Second generation anticoagulant rodenticide residues in red kites 2022

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Issue Number 1

Date 24/05/2024

Title Second generation anticoagulant rodenticide residues in red kites 2022

Client Natural England

UKCEH reference Project No. 09017

This report should be cited as S. Ozaki, E.A. Barnett**, H. Carter, J.S. Chaplow, S. Charman**, M.G. Pereira, E.D. Potter, A.W. Sainsbury*, T. Shadbolt*, D. Sleep, E.A. Sharp***, and L.A. Walker. Second generation anticoagulant rodenticide residues in red kites 2022. UKCEH contract report to Natural England, pp. 34

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Date 24/05/2024

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1 Executive Summary

Second-generation anticoagulant rodenticides (SGARs) can be toxic to all mammals and birds if consumed. Various studies have shown that, in Britain, there is widespread exposure to SGARs in a diverse range of predatory mammals and birds, including red kites (*Milvus milvus*) which scavenge dead rats, a target species for rodent control. The Wildlife Incident Investigation Scheme[1] (WIIS) and the Predatory Bird Monitoring Scheme (PBMS) have shown that some mortalities result from this secondary exposure.

In the present study, we analysed liver SGAR residues in 14 red kites that had been found dead in Britain in 2022. One bird collected in 2021 was also chemically analysed and added to the time trend analysis of this report. The carcasses were submitted to and necropsied by the Disease Risk Analysis and Health Surveillance (DRAHS) programme, the PBMS, and the WIIS for England & Wales. In 2022, there were no birds received from Scotland (i.e., no bird from the WIIS for Scotland and the Raptor Health Scotland study). All these organisations are partners in the WILDCOMS (Wildlife Disease & Contaminant Monitoring & Surveillance Network) network that promotes collaboration among surveillance schemes that monitor disease and contaminants in vertebrate wildlife in the UK.

The UK Rodenticide Stewardship Regime (hereafter referred to as the stewardship scheme) began to come into force in mid-2016 as re-registration of products for use in the UK was approved by the HSE; full implementation of the scheme was in early 2018. The key aim of this stewardship initiative is to support competence among all users of professional SGAR products. A potential benefit of this may be the reduced exposure of non-target wildlife to anticoagulant rodenticides. However, the number and density of SGAR-contaminated rats may remain unchanged although diligent searching, removal, and safe disposal of poisoned rats, as promoted by the stewardship regime, might be expected to reduce the availability of poisoned dead rats to red kites (and other scavengers) and thereby reduce the proportion of birds that are exposed and/or the magnitude of exposure. Concomitant with the stewardship scheme was a relaxation of the indoor-use-only-restriction applied to brodifacoum, flocoumafen, and difethialone, the three most acutely toxic SGARs to use indoor and outdoor around buildings. Any consequent increase in outdoor use of these three SGARs could increase the risk of secondary exposure in red kites. We therefore compared the data in the current report with that collected in 2015 and 2016 to determine if there was any evidence of a change in pattern or magnitude of exposure in red kites that might be connected to stewardship and/or change in usage restriction.

All of the 14 red kites from England & Wales in 2022 had detectable liver residues of at least one type of SGAR. Brodifacoum, difenacoum, and bromadiolone were each detected in 13, 13 and 6 red kites, respectively. Difethialone was found in two individuals while flocoumafen was detected in no bird.

The proportion of analysed red kites exposed to SGARs in 2015 (91%), 2016 (90%), 2017 (96%), 2018 (100%), 2019 (91%), 2020 (88%), 2021 (98%), and 2022 (100%) was similar at circa 90% or more; the higher percentages in 2017 and 2018 were

[1] https://www.hse.gov.uk/pesticides/reducing-environmental-impact/wildlife/wildlife-incident-investigation-scheme.htm

principally due to a greater proportion of birds from Scotland containing residues than observed in other years. However, all red kites from England and Wales were exposed to SGARs in 2022. Difenacoum, brodifacoum, and bromadiolone were the most prevalent compounds (detected in 87%, 87%, and 75% of red kites across the eight years for each compound, respectively). On average, there were detectable residues of two different SGARs in each red kite liver likely demonstrating multiple exposures.

Sum liver SGAR concentrations in birds from 2022 ranged between 30 and 988 ng/g wet weight (arithmetic mean: 380 ng/g wet weight, median 257.9 ng/g wet weight). Necropsy examinations indicated that two red kites showed signs of being poisoned by SGARs (i.e., showing internal haemorrhaging that is not associated with detectable trauma and also having detectable liver SGAR concentrations). These samples accounted for 17% of the red kites of the year excluding uncertain poisoning cases. These two birds had sum SGAR liver concentrations of 544.2 and 661.6 ng/g wet weight. SGARs were considered a contributory cause of death resulting from unspecified use in these cases. SGARs were a contributory cause of death in 16% of the red kite cases examined across all eight years. Over the period 2015 to 2022, a reduction has been observed in the percentage of red kites examined that were diagnosed as birds in which SGARs were implicated as a contributory cause of death. However, given that the WIIS scheme specifically examines suspected poisoning incidents, it is likely that poisoned birds are over represented in this sample compared to the population as a whole in all eight years. Due to these reasons, caution should be used when interpreting evident changes in poisoned bird rates due to the opportunistic sampling methods used in this study that may lead to over reporting of poisoned birds. Those rates being subject to variations in relative contribution of the WIIS and PBMS to each year's sample. It should be noted that sub-lethal poisoning due to SGAR exposure is not considered in this report.

There were statistically significant differences between years in median summed SGAR residues for non-poisoned birds and in all red kites combined with poisoned and non-poisoned birds. The magnitude of accumulated summed SGAR residues, particularly sum of brodifacoum, flocoumafen, and difethialone concentrations, was significantly higher in 2022 than in 2019. Given low occurrence and low concentrations of flocoumafen and difethialone residues, it is demonstrated that the magnitude of brodifacoum residues has increased over recent years.

Data on presence/absence of detectable brodifacoum, flocoumafen or difethialone residues were compared for 2015/2016 and 2017/18/19/20/21/22. The proportion of red kites with detectable residues of these three SGARs was not significantly different between 2015/2016 (82%) and 2017/18/19/20/21/22 (89%). Similarly, there was no significant difference in the proportion of red kites with detectable liver difenacoum or bromadiolone residues (90% in 2015/2016 vs. 94% in 2017/18/19/20/21/22). Since the implementation of the stewardship regime, no difference in exposure pattern relating to active ingredients has been detected with the exception of an increase in the concentrations of brodifacoum.

Spatial analysis by county/region indicated that across the monitoring period highest exposure to SGARs in red kites appeared to be around the Berkshire/Hampshire and, to a lesser extent, North Yorkshire.

Our findings do not indicate that there has been a consistent broad scale change in exposure in red kites to SGARs following implementation of stewardship in terms of either the proportion of the sample exposed or the magnitude of sum SGARs residues detected. However, there is evidence that the proportion of red kites in which SGARs were implicated as a contributory mortality factor has decreased in more recent years. Alternative approaches to monitoring SGARs in red kites could be considered that analyse a random but representative sample, and as part of such a programme there may also be value in monitoring SGARs in the blood of tracked individuals. Brodifacoum exposure has increased in recent years, but whether this change in exposure has been caused by the relaxation of usage restrictions on brodifacoum, difethialone and flocoumafen is still a question to be addressed.

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 red kites (*Milvus milvus*) in Britain. The red kite population in the UK increased by ~2000% over the period 1995 to 2019 (Harris et al. 2020) largely because of successful reintroduction programmes. The background to, rationale for, and aims of the study remain unchanged from those described in previous reports (Walker et al., 2016; 2017; 2018; 2019; 2021a; 2021b). 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 Second generation anticoagulant rodenticides (SGARs) in predatory birds

Previous studies have shown that there is widespread exposure to second generation anticoagulant rodenticides (SGARs) in a diverse range of wildlife, including mammalian and avian insectivores, omnivores and carnivores, in Britain (see Predatory Bird Monitoring Scheme (PBMS) reports; Newton et al., 1999a; Dowding et al., 2010; McDonald et al., 1998; Ruiz-Suárez et al., 2016; Sainsbury et al., 2018; Shore et al., 2003a; 2003b; 2006; 2015; Walker et al., 2008a; 2008b). This is also true in many other countries around the world (van den Brink et al., 2018).

The UK Centre for Ecology & Hydrology's (UKCEH) Predatory Bird Monitoring Scheme (PBMS; https://pbms.ceh.ac.uk/) measures liver SGAR residues in a range of predatory birds to determine the scale and severity of secondary exposure to SGARs in Britain. Our residue studies on barn owls (*Tyto alba*) (Walker et al., 2022) provide data on exposure in a species that feeds predominantly on non-target rodents (i.e., rodent species excluding brown rat, *Rattus norvegicus*, and house mouse, *Mus musculus*) and so provide information on exposure and poisoning mediated through this pathway. This work is used as part of the monitoring undertaken by the industry-led stewardship scheme for anticoagulant rodenticides (Buckle et al., 2017). However, studies on barn owls provide little or no information on exposure resulting from predation of rodents that are the target of anticoagulant rodenticide (AR) control, such as the brown rat.

The red kite is a conservation priority species that was reintroduced to England and Scotland in the late 20th/early 21st centuries as part of an official species recovery programme (Carter & Grice 2002). Since these reintroductions, the UK red kite population has significantly increased with an expanding distribution (Harris et al., 2020). Red kites are scavengers and their diet typically, but not exclusively, includes dead rats. A study of non-breeding diet in the Midlands observed 6% of feeding observations included rats and 27% of winter pellets contained rat remains (Carter & Grice, 2002). This propensity to feed on rodents that are the target of AR control may increase the likelihood of exposure, and periodic studies on another rat-feeding predator, the polecat (*Mustela putorius*), has shown that, while the population has increased and its distribution has expanded, secondary exposure to ARs has increased in this species in Britain over the last 25 years (Sainsbury et al., 2018; Shore et al., 2003a). SGAR-induced deaths of red kites have been documented as part of the WIIS reporting[2].

