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Probabilistic approach reveals the toxicity threshold values of free-living raptors in Great Britain, United Kingdom, for the lethal effect of second-generation anticoagulant rodenticides

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Abstract

Second-generation anticoagulant rodenticides (SGARs) are recognised as an effective tool for rodent pest control. However, the use of SGARs enhances their exposure of non-target species and can cause lethal and sublethal effects on these species, including free-living raptors. Although certain liver SGAR residue concentrations from laboratory experimental studies have been considered as conventional thresholds, determining the threshold values for free-living predatory birds remains a challenge. In this study, we estimated the toxicity threshold values for liver SGAR residues associated with lethal SGAR poisoning (i.e., mortality by coagulopathy due to SGAR contamination), using a probabilistic modelling approach with data of red kites (*Milvus milvus*) and common buzzards (*Buteo buteo*) from Great Britain, United Kingdom. We also assessed factors influencing the relationship between lethal SGAR poisoning and liver SGAR concentrations.

From our results, we suggest 55 and 40 ng/g wet weight of the sum of liver SGAR residues as the toxicity threshold values for red kites and buzzards, respectively, assuming 1% of extra mortality as a negligible effect on the population size. The estimated threshold values did not significantly differ by the sex, age class, season, or British country. However, the threshold values for red kites, but not for buzzards, significantly increased over years, which suggests a decrease in their susceptibility to lethal toxicosis. The reason for this trend is unclear, although improved nutrition or physical conditions of red kites could be possible factors. Further studies are still needed to understand the impacts of SGAR exposure on free-living predatory birds.

Keywords

Conservation; Model-based recursive partitioning; Risk management; Wildlife; Common buzzard *Buteo buteo*; Red kite *Milvus milvus*

1 Introduction

Anticoagulant rodenticides (ARs) are recognised as an effective and cost-efficient tool for controlling rodent pest species in residential, commercial, and agricultural settings (Jacob and Buckle, 2018). The second-generation ARs (SGARs) are now widely used in the United Kingdom (UK) to control rodents that have evolved anticoagulant resistance, such as the brown rat *Rattus norvegicus* and house mouse *Mus musculus* (Buckle, 2013). Compared to first-generation ARs (FGARs), SGARs are lethal at low doses, often in a single feeding, and are more persistent in the environment and vertebrate tissues (Erickson and Urban, 2004; Horak et al., 2018; Nakayama et al., 2019). However, rodents consuming a lethal dose of SGARs can survive for several days and continue feeding on the AR-containing bait (Cox and Smith, 1992). This together, with the longer half-lives of SGARs in rodent tissues compared to FGARs, increases the potential for SGAR exposure of non-target species, particularly in predators of rodent prey, and the risk of secondary poisoning (López-Perea and Mateo, 2018). Despite several restrictive measures or regulations for the professional and amateur use of SGARs, the risk of secondary poisoning by SGARs remains a serious threat to wild vertebrates in many countries (e.g., Carrillo-Hidalgo et al., 2024; Elliott et al., 2022; Moriceau et al., 2022; Murray, 2020; Ozaki et al., 2024b).

The toxicity of ARs is based on their inhibition of vitamin K regeneration and the biosynthesis of clotting factors in the liver of mammals and birds, resulting in coagulopathy and internal haemorrhage (Rattner et al., 2014; Rattner and Mastrota, 2018). The diagnosis of AR poisoning for field vertebrates is based on evidence of physiological symptom signs and confirmation of AR residues in the body (Murray, 2018). However, there remains an important gap in our scientific knowledge for the accurate determination of a threshold residue concentration that causes death by coagulopathy in non-target species. Although several SGAR residue concentrations from field or experimental observations, such as 100 or

200 ng/g wet weight (ww) in the liver, have been conventionally cited as AR toxicity threshold levels for predatory birds (e.g., Badry et al., 2021; Carrillo-Hidalgo et al., 2024; Christensen et al., 2012; Fourel et al., 2024; George et al., 2024; López-Perea et al., 2015; Molenaar et al., 2017; Moriceau et al., 2022; Walker et al., 2008), SGAR residues higher than these values have also been reported in free-living predatory bird samples without any physiological signs of SGAR poisoning (e.g., George et al., 2024; Moriceau et al., 2022; Ozaki et al., 2024a, 2023). These observations indicate high inter- and intraspecific variations, as well as possible changes over years, in susceptibility to ARs.

As the conventional threshold values were mainly generated from a small number of samples, an approach based on data from a large number of field samples would be necessary to assess a relevant AR toxicity threshold residue concentration for free-living predatory birds. Some studies have predicted toxicity threshold values for SGAR residues by probabilistic modelling with large datasets (Elliott et al., 2024; Thomas et al., 2011). However, SGAR threshold values from probabilistic modelling vary across these studies, even within the same predatory bird family or species, which may result from differences in sensitivity to anticoagulants due to biological and physiological conditions of individual birds (Rattner and Harvey, 2021). For example, laboratory experiments have shown differences in anticoagulant rodenticide metabolism between sexes in animals such as leghorn chickens (*Gallus gallus domesticus*) (Watanabe et al., 2015) and Sprague-Dawley rats (*R. n. domestica*) (Zhu and Shin, 2005). Nutritional status can also affect AR toxicity, as the importance of dietary vitamin K intake on AR sensitivity has been demonstrated in humans (e.g., Holmes et al., 2012) and rats (e.g., Greaves and Ayres, 1973). Another possible factor affecting toxicity threshold levels based on a probabilistic approach is a difference in temporal exposure trends among SGAR active substances. Increasing trends in exposure of predatory birds to brodifacoum, a more toxic active substance than bromadiolone or difenacoum, have been reported in numerous European countries (e.g.,

