UK Centre for Ecology & Hydrology

Second generation anticoagulant rodenticide residues in red kites 2020

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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 25 red kites that had been found dead in Britain in 2020. The carcasses were submitted to and necropsied by the Disease Risk Analysis and Health Surveillance (DRAHS) programme, the PBMS, the WIIS for England & Wales, the WIIS for Scotland and the Raptor Health Scotland study. All the 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 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 SGAR users, 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 previously applied to brodifacoum, flocoumafen and difethialone, the three most acutely toxic SGARs. 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 but one of the 21 red kites from England & Wales and two of the four red kites from Scotland had detectable liver residues of at least one type of SGAR. When considering the sample of red kites as a whole, brodifacoum, difenacoum and bromadiolone were each detected in 21, 19 and 17 red kites, respectively. Difethialone was found in four individuals while flocoumafen was detected in one bird.

The proportion of analysed red kites exposed to SGARs in 2015 (91%), 2016 (90%) 2017 (96%), 2018 (100%) 2019 (91%) and 2020 (88%) was similar at *circa* 88% or more; the higher percentages in 2017 and 2018 were principally due to a greater proportion of birds from Scotland containing residues than observe in other years. Difenacoum, brodifacoum, and bromodialone were the most prevalent compounds

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

(detected in 86%, 85%, and 76% of red kites across the six years for each compound, respectively). On average, there were detectable residues of three different SGARs in each red kite liver likely demonstrating multiple exposures.

Sum liver SGAR concentrations in birds from 2020 ranged between non-detectable and 1086 ng/g wet weight (arithmetic mean: 371 ng/g wet weight, median 307 ng/g wet weight). Necropsy examinations indicated that three (13%) of red kites examined had internal haemorrhaging that was not associated with detectable trauma and also had detectable liver SGAR concentrations. These birds had sum SGAR liver concentrations of 663, 905 and 1086 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 17% of the red kite cases examined across all six years. Over the period 2015 to 2020, 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 six years. Due to these reasons, caution should be used when interpreting evident changes in mortality rates due to the sampling protocols used in this study that may lead to over reporting of mortality rates, and those rates being subject to variations in relative contribution of the WIIS and PBMS to each year's sample.

There was no statistically significant difference between years, irrespective of cause of death, in median summed SGAR residues, and no evidence that the magnitude of accumulated summed SGAR residues has changed consistently over time. Sum bromadiolone and difenacoum concentrations were lower in 2016 than 2015, however, there was no difference for sum brodifacoum, flocoumafen, and difethialone concentrations.

Data on presence/absence of detectable brodifacoum, flocoumafen or difethialone residues were compared for 2015/16 and 2017/18/19/20. The proportion of red kites with detectable residues of these three SGARs was 82% in 2015/16 and similar proportions were observed in 2017/18/19/20 (86%). Similarly, there was no significant difference in the proportion of red kites with detectable liver difenacoum or bromadiolone residues (90% in 2015/16 vs. 93% in 2017/18/19/20). Since the implementation of the stewardship regime no difference in exposure pattern relating to active ingredient has been detected.

Our findings do not indicate that there has been a 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. There is some evidence (depending upon the statistical approach used) 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 analyses 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. There was no clear evidence that relaxation of usage restrictions on brodifacoum, difethialone and flocoumafen has altered the pattern of residues for these compounds in red kites to date. However, data following full implementation of the rodenticide stewardship scheme is currently limited to four years.

2 Introduction

The current report is the fifth 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 approaching 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). 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., 1999; Dowding et al., 2010, McDonald et al., 1998, Ruiz-Suárez et al., 2016; Sainsbury et al., 2018; Shore et al., 2003a,b, 2006, 2015; Walker et al., 2008a,b). 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., 2020) provide data on exposure in a species that feeds predominantly on non-target rodents (i.e. rodent species excluding brown rat, *Rattus norveg*icus, 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 (*Rattus norvegicus*).

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 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 of rats as the objective of baiting is to expose rats and house mice and so the number and density of AR-contaminated rats may be maintained. However, diligent searching, removal and safe disposal of poisoned rats is promoted by the stewardship scheme. This might be expected to reduce the availability of poisoned rats to red kites and other scavengers and thereby reduce risk of exposure. Moreover, the red kite is not exclusively a scavenger on rat carcases but 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 particular 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. 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 outdoor permission may increase these three SGARs frequency of use, especially in areas where there is resistance to bromadiolone and difenacoum (Jones et al., 2019). This change may subsequently increase secondary exposure of red kites to these three SGARs, but fewer baits for a shorter time may be necessary for control of target species compared to using resisted active ingredients such as difenacoum or bromadiolone (Buckle et al. 2020). Although all SGARs are highly toxic to vertebrates, brodifacoum, flocoumafen and difethialone typically are the most acutely toxic (Erickson & Urban 2004). 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, 2021). 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. Occasional red kite necropsies are conducted by the PBMS. Analysis of liver SGARs is undertaken by the PBMS.