The stewardship scheme for professional use of 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. The impact of stewardship on the likelihood of secondary exposure and poisoning may differ for barn owls and red kites. Better knowledge and implementation of best practice in AR use, for instance such as reduction/cessation of permanent baiting, would be expected to reduce the time period over which bait is available to and taken up by non-target rodents and so reduce the likelihood of secondary exposure in their predators (such as barn owls). However, there may be no similar change in exposure of predators. The objective of baiting is to expose target rodents, rats and house mice, to AR, and so the number and density of AR-contaminated rats may be maintained. Diligent searching, removal and safe disposal of poisoned rats is therefore promoted by the stewardship scheme aiming to reduce the availability of poisoned rats to red kites and other scavengers and thereby reduce risk of exposure. Although the red kite is not exclusively a scavenger on rat carcases, other potentially contaminated non-target rodents may be consumed by red kites (Carter & Grice, 2002), and hence exposure via this route may be reduced by best practice anticoagulant rodenticide use.

An additional factor that may affect the exposure of red kites to SGARs is the relaxation of the restriction of indoor use only that had been applied to brodifacoum, flocoumafen, and difethialone. The restrictions on the use of all (five) SGARs authorised for use in the UK was harmonised as contemporary risk assessment showed that the science did not support different restrictions (CRRU, 2015). This change was implemented simultaneously with the stewardship scheme at the time of product re-registration, 2015 – 2017. Although all SGARs are highly toxic to vertebrates, brodifacoum, flocoumafen, and difethialone typically are the most acutely toxic (Erickson & Urban, 2004), and these three SGARs can now be used in and around buildings, although UK applications for open area use have not been made to date. This change to in and around buildings permission may increase use of these three SGARs, especially in areas where there is resistance to bromadiolone and difenacoum (Jones et al., 2019). This in and around building use may subsequently increase secondary exposure of red kites to these three SGARs. In contrast, the higher effectiveness of these three SGARs may allow users to apply fewer baits for a shorter time to control target species, particularly resistant rodents, compared to using difenacoum or bromadiolone, which may result in a decrease in secondary exposure of wild predators (Buckle et al. 2020). Consumption of rats poisoned by these compounds may present the most significant risk of secondary poisoning to red kites.

The development of the PBMS monitoring of SGAR residues in red kites, in collaboration with the Disease Risk Analysis and Health Surveillance (DRAHS) programme, run by the Institute of Zoology (IoZ), has been described in previous reports in this series (Walker et al., 2016; 2017; 2018; 2019; 2021a; 2021b; Ozaki et al., *In press*). Tissue samples are submitted to PBMS following post-mortem examinations of kites undertaken by IoZ, who conduct health surveillance of red kites and other reintroduced species as part of the collaborative DRAHS research project.

[2] https://www.hse.gov.uk/pesticides/resources/W/wiis-quarterly.xlsx; last accessed 21/11/2021

Occasional red kite necropsies are conducted by the PBMS. Analysis of liver SGARs is undertaken by the PBMS.

SGAR residues in red kites from England & Wales that are suspected of being poisoned are analysed and reported by Fera Science as part of the Wildlife Incident Investigation Scheme (WIIS) for England & Wales, delivered by Natural England in England and Natural Resources Wales in Wales. The WIIS is a post-registration monitoring scheme designed to inform the pesticide approval process, and investigates the death or illness of wildlife, pets and beneficial invertebrates that may have resulted from pesticide poisoning. Monitoring through the WIIS for England & Wales and PBMS/DRAHS is complementary in that carcasses/tissues of red kites that died in England & Wales are exchanged so that birds suspected of being poisoned are analysed by WIIS, while birds that would not qualify for analysis under the WIIS (typically because poisoning is not suspected) are analysed by the PBMS.

The WIIS for Scotland is run by SASA (formerly known as Science & Advice for Scottish Agriculture) and examines SGAR residues in any raptors found dead in Scotland. Red kite carcasses from Scotland that are offered to the PBMS are redirected so that they are submitted to the Raptor Health Scotland study for post-mortem investigation and then onto SASA for chemical analysis. WIIS data (for England & Wales and for Scotland) are collated and published quarterly online[3].

Data for birds that died in 2022 and analysed by the WIIS (England & Wales) have been made available for the current report so that they can be examined alongside the data obtained through the DRAHS/PBMS. This has been done so as to present as full a picture as possible for SGAR exposure in red kites in Britain. This complex collaboration between five separate organisations/schemes (PBMS, DRAHS, WIIS for England & Wales, Raptor Health Scotland and the WIIS for Scotland) has been facilitated by the WILDCOMS network (https://www.wildcoms.org.uk/), in which all are partners.

2.2 Aims of the current study

Our aims were to report the liver SGAR residues in red kites found dead in 2022 and submitted to the DRAHS/PBMS and WIIS for England & Wales for analysis.

We describe the current incidence, magnitude, and likely toxicological significance of the liver SGAR residues detected in these birds in 2022 and compare our data with those for kites that died between 2015 and 2021 (Walker et al., 2017; 2018; 2019; 2021a; 2021b; Ozaki et al., *In press*). This timeframe spans the implementation of the stewardship programme for anticoagulant rodenticides and the concurrent relaxation of ‘indoor use only’ restrictions for brodifacoum, flocoumafen, and difethialone.

[3] https://www.hse.gov.uk/pesticides/reducing-environmental-impact/wildlife/wildlife-incident-investigation-scheme.htms

3 Methods

The carcasses of 14 red kites that died in 2022 were collected as part of the PBMS or the DRAHS programmes, and WIIS for England & Wales (Table 1). Another red kite (adult female) collected in 2021 chemically was analysed in 2022. This bird was added to the bird from 2021 for the time trend analysis of this report. Both PBMS and DRAHS projects rely on citizen science in that members of the public send in dead birds that they find. However, due to avian influenza widely spread in wild birds, the public was restricted to collect dead wild birds. Therefore, we collected a lower number of birds in 2022 compared with the previous years. WIIS incidents are usually reported by a variety of stakeholders that also include members of the public. However, there was no bird from WIIS for Scotland and the Raptor Health Scotland study in 2022.

Therefore, all red kite carcasses (100%) were from England and Wales in 2022. Juveniles, when age was characterized, were individuals determined to have hatched in the current or previous year, as assessed from plumage characteristics (Molenaar et al., 2017) (Table1).

Table 1. Number of red kites examined in each demographic group for individuals found dead in 2022.

	Adult	Juvenile	Unknown
Male	2	0	2
Female	5	1	3
Unknown	0	0	1

All carcasses were subject to a post-mortem examination and various tissue samples, including the liver, were excised, and stored at -20ºC. Post-mortem examinations were conducted by wildlife veterinarians or trained pathology staff at the Institute of Zoology, the Animal Plant Health Agency, SAC Consulting: Veterinary Services (on behalf of UKCEH and SASA) and Fera Science, respectively.

Protocols of PM examination varied among laboratories, but the diagnosis of SGAR poisoning were conducted in accordance with the principles and methods described in Murray (2018). Non-trauma related macroscopic haemorrhaging was noted during all necropsies. If such haemorrhaging was present and if SGAR residues were detected in the liver, of any magnitude, birds were classed as individuals poisoned by SGARs: SGARs were directly implicated as a contributory cause of death. Therefore, cause of death was not diagnosed based on SGAR residue concentration alone, even if concentrations were elevated. All diagnoses of SGAR poisoning proposed by other laboratories were discussed and agreed between UKCEH and the other laboratories. The nature of the sampling and necropsy methods for both the PBMS and WIIS birds mean that microscopic haemorrhaging and other sublethal effects would not be detected. Red kites for which SGARs were implicated as a contributory cause of death are considered as ‘poisoned birds’ in this report. Birds with haemorrhaging related to trauma and detected levels of SGARs may also have been detrimentally affected by exposure to SGARs but would not be classified as SGAR poisoned birds for the purposes of this report.

Liver SGAR residues in kites submitted to the PBMS were quantified by Liquid Chromatography Mass Spectrometry (LC-MS/MS); analytical methods are outlined in the report by Shore et al. (2018). The methods used by Fera Science and SASA as part of the WIIS are similar in principle to those used by the PBMS but the precise methodology, limits of detection and recoveries differ to some extent (limits of detection and recoveries for the different laboratories are given in Appendix 1). Anticoagulant rodenticide residues are reported for compounds individually and as the sum of all compounds (ΣSGARs) and concentrations are expressed as ng/g wet weight (wet wt.).

Data were statistically analysed in the R environment version 4.3.1 (R Core Team, 2022). Throughout this report analyses with P-values less than 0.05 are considered to be statistically significant. For calculation of sum concentrations values below the limit of detection (LoD) were assigned a value of 0.

4 Results

4.1 Liver SGAR residues in red kites that died in 2022

Of the 14 red kites found dead in 2022, all had detectable concentrations of one or more SGARs in their liver (Table 2). Bromadiolone (detected in 43% of red kites in the sample), difenacoum (93%), and brodifacoum (93%) were the most prevalent residues detected. Difethialone was found in two birds (14%), and flocoumafen was detected in no red kite from 2022. Sum SGAR concentrations ranged between non-detectable to 988 ng/g wet wt. with a median of 258 ng/g wet wt.

Post-mortem examinations indicated that two of the 14 of the red kites found dead in 2022 had internal haemorrhaging that was not associated with detectable trauma. Both birds had comparatively high liver summed SGAR residues of 544.2 and 661.6 ng/g wet wt. (Table 2). Anticoagulant rodenticides were considered to be a contributory cause of death of these two birds. Another bird had a relatively high summed SGAR concentration (988 ng/g wet wt.) and showed signs of haemorrhaging. However, there were also signs of other physical trauma that may have led to the observed haemorrhaging, therefore the contribution of SGAR exposure to the death of this bird was uncertain and so the results of this bird have been excluded from statistical analysis describing and comparing poisoned and non-poisoned birds.

Two dead red kites from Fera science (Bird codes RK_22_04 and RK_22_06) showed signs of haemorrhaging with clear trauma or shot wound. Sample RK_22_04 had blood on head with no detectable fracture or lesions associated with blood. Haemorrhage with bruising and blood clots affected tissues of the whole of the left leg. Sample RK_22_06 showed circular holes about 2mm diameter in skin distal to keel to the right of the midline and on dorsum, medial to the left hip, which mean that this bird must have been shot. Moreover, there was very large blood clot about 5cm diameter in the peritoneal cavity, caudal to the liver. A large blood clot about 3cm diameter was also observed adjacent to right kidney, and small amount of free blood was in the peritoneal cavity. The right kidney was dark red and haemorrhagic. Despite haemorrhaging being observed in both birds and SGAR residues being present in their livers, there was sufficient evidence of other likely causes of the haemorrhaging and so anticoagulant rodenticides were not considered to be a contributory cause of death for these birds.