Carrillo-Hidalgo et al., 2024; Moriceau et al., 2022; Ozaki et al., 2024b). The SGAR threshold values from modelling may differ over years due to changes in the proportions of SGAR active substances with different toxicities. However, the previous probabilistic modelling studies did not consider the potential influence of these factors. It is therefore unclear if the SGAR threshold values from probabilistic modelling would differ by sex, age, or other variables.

This study first aims to evaluate toxicity threshold values associated with death by coagulopathy due to SGARs, based on a probabilistic modelling approach, for free-living predatory birds in the UK where five SGARs (bromadiolone, difenacoum, brodifacoum, difethialone, and flocoumafen) are authorised for use. We used data of two generalist raptor species, red kites (*Milvus milvus*) and common buzzards (*Buteo buteo*), collected across Great Britain for several years. This study also aims to identify factors influencing the estimated relationship between death by coagulopathy and SGAR residue concentrations. We especially focused on changes in the relationship over years. Given the increasing trend of more toxic brodifacoum residues in free-living raptors, we hypothesise that a lower level of the sum of SGAR residues would be associated with death by coagulopathy in recent years.

2 Materials and methods:

2.1 Sample collection

In this study, we used carcasses of free-living red kites and common buzzards. Both species are medium-size and residential raptors in the UK (Carter, 2007; Tubbs, 1974; Walls and Kenward, 2020). The buzzard is slightly smaller than the red kite: Male red kites weigh approximately 1000g and females approximately 1200g (Carter, 2007), whereas male buzzards weigh approximately 800g and females approximately 1000g (Walls and Kenward, 2020). Both species are also considered generalist and opportunist scavengers and known to be at high risk of AR poisoning given the frequency of their foraging on (dead) mammals (Coeurdassier et al., 2012; López-Perea and Mateo, 2018; Ozaki et al., 2025). There was a sufficient number of samples considered to be SGAR-poisoned in the collection of their carcasses.

The carcasses of 263 red kites collected from across Great Britain (England, Scotland, and Wales) from 2015 to 2022 and carcasses of 159 buzzards collected from across England from 2009 to 2021 were used for the study. Supplementary Information (SI) Table 1 summarises the number of samples per year. The buzzard samples were collected by the Wildlife Incident Investigation Scheme (WIIS) and the Predatory Bird Monitoring Scheme (PBMS), whereas the red kite samples were collected as part of the PBMS, Disease Risk Analysis and Health Surveillance (DRAHS) project at the Zoological Society of London (ZSL), WIIS for England & Wales, and the Raptor Health Scotland/WIIS for Scotland schemes. Both PBMS and DRAHS programmes heavily rely on citizen science, in which members of the public send dead birds that they find. WIIS incidents are usually reported by a variety of stakeholders that also include members of the public.

All carcasses were subject to a post-mortem (PM) examination, and various tissue samples, including the liver, were excised and stored at -20ºC. PM examinations of buzzards were conducted by wildlife veterinarians from the Animal Plant Health Agency (APHA) as part of the WIIS, whereas those examinations of red kites were conducted by wildlife veterinarians at ZSL, the APHA (on behalf of Fera Science), SAC Consulting: Veterinary Services (on behalf of SASA), respectively. This study received a positive review from the UKCEH Animal Research Ethics Committee prior to the collection of birds. The information from the PM examination differed between the two species. For red kites, age and sex of carcasses, as well as the date and the location of the carcass collection, were identified and recorded. Juveniles were defined as individuals determined to have hatched in the current or previous year, as assessed from plumage characteristics (Molenaar et al., 2017). For buzzard carcasses, no information about age and sex has been recorded, but only the date and the location of the collection were provided.

In this study, 'lethal SGAR poisoning' was defined as 'death due to AR-induced internal haemorrhage.' A diagnosis of SGAR poisoning was determined in accordance with the principles and methods described in Murray (2018) for both species, assigning each bird as either poisoned or non-poisoned. The presence of macroscopic haemorrhaging unrelated to external trauma was noted. If such haemorrhaging without evidence of trauma was present in a bird and if SGAR residues were detected in its liver, of any magnitude, the given bird was classed as an individual poisoned by SGARs (i.e., SGARs were directly implicated as a contributory cause of death). Therefore, SGAR poisoning was not diagnosed by SGAR residue concentration alone, even if concentrations were elevated. Reports on poisoned/non-poisoned birds from different institutes were revised and re-assigned based on this approach. Samples that could be assigned as neither poisoned nor non-poisoned (e.g., SGAR-detected samples with macroscopic haemorrhaging potentially due to physical trauma) were designated as uncertain cases.

