Anticoagulant rodenticides in red kites (*Milvus milvus)* in Britain in 2017 and 2018

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1. Executive Summary

Second generation anticoagulant rodenticides (SGARs) can be toxic to all mammals and birds. 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 Monitoring Scheme (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 77 red kites that had been found dead in Britain in either 2017 or 2018. 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; the livers from the kites were subsequently analysed for SGAR residues. All the organisations are partners in the WILDCOMS network that promotes collaboration among surveillance schemes that monitor disease and contaminants in vertebrate wildlife.

All of the 66 kites from England & Wales and 10 of the 11 red kites from Scotland had detectable liver residues of at least one SGAR. When considering the sample of kites as a whole, brodifacoum, difenacoum and bromadiolone were each detected in 73, 71 and 60 kites, respectively. Difethialone was found in 11 individuals while flocoumafen was detected in only one bird.

Sum liver SGAR concentrations ranged between non-detected and 1218 ng/g wet wt. (arithmetic mean: 246 ng/g, median 154 ng/g). Post-mortem examinations indicated that 13 (16.8%) of red kites examined had internal haemorrhaging that was not associated with detectable trauma and had detectable liver SGAR concentrations. These birds had sum SGAR liver concentrations that ranged from 135 ng/g wet weight to 1218 ng/g wet weight. SGARs were considered a contributory cause of death in these cases.

The stewardship scheme for anticoagulant rodenticides came fully into force in mid-2016 as re-registration of products for use in the UK was completed. A key aim is to reduce exposure of non-target wildlife to anticoagulant rodenticides but stewardship also aims to maintain efficacious rat control and so the number and density of AR-contaminated rats may remain unchanged. However, diligent searching, removal and safe disposal of poisoned rats, as promoted by stewardship, 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 stewardship 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.

The proportion of red kites exposed to SGARs in 2015 (90.6%), 2016 (89.6%) 2017 (96,4%) and 2018 (100%) was always 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 (89% of red kites across the four years for each compound) along with bromadiolone (75%). On average, there were residues of three different SGARs in each kite liver. There was no significant difference between years in liver sum (Σ) SGAR concentrations.

We investigated if there was a change between years in the exposure of red kites to brodifacoum, flocoumafen and difethialone, the compounds for which *indoor only* usage restrictions were relaxed in 2016. To enable statistical analysis of data on residue prevalence, it was necessary to pool the data into two-year blocks. Data on presence/absence of detectable brodifacoum, flocoumafen or difethialone residues were therefore compared for 2015/16 (pre and year of implementation of change in usage restriction) and 2017/18 (post-change in usage restriction). The proportion of red kites with detectable residues was 82% (50 out of 61 red kites) in 2015/16 but significantly higher (95%; 73 out of 77 red kites) in 2017/18. However, there was also an increase [albeit not statistically significant] in the proportion of red kites with detectable liver difenacoum or bromadiolone residues (90% in 2015/16 vs. 97% in 2017/18). Therefore, these data may simply reflect an increase in the prevalence of exposure to SGARs generally rather than any effect of change in usage restriction. There was no difference between the four years in the summed magnitude of liver brodifacoum, difethialone and flocoumafen concentrations.

The percentage of red kites examined that were diagnosed as birds in which SGARs were implicated as a contributory cause of death did not differ significantly between individual years nor show a significant trend across the years; the overall average across the four years was 22%. However, if data were pooled by pairs of years (2017/8 vs 2015/16), the proportion of red kites for which SGARs were implicated as a contributory cause of death was lower (18%) in 2017/18 than in 2015/16 (33%) for red kites from England & Wales.

Our findings do not indicate that there has been any reduction in exposure in red kites to SGARs following implementation of stewardship, in terms of either the proportion of individuals exposed or the magnitude of 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 was no clear evidence that relaxation of usage restrictions on brodifacoum, difethialone and flocoumafen has altered the pattern of residue accumulation in red kites to date.

2. Introduction

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 predators 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 Centre for Ecology & Hydrology's (CEH) Predatory Bird Monitoring Scheme (PBMS) 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 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 http://www.thinkwildlife.org/stewardship-regime/. 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 Century as part of an official species recovery programme (Carter and Grice 2002). Red kites are scavengers and their diet typically includes dead rats. 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 secondary exposure to ARs increased in this species in Britain in the last 25 years (Sainsbury *et al*., 2018; Shore et al., 2003a). SGAR-induced deaths of red kites have been documented.

The stewardship scheme for anticoagulant rodenticides came fully into force in mid-2016 as re-registration of products for use in the UK was completed. 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 in rat predators. This is because stewardship aims to maintain efficacious rat control 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 stewardship. This might be expected to reduce the availability of poisoned rats to red kites and other scavengers and thereby reduce exposure.

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. This change was implemented simultaneously with stewardship at the time of product re-registration. These three SGARs can now be used *in and around buildings,* [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 (Prescott et al., 2018), and subsequently increase secondary exposure of red kites. 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, 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 ZSL, 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.

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. 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 and examines SGAR residues in any raptors found dead in Scotland. 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 on line[2].

Data for birds that died in 2017 and 2018 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, 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 2017 and 2018 and submitted to the DRAHS/PBMS, WIIS for England & Wales, or the WIIS for Scotland for analysis.

The report covers two years (unlike in previous years) as there was an opportunity that samples collected throughout 2018 could be analysed earlier than has previously been possible and so data for 2018 were available before data for 2017 had been reported. We describe the current

[2] http://www.hse.gov.uk/pesticides/topics/reducing-environmental-impact/wildlife/wiis-quarterly-reports.htm

incidence, magnitude and likely toxicological significance of the liver SGAR residues detected in these birds in both 2017 and 2018 and compare our data with those for kites that died in 2015 and 2016 (Walker et al., 2017, 2018). This timeframe spans the implementation of a stewardship programme for anticoagulant rodenticides and the concurrent relaxation of restrictions to indoor use only for brodifacoum, flocoumafen and difethialone.

3. Methods

The carcasses of 28 and 49 red kites that died in 2017 and 2018, respectively, were collected as part of either 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.

Table 1. Number of red kites examined in each demographic group for individuals found dead in 2017 and 2018.