Table 2. Concentrations of second-generation anticoagulant rodenticides (SGARs) in the livers of red kites found dead in 2022[#]

Scheme	Incident/ Bird code	SGAR contributed to. causes of death	Month of death	Sex	Age	Location	Concentration of SGAR (ng/g wet wt.)					
							Brom	Difen	Floc	Brod	Difeth	ΣSGARs
WIIS	RK_22_01	No	Feb	M	U	Glamorgan	32.0	16.0	0.0	36.0	0.0	84.0
WIIS	RK_22_02	Uncertain	Jan	M	U	West Yorkshire	0.0	21.0	0.0	210.0	0.0	231.0
WIIS	RK_22_03	No	Feb	F	U	Dorset	11.0	13.0	0.0	34.0	0.0	58.0
WIIS	RK_22_04	No	Mar	M	Adult	County Durham	0.0	75.0	0.0	790.0	0.0	865.0
WIIS	RK_22_05	Uncertain	Mar	U	U	Leicestershire	0.0	48.0	0.0	940.0	0.0	988.0
WIIS	RK_22_06	No	Apr	F	U	Cardiganshire	0.4	39.0	0.0	220.0	0.0	259.4
WIIS	RK_22_07	No	Sep	F	U	Denbighshire	0.0	12.0	0.0	18.0	0.0	30.0
PBMS/IoZ	22840	Yes	Mar	F	Adult	Oxfordshire	0.0	0.0	0.0	530.0	14.3	544.2
PBMS/IoZ	22866	Yes	Apr	F	Adult	Dorset	16.3	6.2	0.0	639.1	0.0	661.6
PBMS/IoZ	22872	No	Apr	F	Adult	Hertfordshire	8.5	15.9	0.0	368.1	0.0	392.5
PBMS/IoZ	22918	No	Jun	F	Juv.	Carmarthenshire	2.9	92.6	0.0	0.0	0.0	95.5
PBMS/IoZ	22925	No	Jul	M	Adult	Monmouthshire	0.0	10.9	0.0	245.5	0.0	256.5
PBMS/IoZ	23098	No	Aug	F	Adult	North Hampshire	0.0	15.1	0.0	96.6	0.0	111.7
PBMS/IoZ	23255	No	Feb	F	Adult	Cambridgeshire	0.0	53.3	0.0	669.2	19.5	742.0
PBMS/IoZ#	23256	No	Apr	F	Adult	Cambridgeshire	69.4	6.5	0.0	320.1	210.4	606.4

M – male; F- female; U – sex or age not determined; Juv. – First-year juvenile; Brom – bromadiolone; Difen – difenacoum; Floc – flocoumafen; Brod – brodifacoum; Difeth – difethialone. # - One bird (Bird code 23256) was collected in the previous year (2021) but chemically analysed with the birds from 2022. Values under LoD were replaced by 0. Birds with signs of haemorrhaging but not associated with physical trauma and with detected SGAR residues are highlighted in yellow and were classed as birds for which SGARs are implicated as a contributory cause of death.

4.2 Trend in exposure over time

We examined whether the exposure patterns in red kites and the proportion of birds for which SGARs were implicated as a contributory cause of death has changed over the eight years in which residue data across the five surveillance and monitoring schemes have been combined. This period spans the year (2016) when AR stewardship commenced and usage restrictions on brodifacoum, flocoumafen, and difethialone were harmonised.

The proportion of birds with one or more detectable liver SGAR liver residue ranged between 88% (in 2020) and 100% (in 2018 and 2022) across the monitoring period. It was not possible to analyse if the difference between individual years was statistically significant because the underlying assumptions of a Chi-squared test were not met (values below five in the “expected” cells). Therefore, we pooled samples into groups of years that represented as closely as possible “pre-stewardship implementation——2015/2016” and “post-stewardship implementation——2017/18/19/20/21/22”. The proportion of red kites with detected residues was 193/202 (96%) in post-stewardship years, which was not significantly different (Fisher’s Exact Test, P=0.122) to the equivalent proportion (55/61=90%) in pre-stewardship years. Over the whole period of 2015 to 2022, 248 out of the 263 kites examined (94%) had at least one detectable liver residue and the median number of different compounds detected in the liver was two.

In terms of the magnitude of cumulative exposure, we calculated the summed SGAR concentrations (ΣSGARs) in each red kite and compared concentrations in: (i) birds for which SGARs were implicated as a contributory cause of death (poisoned); (ii) birds for which SGARs were not implicated as a contributory cause of death (non-poisoned), and (iii) all red kites combined (Figure 1). There was no statistically significant difference between years for (i) poisoned birds (Kruskal-Wallis test: KW=13.6, P=0.06) In contrast, there were statistically significant differences for (ii) non-poisoned birds (KW=28.5, P<0.001) and (iii) all red kites (KW=28.3, P<0.001). These differences were however not consistent. Summed SGAR concentrations in non-poisoned birds were significantly higher in 2021 than in 2016, 2017, and 2019. For all red kites combined, ΣSGAR concentrations were significantly higher in 2021 than in 2016, 2017, 2018, and 2019. These results may indicate that the magnitude of accumulated ΣSGAR residues has slightly increased over recent years (see also Figure 6).

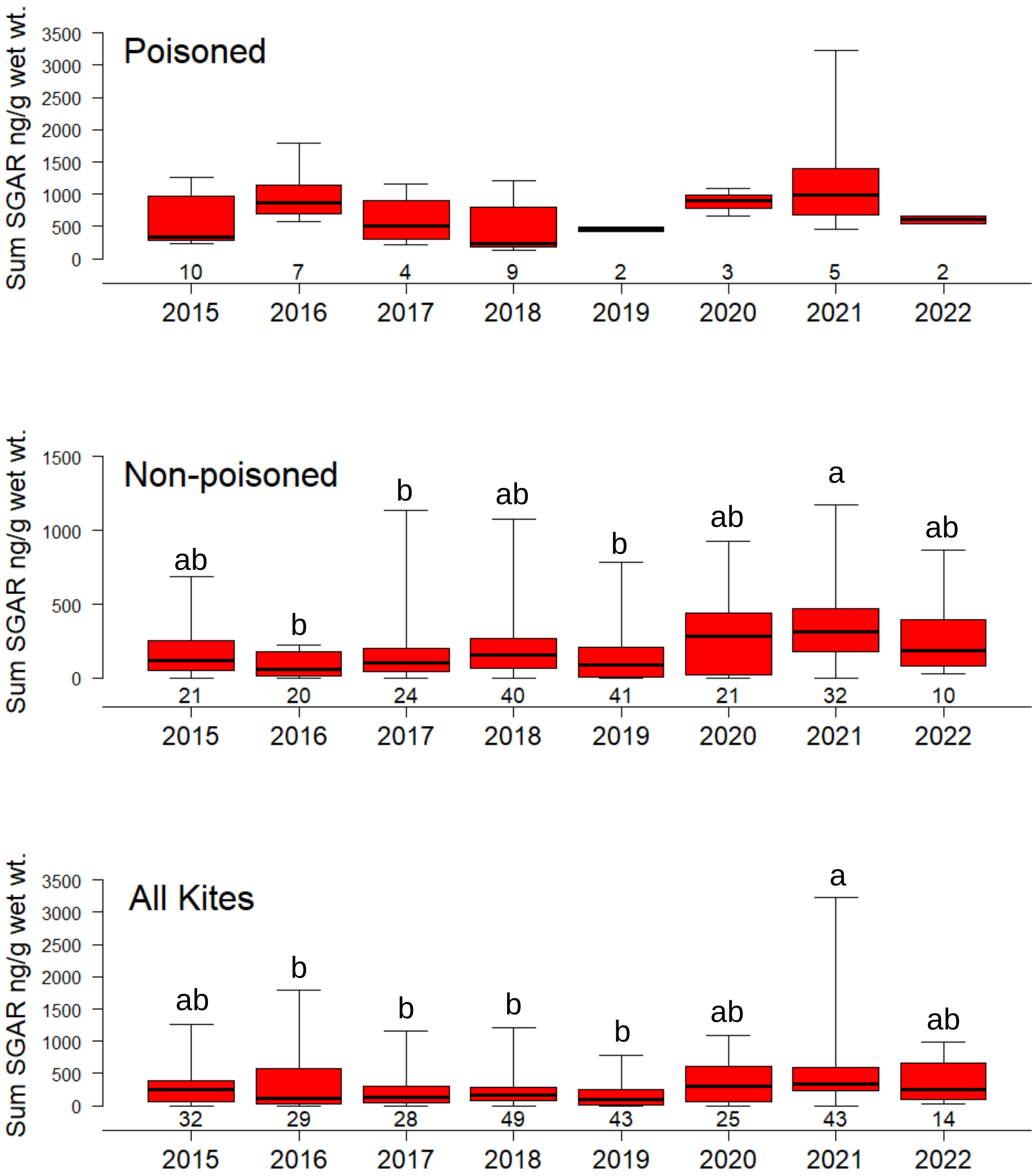

Figure 1. Box and Whisker plots showing median, interquartile range and minimum/maximum range of sum of SGAR concentrations (ΣSGARs) in red kites that died with haemorrhaging disassociated with physical trauma (SGARs implicated in death; 'Poisoned'), those died from other causes (SGARs not implicated in death; 'non-poisoned') and in all red kites combined. Sample numbers are shown above the x-axis for each group. The birds with uncertain poisoning signs were excluded from the analysis on poisoned and non-poisoned birds, namely one, two, one, six, and two birds from the 2015, 2016, 2020, 2021, and 2022 cohort, respectively. Note that one birds was added to 2021 from the previous report. Significant differences ($P<0.05$) between years are indicated by different letters. There was no significant difference in multiple comparisons of years in ΣSGAR in poisoned birds.

We examined whether there was evidence of a change over time in the exposure of birds to the three SGARs that, before 2016, were restricted to indoor use only: brodifacoum, flocoumafen, or difethialone. We analysed whether there were differences between years in either the proportion of birds that contained residues of one or more of these three SGARs or the summed magnitude of residues for those three compounds.

All red kites that had detectable liver residues of flocoumafen or difethialone also had detectable residues of brodifacoum (Table 2), and so the analysis of the proportion of kites with residues was conducted just for brodifacoum. The numbers (%) with detectable liver brodifacoum concentrations were 27 (84% of the sample), 23 (79%), 27 (96%), 46 (94%), 30 (70%), 21 (84%), 42 (98%), and 13 (93%) in 2015, 2016, 2017, 2018, 2019, 2020, 2021, and 2022 respectively (Figure 2).

