study

The rationale for using data on SGAR residues in barn owls that died between 2006 and 2012 was because all measurements had been made using Liquid Chromatography Mass Spectrometry (LCMS), which is more sensitive than older fluorescence methods in terms of detecting residues (Dowding, et al., 2010; Shore, et al., 2015).

The aim of the current study was to measure SGAR exposure in barn owls that died in 2016, the year in which changes in restrictions on use and the roll out of stewardship were fully implemented. We compared SGAR residues in a sample of 100 barn owls that died in 2016 with those in barn owls that died between 2006 and 2012 (baseline years). We also include, for information purposes only, summaries of the data obtained for birds that died in 2015

3 Methods

We analysed 100 barn owls for liver SGAR residues. The owls were collected as part of the Predatory Bird Monitoring Scheme (PBMS). Carcasses were submitted to the PBMS by members of the public throughout the year and were from across the whole of Britain, although predominantly England and Wales, as in previous years (Figure 1). All barn owls received by the PBMS were autopsied and they were found to have died from various causes, but mainly from road traffic collisions or starvation. Any haemorrhaging detected at post-mortem in birds was always associated with signs of trauma and so there was no clear evidence that any individual had died from anticoagulant rodenticide poisoning. Liver subsamples were analysed for difenacoum, bromadiolone, brodifacoum, flocoumafen and difethialone.

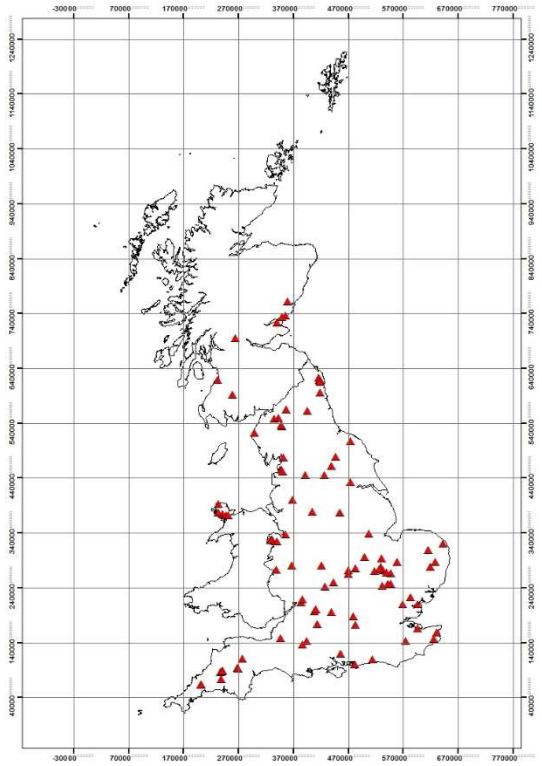

Figure 1. Provenance of the barn owls that died in 2016 and were analysed for liver SGAR residues

The composition of the 100 birds collected in 2016 was 23 adults (10 males, 13 females) and 77 first-years (42 males, 35 females); first year birds were individuals hatched in the current or previous year. Overall the percentage of adults in the 2016 sample was 23% and so within the confidence limits of the baseline datatset (mean: 29.5%, 95% confidence limits: 20.4 – 38.7%). Age is known to have an effect on the magnitude of residues accumulated by barn owls (Walker, et al., 2014) and consistency between years in the proportion of adults in the sample is important.

Chemical determination of residues was by Liquid Chromatography Mass Spectrometry and a summary of the analytical methods can be found in Appendix 1 of this report. To avoid the use of excessively small numbers, AR concentrations in this report are given as ng/g wet weight (ww) throughout. Data used from the report by Shore et al. (2014) were multiplied by 1000 to convert them from µg/g ww to ng/g ww; for example, 0.1 µg/g ww is equivalent to 100 ng/g ww. Limits of detection (LoD) for each compound were 1.5 ng/g ww for all compounds except difethialone that had a LoD of 2.9 ng/g ww. Mean (± SD) recovery for deuterated bromadiolone and brodifacoum that was added to each of the 100 samples was 65.3±9.0 and 64.3±7.9%, respectively.

