UK Centre for Ecology & Hydrology

Second generation anticoagulant rodenticide residues in barn owls 2022

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Date 24/10/2023

Title Second generation anticoagulant rodenticide residues in barn owls 2022

Client Campaign for Responsible Rodenticide Use

UKCEH reference Project No. 05766

This report should be cited as Ozaki, S., Carter, H. Chaplow, J.S., Dodd, B.A, Pereira, M.G., Potter, E.D., Sleep, D., Toon, B., and Walker, L.A., 2023. Second generation anticoagulant rodenticide residues in barn owls 2022. UKCEH contract report to the Campaign for Responsible Rodenticide Use (CRRU) UK, pp. 26

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1 Executive Summary

The current report is the eighth 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 88 barn owls that died in 2022 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 in 2016.

As in the baseline years, the compounds detected most frequently in barn owls that died in 2022 were brodifacoum, bromadiolone, and difenacoum. Overall, 79.5% 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 2022 and the baseline years (3% vs 0%). In contrast, there was a significantly higher proportion of barn owls with detectable liver residues of difethialone in 2022 compared to baseline years (6.8% vs 0.3%), but this proportion was lower than in some of the intervening years (2016-2021).

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 summed SGARs (ΣSGARs). There was a significantly higher proportion of birds with "high" concentrations of brodifacoum detected in their livers in 2022 than in the 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 2022 in the concentrations of "high" residues for all SGAR residues, including ΣSGARs. In contrast, "low" bromadiolone and difenacoum residues were significantly lower in birds from 2022 than in the baseline years, while "low" brodifacoum residues were significantly higher in birds from 2022 than in the baseline years.

Overall, there were significant differences in liver SGAR accumulation between barn owls that died in baseline years and in 2022: significant reductions of bromadiolone and difenacoum and an increase in brodifacoum residues from 2016. However, the lack of significant reductions in ΣSGAR residues in barn owls in 2022 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 eighth 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/) show 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 1995-97, 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

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

12,000 breeding pairs (Prescott et al., 2019). Additionally in 2015 the barn owl population status in the UK was moved from Amber list to Green list on the UK Bird of Conservation Concern assessment and IUCN threat status category of Least Concern, which indicates 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 (Buckle et al., 2021).

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). 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 involves five elements:

- A periodic survey of the knowledge, attitudes, and practices of all professional rodenticide users in order to observe changes over time. A baseline survey had been conducted 2015 in advance of regime implementation and follow-up studies were undertaken in 2017, 2020 and 2023.
- 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; Buckle et al., 2023).
- 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.

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 2022. In this report, we compare SGAR residues in a sample of 88 barn owls that died in 2022 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 88 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). Usually, 100 birds would have been analysed, however, submissions to the PBMS were reduced, and some specimens had to be discarded, due to highly pathogenic avian influenza being present in predatory bird species. 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. Therefore, for all birds, there was no clear evidence that those individuals had died from anticoagulant rodenticide poisoning.

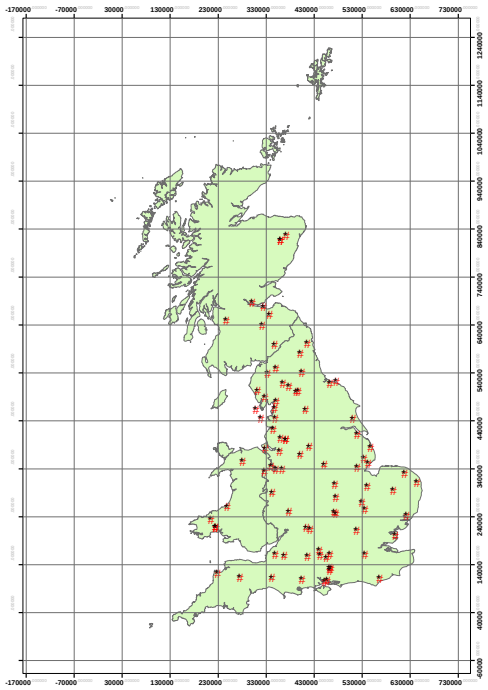

Figure 1. Provenance of the 88 barn owls that died in 2022 and were analysed for liver SGAR residues.

