UK Centre for Ecology & Hydrology

Second generation anticoagulant rodenticide residues in red kites 2019

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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 43 red kites that had been found dead in Britain in 2019. 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 two of the 31 kites from England & Wales and 10 of the 12 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 30, 35 and 37 red kites respectively. Difethialone was found in seven individuals while flocoumafen was not detected in any bird analysed.

The proportion of analysed red kites exposed to SGARs in 2015 (91%), 2016 (90%) 2017 (96%), 2018 (100%) and 2019 (91%) was similar at *circa* 90% or more; the higher percentages in 2017 and 2018 were principally due to a greater proportion of birds from Scotland containing residues. Brodifacoum and difenacoum were the most prevalent compounds (detected in 85% and 87% of red kites across the five years for

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

each compound respectively) along with bromadiolone (77%). 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 2019 ranged between non-detectable and 786 ng/g wet weight (arithmetic mean: 168 ng/g wet weight., median 93 ng/g wet weight.). Post-mortem examinations indicated that two (5%) 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 424 and 491 ng/g wet weight. SGARs were considered a contributory cause of death in these cases. SGARs were a contributory cause of death in 18% of the red kite cases examined across all five years. Over the period 2015 to 2019, 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 results may give an overestimate of incidence of poisoning than that which occurs in the population as a whole.

There was no significant difference between years in liver sum (Σ) SGAR concentrations. There were however statistically significant differences among years in combined Σbromadiolone and difenacoum concentrations, with lower residues being observed in 2019 compared to 2015 (i.e. prior to the implementation of the stewardship regime). However, in 2017 and 2018 combined Σbromadiolone and difenacoum concentrations were similar to 2015. Therefore, based on the data available to date it is not possible to identify a trend in exposure to bromadiolone and difenacoum since the implementation of the stewardship regime.

We also investigated if there was a change between years in the exposure of red kites to brodifacoum, flocoumafen and difethialone by pooling the data into multiple year blocks. Data on presence/absence of detectable brodifacoum, flocoumafen or difethialone residues were compared for 2015/16 and 2017/18/19. The proportion of red kites with detectable residues of these three SGARs was 82% (50 out of 61 red kites) in 2015/16 and similar proportions were observed in 2017/18/19 (86%; 103 out of 120 red kites). Similarly, there was no significant difference in the proportion of red kites with detectable liver difenacoum or bromadiolone residues (90% in 2015/16 vs. 95% in 2017/18/19).

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 individuals 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. There is also some evidence that the magnitude of concentrations of bromadiolone and difenacoum, when summed together, has decreased and if this were seen in future years then this would indicate a reduction in exposure to these SGARs. 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.

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*) (Shore et al., 2019) provide data on exposure in a species that feeds predominantly on non-target rodents (i.e. rodent species excluding brown rat, *Rattus norvegi*cus, 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 may increase their frequency of use, especially in areas where there is resistance to bromadiolone and difenacoum (Jones et al., 2019). This may subsequently increase secondary exposure of red kites to these 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). Tissue samples are submitted to PBMS following post mortems of kites undertaken by IoZ, who conduct health

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

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.

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 2019 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 2019 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 2019 and compare our data with those for kites that died between 2015 and 2018 (Walker et al., 2017, 2018, 2019). This timeframe spans the implementation of the stewardship programme for

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

anticoagulant rodenticides and the concurrent relaxation of ‘indoor use only’ restrictions for brodifacoum, flocoumafen and difethialone.

3 Methods

The carcasses of 43 red kites that died in 2019 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 (72%) 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 2019.

	Adult	First-year	Unknown
Male	5	1	9
Female	4	0	4
Unknown	3	3	14

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 2019

Of the 43 red kites found dead in 2019, all but four had detectable concentrations of one or more SGARs in their liver (Table 2); two of the birds with non-detected residues were from Scotland, one from North Yorkshire and one from Denbighshire. Bromadiolone (detected in 86% of red kites in the sample), difenacoum (81%) and brodifacoum (69%) were the most prevalent residues detected. Difethialone was found in seven birds (16%) but flocoumafen was not detected in any red kites from 2019. Sum SGAR concentrations ranged between non-detectable to 787 ng/g wet wt. with a median of 93 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=0.73) with detected residues of at least one SGAR in 2019. Two of the 31 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 2 of the 43 (5%) of the red kites found dead in 2019 had internal haemorrhaging that was not associated with detectable trauma, these birds had comparatively high liver SGAR residues of 424 and 491 ng/g wet wt. (Table 2). Anticoagulant rodenticides were considered to be a contributory cause of death of these two birds.