2.2 Chemical analysis

At the UKCEH Lancaster site, a sub-sample (0.25g) of each liver was thawed, weighed accurately, ground and dried with anhydrous sodium sulphate. Each sample was spiked with labelled standards (d^5-Bromodialone, and d^4-Brodifacoum, QMx Laboratories Ltd). Chloroform: acetone (1:1 v/v) was added to each sample, and the samples were thoroughly mixed using a vortex. Samples were extracted on a mechanical shaker (Stuart SF1, Bibby Scientific) for one hour, then centrifuged at 5000 rpm (4696 g-force) for 5 minutes. The supernatant was transferred to a clean tube. This process was repeated with clean solvent, but the second time, samples were on the mechanical shaker for only 30 minutes. The combined extract was evaporated to dryness using a parallel evaporator (Büchi Syncore, Switzerland), re-dissolved in chloroform:acetone (1:1; v/v), and filtered (0.2 mm Polytetrafluoroethylene, PTFE, filter). The filtered sample was evaporated to dryness and re-dissolved in acetone:dichloromethane (1:23; v/v). The sample was re-filtered (0.2 mm PTFE filter) and then cleaned using automated size exclusion chromatography (Agilent 1200 HPLC system). The clean extract was evaporated, and the residue was re-suspended in chloroform:acetone:acetonitrile (1:1:8; v/v). The extract was further cleaned using solid phase extraction cartridges (ISOLUTE® SI 500mg, 6ml). The cartridges were washed with methanol and activated with acetonitrile. The samples were eluted with acetonitrile, and this solvent was then exchanged for the mobile phase.

Analysis was performed using a 'Acquity' UPLC coupled to a triple quadrupole 'Xevo TQ-XS' mass spectrometer (Waters Ltd, Wilmslow, UK) interfaced with a 'Unispray' source in negative polarity mode and operated with Masslynx software™ (V.4.2). Analyte separation (1 µL inj. volume) was performed on an Acquity UPLC BEH C18 column (Waters, 1.7 µm particle size, 100 mm x 3mm I.D.) using a H_2O:MeOH mobile phase gradient. The analytes were eluted from the column using a programme which mixed different ratios of mobile phase A: 0.77g/L Ammonium

acetate in water and Mobile phase B: 0.77g/L Ammonium acetate in Methanol at a rate of 0.3 ml min^{-1}. Gradient elution started from 70% A and 30% B, increased to 65% B in 3 minutes and held until 9 minutes then ramped to 75% B at 12 minutes and finally to 98% B at 19 minutes, held for 1.5 minutes and then returned to starting conditions.

MS/MS was performed in multiple reaction mode (MRM) using Unispray in the negative mode, and characteristic ion fragments were monitored for each compound. Argon was used as collision gas. Chromatographic peaks were integrated using Masslynx™ which was also used to generate linear calibration curves with R^2>0.99. The rodenticides standards (Dr Ehrenstorfer, LGC Group, Teddington, UK) were matrix matched.

The performance of the method was assessed in terms of the limit of quantification (LoQ), the recovery of the internal standards for the analytes and linearity. Recovery for the total procedure was calculated using the labelled standards.

A similar method was used by Fera Science until 2020. A QuEChERS (Quick Easy Cheap Effective Rugged Safe) extraction method was used by Fera Science since 2020 and by SASA. Details of QuEChERS method are described in Supplementary Information. The recovery rates for spikes used in analysis were within the range from 49 to 105%. Limits of quantification for the different laboratories in each year are given in SI Table 2. SGAR residues were reported for individual compounds (bromadiolone, difenacoum, brodifacoum, difethialone, and flocoumafen), and the sum of all five SGAR compounds (ΣSGARs) was calculated per sample. For calculating ΣSGARs, concentrations under the LoQ (<LoQ) of each active substance were converted into 0 ng/g ww. Concentrations are expressed as ng/g wet weight (ww).

2.3 Data analysis

2.3.1 Exposure of British red kites and buzzards to SGARs

The detection rate and magnitude of SGARs residues were statistically compared between red kites and buzzards. The differences in the detection rates were tested using a Fisher exact test for each active substance and ΣSGARs. The magnitude of liver residues was statistically compared between the two species using a non-parametric Wilcoxon Mann-Witney test for each active substance and ΣSGARs.

For red kites, we statistically compared the magnitude of SGARs residues between sexes and age classes using a Wilcoxon Mann-Witney test, excluding samples of unidentified sex, and age class, respectively. The magnitude was also compared across four seasons and three British countries (England, Scotland, and Wales) using a non-parametric Kruskal-Wallis test, excluding samples with non-recorded collection season and country, respectively. For buzzards, we could not test the differences between sexes and age classes, as well as among countries, because of a lack of information due to differences in protocols among collection schemes. Only the difference across seasons was tested using a Kruskal-Wallis test, excluding samples with non-recorded collection season.