The composition of the 88 birds collected in 2022 was 23 adults (16 males, 7 females) and 65 first year birds (29 males, 34 females); first year birds were individuals hatched in the current or previous year. Overall, the percentage of adults in the 2022 sample was 26.1% 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 88 samples was 81.4±12.0 and 75.7±9.1%, 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 2022 (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 2022 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 2022 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 2022 for information (Figures 3-8). Time trend analysis was conducted on prevalence and magnitude of detected residues with generalised linear regression and Spearman’s rank correlation test, respectively.

4 Results

4.1 General summary of liver SGAR residue data for 2022 owls

As in the baseline and subsequent years, the compounds detected most frequently in barn owls that died in 2022 were bromadiolone, difenacoum and brodifacoum. Between 25% and 67% of 2022 owls contained detectable residues of each of these compounds (Table 1). Overall, 79.5% of owls in 2022 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 44.3% of the owls in 2022 had multiple compounds in their livers.

Table 1. Proportion of barn owls that died in 2022 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	49	66	29	18	49
detected	39	22	59	70	39
% detected	44.3%	25%	67%	79.5%	44.3%

[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 2022 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 2022 than in baseline years (Fisher exact test, $P<0.001$). Flocoumafen had the same level of prevalence in 2022 as in baseline years, namely no bird had detectable residues of this compound (Table 2).

Generalised linear regression analysis on the proportion of barn owls that had detected residues indicated that there was no significant time trend for flocoumafen. However, the same analysis for the other SGARs indicates that proportions of birds with detected residues significantly increased over the years for brodifacoum and difethialone but significantly decreased for bromadiolone and difenacoum (Figure 3).

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

	Flocoumafen		Difethialone	
	Baseline	**2022**	**Baseline**	**2022**
non-detected	383	88	394	82
detected	12	0	1	6
% Detected	3%	0%	0.3%	6.8%
***P-value*[1]**	*0.136*		*<0.001*	

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

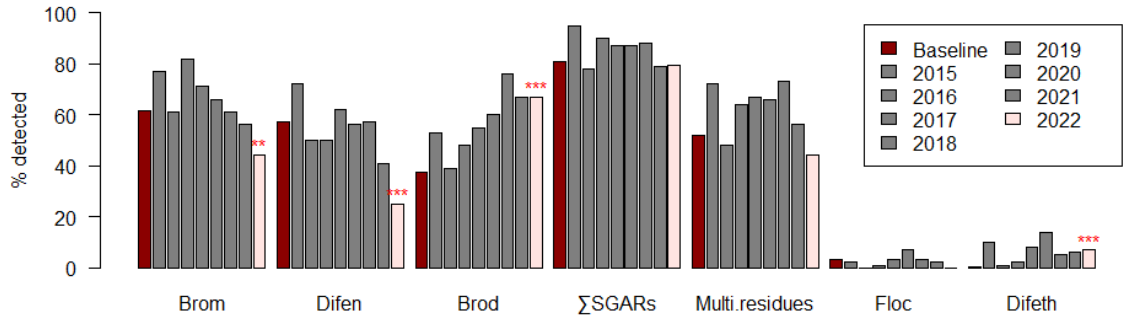

Figure 2. Percentage of barn owls with detected residues of SGARs in their liver. No birds found in 2016 had detectable residues of flocoumafen in their liver. Brom: bromadiolone; Difen: difenacoum; Brod: brodifacoum; Floc: flocoumafen; Difeth: difethialone. Statically significant differences between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

