Second Generation Anticoagulant Rodenticides (SGARs) in Mammals and Predatory Birds

An interspecies comparison

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Contents

1 Executive Summary

Avian and mammalian predators and scavengers are widely exposed to second generation anticoagulant rodenticides (SGARs). Their exposure is likely to be due to feeding on SGAR contaminated prey (i.e., secondary exposure), which can often include non-target animals.

Although the use of the more acutely toxic SGARs, brodifacoum, flocoumafen, and difethialone, was historically more restricted than difenacoum and bromadiolone in the United Kingdom (UK), the conditions for use of the five SGARs were revised and modified in 2015, including an extension of the use of the three more acutely toxic SGARs in outdoor situations, which could subsequently increase secondary exposure of wildlife to some SGARs. The changes in authorisations for the use of rodenticides have been accompanied by the development and implementation of an industry-led stewardship regime, which is intended to coordinate and deliver best rodenticide practices and thereby minimise exposure of wildlife to SGARs. Recent monitoring outcomes from the stewardship regime showed contrasting trends among active ingredients, which might be attributable to the influence of either or both the regulation change of the active ingredients' use and the stewardship regime implementation. To better understand the impact of the regulation change and the effectiveness of the stewardship regime, further analysis of data from various monitoring programmes is needed.

Results from monitoring SGARs residues in wildlife, such as the magnitude or the temporal trends of SGARs residues, are not always the same between different species. The outcomes of monitoring depend on the species studied because animals have different feeding or hunting strategies and preferred habitats, which determine both the proportion of target/non-target species and/or SGAR contaminated food in their diet. In addition, the sensitivity to the SGAR toxicity varies among predator species.

To fill these knowledge gaps, the present report aims to (i) summarise the current information and compare the magnitude of SGAR residues in various mammals and predatory birds collected from across the UK, and (ii) assess temporal changes in SGAR residues in relation to the change in authorisation for active ingredient registration and the implementation of the stewardship regime.

Our results showed that SGARs residues were significantly higher in '*generalist and scavenger'* birds and mammals than in other animal groups in general, like '*bird feeding' birds*. Among the animals in the category '*generalist and scavenger species*', the red fox showed higher SGARs residues than birds. Bird feeding predators, such as the sparrowhawk and peregrine falcon, had lower SGARs residues than '*small mammal feeding*' and '*omnivore*' animals that showed intermediate levels of SGARs residues.

In most species, summed SGAR residue concentrations (ΣSGARs) have significantly increased over time. Three active ingredients, bromadiolone, difenacoum and brodifacoum, generally showed significant increasing trends over more than ten years. However, increases in ΣSGARs seem to be largely driven by increases in brodifacoum residues. In contrast, difenacoum residues in barn owls have recently shown a decreasing trend.

Both the occurrence and the magnitude of SGARs residues showed significant increases after the SGAR regulation change. Increases in the occurrence and the

magnitude of bromadiolone were observed in '*bird feeding' predators*, whereas increases in the occurrence and the magnitude of brodifacoum were observed in most of the species.

These outcomes would suggest that both changes in the usage of brodifacoum-containing products and the difference in ecological factors, such as the diet, could have led to such an increasing exposure pattern. Moreover, general increasing trends in summed SGARs residues might be modified between pre- and post-SGAR regulation change and the implementation of the stewardship regime. Nonetheless, exposure to SGARs has continuously increased in some species, such as the red kite and buzzard, over both the long term and since the introduction of the new measures in 2015/2016.

The present study has summarised data collected from various mammalian and avian species, mainly predatory birds and has assessed differences in SGARs residues between them and the influence of the regulation change for usage of different active ingredients and the implementation of the stewardship regime on trends. The main recommendations for further studies about monitoring of exposure to SGARs in predatory birds and mammals are to determine ecological factors significantly driving the uptake of SGARs and to integrate them into monitoring. Our findings will contribute to further approaches to monitoring of SGARs in predatory birds and mammals.

2 Introduction

2.1 Changes in SGAR authorisations and implementation of stewardship

Avian and mammalian predators and scavengers in rural Britain are widely exposed to second-generation anticoagulant rodenticides (SGARs) (Dowding et al., 2010; Hughes et al., 2013; McDonald et al., 1998; Newton et al., 1999; Ruiz-Suárez et al., 2014; Shore et al., 2003a, 2003b; Walker et al., 2014, 2008a, 2008b). Exposure of predator and scavenger birds is likely to include feeding on either or both 'target' and 'non-target' small mammal species contaminated by SGARs (Geduhn et al., 2016; Rattner et al., 2014; Shore et al., 2015). The target species for anticoagulant rodenticides are certain rodents, such as brown rat (*Rattus norvegicus*) and house mouse (*Mus musculus*), but non-target species, primarily wood mice (*Apodemus sylvaticus*) and bank voles (*Myodes glareolus*) in Britain, also feed on rodenticide bait they encounter (Brakes and Smith, 2005; Tosh et al., 2012). This exposure scenario may be most significant where SGARs are used around buildings and in open areas.

Currently, five SGARs are authorised in the United Kingdom (UK) - difenacoum, bromadiolone, brodifacoum, flocoumafen and difethialone. Among the five SGARs, only difenacoum and bromadiolone were historically authorised for the use '*indoor*', '*in and around buildings*' and in '*open areas*'[1] in Britain (Buckle, 2013). In contrast, the other three compounds (i.e., brodifacoum, flocoumafen, and difethialone) are more acutely toxic than difenacoum or bromadiolone (Erickson and Urban, 2004; Health and Safety Executive, 2015) and were restricted to '*indoor*' use only as a mitigation measure to reduce unintentional primary and secondary exposure and poisoning of non-target species. However, the restrictions on the use of all five SGARs in the UK were revised, and a new restriction for sale and professional use was step by step applied in 2015/2016 (Health and Safety Executive, 2015). For example, the 1st of June 2015 was set as the deadline for all existing authorisation holders to apply for outdoor use under stewardship (below), and the 1st of June 2016 was for the deadline for ceasing use of anticoagulant rodenticide products for professional outdoor use where this has not been applied for under stewardship. The use of brodifacoum, flocoumafen, and difethialone is now authorised both '*indoor*' and '*in and around*

[1] 'Indoor' is defined as a situation where the rodenticide is placed within a building or other enclosed structure and where the target rodent is living or feeding predominantly within that building or structure and behind closed doors. Note that if rodents living outside a building can move freely to where the rodenticide is laid within the building, then products restricted to use indoors should not be used.

'In and around buildings' is understood to include the entire building that is the subject of the treatment, or those areas of it that are infested, as well as the infested area around the building that needs to be treated to deal with the rodents that are moving into the building from outside.

'Open area' is an area that fit neither of the two preceding definitions and is an urban, suburban, or rural space that is not directly associated with a building. This includes farmland, parks, golf courses, as well as places for game rearing or outside food stores.

Sources:

Health and Safety Executive (2012). Environmental risk mitigation measures for second-generation anticoagulant rodenticides proposed by the UK.

European Commission (2009). Risk mitigation measures for anticoagulants used as rodenticides.

buildings', including '*sewers*' (based on '*UK authorised biocide products*' by Health and Safety Executive; https://www.hse.gov.uk/biocides/uk-authorised-biocidal-products.htm; data accessed on 27/06/2023)[2]. This change in authorisation might lead to an increase in the frequency or nature of use of the three more toxic products, especially where there are target rodents with resistance to bromadiolone and difenacoum (Jones et al., 2019). It is important to note that, although Health and Safety Executive has stated it would consider applications for open area use of products containing brodifacoum, difethialone and flocoumafen, industry has chosen not to make such applications. The industry has voluntarily agreed not to make such applications (Buckle et al., 2021).

A review of the available ecotoxicological data concluded that despite the differences in acute toxicity, the five SGARs were indistinguishable in terms of environmental toxicity (i.e., risks to non-target species) and that they should be treated in the same way in terms of authorisation in the UK (Health and Safety Executive, 2012). This led to a question about how the efficiency of the authorisations is assessed. The changes in authorisations for rodenticides have been accompanied by the development and implementation of an industry-led stewardship regime (http://www.thinkwildlife.org/stewardship-regime/). This stewardship regime commenced in 2015, intending to harmonise and simplify product labels, coordinate and deliver best practices in the use of rodenticides, and thereby minimise and reduce exposure of wildlife to SGARs and the risk of rodenticides to non-target species from current levels (Buckle et al., 2017).

Recent reports from the stewardship regime showed contrasting trends among active ingredients (Ozaki et al., 2022). For example, among barn owls (*Tyto alba*) having "low" levels of SGAR residue concentrations (i.e., liver concentration <100 ng/g ww), residues of bromadiolone and difenacoum were significantly lower in 2021 than in 2015/2016 (set up as the baseline years before the implementation of the stewardship regime). In contrast, residues of brodifacoum were significantly higher in 2021 than in 2015/2016. A similar trend was also observed in the red kite (Ozaki et al., 2024b, 2024a); Liver brodifacoum residues increased over years, whereas the proportion of birds with detectable bromadiolone and difenacoum residues remained at a similar level over the same period. These results may be from a possible shift of the usage or the practices among active ingredients since the change in rodenticide authorisations and the implementation of the stewardship regime and/or from different SGAR exposure or accumulation capacity among species. The variation in SGAR residues among species has been reported in other studies (López-Perea and Mateo, 2018), meanwhile exposure to SGARs depends on the prey composition of their diet (Geduhn et al., 2016). To elucidate changes in time trends among active ingredients, we need to compare monitoring of different predator species.