Year:	2017			2018		
	Adult	Juvenile	Unknown	Adult	Juvenile	Unknown
Male	7	2	0	15	2	1
Female	4	2	3	8	1	3
Unknown	1	1	8	3	4	12

The provenance of the 77 carcasses is shown in Figure 1. The majority (86% in both of 2017 and 2018) were from England and Wales. Juveniles, when age was characterized, were individuals hatched in the current or previous year, as assessed from plumage characteristics (Molenaar et al., 2017).

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 CEH, Fera Science and SASA, respectively. During the necropsy, 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; 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 will 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).

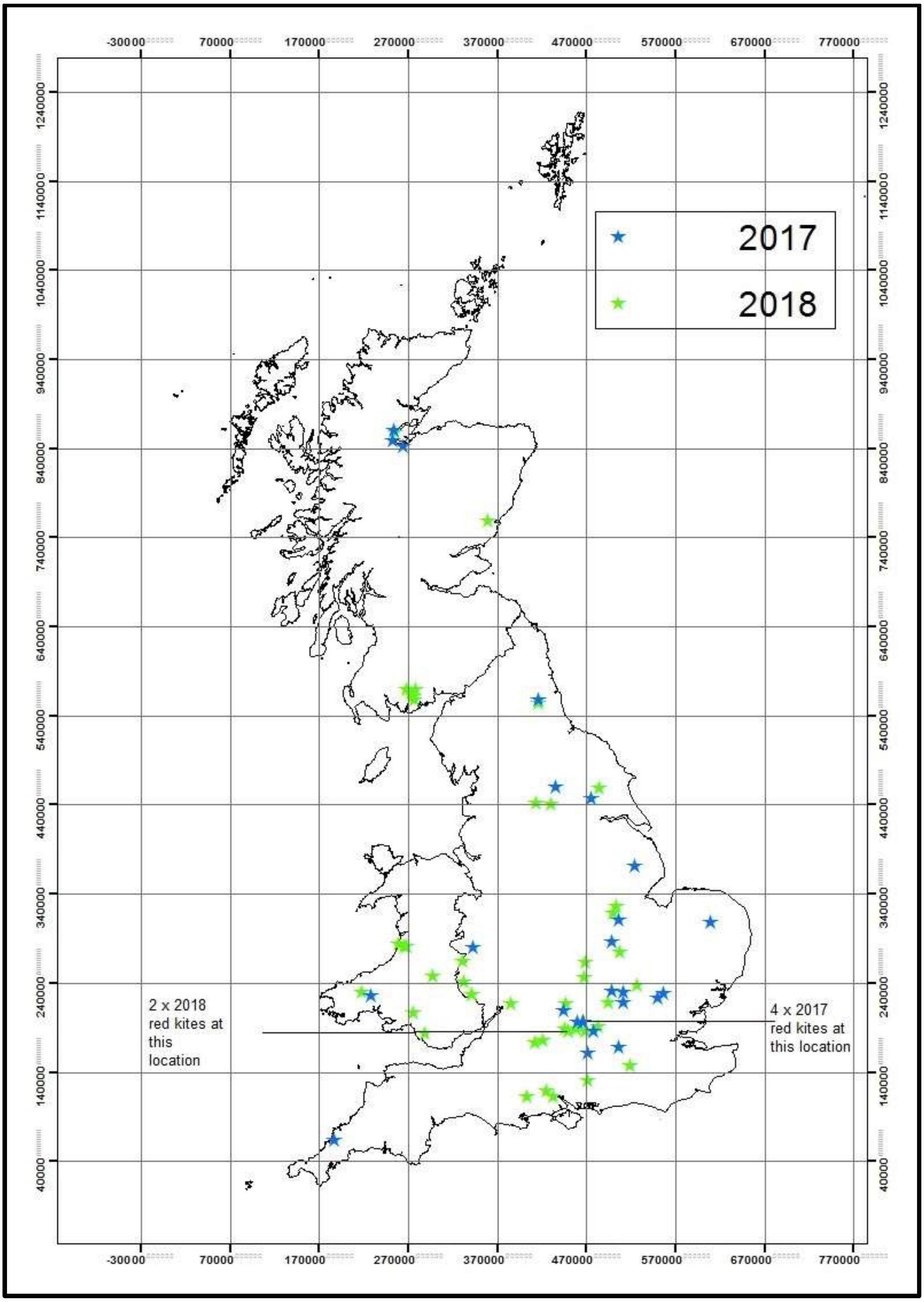

Figure 1. Location of red kites, *Milvus milvus*, found dead in 2017 and 2018 for which livers were analysed for second generation anticoagulant rodenticides (SGARs) by the PBMS, SASA and Fera

4. Results and Discussion

4.1 Liver SGAR residues in red kites that died in 2017 and 2018

Of the 28 red kites found dead in 2017, all but one had detectable concentrations of one or more SGARs in their liver (Table 2); the single bird with non-detected residues was a male juvenile from the Highlands of Scotland (bird no/ 17027/1). Brodifacoum (96% of kites in the sample) difenacoum (89%) and bromadiolone (82%) were the most prevalent residues detected. Difethialone was found in three birds (11%) but flocoumafen was not detected in any kites from 2017. Sum SGAR concentrations ranged between non-detected to 1150 ng/g wet wt. with a median of 125 ng/g wet wt.

All of the 49 red kites found dead in 2018 had detectable residues of at least one SGAR in their liver (Table 3). As with birds that died in 2017, brodifacoum (94% of kites in the sample), difenacoum (94%) and bromadiolone (76%) were the most prevalent residues. Difethialone and flocoumafen were present in 16.3% and 2% of birds respectively. Sum SGAR concentrations ranged between 1.7 and 1218 ng/g wet wt. with a median of 167.0 ng/g wet wt.

Although the limit of detection for the analysis of SGARs was slightly higher for samples from Scotland (3ng/g wet wt., Appendix 1), applying this higher rate to birds from England & Wales would have made no difference to the reported % of birds with detected residues of at least one SGAR in 2017. One of the 42 kites that died in 2018 in England & Wales would have been classed as having non-detected residues if the higher detection limit was applied.

Post mortem examinations indicated that 4/28 (14%) and 9/49 (18%) of the kites found dead in 2017 and 2018, respectively, had internal haemorrhaging that was not associated with detectable trauma, and had detectable liver SGAR residues (Tables 2 and 3). Anticoagulant rodenticides were considered to be a contributory cause of death of these birds.

