UK Centre for Ecology & Hydrology

Second generation anticoagulant rodenticide residues in red kites 2021

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Contents

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 42 red kites that had been found dead in Britain in 2021. 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 (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 39 red kites from England & Wales and two of the three 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 41, 39, and 32 red kites, respectively. Difethialone was found in four 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%), and 2021 (98%) was similar at circa 88% or more. Difenacoum, brodifacoum, and bromadiolone were the most prevalent compounds (detected in 87%, 87%, and 76% of red kites across the seven

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

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 2021 ranged between non-detectable and 3223.7 ng/g wet weight (arithmetic mean: 482 ng/g wet weight, median 334.4 ng/g wet weight). Necropsy examinations indicated that five 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 14% of the red kites of this year excluding uncertain poisoning cases. These five birds had sum SGAR liver concentrations of 463.5, 684, 990, 1405.9, and 3223.7 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 seven years. Over the period 2015 to 2021, 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 seven 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 were statistically significant differences between years in median summed SGAR residues, irrespective of cause of death. The magnitude of accumulated summed SGAR residues, particularly sum of brodifacoum, flocoumafen, and difethialone concentrations, was significantly higher in 2021 than in many of the previous years. Given low occurrence and low concentrations of flocoumafen and difethialone residues, it is likely 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. The proportion of red kites with detectable residues of these three SGARs was not significantly different between in 2015/2016 (82%) and in 2017/18/19/20/21 (88%). 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). Since the implementation of the stewardship regime, no difference in exposure pattern relating to active ingredient 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 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 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, when considered collectively but brodifacoum exposure has increased in recent years.

2 Introduction

The current report is the sixth 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, 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,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., 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 (*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 professional use 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 in and around buildings 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, 2019, 2021a, 2021b). 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 21/11/2021

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 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 2021 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 2021 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 2021 and compare our data with those for kites that died between 2015 and 2020 (Walker et al., 2017, 2018, 2019, 2021a, 2021b). 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 42 red kites that died in 2021 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 (93%) 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 2021.

	Adult	First-year	Unknown
Male	3	2	9
Female	14	2	10
Unknown	1	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 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 in the R environment version 4.2.1 (R Core Team, 2020). 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 2021

Of the 42 red kites found dead in 2021, all but one had detectable concentrations of one or more SGARs in their liver (Table 2). Bromadiolone (detected in 76% of red kites in the sample), difenacoum (93%), and brodifacoum (98%) were the most prevalent residues detected. Difethialone was found in four birds (10%), and flocoumafen was detected in no red kite from 2021. Sum SGAR concentrations ranged between non-detectable to 3223.7 ng/g wet wt. with a median of 334 ng/g wet weight.

Although the limit of detection (LoD) 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 2021. None of the 39 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 five of the 42 of the red kites found dead in 2021 had internal haemorrhaging that was not associated with detectable trauma. All five birds had comparatively high liver summed SGAR residues of 463.5, 684, 990, 1405.9, and 3223.7 ng/g wet wt. (Table 2). Anticoagulant rodenticides were considered to be a contributory cause of death of these five birds. One other bird had a relatively high summed SGAR concentration (825 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.

It is also worth to note that two dead red kites from Fera Science ‘RK_21_08’ and ‘RK_21_11’ showed high levels of bendiocarb in the tissues. It is therefore suspected that they might be poisoned with this compound.

Table 2. Concentrations of second-generation anticoagulant rodenticides (SGARs) in the livers of red kites found dead in 2021

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
Fera Science	RK_21_01	No	Mar	F	U	North Yorkshire	9.0	11.0	0.0	48.0	0.0	68.0
Fera Science	RK_21_02	No	Nov	M	U	North Yorkshire	25.0	130.0	0.0	20.0	0.0	175.0
Fera Science	RK_21_03	No	Feb	F	U	County Durham	61.0	95.0	0.0	20.0	0.0	176.0
Fera Science	RK_21_04	No	Nov	M	U	North Yorkshire	0.0	2.7	0.0	180.0	0.0	182.7
Fera Science	RK_21_05	No	Feb	M	U	Northumberland	14.0	99.0	0.0	220.0	0.0	333.0
Fera Science	RK_21_06	No	Mar	M	U	North Yorkshire	1.0	32.0	0.0	400.0	0.0	433.0
Fera Science	RK_21_07	Uncertain	Nov	M	U	County Durham	5.6	15.0	0.0	640.0	0.0	660.6
Fera Science	RK_21_08	No	Jan	F	U	North Yorkshire	0.0	74.0	0.0	1100.0	0.0	1174.0
Fera Science	RK_21_09	No	Apr	M	U	Glamorgan	1.7	24.0	0.0	80.0	0.0	105.7
Fera Science	RK_21_10	No	Apr	F	Adult	Cardiganshire	45.0	42.0	0.0	6.0	0.0	93.0
Fera Science	RK_21_11	No	Feb	F	U	Denbighshire	13.0	10.0	0.0	590.0	0.0	613.0
Fera Science	RK_21_12	Yes	Mar	F	U	Oxfordshire	0.0	0.0	0.0	990.0	0.0	990.0
Fera Science	RK_21_13	Yes	Feb	F	U	West Yorkshire	0.0	5.9	0.0	1400.0	0.0	1405.9
Fera Science	RK_21_14	Yes	Apr	F	U	Hampshire	3.7	20.0	0.0	3200.0	0.0	3223.7
Fera Science	RK_21_15	No	Jul	M	U	West Yorkshire	54.0	12.0	0.0	150.0	0.0	216.0
Fera Science	RK_21_16	No	Mar	F	Adult	Norfolk	38.0	120.0	0.0	110.0	0.0	268.0
Fera Science	RK_21_17	Uncertain	Feb	M	U	Wiltshire	37.0	8.0	0.0	570.0	0.0	615.0
Fera Science	RK_21_18	Uncertain	May	F	Adult	West Sussex	17.0	58.0	0.0	750.0	0.0	825.0
Fera Science	RK_21_19	No	May	M	U	Warwickshire	56.0	120.0	0.0	210.0	0.0	386.0
Fera Science	RK_21_20	No	Jul	F	U	Berkshire	77.0	9.2	0.0	500.0	0.0	586.2

M – male; F- female; U – sex or age not determined; F. year – First year; Brom – bromadiolone; Difen – difenacoum; Floc – flocoumafen; Brod – brodifacoum; Difeth – difethialone. Values under LoD were replaced by 0. 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 2021.