Shore et al. (2014) outlined how new data on residues should be compared to the baseline dataset. For statistical reasons, this involves dividing the residue data into populations of <100 ng (so called “low” residues) and >100 ng/g ww (“high” residues) and analyzing the two

separately. Barn owls carrying residues of flocoumafen and difethialone were too infrequent in the baseline dataset to permit statistical comparison in subsequent years of changes in the levels of residues for these two compounds. Therefore it was recommended that the following comparisons should be made:

a) Change in the ratio of birds with detectable residues of flocoumafen and difethialone
b) Changes in the ratio *number of owls with "high" concentrations: number of owls with "low" concentrations* for brodifacoum, difenacoum, bromadiolone, ∑SGARs
c) Change in "low" and "high" concentrations of brodifacoum, difenacoum, bromadiolone, and summed SGARs (∑SGARs)

A summary of the proportion of birds with detectable residues in 2016 is given in Section 4.1. This was done for all compounds individually, including flocoumafen and difethialone which is the metric described in (a) above, and for ∑SGARs. The above metrics for (b) and (c) are reported in sections 4.2 and 4.3, respectively. Comparisons between proportions of birds containing residues were by Fisher's Exact test and comparisons of liver SGAR concentrations between owls that died in baseline years and in 2016 were conducted by Mann-Whitney U tests. A probability level of $P<0.05$ was taken as statistically significant. In this report the method of calculation of 25^{th} and 75^{th} percentile of residue values for tables 4 and 5 has been amended to a method that is consistent with a wider range of statistical packages (e.g. SAS method 4, Minitab default, Excel Quartile.EXC method).

4 Results

4.1 General summary of liver SGAR residue data for 2016 owls

As in the baseline years, the compounds detected most frequently in barn owls that died in 2016 were bromadiolone, difenacoum and brodifacoum with between 39% and 61% of owls in 2016 containing detectable residues of each compound (Table 1). Overall, 78% of owls had detectable liver residues of one or more SGAR and almost half had liver residues of more than one compound. The overall prevalence of residues, as judged from the % of owls with ≥ 1 residue, was lower in owls in 2016 (78%) compared with the relatively high value of 94% in 2015, and slightly lower than the overall value (81%) for baseline years (Figure 2).

Table 1. Proportion of barn owls that died in 2016 and had non-detected and detected liver bromadiolone, difenacoum, brodifacoum, ∑SGARs and multiple SGAR residue

	Bromadiolone	Difenacoum	Brodifacoum	∑SGARs	multiple residues
non-detected	39	50	61	22	52
detected	61	50	39	78	48
% detected	61.0%	50.0%	39.0%	78.0%	48.0%

One of the comparator metrics for stewardship is to compare the proportion of 2016 barn owls containing flocoumafen and difethialone with that for owls in baseline years. There was no difference between owls from baseline years and from 2016 in the frequency of detection of difethialone and flocoumafen (Table 2).

Table 2. Proportion of barn owls that had non-detected and detected liver concentrations of flocoumafen and difethialone

	Flocoumafen		Difethialone	
	Baseline	2016	Baseline	2016
non-detected	383	100	394	99
detected	12	0	1	1
% Detected	3%	0%	0.3%	1%
P-value[1]	*0.137*		*0.364*	

[1] P-value determined by Fisher's exact test., P<0.05 considered statistically significant.

The general pattern for all compounds was that the frequency of residue detection in barn owls was lower in the 2016 than in 2015 and similar to that in the baseline years (Figure 2).