2.3.2 Proportion of samples assigned as poisoned

The proportions of samples assigned as poisoned and as non-poisoned were statistically compared, excluding uncertain poisoned cased. For red kites, we compared the proportions between sexes and age classes using a Fisher exact text. We also compared the proportions across four seasons and the three countries using a chi-squared test. For buzzards, only the difference across seasons was tested using a chi-squared test. The difference

in ΣSGAR concentrations between samples assigned as poisoned and as non-poisoned were tested using a Wilcoxon Mann-Witney test for each species. Similar to the tests for SGAR exposure, samples of unknown sex, age class, season or country were excluded from each test.

2.3.3 Probabilistic modelling

A probabilistic model was built using logistic regression with birds classed into poisoned and non-poisoned cases. Birds classed as uncertain were excluded from the model. To predict the probability of lethal SGAR poisoning along with liver ΣSGARs residue concentrations, we considered poisoned birds as 1, whereas non-poisoned birds as 0 as a binary response variable in the logistic regression. Given that liver ΣSGARs residues are usually skewed, a logarithmical transformation was applied. Samples with ΣSGARs <LoQ (i.e. all active substances were <LoQ) were excluded from the data like the studies of Thomas et al. (2011) and Elliott et al. (2024). We also carried out the same probabilistic approach with the data including samples with ΣSGARs <LoQ to check how the threshold values would change. In this model, <LoQ was replaced with the minimum value of observed ΣSGARs (1.21 ng/g ww). The significance of the explanatory variable (i.e., liver ΣSGARs residue concentrations) was assessed by the likelihood ratio (LR) test (Zuur et al., 2009).

2.3.4 Factors influencing probabilistic models

To assess whether and what factors significantly influence the probabilistic model, we applied model-based recursive partitioning (MOB; Zeileis et al., 2008). The aim of this analysis is not to find factors significantly affecting the response variable of the model (i.e., poisoned/non-poisoned) but to seek factors significantly modifying the relationship between lethal SGAR poisoning and liver ΣSGARs residues in the probabilistic model.

The principle of MOB is a statistical recursive binary partitioning of data. After establishing a regression model and selecting partitioning variables to involve, this analysis assesses whether the given model established on the whole data is more stable than the same model established on two datasets separated by one of the partitioning variables. Permutation tests by Strasser and Weber (1999) were applied for the significance of each splitting variable. If there is a significant partitioning variable, the analysis also computes the split point (for quantitative variables) or split class (for qualitative variables) that optimizes the model established with two-sprit data. If several significant splitting variables are detected, the splitting variable most significantly optimising the model will be selected. Those steps are recursively performed to each of the two regression models using the split data, until no significant data-splitting is observed.

In this study, MOB was first applied to the probabilistic model established with the red kite data to assess influence of the age, sex, and country. These three variables are chosen as partitioning variables, and red kite samples with unknown sex, age and/or collection location were excluded. The partitioning variable 'country' in this analysis was composed of two classes, 'England' and 'the others (Wales and Scotland)', due to a small number of poisoned samples from each of Wales and Scotland. MOB was then applied to the probabilistic model with data including both species to assess influence the species, season, and collection year. In this analysis, species, season, and collection year were chosen as partitioning variables, and samples with unknown collection date and location were excluded.

All statistical analyses were computed using the statistical software R (ver. 4.5.0; R Core Team, 2025). Logistic regression models were established by the "*glm*" function in the basic "*stats*" package. MOB was performed by the "*glmtree*" function in the "*partykit*" package.

3 Results

3.1 Exposure of British red kites and buzzards to SGARs

At least one SGAR was detected in the liver of 94.3% of red kites (248/263 samples) and 88.7% of buzzards (141/159) (Table 1). The detection rates of bromadiolone, difenacoum, brodifacoum, difethialone, and flocoumafen were 74.5, 87.5, 87.1, 16.3, and 0.8% for red kites and 65.4, 73.0, 61.0, 3.8, and 3.1% for buzzards, respectively. The detection rate of ΣSGARs and each active substance was significantly higher in red kites than buzzards (Fisher exact text; p-value <0.05), except for the detection rate of flocoumafen that was not significantly different between the two species (p-value = 0.11).

The median values of ΣSGARs in red kites and buzzards were 194.5 and 35.7 ng/g ww, respectively (range: <LoQ – 3223.7 and <LoQ – 4433.0 ng/g ww; Table 1). When we removed samples with ΣSGARs <LoQ, the median values of ΣSGARs were 216.8 and 43.0 ng/g ww, respectively. The medians of bromadiolone, difenacoum, and brodifaoum concentrations in red kites were 7.3, 21.0, and 43.9 ng/g ww, whereas the medians of these active substances in buzzards were 1.3, 4.3, and 1.7 ng/g ww ng/g ww, respectively. The magnitude of ΣSGARs and each active substance was significantly higher in red kites than buzzards (Wilcoxon Mann-Witney test; p-value <0.001), except for concentrations of flocaumafen that were not significantly different (p-value = 0.06).

For red kites, concentrations of ΣSGARs were significantly higher in females than males (Wilcoxon Mann-Witney test; p-value <0.001). There was no significant difference in ΣSGAR residue between two age classes (p-value = 0.13) and between the four seasons (Kruskal-Wallis test; p-value = 0.06). Concentrations of ΣSGARs were significantly higher in England than Wales and Scotland (Kruskal-Wallis test; p-value <0.001).