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
PBMS/IoZ	22487	No	Unknown	F	Adult	North Hampshire	8.4	13.1	0.0	109.9	0.0	131.4
PBMS/IoZ	22489	No	Mar	M	Adult	North Hampshire	0.0	24.8	0.0	310.2	0.0	335.0
PBMS/IoZ	22502	No	Jan	F	Adult	Powys, Wales	44.5	84.0	0.0	186.1	0.0	314.7
PBMS/IoZ	22503	Uncertain	Mar	M	F. year	Shropshire	7.3	103.1	0.0	28.6	0.0	139.0
PBMS/IoZ	22508	No	Apr	F	Adult	Cambridgeshire	212.1	29.8	0.0	87.9	0.0	329.9
PBMS/IoZ	22712	No	Apr	F	Juvenile	Dorset	2.6	25.4	0.0	476.7	0.0	504.6
PBMS/IoZ	22707	Uncertain	Feb	F	Adult	West Berkshire	2.5	7.1	0.0	514.4	6.4	530.4
PBMS/IoZ	22609	No	Jul	M	Adult	Surrey	51.8	19.5	0.0	24.7	0.0	96.0
PBMS/IoZ	22708	Uncertain	Apr	F	Adult	Durham	44.6	14.0	0.0	330.7	3.7	393.0
PBMS/IoZ	22710	No	Mar	F	Adult	Staffordshire	0.0	7.0	0.0	245.3	0.0	252.2
PBMS/IoZ	22778	No	May	M	Adult	Warwickshire	21.0	67.8	0.0	245.0	0.0	333.8
PBMS/IoZ	22371	Yes	Apr	F	Adult	North Essex	0.0	158.0	0.0	305.5	0.0	463.5
PBMS/IoZ	22714	No	Mar	F	Adult	Oxfordshire	186.9	39.7	0.0	63.0	0.0	289.6
PBMS/IoZ	22715	No	Jan	U	Adult	Cambridgeshire	15.3	57.4	0.0	211.8	0.0	284.5
PBMS/IoZ	22706	No	Apr	F	Adult	Bedfordshire	9.5	27.2	0.0	149.4	412.6	598.7
PBMS/IoZ	22515	No	Aug	M	Juvenile	Shropshire	30.3	3.9	0.0	270.2	0.0	304.4
PBMS/IoZ	22709	No	Apr	F	Juvenile	Oxfordshire	0.0	2.5	0.0	515.7	0.0	518.1
PBMS/IoZ	22711	No	Apr	F	Adult	Dorset	8.7	79.1	0.0	446.6	0.0	534.4
PBMS/IoZ	22713	No	Apr	F	Adult	Durham	275.7	29.9	0.0	82.5	3.4	391.5
WIIS SASA	RK_21_21	No	May	F	U	Highland	6.0	0.0	0.0	280.0	0.0	286.0
WIIS SASA	RK_21_22	Yes	May	F	U	Highland	0.0	165.0	0.0	519.0	0.0	684.0
WIIS SASA	RK_21_23	No	May	U	U	Tayside	0.0	0.0	0.0	0.0	0.0	0.0

M – male; F- female; U – sex or age not determined; F. year – First year; Brom – bromadiolone; Difen – difenacoum; Floc – flocoumafen; Brod – brodifacoum; Difeth – difethialone. Values under LoD were replaced by 0. 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 seven 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––2017/18/19/20/21”. The proportion of red kites with detected residues was 178/187 (95%) in post-stewardship years (2017/18/19/20/21), which was not significantly different (Fisher’s Exact Test, $P = 0.212$) to the equivalent proportion (55/61 = 90%) in pre-stewardship years (2015/2016). Over the whole period of 2015 to 2021, 233 out of the 248 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 summed SGAR concentrations (ΣSGAR) 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 a statistically significant difference between years for all analyses: (i) Kruskal-Wallis test: $KW = 12.8$, $P < 0.047$; (ii) $KW = 26.0$, $P < 0.001$; (iii) $KW = 25.9$, $P < 0.001$). These differences were however not consistent. For poisoned birds, Dunn's Kruskal-Wallis multiple comparisons showed no significant difference of ΣSGAR concentrations between each year (p-value adjusted by the Holm's correction >0.05). In contrast, ΣSGAR 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 did not change even if the two birds suspected to be poisoned with bendiocarb were excluded from the analysis. Therefore, these results may indicate that the magnitude of accumulated summed SGAR residues has slightly increased over recent years (see also Figure 6).

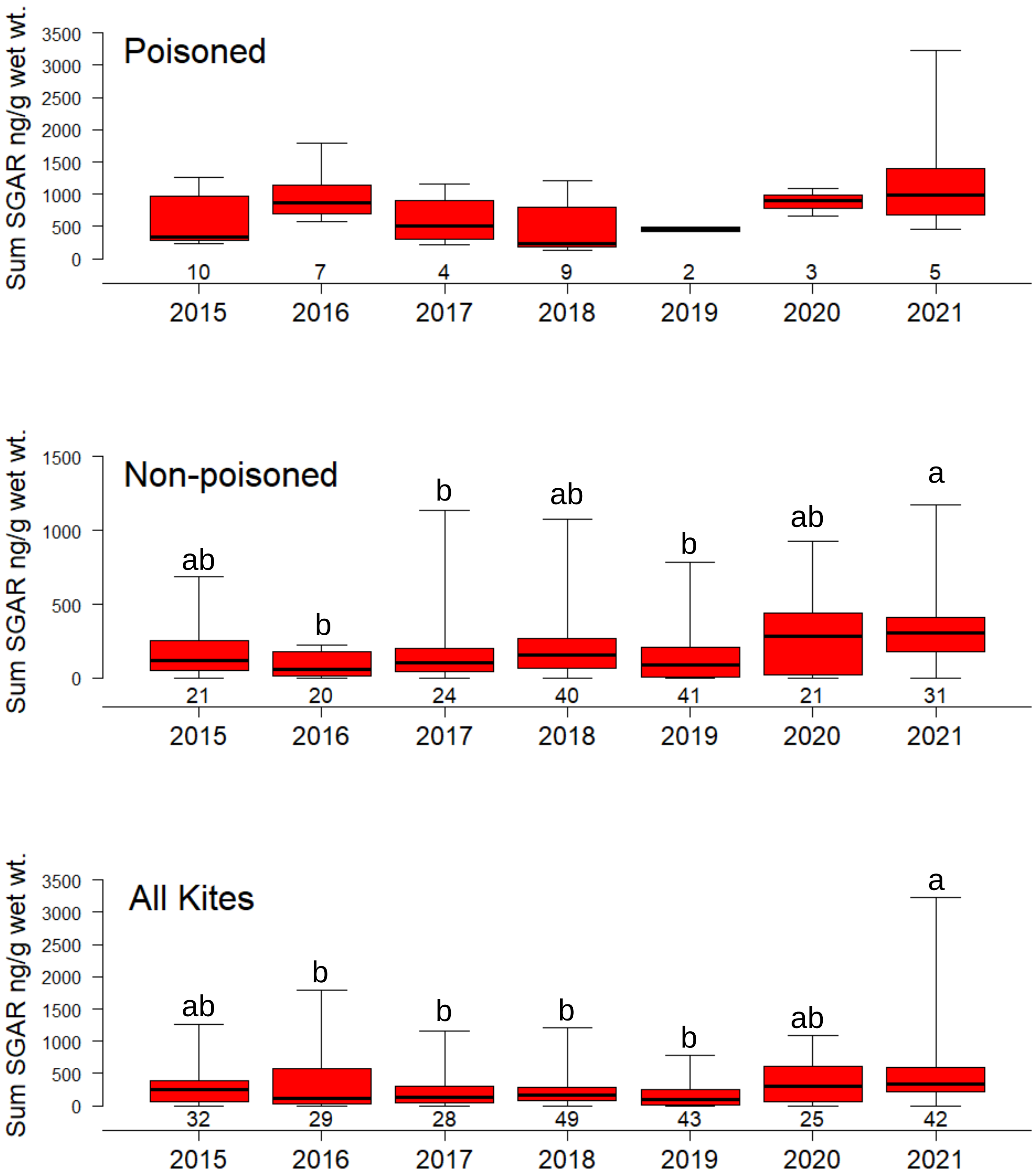

Figure 1. Box and Whisker plots showing median, interquartile range and minimum/maximum range of sum of SGARs concentrations (ΣSGAR) in red kites that died with haemorrhaging disassociated with physical trauma (SGARs implicated in death; 'Poisoned'), those died from other causes (SGARs not implicated 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, and six birds from the 2015, 2016, 2020, and 2021 cohort, respectively. 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%), and 41 (98%) in 2015, 2016, 2017, 2018, 2019, 2020, and 2021, respectively (Figure 2).