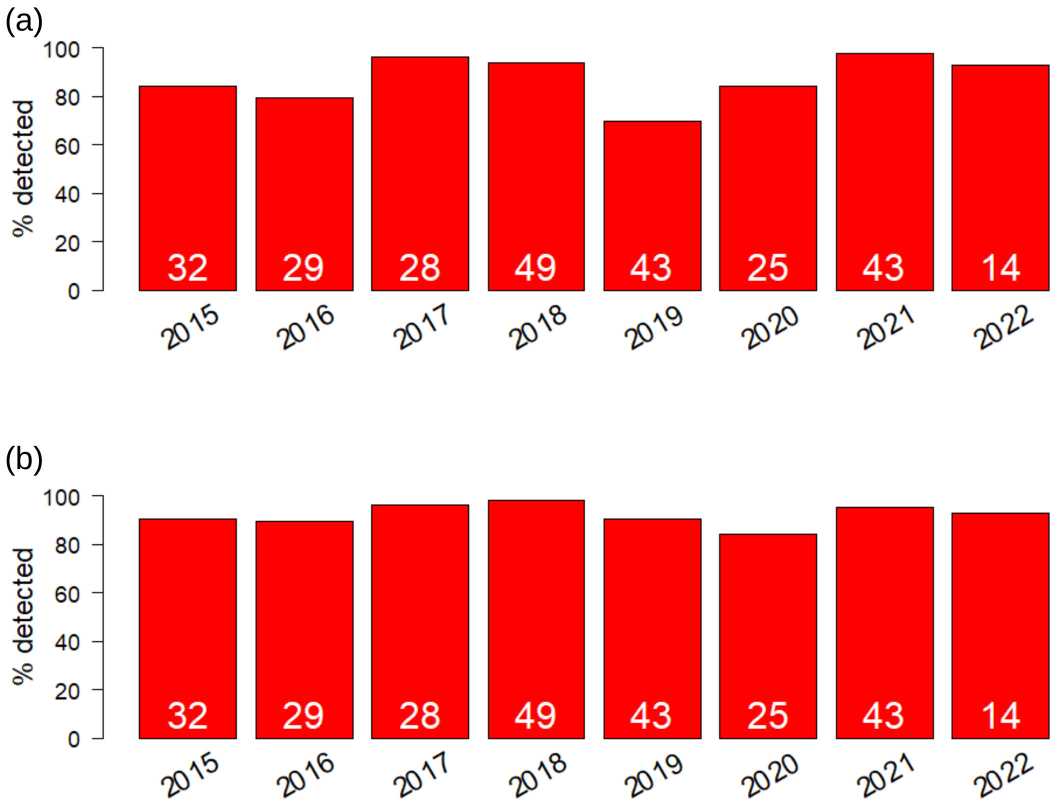

Figure 2. The percentage of red kites found dead between 2015 and 2022 that had detectable concentrations of brodifacoum, flocoumafen, and/or difethialone (a) or difenacoum and/or bromadiolone (b) in their livers. Total sample numbers are shown in the bars.

As when comparing incidence of any SGAR, it was not possible to analyse if there was statistically significant variation in the percentage of birds with detectable residues of brodifacoum (and hence flocoumafen, and difethialone) between individual years. We therefore again pooled samples into “pre-stewardship implementation—2015/2016” and “post-stewardship implementation—2017/18/19/20/21/22” year blocks. The proportion of birds with brodifacoum, flocoumafen or difethialone residues in the liver was 50/61 (82%) and 179/202 (89%) in pre- and post-stewardship years, respectively. There was no significant difference between year groups (Fisher’s Exact test; P=0.193). Similarly, there was no significant difference between these year groups in the proportion of kites that had liver difenacoum or bromadiolone residues (90% in pre- vs. 94% in post-stewardship, Fisher’s Exact test; P=0.399; Figure 2).

In contrast, there were significant differences among years in the sum of brodifacoum, flocoumafen, and difethialone liver concentrations (KW=40.4, P<0.001; Figure 3). The sum in 2021 was significantly higher than all the previous years except 2020, and the sum in 2022 was significantly higher than in 2019 (Dunn’s multiple comparison test, adjusted P-value <0.05). The same trend was observed for only brodifacoum liver concentrations: brodifacoum concentrations were significantly different among years (KW=46.1, P<0.001), and concentrations in 2021 were significantly higher than all the previous years except 2020, while concentrations in 2022 was significantly higher than in 2019 (Dunn’s multiple comparison test, adjusted P-value<0.05; Figure 4). The results indicate that the magnitude of SGAR concentration in birds that are exposed to the three ‘previously indoor use only’ SGARs, particularly brodifacoum was higher in later years.

There were also significant differences among years in the sum of bromadiolone and difenacoum concentrations (KW=15.7, P=0.03; Figure 3). However, Dunn’s multiple comparison test with Holm p-value correction shows no significant difference between each year. There is little evidence that the proportion of birds exposed to these two SGARs has changed over time.

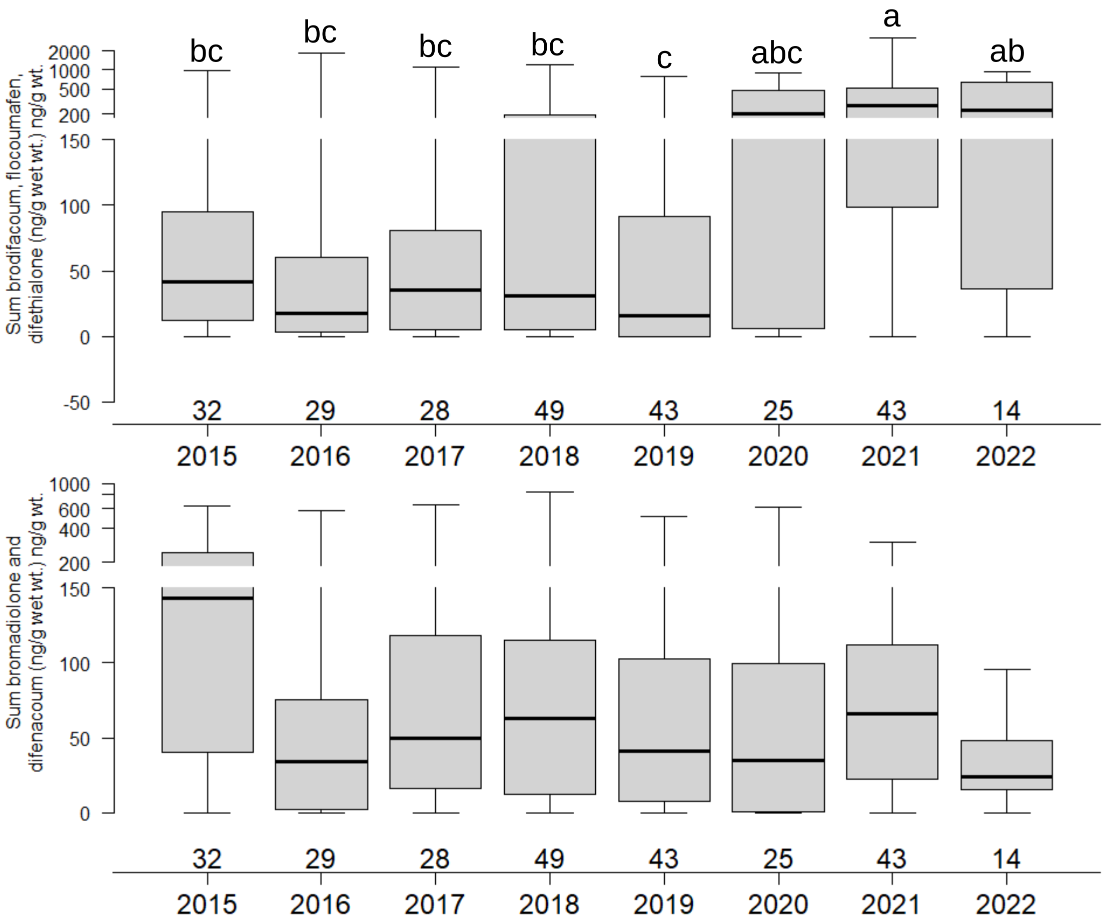

Figure 3. The liver sum concentrations of brodifacoum, flocoumafen, and difethialone (top) and liver sum concentrations of bromadiolone and difenacoum (bottom) in all red kites found dead between 2015 and 2022. For sum of brodifacoum, flocoumafen, and difethialone concentrations, significant (P<0.05) differences between years are indicated by different letters. There was no significant in multiple comparisons of years in the sum of bromadiolone and difenacoum concentrations. Total sample numbers are shown above the x-axis.

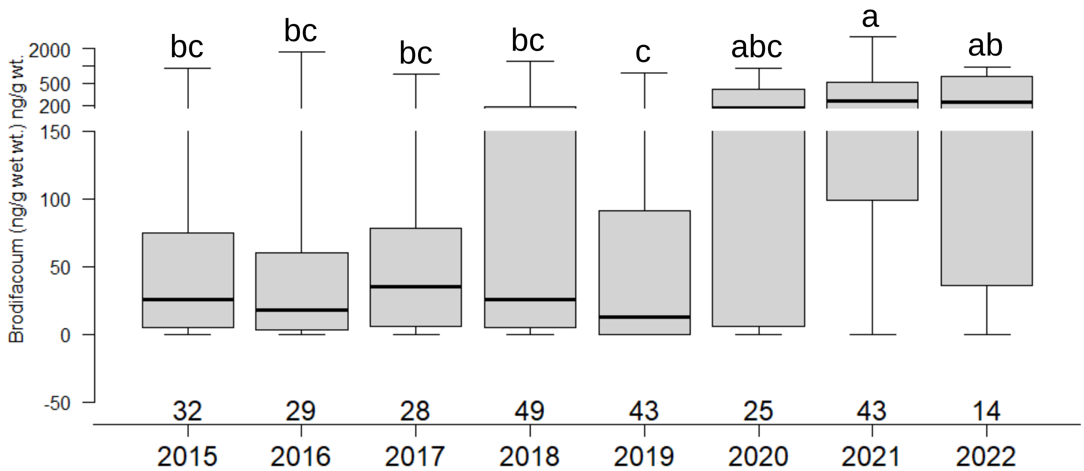

Figure 4. The liver concentrations of brodifacoum in all red kites found dead between 2015 and 2022. Significant (P<0.05) differences between years are indicated by different letters. Total sample numbers are shown above the x-axis.