Table 2. Concentrations of second generation anticoagulant rodenticides (SGARs) in the livers of red kites found dead in 2019.

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	21635	No	July	U	Adult	Oxfordshire	5.1	6.6	ND	759.9	15.1	786.7
PBMS/IoZ	21632	No	July	U	U	Huntingdonshire	22.6	47.8	ND	3.5	ND	74.0
PBMS/IoZ	21630	No	May	U	First year	Surrey	2.6	431.5	ND	81.3	ND	515.4
PBMS/IoZ	21629	No	May	F	Adult	Worcestershire	2.9	188.3	ND	84.7	ND	275.9
PBMS/IoZ	21627	No	Aug	U	First year	Cardiganshire	140.1	4.4	ND	ND	ND	144.4
PBMS/IoZ	21628	No	May	M	Adult	Buckinghamshire	48.5	50.8	ND	246.2	ND	345.5
PBMS/IoZ	20779	No	Jab	F	Adult	Unknown	2.1	98.5	ND	293.8	ND	394.4
PBMS/IoZ	20847	No	Feb	F	Adult	Merionethshire	ND	18.1	ND	28.6	ND	46.7
PBMS/IoZ	20905	No	May	M	Adult	South Hampshire	76.4	280.5	ND	90.7	ND	447.5
PBMS/IoZ	21637	No	Nov	U	First year	South Hampshire	3.7	34.5	ND	97.7	ND	136.0
PBMS/IoZ	21633	No	May	M	Adult	West Suffolk	146.9	191.4	ND	97.6	ND	435.9
PBMS/IoZ	21631	No	Mar	M	First year	North Wiltshire	14.2	52.1	ND	163.8	3.0	233.2
SASA	19061	No	Apr	M	U	Central Scotland	59.0	9.0	ND	33.0	ND	101.0
SASA	19068/1	No	May	F	U	D&G	14.0	63.0	ND	5.0	11.0	93.0
SASA	19068/2	No	May	M	U	D&G	ND	ND	ND	ND	ND	ND
SASA	19068/3	No	May	U	U	D&G	5.0	ND	ND	ND	ND	5.0
SASA	19073	No	May	F	Adult	D&G	29.0	177.0	ND	ND	ND	206.0
SASA	19074/2	No	Jun	M	U	D&G	3.0	13.0	ND	ND	ND	16.0
SASA	19105	No	Aug	M	Adult	Grampian	160.0	4.0	ND	ND	ND	164.0
SASA	19123	No	Aug	M	U	D&G	12.0	6.0	ND	ND	ND	18.0
SASA	19169	No	Nov	U	U	D&G	ND	ND	ND	ND	ND	ND
SASA	19190	No	Dec	U	U	D&G	31.0	ND	ND	159.0	ND	190.0
SASA	19191	No	Dec	U	U	D&G	26.0	46.0	ND	ND	ND	72.0
SASA	19200	No	Dec	F	U	D&G	96.0	12.0	ND	11.0	ND	119.0
Fera Sci.	8 99303	No	Jan	U	U	West Yorkshire	75.0	26.0	ND	13.0	ND	114.0

Table 2. Concentrations of second generation anticoagulant rodenticides (SGARs) in the livers of red kites found dead in 2019.