[2] The products containing bromadiolone and/or difenacoum could be used in open areas after the new restriction, but this situation has recently changed: their sale for use in open areas and waste dumps ceased on the 4th of July 2024, and the use in open areas and waste dumps was authorised until the 31st of December 2024. It is now illegal to use any SGAR product to treat a rodent infestation not associated with a building.

2.2 Aims of this report

Given the knowledge gaps related to monitoring summarised above, the present report aims to:

(i) Summarise the current information on the SGAR contamination of various mammals and predatory birds from across the UK and compare the magnitude of SGAR residues among species, and

(ii) Compare temporal changes in SGAR residues in predatory birds and mammals and assess whether and how trends changed following the change in SGAR authorisation and/or the start of the stewardship regime.

3 Methods

3.1 Interspecies comparison of exposure in predatory birds and mammals

We used several data sets on concentrations of SGAR residues in the livers of predatory birds and mammals found dead in the UK, primary as a result of collisions or starvation. There were three main sources of data:

(i) Data generated by the Predatory Birds Monitoring Scheme (PBMS) and associated projects, namely Life APEX (https://lifeapex.eu/) and European Raptor Biomoniotoring Facility (ERBF; https://erbfacility.eu/) from UK Centre for Ecology and Hydrology (UKCEH);

(ii) Data generated by the Wildlife Incidents Investigation Scheme (WIIS) for birds found dead in England and Wales, which had been compiled by Natural England (NE);

(iii) Data reported in published papers and reports, such as Broughton et al., (2022).

All data were passed to or gathered by UKCEH for this study. All of the three sources were used for establishing a summary of descriptive statistics for exposure of wildlife to SGARs. We used only datasets from the sources (i) and (ii) for the statistical tests: predatory birds and mammals submitted to WIIS and PBMS. We excluded from the study the barn owls found dead in 2015 onwards that have been collected specifically for the rodenticide stewardship monitoring purposes (Table 1). While sub-samples of bird and mammal tissues that have been analysed as part of WIIS are usually shared with the PBMS, these sub-samples were excluded from the PBMS monitoring analysis source group (i) in this report. For PBMS reports on red kite data from WIIS were combined with analysis of mutually exclusive samples.

Table 1. Summary of the data gathered and used in the present study.

Data	Source	Species	Usage in this report	Notes
(i) Data from PBMS & associated projects	PBMS Life APEX ERBF	Red kite Buzzard Tawny owl	Data were used for the statistical analysis	Including red kite samples from WIIS
(ii) Data from WIIS (England & Wales only)	WIIS	Peregrine falcon Sparrowhawk Barn owl# Tawny owl Buzzard Fox Badger Grey squirrel Hedgehog	Data were used for the statistical analysis	The data include incident number, date when animal(s) were found, concentrations of the five SGARs if detected, county and, if available geogrid reference, where the animal was found, and other details.
(iii) Data reported in papers & reports	PBMS Literature Literature Literature	Barn Owl Sparrowhawk Sparrowhawk Kestrel	Only descriptive statistical results reported are used in this study	Ozaki et al., 2022 Broughton et al., 2022 Hughes et al., 2013 Roos et al., 2021

Barn owls found dead in 2015 onwards, which have been collected for another contract, were excluded from the study.

The mean, standard error for the mean, median, interquartile range (i.e., 1st – 3rd quartiles), minimum and maximum of ΣSGARs were calculated (if dataset is available for this study) or taken from a reference as descriptive statistics. This summary was established by species and, if there are several datasets/references for the same species, by each dataset/reference. For some datasets/references, we particularly focussed on the period from 2017 to 2021 because they are the years since rodenticide stewardship has been implemented in an effort to reduce exposure in wildlife. For the red kite, we used only the dataset from the PBMS reports because this dataset includes the analyses of the red kites submitted to WIIS.

For statistical tests, predatory birds and mammals were classed into four feeding types: (i) '*bird feeding species*': animals predominantly feeding on live bird prey, (ii) '*small mammal feeding species*': animals predominantly feeding on live small mammal prey, (iii) '*Generalist feeding and scavenger species*', and (vi) '*omnivores*'. Significant differences between ΣSGARs among the feeding types or species was statistically checked by the non-parametric Kruskal-Wallis test. When there was a significant difference, we also carried out the Dunn's Kruskal-Wallis multiple comparisons.

3.2 Time trends in liver SGAR concentrations

Time trends of SGAR residues were separately analysed by species and, in cases where there were several datasets for the same species, also by dataset. The significance of correlation between the SGAR residue and year was checked by the Spearman rank correlation test. General time trends of the SGAR residues were traced on each scatter plot using the smoothing function LOWESS (Locally Weighted Scatterplot Smoothing; Cleveland, 1979) and then visually inspected. This analysis was conducted for ΣSGARs and for each active ingredient, except for flocoumafen and difethialone because only few samples showed a detectable level of flocoumafen and/or difethialone residues. For the other active ingredients (i.e., bromadiolone, difenacoum, and brodifacoum), residue values lower than the limit of quantification (<LoQ) were replaced with half of the minimum value of each active ingredient. This replacement of <LoQ does not affect the results of the Spearman rank correlation test.

In this report, we also focused on differences in exposure of animals to SGARs before and after the SGAR regulation change in 2015/2016, which was concurrent with the implementation of Stewardship regime. The proportion of individuals that had detectable SGAR residues was compared between pre- (up to 2015) and post-regulation change (from 2016) per species for each of the five active ingredients and ΣSGARs, by the Fisher exact test. The magnitude of SGAR residues between pre- and post-2015 years was also compared by the Wilcoxon Mann-Whitney test. The time trend of SGAR residues after the regulation change (from 2016) were also assessed.

4 Results

4.1 Magnitude and prevalence of exposure to SGARs in predatory birds and mammals

Table 2 presents the summary of descriptive statistics for ΣSGARs in the liver of predatory birds and mammals. Some datasets were described by two different monitoring periods, according to the stewardship period, to facilitate the comparison among species.

It is evident that a wide range of species were exposed to SGARs, including species that predate mainly on small mammals (e.g., barn owl; *Tyto alba*) or avian prey (e.g., sparrowhawk; *Accipiter nisus*) and more generalist predators and scavengers, such as the red fox (*Vulpes vulpes*), common buzzard (*Buteo buteo*), and red kite (*Milvus milvus*).

Some of the datasets did not show the same temporal range. Although a direct comparison is problematic for that reason, both the mean and median of concentrations were in general lowest in the predators of birds, such as sparrowhawks (means = 25 – 60 ng/g ww) and peregrine falcons (means = 13 ng/g ww), intermediate in the small mammal predators, such as barn owls (means = 63 –96 ng/g ww) and tawny owl (means = 86 – 109 ng/g ww) and highest in the generalist predators and scavengers, such as red kites (means = 190 – 200 ng/g ww). Nonetheless, the buzzard is a generalist predator but showed a similar range of SGAR residues to the small mammal predators (means = 59 – 105 ng/g ww). A statistical test with the available data justified this observation about the difference due to the feeding types: ΣSGARs were significantly highest in the generalist and scavenger birds and lowest in the birds predominantly feeding on avian prey (Kruskal Wallis test p-value <0.001; Fig. 2). (NB. Multiple comparisons between species are shown in section 4.2.2.)

The red fox, a generalist predator and scavenging mammal, showed a very high level of liver ΣSGARs (mean = 993 ng/g ww). The badger (*Meles meles*), grey squirrel (*Sciurus carolinensis*), and Hedgehog (*Erinaceus europaeus*) are the mammals that are most commonly reported by WIIS after the red fox. These three species are omnivorous mammals and had significantly lower ΣSGARs than red foxes (Kruskal Wallis test p-value <0.001; Fig. 2). Although there was no significant difference among the three species, liver SGAR concentrations in hedgehogs submitted to WIIS were generally lower (mean = 12.5; maximum = 61 ng/g ww) than the two others, and SGAR residues were detected in 55% of its samples (6/11). The majority of grey squirrels (56%; 9/16) showed SGAR residues <LoQ. However, a few samples had high SGAR residues (mean = 1175 ng/g ww), and four of 16 samples (25%) showed very high liver SGAR residues ranging between 811 and 6500 ng/g ww (Fig. 4). Summed SGAR concentrations in badgers were intermediate between the two other species (mean = 186; maximum = 2843 ng/g ww), with relatively high concentrations (>500 ng/g) in some samples.