Table 2. Concentrations of second generation anticoagulant rodenticides (SGARs) in the livers of red kites found dead in 2017.

Scheme	Incident/ Bird code	SGAR contributory cause of death	Month of death	Sex	Age	Location	Concentration of SGAR (ng/g wet wt.)					
							Brom	Difen	Floc	Brod	Difeth	ΣSGARs
PBMS/IoZ	19778	No	Nov	M	Juvenile	Carmarthenshire	8.7	3.8	ND	364	ND	376.4
PBMS/IoZ	19795	No	Nov	M	Adult	Berkshire	90.2	22.0	ND	26.0	ND	138.2
PBMS/IoZ	19933	No	Feb	F	Adult	Bedfordshire	38.8	86.3	ND	63.0	ND	188.0
PBMS/IoZ	19934	No	Jan	F	Juvenile	Hertfordshire	94.3	45.4	ND	2.2	ND	142.0
PBMS/IoZ	19935	No	Jan	U	Adult	Lincolnshire	10.9	52.4	ND	392	683	1139
PBMS/IoZ	19936	Yes	Jan	F	Adult	Tyne & Wear	ND	26.5	ND	81.6	1042	1150
PBMS/IoZ	19937	No	Feb	M	Adult	Oxfordshire	ND	1.8	ND	718	36.6	756.7
PBMS/IoZ	19939	Yes	Apr	M	Adult	West Yorkshire	85.8	82.7	ND	55.6	ND	224.1
PBMS/IoZ	19940	No	Apr	F	Adult	Essex	21.2	2.1	ND	15.5	ND	38.8
PBMS/IoZ	19941	Yes	May	M	Adult	Northamptonshire	17.9	286.6	ND	66.0	ND	370.4
PBMS/IoZ	19943	No	Apr	M	Adult	Hertfordshire	19.6	152.0	ND	43.9	ND	215.6
PBMS/IoZ	20708	No	Oct	U	Juvenile	Surrey	ND	13.5	ND	374	ND	387.8
PBMS/IoZ	20709	No	Nov	M	Adult	Hertfordshire	7.4	10.2	ND	4.8	ND	22.4
SASA	17027/1	No	Mar	M	Juvenile	Highland	ND	ND	ND	ND	ND	ND
SASA	17119/1	No	Aug	M	Adult	Highland	75.0	11.0	ND	140	ND	226.0
SASA	17121/1	No	Aug	F	Juvenile	Highland	8.0	43.0	ND	2.0	ND	53.0
SASA	17141/1	No	Oct	F	Adult	D&G	2.0	38.0	ND	6.0	ND	46.0
Fera Science	5	No	Dec	U	U	North Yorkshire	12.0	41.0	ND	52.0	ND	105.0
Fera Science	16	No	Mar	U	U	Shropshire	ND	49.0	ND	55.0	ND	104.0
Fera Science	20	No	April	F	U	Lincolnshire	68.0	68.0	ND	15.0	ND	151.0
Fera Science	21	No	Apr	F	U	Oxfordshire	17.0	7.0	ND	88.0	ND	112.0
Fera Science	22	No	Apr	U	U	Cornwall	0.4	18.0	ND	6.2	ND	24.6
Fera Science	27	No	Feb	F	U	Oxfordshire	2.0	13.0	ND	74.0	ND	89.0
Fera Science	63	No	Oct	U	U	Norfolk	52.0	1ND	ND	3.0	ND	65.0
Fera Science	55/3	No	Sep	U	U	Oxfordshire	0.3	ND	ND	5.0	ND	5.3
Fera Science	55/4	Yes	Sep	U	U	Oxfordshire	650	1.0	ND	8.0	ND	659.0
Fera Science	55/5	No	Sep	U	U	Oxfordshire	0.7	ND	ND	5.0	ND	5.7
Fera Science	55/6	No	Sep	U	U	Oxfordshire	7.0	58.0	ND	18.0	ND	83.0

M – male; F- female; U – sex or age not determined; ND = non-detected; Brom – bromadiolone; Difen – difenacoum; Floc – flocoumafen; Brod – brodifacoum; Difeth – difethiolone. 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 3. Concentrations of second generation anticoagulant rodenticides (SGARs) in the livers of red kites found dead in 2018.

Scheme	Incident/ Bird code	SGAR contributory cause of death	Month of death	Sex	Age	Location	Concentration of SGAR (ng/g wet wt.)					
							Brom	Difen	Floc	Brod	Difeth	Σ SGARs
PBMS/IoZ	20065	No	Mar	M	Adult	Oxfordshire	97.8	8.8	ND	319.6	ND	426.2
PBMS/IoZ	20129	No	Apr	F	Adult	Oxfordshire	ND	6.7	ND	272.8	ND	279.4
PBMS/IoZ	20357	No	Sep	F	Adult	Herefordshire	4.4	144.4	ND	135.2	ND	283.9
PBMS/IoZ	20387	No	Jul	U	U	Cardiganshire	ND	2.2	ND	4.9	ND	7.1
PBMS/IoZ	20388	No	Sep	U	U	Cardiganshire	2.7	4.4	ND	192.4	ND	199.5
PBMS/IoZ	20418	No	Jul	U	Adult	Hampshire	2.7	7.0	ND	1020	46.0	1076
PBMS/IoZ	20710	No	Jan	M	Adult		11.7	99.4	ND	53.4	ND	164.4
PBMS/IoZ	20711	Yes	Jan	F	First year	Dorset	44.1	20.3	ND	201.4	ND	265.8
PBMS/IoZ	20712	Yes	Feb	F	Adult	Hertfordshire	10.3	33.5	ND	96.3	ND	140.1
PBMS/IoZ	20713	No	Mar	U	Adult		ND	4.0	ND	189.9	ND	193.9
PBMS/IoZ	20714	No	Mar	M	Adult	Gloucestershire	ND	12.6	ND	3.4	ND	16.1
PBMS/IoZ	20715	No	Mar	M	Adult	Buckinghamshire	ND	5.4	ND	46.8	ND	52.1
PBMS/IoZ	20716	No	Mar	U	Juvenile	Herefordshire	1.7	107.1	ND	24.6	ND	133.4
PBMS/IoZ	20717	No	Apr	M	Adult	Breconshire	ND	2.7	ND	6.7	ND	9.4
PBMS/IoZ	20718	No	Apr	M	Juvenile		93.6	8.5	ND	7.0	ND	109.0
PBMS/IoZ	20719	No	Jul	M	Adult	Hampshire	ND	3.0	ND	734.9	ND	737.9
PBMS/IoZ	20720	No	Aug	U	Juvenile	Surrey	50.9	12.1	ND	8.1	283.3	354.4
PBMS/IoZ	20721	No	Aug	M	Juvenile	Northamptonshire	4.3	9.7	ND	242.1	ND	256.1
PBMS/IoZ	20722	Yes	Oct	M	Adult	Hampshire	ND	20.6	ND	754.5	26.3	801.5
PBMS/IoZ	20723	Yes	Oct	F	Adult	Lincolnshire	3.0	100.7	2.8	28.1	ND	134.6
PBMS/IoZ	20724	No	Oct	F	Adult	Wiltshire	18.5	2.1	ND	197.0	ND	217.6
PBMS/IoZ	20725	No	Dec	M	Adult	Herefordshire	2.7	74.0	ND	70.0	7.5	154.2
PBMS/IoZ	20726	No	Oct	F	Adult	Huntingdonshire	14.9	102.2	ND	13.9	ND	131.0
PBMS/IoZ	20727	No	Oct	U	Juvenile	Wiltshire	51.0	103.0	ND	15.4	ND	169.4
PBMS/IoZ	20846	No	Apr	M	Adult	Glamorgan	7.6	320.8	ND	4.9	ND	333.3