[2] https://www.hse.gov.uk/pesticides/resources/W/wiis-quarterly.xlsx; last accessed 05/05/2021

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 complimentary 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 Science & Advice for Scottish Agriculture (SASA) 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 2020 and analysed by the WIIS (England & Wales and Scotland) 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 2020 and submitted to the DRAHS/PBMS, WIIS for England & Wales, or the WIIS for Scotland for analysis.

We describe the current incidence, magnitude and likely toxicological significance of the liver SGAR residues detected in these birds in 2020 and compare our data with those for kites that died between 2015 and 2019 (Walker et al., 2017, 2018, 2019, 2021). 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.htm

3 Methods

The carcasses of 25 red kites that died in 2020 were collected as part of the PBMS or the DRAHS programmes, WIIS for England & Wales, or the Raptor Health Scotland/WIIS for Scotland schemes (Table 1). Both PBMS and DRAHS projects rely on citizen science in that members of the public send in dead birds that they find. WIIS incidents are reported by a variety of stakeholders that also include members of the public.

The majority of red kite carcasses (84%) were from England and Wales. 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).

Table 1. Number of red kites examined in each demographic group for individuals found dead in 2020.

	Adult	First-year	Unknown
Male	2	1	5
Female	5	1	7
Unknown	2	1	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), Fera Science and SASA, respectively. Protocols varied among laboratories but during all necropsies, non-trauma related macroscopic haemorrhaging that was consistent with AR-induced anticoagulation was noted. Birds were classed as individuals in which SGARs were implicated as a contributory cause of death only if such haemorrhaging was present and if AR residues (of any magnitude) were detected in the liver.

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 using Minitab 16.1 (Minitab Ltd., Coventry, U.K.) and illustrated using GraphPad Prism version 5.04 for Windows (GraphPad Software, San Diego, USA). Throughout this report analyses with P-values less than 0.05 are considered to be statistically significant.

4 Results

4.1 Liver SGAR residues in red kites that died in 2020

Of the 25 red kites found dead in 2020, all but three had detectable concentrations of one or more SGARs in their liver (Table 2). Bromadiolone (detected in 68% of red kites in the sample), difenacoum (76%) and brodifacoum (84%) were the most prevalent residues detected. Difethialone was found in four birds (16%) and flocoumafen was detected in one red kite from 2020. Sum SGAR concentrations ranged between non-detectable to 1086 ng/g wet wt. with a median of 308 ng/g wet weight.

Although the limit of detection for the analysis of SGARs was slightly higher for samples from Scotland (Appendix 1), applying this LoD to birds from England & Wales would have made no significant difference to the reported percentage of birds (Fishers exact test, P=1.0) with detected residues of at least one SGAR in 2020. One of the 21 red kites in England & Wales would have been classed as having non-detected residues if the higher detection limit was applied.

Post-mortem examinations indicated that three of the 25 of the red kites found dead in 2020 had internal haemorrhaging that was not associated with detectable trauma. All three birds had comparatively high liver sumSGAR residues of 663, 905, and 1086 ng/g wet wt. (Table 2). Anticoagulant rodenticides were considered to be a contributory cause of death of these three birds. One other bird had a relatively high sumSGAR concentration (761 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.

Table 2. Concentrations of second generation anticoagulant rodenticides (SGARs) in the livers of red kites found dead in 2020.

Scheme	Incident/ Bird code	SGAR contrib. cause of death	Month of death	Sex	Age	Location	Concentration of SGAR (ng/g wet wt.)					
							Brom	Difen	Floc	Brod	Difeth	ΣSGARs
Fera Science	100165	No	Dec	F	Adult	W. Sussex	98	92	0	300	0	490
Fera Science	100129	No	Nov	M	U	Dorset	4.4	23	0	900	0	927
Fera Science	100148	No	Oct	M	U	N Yorks.	0	0.7	0	15	0	15.7
Fera Science	100009	Yes	Jul	U	Adult	N Yorks	3.4	620	0	40	0	663
Fera Science	100008	Yes	Jul	U	Juvenile	N Yorks..	16	450	0	620	0	1086
Fera Science	99929	Yes	Apr	F	U	N Yorks.	32	2.7	0	380	490	905
Fera Science	99873	No	Apr	F	Adult	Lincs.	250	62	0	32	0	344
Fera Science	100006	No	Apr	M	U	Lincs.	13	140	0	290	0	443
Fera Science	99988	No	Apr	F	U	N Yorks.	9.1	76	0	200	0	285
Fera Science	99810	No	Jan	U	Adult	Beds.	7.6	30	0	270	0	308
Fera Science	99862	No	Mar	F	U	W Yorks	49	5.5	0	1	0	55.5
Fera Science	99866	Uncertain	Mar	F	U	Oxfordshire	0	0.95	0	750	9.9	761
Fera Science	99903	No	Mar	M	U	Powys	0	0	0	0	0	0
Fera Science	99904	No	Mar	M	U	Powys	0.61	0	0	0.6	0	1.21
Fera Science	99952	No	May	F	U	Carmarthenshire	0	0	0	70	0	70