***Table 2**. Summary of the descriptive statistics for liver Summed SGAR (ΣSGAR) concentrations (ng/g ww) in predatory bird and mammals. The period from 2017 to 2021 is particularly focused on for (i) barn owls from the CRRU annual report, (ii) red kites from the PBMS data, and (iii) buzzards from Life APEX and WIIS data. SEM: standard error for the mean; IQR: Interquartile range (i.e., 1st – 3rd quartiles), ND: none of the five SGAR was detected.*

Species *(Source of data)*	**Year range**	**N**	**Temporal trend**	**Mean (SEM)**	**Median (IQR)**	**Min**	**Max**	**Notes**
Predominantly bird feeding species:								
Peregrine falcon *(WIIS)*	2005-21	47	No	13 (3.5)	2.8 (ND-12)	ND	93	England & Wales
Sparrowhawk *(WIIS)*	2005-21	25	No	29 (6.6)	13 (ND-49)	ND	89	England & Wales
Sparrowhawk (Broughton et al., 2022)	1995-15	210	Some active ingredient and regional specific increases	25 (1.8)	16		157	
Sparrowhawk (Hughes et al., 2013)	2000-10	37	Not assessed	60 (16)	35	ND	-	Scotland only
Predominantly small mammal feeding species:								
Barn owl (Ozaki et al., 2022)	2006-21	1384	No	63 (4)	21 (5-66)	ND	1384	Data are not available for the analysis of this report
(only post-stewardship)	*2017-21*	*500*	*No*	*66 (5)*	*20 (4-78)*	*ND*	*711*	
Barn owl *(WIIS)*	2006-21	49	Increasing	96 (27)	6.9 (ND-79)	ND	840	England & Wales
Tawny owl *(European Raptor Biomonitoring Facility)*	2015-19	79	no	86 (17)	22 (2.8-73)	ND	793	Unpublished data
Tawny owl *(WIIS)*	2005-21	22	Increasing	109 (44)	17 (0.6-108)	ND	920	England & Wales
Kestrel (Roos et al., 2021)	2006-11	88	Not assessed	246 (31)	-	-	-	

Generalist feeding and scavenger species:								
Red kite *(PBMS)* *Pre-stewardship*	2015 &16	61		325 (50)	200 (52-446)	ND	1800	Including data from WIIS
Post-stewardship	2017-21	187	Some indication of increasing brodifacoum concentrations	298 (26)	190 (71-395)	ND	3224	Including data from WIIS
Buzzard *(Life APEX)*	2001-19	72	-	59 (11)	22 (7-88)	ND	474	Unpublished data
(only post-stewardship)	*2017-19*	*18*	-	*115 (26)*	*103.5 (14-187)*	*ND*	*417*	
Buzzard *(WIIS)*	2005-21	319	Increasing	105 (18)	31 (2.5-91)	ND	4433	England & Wales
(only post-stewardship)	*2017-21*	*142*	*Increasing*	*165 (36)*	*44 (9-171)*	*ND*	*4433*	
Fox *(WIIS)*	2006-21	127	Increasing	993 (230)	231 (35-1366)	ND	21140	England & Wales
Omnivores								
Badger *(WIIS)*	2006-21	55	no	186 (73)	80 (ND-139)	-	2843	England & Wales
(only post-stewardship)	*2017-21*	*15*	*no*	*203 (169)*	*7.9 (ND-77)*	-	*2563*	
Grey Squirrel *(WIIS)*	2006-21	16	no	1175 (602)	ND (ND-262)	-	6500	England & Wales
Hedgehog *(WIIS)*	2007-21	11	no	12.5 (6.1)	0.9 (ND-21.5)	-	61	England & Wales

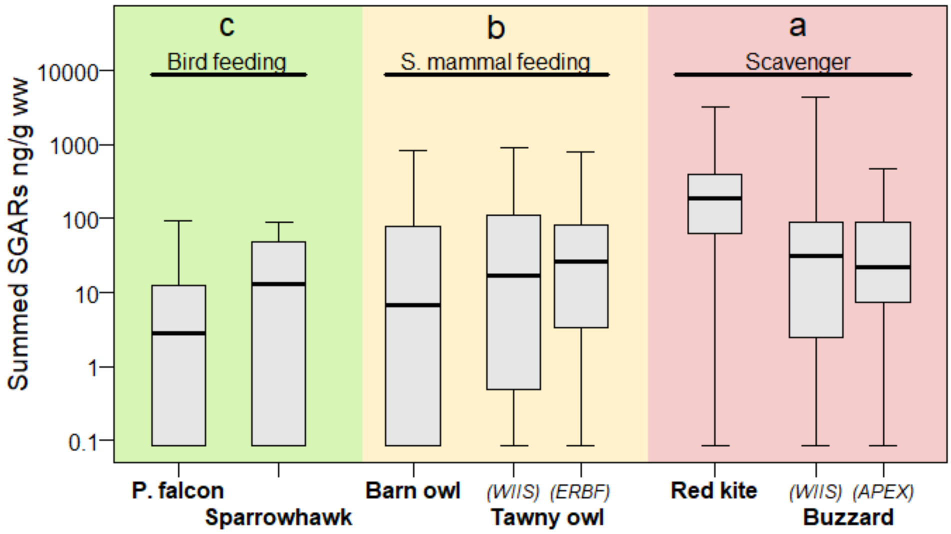


Figure 2*. Box and Whisker plots showing median, interquartile range and minimum/maximum range of summed SGAR concentrations (ΣSGARs) in predatory birds. Significant differences between feeding types (i.e., predominantly bird or small mammal feeding, or scavenger birds) are indicated by different letters (multiple comparison p-value <0.05). Non-detected SGAR values are replaced by half of the minimum ΣSGARs for logarithmically transformed ΣSGARs.*

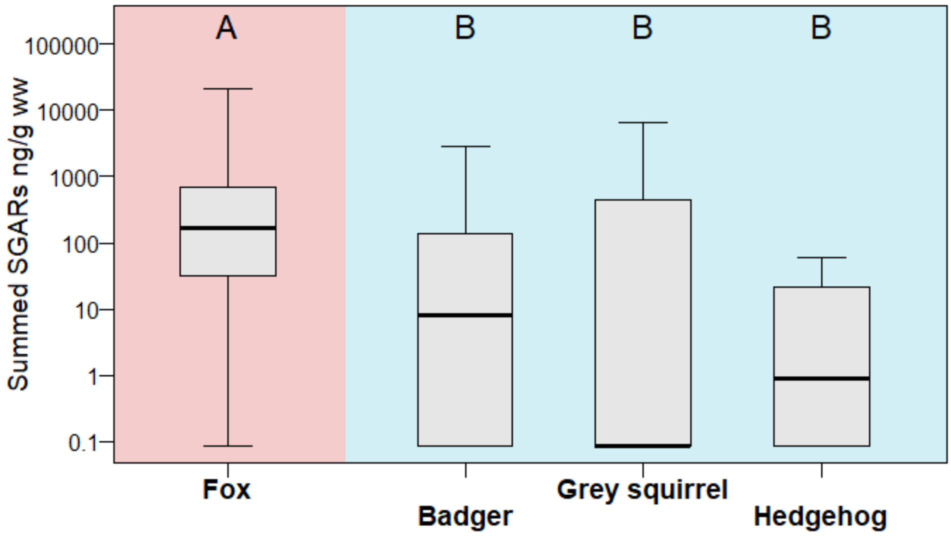


Figure 3*. Box and Whisker plots showing median, interquartile range and minimum/maximum range of summed SGAR concentrations (ΣSGARs) in generalist and scavenger (fox) and omnivore (badger, grey squirrel, and Hedgehog) mammals. Significant differences between species are indicated by different letters (multiple comparison p-value <0.05). Non-detected SGAR values are replaced by half of the minimum ΣSGARs for logarithmically transforming ΣSGARs.*

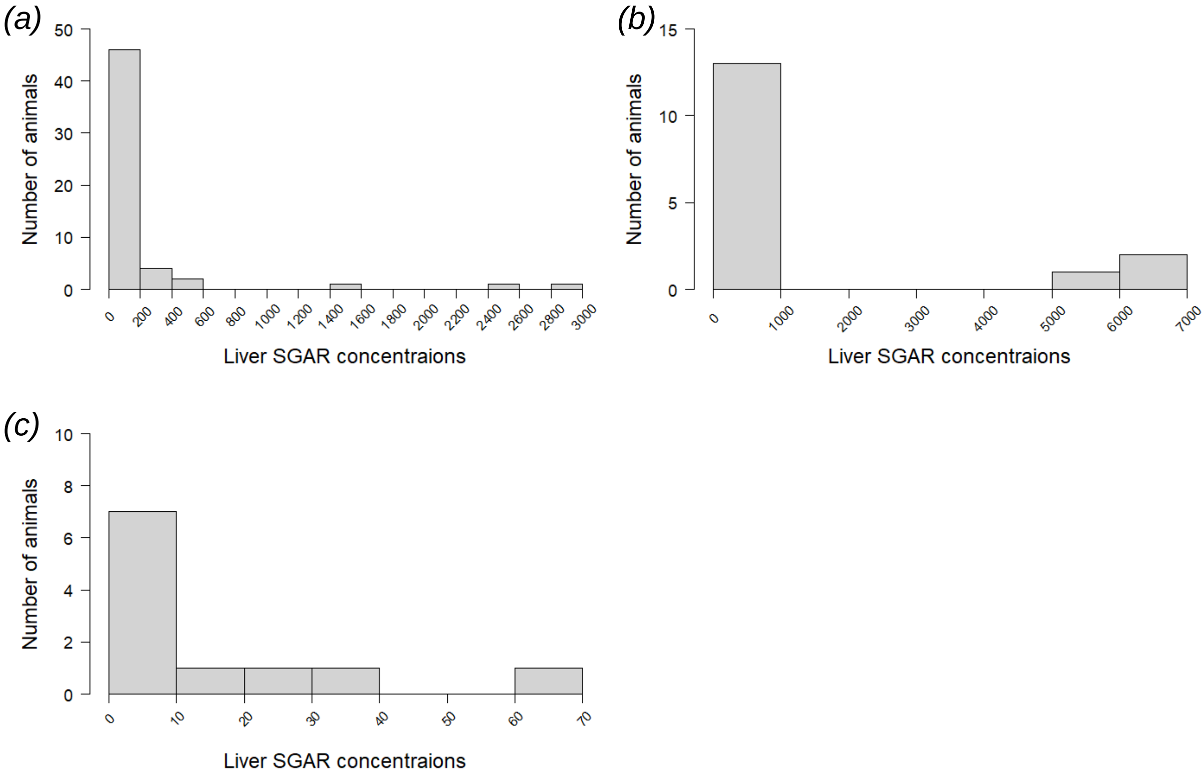


Figure 4*. Histograms for the number of (a) badgers, (b) squirrels, and (c) hedgehogs submitted to the WIIS by summed SGAR concentrations (ΣSGARs) in the liver.*