M – male; F- female; U – sex or age not determined; ND = non-detected; Brom – bromadiolone; Difen – difenacoum; Floc – flocoumafen; Brod – brodifacoum; Difeth – difethiolone. 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 3 contd. Concentrations of second generation anticoagulant rodenticides (SGARs) in the livers of red kites found dead in 2018.

Lab	Incident/ Bird code	SGAR contributory cause of death	Month of death	Sex	Age	Location	Concentration of SGAR (ng/g wet wt.)					
							Brom	Difen	Floc	Brod	Difeth	Σ SGARs
PBMS/IoZ	20848	No	Apr	F	Adult	Pembrokeshire	5.5	160.2	ND	2.9	ND	168.5
PBMS/IoZ	20849	No	Apr	U	Subadult	Durham	9.2	105.9	ND	22.4	ND	137.5
PBMS/IoZ	20850	No	Apr	M	Adult	Cardiganshire	3.4	64.7	ND	2.8	ND	70.9
PBMS/IoZ	20851	No	Jul	M	Adult	Oxfordshire	12.7	ND	ND	32.8	27.7	73.2
PBMS/IoZ	20852	No	Aug	F	Adult	Northamptonshire	ND	17.5	ND	25.8	344.0	387.2
SASA	18010	No	Jan	U	U	D&G	3.0	18.0	ND	33.0	ND	54.0
SASA	18050	No	Apr	M	Adult	D&G	22.0	0.0	ND	0.0	ND	22.0
SASA	18061	No	Apr	U	Adult	D&G	234.0	58.0	ND	25.0	ND	317.0
SASA	18065	Yes	Apr	U	U	Highland	5.0	833.0	ND	9.0	ND	847.0
SASA	18072	No	Apr	M	Adult	D&G	4.0	59.0	ND	0.0	ND	63.0
SASA	18086	No	May	F	U	D&G	178.0	81.0	ND	4.0	ND	263.0
SASA	18177	No	Dec	M	Adult	Tayside	ND	135.0	ND	0.0	ND	135.0
Fera Science	9	No	Feb	U	U	Lincolnshire	11.0	55.0	ND	6.8	ND	72.8
Fera Science	17	No	Mar	U	U	Oxfordshire	140.0	6.9	ND	320.0	43.0	509.9
Fera Science	17	No	Mar	U	U	Oxfordshire	4.2	55.0	ND	100.0	ND	159.2
Fera Science	18	Yes	Feb	U	U	Oxfordshire	0.5	10.0	ND	1200	7.1	1217.6
Fera Science	28	No	Apr	F	U	Herefordshire	92.0	4.0	ND	1.1	ND	97.1
Fera Science	29	Yes	Mar	U	U	West Yorkshire	1.8	2.7	ND	190.0	ND	194.5
Fera Science	39	No	Apr	M	Adult	Glamorgan	ND	13.0	ND	1.1	ND	14.1
Fera Science	41	Yes	Apr	F	U	West Yorkshire	91.0	140.0	ND	1.5	ND	232.5
Fera Science	68	No	Jul	U	U	Cardiganshire	ND	ND	ND	1.7	ND	1.7
Fera Science	81	No	Sep	U	U	Northamptonshire	120.0	21.0	ND	26.0	ND	167.0
Fera Science	87	Yes	Oct	U	U	Hampshire	0.3	60.0	ND	130.0	ND	190.3
Fera Science	99	No	Dec	M	U	North Yorkshire	0.2	11.0	ND	13.0	ND	24.2

M – male; F- female; U – sex or age not determined; ND = non-detected; Brom – bromadiolone; Difen – difenacoum; Floc – flocoumafen; Brod – brodifacoum; Difeth – difethiolone. 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 Trends in exposure over time

We examined whether the exposure patterns in 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, and between 95 and 100% in each of 2017 and 2018. 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 pairs of years that represented as closely as possible "pre and stewardship implementation—2015/2016" and "post-stewardship implementation—2017/18". The proportion of red kites with detected residues was 76/77 (99%) in 2017/18, significantly higher (Fisher's goodness of Fit test, P=0.044) than the equivalent proportion (55/61 = 90%) in 2015/16. Over the whole period, 131 out of the 138 kites examined had at least one detectable liver residue and the median number of different compounds detected in the liver was three.

It is possible 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 long tissue half-lives) is likely to increase with age. 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, and so was not elevated in the latter two years when the proportion of birds with detectable residues was highest. Furthermore, there were 9 red kites from England & Wales in 2017 and 2018 that were classed as juveniles and all had detectable liver SGAR residues and an average (median) of three different SGARs present in the liver (Tables 2 and 3). This was the same as for adults from the same region and years. 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 2). There was no statistically significant difference between years in any of the three analyses (Kruskal-Wallis test: $KW \leq 7.63$, $P>0.05$) and no evidence that the magnitude of accumulated summed AR residues has changed consistently over time. Exclusion of known juvenile birds from the analyses, on the basis that they may have accumulated lower residues and were not exactly evenly distributed across each of the years, did not alter the statistical outcomes.