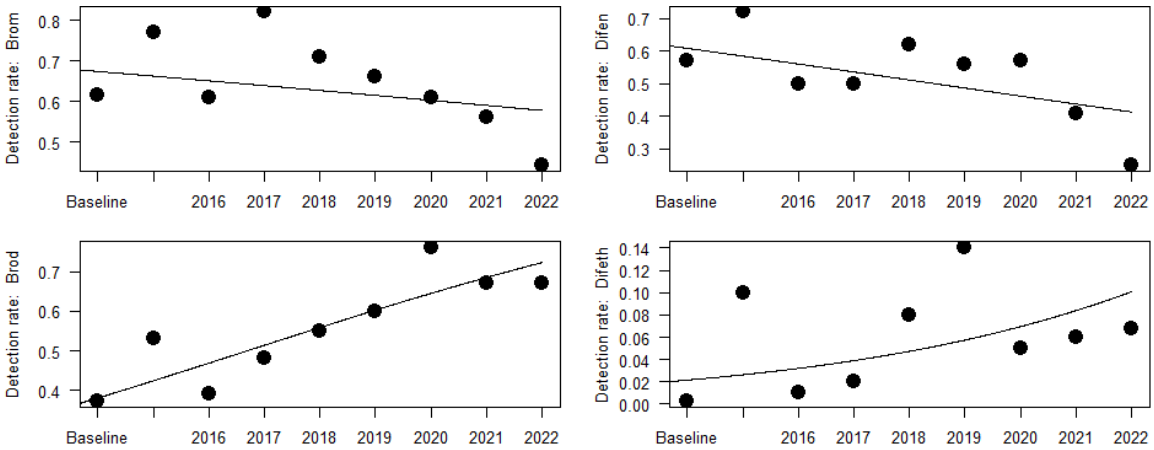

Figure 3. Generalised linear regression analysis on the relationship between the proportion of barn owls with detected SGARs residues and years. Only significant results (P≤0.017) are shown. Brom: bromadiolone; Difen: difenacoum; Brod: brodifacoum; Floc: flocoumafen; Difeth: difethialone.

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 2022 in the ratio of birds with “low” (<100 ng/g wet wt.) vs “high” (>100 ng/g wet wt.) (P≥0.146). However, there was a significantly higher proportion of birds with “high” concentrations of brodifacoum in 2022 compared to the baseline years (P=0.437; 12.5% vs 3.5%, respectively; Table 3 and Figure 4).

The percentages of barn owls with “high” residues among birds with detected SGAR residues in all 9 monitoring years/periods are shown in Figure 4. The percentage for brodifacoum exceeded 10%, and the value for ΣSGARs exceeded 20% in 2022. The percentages were below 10% for bromadiolone and difenacoum for all monitoring years.

Generalised linear regression analysis on the percentages of barn owls with “high” residues indicated that there was no statistically significant time trend for bromadiolone. In contrast, the analysis indicated a statistically significant increasing time trend for brodifacoum and a significant decreasing trend for difenacoum from the baseline years to 2022 (Figure 5).

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.

Conc.	Bromadiolone		Difenacoum		Brodifacoum		∑SGAR	
	Baseline	2022	Baseline	2022	Baseline	2022	Baseline	2022
<100 ng/g "low"	376	83	375	87	381	77	329	70
>100 ng/g "high"	19	5	20	1	14	11	66	18
% high	4.8%	5.7%	5.1%	1.1%	3.5%	12.5%	16.7%	20.5%
***P-value*[1]**	0.786		0.146		0.002		*0.439*	

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

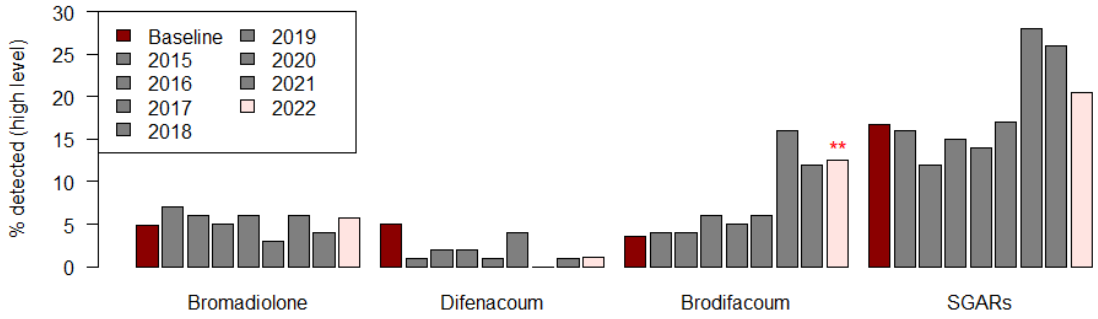

Figure 4. Percentage of barn owls with "high" (>100 ng/g wet wt.) liver SGAR concentrations. No birds found in 2020 had "high" residues of difenacoum in their liver. Statically significant differences between baseline and the most recent year are indicated: * = P<0.05, ** = P<0.01, *** = P<0.001.

