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Second generation anticoagulant rodenticide residues in barn owls 2021

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Contents

1 Executive Summary

The current report is the seventh in a series of annual reports that describe the monitoring of second-generation anticoagulant rodenticide (SGAR) liver residues in barn owls *Tyto alba* in Britain. This work is an element of an overarching monitoring programme undertaken to track the outcomes of stewardship activities associated with the use of anticoagulant rodenticides. The barn owl is used for exposure monitoring as it is considered a sentinel for species that are generalist predators of small mammals in rural areas. The specific work reported here is the measurement of liver SGAR residues in 100 barn owls that died in 2021 at locations across Britain. The residue data are 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.

As in the baseline years, the compounds detected most frequently in barn owls that died in 2021 were brodifacoum, bromadiolone, and difenacoum. Overall, 79% of the owls had detectable liver residues of one or more SGAR.

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 flocoumafen between the baseline years and 2021. There was a significantly higher proportion of barn owls with detectable liver residues of difethialone in 2021 compared to baseline years (6% vs 0.3%), but it was lower than in some of the intervening years (2016-2019).

The ratio of birds with "low" (<100 ng/g wet weight (wet wt.) vs "high" (>100 ng/g wet wt.) concentrations for any single SGAR or for ΣSGARs. There were significantly higher proportion of birds from 2021 with "high" concentrations of brodifacoum and summed SGARs (ΣSGARs) detected in their livers compared to baseline years.

Average concentrations of brodifacoum, difenacoum, bromadiolone and ΣSGARs in the cohort of owls with "low" residues (<100 ng/g wet wt.) and "high" residues (>100 ng/g wet wt.). There was no significant difference between barn owls from baseline years and from 2021 in the concentrations of "high" residues for all SGAR residues, including ΣSGARs. In contrast, "low" bromadiolone and difenacoum residues were significantly lower in 2021 than baseline years, while "low" brodifacoum residues were significantly higher in 2021 than baseline years.

Overall, there were significant differences in liver SGAR accumulation between barn owls that died in baseline years and in 2021: a potential reduction of bromadiolone and difenacoum and an increase of brodifacoum residues from 2016. However, the lack of significant reductions in sum of SGAR residues in barn owls in 2021 suggests that full implementation of stewardship since 2018 has yet to result in a statistically significant reduction in exposure of barn owls to SGARs.

2 Introduction

The current report is the seventh in a series of annual reports describing the magnitude of second-generation anticoagulant rodenticide (SGAR) liver residues in barn owls *Tyto alba* in Britain. The background to, rationale for, and aims of the study remain unchanged from those described in previous reports. They are repeated here in Sections 2.1-2.3 so that the current report can be read as a stand-alone publication.

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

Avian and mammalian predators and scavengers in rural Britain are widely 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; Sainsbury et al., 2018). Defra's Wildlife Incident Investigation Scheme (WIIS)[1] and the Predatory Bird Monitoring Scheme (PBMS- http://pbms.ceh.ac.uk/) have suggest that exposure can lead to some mortalities. 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). 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 historically were the only SGARs licensed for in and around buildings and open area use in Britain) in barn owl livers in past years is consistent with this assumption. However, these SGARs were also the most widely used compounds in Britain and residues in predators may simply reflect predominant usage (Shore, et al., 2015).

The barn owl can be considered as a sentinel for demonstrating exposure to SGARs in generalist predators of small mammals in rural areas in the UK and elsewhere; SGAR residues have been detected in this species in Canada, Denmark, France, and Spain (López-Perea & Mateo, 2018). Monitoring of liver SGAR residues in barn owls in Britain has demonstrated increases in exposure largely through the 1980s and 1990s, and current widespread prevalence of residues (Walker, et al., 2014). However, there is no evidence of an associated adverse effect on barn owl populations. Previous declines in barn owl numbers are more likely to have been the indirect consequence of the earlier use of organochlorine pesticides and subsequent changes in the agricultural management of grassland (Smith and Shore, 2015). At the last comprehensive census of the population conducted during the period 1995-

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

97, there was an estimated 4,000 breeding pairs of barn owls in the UK (Toms et al., 2001). More recently, the UK population has been estimated to be in the range 9,000 to 12,000 breeding pairs (Prescott et al., 2019). Additionally in 2015 the barn owl population status in the UK was moved from amber to green on the UK Bird of Conservation Concern assessment and IUCN threat status category of Least Concern indicating that the species occurs regularly in the UK (Stansbury et al., 2021).

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. As previously stated, only difenacoum and bromadiolone were historically 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 reduce 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 in the UK (Health & Safety Executive, 2012). This led to a change in the way authorisations are assessed and all five SGARs are currently eligible for broadly similar authorisations that can include in and around buildings and, potentially, open area use. However, industry has voluntarily agreed to make no applications for authorisations for the use of brodifacoum, difethialone and flocoumafen in open areas (A. Buckle pers. comm.).