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 Sci.	9 99308	No	Jan	U	Adult	Cambridgeshire	ND	10.0	ND	ND	ND	10.0
Fera Sci.	18 99338	No	Jan	F	U	Wiltshire	0.7	1.0	ND	74.0	ND	75.7
Fera Sci.	42 99472	Yes	Apr	U	U	West Yorkshire	62.0	42.0	ND	300.0	20.0	424.0
Fera Sci.	44 99534	No	Apr	M	Adult	Lincolnshire	2.6	510.0	ND	92.0	ND	604.6
Fera Sci.	46 99527	No	May	U	Adult	Gloucestershire	6.3	6.6	ND	74.0	ND	86.9
Fera Sci.	55 99560	No	Mar	M	U	North Yorkshire	ND	ND	ND	ND	ND	ND
Fera Sci.	70 99652	No	Jun	U	U	North Yorkshire	39.0	1.8	ND	28.0	ND	68.8
Fera Sci.	81 99724	No	Sept	U	U	Northamptonshire	0.8	ND	ND	0.6	ND	1.4
Fera Sci.	81 99725	No	Sept	U	U	Northamptonshire	1.2	1.0	ND	5.9	ND	8.1
Fera Sci.	81 99726	No	Sept	U	U	Northamptonshire	1.7	ND	ND	18.0	ND	19.7
Fera Sci.	81 99727	No	Sept	U	U	Northamptonshire	6.1	2.6	ND	0.1	0.4	9.2
Fera Sci.	81 99748	No	Sept	U	U	Northamptonshire	1.0	0.8	ND	0.2	0.1	2.1
Fera Sci.	84 99770	No	Sept	M	U	East Yorkshire	70.0	57.0	ND	210.0	ND	337.0
Fera Sci.	87 99756	No	Sept	U	U	Denbighshire	ND	ND	ND	ND	ND	ND
Fera Sci.	93 99766	No	Nov	F	U	Denbighshire	49.0	2.1	ND	64.0	ND	115.1
Fera Sci.	94 99767	No	May	M	U	West Yorkshire	3.3	57.0	ND	ND	ND	60.3
Fera Sci.	94 99768	Yes	May	M	U	West Yorkshire	3.2	28.0	ND	460.0	ND	491.2
Fera Sci.	94 99769	No	May	M	U	West Yorkshire	4.3	1.6	ND	1.0	ND	6.8
Fera Sci.	94 99769	No	May	M	U	West Yorkshire	4.3	1.6	ND	1.0	ND	6.8

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 four 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 relaxed.

The proportion of birds with one or more detectable liver SGAR liver residue was approximately 90% in each of 2015 and 2016, between 95 and 100% in each of 2017 and 2018 and 91% in 2019. 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”. The proportion of red kites with detected residues was 115/120 (96%) in 2017/18/19, which was not significantly different (Fisher’s Exact Test, P=0.186) to the equivalent proportion (55/61 = 90%) in 2015/16. Over the whole period of 2015 to 2019, 170 out of the 181 kites examined had at least one detectable liver residue and the median number of different compounds detected in the liver was three.

In our previous report (Walker et al., 2019) considering only data to 2018 there appeared to be a difference between exposure in red kites pre- and post- stewardship implementation ($P<0.05$). It might be suggested that some variation between years in the proportion of red kites with detectable residues may be attributable to variation in the proportion of adults to juveniles in the sample. This is because the likelihood of, and extent to which, kites consume contaminated rodents and bioaccumulate residues (which have liver half-lives of 10’s to 100’s of days; Vandenbroucke et al. 2008) is likely to increase with age (typical lifespan of a red kite is four years (Robinson 2005)). (http://www.bto.org/birdfacts, accessed on 10 August 2021).However, the proportion of red kite sample that were known to be adults was 59%, 55%, 43% and 53% in 2015, 2016, 2017 and 2018, respectively. Consequently, the percentage of adults was not elevated in 2017 and 2018, when the proportion of birds with detectable residues was highest, compared to the two pre-stewardship years. It should be noted that that there were a large number of birds for which age had not been determined in 2019 (Table 2). Furthermore, there were four red kites from England & Wales in 2019 that were classed as juveniles, all had detectable liver SGAR residues with an average (median) of three different SGARs present in the liver (Table 2) the same as observed for adult birds from England & Wales in 2019. Thus, we have no evidence that age class explained variation between years in the proportion of red kites with detectable residues.