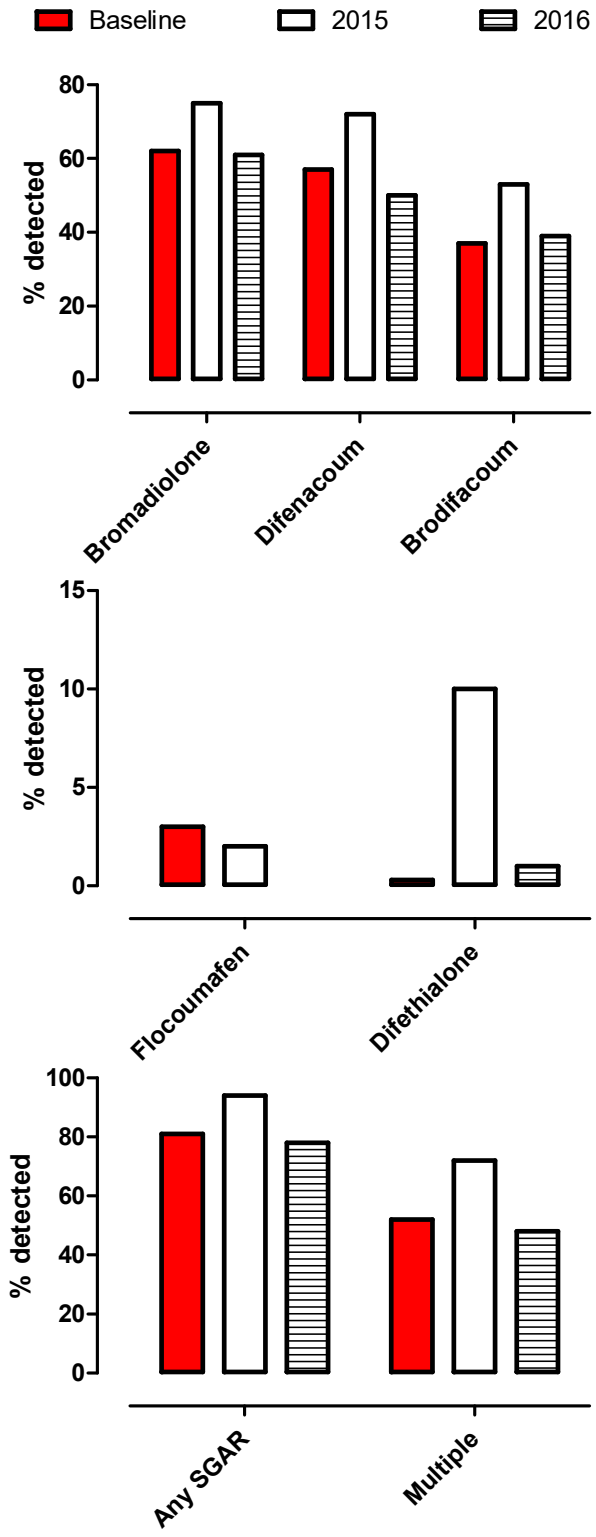

Figure 2. Percentage of barn owls with detected residues of SGARs in their liver. No birds found in 2016 had detectable residues of flocoumafen in their liver.

4.2 Number of owls with liver AR residues above and below 100 ng/g ww

This analysis was conducted for brodifacoum, difenacoum, bromadiolone and ∑SGARs only as there were too few owls with flocoumafen and difethialone residues in the baseline years to conduct this analysis.

There was no significant difference between barn owls from baseline years and from 2016 in the ratio of *birds with "low" (non-detected and <100 ng/g wet wt.) vs "high" (>100 ng/g wet wt.) concentrations* for any single SGAR or for ∑SGARs (Table 3 & Figure 3).