4.3 Trends in poisoning over time

The percentage of birds from 2022 for which SGARs was diagnosed as a contributory factor in their cause of death (Table 3) was 17% with this value ranging between 5% and 32% during the monitoring period. However, the numbers of red kites in "expected cells" in the Chi-squared tests were low (i.e., n<5). We therefore also compared data when pooled into groups of years (pre-stewardship vs post-stewardship) as in Section 4.2. In this analysis, the proportion of red kites in which SGARs were implicated as a cause of death was significantly lower in post-stewardship than in pre-stewardship years for England & Wales (Fisher's Exact test: P=0.002) and Britain as a whole (Fisher's Exact test: P=0.008).

Table 3. Number (% of total excluding uncertain contributory cause of death) of red kites that showed signs of haemorrhaging without associated physical trauma and that had one or more detectable liver SGAR residue (SGARs implicated).

	Number (%) of red kites in which SGARs were implicated/not implicated[1] as a contributing cause of death (poisoning)							
	England & Wales				Britain			
Year	SGAR poisoned	un-certain	not poisoned	Total	SGAR poisoned	un-certain	not poisoned	Total
2015	9(36%)	1	16	26	10(32%)	1	21	32
2016	7(35%)	2	13	22	7(26%)	2	20	29
2017	4(17%)	0	20	24	4(14%)	0	24	28
2018	8(19%)	0	34	42	9(18%)	0	40	49
2019	2(6%)	0	29	31	2(5%)	0	41	43
2020	3(15%)	1	17	21	3(13%)	1	21	25
2021	4(12%)	6	30	40	5(14%)	6	32	43
2022	2(17%)	2	10	14	2(17%)	2	10	14
Total	**39(19%)**	**12**	**169**	**220**	**42(17%)**	**12**	**209**	**263**

[1]Not implicated – red kites with no detected haemorrhaging, with haemorrhaging associated with trauma, and/or no-detected liver SGAR residue.

As shown in Figure 1, sum of SGAR liver concentrations in poisoned birds did not significantly differ among years (KW=13.6, P=0.06). All birds for which SGARs were implicated as a contributing cause of death had residues of between one and four different SGARs in their livers. We examined what proportion of the summed residue was comprised of brodifacoum, flocoumafen, and difethialone and whether this proportion varied between years. On average, 77% (median value) of the ΣSGAR liver residues in poisoned birds was comprised of brodifacoum, flocoumafen, and difethialone. This proportion significantly differed among years (Figure 5; KW=14.1; P<0.05), but Dunn's multiple comparison test with Holm p-value correction shows no significant difference between each year. Although not statistically significant, Figure 5 shows a recent increase in the proportion of brodifacoum, flocoumafen, and difethialone in liver ΣSGAR residues in poisoned bird. This may suggest these three SGARs make a higher contribution to the sumSGAR concentration in poisoned birds in recent years.

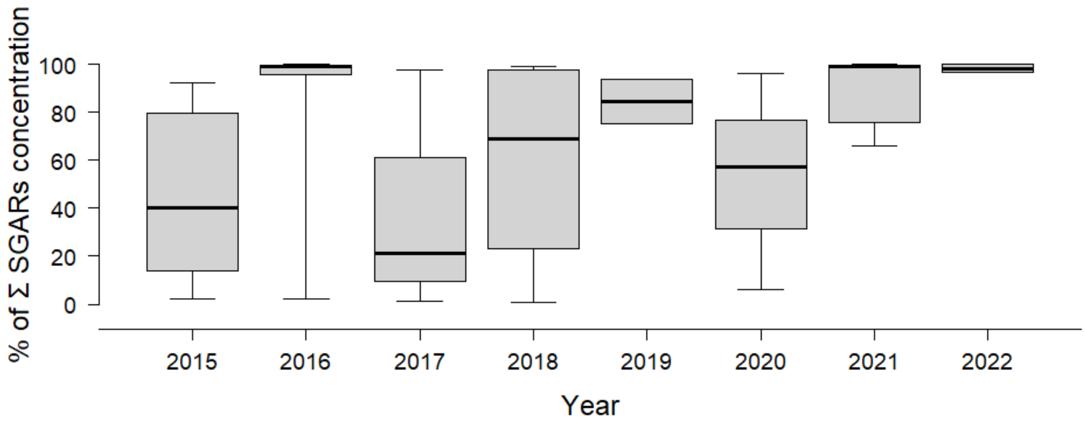

Figure 5. Box and Whiskers plot showing median, interquartile range and minimum/maximum range in sum of brodifacoum, flocoumafen, and difethialone concentrations expressed as a percentage of sum of SGAR concentrations for red kites for which SGARs were implicated as a contributing cause of death between 2015 and 2022. There was no significant difference in multiple comparisons of pairs of years.

As in previous reports in this series, we pooled data across years to improve characterisation of liver residues in birds in which SGARs were considered a contributory cause of death (Figure 6). The minimum, 1st quartile (Q1), median, 3rd quartile (Q3), and maximum ΣSGAR concentrations in poisoned birds were 134.6, 289.4, 660.3, 983.1, and 3223.7 ng/g wet wt., respectively. Overall, the median ΣSGAR concentration in those red kites was almost 4.9-fold higher than that of birds that had died from a variety of other causes. Liver residues in red kites poisoned by SGARs were significantly higher than residues in non-poisoned red kites (Mann-Whitney U test, U=1195/7583, $P<0.001$). Despite being partly overlapped potentially due to inter-individual susceptibility to SGARs, liver residues considerably differed between the two groups of kites (Figure 6).

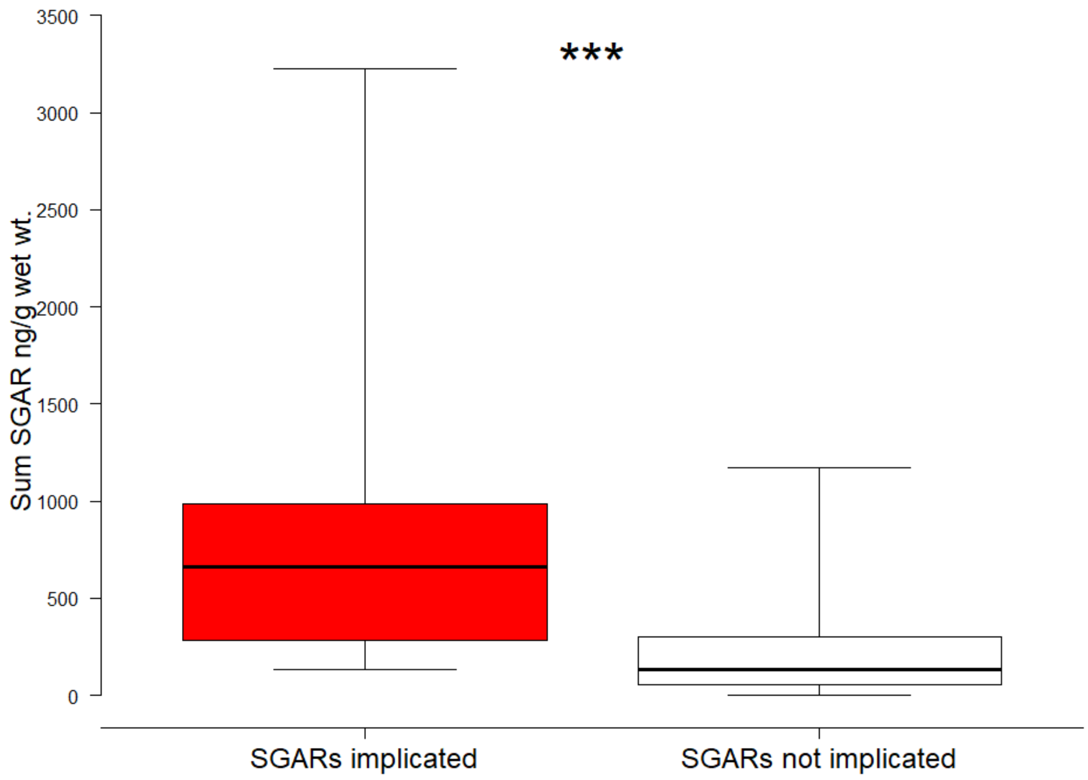

Figure 6. Box and Whiskers plot showing median, interquartile range and minimum/maximum range of sum of SGAR concentrations in red kites that died between 2015 and 2022, with haemorrhaging not associated with physical trauma (SGARs implicated as a contributory cause of mortality; n=42) and those that were diagnosed to have died from causes unrelated to SGARs (SGARs not implicated as a contributory cause of mortality; n=209). The difference in median concentrations between the two groups was statistically significant (Mann-Whitney U test, U=1195/7583, P<0.001).

5 Spatial Analysis

We assessed the importance of exposure by count to determine some hot spots of SGAR exposure. The number of birds from counties or regions has differed year by year, red kites have been mainly from Oxfordshire (n=22 from 2015 to 2022), North Yorkshire (n=17), Northamptonshire (n=15), West Yorkshire (n=14), and Hampshire (n=13) in England, Ceredigion in Wales (n=11), and the regions “Dumfries and Galloway” (n=20) and “Highland” (n=12) in Scotland (Figure 7a). To avoid biases from different sample numbers by county, we assessed and represent on maps the maximum ΣSGAR concentrations (Figure 7b) and the proportion of poisoned red kites (Figure 7c) by county during the monitoring period 2015 – 2022.

The maximum value of liver ΣSGAR concentrations was observed in Hampshire (3223 ng/g wet wt.), followed by Berkshire (1800 ng/g wet wt.) and West Yorkshire (1406 ng/g wet wt.). The maximum ΣSGAR concentrations per county higher than the 3rd quartile of ΣSGAR concentrations in poisoned birds (983.1 ng/g wet wt.) were also observed in South East (Oxfordshire, 1218 ng/g wet wt.), East Midland (Northamptonshire, 1267 ng/g wet wt.; Leicestershire, 988 ng/g wet wt.; Lincolnshire, 1138 ng/g wet wt.), Yorks/Humber (North Yorkshire, 1174 ng/g wet wt.), and North East (Tyne and Wear, 1150 ng/g wet wt.) in England, as well as Wales (Powys, 1065 ng/g wet wt.).