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; (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: $KW \leq 8.33$, $P>0.05$) and no evidence that the magnitude of accumulated summed SGAR residues has changed consistently over time (see also Figure 5). Analysing known adults and birds of unknown age, on the

basis that juvenile birds may have accumulated lower residues and were not evenly distributed across each of the years, did not alter the statistical outcomes.

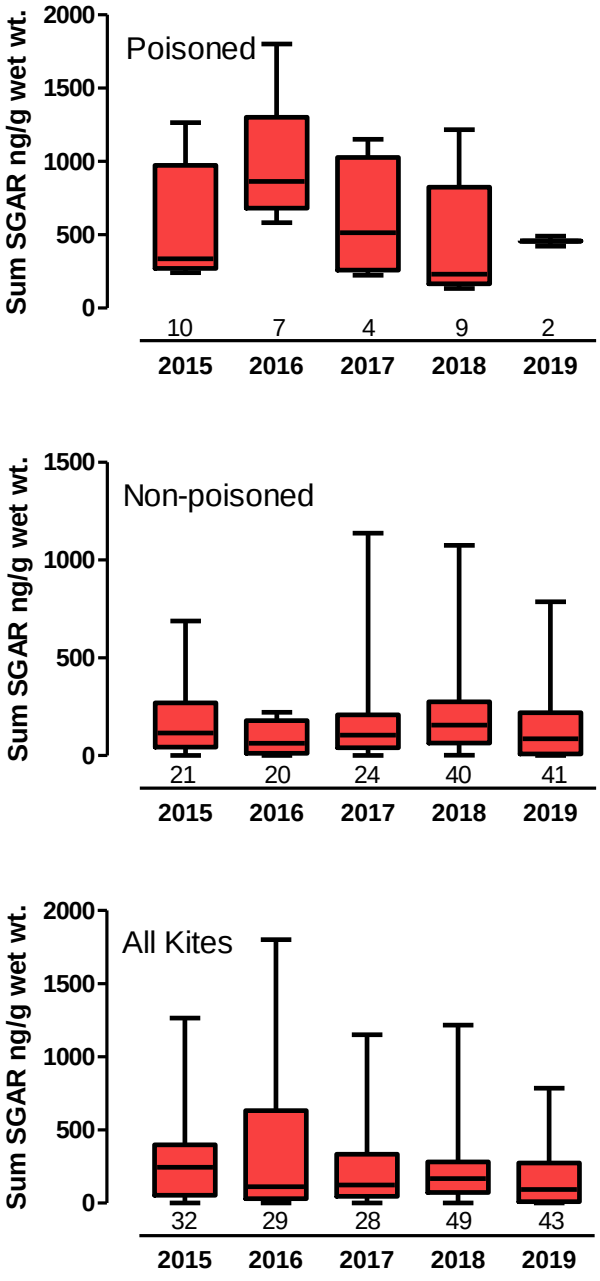

Figure 1. Box and Whisker plots showing median, interquartile range and minimum/maximum range of sum (Σ)SGAR concentrations in red kites that died with haemorrhaging dis~~un~~associated with physical trauma (SGARs implicated in death; 'Poisoned'), those died from other causes (SGARs not implicated in death; 'Non-poisoned') and in all red kites combined. Sample numbers are shown above the x-axis for each group. One bird from the 2015 cohort and two from the 2016 cohort 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 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 (and hence flocoumafen or difethialone) concentrations were 27 (84% of the sample), 23 (79%), 27 (96%), 46 (94%) and 30 (69%) in 2015, 2016, 2017, 2018 and 2019 respectively (Figure 2).