Table 3. Number of barn owls that had "low" (non-detected and <100 ng/g ww) and "high" (>100 ng/g ww) concentrations of SGARs in their liver

	Bromadiolone		Difenacoum		Brodifacoum		∑SGAR	
Conc.	Baseline	2016	Baseline	2016	Baseline	2016	Baseline	2016
<100 ng/g "low"	376	94	375	98	381	96	329	88
>100 ng/g "high"	19	6	20	2	14	4	66	12
% high	4.8%	6.0%	5.1%	2.0%	3.5%	4.0%	16.7%	12%
P-value[1]	*0.612*		*0.277*		*0.769*		*0.285*	

[1] P-value determined by Fisher's exact test., P<0.05 are considered statistically significant

The percentage of owls with "high" residues in all three monitoring periods (baseline years, 2015, 2016) are summarised in Figure 3.

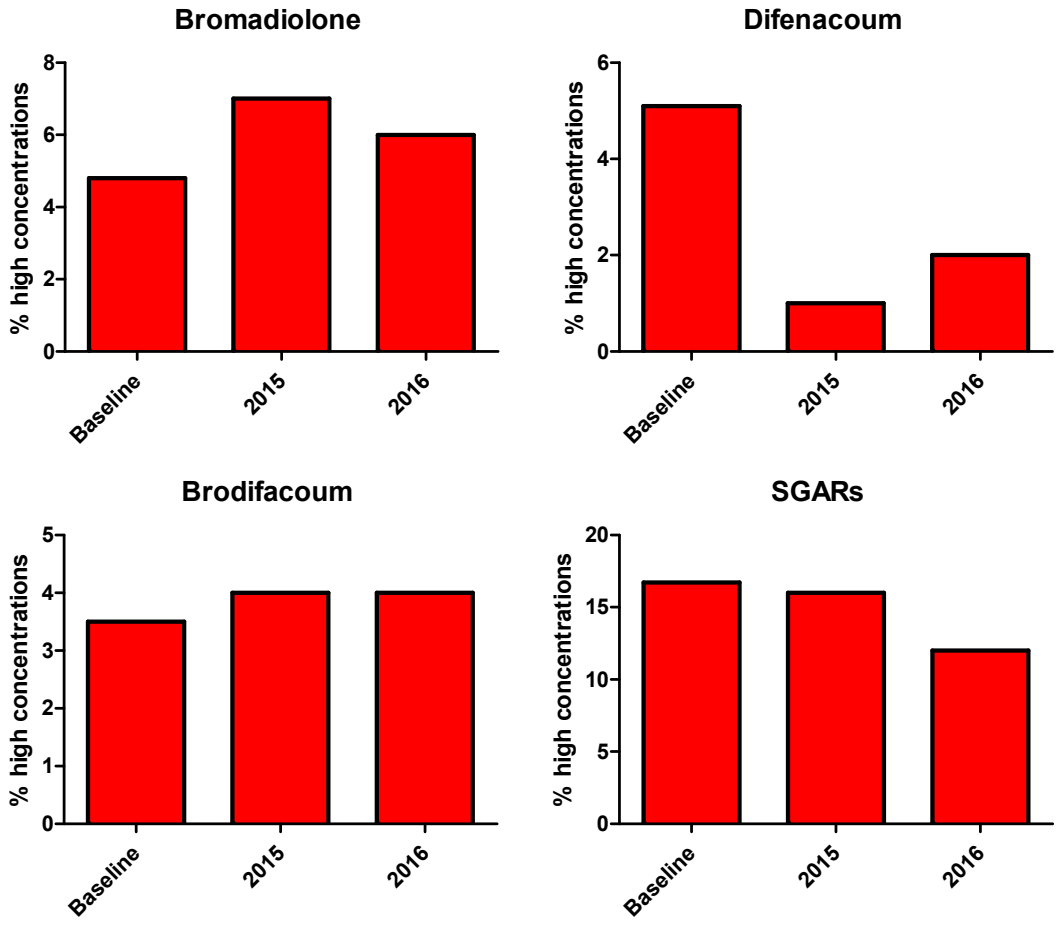

Figure 3. Proportion of barn owls that had "high" (>100 ng/g ww) concentrations of SGARs in their liver.