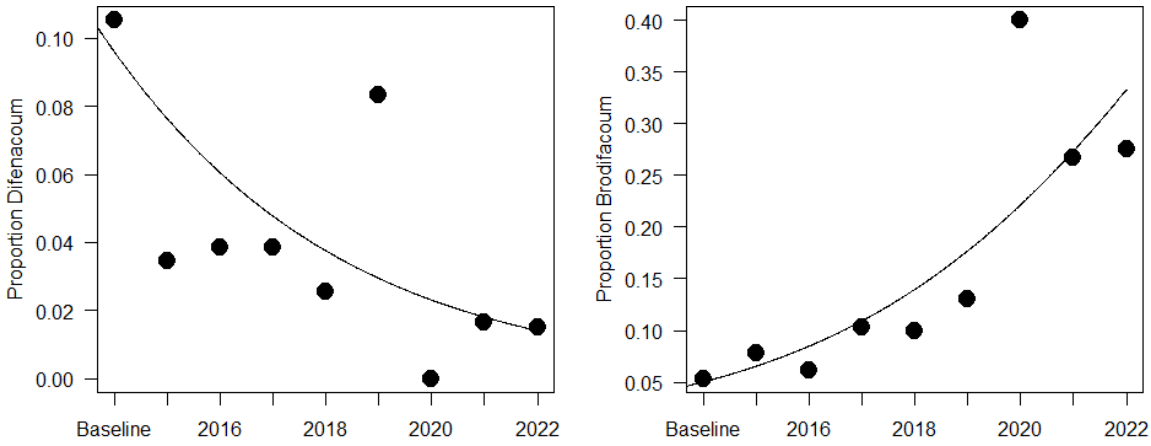

Figure 5. Generalised linear regression analysis on the relationship between the proportion of barn owls with "high" SGARs residues among birds with detected SGAR residues and years. Only significant results (P≤0.002) are shown.

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 2022 than the baseline years, whereas "low" residues of brodifacoum were significantly higher in 2022 than the baseline years (Table 4). For the magnitude of "high" residues of difenacoum, there were too few birds to test whether the magnitude was significantly different between the two periods for difenacoum. For the magnitude of "high" residues of brodifacoum and bromadiolone, there was no significant difference between 2022 and the baseline years (P≥0.406).

Although comparison between the baseline and current years is the metric required for stewardship monitoring, change over years can also be informative and is shown in Figures 6 and 7. "Low" residues of brodifacoum show a clear increasing trend over time from the baseline years (Figure 6). In fact, "low" brodifacoum residues in 2015, 2017, 2018, 2019, 2020, 2021, and 2022 were significantly higher than baseline years. Spearman's rank correlation analysis on the median concentrations of the years also indicates a statistically significant and positive relationship (Spearman's correlation index=0.865, P=0.003). However, "High" residues of brodifacoum shows no significant correlation (Figure 7). In contrast, "low" bromadiolone residues show a decreasing trend over time. Although the differences were not significant, median "low" concentrations of bromadiolone decreased in the years 2016 and 2017 compared to the baseline years, were then similar to the baseline in 2018 but tended to decrease from 2018 onwards (Figure 6). Significantly lower concentrations of bromadiolone were observed in 2021 and 2022. "High" bromadiolone residues also show an analogous temporal change, but no single monitoring year had significantly lower concentrations than the baseline years. However, Spearman's correlation tests indicates that the trends for both "low" and "high" bromadiolone residues have significantly declined

(correlation index=-0.767 and -0.700, P=0.021 and 0.043, respectively). For difenacoum, no significant time trend was observed for both “low” and “high” residues.