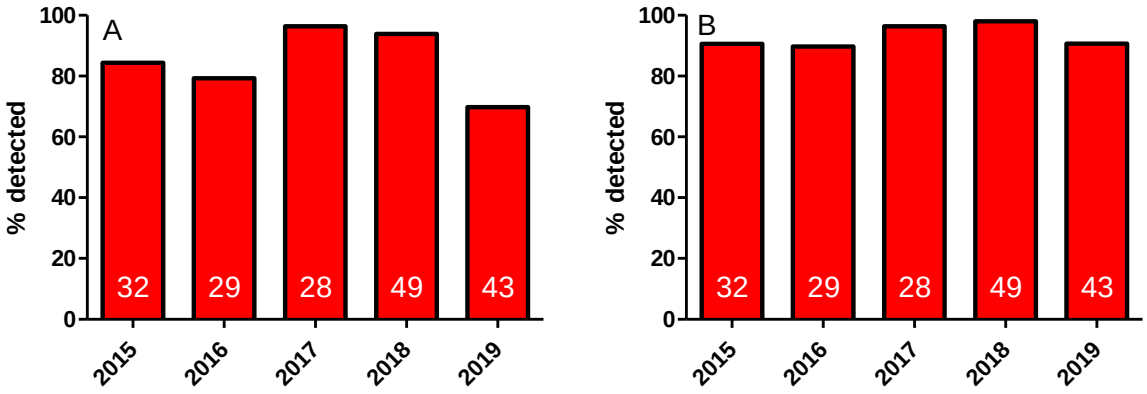

Figure 2. The percentage of red kites found dead between 2015 and 2019 that had detectable concentrations of brodifacoum, flocoumafen, and/or difethialone (A) or difenacoum and/or bromadiolone (B) in their livers. Total sample numbers are shown in the bars.

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” year blocks. The proportion of birds with liver brodifacoum residues was 50/61 (82%) and 103/120 (86%) in 2015/16 and 2017/18/19 respectively. Again unlike the same analysis from our previous report 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=0.52). 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. 95% in 2017/18/19, Fisher’s Exact test; P=0.14; Figure 2). There was no significant difference among years in the sum brodifacoum, flocoumafen and difethialone liver concentration (Kruskal-Wallis test:4.41, P=0.35) but there were significant differences between years for sum bromadiolone and difenacoum concentrations (K-W: 11.60, P=0.02; Figure 3). However, these differences were not consistent with only 2016 and 2019 having lower

sum bromadiolone and difenacoum concentrations compared to 2015. Therefore, there is little evidence to suggest that the proportion of birds that are exposed to brodifacoum, bromadiolone or difenacoum has changed since the harmonisation of usage restrictions but there may be some evidence of reduced residue magnitude for bromadiolone and difenacoum combined.

4.3 Trends in poisoning over time

The percentages of birds from 2019 for which SGARs was diagnosed as a contributory factor in their cause of death (Table 3) was lower than that observed in birds from previous years 2015 to 2018. This difference between years was not statistically significant for red kites from England & Wales, but was statistically significant from Britain as a whole, when analysis was conducted as standard Chi Squared test (England & Wales: $\chi^2 = 9.079$, d.f.=4, P=0.06; Britain $\chi^2 = 10.33$, d.f.=4, P=0.035). However, 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: $\chi^2 = 8.181$, d.f.=1, P=0.004; Britain: $\chi^2 = 8.615$, 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) 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 than in 2015/16 for England & Wales (Fisher's Exact test: P=0.007) and Britain as a whole (Fisher's Exact test: P=0.011).

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 implicated	un-certain	not implicated	total	SGARs implicated	un-certain	not implicated	total
2015	9 (35%)	1	16	26	10 (31%)	1	21	32
2016	7 (32%)	2	13	22	7 (24%)	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
Total	30 (21%)	3	83	146	32 (18%)	3	105	181