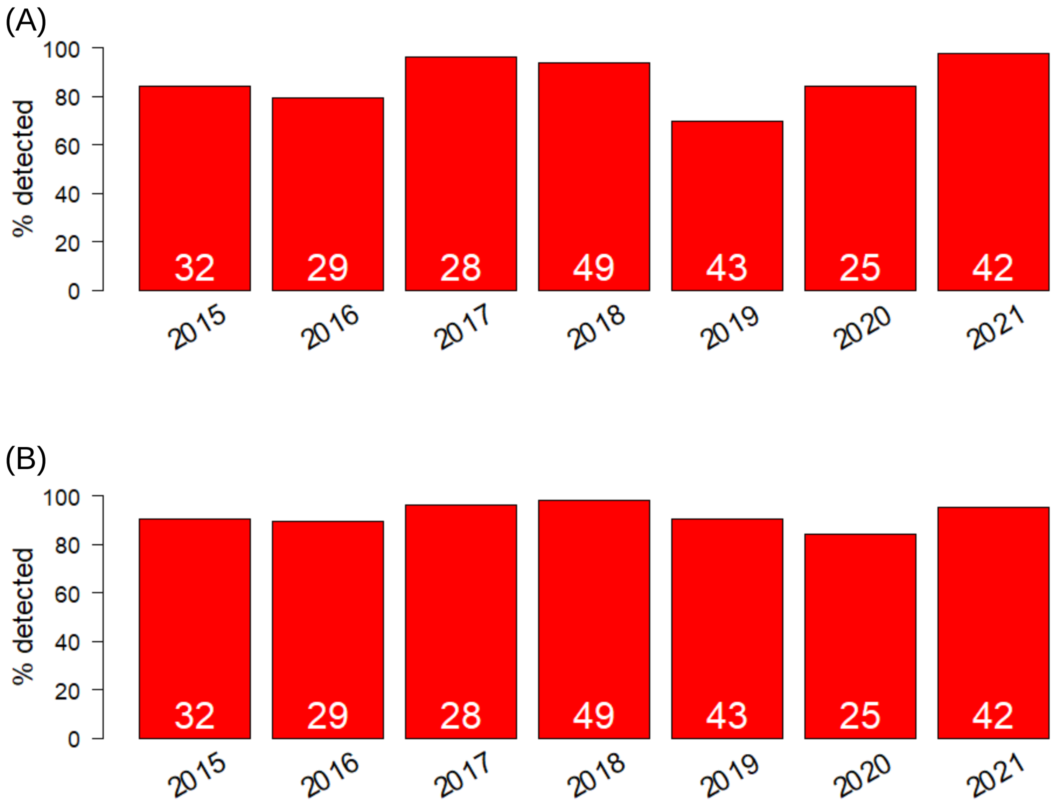

Figure 2. The percentage of red kites found dead between 2015 and 2021 that had detectable concentrations of brodifacoum, flocoumafen, and/or difethialone (A) or difenacoum and/or bromadiolone (B) in their livers. Total sample numbers are shown in the bars.

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” year blocks. The proportion of birds with liver brodifacoum residues was 50/61 (82%) and 165/187 (88%) in 2015/2016 and 2017/18/19/20/21, 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 = 0.276). Similarly, there was no significant difference between these year groups in the proportion of kites that had liver difenacoum or bromadiolone residues (90% in 2015/2016 vs. 94% in 2017/18/19/20/21, Fisher’s Exact test; P = 0.397; Figure 2).

In contrast, there were significant differences among years in the sum of brodifacoum, flocoumafen, and difethialone liver concentrations (KW = 34.4, P <0.001; Figure 3). The sum in 2021 was significantly higher than all the previous years except 2020 (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 = 38.9, P <0.001), and Brodifacoum concentrations in 2021 were significantly higher than all the previous years except 2020. (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, increased since 2019.

There were also significant differences among years in the sum of bromadiolone and difenacoum concentrations (KW = 13.2, P = 0.041; 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 bird exposed to these two SGARs has changed over time.

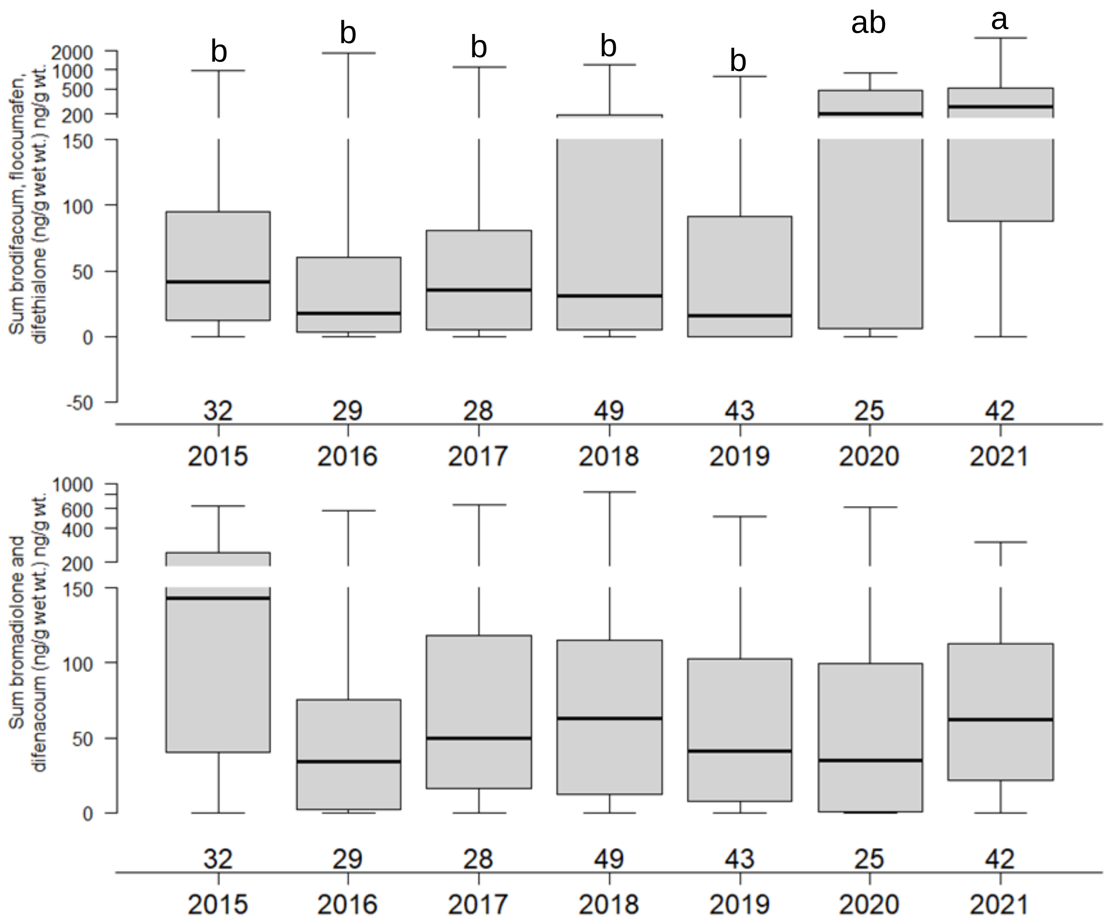

Figure 3. The liver sum concentrations of brodifacoum, flocoumafen, and difethialone (top) and liver sum concentrations of bromadiolone and difenacoum (bottom) in all red kites found dead between 2015 and 2021. For sum of brodifacoum, flocoumafen, and difethialone concentrations, significant (P<0.05) 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.