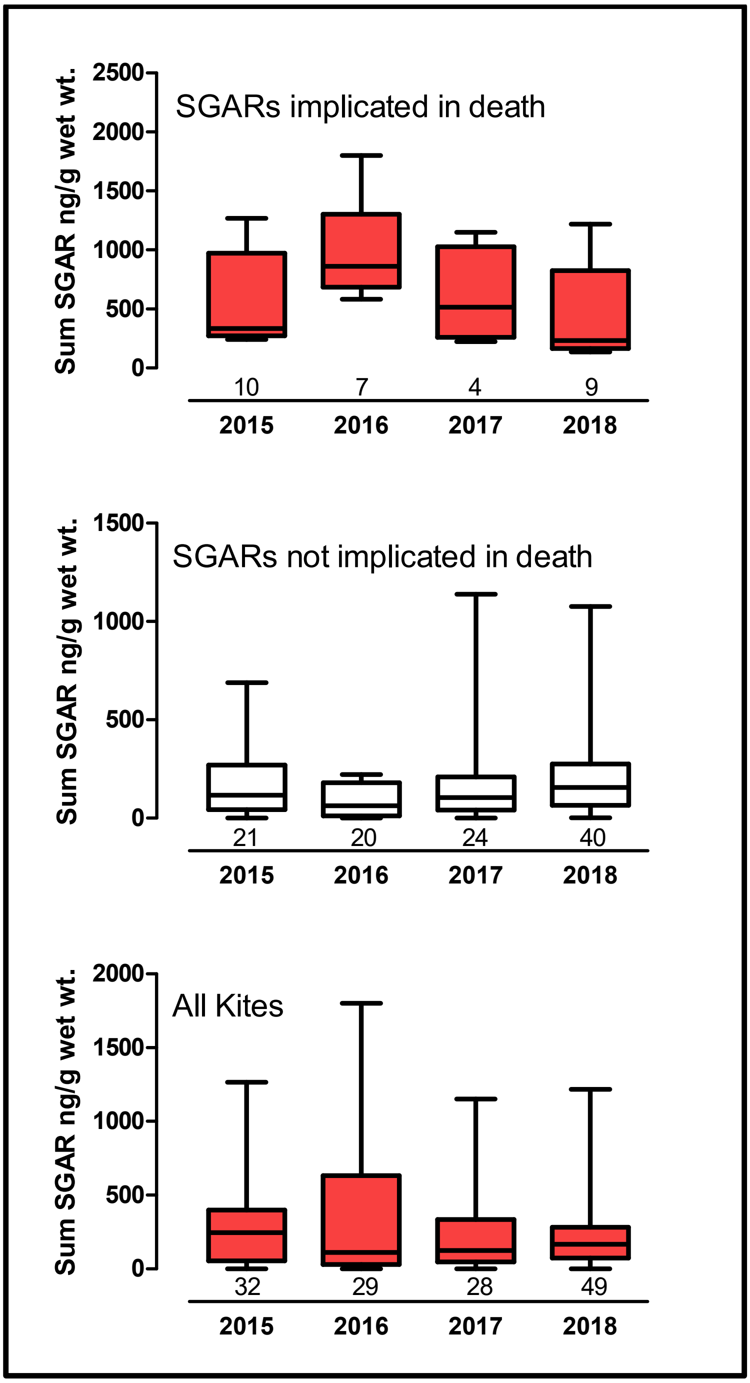

Figure 2. Box and Whiskers plot showing median, interquartile range and minimum/maximum range of sum (Σ) SGAR concentrations in red kites that died with haemorrhaging unassociated with physical trauma (SGARs implicated in death), those died from other causes (SGARs not implicated in death) and in all kites combined. Sample numbers are shown near the x-axis for each group. Three birds (numbers 19118 from the 2015 cohort and numbers 18965 and 33a 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.

In fact, all kites that had liver residues of flocoumafen or difethialone also had detectable residues of brodifacoum (Tables 2 and 3) 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%) and 46 (94%) in 2015, 2016, 2017 and 2018 respectively (Figure 3). It was not possible to analyse if there was statistically significant variation in proportions between individual years because the underlying assumptions of a Chi Squared test were not met (values in expected cells of less than five). We therefore again pooled samples into "pre and stewardship implementation—2015/2016" and "post-stewardship implementation—2017/18 year blocks. The proportion of birds with liver brodifacoum residues was 50/61 (82%) and 73/77 (95%) in 2015/16 and 2017/18 respectively and the difference between year groups was significant (Fisher's Exact test; P=0.025). However, there was also some increase across these pairs of years in the proportion of kites that had liver difenacoum or bromadiolone residues (90% in 2015/16 vs. 97% in 2017/18, albeit not statistically significant: Fisher's Exact test; P=0.14; Figure 3). It is therefore uncertain whether the greater proportion of kites with liver brodifacoum residues in 2017-2018 reflected increased exposure to this compound relative to other SGARs, or simply reflected more wide scale exposure of red kites to SGARs generally.

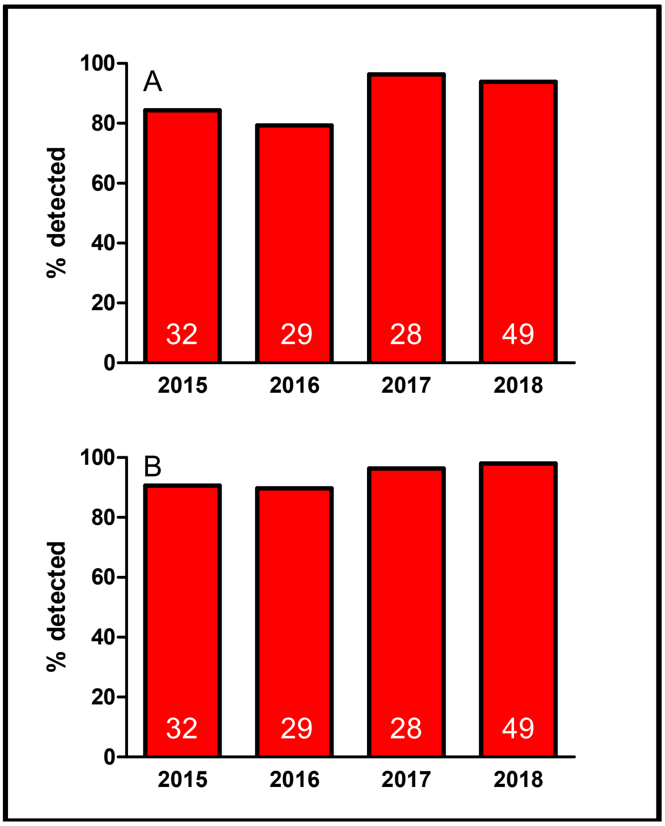

Figure 3. The percentage of red kites found dead between 2015 and 2018 that had detectable concentrations of brodifacoum (A) or either difenacoum and/or bromadiolone (B) in their livers.