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 minimise (and reduce from current levels) exposure and risk to non-target species (Buckle et al., 2017). A 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 involves 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 had been conducted in advance of regime implementation and follow-up studies were undertaken in 2017 and 2021.
- An annual report of WIIS data concerning vertebrate pesticides used in the UK.
- Reviews of the current state of knowledge of the distribution, severity and practical implications of anticoagulant resistance in UK rodents (Jones et al., 2019; Buckle et al., 2020; Buckle et al., 2022).

- SGAR residues in the livers of barn owls from across Britain are monitored annually to determine whether there has been any change in exposure in this wildlife sentinel.
- Although not a formal monitoring requirement, the breeding success at 130 selected barn owl nest sites located across five regions of the UK are monitored to determine year on year fluctuations in nest productivity (see Prescott et al., 2019). This is to examine certain barn owl breeding parameters in the presence of the SGAR residues found in the UK barn owl population.

This report relates to 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 (i.e., before the implementation of stewardship). 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 is currently conducted in support of stewardship and uses birds that died in 2016 and in later years—changes in authorisations and implementation of stewardship relate to 2016 and thereafter. This report considers birds that died in 2021.

2.3 Aims of the current study

The rationale for using data on SGAR residues in barn owls that died between 2006 and 2012 as a baseline measurement against which future changes would be assessed is described by Shore et al. (2014). This time period was chosen partly 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 current report describes liver SGAR concentrations in barn owls that died in 2021. In this report, we compare SGAR residues in a sample of 100 barn owls that died in 2021 with those in barn owls that died between the 2006 and 2012 (baseline) years. We also include, for information purposes, summaries of the data obtained for birds that died in 2015 (pre-stewardship) and the intervening years. The stewardship scheme for anticoagulant rodenticides came into force in mid-2016 as re-registration of products for use in the UK was completed with a requirement for proof of competence at point of sale. Further stewardship measures came into effect in 2017 and 2018.

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 (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. In all but three birds, any haemorrhaging detected at post-mortem in birds was always associated with signs of trauma, and there was no clear evidence that those 97 individuals had died from anticoagulant rodenticide poisoning. One of the three remaining birds showed haemorrhaging in the body cavity, liver, and kidneys with possible trauma. The death of the bird could be due to anticoagulant rodenticide poisoning. Another showed bruising on right pectoral muscle and wing with subcutaneous haemorrhage, and the other showed haemorrhage in right wing muscle. However, cause of haemorrhaging in both birds was unclear from circumstance because no clear fracture was observed in them.

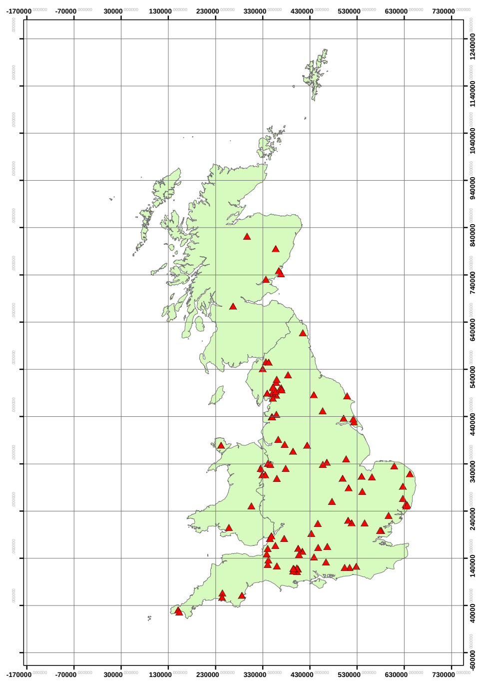

Figure 1. Provenance of the barn owls that died in 2021 and were analysed for liver SGAR residues

The composition of the 100 birds collected in 2021 was 33 adults (19 males, 14 females) and 67 first-years (41 males, 23 females, and three individuals of unknown sex); first year birds were individuals hatched in the current or previous year. Overall, the percentage of adults in the 2021 sample was 33% and so within the confidence limits of the baseline dataset (mean: 29.5%, 95% confidence limits: 20.4 – 38.7%). Age has 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 therefore important. For birds received by the PBMS and not analysed, tissue samples are retained in the PBMS tissue archive.