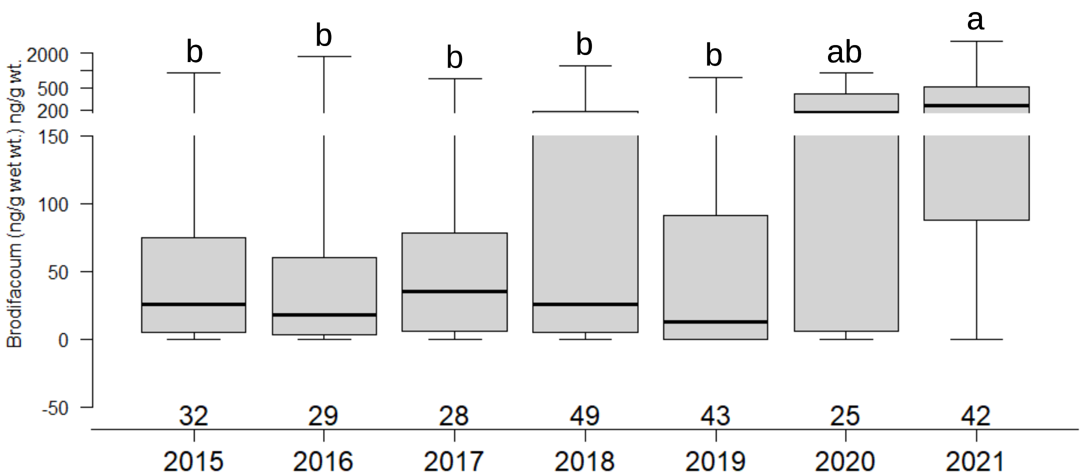

Figure 4. The liver sum concentrations of brodifacoum in all red kites found dead between 2015 and 2021. Significant (P<0.05) differences between years are indicated by different letters. Total sample numbers are shown above the x-axis.

4.3 Trends in poisoning over time

The percentage of birds from 2021 for which SGARs was diagnosed as a contributory factor in their cause of death (Table 3) was 14% with this value ranging between 5% and 32% during the monitoring period. When analysis was conducted as standard Chi-squared test the difference among years was not statistically significant for red kites from England and Wales and for Britain as a whole (England & Wales: $\chi 2 = 12.5$, df = 6, P = 0.051; Britain $\chi 2 = 12.19$, df = 6, P = 0.058). When the Chi-squared test was conducted as a trend analysis of percentages, for both England & Wales and Britain, these were significantly lower in later years (England & Wales: Z = -2.91, df = 1, P = 0.004; Britain: Z = -2.62, df = 1, P = 0.009). However, the numbers of red kites in "expected cells" in the Chi-squared tests were low (i.e., <5). We therefore also compared data when pooled into groups of years (2015/2016 vs 2017/18/19/20/21) 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 2017/18/19/20/21 than in 2015/2016 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 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	29	31	2(5%)	0	41	43
2020	3(15%)	1	17	21	3(13%)	1	21	25
2021	4(12%)	6	29	39	5(14%)	6	31	42
Total	**37(19%)**	**10**	**158**	**205**	**40(17%)**	**10**	**198**	**248**

[1]Not impacted – 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 significantly differ among years. All birds for which SGARs were implicated in 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, 76% (median value) of the ΣSGAR liver residues in poisoned birds was comprised of brodifacoum, flocoumafen, and difethialone. This proportion did not significantly differ among years (Figure 5; KW = 11.63; P = 0.07).

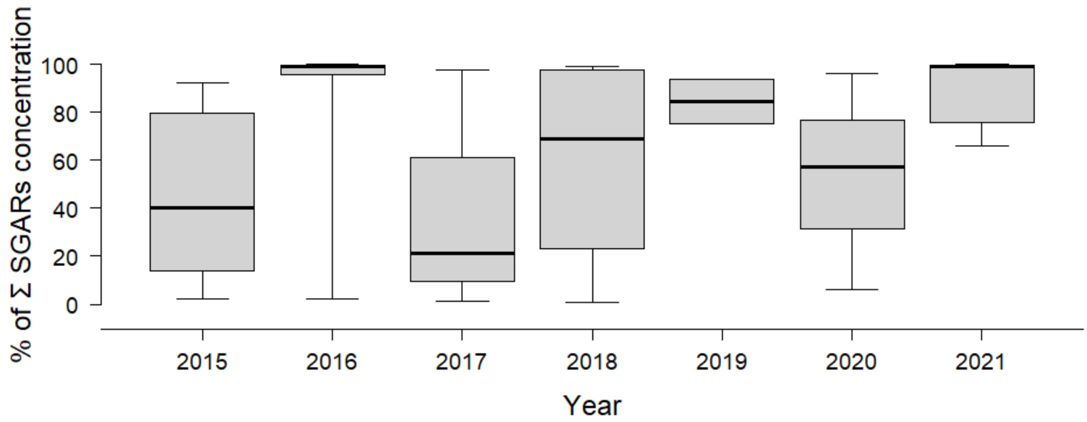

Figure 5. Box and Whiskers plot showing median, interquartile range and minimum/maximum range in sum of brodifacoum, flocoumafen, and difethialone concentrations expressed as a percentage of sum of SGAR concentrations for red kites that died between 2015 and 2021 for which SGARs were implicated in death. 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). 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. Only 4% of red kites that died from causes unrelated to SGARs had liver ΣSGAR residues >700 ng/g wet wt. compared to 42% 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); this bird was found in 2018. Liver residues in red kites poisoned by SGARs were significantly higher than residues in non-poisoned red kites (Mann-Whitney U test, U = 1087/6873, 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). 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).