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

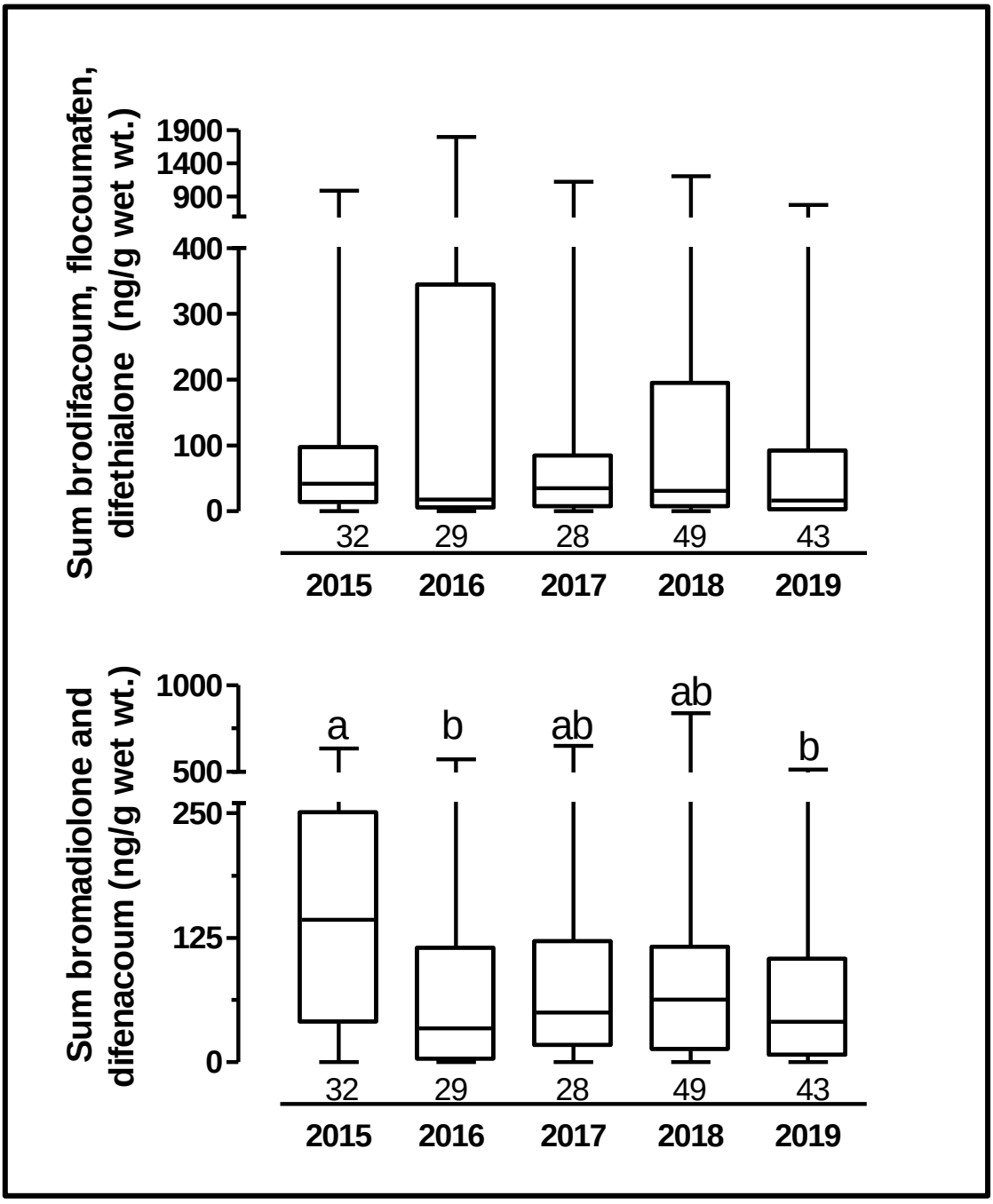

Figure 3. The liver sum concentrations of brodifacoum, flocoumafen and difethialone (top) and liver sum concentrations of bromadiolone and difenacoum (bottom) in all red kites found dead between 2015 and 2019. For sum bromadiolone and difenacoum concentrations significant ($P<0.05$) differences between 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 2, Kruskal-Wallis=8.327, P=0.080). 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, 76% (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= 8.43; P=0.077) with no consistent pattern across years.