M – male; F- female; U – sex or age not determined; ND = non-detected; Brom – bromadiolone; Difen – difenacoum; Floc – flocoumafen; Brod – brodifacoum; Difeth – difethialone; D&G – Dumfries & Galloway. Birds with signs of haemorrhaging unassociated 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.

Table 2 continued. Concentrations of second generation anticoagulant rodenticides (SGARs) in the livers of red kites found dead in 2020.

Scheme	Incident/ Bird code	SGAR contrib. cause of death	Month of death	Sex	Age	Location	Concentration of SGAR (ng/g wet wt.)					
							Brom	Difen	Floc	Brod	Difeth	ΣSGARs
PBMS/IoZ	21765	No	Mar	M	Adult	Cambs	3.3	53.8	4.1	180.9	50.9	293.0
PBMS/IoZ	22134	No	Dec	F	Adult	Yorkshire	17.2	82.4	0.0	472.5	0	572.1
PBMS/IoZ	22159	No	Dec	M	Adult	Warwickshire	1.5	5.1	0.0	607.3	0	613.9
PBMS/IoZ	22501	No	Nov	F	Adult	Carmarthens.	0.0	9.5	0.0	180.3	0	189.8
PBMS/IoZ	22506	No	U	F	Adult	Norfollk	45.6	159.3	0.0	177.6	36.8	419.4
WIIS SASA	20044	No	Apr	F	Juvenile	Tayside	0.0	0.0	0.0	0.0	0.0	0.0
WIIS SASA	20045	No	May	F	U	Central	46.0	30.0	0.0	6.0	0.0	82.0
WIIS SASA	20066	No	Mar	U	U	D&G	0.0	0.0	0.0	0.0	0.0	0.0
WIIS SASA	20145	No	Oct	F	U	Highland	25.0	0.0	0.0	0.0	0.0	25.0

M – male; F- female; U – sex or age not determined; ND = non-detected; Brom – bromadiolone; Difen – difenacoum; Floc – flocoumafen; Brod – brodifacoum; Difeth – difethialone; D&G – Dumfries & Galloway. Birds with signs of haemorrhaging unassociated 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 six 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 was between 88% (in 2020) and 100% (in 2018) 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”. The proportion of red kites with detected residues was 137/145 (96%) in 2017/18/19/20, which was not significantly different (Fisher’s Exact Test, $P=0.362$) to the equivalent proportion (55/61 = 90%) in 2015/16. Over the whole period of 2015 to 2020, 192 out of the 206 kites examined had at least one detectable liver residue and the median number of different compounds detected in the liver was three.

In terms of the magnitude of cumulative exposure, we calculated the sum (Σ)SGAR concentrations 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, and (iii) all red kites combined (Figure 1). There was no statistically significant difference between years in any of the three analyses (Kruskal-Wallis test: $KW \leq 9.82$, $P>0.05$) and no evidence that the magnitude of accumulated summed SGAR residues has changed consistently over time (see also Figure 5).