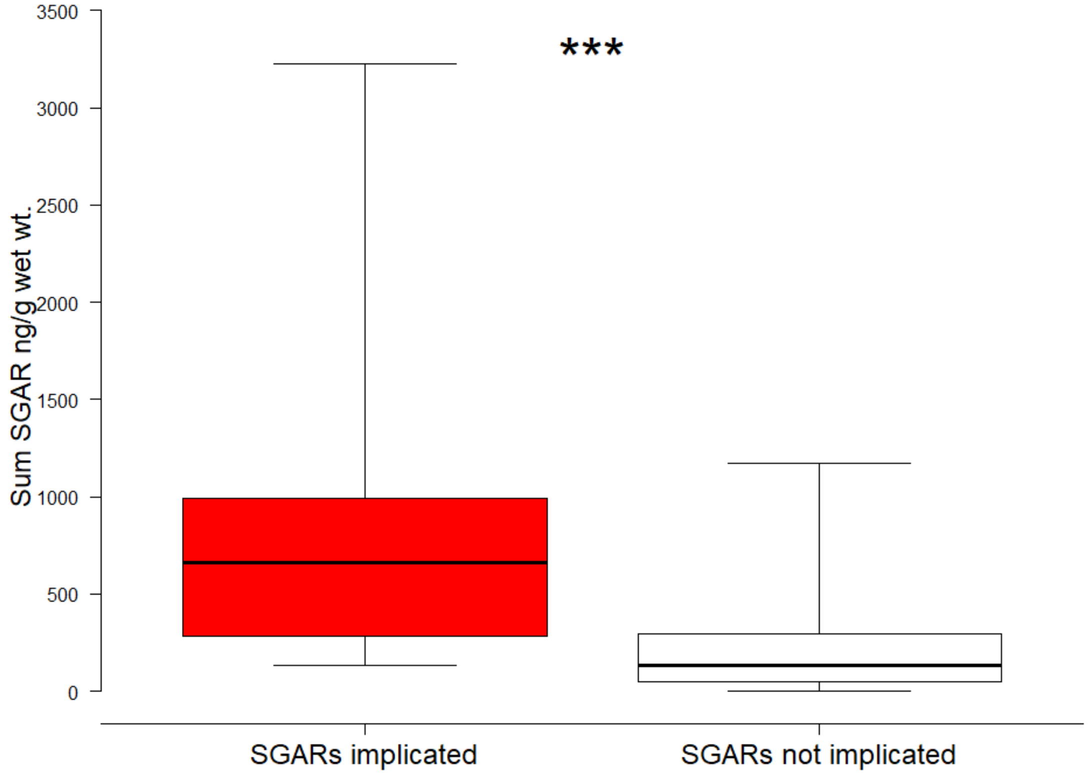

Figure 6. Box and Whiskers plot showing median, interquartile range and minimum/maximum range of sum of SGAR concentrations in red kites that died between 2015 and 2021, with haemorrhaging not associated with physical trauma (SGARs implicated as a contributory cause of mortality; n = 40) and those that were diagnosed to have died from causes unrelated to SGARs (SGARs not implicated as a contributory cause of mortality; n = 198). The difference in median concentrations between the two groups was statistically significant (Mann-Whitney U test, U = 1067/6853, P <0.001).

5 Spatial Analysis

Although the number of birds from counties/regions of the UK has differed year by year, red kites have been mainly from Oxfordshire, Northamptonshire, North Yorkshire, West Yorkshire, and Hampshire in England, Ceredigion in Wales, and the regions “Dumfries and Galloway” and “Highland” in Scotland (Figure 7a).

The number of poisoned birds during the monitoring period (2015 – 2021) was highest in West Yorkshire (poisoned birds = 6), followed by Berkshire (4) and Northamptonshire (4) (Figure 7b). In many years of the monitoring period, poisoned birds were identified in West Yorkshire (such as 2018, 2019, 2021; data for individual years are shown in Appendix 3). There were no other counties where poisoned birds were consistently identified. In Wales, poisoned birds were identified only in Vale of Glamorgan (2). In Scotland, poisoned birds were observed in Highland (2) and Grampian (1).

Figure 7c shows the maximum value of the sum SGAR concentrations by county/region. A high value of the monitoring period was observed in Hampshire (3200 ng/g ww Sum SGARs), followed by Berkshire (1800 ng/g ww) (Figure 3). However, there is a variation of exposure between years: the highest magnitude of exposure was observed in 2016 in Berkshire, whereas it was observed in 2021 in Hampshire (Appendix 4).

We also counted the number of birds with sum SGAR concentrations higher than 700 ng/g ww (Figure 4). In the monitoring period, the number reached five in Oxfordshire, four in Berkshire and Hampshire, and three in North Yorkshire and Northamptonshire. Birds with ΣSGARs > 700 ng/g ww have been observed every year since 2017 in Oxfordshire (Appendix 5). There were no other counties where Birds with ΣSGARs > 700 ng/g ww were constantly observed.

Overall, there are two hot spots of exposure of red kites to SGARs: central - southern part of England (Northamptonshire, Berkshire, Oxfordshire, Hampshire) and the central part of Northern England (North and West Yorkshire). However, there are some differences between the two areas: ΣSGAR concentrations were higher in the former part (Northamptonshire, Berkshire, Oxfordshire, Hampshire) than in the latter part (North and West Yorkshire), whereas the number of poisoned birds was higher in the latter part than in the former part. These results might suggest a difference in the exposure pattern (e.g., type of SGARs, human activities related to SGARs, toxicity of SGARs, or the ecology of rodents) and/or the resistance of red kites to contaminants between these two hot spots.

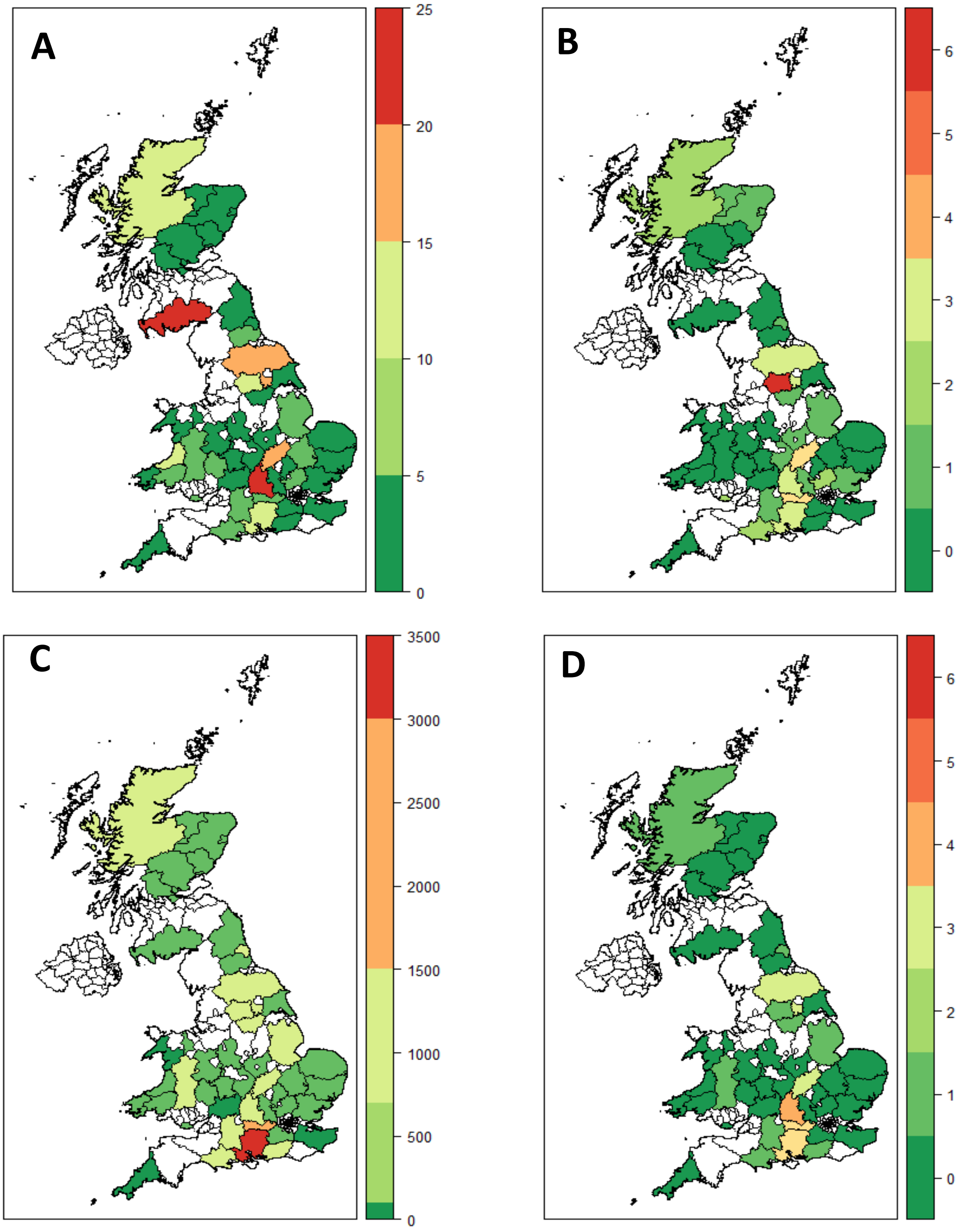

Figure 7. Maps summarising data from red kites that had died between 2015 and 2021. Data show are (A) number of samples collected, (B) number of poisoned birds, (C) maximum value of sum SGAR hepatic concentrations (ng/g ww), and (D) Number of samples > 700ng/g ΣSGAR ww. Hepatic concentrations by county (or by region in Scotland). Counties where no bird has been collected are blank. Data for individual years are shown in Appendices 2 to 5.

