Second generation anticoagulant rodenticide residues in barn owls 2015

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1 Executive Summary

A wide range of avian and mammalian predators and scavengers in rural Britain are 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. Annual monitoring of liver SGAR residues in some 100 barn owls per year will be conducted in support of stewardship using birds that die in 2016 and in subsequent years. Annual measurements of SGAR liver concentrations in barn owls will be compared with those for 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 because all measurements had been made using the same analytical techniques, there had been little clear change in exposure over time, and the data were the most recent available. However, lack of data for birds that died between 2013 and 2015 meant that there were no data available for SGAR exposure in barn owls immediately prior to change in authorisations and implementation of stewardship. The aim of the current study was to measure SGAR exposure in barn owls that died in 2015, immediately prior to changes in authorisations and stewardship, and to determine if they were similar to concentrations measured in barn owls from baseline years. A secondary purpose of this project was to provide an exemplar analysis and report of residues that could be broadly followed in future years of monitoring.

As in the baseline years, the compounds detected most frequently in barn owls that died in 2015 were bromadiolone, difenacoum and brodifacoum. Overall, most birds (95% of the sample) 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 2015 and in baseline years.

- *Numbers of barn owls containing detectable residues of flocoumafen and difethialone.* The proportion of barn owls with detectable liver residues of difethialone was significantly higher in 2015 than in baseline years but there was no such difference for flocoumafen. The rise in difethialone detections probably reflected the recent entry of this compound into the UK market.
- *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 2015 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 2015 in the concentrations of either low or high residues for bromadiolone and difenacoum, for high brodifacoum

residues, or for ∑SGARs. The median low brodifacoum concentration in birds that died in 2015 was marginally but significantly higher than in barn owls from baseline years. This reflected an increase in the proportion of owls with detectable brodifacoum residues. The median low brodifacoum concentration in 2015 owls was around the detection limit

Overall, the lack of major differences in residue data between birds that died in 2015 and those that died in baseline years suggests that the baseline dataset is largely suitable for assessing future changes that may be associated with new SGAR authorisations and stewardship, perhaps with the exception of difethialone detections. In term of the concentration data, it may be worthwhile pooling the baseline and 2015 concentration data to create a revised baseline against which to judge future changes.

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) (Dowding et al., 2010; Hughes et al., 2013; McDonald et al., 1998; Newton et al., 1999; Ruiz-Suárez et al., 2016; Shore et al., 2003a; Shore et al., 2003b; Shore et al., 2006; Walker et al., 2014; Walker et al., 2008a; Walker et al., 2008b). 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 thought to be due to feeding on non-target small mammal species (Geduhn et al., 2016; Rattner et al., 2014; Shore et al., 2015). In Britain, such non-target species are likely to be 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 is most likely to be significant where SGARs are used around buildings and in open areas. The prevalence 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 Britain - 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 now potentially eligible for similar authorisations and could include *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 form current levels) exposure and risk to non-target species from ARs. The stewardship scheme in the UK is led 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 three 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 of selected barn owl populations will be studied to determine impacts, if any, of rodenticide use.
- 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). This 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 die 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 (a) 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) and (b) more recent data to incorporate into the baseline data were not available. Lack of data for birds that died between 2013 and 2015 means that it is unclear if levels of exposure immediately prior to change in authorisations and implementation of stewardship were similar to those measured in birds between 2006 and 2012.

The aim of the current study was to measure SGAR exposure in barn owls immediately prior to changes in authorisations and stewardship. This involved comparing SGAR residues in a sample of 100 barn owls that died in 2015 with those in barn owls that died between 2006 and 2012 (baseline years). A secondary purpose of this project was to provide an exemplar analysis and report of residues that could be broadly followed in future years.

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 hemorrhaging 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.