Examination of the summed magnitude of liver concentrations of brodifacoum, difethialone and flocoumafen (Figure 4) revealed no significant difference between years (KW= 1.032, P=0.79). However, this was not true for the summed concentrations of bromadiolone and difenacoum which did vary between years (KW = 10.76, P=0.013) with median concentrations in 2015 generally higher than subsequent years, although post-hoc comparisons (Dunn's Multiple Comparison test) indicated that only the difference between 2015 and 2016 was statistically significant.

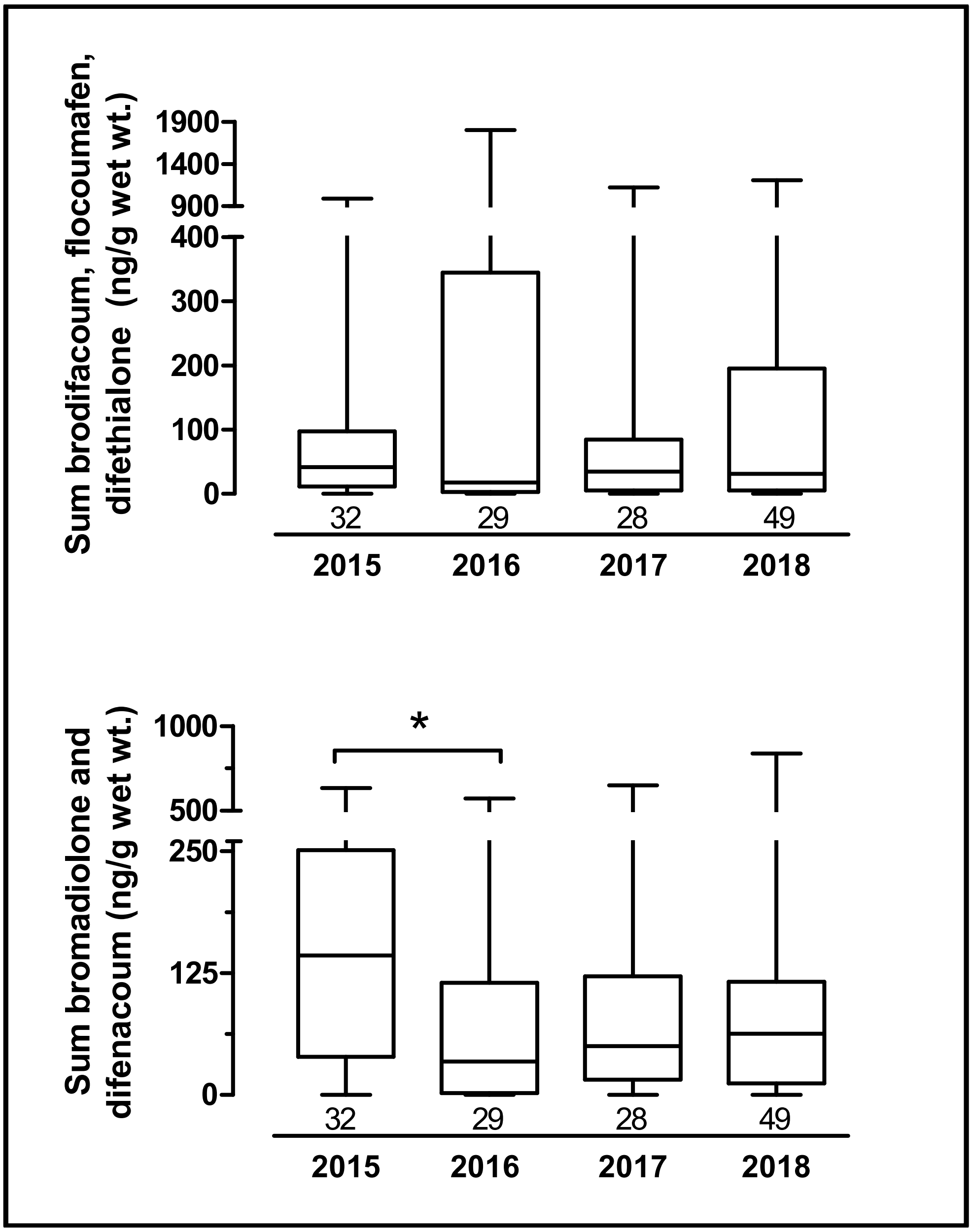

Figure 4. The liver sum concentrations of brodifacoum, flocoumafen and difethialone (top graph) and liver sum concentrations of bromadiolone and difenacoum (bottom graph) in all red kites found dead between 2015 and 2018.

Overall, our temporal analysis of exposure indicates that the overall proportion of kites exposed to SGARs was greater in 2017 and 2018 than in 2016 and 2015. This was almost completely due to an increase in the proportion of birds from Scotland that contained residues, with 8 out of 13 kites (62%) in 2015/16 with residues compared with 11 out of 12 (92%) in 2017/18. In contrast, almost all kites from England and Wales had detectable liver residues in all four years. There was no indication of a clear change in the magnitude or scale of exposure of red kites to brodifacoum, flocoumafen and difethialone over the period in which stewardship was introduced, despite these compounds becoming available for use both *in and around buildings* since 2016. In fact, across the whole four years, brodifacoum and difenacoum were the most prevalent compounds found in red kite livers (123/138 birds-89% for each compound) and bromadiolone was found in 75% of birds.