4.2 Time trends of SGAR residues in predatory birds and mammals

4.2.1 General trends:

Summed SGARs in predatory animals: The magnitude of ΣSGARs in general significantly increased during the whole monitoring period (Fig. 5a; Table3). Only ΣSGARs in tawny owls collected by ERBF did not show a significant temporal trend. Exposure of bird feeding predators was well correlated with years compared to small mammal feeding or generalist and scavengers because the former group showed a higher Spearman's correlation coefficient (r_s) than the latter. For example, r_s was 0.51 and 0.73 for peregrine falcons and sparrowhawks, respectively, whereas r_s was 0.15 and 0.24 for red kites and foxes. Summed SGAR concentrations in buzzards also significantly increased over year. A significant increase was observed in ΣSGARs in buzzards submitted to the PBMS between 2001 and 2019 (r_s= 0.41) and in buzzards submitted to the WIIS scheme between 2006 and 2021 (r_s= 0.32).

Although significantly increasing through the monitoring period, a visual inspection suggests that time trends of exposure to ΣSGARs may have changed in 2012 – 2017. During the period 2015 – 2017, increasing trends of sparrowhawks, peregrine falcons, and buzzards submitted to WIIS became stagnant. WIIS buzzards however showed a significant increase in ΣSGARs after the regulation change (r_s= 0.23) (Table 3).

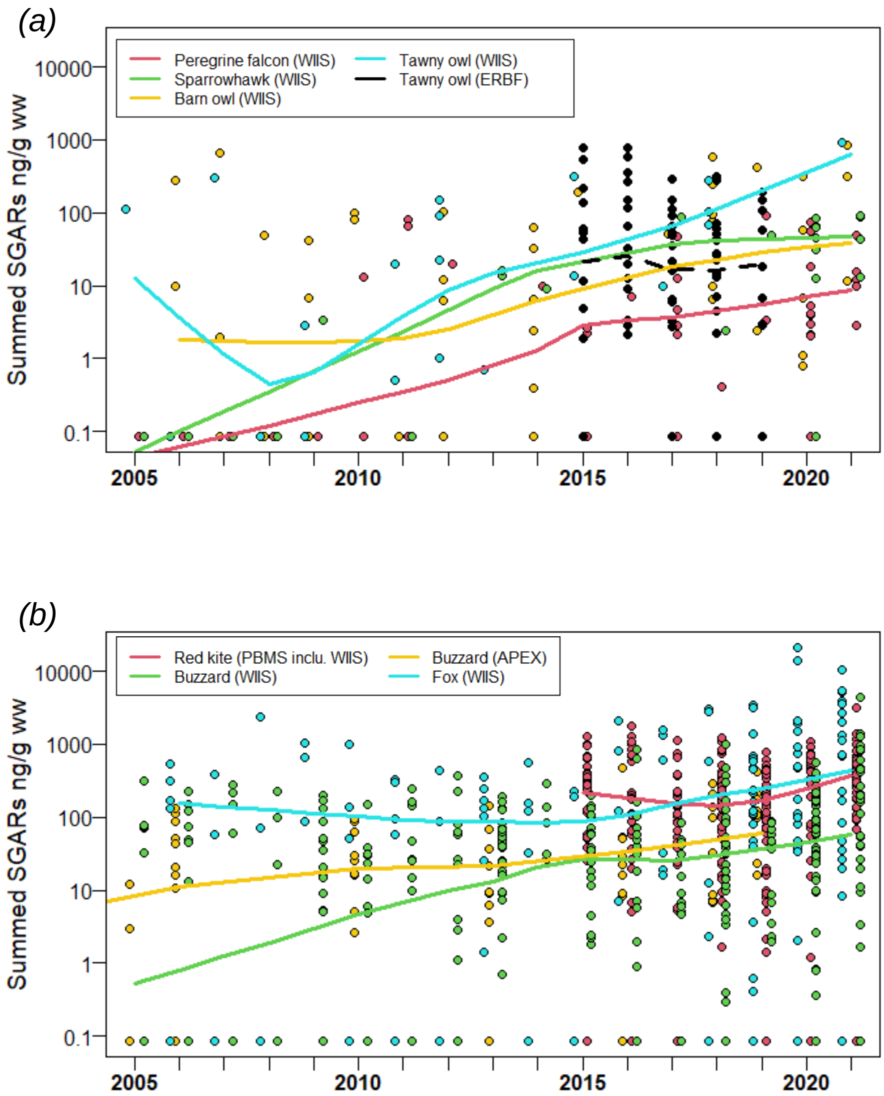
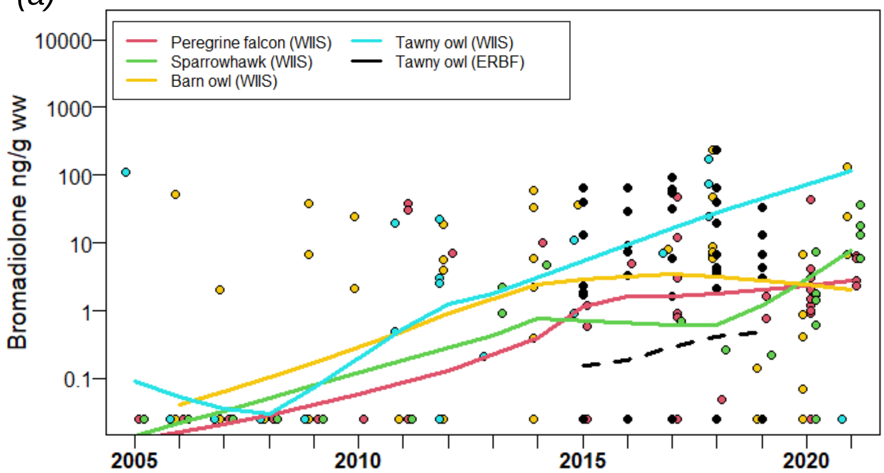


***Figure 5**. Time trend of ΣSGARs over time (a) in bird or small mammal feeding predators (i.e., peregrine falcons, sparrowhawks, barn owls, and tawny owls) and (b) in generalist and scavengers (i.e., red kites, buzzards, and foxes). Solid lines represent significant time trends by the Spearman's correlation test (p-value <0.05), while dotted lines represent non-significant trends.*

Bromadiolone in predatory animals: Time trends of bromadiolone were more contrasted between the two types of feeding than ΣSGARs (Fig. 6; Table 3). Except for ERBF tawny owls, all birds- and small mammals feeding species showed a significant increasing time trend with relatively high r_S: peregrine falcons (r_S= 0.51), sparrowhawks (r_S= 0.74), barn owls (r_S= 0.40), WIIS tawny owls (r_S= 0.46). In contrast, only foxes showed a significant increasing trend (r_S= 0.20) among scavengers.

A visual inspection suggests that time trends of bromadiolone concentrations also changed in years around 2015. Concentrations in peregrine falcons and barn owls increased up to 2014, after which concentrations levelled off. Sparrowhawks showed an increasing trend after the regulation change (r_S = 0.71; Table 3). However, the magnitude of bromadiolone seems to decrease, or at least level off during 2014 – 2018 and then increased.

(a)



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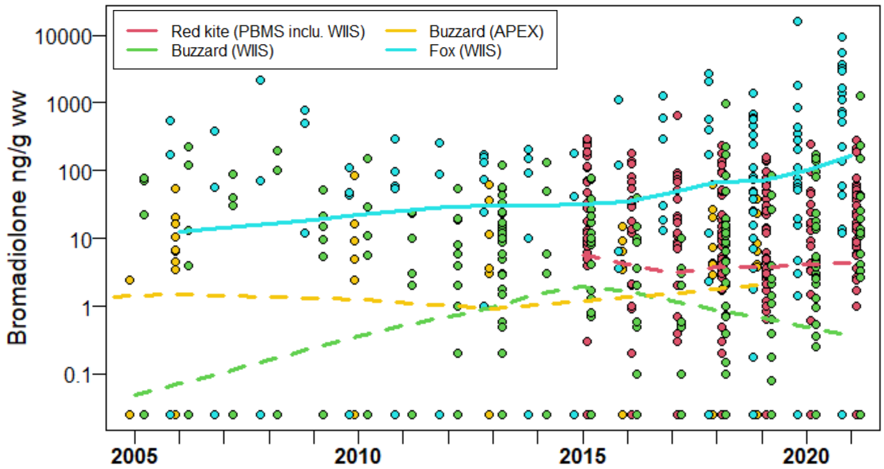
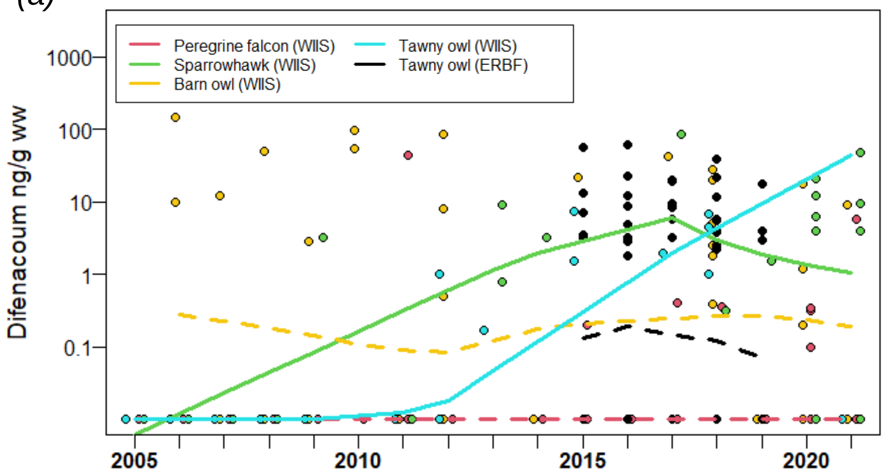