Liver subsamples were analysed for difenacoum, bromadiolone, brodifacoum, flocoumafen and difethialone. 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. AR concentrations in this report are given as ng/g wet weight (wet wt.) throughout. Data used from the report by Shore et al. (2014) were multiplied by 1000 to convert them from µg/g wet wt. to ng/g wet wt.; for example, 0.1 µg/g wet wt. is equivalent to 100 ng/g wet wt.. Limits of detection (LoD) for each compound were 1.5 ng/g wet wt. for all compounds except difethialone that had a LoD of 3.0 ng/g wet wt., which is consistent over the baseline and monitoring period. Mean (± SD) recovery for deuterated bromadiolone and brodifacoum standards that were added to each of the 100 samples was 74.3±9.9 and 76.3±8.5%, 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 two populations: (i) so called “low” residues which are <100 ng/g wet wt. and include non-detected values (assigned a numerical value of zero), and (ii) “high” residues which are >100 ng/g wet weight. These two datasets were analysed separately. This approach was used for liver difenacoum, bromadiolone and brodifacoum residues and for summed concentrations (ΣSGARs); summed residues were calculated as the arithmetic sum of the residues of any of the five SGARs that were measured. For flocoumafen and difethialone, there were few barn owls in the baseline dataset with liver residues of either compound and statistical comparison with concentrations in later years was not possible. Change in exposure to each of these two compounds was assessed through comparison of the proportion of birds with detectable residues in baseline and in subsequent years.

Overall, three metrics of change were assessed as per Shore et al. (2014):

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 of flocoumafen and difethialone in 2021 (metric (a)) is given in Section 4.1. This metric is also given for the other SGARs and for ΣSGARs but for information only. The above metrics for (b) and (c) are reported in sections 4.2 and 4.3, respectively. Comparisons between baseline years and 2021 for the proportions of birds that had detectable residues were by Fisher’s Exact test. Comparisons of liver SGAR concentrations between owls that died in baseline years and in 2021 were conducted by Mann-Whitney U tests. A probability level of P<0.05 was taken as statistically significant.

Although comparison between the baseline and current year is the metric required for stewardship reporting, change over years can also be informative and the change in metrics from baseline is shown for years 2015 to 2021 for information (Figures 3-6). Time trend analysis was conducted on prevalence and magnitude of detected residues and the magnitude of residues.

4 Results

4.1 General summary of liver SGAR residue data for 2020 owls

As in the baseline and subsequent years, the compounds detected most frequently in barn owls that died in 2021 were bromadiolone, difenacoum, and brodifacoum. Between 41% and 67% of 2021 owls contained detectable residues of each of these compounds (Table 1). Overall, 79% of owls in 2021 had detectable liver residues of one or more SGAR. The equivalent figure in the baseline years was 81% and it has varied between 78% (2016) and 94% (2015) subsequently (Figure 2). Some 56% of the owls in 2021 had multiple compounds in their livers. This is the first year, since the monitoring, that the proportion detectable liver residues of difenacoum was significantly lower than in baseline years (Figure 1). In contrast, the proportion of detectable liver brodifacoum residues was significantly higher than in baseline years, like the previous year 2020.

Table 1. Proportion of barn owls that died in 2021 and had non-detected and detected liver bromadiolone, difenacoum, brodifacoum, ΣSGARs, and multiple SGAR residues**.**

	Brom[1]	Difen[1]	Brod[1]	∑SGARs	multiple residues
non-detected	44	59	33	21	44
detected	56	41	67	79	56
% detected	56%	41%	67%	79%	56%

[1] Brom: bromadiolone, Difen: difenacoum, Brod: brodifacoum

One of the metrics for stewardship is the proportion of barn owls with detectable liver flocoumafen or difethialone residues in 2021 compared with in baseline years. Similarly to previous monitoring years, except 2016, there was a significantly higher proportion of birds with detectable liver residues of difethialone in 2021 than in baseline years (Fisher exact test, $P<0.001$). Flocoumafen had the same level of prevalence in 2021 as in baseline years, namely 2% of birds had detectable residues of this compound (Table 2).

Linear regression analysis on the arcsine square-root transformed proportion of barn owls that had detected residues indicated that for both flocoumafen and difethialone there was not a significant time trend ($F_{1,6} \leq 1.82$, $P \geq 0.226$). However, the same analysis for the other SGARs indicates that the proportion of birds with detected brodifacoum residues significantly increased over time ($F_{1,6}=17.3$, $P=0.006$, $R^2 = 0.742$; Figure 3).

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

	Flocoumafen		Difethialone	
	Baseline	**2021**	**Baseline**	**2021**
non-detected	383	98	394	94
detected	12	2	1	6
% Detected	3%	2%	0.3%	6%
P-value[1]	*0.746*		*<0.001*	

[1] P-value determined by Fisher's exact test.