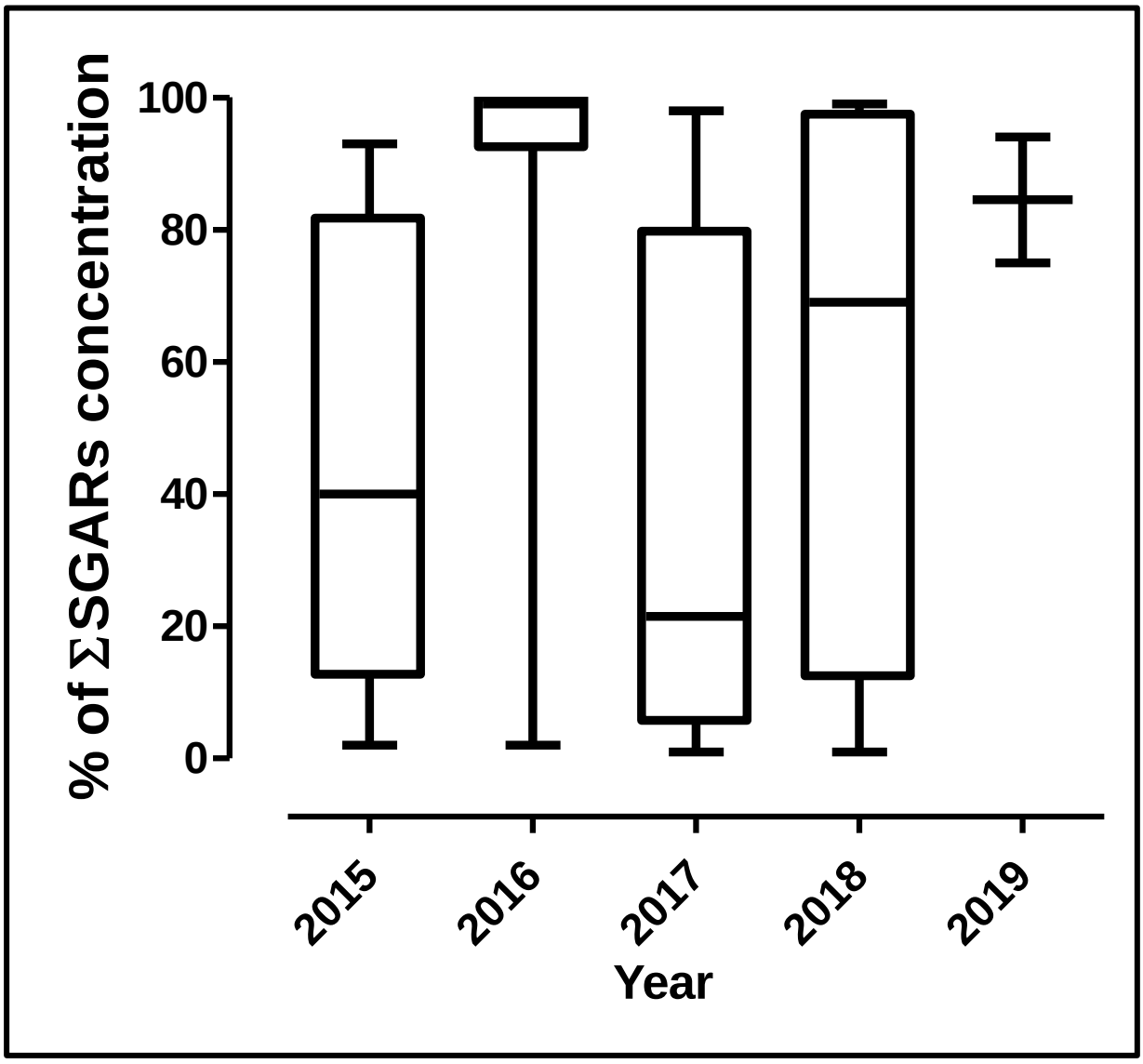

Figure 4. Box and Whiskers plot showing median, interquartile range and minimum/maximum range in sum brodifacoum, flocoumafen and difethialone concentrations expressed as a percentage of (Σ)SGAR concentrations for red kites that died between 2015 and 2019 for which SGARs were implicated in death.

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 4.7 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 34% for birds in which SGARs were implicated in their death. No red kites 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 6), 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).