Figure 6*. Time trend of bromadiolone over time (a) in bird or small mammal feeding predators (i.e., peregrine falcons, sparrowhawks, barn owls, and tawny owls) and (b) in generalist and scavengers (i.e., red kites, buzzards, and foxes). Solid lines represent significant time trends by the Spearman's correlation test (p-value <0.05), while dotted lines represent non-significant trends.*

Difenacoum in predatory animals: The magnitude of difenacoum residues significantly increased in sparrowhawks (r_s= 0.48), tawny owls (r_s= 0.70), and buzzards submitted to WIIS (r_s= 0.29) and APEX (r_s= 0.17) (Fig. 7). However, the magnitude of difenacoum residues in buzzards and sparrowhawks did not significantly increased since 2016, and difenacoum residues in barn owls significantly decreased after the regulation change (Table 3). In contrast, the magnitude of difenacoum in WIIS tawny owls sharply increased since 2011 or 2012.

(a)



(b)

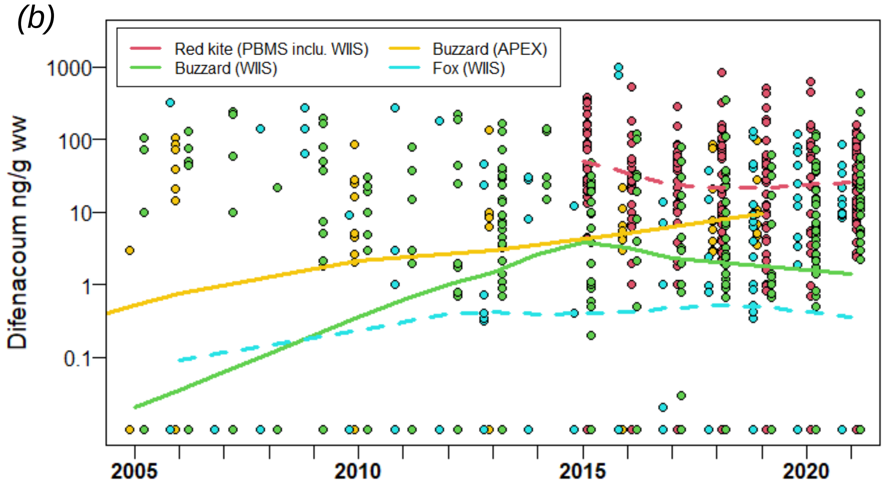


***Figure 7**. Time trend of difenacoum over time (a) in bird or small mammal feeding predators (i.e., peregrine falcons, sparrowhawks, barn owls, and tawny owls) and (b) in generalist and scavengers (i.e., red kites, buzzards, and foxes). Solid lines represent significant time trends by the Spearman's correlation test (p-value <0.05), while dotted lines represent non-significant trends.*

Brodifacoum in predatory animals: The magnitude of brodifacoum has significantly increased in predatory birds and mammals, except for ERBF tawny owls (Fig. 8; Table 3). The Spearman's correlation coefficient r_s for APEX buzzards was 0.27, but the other species showed relatively high r_s, around 0.40 – 0.60.

A visual inspection of these relationships suggests that changes took place in increasing trend rates over the monitoring period of some species. The magnitude of brodifacoum residues in WIIS buzzards increased constantly, whereas the increase trends in brodifacoum residues in the buzzards as part of the Life APEX project showed a sharp increase since around 2014. The trend of foxes was relatively constant, but their trend after 2016 was not significant (Table 3). The trends of brodifacoum residues in peregrine falcons, sparrowhawks, and barn owls after the regulation change were not significant.

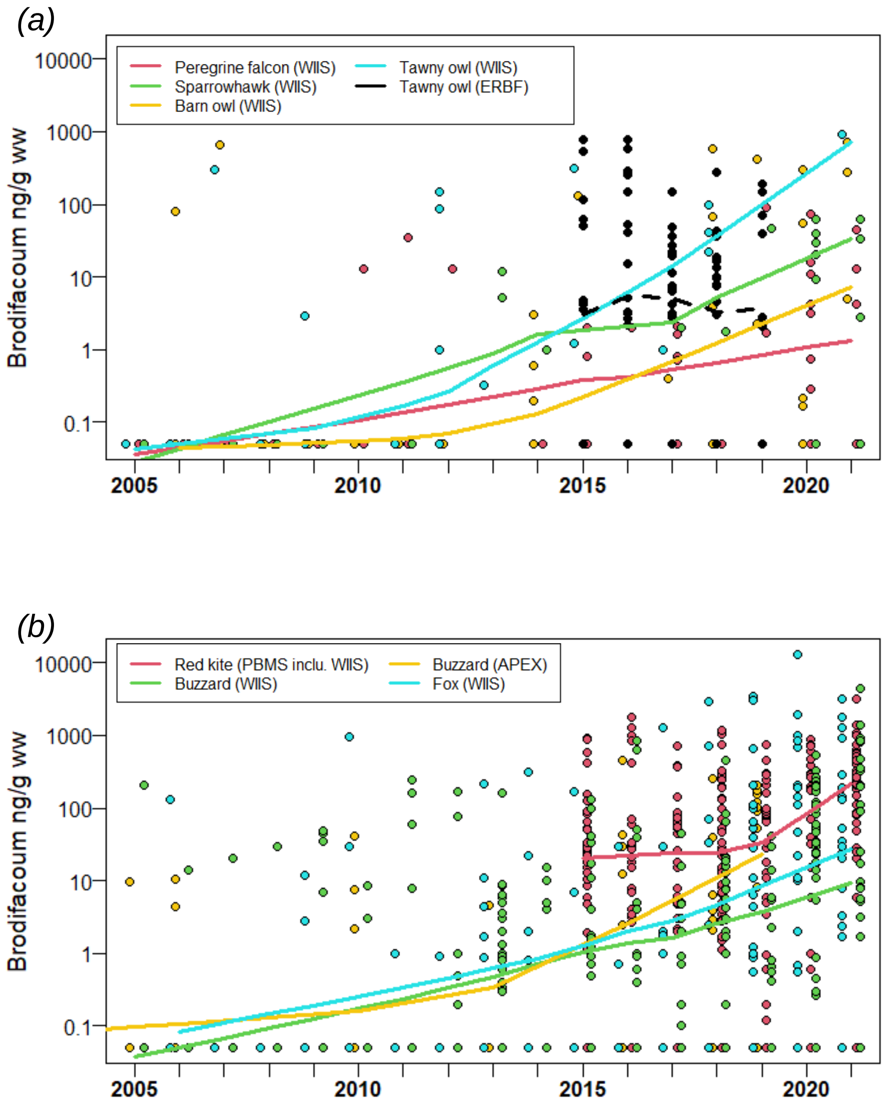


***Figure 8**. Time trend of brodifacoum over time (a) in bird or small mammal feeding predators (i.e., peregrine falcons, sparrowhawks, barn owls, and tawny owls) and (b) in generalist and scavengers (i.e., red kites, buzzards, and foxes). Solid lines represent significant time trends by the Spearman's correlation test (p-value <0.05), while dotted lines represent non-significant trends.*

SGARs in omnivorous mammals: There was no evident time trend in ΣSGARs, bromadiolone, and difenacoum residues in badgers, squirrels, or hedgehogs (p-value >0.05). However, the magnitude of brodifacoum residues significantly increased over years in both badger (r_s= 0.40) and in hedgehogs (r_s= 0.68) (Fig.9; Table 3). No significant trend was observed after the regulation change (Table 3).