4.3 Trends in poisoning over time

The proportions of birds from both 2017 and 2018 for which SGARs were diagnosed as a contributory factor in their cause of death (Table 4) were lower than those observed in birds from 2015 and 2016. However, this difference between years was not statistically significant for red kites from England & Wales, or from Britain as a whole, irrespective of whether analysis was conducted as standard Chi Squared test (England & Wales: $\chi^2 = 3.555$, d.f.=3, P=0.31; Britain $\chi^2 = 3.286$, d.f.=3, P=0.35) or as a trend analysis (Chi Squared test for trends: England & Wales: $\chi^2 = 2.931$, d.f.=1, P=0.09; Britain: $\chi^2 = 2.248$, d.f.=1, P=0.13). 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 pairs of years (2015/2016 vs 2017/18) 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 than in 2015/16 for England & Wales (Fisher's Exact test: P=0.047) but not for Britain as a whole (Fisher's Exact test: P=0.098).

Table 4. 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 (%) red kites in which SGARs implicated/not implicated as a mortality factor							
	England & Wales				Great Britain			
Year	SGARs implicated	uncertain	SGARs not implicated[1]	total	SGARs implicated	uncertain	SGARs not implicated[1]	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
Total	28 (25%)	3	83	114	30 (22%)	3	105	138

[1]Red kites with no detected haemorrhaging, with haemorrhaging associated with trauma, and/or no-detected liver SGAR residue

Sum SGAR liver concentrations in poisoned birds did not differ significantly among years (Figure 2, Kruskal-Wallis=7.628, P=0.054). All poisoned birds 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, 72% (median value) of the ΣSGAR liver residue in poisoned birds was comprised of brodifacoum, flocoumafen and difethialone. This proportion differed between years (Figure 5; KW= 8.04; P=0.045) but post-hoc Dunn's test could not distinguish between pairs of years and there was no consistent pattern across years.

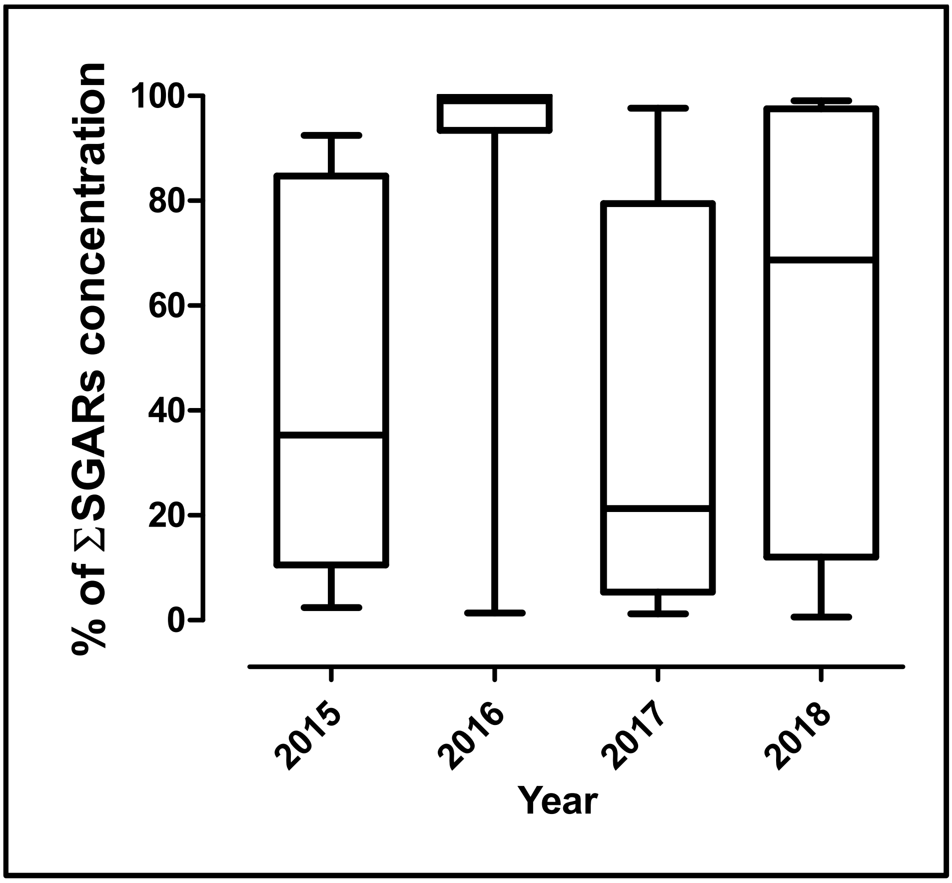

Figure 5. Box and Whiskers plot showing median, interquartile range and minimum/maximum range of percentage of (Σ)SGAR concentrations that consist of brodifacoum, flocoumafen and difethialone in red kites that died between 2015 and 2018.

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 4.6 fold higher than that of birds that had died from a variety of other causes. Only 5% of red kites that died from causes unrelated to SGARs had liver ΣSGAR residues >700 ng/g wet wt. 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), presumably at least in part reflecting inter-individual susceptibility to SGARs. There does not appear to be a clear diagnostic threshold for residues that is indicative of SGAR poisoning in red kites or, in fact, other species (Thomas *et al.* 2011). The probabilistic approaches to interpreting the significance of liver residues, as proposed by Thomas *et al.* (2011), may be a better means of understanding the likely impact of ΣSGAR residues in this range at least. The current dataset may also be useful in testing the validity of such probabilistic approaches.