6 Power analysis

Based on the data for the ΣSGAR liver residues in red kites from 2015 to 2020, we conducted the power analysis. For this analysis, logarithmically transformed ΣSGAR liver residues (base = 10) were used. The number of red kites found dead by year was fixed to the average number of 35, and we assumed that the geometric mean ΣSGAR liver residues would have consistently increased from 126.2 in 2015 to 308.8 in 2021 (i.e., residues have increased 2.45 times for 6 years).

After 1000 simulations, the power of the dataset was about 69 %. Assuming that the current increase constantly continues, the power monitoring for 10 years would reach 95 %.

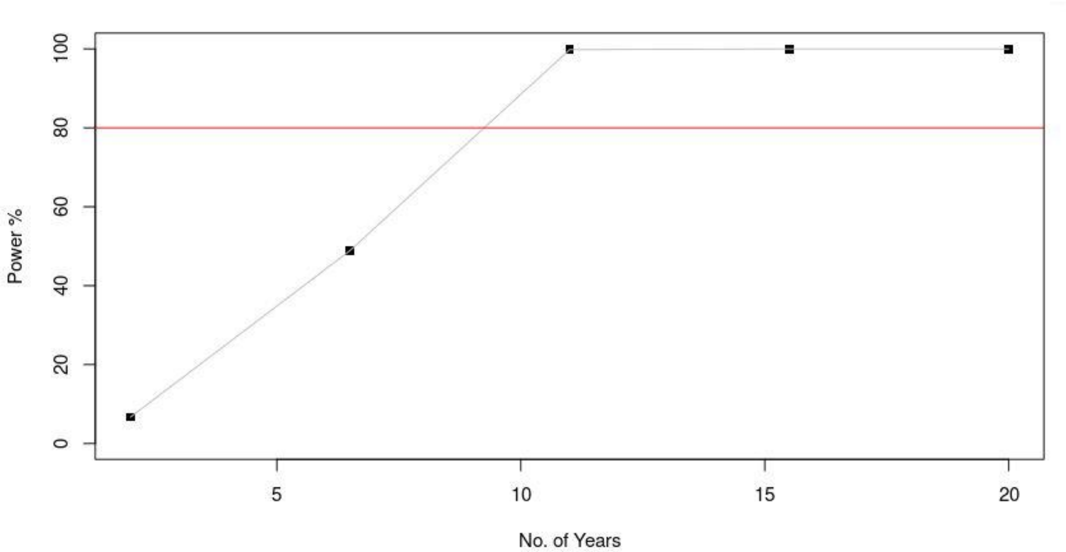

Figure 8. Relationship between the power and the number of years based on the ΣSGAR liver residues in red kites found dead from 2015 and 2021. Logarithmically transformed ΣSGAR liver residues (base = 10) are used for the analysis. The number of red kites found dead by year is fixed to 35 (the average number), and ΣSGAR liver residues are supposed to increase 2.45 times for 6 years (geometric mean ΣSGAR liver residues = 126.2 and 308.8 in 2015 and 2021, respectively).

7 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 42 red kites from England, Wales, and Scotland, found dead in 2021, all but one had been exposed to SGARs. In five cases (12% of total excluding uncertain contributory cause of death), SGARs were implicated as a contributory cause of death. For the red kites in which SGARs were considered a contributory cause of death (three Fera Science samples: RK_21_12, RK_21_13, and RK_21_14; one PBMS/IoZ sample: 22371; one WIIS SASA sample: RK_21_22 – Table 2), death was associated with “unspecified” SGAR use.

Difenacoum, brodifacoum, and bromodialone were the most prevalent compounds (detected in 87%, 87%, and 76% of red kites across the seven 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 (2015–2021), the proportion of red kites with detectable liver SGAR residues remains at >88%. There were statistically significant differences between years, and the magnitude of accumulated summed SGAR residues has generally been higher in recent years. There was no clear difference for sum bromadiolone and difenacoum. Meanwhile, sum of brodifacoum, flocoumafen, and difethialone concentrations, and even only brodifacoum concentrations, were statistically higher in 2021 than the previous years except 2020. The results clearly indicate an increasing trend of brodifacoum residues in red kites in recent years.

Given the result from the power analysis, additional monitoring years (10 years in total) for which we have combined data from different monitoring schemes would be required to detect increase in concentrations, assuming the magnitude of non-significant increase continues over that extended 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 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. Potential change in the proportion of birds in which SGARs were diagnosed as a contributory mortality factor is difficult to determine. 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 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. 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.

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., 2023).

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 seven 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; b). 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 residues 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. Where possible it should be considered how persistence of the individual active ingredients may impact such analysis. 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.