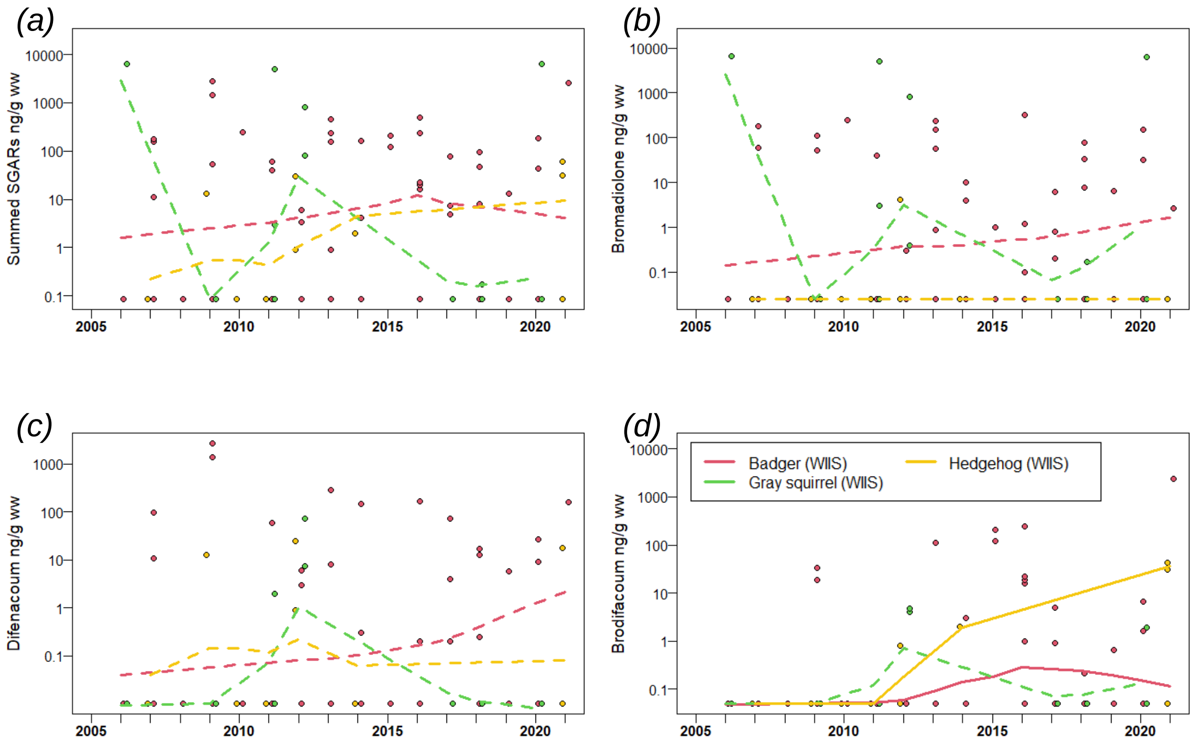


***Figure 9**. Time trend of ΣSGARs (a), bromadiolone (b), difenacoum (c), and brodifacoum (d) over time in badgers, squirrels, or hedgehogs. Solid lines represent significant time trends by the Spearman's correlation test (p-value <0.05), while dotted lines represent non-significant trends.*

Table 3. *Results of the Spearman's correlation coefficient and its p-value for the relationship between SGAR residues in the liver of predatory birds and mammals (i.e., ΣSGARs, bromadiolone, difenacoum, and brodifacoum) and monitoring years for whole monitoring period and after the regulation change (i.e., after 2016). Significant correlations (p-value <0.05) are highlighted in bold letters. Correlation analysis after the regulation change was not carried out for data with small number of specimens for after the regulation change (e.g., Tawny owls from WIIS).*

	ΣSGARs				Bromadiolone				Difenacoum				Brodifacoum			
	Whole period		2016-		Whole period		2016-		Whole period		2016-		Whole period		2016-	
	r	*p-value*	*r*	*p-value*	*r*	*p-value*	*r*	*p-value*	*r*	*p-value*	*r*	*p-value*	*r*	*p-value*	*r*	*p-value*
Peregrine falcon	n = 47		n = 26													
(2005 - 2021)	**0.51**	**<0.001**	0.15	0.45	**0.51**	**<0.001**	0.14	0.49	0.22	0.15	0.04	0.83	**0.42**	**0.003**	0.14	0.50
Sparrowhawk	n = 25		n = 14													
(2005 - 2021)	**0.74**	**<0.001**	0.18	0.55	**0.75**	**<0.001**	**0.71**	**0.004**	**0.49**	**0.01**	0.00	0.98	**0.59**	**0.002**	0.11	0.71
Barn owl	n = 31		n = 18													
(2006 - 2021)	**0.36**	**0.01**	-0.02	0.93	**0.40**	**<0.001**	-0.18	0.47	0.00	0.95	**-0.56**	**0.02**	**0.54**	**<0.001**	0.29	0.24
Tawny owl (WIIS)	n = 49		n = 5													
(2005 - 2021)	**0.49**	**0.02**			**0.46**	**0.03**			**0.70**	**<0.001**			**0.61**	**0.003**		
Tawny owl (ERBF)	n = 79		n = 79													
(2015 - 2019)	-0.06	0.59	-0.15	0.23	0.12	0.29	0.11	0.40	-0.05	0.64	-0.15	0.24	-0.02	0.83	-0.10	0.43
Red kite	n = 248		n = 216													
(2015 - 2021)	**0.15**	**0.02**	**0.21**	**0.001**	-0.03	0.60	0.12	0.47	-0.09	0.13	0.02	0.79	**0.27**	**<0.001**	**0.29**	**<0.001**
Buzzard (WIIS)	n = 319		n = 157													
(2005 - 2021)	**0.32**	**<0.001**	**0.23**	**0.006**	0.10	0.07	-0.11	0.16	**0.17**	**0.002**	0.00	0.96	**0.46**	**<0.001**	**0.19**	**0.02**
Buzzard (APEX)	n = 72		n = 27													
(2001 - 2019)	**0.41**	**<0.001**	0.29	0.24	0.02	0.89	0.11	0.58	**0.29**	**0.01**	0.19	0.34	**0.56**	**<0.001**	**0.38**	**0.05**
Fox	n = 127		n = 83													
(2006 - 2021)	**0.24**	**0.005**	0.13	0.23	**0.20**	**0.02**	0.15	0.19	0.03	0.77	-0.05	0.64	**0.42**	**<0.001**	0.19	0.08
Badger	n = 55		n = 21													
(2006 - 2021)	0.11	0.43	-0.12	0.60	0.14	0.29	0.11	0.64	0.17	0.21	0.18	0.44	**0.40**	**0.002**	-0.25	0.27
Gray squirrel	n = 16		n = 6													
(2006 - 2021)	-0.05	0.83			-0.07	0.79			-0.02	0.95			0.29	0.27		
Hedgehog	n = 11		n = 3													
(2007 - 2021)	0.5	0.11			0.05	0.88			0.10	0.77			**0.68**	**0.02**		

4.2.2 Pre- and post-regulation change

The proportion of samples with detected SGAR residues significantly increased after 2015 in many predatory birds and mammals (Table 4). The proportions particularly increased in bird-feeding birds, such as peregrine falcons (from 33 to 88%; Fisher text p-value <0.001), sparrowhawks (from 36 to 93%; p-value <0.01), and barn owls (from 58 to 100%; p-value <0.001). The proportion of samples with detected ΣSGARs also significantly increased in buzzards submitted to WIIS and foxes (from 70 to 91% and from 75 to 93%, respectively), although their proportion was also high before 2015. Similar trends were observed in the proportion of samples with detected bromadiolone residues. In contrast, the proportion of samples with detected difenacoum residues significantly increased in tawny owls submitted to WIIS (from 24 to 80%; p-value <0.05), buzzards submitted to WIIS (from 58 to 73%; p-value <0.01), and buzzards submitted to the Life APEX project (from 58 to 96%; p-value <0.001). Of the five SGARs, brodifacoum showed significant increases in the proportion of samples with detected in the most species: peregrine falcons, sparrowhawks, barn owls, buzzards, foxes, and badgers. The proportion of these species were around 20 – 40% before 2015 but increased to 60 – 80% after 2015. No significant change was observed in the proportion of samples with detected flocoumafen and difethialone residues. The proportion of samples with detected flocoumafen residues were low (<7%) for both before and after 2015. The proportion of samples with detected difethialone residues was also low (<10%) for both before and after 2015, except for red kites (pre-2015: 28%: post-2015: 15%) and foxes in post-2015 years (17%).

Differences in liver SGAR concentrations pre- and post-2015 might be reflected by these differences in proportions of samples with detected SGAR residues (Fig. 10). Summed SGAR concentrations significantly increased from pre- to post-2015 years in peregrine falcons, sparrowhawks, barn owls, buzzards, and foxes. However, the active ingredients causing these increases in ΣSGARs differ among species. In bird feeding predators (i.e., peregrine falcons and sparrowhawks) and barn owls, the mean liver concentrations of both bromadiolone and brodifacoum significantly increased from pre- to post-2015 years. Difenacoum concentrations in sparrowhawks also significantly differed. Among the scavengers (i.e., red kites, buzzards, and foxes), bromadiolone concentrations showed a significant increase from pre- to post-2015 years only in foxes. Brodifacoum concentrations significantly increased in buzzards and foxes but showed a significant decrease in red kites, where liver brodifacoum residues were higher before 2015. Difenacoum residues in red kites significantly decreased from pre- to post-2015. In tawny owls submitted to WIIS, both difenacoum and brodifacoum residues significantly increased from pre- to post-2015 years. There was also a significant increase in brodifacoum residues in badgers from pre- to post-2015 years.

Like ΣSGARs, the residues in the liver of the three active ingredients were highest in certain scavenger species and tended to be lowest in predominantly bird feeding predators.