When the proportion of poisoned birds were compared to all birds including uncertain cases during the monitoring period (2015 – 2022), the proportion was 100% in Tyne and Wear and South Yorkshire, only one bird had been collected in these two counties though. The proportion was also high in Essex (50%; 1/2 samples), Berkshire (44.4%; 4/9 samples), Dorset (42.9%; 3/7 samples), and West Yorkshire (42.9%; 6/14 samples) (Figure 7b). The proportion ranged between 20 – 40 % in the middle part of South England, namely Hampshire (23.1%; 3/13), Buckinghamshire (25.0%; 1/4), Hertfordshire (28.6%; 2/7), Leicestershire (33.3%; 1/3), Northamptonshire (26.7%; 4/15), and Warwickshire (25.0%; 1/4), as well as Grampian (Aberdeen city, Aberdeenshire, and Moray) in Scotland (33.0%; 1/3). When the proportion of poisoned birds were compared to all birds excluding uncertain cases, the proportion of poisoned birds increase in Berkshire (57.1%, 4/7), West Yorkshire (46.2%; 6/13), Hampshire (25.0%; 3/12), Leicestershire (50.0%; 1/2), Oxfordshire (19.0%, 4/21), and Wiltshire (14.3%, 1/7).

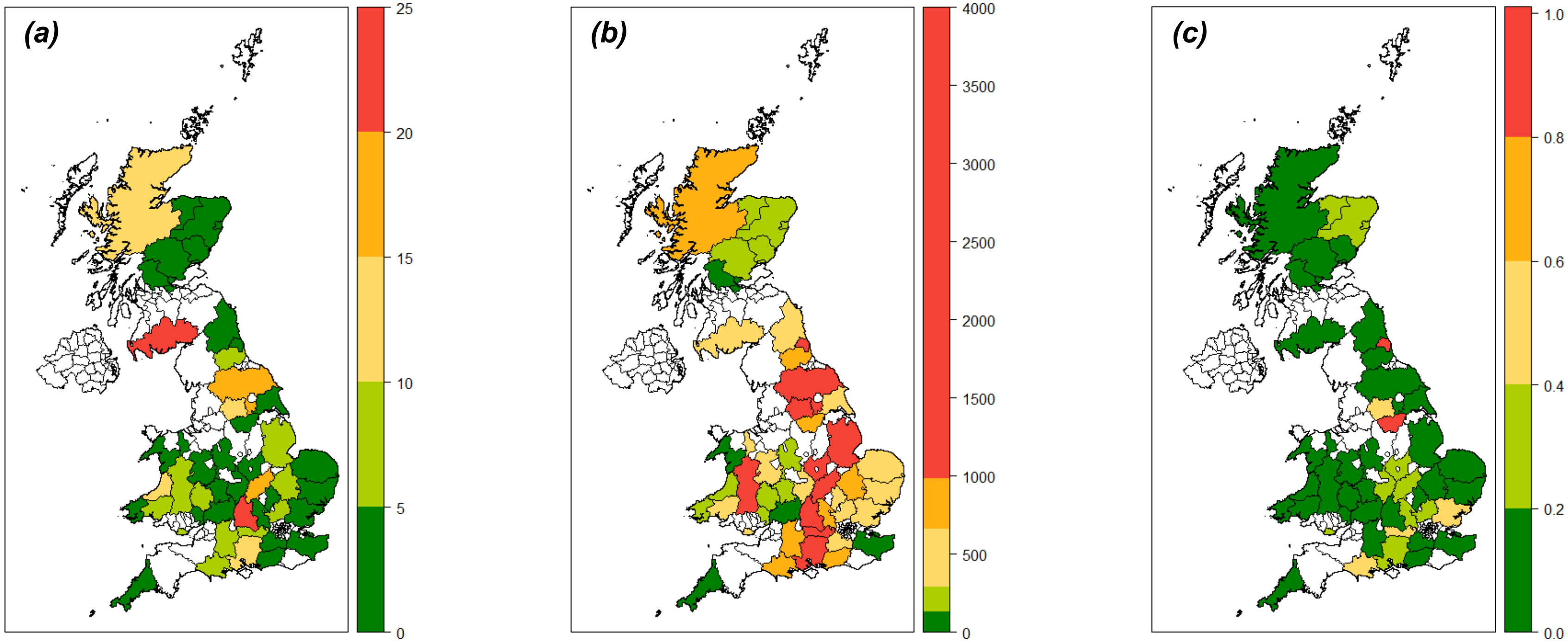

Figure 7. Maps summarising data from red kites that had died between 2015 and 2022 by county/region: (a) number of birds collected; (b) maximum value of liver ΣSGAR concentrations (ng/g wet wt.); and (c) proportion of poisoned birds compared to all birds excluding uncertain cases. The colouring in Figure 7b corresponds to the summary statistics of ΣSGAR residues in poisoned birds, i.e., <LoD – minimum: green; minimum – Q1: light green; Q1 – median: yellow; median – Q3: orange; Q3 – maximum: red. Counties/regions where no bird has been collected are coloured in white in all maps.

6 Power analysis

Based on the data for the ΣSGAR liver residues in red kites from 2015 to 2022, we conducted the power analysis. For this analysis, logarithmically transformed ΣSGAR liver residues (base = 10) were used. The power analysis requires a sample number and a hypothetical constant temporal change in values. We therefore fixed the number of red kites found dead by year to the average number of 33 during the period and assumed that the geometric mean ΣSGAR liver residues would have consistently increased from 126.2 in 2015 to 240.4 ng/g wet wt. in 2022 (i.e., residues have increased 1.9 times over 7 years) based on the geometric mean of these years.

After 1000 simulations, the power of the dataset was about 10%. Supposed that the current increase constantly continues, the power monitoring for 20 years would reach 80% (Figure 8). Compared with the results in the previous report, the power of the dataset was reduced due to the lower geometric mean ΣSGAR liver residues and fewer samples in 2022. If the sample number remains low in the following years, an extended monitoring period would be needed to obtain enough statistical power to detect similar annual changes in ΣSGAR liver residues.

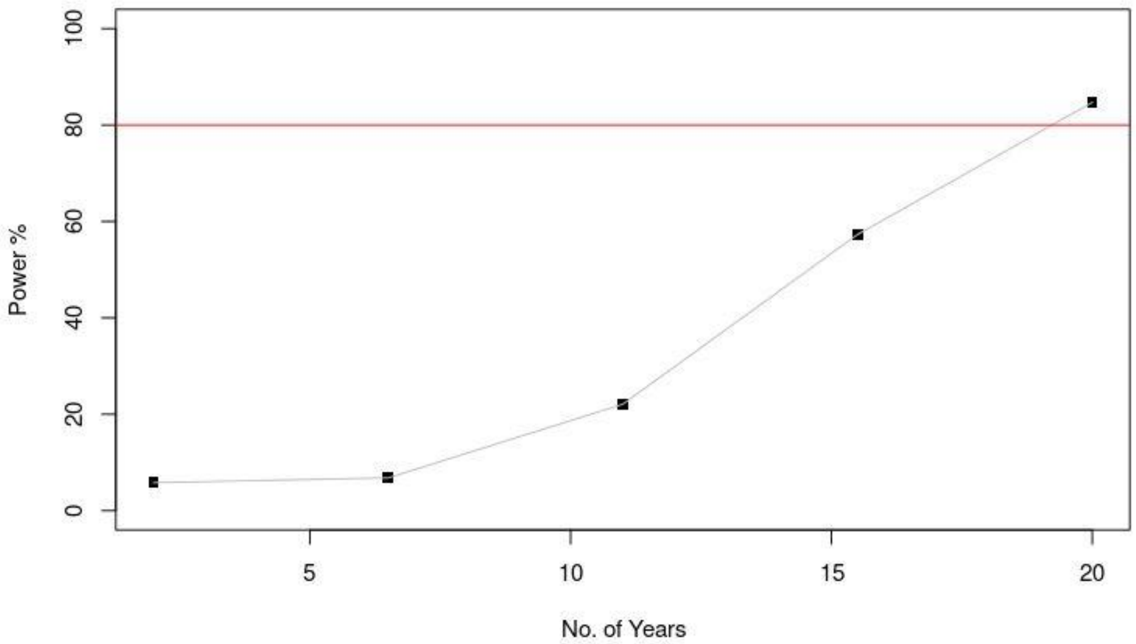

Figure 8. Relationship between the power and the number of years based on the ΣSGAR liver residues in red kites found dead from 2015 and 2022. Logarithmically transformed ΣSGAR liver residues (base = 10) are used for the analysis. The number of red kites found dead by year is fixed to 33 (the average number), and ΣSGAR liver residues are supposed to increase 1.9 times for 7 years (geometric mean ΣSGAR liver residues=126.2 and 240.4 in 2015 and 2022, respectively).

7 Analysis of the impact of avian influenza on residues data profiles

Since 2022, the number of red kite carcases or tissues received by the Predatory Bird Monitoring Scheme (PBMS) and the Wildlife Incident Investigation Scheme (WIIS) has declined, principally due to the presence of highly pathogenic avian influenza (HPAI) in wild bird populations. This reduction may be due to two factors namely (i) in 2022 advice from Defra was published advising the public not to pick up dead birds which may have led to fewer carcases being collected, and (ii) some birds that were collected subsequently tested positive for HPAI and so were destroyed and not available for SGAR analysis. The numbers of birds/tissue sets received by the PBMS fell from a 5-year average (2017 to 2021) of 614 to 461 in 2022, a 25% reduction in birds received. Specifically for red kites, the number of birds for which SGAR concentrations are available for these reports has declined in 2022 to 14 compared to an average between 2015 and 2021 of 36 birds, a 60% decline. As discussed in the power analysis section of this report, such overall reductions in sample numbers will reduce our ability to detect statistically significant changes in exposure to SGARs in the red kite. However, another impact of changes to submissions of red kites due to HPAI is that if it affects the proportion of sample that originates through either the PBMS or WIIS, this may lead to inter-year variation in liver concentrations if those residues differ between those two sources of samples. Therefore, we investigated whether there are such differences in ΣSGAR concentrations, both in terms of average concentrations and trends in those concentrations, between PBMS and WIIS samples.

We have pooled SGAR residues data from different laboratories, which might mix different red kite populations. We therefore compared SGAR residues in red kites from PBMS and WIIS (Fera Science for England and Wales and Science & Advice for Scottish Agriculture for Scotland). Overall, the median ΣSGAR concentration in red kites from PBMS was almost three times higher than ΣSGARs in birds from WIIS (median=291 and 105 ng/g wet wt., respectively). The difference was statistically significant (Mann-Whitney U test, U= 5255.5/11796.5, P<0.001) (Figure 9a). When the same analysis was carried out for each active ingredient, bromadiolone, difenacoum and brodifacoum concentrations were also significantly higher in birds from PBMS than in birds from WIIS (Mann-Whitney test U = 7273/9779, 6273.5/10778.5, and 5765/11287; P = 0.04, <0.001, and <0.001, respectively) (Figure 9b, c, d). The median values of these SGARs in birds from the PBMS were 9.0, 29.2, and 89.3 ng/g wet wt., respectively, whereas those in birds from WIIS were 5.0, 13.0, and 18.0 ng/g wet wt., respectively. Difethialone residues were detected only in 30 birds from PBMS. The median value of Difethialone residues from PBMS and WIIS was 0.0 ng/g wet wt., but concentrations were significantly higher in birds from PBMS than from WIIS (Mann-Whitney test U = 6991/10061; P < 0.001). Flocoumafen residues were detected only in 2 birds from PBMS, and the difference between PBMS and WIIS was not statistically tested.