For buzzards, there was no significant difference between seasons (Kruskal-Wallis test; p-value = 0.66). Liver ΣSGAR residue concentrations per year are graphically represented in SI Figure.

3.2 Poisoned red kites and buzzards

The numbers of red kites assigned as poisoned or non-poisoned by PM ("poisoned samples" and "non-poisoned samples" hereinafter) were 42 and 209, accounting for 16.0% and 79.5% of the red kite samples, respectively. Twelve red kites (4.6%) could not be assigned as poisoned or non-poisoned, considered as uncertain. The proportion of poisoned samples was significantly higher in females than in males (Fisher exact test; p-value = 0.02), and the proportion of poisoned birds was significantly higher in England than Wales and Scotland (chi-squared test; p-value = 0.009). No significant difference in the proportion of poisoned individuals was observed between age classes and between seasons.

The numbers of poisoned and non-poisoned buzzards were 23 and 134, accounting for 14.5% and 84.3% of the buzzard samples, respectively. There were two uncertain buzzards (1.3%). The differences in the proportion of poisoned samples did not significantly differ among seasons.

Concentrations of ΣSGARs in the liver significantly differed between poisoned and non-poisoned samples. For red kites, ΣSGARs in poisoned samples (median: 660.3 ng/g ww; range: 134.6 – 3223.7 ng/g ww) were significantly higher than ΣSGARs in non-poisoned samples (median: 135.9 ng/g ww; range: <LoQ – 1174.0 ng/g ww) (Wilcoxon Mann-Witney test; p-value <0.001). Likewise, ΣSGARs in poisoned buzzards (median: 285.0 ng/g ww; range: 137.0 – 4433.0 ng/g ww) were significantly higher than ΣSGARs in non-poisoned ones (median: 22.0 ng/g ww; range: <LoQ – 1304.9 ng/g ww) (p-value <0.001). Supplementary Information Figure also represents ΣSGAR residue concentrations of each poisoned and non-poisoned sample.

The proportion of the SGAR poisoned samples did not significantly differ between the two species (Fisher exact test; p-value = 0.78).

3.3 Probabilistic model

The logistic models for both species showed that lethal SGAR poisoning was significantly explained by logarithmically transformed liver ΣSGAR residue concentrations (LR test; p-value <0.001 for both species). The model with 236 red kite data from 2015 to 2022 (excluding uncertain poisoned samples and samples with ΣSGAR <LoQ) indicated that the threshold values for the probability of 0.01, 0.05, 0.10, 0.20, and 0.50 of death due to SGAR poisoning (i.e., 1%, 5%, 10%, 20% and 50% of lethal SGAR poisoning) were 54.2, 138.5, 211.9, 335.9, and 738.7 ng/g ww of ΣSGAR residue concentrations in the liver, respectively (Figure 1a, Table 2). Meanwhile, the model with 139 buzzards from 2009 to 2021 (excluding uncertain poisoned samples and samples with ΣSGAR <LoQ) indicated that threshold values for these probabilities were 38.2, 67.4, 87.2, 115.3, and 185.8 ng/g ww of ΣSGAR concentrations in the liver, respectively (Figure 1b, Table 2). The medians of liver ΣSGAR residue concentrations for red kites and buzzards, 194.5 and 35.7 ng/g ww, respectively, correspond to the probability of 9% and 0.8% of the death due to SGAR poisoning, respectively.

The threshold values obtained from the data including samples with ΣSGAR <LoQ (replaced with the minimum ΣSGARs measured) were almost identical to the above-mentioned values, and they are the same at the second decimal level.

3.4 Factors influencing probabilistic models

The MOB for 114 red kites showed that the relationship between lethal poisoning cases and liver ΣSGAR residues did not significantly differ by sex, age, or British country. However, MOB with the data for both species (N = 319: 212 red kites and 107 buzzard) demonstrated significant factors modifying the relationship (Figure 2). First, the relationship differed between buzzards and red kites (p = 0.001). Our analysis continued to assess the significance of partitioning variables by each species, and the relationship significantly differed between the periods 2015 – 2018 and 2019 – 2021 for red kites (p-value = 0.007), but not for buzzards (Figure 2). The number of red kite samples in these two periods (2015 – 2018 and 2019 – 2021) was 119 and 93, respectively. These numbers did not significantly differ from 1:1 (chi-squared = 3.18, df = 1, p-value = 0.074). Season did not significantly affect the probabilistic model. After applying MOB, 1%, 5%, 10%, 20% and 50% of lethal SGAR poisoning for buzzards were 34.9, 64.6, 85.6, 115.6, and 194.0 ng/g ww of ΣSGAR (Table 3). For the red kites, the same probabilities of lethal SGAR poisoning were 35.5, 90.3, 137.7, 217.6, and 475.9 ng/g ww of ΣSGARs in the periods of 2015 – 2018 and 220.6, 361.3, 451.7, 575.5, and 871.0 ng/g ww of ΣSGARs in the periods of 2019 – 2021 (Table 3).