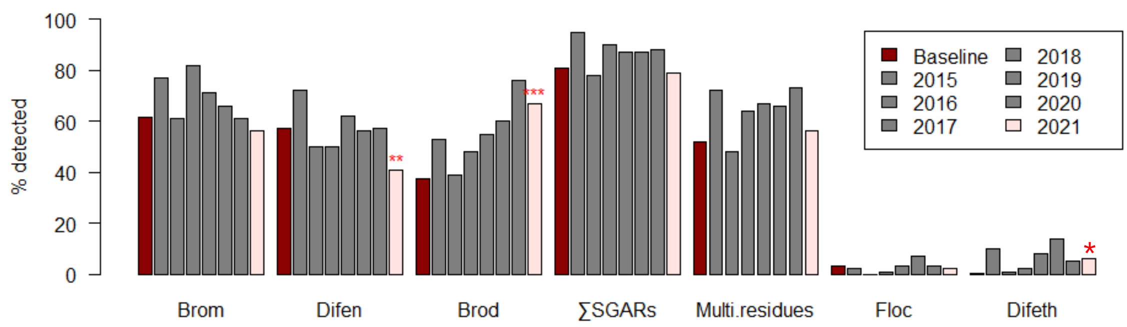

Figure 2. Percentage of barn owls with detected residues of SGARs in their liver. No birds found in 2016 had detectable residues of flocoumafen in their liver. Brom: bromadiolone; Difen: difenacoum; Brod: brodifacoum; Floc: flocoumafen, Difeth: difethialone. Statically significant differences between baseline and the most recent year are indicated: * = P<0.05, ** = P<0.01, *** = P<0.001.

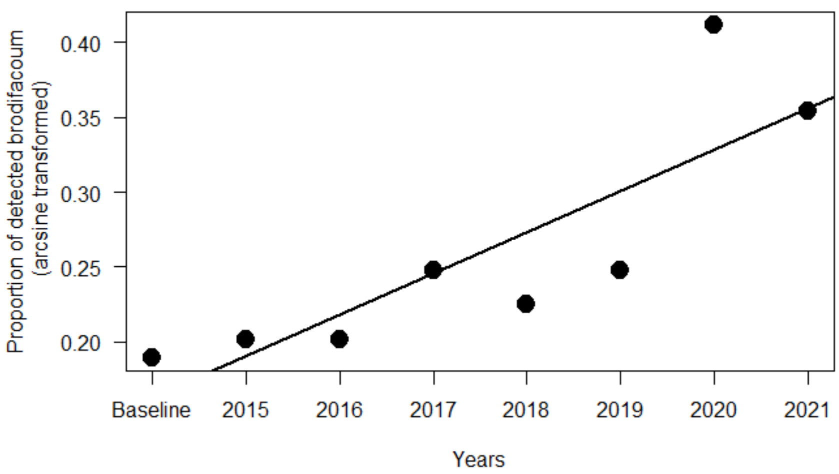

Figure 3. Linear regression analysis on the relationship between the arcsine square-root transformed proportion of barn owls and years ($F_{1,6}$=17.3, P=0.006, R^2 = 0.742).

4.2 Number of owls with liver AR residues above and below 100 ng/g wet wt.

This analysis was conducted for brodifacoum, difenacoum, bromadiolone and ΣSGARs only.

For bromadiolone and difenacoum there was no significant difference between barn owls from baseline years and 2021 in the ratio of birds with "low" (<100 ng/g wet wt.) vs "high" (>100 ng/g wet wt.). However, there was a significantly higher proportion of birds with "high" concentrations of brodifacoum and ΣSGARs (Table 3).

The percentages of owls with "high" residues in all eight monitoring years/periods are shown in Figure 4. Like the previous year, the values for brodifacoum, exceeded 10% and that the value for ΣSGARs exceeded 25%.

Spearman rank correlation analysis showed a positive relationship between proportion of birds with "high" brodifacoum concentrations and year for baseline through to 2021 (Spearman r=0.928, P=0.001). There was no statistically significant correlation for other SGARs.

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

	Bromadiolone		**Difenacoum**		**Brodifacoum**		**∑SGAR**	
Conc.	Baseline	2021	Baseline	2021	Baseline	2021	Baseline	2021
<100 ng/g "low"	376	96	375	99	381	88	329	74
>100 ng/g "high"	19	4	20	1	14	12	66	26
% high	4.8%	4%	5.1%	1%	3.5%	12%	16.7%	26%
***P-value*[1]**	0.999		0.094		0.002		*0.043*	