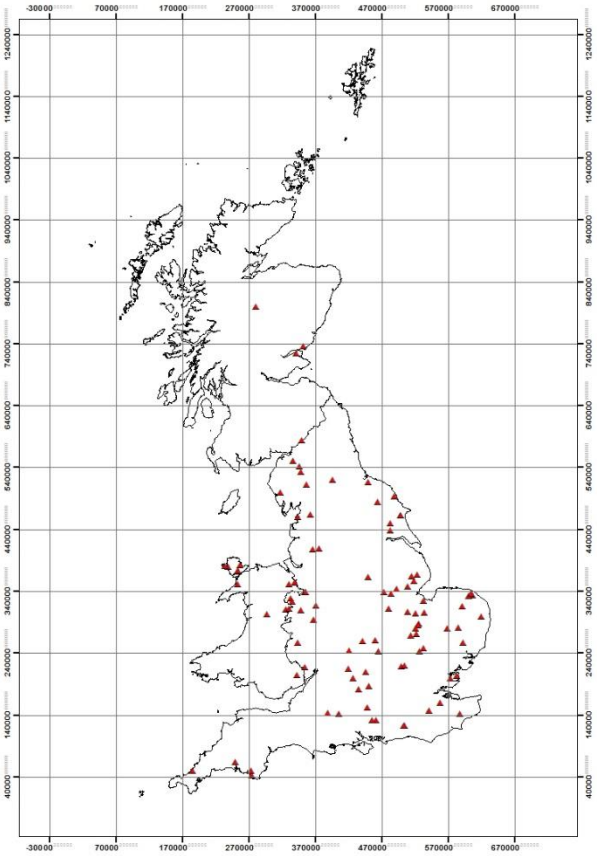

Figure 1. Provenance of the barn owls that died in 2015 and were analysed for liver SGAR residues

The composition of the 100 birds collected in 2015 was 27 adults (9 males, 18 females) and 73 first-years (36 males, 37 females); first year birds were individuals hatched in the current or previous year. Overall the percentage of adults in the 2015 sample was 27% and so was close to the mean for the baseline datatset of 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 downloaded at http://pbms.ceh.ac.uk/docs/AnnualReports/PBMS_Rodenticides_Methods.pdf. 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 for each compound were 1.6 ng/g ww for all compounds, similar to previous years. Recoveries of native or deuterated (bromadiolone) compounds from spiked chicken livers were on average between 70% and 89% depending on compounds and were consistent between batches of samples with standard errors less than 10% of mean values; average deuterated recovery for bromadiolone in samples was 58.1±1.7%.

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. It was recommended that the following comparisons should be made:

a) Change in the ratio of birds with detectable residues of flocoumafen and difethiolone
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 2015 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 2015 were conducted by Mann-Whitney U tests. A probability level of $P<0.05$ was taken as statistically significant.

4 Results

4.1 Summary liver SGAR residue data for 2015 owls

As in the baseline years, the compounds detected most frequently in barn owls that died in 2015 were bromadiolone, difenacoum and brodifacoum with between approximately 50% and 80% of owls in 2015 containing detectable residues of each compound (Table 1). Overall, most birds (94% of the sample) had detectable liver residues of one or more SGAR and almost three-quarters had liver residues of more than one compound.

Table 1. Proportion of barn owls that died in 2015 and had non-detected and detected liver bromadiolone, difenacoum, brodifacoum, ∑SGARs and multiple SGAR residue

	Bromadiolone	Difenacoum	Brodifacoum	∑SGARs	multiple residues
non-detected	23	28	47	6	28
detected	77	72	53	94	72
% detected	77%	72%	53%	94%	72.0%

One of the comparator metrics for stewardship is to compare the proportion of 2015 barn owls containing flocoumafen and difethialone with that for owls in baseline years. The proportion of barn owls with detectable liver residues of difethialone was significantly higher in 2015 than in baseline years. There was no difference in the frequency of detection of flocoumafen (Table 2).

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

	Flocoumafen		Difethialone	
	Baseline	2015	Baseline	2015
non-detected	383	98	394	90
detected	12	2	1	10
% Detected	3%	2%	0.3%	10%
P-value[1]	*0.745*		***<0.001***	

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

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. There were too few owls with detected residues in the baseline years to conduct this analysis

for flocoumafen and difethialone. There was no significant difference between barn owls from baseline years and from 2015 in the ratio of birds with low (<100 ng/g wet wt.) vs high (>100 ng/g wet wt.) concentrations for any single SGAR or for ∑SGARs (Table 3).

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

	Bromadiolone		Difenacoum		Brodifacoum		∑SGAR	
Conc.	Baseline	2015	Baseline	2015	Baseline	2015	Baseline	2015
<100 ng/g "low"	376	93	375	99	381	96	329	84
>100 ng/g "high"	19	7	20	1	14	4	66	16
% high	4.8%	7.0%	5.1%	1.0%	3.5%	4.0%	17%	16%
P-value[1]	*0.450*		*0.094*		*0.769*		*1.000*	

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

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 2015 in the concentrations of either low or high residues for bromadiolone and difenacoum (Table 4), for high brodifacoum residues (Table 4), or for ∑SGARs (Table 5). There was a marginal but statistically significant difference in the low brodifacoum concentrations (Table 4), concentrations. The median low brodifacoum concentration in birds that died in 2015 was around the detection limit whereas it was below the detection limit in owls from baseline years.