Table 4. *Proportion of predatory birds and mammals with detectable liver SGAR residues (i.e., ΣSGARs, bromadiolone, difenacoum, brodifacoum, flocoumafen, and difethialone) before and after the regulation change (i.e., – 2015 and 2016 –, respectively). The number of birds with detected liver SGAR residues and the total number are indicated below each proportion. A significant difference in the proportions between pre- and post-change is indicated by bold letters and asterisks (Fisher exact test p-value; *: <0.05, **: <0.01, ***:<0.001).*

Species	**ΣSGARs**			**Bromadiolone**			**Difenacoum**			**Brodifacoum**			**Flocoumafen**		**Difethialone**	
	pre-	*post-*		*pre-*	*post-*		*pre-*	*post-*		*pre-*	*post-*		*pre-*	*post-*	*pre-*	*post-*
Peregrine	**33%**	**88%**	***	**29%**	**85%**	***	10%	23%		**24%**	**65%**	**	0%	4%	0%	4%
(from WIIS)	*7/21*	*23/26*		*6/21*	*22/26*		*2/21*	*6/26*		*5/21*	*17/26*		*0/21*	*1/26*	*0/21*	*1/26*
Sparrowhawk	**36%**	**93%**	**	**27%**	**86%**	**	36%	71%		**27%**	**79%**	*	0%	0%	0%	14%
(from WIIS)	*4/11*	*13/14*		*3/11*	*12/14*		*4/11*	*10/14*		*3/11*	*11/14*		*0/11*	*0/14*	*0/11*	*2/14*
Barn owl	**58%**	**100%**	**	**48%**	**89%**	**	35%	61%		**19%**	**78%**	***	0%	0%	0%	0%
(from WIIS)	*18/31*	*18/18*		*15/31*	*16/18*		*11/31*	*11/18*		*6/31*	*14/18*		*0/31*	*0/18*	*0/31*	*0/18*
Tawny owl	71%	100%		53%	80%		**24%**	**80%**	*	47%	100%		0%	0%	0%	0%
(from WIIS)	*12/17*	*5/5*		*9/17*	*4/5*		*4/17*	*4/5*		*8/17*	*5/5*		*0/17*	*0/5*	*0/17*	*0/5*
Tawny owl	75%	89%		38%	43%		38%	44%		56%	75%		6%	2%	6%	10%
(from ERBF)	*12/16*	*56/63*		*6/16*	*27/63*		*6/16*	*28/63*		*9/16*	*47/63*		*1/16*	*1/63*	*1/16*	*6/63*
Red kite	91%	94%		78%	76%		91%	86%		84%	87%		0%	1%	28%	15%
(from PBMS)	*29/32*	*204/216*		*25/32*	*164/216*		*29/32*	*186/216*		*27/32*	*188/216*		*0/32*	*2/216*	*9/32*	*32/216*
Buzzard	**70%**	**91%**	***	52%	61%		**58%**	**73%**	**	**36%**	**71%**	***	1%	4%	1%	4%
(from WIIS)	*113/162*	*143/157*		*84/162*	*96/157*		*94/162*	*115/157*		*59/162*	*112/157*		*2/162*	*6/157*	*2/162*	*7/157*
Buzzard	80%	96%		62%	70%		**58%**	**96%**	***	**22%**	**74%**	***	0%	7%	2%	7%
(from APEX)	*36/45*	*26/27*		*28/45*	*19/27*		*26/45*	*26/27*		*10/45*	*20/27*		*0/45*	*2/27*	*1/45*	*2/27*
Fox	**75%**	**93%**	*	**70%**	**88%**	*	48%	57%		**41%**	**77%**	***	0%	1%	5%	17%
(from WIIS)	*33/44*	*77/83*		*31/44*	*73/83*		*21/44*	*47/83*		*18/44*	*64/83*		*0/44*	*1/83*	*2/44*	*14/83*
Badger	56%	71%		41%	62%		32%	57%		**18%**	**62%**	**	0%	0%	0%	5%
(from WIIS)	*19/34*	*15/21*		*14/34*	*13/21*		*11/34*	*12/21*		*6/34*	*13/21*		*0/34*	*0/21*	*0/34*	*1/21*

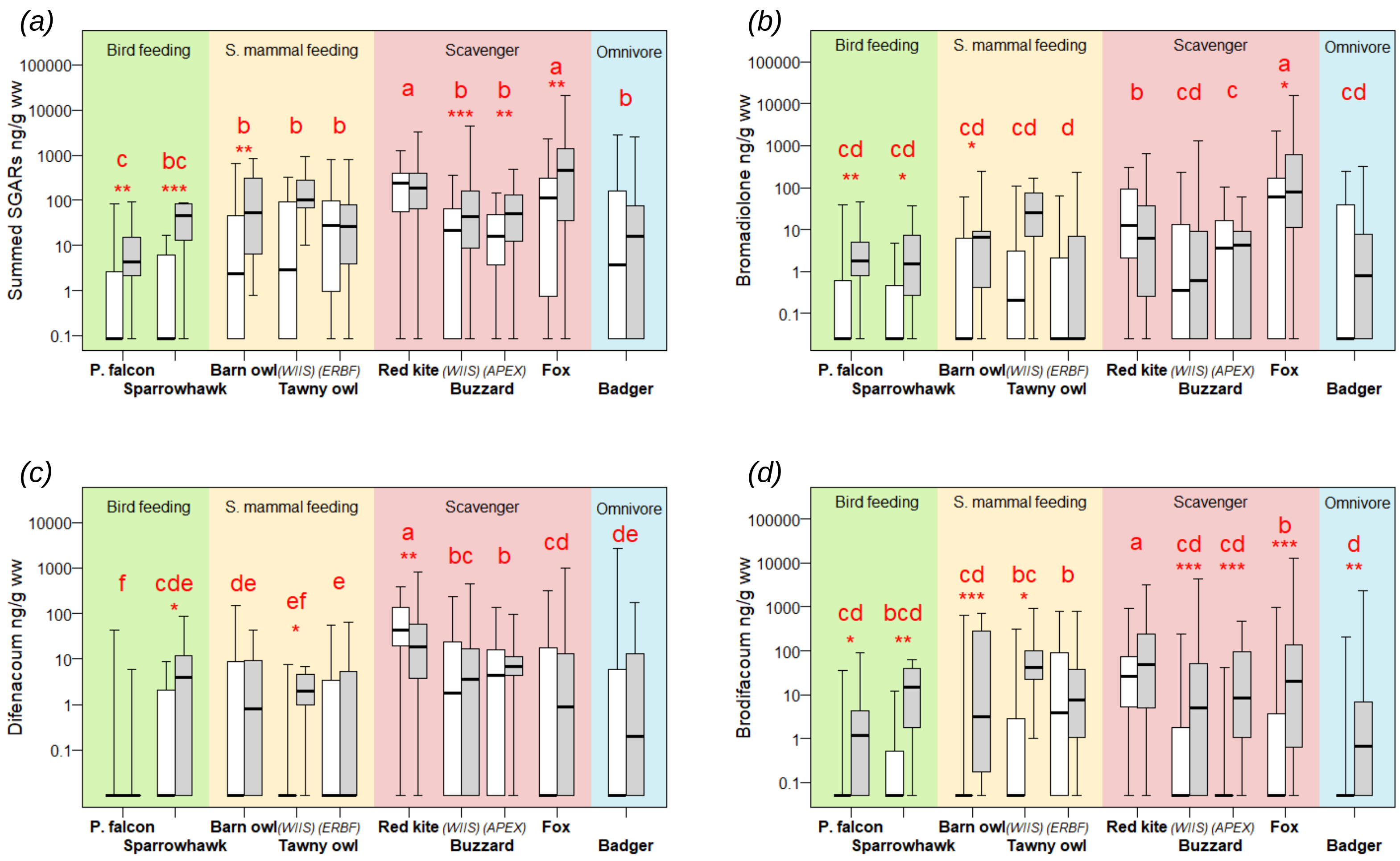


Figure 10*. Box and Whisker plots showing median, interquartile range and minimum/maximum range of (a) ΣSGARs, (b) bromadiolone, (c) difenacoum, and (d) brodifacoum before 2015 (white boxes) and after 2015(grey boxes) in predatory birds and mammals. Significant differences between pre- and post-2015 for each species are indicated by asterisks (Wilcoxon test p-value; *: <0.05, **: <0.01, ***:<0.001). Significant differences between species are indicated by different letters (multiple comparison p-value <0.05). SGAR values <LoQ are replaced with half of the minimum ΣSGARs for logarithmically transformed ΣSGARs.*

Discussion

4.3 Magnitude, prevalence, and time trend of SGARs in predatory birds and mammals

This report and previously published results indicate that a broad range of species are exposed to SGARs, with a high proportion of individuals accumulating a detectable level in their livers. In terms of the magnitude, predatory mammals, such as the red fox, tend to accumulate higher concentrations than predatory birds. Of the predatory birds, the highest level of concentrations was detected in the red kite, followed by the common buzzard, i.e., generalists and scavenging species. Small mammal feeding species, such as the barn owl, kestrel, and tawny owl, tended to have intermediate concentrations in general. Bird feeding species, the peregrine falcon and sparrowhawk, had lower concentrations than the others.