4.3 Concentrations of brodifacoum, difenacoum, bromadiolone and ∑SGARs in the cohort of owls with residues <100 ng/g wet weight ("low" residues) and >100 ng/g wet weight ("high" residues)

There was no significant difference between barn owls from baseline years and from 2016 in the concentrations of either "low" or "high" residues for bromadiolone and brodifacoum (Table 4), or for ∑SGARs (Table 5)**.** The median "low" difenacoum concentration in birds that died in 2016 was significantly lower than in owls from baseline years (Table 4). Only two barn owls had detected "high" residues of difenacoum and so it was not possible to compare between concentrations for the baseline years and 2016.

Table 4. Median, 25th percentile (Q1), and 75th percentile (Q3) concentrations (ng/g ww) of bromadiolone, difenacoum and brodifacoum in barn owl livers. Non-detected values were assigned a score of zero.

		Bromadiolone			Difenacoum			Brodifacoum		
Conc.		Median	Q1	Q3	Median	Q1	Q3	Median	Q1	Q3
< 100	Baseline	5.0	0.0	17.8	3.1	0.0	12.3	0.0	0.0	5.9
ng/g ww	2016	2.5	0.0	13.0	0.0	0.0	5.1	0.0	0.0	2.8
(low)	MW value[1]	16238			15254			18189		
	P-value	*0.208*			*0.006*			*0.924*		
> 100	Baseline	179	114	224	136	115	160	347	133	923
ng/g ww	2016	135	123	160	138	-	-	562	243	590
(high)	MW value[1]	44.00			-			25.00		
	P-value	*0.426*			-			*0.791*		

[1] Mann-Whitney U value

Table 5. Median, 25th percentile (Q1), and 75th percentile (Q3) concentrations (ng/g ww) of ∑SGARs in barn owl livers. Non-detected values were assigned a score of zero.

		Sum SGAR		
Conc.		Median	Q1	Q3
"Low"	Baseline	15.4	2.8	38.5
	2016	9.3	0.4	32.5
	MW value[1]	13296		
	P-value	*0.237*		
"High"	Baseline	171	123	272
	2016	213	146	470
	MW value[1]	293		
	P-value	*0.156*		

[1]Mann-Whitney U value

5 Discussion

Overall, there were few differences in liver SGAR accumulation between barn owls that died in baseline years and those that died in 2016. As in baseline years, the prevalence of residues in barn owls in 2016 remained widespread; most residues (88% for ∑SGARs) were <100 ng/g wet wt. The only significant differences between owls that died in 2016 and those that died in baseline years were that the median "low" difenacoum concentration was lower in 2016 birds. This reflected the fact that, amongst barn owls in the low difenacoum cohort, the proportion with liver difenacoum concentrations above the detection limit was lower in 2016 (50%) than in baseline years (average of 57%). The result was that the median value decreased from close to the detection limit in baseline years to non-detected in 2016. The reduction in "low" difenacoum residues may be indicative of a reduction in exposure to this compound, but future monitoring is needed to determine whether any such change is sustained.

Shore et al. (2016) suggested that baseline years may underestimate the current extent of usage (and associated exposure of non-target wildlife) of difethialone. This was because the proportion of birds with detectable liver residues of difethialone, albeit relatively small (≤10%), was higher in 2015 than in baseline years. However, the prevalence of difethialone residues in barn owls in 2016 was lower than in 2015 and, in fact, similar to that in baseline years.

Overall, the lack of difference in SGAR accumulation by barn owls in 2016 compared within baseline years suggests that full implementation of stewardship in 2016 has yet to be reflected by a reduction in exposure in barn owls. This is hardly surprising given that full implementation of stewardship did not occur until the middle of 2016, although it may be encouraging that residues were generally a little less prevalent in owls in 2016 than in 2015. It may be expected for cultural and ecological reasons that there will be time-lags between implementation of stewardship, change in use patterns, and detection of change in SGAR accumulation in barn owls. Indeed, the likely time-lag for such detection based solely on the variability of residues between barn owls, was highlighted in the report by Shore et al., (2014). Additional years of monitoring will increasingly reveal the impact of stewardship on SGAR exposure and accumulation in barn owls.