Table 4. Median, 25th percentile (Q1), and 75th percentile (Q3) concentrations (ng/g ww) of bromadiolone, difenacoum and brodifacoum in barn owl livers

		Bromadiolone			Difenacoum			Brodifacoum		
Conc.		Median	Q1	Q3	Median	Q1	Q3	Median	Q1	Q3
< 100	Baseline	5.0	0.0	17.7	3.1	0.0	12.2	0.0	0.0	5.9
ng/g ww	2015	5.4	0.0	17.3	2.7	0.0	9.4	0.8*	0.0	6.5
(low)	MW value[1]	16300			18127			16016		
	P-value	*0.300*			*0.709*			***0.031***		
> 100	Baseline	179	115	219	136	122	155	347	141	865
ng/g ww	2015	180	145	200	128	-	-	147	128	268
(high)	MW value[1]	63.00			-			17.00		
	P-value	*0.862*			-			*0.265*		

*Median value appears anomalous because it is below the limit of detection. This is because the number of barn owls with low brodifacoum concentrations was 96 (Table 3) and so the median was the mid-point between the 48th and 49th highest concentrations which were non-detected (taken as zero) and 1.6 ng/g ww (detection limit), respectively.

Table 5. Median, 25th percentile (Q1), and 75th percentile (Q3) concentrations (ng/g ww) of ∑SGARs in barn owl livers

		Sum SGAR		
Conc.		Median	Q1	Q3
Low	Baseline	15.4	2.9	38.5
	2015	15.4	6.8	36.5
	MW value[1]	12822		
	P-value	*0.306*		
High	Baseline	171	124	266
	2015	187	133	216
	MW value[1]	511.0		
	P-value	*0.847*		

[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 2015. The presence of residues was widespread in barn owls. Most residues (84% for ∑SGARs) were <100 ng/g wet wt. in birds that died in 2015. The only significant differences between owls that died in 2015 and those that died in baseline years were that the median "low" brodifacoum concentration and the proportion of birds with detectable liver difethialone residues were both higher in 2015 birds. The slight increase in median low brodifacoum concentration simply reflected the fact that, amongst barn owls in the low brodifacoum cohort, the proportion with liver brodifacoum concentrations above the detection limit was greater in 2015 (53%) than in baseline years (average of 35%). The result was that the median value increased from non-detected in baseline years to around the detection limit in 2015. The increased detection frequency of difethialone in 2015 owls most probably reflected the relative recent entry of this compound into the UK market.

The lack of major differences in residue data between birds that died in 2015 and those that died in baseline years suggests that the baseline dataset is largely suitable for assessing future changes that may be associated with new SGAR authorisations and stewardship. A caveat may be that data from baseline years may well underestimate the current extent of usage (and associated exposure of non-target wildlife) of difethialone, as the proportion of birds with detectable liver residues of difethialone was higher in 2015 than in baseline years. Overall however, it may be reasonable to pool the baseline and 2015 concentration data at least to create a revised baseline against which to judge future changes. An advantage would be that larger datasets are typically more sensitive for detecting change, as the influence of outlier data on overall variance within the dataset is usually smaller. This would likely be the case if liver residue data were pooled; the 5^{th} and 95^{th} percentile of the whole ∑SGAR residues dataset are 0 and 221 ng/g ww when data are pooled and 0 and 232 ng/g ww for the baseline data alone.

The secondary aim of the current study was to produce an exemplar report that would demonstrate how the results of future monitoring are likely to be presented. In the current report, we have simply compared data from measurements made on owls that died in 2015 with those from baseline years using a two sample and goodness-of-fit statistical tests. Tabulation was the easiest and most suitable format for presentation of the summary data. We compared the metrics described by Shore et al. (2014) and used non-parametric analyses because the data were not Normal or even log-Normal in distribution. It is proposed that a similar reporting approach will be used in future, although with increasing years, a parametric or non-parametric analysis of variance approach (that would incorporate data for all years) may be utilised. When data for multiple year are available, data may be better presented in figures rather than tables and time-trends in the data will also be analysed for to determine if there is evidence for consistent change over years.

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