All these results indicate that scavengers are relatively highly exposed to SGARs. Among the scavengers, the magnitude of SGAR residues was higher in mammals (i.e., the red fox) than in birds, although the proportion of samples with detected SGARs were at a similar level among these scavengers. These findings concur with the review of López-Perea and Mateo (2018), which showed similar occurrence of SGARs but higher SGAR concentrations in generalist mammals compared to generalist birds. The red fox is a scavenger that can live in the urban habitat. Geduhn et al. (2015) observed a positive correlation between rodenticide residue occurrence and the percentage of urban areas, which suggests that urban habitats might provide SGAR-contaminated food. The reason for this correlation remains unclear but may be owing to the difference in the diet, wider opportunities to encounter SGAR contaminated rodents or their carcasses, and/or higher probability of rodenticide resistant rodents in urban environments.

For mammals, omnivores and insectivores, such as the badger, grey squirrel, and hedgehog, tended to have lower liver ΣSGAR residues than the scavenger mammal red fox. Dowding et al. (2010) reported that 22.5% of hedgehogs collected across the UK between 2004 and 2006 were contaminated with one or more SGARs when the liver was analysed by high performance liquid chromatography coupled with fluorescence detection (HPLC). They noted that the SGAR detection rate increased up to 69% with liquid-chromatography mass spectrometry (LCMS). Compared to the rates observed in this study, these SGAR detection rates in hedgehogs are at a lower or similar level of the rate of badgers, depending on analytical methods, but lower than the rate of foxes in both cases.

The increasing trends in exposure to SGARs, particularly exposure to brodifacoum, were generally observed in this study. Moreover, both the magnitude of SGARs residues and the proportion of samples with detected SGARs of many species increased between before and after the regulation change. Nonetheless, details of these changes in the magnitude of SGARs differed among species and active ingredients. Bird-feeding species particularly showed a large increase in the proportion of samples with detected SGARs. The proportion of bird-feeding species after the regulation change reached at a comparable level of that of the other feeding groups. In contrast, the magnitude of their SGAR residues remained lower than those for scavengers. Interestingly, the difference in the magnitude of bromadiolone and difenacoum, i.e., historically widely used active ingredients, between before and after the regulation change were remarkable in the bird feeding predators compared to the small mammal feeding or scavenger predators. Given the results of Dowding et al. (2010), the detection rate widely differs between analytical methods because of difference in the limit of detection or quantification. The change or difference in detection rate might be owing to the technical advance or different methodology between laboratories. However, the LoQ values were quite small compared to detected SGAR residues in many samples. For instance, the PBMS-related studies use around 1.5 – 3.0 ng/kg ww as a LoQ value of each active ingredient by LCMS, while WIIS England & Wales gave 0.8 ng/kg ww as a LoQ value of each ingredient (e.g., Ozaki et al., 2022; Walker et al., 2021). Therefore, the difference in LoQ between analytical method did not remarkably affect the results of statistical analysis. Our results suggest that the SGAR contamination has been widely spread but at a low SGAR residue level among the bird prey, i.e., small birds' populations, compared to the small mammal prey, i.e., rodents' populations including target species. The presence and accumulation of SGARs in bird feeding species have been shown in other studies (Hughes et al., 2013; Ruiz-Suárez et al., 2014; Walker et al., 2014), as well as in their prey, such as passerines and/or invertebrates (e.g., Alomar et al., 2018; Elliott et al., 2014; Walther et al., 2021) through various exposure routes (Elliott et al., 2014; Rattner et al., 2014). Our findings provide further evidence for potentially widespread transfer of SGARs within food webs through other organisms than small mammals, such as passerines or invertebrates.

For both scavenging and small mammal feeding species, notably barn owls, red kites, and common buzzards, there is evidence, especially from studies that used samples analysed as part of the PBMS and WIIS schemes, that exposure to SGARs has in general increased after the regulation change or the implementation of the stewardship regime (e.g., Ozaki et al., 2022; Walker et al., 2019, 2021). Interestingly, the findings in this report indicate that the increases in ΣSGARs after the regulation change in certain species, such as the red kite and common buzzard, appeared to be largely driven by increases in brodifacoum concentrations with unchanged time trends in the other active ingredients. Ozaki et al. (2022) reported that exposure of barn owls to SGARs in 2021 also increased after the stewardship implementation compared to the baseline period of 2006 to 2012, with the main driver for this increase being an increase in exposure to brodifacoum. This would suggest that changes in the levels of usage, the purpose or way in which brodifacoum containing products are used (from indoor only to in and around buildings), or changes in diet across these species has led to increased exposure to this compound.

The concurrent dates of the change in the authorisation relating to usage labelling for SGAR containing products and the implementation of the rodenticide stewardship regime make it problematic to distinguish whether any observed changes in exposure to SGARs in wildlife is due to one of these factors alone. It is unlikely that the best practice measures promoted by the rodenticide stewardship regime would lead to increased exposure in wildlife. However, our findings suggest some indications of increases in exposure over both the long term and since the introduction of these measures in some species, such as the red kite.

Several possibilities could cause such increases in exposure of mammals and birds since 2005 and exposure of scavenger birds after the regulation change. Geduhn et al. (2016) demonstrated that exposure risk of predators depends on the variation in prey composition. Changes in diet could possibly explain, at least in part, the observed increases in exposure in some predatory birds and mammals. However, it is unlikely that change in diet is the case across all species studied in this report, as they have very different diets. Another possible driver contributing to the increases in exposure may be SGAR resistance in target pest species, such as the brown rat and house mouse. Buckle et al. (2022) reported a high proportion of target species (*R. norvegicus* and *M. musculus*) with resistance to bromadiolone and difenacoum in some UK areas like Berkshire, which may lead to prolonged use of these ingredients or an increase in the use of more acutely toxic ingredients like brodifacoum. Regarding rodents resistant to SGARs, Ozaki et al. (2024a, 2024b) demonstrated, independently from the study of Buckle et al. (2022), that the areas where resistant rodents were reported could be a focus of high exposure of red kites to SGARs and concluded that resistance of target species may lead to accumulation of higher SGARs in their tissues. Although the region is an important ecological factor driving uptake of rodenticides (Broughton et al., 2022), we did not consider this factor in this report. Considering the time trends after the regulation change, there might also be sampling biases such as difference in sampling regions. Other possible ecological drivers for the SGARs uptake that we did not consider in this report are the season, i.e., month of sampling (Shore et al., 2003a), and landscape, i.e., preferred habitats (Hindmarch and Elliott, 2018). It is needed to integrate these ecological drivers into further studies and minimise potential biases in the datasets.

5 Conclusion

This report assessed SGARs residues in predatory birds and mammals across the UK. Based on data from various sources, SGARs residues in the liver of a range species were summarised. SGARs residues significantly differed among species and feeding types, but SGARs residues significantly increased over years in most of the species examined. General increasing trends in SGARs residues are concurrent with the change in authorisation and usage labelling of SGAR containing products and the implementation of the rodenticide stewardship regime, which are concurrent in the history. However, the recent increases in ΣSGARs in some species, particularly scavenger species, seems to be largely driven by increases in brodifacoum residues, while difenacoum residues had a tendency to decrease in certain species after the regulation change. Such a contrast among active ingredients could potentially result from changes in the levels and the purpose of the SGAR usage, or possible changes in other environmental factors driving the SGAR uptake like the diet. Moreover, the time trend of the SGAR exposure pattern differed between feeding types. Increases in the magnitude of bromadiolone residues were remarkably observed in bird feeding predators compared to other types, such as scavengers. Such contrasting exposure patterns possibly result from different exposure patterns and scenarios, which might be linked to both the use of each active ingredient and environmental drivers for the SGAR uptake.

Recommendations:

- The findings of this study highlight the importance for determining ecological factors significantly driving the uptake of SGARs. Integrating them into monitoring and the probabilistic model would improve their outcomes.
- Time trend of exposure to SGARs might be feeding type- or species-specific, which means that some particular species would be used to focus on a specific hypothesis. For instance, bird feeding predators, like sparrowhawk, would be pertinent species to assess '*whether and how SGARs are spread in non-target species other than rodents, such as small birds' populations*.' To achieve this goal, the time trend should be compared among species after improving monitoring outputs by determining the significant ecological factors.

Overall, the present study provides the information about the difference in SGARs residues among mammals and predatory bird species, the time trend of exposure to SGARs, and potential influence of the change in authorisation and the harmonisation for usage labelling, which were concurrent with the implementation of the stewardship regime.

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8 Glossary

Active ingredient: Chemicals in pesticide products that kill, control, or repel pests. In this report this term is a synonym of each rodenticide chemical.

Acute toxicity: Adverse effects of a substance that result in a short period of time.

Dry weight: Weight of organism or tissue after all the water has been removed.

Generalist (species): species able to make use of a variety of different resources, a heterotroph with a varied diet.

Limit of Quantification: the lowest possible concentration of the analyte that can be quantified by the method in a reliable way.

Multiple comparisons: Analysis of all possible pairwise comparisons.

Post-mortem examination: Examination of a dead body, by dissection, to determine the cause of death and other biological parameters (= **Necropsy**).

P-value: Probability of obtaining the result observed and more extreme results under the assumption that the null hypothesis is correct. A very small p-value means that the observed outcome would be very unlikely under the null hypothesis.

Scavenger (animal): Animals that consume dead organisms which have died from other causes than predation or have been killed by other predators.

Starvation: State of a severe deficiency in calorific energy intake or food.

Statistical power: probability that the test correctly rejects the null hypothesis when a specific alternative hypothesis is true.

Target species: Species which are object of a hunting. In this report, they are object of an eradicating or a control their population by rodenticides.

Wet weight: Weight of organism or tissue containing the water.

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