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

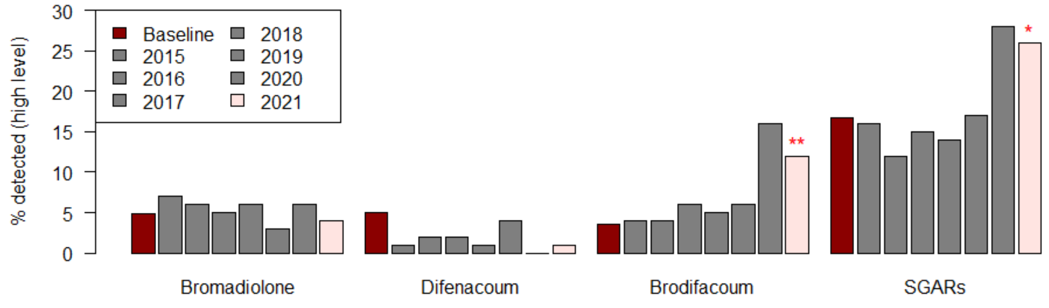

Figure 4. Proportion of barn owls with "high" (>100 ng/g wet wt.) liver SGAR concentrations. No birds found in 2020 had "high" residues of difenacoum in their liver. Statically significant differences between baseline and the most recent year are indicated: * = P<0.05, ** = P<0.01, *** = P<0.001.

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)

For individual SGAR active ingredients, "low" residues of bromadiolone and difenacoum were significantly lower in 2021 than baseline years, whereas "low" residues of brodifacoum were significantly higher in 2021 than baseline. For the magnitude of "high" residues, there were too few birds in order test whether the magnitude was significantly different between the two years for bromadiolone and difenacoum. For the magnitude of "high" residues of brodifacoum, there was no significant difference between 2021 and baseline years.

Although comparison between the baseline and current year is the metric required for stewardship monitoring, change over years can also be informative and is shown in Figures 5 and 6. For "low" residues of bromadiolone and difenacoum, there were no apparent temporal trends: the median concentrations for "low" concentrations for these two active ingredients were low in year 2016 and 2017, then became at the same level as baseline in 2018, but tended to decrease from 2018 onwards (Figure 5). In contrast, "low" residues of brodifacoum clearly show an increasing trend over time from 2016. In fact, "low" brodifacoum residues in 2015, 2017, 2018, 2019, 2020, and 2021 were significantly higher than baseline years, and Spearman rank correlation analysis on the median concentrations of the years showed a statistically significant and positive relationship (Spearman r=0.854, P=0.007). Contrary to "low" residues, the descriptive statistics for "high" concentrations have generally been similar among years (Figure 6).

For ΣSGARs, there was no significant difference between barn owls from baseline years and 2021 in the magnitude of "high" residues (Tables 5 and Figure 6).

However, the magnitude of "low" ΣSGARs residues were lower in 2021 than baseline years but this was not statistically significant (P = 0.054) (Tables 5 and Figure 5).

Although no significant difference was observed in the magnitude of ΣSGARs, the magnitude of "low" ΣSGAR excluding brodifacoum residues in 2016 and 2021 were significantly lower than baseline years (Figure 7). However, Spearman rank correlation analysis on the median of "low" ΣSGAR excluding brodifacoum residues of the years showed no statistically significant relationship over years (Spearman r=-0.643, P= 0.0962). No difference was observed in the magnitude of "high" ΣSGAR excluding brodifacoum residues.

Table 4. Median, 25th percentile (Q1), and 75th percentile (Q3) concentrations (ng/g wet wt.) of bromadiolone, difenacoum and brodifacoum in barn owl livers. Non-detected values were assigned a score of zero. Sample numbers (N) given in Table 3.

		Bromadiolone[2]			**Difenacoum**[2]			**Brodifacoum**		
Conc.		Median	Q1	Q3	Median	Q1	Q3	Median	Q1	Q3
< 100 ng/g wet wt. (low)	Baseline	5.0	0.0	17.8	3.1	0.0	12.3	0.0	0.0	5.9
	2021	2.0	0.0	8.3	0.0	0.0	2.9	4.4	0.0	15.7
	MW value[1]	20639 / 15457			22916 / 14209			11771 / 21757		
	P-value	0.024			<0.001			<0.001		
> 100 ng/g wet wt. (high)	Baseline	179	114	224	136	115	160	347	133	923
	2021	147	-	-	157	-	-	195	134	410
	MW value[1]							105/63		
	P-value							0.297		

[1] Mann-Whitney U values
[2] None of the 100 barn owls tested had detected "high" residues of difenacoum and so it was not possible to compare between concentrations for the baseline years and 2021 for this compound.

Table 5. Median, 25th percentile (Q1), and 75th percentile (Q3) concentrations (ng/g wet wt.) of ∑SGARs in barn owl livers. Non-detected values were assigned a score of zero. Sample numbers (N) given in Table 3.