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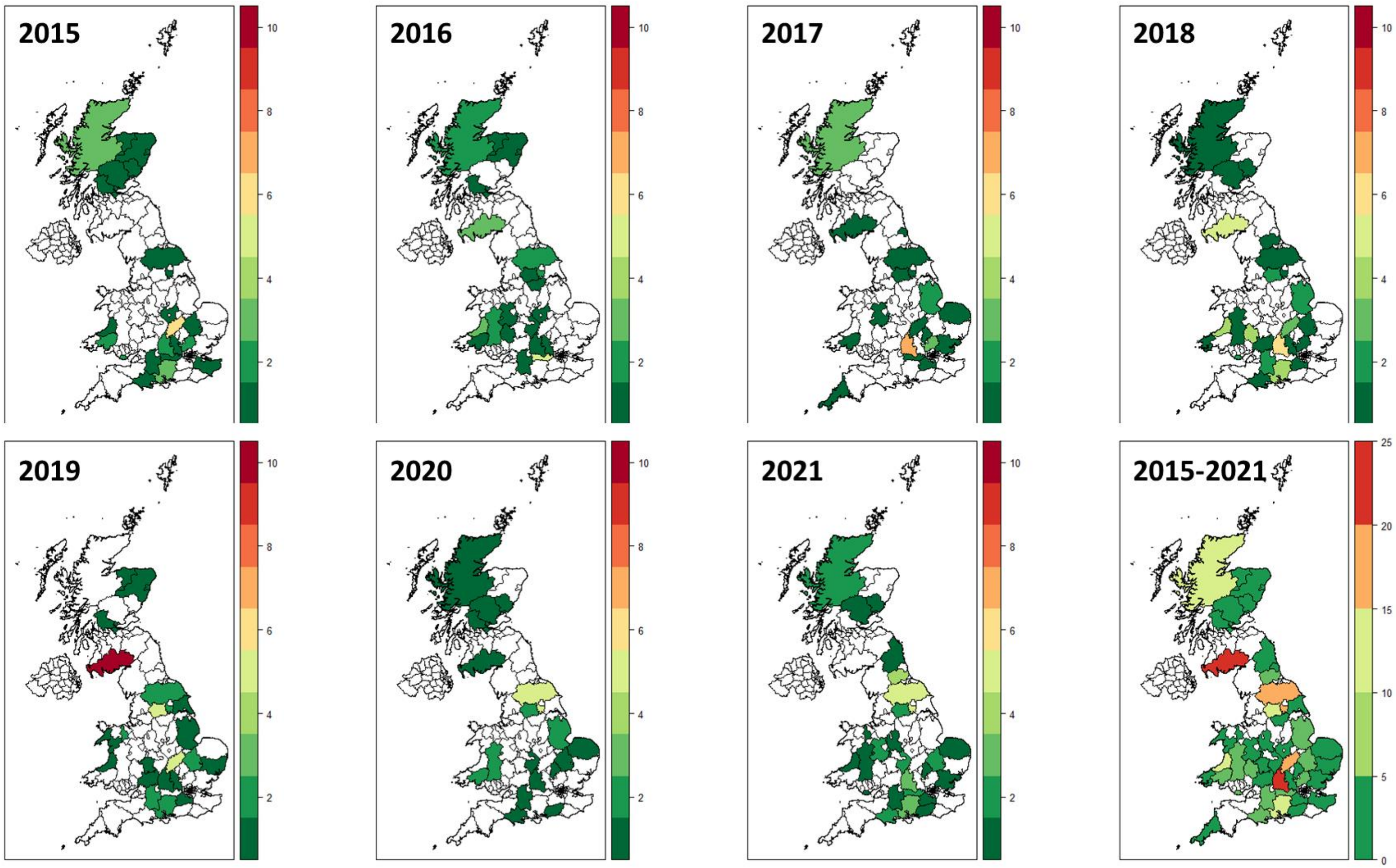
Appendix 1 – Summary of limits of detection and spiked standard recoveries for anticoagulant rodenticides by LC-MS/MS analysis across schemes

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.

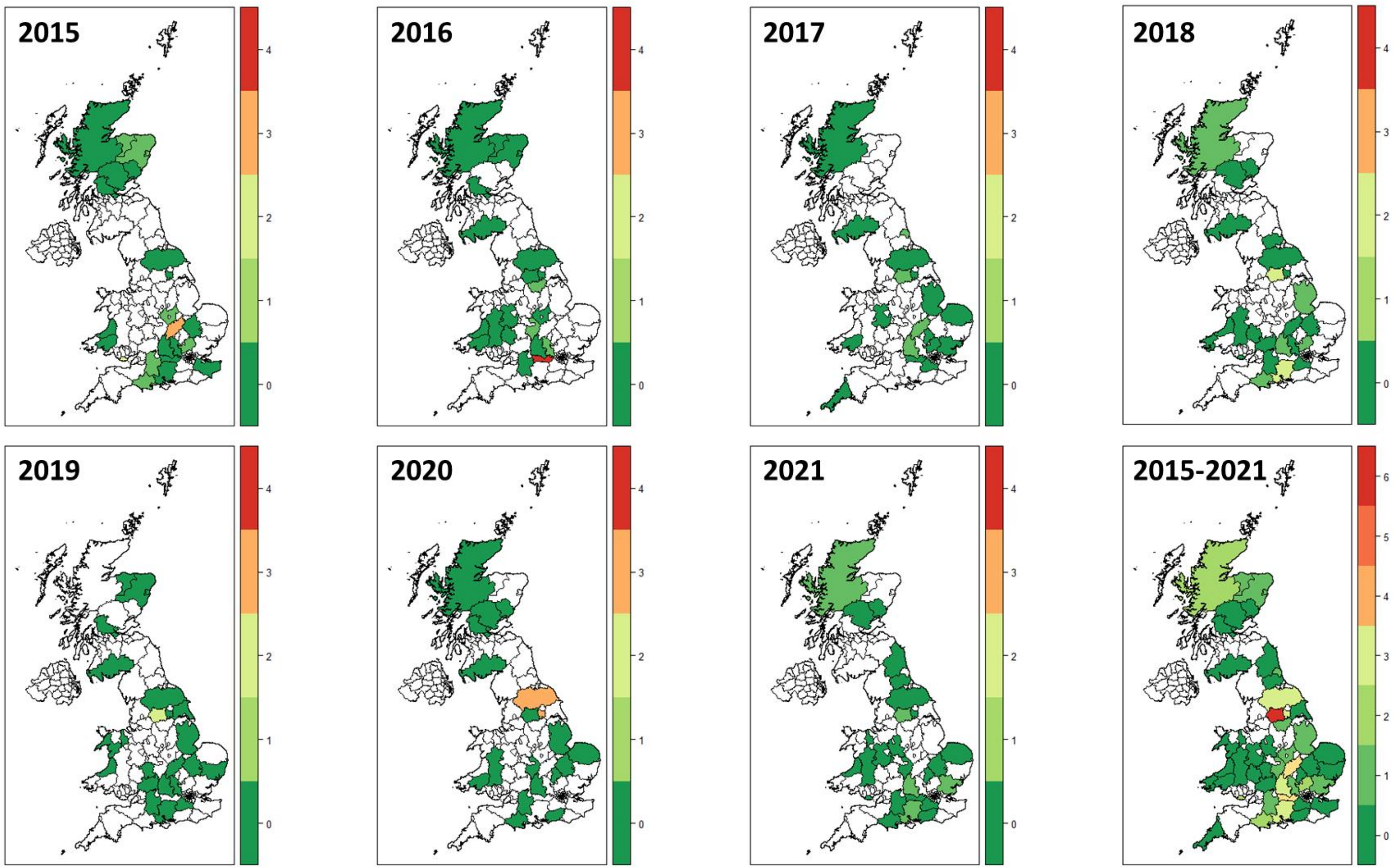
	UKCEH		Fera Science		SASA	
	LoD	% Spike recovery#	LoD	Typical % Spike recovery	LoD	Typical % Spike recovery*
Brodifacoum	1.5	77.1	0.8	64	2	80.7
Bromadiolone	1.5	76.2	0.8	94	2	82.7
Difenacoum	1.5	-	0.8	94	2	85.0
Flocoumafen	1.5	-	0.8	105	2	77.7
Difethialone	2.8	-	0.8	83	2	79.3