For ΣSGARs, there was no significant difference between barn owls from the baseline years and 2022 in the magnitude of “high” and “low” residues (Tables 5 and Figure 7).

No significant difference was observed in the magnitude of ΣSGARs between the baseline years and 2022. However, the magnitude of “low” ΣSGAR excluding brodifacoum residues in 2016, 2021 and 2022 were significantly lower than baseline years (Figure 8). When the temporal trend of the magnitude ΣSGAR excluding brodifacoum residues was assessed by Spearman rank correlation analysis, both the medians of “low” and “high” residue categories indicate statistically significant decreasing trends over the monitoring period (Spearman r=-0.750 and -0.816, P=0.025 and 0.011, respectively).

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			**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
	2022	0.0	0.0	4.1	0.0	0.0	0.0	5.7	0.0	15.9
	MW value[1]	19301/11907			22097/10528			10026/19311		
	P-value	<0.001			<0.001			<0.001		
> 100 ng/g wet wt. (high)	Baseline	179	114	224	136	115	160	347	133	923
	2022	116	106	214	113	ND	ND	387	148	516
	MW value[1]	60/35			ND			84/70		
	P-value	*0.406*			*ND*			0.727		

[1] Mann-Whitney U values

[2] Only one of the 88 barn owls tested had detected “high” residues of difenacoum. Therefore, it was not possible to compare between concentrations for the baseline years and 2022 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
	2022	12.9	0.0	35.9
	MW value[1]	12324/10706		
	P-value	0.353		
"High"	Baseline	171	123	272
	2022	197	145	433
	MW value[1]	450/738		
	P-value	0.118		