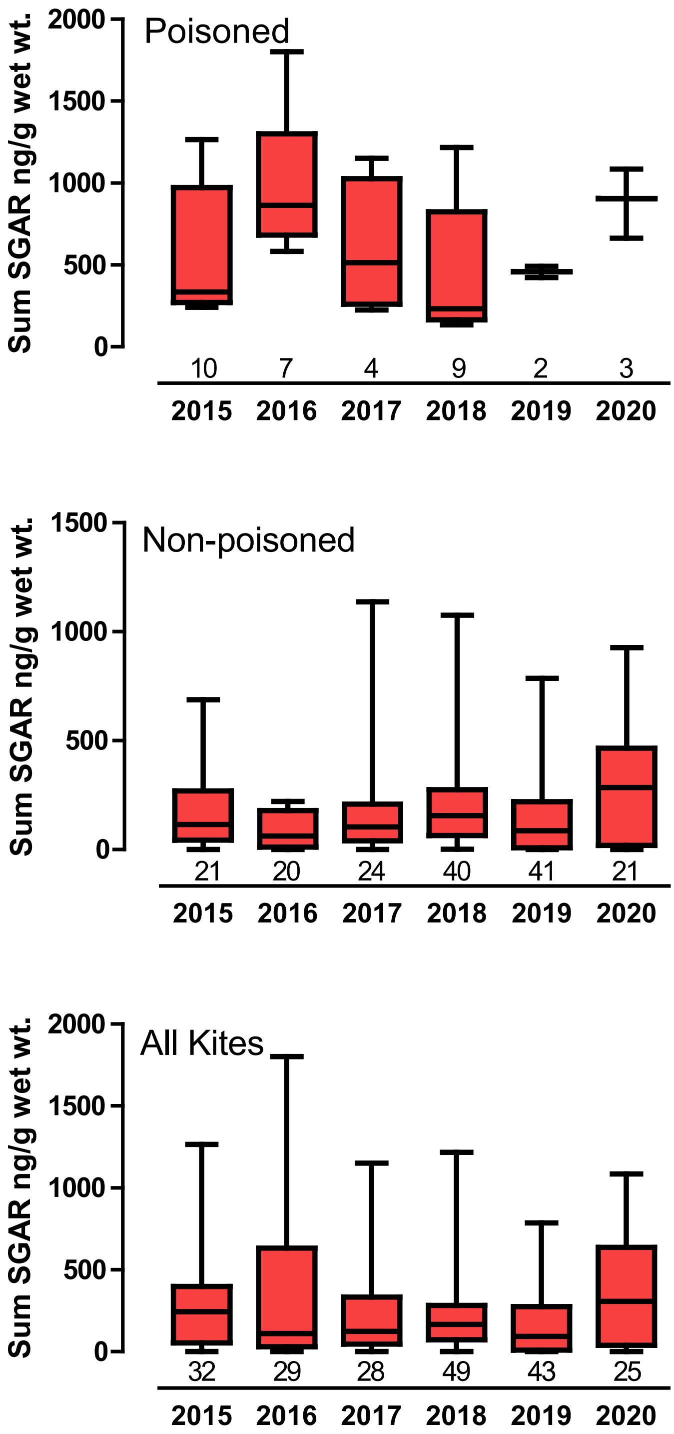


Figure 1. Box and Whisker plots showing median, interquartile range and minimum/maximum range of sum (Σ)SGAR concentrations in red kites that died with haemorrhaging disassociated with physical trauma (SGARs implicated in death; 'Poisoned'), those died from other causes (SGARs not implicated in death; 'Non-poisoned') and in all red kites combined. Sample numbers are shown above the x-axis for each group. One, two and one bird from the 2015, 2016, and 2020 cohort, respectively were excluded from the analysis as it was unclear whether observed haemorrhaging was associated with trauma or not.

We examined whether there was evidence of a change over time in the exposure of birds to brodifacoum, flocoumafen or difethialone, the three SGARs that before 2016 were restricted to indoor use only. 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 (69%), and 21 (84%) in 2015, 2016, 2017, 2018, 2019 and 2020 respectively (Figure 2).

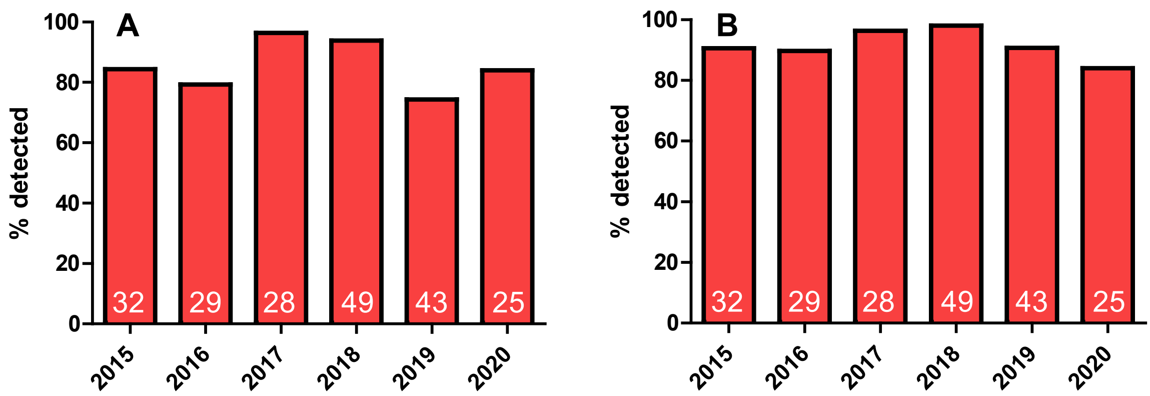


Figure 2. The percentage of red kites found dead between 2015 and 2020 that had detectable concentrations of brodifacoum, flocoumafen, and/or difethialone (A) or difenacoum and/or bromadiolone (B) in their livers. Total sample numbers are shown in the bars.