4 Discussion

4.1 SGAR exposure and the threshold values of red kites and buzzards

Our study demonstrates the relationship between the corresponding probability of lethal SGAR poisoning (i.e., observing signs of direct death by coagulopathy) and SGAR residues in the liver of British red kites and buzzards. The threshold SGAR residue values for 1%, 5%, 10%, 20% and 50% of lethal SGAR poisoning are approximately 55, 140, 210, 340, and 740 ng/g ww for red kites and 40, 70, 90, 120, and 200 ng/g ww for buzzards, respectively. These values from the data excluding the samples with SGAR residue <LoQ were almost identical to the values from the data including the samples with SGAR residue <LoQ, which indicates that the treatment of samples with SGAR residue <LoQ does not significantly affect our probabilistic model.

The two species of raptors markedly vary in their exposure to SGARs. Red kites are significantly more exposed to SGARs than buzzards in our results. Higher exposure level of red kites to SGARs compared to other predatory birds has been reported in various studies, such as studies conducted in the UK (e.g., Hughes et al., 2013; Molenaar et al., 2017; Walker et al., 2008a), Spain (e.g., López-Perea et al., 2019; Sánchez-Barbudo et al., 2012), or France (e.g., Coeurdassier et al., 2014; Moriceau et al., 2022). Anticoagulant rodenticide target rodents, such as brown rats may be an important food source for red kites (Carter, 2007; Ntampakis and Carter, 2005), and Shore et al. (2018) argued that red kites, that feed on rodents and dead small animals, had relatively high liver SGAR residues. Likewise, buzzards have also shown high SGAR detection rates and magnitude in previous studies in various countries (e.g., Christensen et al., 2012; George et al., 2024; Hughes et al., 2013; Lestrade et al., 2021; López-Perea et al., 2015; Moriceau et al., 2022; Ozaki et al., 2024b). However, although buzzards also consume carrion and rodents, they have a

more omnivorous diet, including birds and invertebrates, compared to the diet of red kites (Carter, 2007; Carter and Grice, 2002; Tubbs, 1974; Walls and Kenward, 2020). Higher AR exposure of red kites than buzzards has also been observed in the study of Badry et al. (2022) carried out in Germany and in the study of Moriceau et al. (2022) in France.

It has been suggested that high levels of SGAR exposure of red kites would be expected to increase their risk of poisoning (e.g., Badry et al., 2021; Shore et al., 2018). Hughes et al. (2013) observed higher rates of mortality attributable to rodenticide poisoning (10.5% of carcasses; 12/114) in red kites collected from Scotland during 2000 – 2010 compared to other predatory birds collected in their study. However, the threshold values of buzzards were lower than those of red kites, which indicates that buzzards are more susceptible to SGAR exposure than red kites. Interspecific variation in susceptibility to SGARs in free-living predatory birds has been widely reported (e.g., Elliott et al., 2024; Rattner et al., 2014; Rattner and Mastrota, 2018; Thomas et al., 2011). Despite a higher level of SGAR exposure of red kites, the proportion of the SGAR-poisoned samples did not significantly differ between the two species, probably because buzzards are more susceptible to SGARs. Therefore, the risk of SGAR poisoning results from the compromise between the SGAR exposure level and the susceptibility of each species.

4.2 Factors influencing SGAR exposure and the threshold values

Our results indicate difference in the factors affecting the exposure of red kites to SGARs and those influencing their susceptibility to SGARs. Although the levels of SGAR exposure of red kites significantly differed between sexes and between the British countries, our probabilistic models showed that their susceptibility to SGARs did not significantly differ according to the sex, age class, season or country, but only by years.

Higher detection rate and/or magnitude in samples from England than the other British countries were reported in red kites (Shore et al., 2018), buzzards (Ozaki et al., 2024b), Eurasian sparrowhawks (Broughton et al., 2022) and common kestrels (Roos et al., 2021). These authors argued that such spatial variations might be due to the mode and quantity of the local SGAR use and the diet of predatory birds reflecting the availability of food in the field and competition with other predators. Although SGAR exposure was higher in males than females in sparrowhawks (Broughton et al., 2022) and kestrels (Roos et al., 2021), Ozaki et al. (2024b) reported higher SGAR exposure levels in female buzzards, suspecting that differences in feeding behaviour during incubation and chick-rearing between sexes (males hunt prey and eat its head, while females ingest the remaining body including liver) might explain the observed high SGAR exposure in females. These differences in SGAR exposure between sexes or regions seem to be concorded with a high SGAR poisoning rate, but these factors did not influence the susceptibility to SGARs.

In contrast, our results demonstrated a significant change in the threshold values (i.e., the relationship between death caused by coagulopathy and liver ΣSGAR residues) of red kites over years. In opposition to our hypothesis, however, such a temporal change was only observed for red kites but not buzzards, and the threshold values increased (i.e., the susceptibility to SGARs reduced). Our results indicated that the threshold values of red kites changed between 2018 and 2019. However, because the number of samples did not differ significantly between before and after 2018/2019, this time point may be the middle of a constant temporal change in SGAR susceptibility. We therefore cannot determine whether the threshold values of red kites changed around 2018/2019 or gradually. Nonetheless, to our knowledge, no study has reported a temporal change in the susceptibility of free-living predatory birds to SGARs. A wide spread of rodent populations with AR-resistant genes has been reported in the UK (Buckle, 2013; Buckle et al., 2022, 2020) and other countries (e.g., Carromeu-Santos et al., 2023; Grandemange et al., 2010; McGee et al.,