[1]*Mann-Whitney U values*

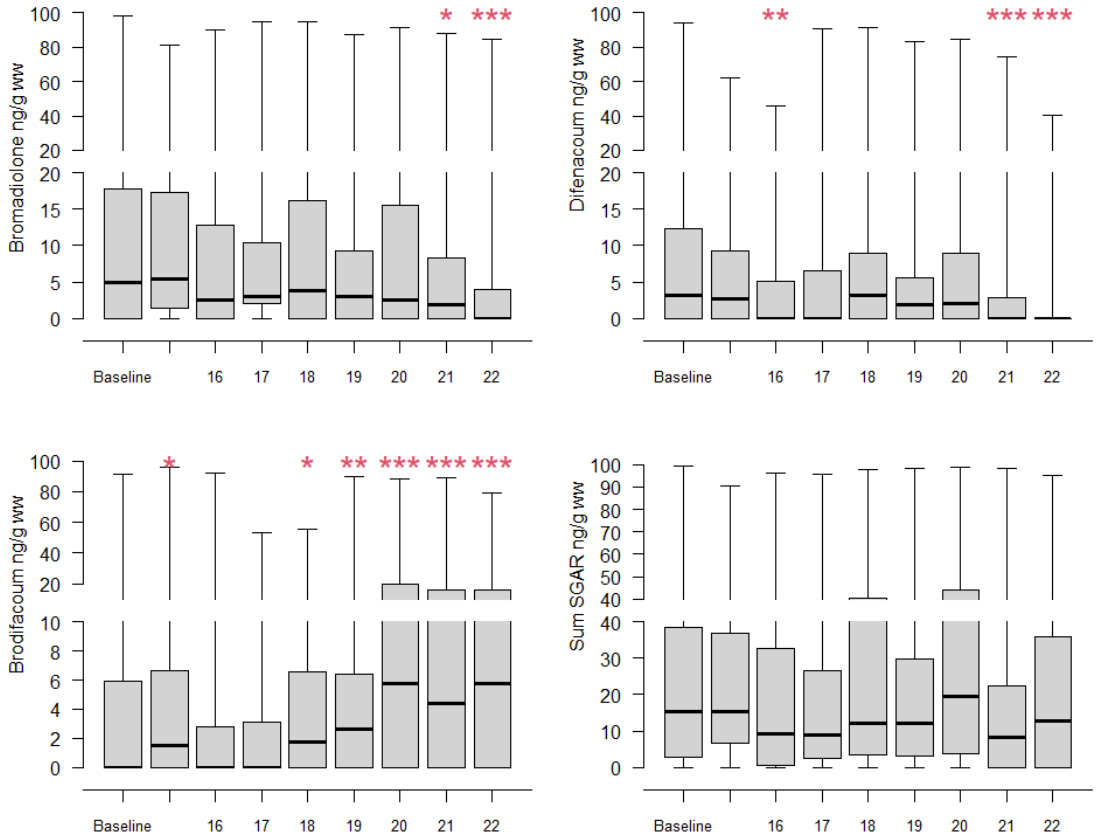

Figure 6. Box and whiskers plot of brodifacoum, difenacoum, bromadiolone and ΣSGARs liver concentrations in the cohort of owls with residues <100 ng/g wet weight ("low" residues) found dead in the 2006-2012 (Baseline), and single years 2015 to 2022. Horizontal line, box and whiskers represent median, 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.

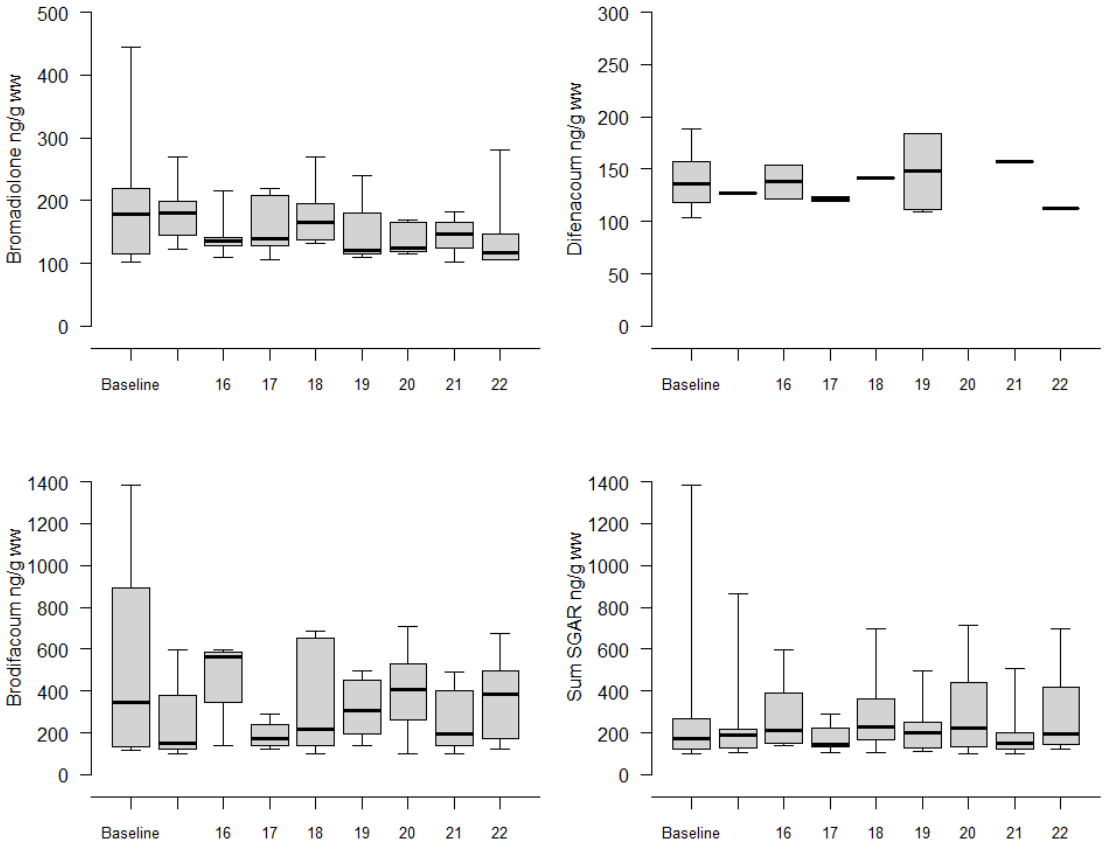

Figure 7. Box and whiskers plot of brodifacoum, difenacoum, bromadiolone and ΣSGARs liver concentrations in the cohort of owls with residues >100 ng/g wet weight ("high" residues) found dead in the 2006-2012 (Baseline), and years 2015 to 2022. Horizontal line, box and whiskers represent median, 25-75th quartile range and minimum maximum range, respectively.