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” year blocks. The proportion of birds with liver brodifacoum residues was 50/61 (82%) and 124/145 (86%) in 2015/16 and 2017/18/19, respectively. Again unlike the same analysis from a previous report that included birds found dead in years up to 2018, that showed a higher frequency of brodifacoum residues in post-stewardship implementation years (Walker *et al*, 2019), there was no significant difference between year groups (Fisher’s Exact test; P=0.53). Similarly there was no significant difference between these year groups in the proportion of kites that had liver difenacoum or bromadiolone residues (90% in 2015/16 vs. 93% in 2017/18/19/20, Fisher’s Exact test; P=0.57; Figure 2). There was no significant difference among years in the sum brodifacoum, flocoumafen and difethialone liver concentration (Kruskal-Wallis test:7.59, P=0.18) but there were significant differences between years for sum bromadiolone and difenacoum concentrations (K-W: 12.18, P=0. 03; Figure 3). However, these differences were not consistent with only 2016 having lower sum bromadiolone and difenacoum concentrations compared to 2015 (Dunn's Multiple Comparison Test, P>0.05). Therefore, there is little evidence to suggest that the proportion of birds that are exposed to brodifacoum, bromadiolone or difenacoum, nor the magnitude of that exposure has changed since the harmonisation of usage restrictions.

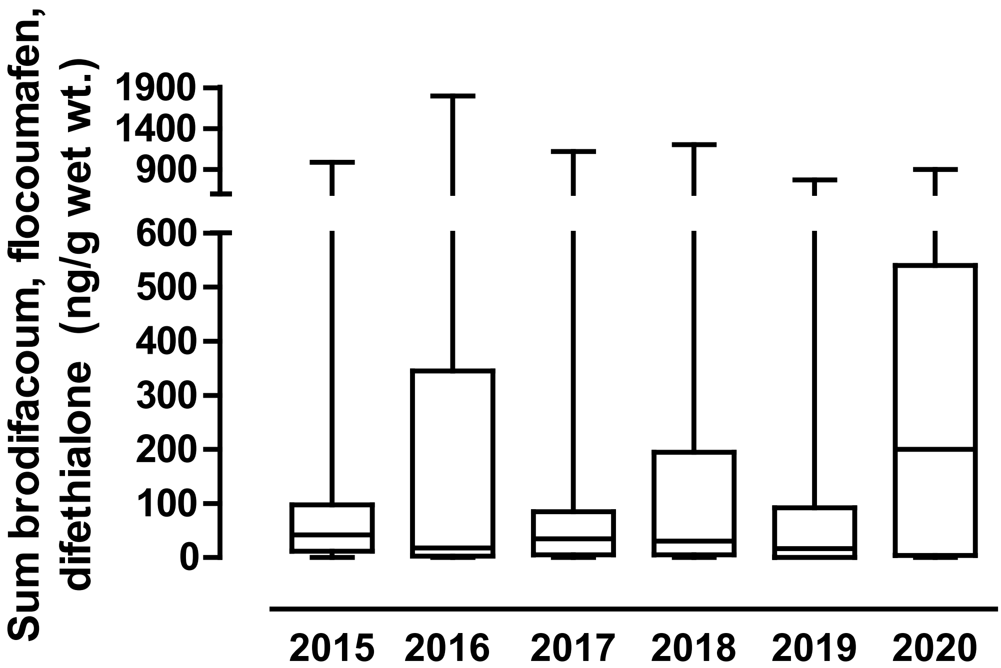
4.3 Trends in poisoning over time

The percentage of birds from 2020 for which SGARs was diagnosed as a contributory factor in their cause of death (Table 3) was 13% with this value ranging between 5% and 32% during the monitoring period. This difference among years was not statistically significant for red kites from England and Wales only. but it was significant for Britain as a whole, when analysis was conducted as standard Chi Squared test (England & Wales: χ^2 = 10.99, d.f.=5, P=0.06; Britain χ^2 = 11.65, d.f.=5, P=0.04). When the Chi Squared test was conducted as a trend analysis percentages for both England & Wales and Britain were significantly lower in later years (England & Wales: $\chi2$ = 8.23, d.f.=1, P=0.004; Britain: $\chi2$ = 8.71, d.f.=1, P=0.003). The data did not violate the underlying assumptions of the Chi Squared test but the numbers of red kites in "expected cells" in the Chi Squared tests were low. We therefore also compared data when pooled into groups of years (2015/2016 vs 2017/18/19/20) 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 2017/18/19/20 than in 2015/16 for England & Wales (Fisher's Exact test: P=0.004) and Britain as a whole (Fisher's Exact test: P=0.007).