2020; Rost et al., 2009). However, such genetic AR-resistant mechanisms are unlikely to explain our results given the life-history characteristics of predatory birds: slower generation turnover, lower fecundity, and longer lifespan compared to rodents. There may be other mechanisms related to the susceptibility to SGARs, such as bird nutritional conditions (Rattner and Harvey, 2021). Vitamin K is a key element of the toxicity mechanisms of ARs. Anticoagulant rodenticides bind tightly to and inactivate vitamin K epoxide reductase, and vitamin K is an antidote to AR intoxication (Rattner et al., 2014; Rattner and Mastrota, 2018). It is possible to hypothesise that the diet of British red kites has shifted to nutrient-rich food or that the health status of the species has increased. Anthropogenic foods are often incorporated into red kite diets, which might explain, at least partly, a possible shift in nutritional quality of red kites and the difference between the two species. Since reintroduction of red kites in the late 1980s, several feeding stations for red kites have been established (Carter, 2007). In parallel, food piracy (i.e., stealing food from other birds are frequently reported in red kites (Carter, 2007). Red kites also benefit from members of the public putting out food for them (Carter and Grice, 2000). Orros and Fellowes (2015) showed that feeding of red kites on supplementary food in domestic gardens was practised by up to one in 20 households in Reading, a city in south England. Although cooked foods usually contain low nutritional value for predatory avian species and potentially harmful additives (Carter, 2007), most householders recently gave some raw meat containing skin and/or bone instead of processed food, which may provide higher nutritional conditions to red kites (Orros and Fellowes, 2014). Although the influence of garden wild bird feeding on small bird populations and communities has been documented (Plummer et al., 2019), the link of the supplementary feeding with our finding, as well as its ecological and conservational consequences, are unproven. However, such anthropogenic influences other than the release of contaminants might be as hidden key factors of the contaminant risk management.

4.3 The threshold value that should be applied in predatory bird conservation.

The probabilistic model provides numerous liver SGAR residue values corresponding to probabilities of lethal SGAR poisoning. Some of our threshold values are similar to those commonly cited as the thresholds (Table 4). Compared with these values in the literature, our value corresponding to 50% of lethal SGAR poisoning for red kites, 740 ng/g ww, is similar to the value proposed by the Rodenticide Registrants Task Force (Table 4). The widely cited threshold values 100 – 200 ng/g ww are associated with 5% – 20% of lethal SGAR poisoning for red kites and buzzards in our results. Therefore, comparing these conventional thresholds with liver SGAR residue concentrations may still be reasonable.

The values derived from the probabilistic modelling approach may be applied to the residue data observed in individuals to assess the additional mortality rate due to contaminants, which could then be used for conservation and risk management purposes. However, a specific probability of lethal SGAR poisoning aiming at the conservation of wild bird populations has not been established. For this purpose, it would ideally be possible to determine a residue value below which impacts on red kite and buzzard populations would not be expected. The Council Directive 79/409/EEC of 2 April 1979 on the conservation of wild birds set 1% of the overall annual natural mortality as the small numbers of loss meeting the condition of a negligible effect on the population size of the species concerned (European Commission, 1993). If we assume lethal effects of chemical contaminants as an additional mortality, this "1% mortality criterion" may be applied to the threshold value for the conservation of populations. From our models, the 1% of lethal poisoning corresponds to 54.2 and 38.2 ng/g ww of liver ΣSGAR residues for British red kites and buzzards, respectively. These values are lower than the conventional values of 100 – 200 ng/g ww but are at a similar level to the threshold proposed by Elliott et al. (2024) for the barn owl in British Columbia, Canada: 40 ng/g ww (Table 4).

However, there are still many biases and uncertainties in our data for the probabilistic approach to determine one definitive threshold value. First, the PM diagnosis of SGAR poisoning may be biased. The interpretation of post-mortem lesions may be hindered in certain circumstances, for example due to decomposition of carcasses or freeze-thaw cycle (Murray, 2018; Roe et al., 2012). The distinction between AR-induced bleeding and simple trauma is also problematic (Murray, 2018; Stone et al., 1999) because macroscopic evidence of bleeding is not always enough to identify subtle physiological effects of ARs (Sánchez-Barbudo et al., 2012). The individuals with haematoma/haemorrhage associated with trauma were discounted from poisoned cases in our data, which can be a large source of bias. Second, the time gap between the ingestion of ARs and death due to AR poisoning causes biases in tissue AR residues. Thomas et al. (2011) pointed out that the threshold SGAR residue values are likely to be both under- and overestimated because of the metabolization of ingested SGARs and the ingestion of a higher dose of SGARs than the lethal level before the death of animals, respectively. Meanwhile, the study of Rattner et al. (2020) with captive American kestrels *Falco sparverius* provided evidence that ARs may have prolonged effects that increase the toxicity of subsequent AR exposure. Exposure of predators to multiple ARs could have protracted and cumulative effects on their health. Thirdly, the relative toxicity among ARs should be considered and integrated into the threshold values. The difference in the toxicity among ARs based on laboratory experiments are widely known (e.g., Nakayama et al., 2019; Rattner et al., 2014). However, it is still in question whether the relative toxicity values from laboratory-based experiments are valid for free-living animals. Elliott et al. (2024) evaluated SGAR toxic equivalence factors (TEFs) for difethialone (setting TEF = 1 for this compound), brodifacoum (TEF = 0.8) and bromadiolone (TEF = 0.5) from heir probabilistic modelling with sample exposed to one active substance. It seems more relevant to use such TEFs, rather than ΣSGAR residues, as the threshold values for free-living predatory birds. However, this approach needs