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

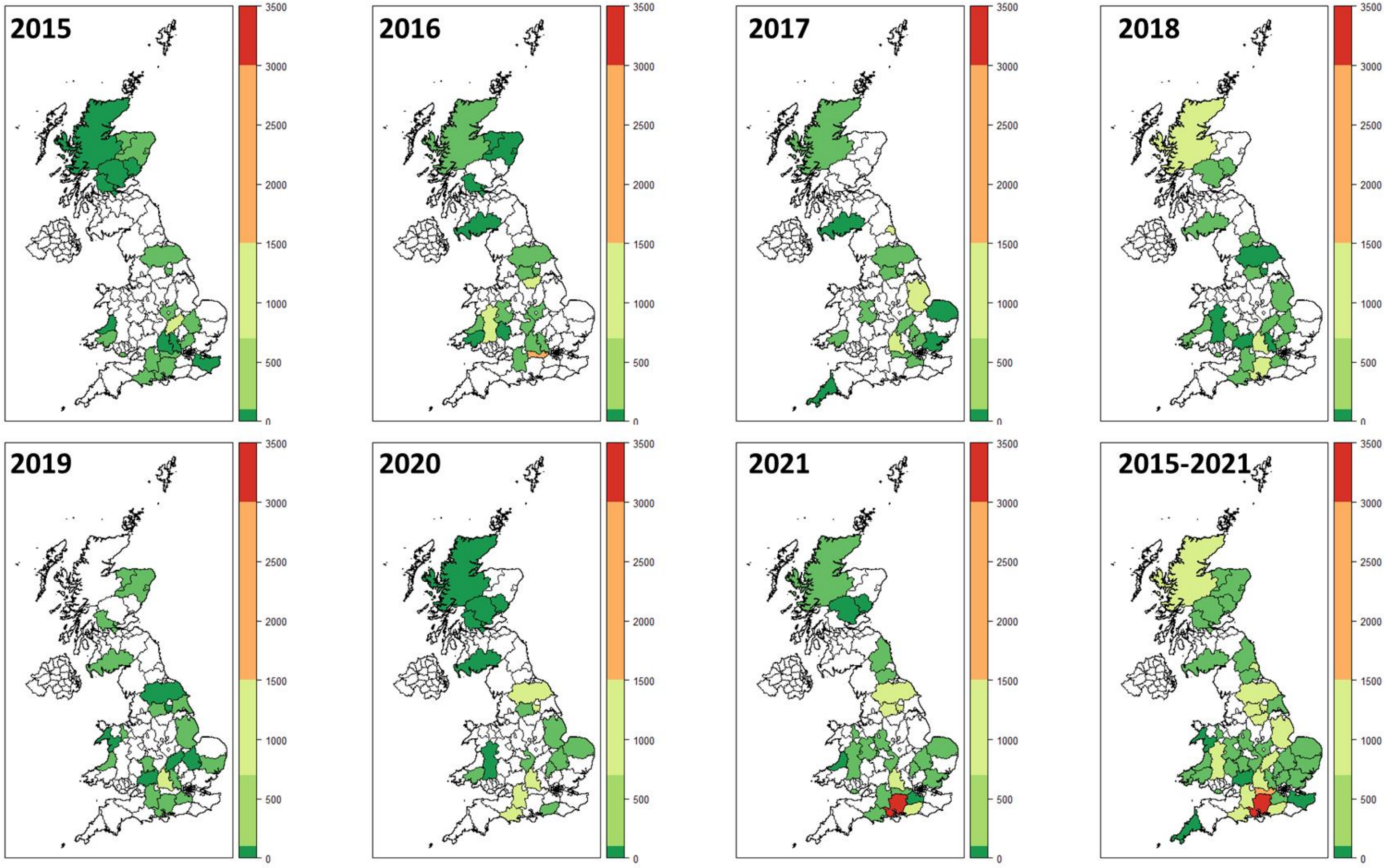
Appendix 2 – Number of samples collected for individual years and in total over the monitoring period by county. Counties where no bird has been collected are blank. The bar on the side of each map indicates the value of the metric presented.



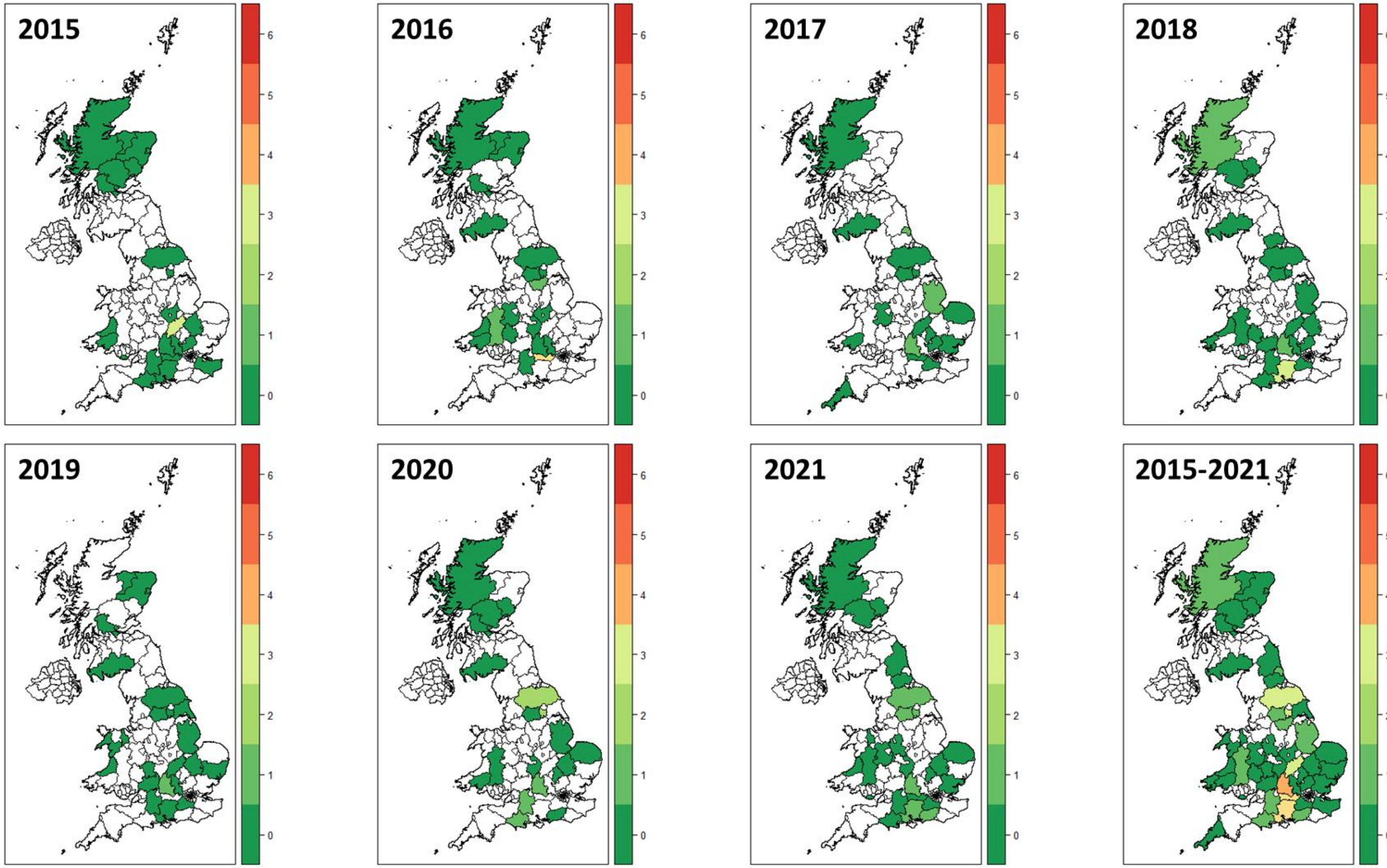
Appendix 3 – Number of poisoned birds for individual years and in total over the monitoring period by county. Counties where no bird has been collected are blank. The bar on the side of each map indicates the value of the metric presented.



Appendix 4 – Maximum value of sum SGAR hepatic concentrations (ng/g ww) by county. Counties where no bird has been collected are blank. The bar on the side of each map indicates the value of the metric presented.



Appendix 5 – Number of samples with liver concentrations greater than 700ng/g ΣSGAR ww by county. Counties where no bird has been collected are blank. The bar on the side of each map indicates the value of the metric presented.



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