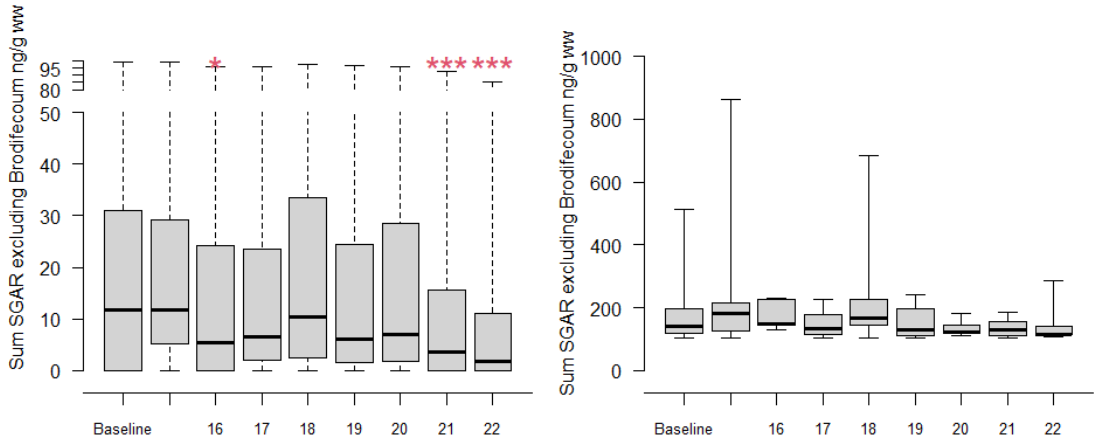

Figure 8. Box and whiskers plot of liver concentrations of ΣSGARs excluding brodifacoum in the cohorts of owls with "high" and "low" residues found dead in the 2006-2012 (Baseline), and single years 2015 to 2022. Horizontal line, box and whiskers represent median, 25-75th quartile range and minimum maximum 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 2022. Particularly, the metrics relating to difenacoum and brodifacoum in 2022 show contrasting results when compared to baseline years. Statistically significant differences between the baseline years and the report year have become more apparent in recent years than at the beginning of the stewardship monitoring. Moreover, the data cumulated during a long period now allow temporal trend analysis to be conducted to clarify the long-term trend of exposure of birds of prey to SGARs. It is therefore evident that the methods used in the scheme, including the numbers of owls examined, are robust and fit for its purpose and should be continued in future.

As in baseline years, residues of one or more active ingredients were present in most barn owls in 2022, but most residues (79.5% for ∑SGARs) were <100 ng/g wet wt. There were statistically significant differences between baseline years and 2022 in terms of prevalence or magnitude of detectable concentrations. The prevalence of difethialone and brodifacoum residues increased, while the prevalence of bromadiolone and difenacoum residues decreased. The increase in difethialone compared of the baseline years reflects that this SGAR was new to the market in the baseline years. However, detection rate of difethialone remained relatively low even in 2022. Meanwhile, a significantly higher proportion of birds had “high” concentrations of brodifacoum compared to the baseline years, and the magnitude of “low” brodifacoum residues had increased over the monitoring period.