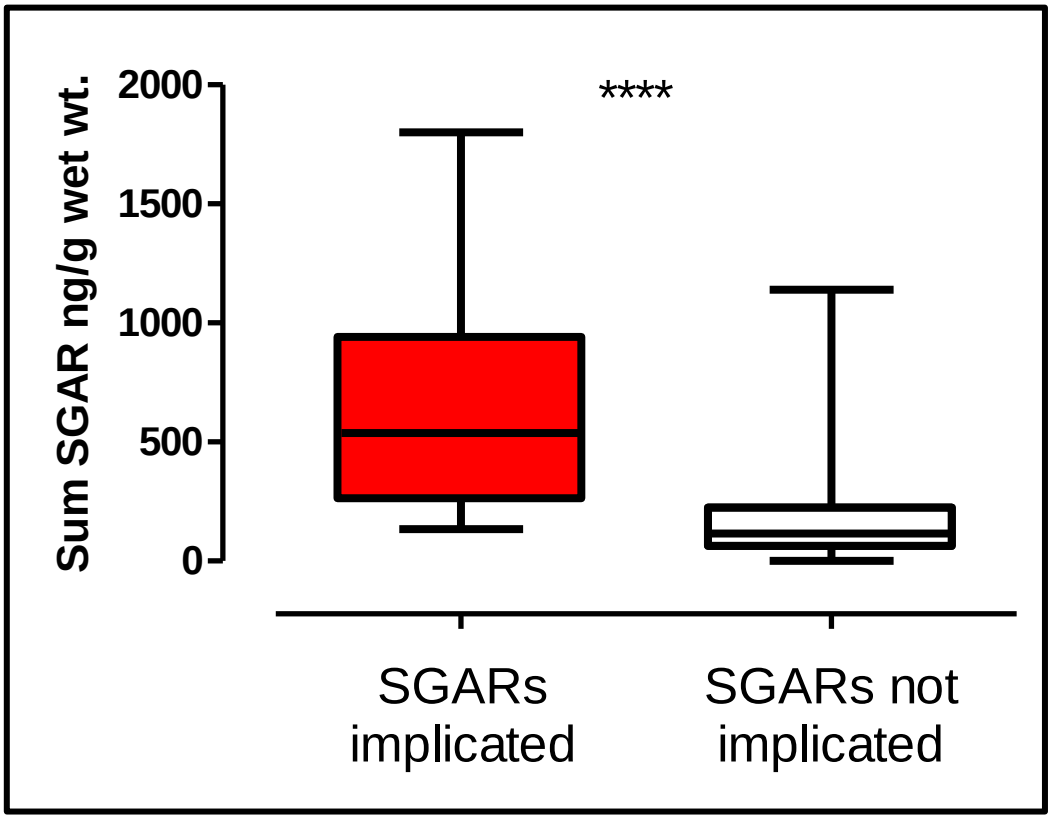

Figure 5. Box and Whiskers plot showing median, interquartile range and minimum/maximum range of sum (Σ)SGAR concentrations in red kites that died between 2015 and 2018, with haemorrhaging not associated with physical trauma (SGARs implicated as a contributory cause of mortality; n=32) and those that were diagnosed to have died from causes unrelated to SGARs (SGARs not implicated as a contributory cause of mortality; n=137). The difference in median concentrations between the two groups was statistically significant (Mann-Whitney U test, U=548, P<0.0001). Birds with non-detected values were excluded from the analysis.

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 43 red kites from England, Wales and Scotland, found dead in 2019, all but four had been exposed to SGARs. In two (5%) cases, SGARs were implicated as a contributory cause of death. For both red kites in which SGARs were considered a contributory cause of death (Fera Science incident codes 42 99472, 94 99768 – Table 2), death was associated with “unspecified” SGAR use.

Potential change in the proportion of birds in which SGARs were diagnosed as a contributory mortality factor is difficult to determine. There was no statistically significant difference among individual years in this proportion nor was there a statistically significant annual trend. However, 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 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. Overall, therefore, there was no clear-cut consistent picture of change overall in exposure, but mortality has declined over the last five years. 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.

With regards change over time (2015-2019), the proportion of red kites with detectable liver SGAR residues remains at >90%. There is little evidence of any consistent change between years in the magnitude of the residues accumulated. Sum bromadiolone and difenacoum concentrations were lower in 2019 than 2015, however, there was no difference for 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 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.

Overall, the very high proportion of red kites exposed to SGARs remains an issue of concern, as is the assessment that SGARs were a contributory cause of death in 18% of the red kite cases examined across all five 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.

6 Acknowledgements

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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	76	0.8	64	3	87
Bromadiolone	1.5	94	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.