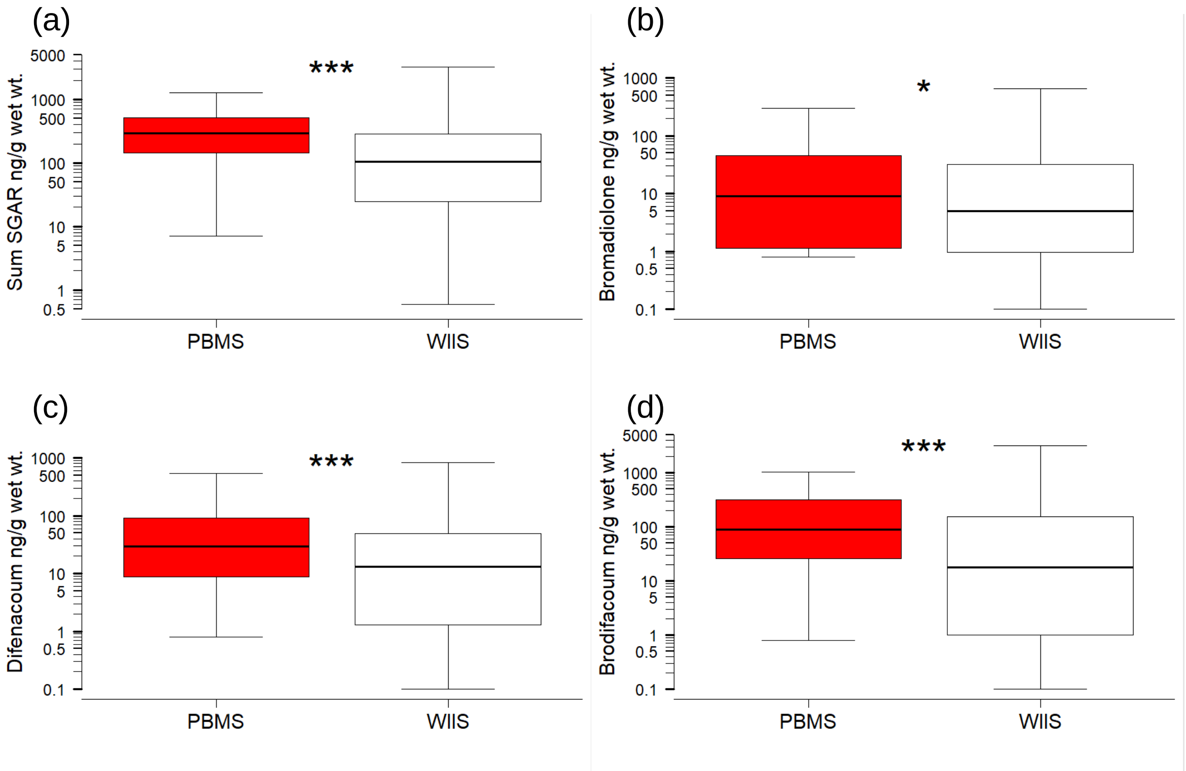

Figure 9. Box and Whiskers plot showing median, interquartile range and minimum/maximum range of SGAR concentrations in red kites that died between 2015 and 2022 from PBMS (n=116) and from WIIS (WIIS Fera Science for England and Wales and WIIS SASA for Scotland; n=147): (a) ΣSGAR, (b) bromadiolone, (c) difethialone, and (d) brodifacoum. Difference of SGAR concentrations was tested by non-parametric Mann-Whitney test, and statically significant differences are indicated by asterisk symbols (*: P<0.05; **: P<0.01; ***: P<0.001). For a graphical representation with a logarithm scale, residues <LoD were replaced by the half of the lowest detected value for each of PBMS and WIIS (a) or by the half of the LoD in each analysis (b, c, d).

When ΣSGAR residues were compared between these groups by year, statistically significant differences were observed in early years of the monitoring (i.e., 2015, 2016, 2017, and 2019), but differences were not statistically significant since 2020 (Figure 10a). Bromadiolone residues did not significantly differ between PBMS and WIIS in each year, whereas difenacoum residues in birds from PBMS in 2019 was significantly higher than in birds from WIIS. Brodifacoum residues in birds from PBMS was significantly higher than in birds from WIIS in 2015, 2017, 2018, and 2019. Difethialone and flocoumafen residues were not compared by year.

The temporal trend of SGAR residues was also differ between birds from PBMS and WIIS. In birds from PBMS, ΣSGAR and difenacoum residues did not significantly differ among years (KW=12.6 and 12.0; P=0.08 and 0.1, respectively). Kruskal Wallis test showed a significant difference among years in difenacoum and brodifacoum residues in birds from PBMS (KW=14.8 and 16.4; P=0.04 and 0.02), but Dunn's multiple comparison test did not detect any significant difference between each combination of two years. In contrast, ΣSGARs in birds from WIIS significantly differed among years (KW=26.0; P<0.0015), and Dunn's multiple comparison test with Holm p-value correction showed higher ΣSGAR residues in WIIS birds in 2021 that in 2016, 2017, and 2019. Bromadiolone residues in WIIS birds significantly differed among years (KW=14.7; P=0.04), but Bromadiolone residues was not significant different between any of two years. Difenacoum residues in WIIS birds did not significantly differ among years (KW=11.7; P=0.1). Brodifacoum residues in WIIS birds significantly differed among years (KW=36.4; P<0.001). Brodifacoum residues in WIIS birds in 2021 was significantly higher than in 2015 – 2019, and residues in 2022 was also significantly higher than in 2019.

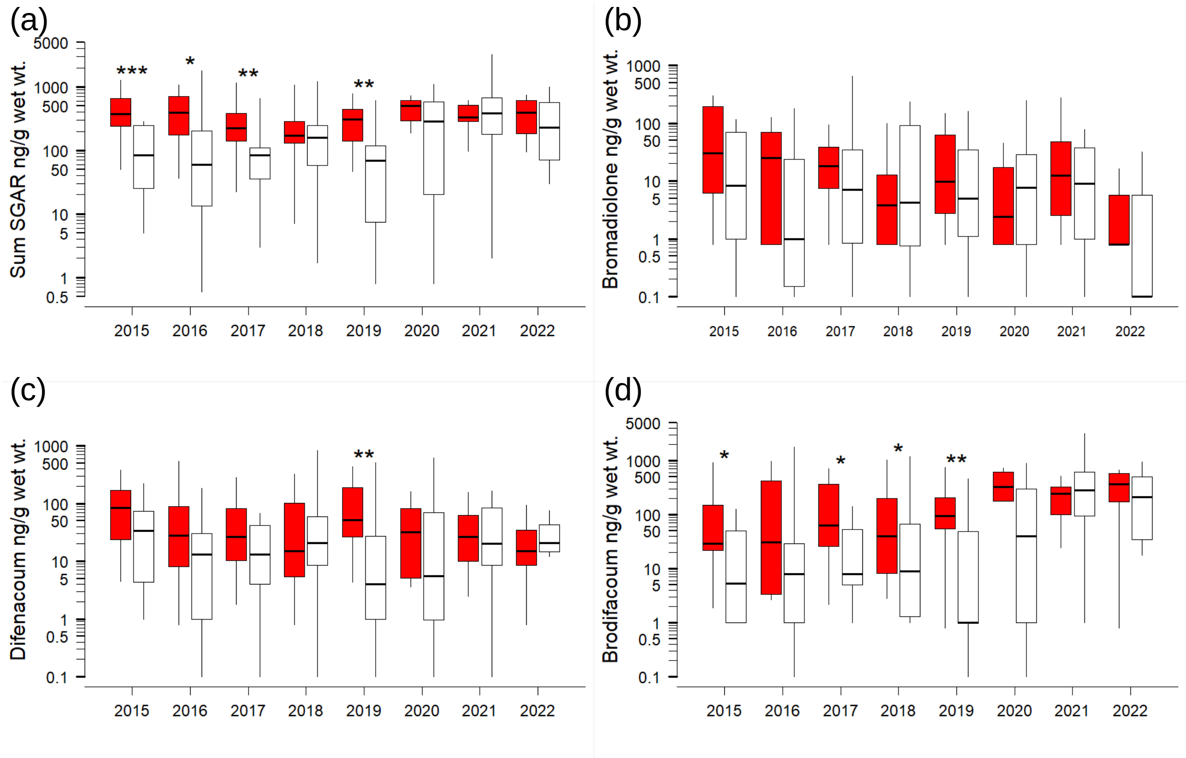

Figure 10. Box and Whiskers plot showing median, interquartile range and minimum/maximum range of sum of SGAR concentrations in red kites collected in each year from between 2015 and 2022 from PBMS (red) and from WIIS (WIIS Fera Science for England and Wales and WIIS for Scotland) (white). Differences of SGAR concentrations between PBMS and WIIS were tested by year by non-parametric Mann-Whitney test, and statically significant differences are indicated by asterisk symbols (*: P<0.05; **: P<0.01; ***: P<0.001). For a graphical representation with a logarithm scale, residues <LoD were replaced by the half of the lowest detected value for each of PBMS and WIIS (a) or by the half of the LoD in each analysis (b, c, d).

Given that the median concentration of ΣSGARs in samples received by the PBMS is significantly higher than those received by the WIIS, a change in ratio of samples received by these two sources could result a change in the exposure characterised by this monitoring. The concentration observed between 2015 and 2022 indicates that if the sample is dominated by WIIS samples, the monitoring would underestimate the exposure compared to the current sample structure. The percentage of samples received from WIIS has varied among years within the monitoring period, ranging between 44% and 80% (Figure 11), and so the concentrations observed in this series of reports includes significant variation of relative proportions of data sources. Therefore, the percentage of samples received from WIIS would need to exceed this range to have a pronounced effect on the ΣSGAR concentrations reported. In an extreme circumstance where samples for this monitoring were received by solely from WIIS or PBMS, the monitoring would under or overestimate ΣSGAR exposure, respectively, in red kites compared to the current monitoring.