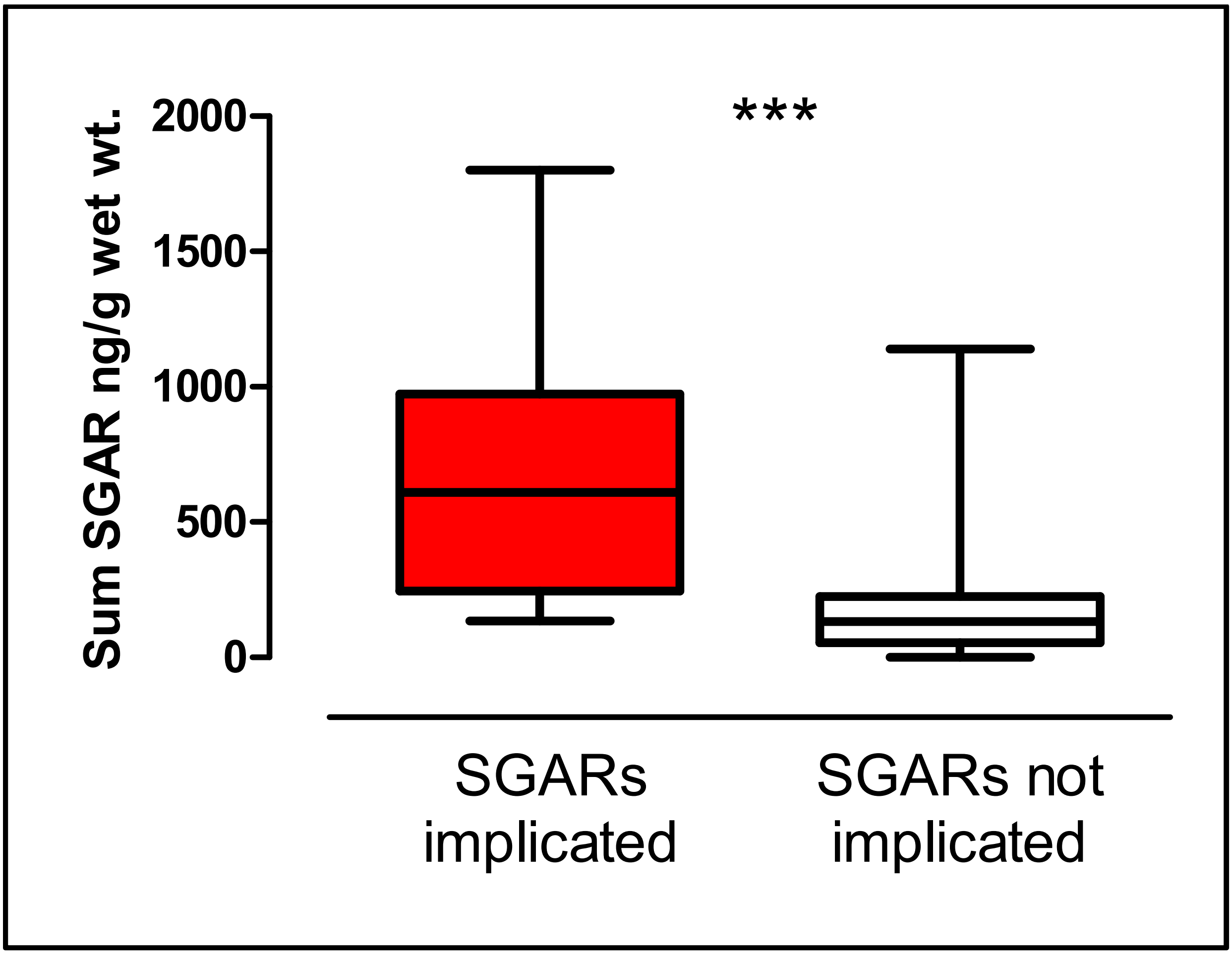

Figure 6. Box and Whiskers plot showing median, interquartile range and minimum/maximum range of sum (Σ)SGAR concentrations in red kites that died between 2015 and 2018, with haemorrhaging not associated with physical trauma (SGARs implicated as a contributory cause of mortality; n=30) and those that were diagnosed to have died from causes unrelated to SGARs (SGARs not implicated as a contributory cause of mortality n=98). The difference in median concentrations between two groups was statistically significant (Mann-Whitney U test, U=386, 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 feed directly on target prey species, such as the brown rat.

Of the 77 red kites from England, Wales and Scotland, found dead in 2017 and 2018 combined, all but one had been exposed to SGARs. In 13 (17%) cases, SGARs were implicated as a contributory cause of death. This prevalence does not include any other type of poisoning that may occur because of illegal use of other pesticides and through exposure to lead (Pain et al., 2007; Molenaar et al., 2017). None of the kites in which SGARs were considered a contributory cause of death and were submitted through the WIIS scheme (Fera Science incident codes 55/4, 18, 29, 41, 87; SASA incident code 18065– Table 2 & 3) were found to have been a consequence of abuse or misuse. They were all associated with "unspecified use" which means that the circumstances of exposure could not be determined. It is unknown whether exposure of the 7 individuals diagnosed as poisoned by PBMS/IoZ were the result of incidental secondary exposure or due to misuse or abuse.

With regards change over time (2015-2018), the proportion of red kites with detectable liver SGAR residues remains at >90% and, if anything, has increased since the introduction of stewardship. There is no evidence of any change between years in the magnitude of the residues accumulated. Potential change in the proportion of birds in which SGARs were diagnosed as a contributory mortality factor is more difficult to determine. There was no statistically significant difference between individual years in this proportion nor was there a statistically significant annual trend. However, annual sample sizes were small and when data were pooled into two year blocks (2017/18 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, but not for Britain as a whole. Overall, therefore, there was no clear-cut consistent picture of change over in exposure and effects over the last four years.

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 likely to be 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 also be a time lag between a change in usage practice and any consequent change in residue accumulation by red kites. It is, therefore, perhaps unsurprising that if stewardship does lead to a change in exposure and/or likelihood of poisoning in red kites, such a change is not manifest only after two years.

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 22% of the red kite cases examined across all four years. We do not know how SGAR-induced mortality affects the population dynamics of red kites but it is clear that any current effects do not prevent population growth as red kite populations in Britain have expanded and continue to do so (Harris et al., 2019).

6. Acknowledgements

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

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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.

This report was peer-reviewed by Richard Saunders, Dr Genevieve Madgwick, Dr. Alastair Burn and Dr Suzane Qassim of Natural England, and by the steering group of the Campaign for Responsible Rodenticide Use.

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

Table 2 Limits of detection (LoD; ng/g wet wt.) and percentage recovery for spikes used in analysis by PBMS (CEH), WIIS England & Wales (Fera Science) and WIIS Scotland (SASA) laboratories.

	CEH		Fera Science		SASA	
	LoD	% Spike recovery#	LoD	Typical % Spike recovery	LoD	Typical % Spike recovery*
Brodifacoum	1.6	65.8	0.8	63.5	3	85
Bromadiolone	1.6	65.2	0.8	94	3	86
Difenacoum	1.6	-	0.8	94	3	90
Flocoumafen	1.6	-	0.8	104.5	3	79
Difethialone	3.1	-	0.8	82.8	3	84

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