		Sum SGAR		
Conc.		Median	Q1	Q3
"Low"	Baseline	15.4	2.8	38.5
	2021	8.4	0.0	22.9
	MW value[1]	13907/10439		
	P-value	0.054		
"High"	Baseline	171	123	272
	2021	149	122	200
	MW value[1]	957/759		
	P-value	0.393		

[1]Mann-Whitney U values

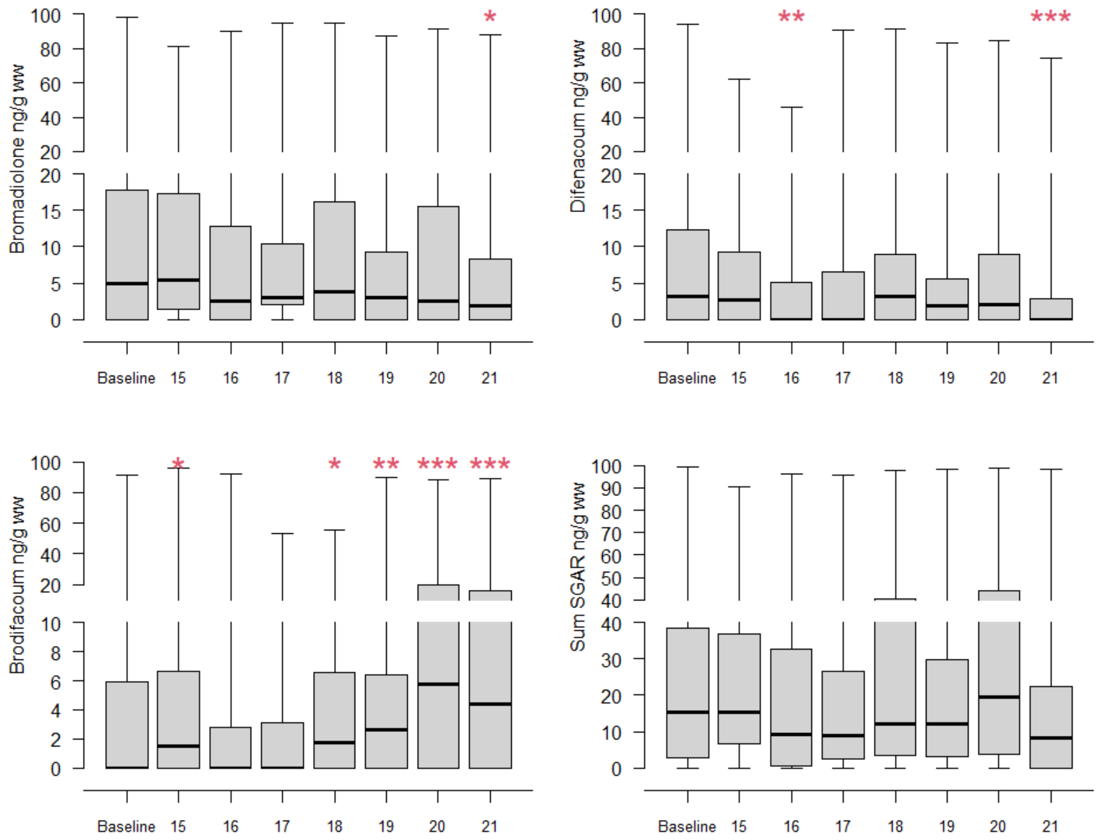

Figure 5. Box and whiskers plot of brodifacoum, difenacoum, bromadiolone and ΣSGARs liver concentrations in the cohort of owls with residues <100 ng/g wet weight ("low" residues) found dead in the 2006-2012 (Baseline), and single years 2015 to 2021. Horizontal line, box and whiskers represent median, 25-75th quartile range and minimum maximum range, respectively. Statically significant differences between baseline and a subsequent year are indicated: * = P<0.05, ** = P<0.01, *** = P<0.001.