As with result from the previous year, the observed increase in the proportion of birds with high residues and in the magnitude of low residues of brodifacoum may reflect a shift in the distribution of brodifacoum residues to higher concentrations compared to baseline years (Figure 9). 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 the magnitude of low brodifacoum residues might be compensating for declines in the other active ingredients at low residues, particularly bromadiolone and difenacoum. With the 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. One of the reasons for the change in active ingredients may be an increasing trend of resistant rodents to SGARs. The latest report on the anticoagulant resistance in UK rats and rice (Buckle et al., 2023) clearly demonstrated an apparent proliferation of resistance mutations in Norway rat populations. Particularly, Y139 SNP (single nucleotide polymorphism), which confers resistance to bromadiolone and difenacoum, was recorded as more widely spread across England, Scotland, and Wales than that observed in the previous survey in 2021-22 (Buckle et al., 2022). The survey also confirms the occurrence of ‘hybrid’ resistance (i.e. carrying various resistance SNPs). More potent SGARs are needed to control such highly resistant rats, which might lead the change in usage.

The lack of reductions in ΣSGAR residues in barn owls in 2022 suggests that implementation of stewardship since 2016 has yet to result in a statistically significant reduction in exposure of barn owls to ΣSGARs. In the case of brodifacoum there is evidence that exposure is increasing. However there have been reductions in exposure to difenacoum and bromadiolone observed in recent years.

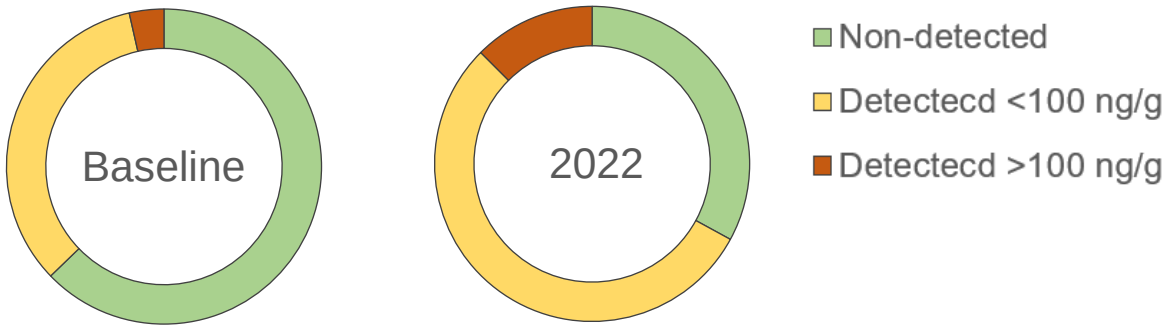

Figure 9. Percentage of birds in baseline years and 2022 that had either non-detected, detected but low residues, or detected high residues of brodifacoum present in their livers.

6 Acknowledgements

This project was funded by the Campaign for Responsible Rodenticide Use (CRRU) UK.

We thank the Predatory Bird Monitoring Scheme (PBMS) for provision of barn owl livers for analysis. The PBMS collection of owl carcasses was supported by the Natural Environment Research Council award number NE/R016429/1 as part of the UK-SCAPE programme delivering National Capability.

The PBMS is a citizen science project and relies on members of the public to submit bird carcasses to the scheme. Their efforts are key to the success of the PBMS and projects, such as the current one, which are dependent on the samples collected, and we thank all members of the public who have sent in bird carcasses.

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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 a parallel evaporator (Büchi Syncore, Switzerland), 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 'Acquity' UPLC coupled to a triple quadrupole 'Xevo TQ-XS' mass spectrometer (Waters Ltd, Wilmslow, UK) interfaced with a 'Unispray' source in negative polarity mode and operated with Masslynx software™ (V.4.2). Analyte separation (1 µL inj. volume) was performed on an Acquity UPLC BEH C18 column (Waters, 1.7 µm particle size, 100 mm x 3mm I.D.) using a H_2O: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 65% B in 3 min and held until 9 min then ramped to 75% B at 12 min and finally to 98% B at 19 min, held for 1.5 min and then returned to starting conditions.

MS/MS was performed in multiple reaction mode (MRM) using Unispray in the negative mode, and characteristic ion fragments were monitored for each compound. Argon was used as collision gas.

Chromatographic peaks were integrated using Masslynx™ which was also used to generate linear calibration curves with R^2>0.99. The rodenticides standards (Dr Ehrenstorfer) were matrix matched.

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. Recovery for the total procedure was calculated using the labelled standards. 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 88 samples was 81.4±12.0 and 75.7±9.1%, respectively.

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