In conclusion, the most significant effect of the presence of HPAI in wild bird populations is a reduction in the number of samples, as a whole, that are available for monitoring SGARs in red kites. Pronounced changes in sample structure would lead to an overestimate of exposure if PBMS samples dominated the sample, and conversely an underestimate of exposure if WIIS samples dominated the sample, compared to current monitoring.

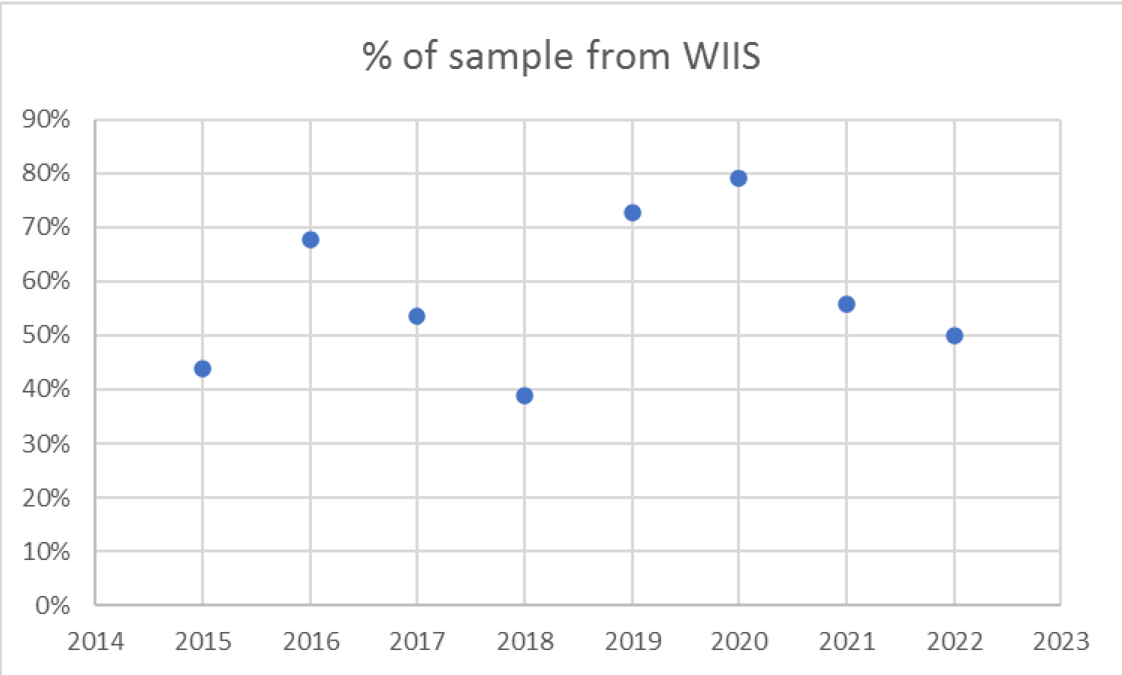

Figure 11. Percentage (%) of samples that were received from WIIS from 2015 to 2022.

8 Conclusions

The monitoring of SGAR residues in red kites remains an important contribution to our understanding of SGAR exposure in wildlife, particularly in relation to predators and scavengers that take a proportion of target prey species, such as the brown rat, as components of their diets.

Of the 14 red kites from England and Wales found dead in 2022, all had been exposed to SGARs. In two cases (14% of total excluding uncertain contributory cause of death), SGARs were implicated as a contributory cause of death.

Difenacoum, brodifacoum, and bromadiolone were the most prevalent compounds (detected in 87%, 87%, and 75% of red kites across the eight years for each compound, respectively). On average, there were detectable residues of three different SGARs in each red kite liver likely demonstrating multiple exposures. With regards change over time (2015–2022), the proportion of red kites with detectable liver SGAR residues ranges 88 – 100%. There were statistically significant differences between years, and the magnitude of accumulated summed SGAR residues has generally been higher in recent years. Although there was no clear difference for sum bromadiolone and difenacoum, the sum of brodifacoum, flocoumafen, and difethialone concentrations, and even only brodifacoum concentrations, were statistically higher in 2021 than the previous years except 2020. Despite a low sample number which reduced the statistical power, birds from 2022 showed statistically higher brodifacoum concentrations than in 2019. The results clearly indicate an increasing trend of brodifacoum residues in red kites in recent years.

Given the result from the power analysis, the number of years for which we have combined data from different monitoring schemes is not yet high enough to detect a temporal trend in SGAR residues. With the current number of red kites found dead and analysed, more monitoring years are required to obtain high statistical power that infers a significant effect through monitoring period. Thus, our ability to detect temporal changes over and above variability related to other factors (such as provenance, age, other mortality factors) is limited currently. Furthermore, many of the birds examined (where age was reported) were adults and so may have liver residues at least partly derived from exposure that occurred months or possibly years previously; the liver half-lives of SGARs are reported to range between approximately one month and just over 300 days (Vandenbroucke et al., 2008). Thus, there may be a time lag between a change in usage practice and any consequent change in residue accumulation by red kites. There were statistically significant differences among individual years in this proportion and there was a statistically significant decreasing annual trend. Annual sample sizes of birds for which SGARs were diagnosed as a contributory mortality factor were small, however when data were pooled into year blocks (2017/18/19/20/21/22 vs 2015/2016), the proportion of red kites in which SGARs were implicated as a cause of death was significantly lower in later than earlier years for birds from England & Wales, and for Britain as a whole. Therefore, while there was no clear-cut consistent picture of change in exposure to SGARs in general, mortality attributed to SGARS showed a decline over the monitoring period. However, given that the WIIS scheme specifically examines suspected poisoning incidents, the relative proportion of birds that have been examined as part of the WIIS scheme may affect year-to-year variation in the proportion of birds for which SGARs were diagnosed as a contributory mortality factor. This potential bias requires further consideration as the dataset available for analysis increases due to population increase and expansion. Furthermore, the pros and cons of monitoring SGAR levels in a random sample of red kites, as is used in barn owl monitoring (Ozaki et al, 2022), should be considered in the future to improve our ability to interpret changes in SGAR levels. The monitoring of SGARs in blood samples from tracked individuals could also be considered as part of such a programme.

Another concern about sampling is the influence of avian influenza. In fact, a lower number of samples in 2022 than the previous years, because of avian influenza, largely affected the statistical power of monitoring. If sampling continues to be restricted, we need to revise monitoring programmes, such as prolonging the sampling period or seeking alternative samples.

The most significant effect of the presence of highly pathogenic avian influenza (HPAI) in wild bird populations is a reduction in the number of samples, as a whole, that are available for monitoring SGARs in red kites. Pronounced changes in sample structure would lead to an overestimate of exposure if PBMS samples dominated the sample, and conversely an underestimate of exposure if WIIS samples dominated the sample, compared to current monitoring.

Spatial analysis, by county/region indicated that across the monitoring period highest exposure of summed SGARs in red kites appeared to be around the Berkshire/Hampshire and, to a lesser extent, North and West Yorkshire areas. The Berkshire/Hampshire area is a long establish foci for bromadiolone and difenacoum resistance in target species, and so this may be a contributory factor to this greater exposure. There have been resistance genotypes detected in North Yorkshire, but these have only been more recently documented (Buckle et al., 2022).

Overall, the high proportion of red kites exposed to SGARs along with observed higher sum of SGAR and the increasing trend of brodifacoum residues observed in recent years remains a concern, as is the assessment that SGARs were a contributory cause of death in 16% of the red kite cases examined across all eight years. Over recent years, the red kite population in Britain has increased considerably (by approaching 2000% in the period 1995 to 2019; Harris et al., 2020), largely as a consequence of reintroduction policies. However, we do not know how SGAR-induced mortality may impact on the population dynamics of red kites. For this point, further research should be addressed on the sensitivity of the red kite to SGAR toxicity and the effects of SGARs on populations. A commonly cited threshold of toxicity is given as “greater than 100-200 mg/kg wet weight” based on a potentially lethal range derived for the barn owl (Newton et al., 1999a; 1999b). However, liver concentrations associated with rodenticide poisoning vary greatly, both among species and among individuals within species (Stone et al., 1999). Further analysis on the diagnosis of various effects by SGAR exposure at the individual level, using such as probabilistic modelling (Thomas et al., 2011) and at the population level, using such as population change modelling, are required. To detect statistically significant time trends of SGAR residues and integrate various parameters like sex and age into models, continued monitoring of SGAR concentrations in this species is recommended.

9 Acknowledgements

We thank all the members of the public who have submitted carcasses to the Predatory Bird Monitoring Scheme (PBMS). Their efforts are key to the success of the scheme.

The PBMS is funded by the Natural Environment Research Council award number NE/R016429/1 as part of the UK-SCaPE programme delivering National Capability. Additional funding to enhance elements of the PBMS core collection activities were provided by Natural England (NE) and the Campaign for Responsible Rodenticide Use (CRRU). The resources for the chemical analysis of red kite samples for the current work and for the production of this report were provided by Natural England.

The Wildlife Incident Investigation Scheme in England is under the policy responsibility of the Chemicals Regulation Division of the Health and Safety Executive (HSE) and the WIIS is run on HSE's behalf by Natural England. In Wales, Scotland and Northern Ireland, the WIIS is run by the Welsh Government, SASA on behalf of the Scottish Government and the Department of Agriculture and Rural Development, respectively.

This report was peer-reviewed by Suzane Michelle Qassim, Graeme Shaw, Rosie J. Lennon, Rebecca Pringle, Helen Donald, and Patrick Shannon-Hughes of Natural England and Tony Sainsbury of Institute of Zoology.

We thank Georgina Gerard, Shaheed Karl Macgregor, and Shinto Kunjamma John for assistance with pathological examinations.

10 References

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Appendix 1 – Summary of limits of detection and spiked standard recoveries for anticoagulant rodenticides by LC-MS/MS analysis across schemes

Limits of detection (LoD; ng/g wet wt.) and percentage recovery for spikes used in analysis by PBMS (UKCEH) and WIIS England & Wales (Fera Science) laboratories.

	UKCEH		Fera Science	
	LoD	% Spike recovery	LoD	Typical % Spike recovery
Brodifacoum	1.5	86.3	0.8	64
Bromadiolone	1.5	72.8	0.8	94
Difenacoum	1.5	-	0.8	94
Flocoumafen	1.5	-	0.8	105
Difethialone	2.8	-	0.8	83

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