Table 3. Number (% of total) 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 mortality factor							
	England & Wales				Britain			
Year	SGARs implicat'd	un-certain	not implicat'd	total	SGARs implicat'd	un-certain	not implicat'd	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	30	32	2 (5%)	0	41	43
2020	3 (15%)	1	17	21	3 (13%)	1	21	25
Total	9 (36%)	1	16	26	10 (32%)	1	21	32

[1]Not impacted - Red kites with no detected haemorrhaging, with haemorrhaging associated with trauma, and/or no-detected liver SGAR residue



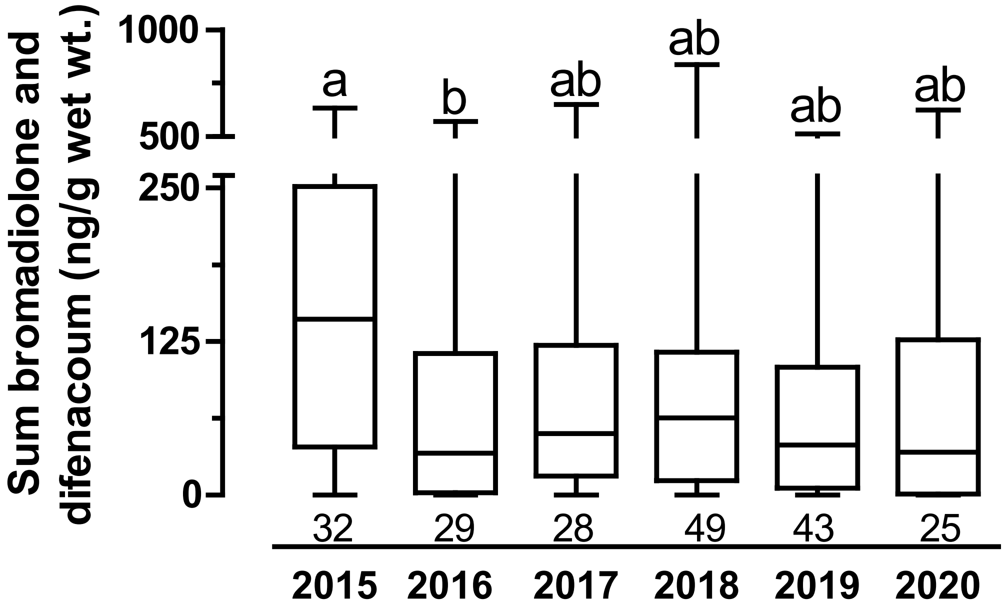


Figure 3. The liver sum concentrations of brodifacoum, flocoumafen and difethialone (top) and liver sum concentrations of bromadiolone and difenacoum (bottom) in all red kites found dead between 2015 and 2020. For sum bromadiolone and difenacoum concentrations significant ($P<0.05$) differences between years are indicated by different letters. There was no significant difference among years in Sum brodifcaoum, flocoumafen, and difethialone concentrations, and so pairwise post-hoc tests are not presented in this figure.

Sum SGAR liver concentrations in poisoned birds did not differ significantly among years (Figure 1, Kruskal-Wallis=9.82, P=0.081). All birds for which SGARs were implicated in death had residues of between two 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, 75% (median value) of the ΣSGAR liver residue in poisoned birds was comprised of brodifacoum, flocoumafen and difethialone. This proportion did not differ among years (Figure 4; KW= 8.56; P=0.13) with no consistent pattern across years.