6 Acknowledgements

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7 References

Brakes, C.R., Smith, R.H., 2005. Exposure of non-target small mammals to rodenticides: short-term effects, recovery and implications for secondary poisoning. Journal of Applied Ecology 42, 118-128.

Buckle, A., Prescott, C., Davies, M., Broome, R. (2017) The UK Rodenticide Stewardship Regime. A model for anticoagulant risk mitigation? In: Proceedings of the Ninth International Conference on Urban Pests. Davies, M., Pfeiffer, C., Robinson, W.H. (Eds.). Aston University, Birmingham, 9-12 July 2017. Pp. 165-170. Available at: http://www.icup2017.org.uk/proceedings/.

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.

Geduhn, A., Esther, A., Schenke, D., Gabriel, D., Jacob, J., 2016. Prey composition modulates exposure risk to anticoagulant rodenticides in a sentinel predator, the barn owl. Science of the Total Environment 544, 150-157.

Health & Safety Executive, 2012. Consideration of the environmental risk from the use of brodifacoum, flocoumafen, difethialone, difenacoum and bromadiolone. p. 23 http://www.hse.gov.uk/biocides/downloads/er-sgar.pdf (last accessed 1st September, 2014)

Hughes, J., Sharp, E., Taylor, M.J., Melton, L., Hartley, G., 2013. Monitoring agricultural rodenticide use and secondary exposure of raptors in Scotland. Ecotoxicology 22, 974-984.

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. Filander Verlag, Fürth, pp. 347-367.

Rattner, B.A., Lazarus, R.S., Elliott, J.E., Shore, R.F., van den Brink, N., 2014. Adverse Outcome Pathway and Risks of Anticoagulant Rodenticides to Predatory Wildlife. Environmental Science & Technology 48, 8433-8445.

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.

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. The Linnean Society, London, pp. 37-50.

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., Walker, L.A., 2015. Monitoring rodenticide residues in wildlife, in: Buckle, A.P., Smith, R.H. (Eds.), Rodent pests and their Control, 2nd edition. CAB International, Wallingford, pp. 346-365.

Shore, R.F., Walker, L.A., Potter, E.D., Pereira, G., 2016. Second generation anticoagulant rodenticide residues in barn owls 2015., CEH contract report to the Campaign for Responsible Rodenticide Use (CRRU) UK. Centre for Ecology and Hydrology, Lancaster, UK, p. 17.
http://pbms.ceh.ac.uk/sites/pbms.ceh.ac.uk/files/stewardship-2015-owls_0.pdf

Tosh, D.G., McDonald, R.A., Bearhop, S., Llewellyn, N.R., Montgomery, W.I., Shore, R.F., 2012. Rodenticide exposure in wood mouse and house mouse populations on farms and potential secondary risk to predators. Ecotoxicology 21, 1325-1332.

Walker, L.A., Chaplow, J.S., Moeckel, C., Pereira, M.G., Potter, E., Shore, R.F., 2014. Anticoagulant rodenticides in predatory birds 2012: a Predatory Bird Monitoring Scheme (PBMS) report. Centre for Ecology & Hydrology, Lancaster, UK p. 18.
https://wiki.ceh.ac.uk/download/attachments/134414860/PBMS%134414820Report%134414820Rodentocide%134202012.pdf?version=134414861&modificationDate=1402491816000&api=v1402491816002

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 – Analytical method for determination of SGARs in liver tissues

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 were 1.5 ng/g ww for all compounds except difethialone that had a LoD of 2.9 ng/g ww. Each liver sample was spiked with deuterated bromadiolone and brodifacoum and mean (± SD) recovery for deuterated bromadiolone and brodifacoum that was added to each of the 100 samples was 65.3±9.0 and 64.3±7.9%, respectively.