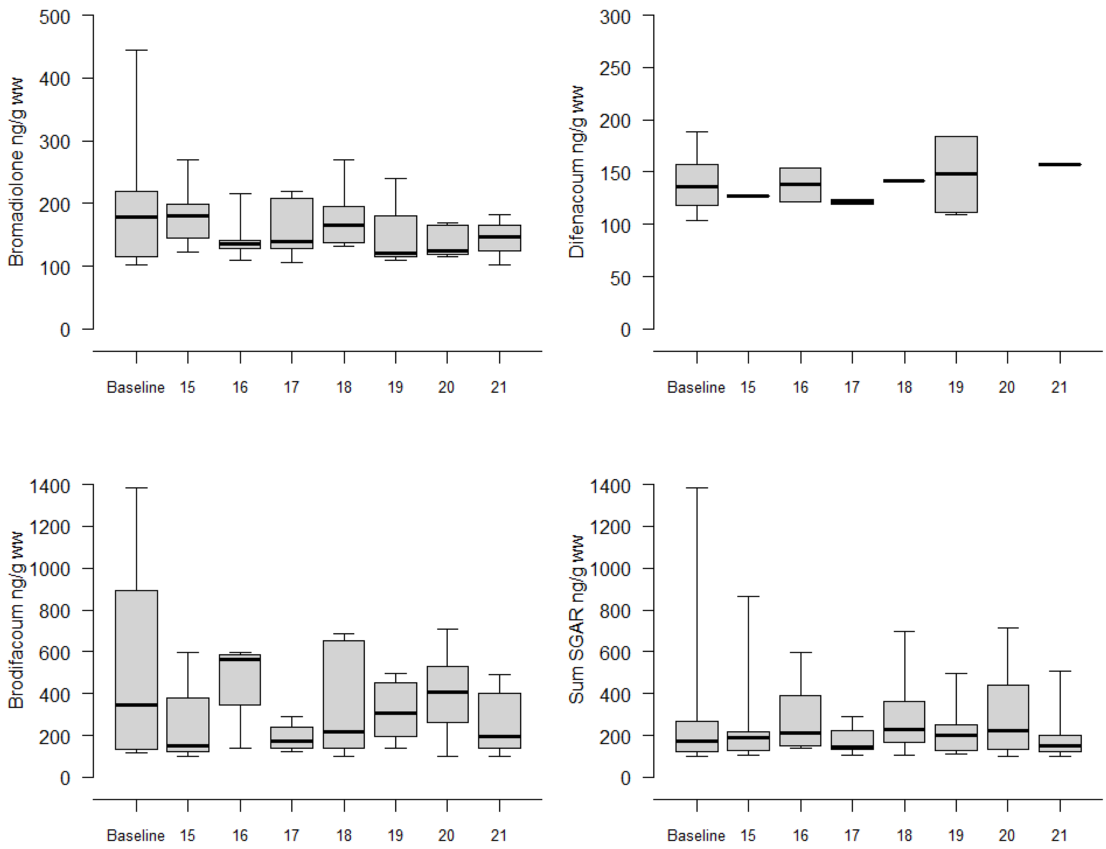

Figure 6. Box and whiskers plot of brodifacoum, difenacoum, bromadiolone and ΣSGARs liver concentrations in the cohort of owls with residues >100 ng/g wet weight ("high" residues) found dead in the 2006-2012 (Baseline), and years 2015 to 2021. Horizontal line, box and whiskers represent median, 25-75th quartile range and minimum maximum range, respectively.

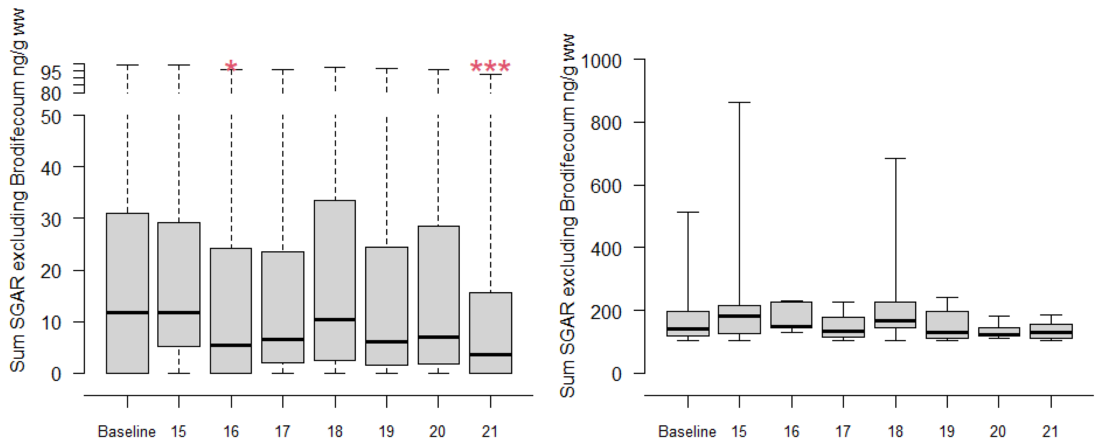

Figure 7. Box and whiskers plot of liver concentrations of ΣSGARs excluding brodifacoum in the cohorts of owls with "high" and "low" residues found dead in the 2006-2012 (Baseline), and single years 2015 to 2021. Horizontal line, box and whiskers represent median, 25-75th quartile range and minimum maximum range, respectively. Statically significant differences between baseline and a subsequent year are indicated: * = P<0.05, ** = P<0.01, *** = P<0.001.

5 Discussion

Overall, we observed significant differences in liver SGAR metrics between barn owls that died in baseline years and those that died in 2021. Particularly, the metrics relating to difenacoum and brodifacoum in 2021 show contrasting results when compared to baseline years.