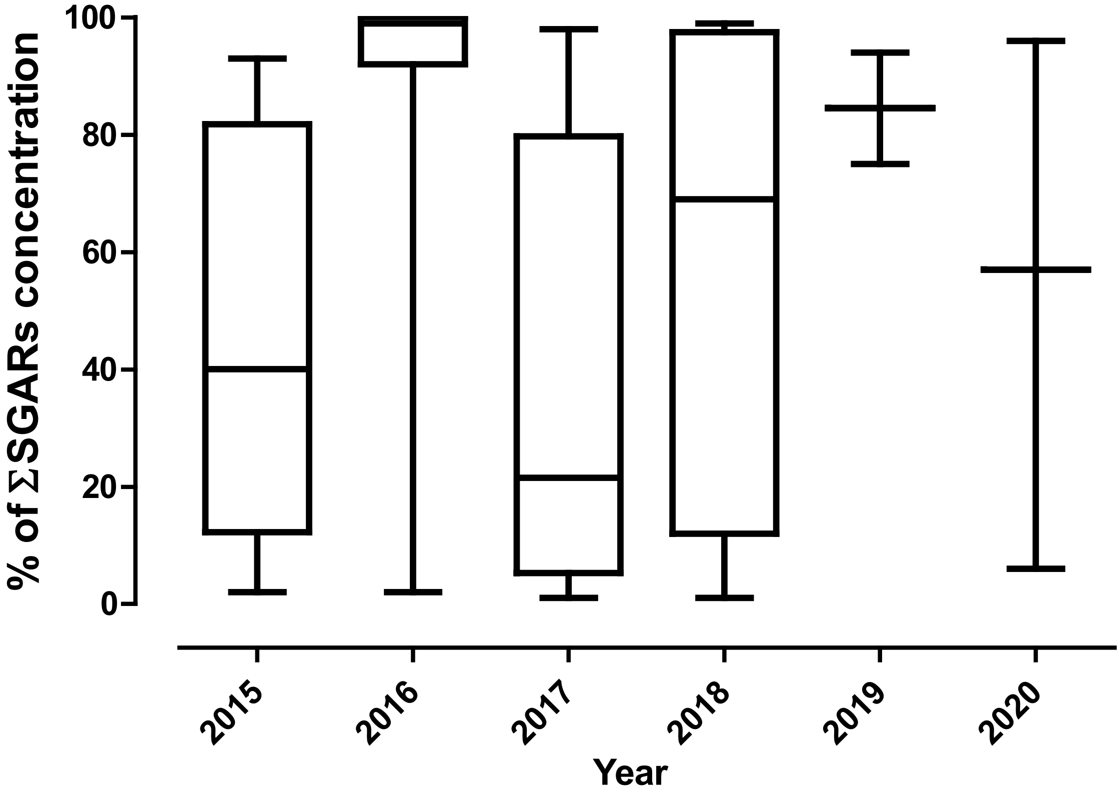


Figure 4. Box and Whiskers plot showing median, interquartile range and minimum/maximum range in sum brodifacoum, flocoumafen and difethialone concentrations expressed as a percentage of (Σ)SGAR concentrations for red kites that died between 2015 and 2020 for which SGARs were implicated in death.

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 5). Overall, the median ΣSGAR concentration in those red kites was almost 5.7 fold higher than that of birds that had died from a variety of other causes. Only 4% of red kites that died from causes unrelated to SGARs had liver ΣSGAR residues >700 ng/g wet wt. compared to 40% for birds in which SGARs were implicated in their death. Only one red kite with liver residues <140 ng/g wet wt. were diagnosed as individuals poisoned by SGARs (none had non-trauma related haemorrhaging). However, there was considerable overlap in liver residues between the two groups of kites (Figure 5), potentially at least in part reflecting inter-individual susceptibility to SGARs. There does not appear to be a clear diagnostic threshold for residues that are indicative of potential SGAR poisoning in red kites or other species (Thomas *et al.* 2011). The current dataset may be useful in testing the validity of the probabilistic approaches to interpreting the significance of liver residues as suggested by Thomas *et al.* (2011).

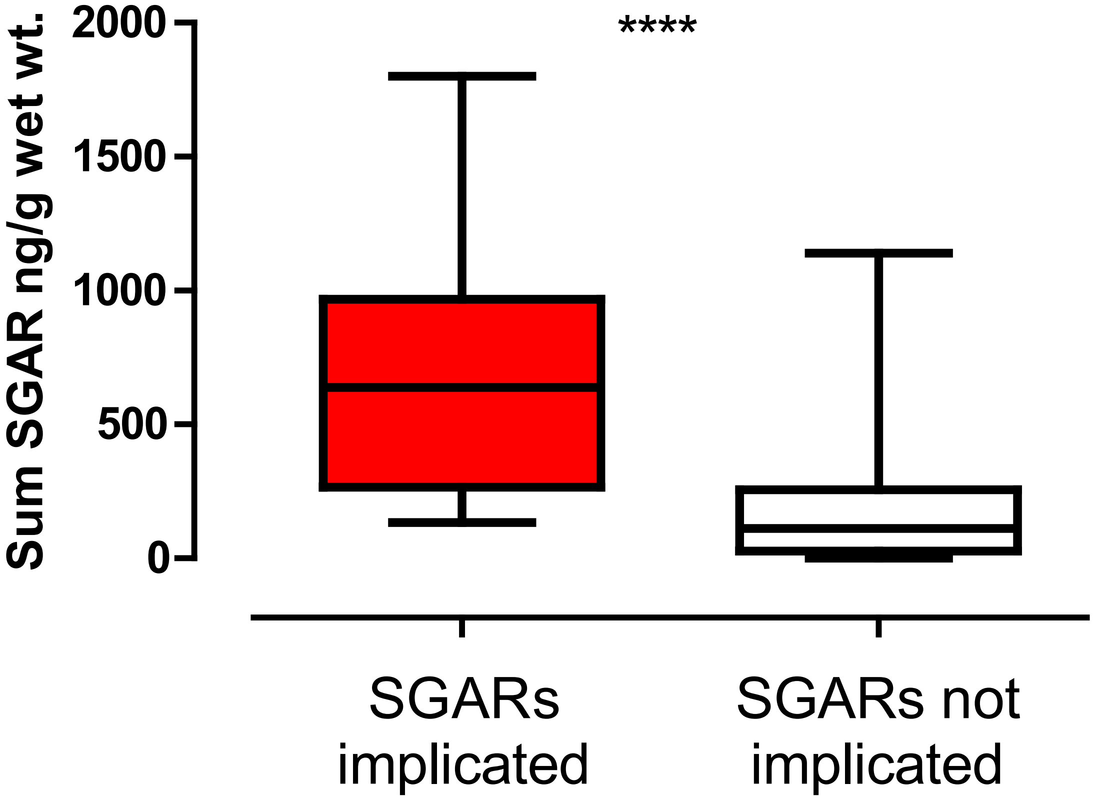


Figure 5. Box and Whiskers plot showing median, interquartile range and minimum/maximum range of sum (Σ)SGAR concentrations in red kites that died between 2015 and 2020, with haemorrhaging not associated with physical trauma (SGARs implicated as a contributory cause of mortality; n=35) and those that were diagnosed to have died from causes unrelated to SGARs (SGARs not implicated as a contributory cause of mortality; n=167). The difference in median concentrations between the two groups was statistically significant (Mann-Whitney U test, U=729, P<0.0001).

5 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 25 red kites from England, Wales and Scotland, found dead in 2020, all but three had been exposed to SGARs. In three (13%) cases, SGARs were implicated as a contributory cause of death. For the red kites in which SGARs were considered a contributory cause of death (Fera Science sample codes 100009, 100008, and 99929 – Table 2), death was associated with “unspecified” SGAR use.

Difenacoum, brodifacoum, and bromodialone were the most prevalent compounds (detected in 86%, 85%, and 76% of red kites across the six 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 (2015-2020), the proportion of red kites with detectable liver SGAR residues remains at >88%. There was no statistically significant difference between years irrespective of cause of death and no evidence that the magnitude of accumulated summed SGAR residues has changed consistently over time. Sum bromadiolone and difenacoum concentrations were lower in 2016 than 2015, however, there was no difference for sum brodifacoum, flocoumafen, and difethialone.

The number of years for which we have combined data from different monitoring schemes is low. 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 for which an age was reported were adults and so may have liver residues at least partly derived from exposures 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 and it is not surprising that we are not yet able to detect any definitive change as a consequence of the stewardship scheme.

Potential change in the proportion of birds in which SGARs were diagnosed as a contributory mortality factor is difficult to determine. There was were statistically significant differences among individual years in this proportion and there was a statistically significant annual trend. Annual sample sizes of birds for which SGARs were diagnosed as a contributory mortality factor were small and when data were pooled into year blocks (2017/18/19/20 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, there was no clear-cut consistent picture of change overaleewll in exposure, but 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. Indeed, in all years the sample was biased towards those individuals that have died, and perhaps not a true representation of the red kite population as a whole. 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 (Walker 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.

Overall, the very high proportion of red kites exposed to SGARs remains a concern, as is the assessment that SGARs were a contributory cause of death in 17% of the red kite cases examined across all six 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 and continued monitoring of SGAR concentrations in this species is recommended.

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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.

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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), WIIS England & Wales (Fera Science) and WIIS Scotland (SASA) laboratories.

	UKCEH		Fera Science		SASA	
	LoD	% Spike recovery#	LoD	Typical % Spike recovery	LoD	Typical % Spike recovery*
Brodifacoum	1.5	72	0.8	64	3	87
Bromadiolone	1.5	73	0.8	94	3	87
Difenacoum	1.5	-	0.8	94	3	86
Flocoumafen	1.5	-	0.8	105	3	79
Difethialone	2.8	-	0.8	83	3	81

* Spiked at 20 ng/g wet wt., # spiked with deuterated spiking solution.