The one bird that showed haemorrhaging in the body cavity at necropsy with possible signs of trauma had ∑SGAR liver concentration of 0 ng/g wet weight. Despite the necropsy observations, it is clear that SGAR exposure did not contribute to the death of this bird. For the two other suspicious birds, ∑SGAR liver concentrations were 159.6 and 380.1 ng/g wet weight. It is uncertain that SGAR exposure contributed to the death of the birds in this year.

As in baseline years, residues of one or more active ingredients were present in most barn owls in 2021, but most residues (74% for ∑SGARs) were < 100 ng/g wet wt. There were statistically significant differences between baseline years and 2021 in terms of prevalence or magnitude of detectable concentrations: The prevalence of difethialone and brodifacoum residues increased, while the prevalence of difenacoum residues were decreased. The increase in difethialone compared of baseline years reflects that this SGAR was new to the market in baseline years. Overall, detection rates remain relatively low even in 2021. A significantly higher proportion of birds had “high” concentrations of brodifacoum compared to baseline years, and the magnitude of “low” residues of brodifacoum had increased over the monitoring period.

As with result from the previous year, an increase in the magnitude of low residues of brodifacoum and an increase in the proportion of birds with high residues of this active ingredient may reflect a shift in the distribution of residues to higher concentrations compared to baseline years (Figure 8). It is evident from our results that exposure to brodifacoum may be increasing. Moreover, our results on brodifacoum residues and sum of the other active ingredient suggest that the increase in low brodifacoum residues might be compensating for declines in the other active ingredients at low resides. With a decrease in exposure to difenacoum and bromadiolone, our results may indicate a change in usage and relative exposure to barn owls for these active ingredients.

Nonetheless, ΣSGARs remain almost unchanged from baseline years. The proportion of birds with “high” ΣSGARs concentrations were higher than baseline years but the magnitude of “low” residues, which represents the majority of owls analysed, was lower in 2021 than baseline years, although this difference was not significant. The lack of reductions in ΣSGAR residues in barn owls in 2021 suggests that implementation of stewardship since 2016 has yet to result in a statistically significant reduction in overall exposure of barn owls to SGARs, and in the case of brodifacoum there is evidence that exposure in increasing.

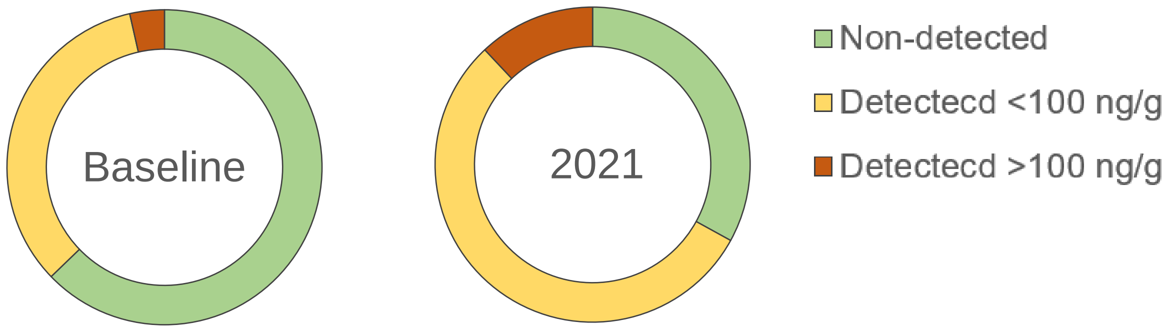

Figure 8. Percentage of birds in baseline years and 2021 that had either non-detected, detected but low residues, or detected high residues of brodifacoum present in their livers.

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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 d^4- 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 (0.2 mm Polytetrafluoroethylene, PTFE, filter). The filtered sample was evaporated to dryness and re – dissolved in acetone: Dichloromethane(1:23; v/v).

The sample was re-filtered (0.2 mm PTFE 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 injection volume) was performed on a Hypersil Gold column (Thermo, 1.9 µm particle size, 50 mm x 2.1mm I.D.) using a H_2O:methanol mobile phase gradient.

The analytes were eluted from the column using a programme which mixed different ratios of mobile phase, A: 0.77 g/L ammonium acetate in water and mobile phase B: 0.77 g/L ammonium acetate in methanol at a rate of 0.3 ml min^{-1}. Gradient elution started from 70% for mobile phase A. For mobile phase B is started at 30%, increased to 60% after 2 min and held until 6 min; it was then ramped to 70% at 8.5 min and finally to 100% 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) were 1.5 ng/g wet wt. for all compounds except for difethialone that had a LoD of 3.0 ng/g wet wt. Each liver sample was spiked with deuterated bromadiolone and brodifacoum and the mean (± SD) recovery for deuterated bromadiolone and brodifacoum that was added to each of the 100 samples was 74.3±9.9 and 76.3±8.5%, respectively.

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