a large data of single exposure for the relative toxicity values to be accurately calculated. Data from wildlife exposed to a single AR active substance are currently limited because many field samples have already been exposed to multiple ARs (e.g., Nakayama et al., 2019). Finally, the impact of chemical poisoning on the population size needs to be elucidated and integrated into the estimation of the toxicity threshold values. So far, only direct lethal effects of the SGAR exposure have been applied to the probabilistic modelling. However, SGAR exposure also causes chronic and sublethal effects of coagulopathy, such as sublethal haemorrhage or haematoma resulting in behavioural and histopathological changes, dysfunction of the immune system, and alteration of body conditions, all of which leads to a decrease in their fitness (Rattner et al., 2014; Rattner and Harvey, 2021; Rattner and Mastrota, 2018). In addition, the "1% mortality criterion" is based on the fact that the mortality parameters are often not known with an accuracy of 1% (European Commission, 1993). In a simulation study on the impact of wind turbine collision of birds, Schippers et al. (2020) demonstrated that even 1% of extra mortality could result in severe consequences in the population dynamics, while the simulation of Duriez et al. (2023) showed that, if there is no immigration, an increase of 5% in annual mortality rate by windfarm collisions would lead to a decline in the lesser kestrel (*Falco naumanni*) populations in France. Meanwhile, it should be considered that predatory bird populations may be affected by other environmental contaminants and factors. For example, Green et al. (2022) estimated 0.4% and 0.2% of annual death rates from lead poisoning in red kites and buzzards, respectively, in Europe. The significance of the extra-mortality by environmental contaminants on the population dynamics will depend on the balance of processes contributing to the population growth rate. After their reintroduction, the populations of British red kites have rapidly recovered (Carter, 2007; Molenaar et al., 2017). Such a high growth rate might allow the species to maintain or recover their population numbers even with higher extra mortality than 1%. However, given the current numerous environmental and

anthropogenic threats, including chemical contaminants, the impacts of 1% of extra-mortality by SGARs on predatory bird populations still needs to be studied further.

5 Conclusion

In this study, we estimated the threshold values for the exposure of British red kites and buzzards to SGAR, based on a probabilistic approach. The liver ΣSGAR residues corresponding to several probabilities of death caused by coagulopathy (i.e., lethal SGAR poisoning) were calculated, and the value for the probability of 1% was chosen as the threshold that would not affect the population size for each species. We therefore suggest 55 and 40 ng/g ww of liver ΣSGAR residues as expected threshold values for SGAR exposure of British red kites and buzzards, respectively.

This study also aimed to identify factors influencing the relationship between lethal SGAR poisoning and liver ΣSGAR residues. Our results demonstrated that the threshold values based on the probabilistic models for red kites increased over years, which means that British red kites might have become less susceptible to SGAR toxicity. However, the reason for the result remains obscure. There are still various potential biases in our data possibly affecting the probabilistic modelling, such as the accuracy of PM diagnosis, the bias in the tissue SGAR accumulation, variation in the AR toxicity, sublethal effects on populations. Moreover, the significance of extra-mortality due to SGARs on population sizes depends on the population growth rate of the given species and the extra-mortality by other environmental and anthropogenic threats. Further research is needed to understand and incorporate these factors into models to determine likely SGAR toxicity thresholds for free-living predatory birds.

Supplementary Information (SI)

SI Analytical method: Quick Easy Cheap Effective Rugged Safe (QuEChERS) extraction method

SI Table 1: Number of collected red kite and buzzard samples per year

SI Table 2: Limit of quantification of each SGAR active substance

SI Figure: Liver ΣSGAR residue concentrations of poisoned and non-poisoned red kites and buzzards per year

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Figure 1: Logistic regression models demonstrating the relationship between probability of lethal SGAR poisoning in red kites (a) and buzzards (b) and ΣSGAR concentrations in the liver. Birds assigned as poisoned by SGARs are represented by points at the top of the plot, while birds assigned as non-poisoned are represented by points at the bottom of the plot. Red line represents probability for lethal poisoning in relation to ΣSGAR concentrations from the logistic regression. Vertical black solid lines represent the threshold ΣSGAR values for the probability of 0.01, 0.05, 0.10, 0.20, and 0.50 for death due to SGAR poisoning. Different zones between these ΣSGAR threshold values are distinguished by colours. Probability of death due to SGAR poisoning <0.01: dark green; 0.01 – 0.05: green; 0.05 – 0.1: yellow green; 0.1 – 0.2: yellow; 0.2 – 0.5: orange; and >0.5: red.

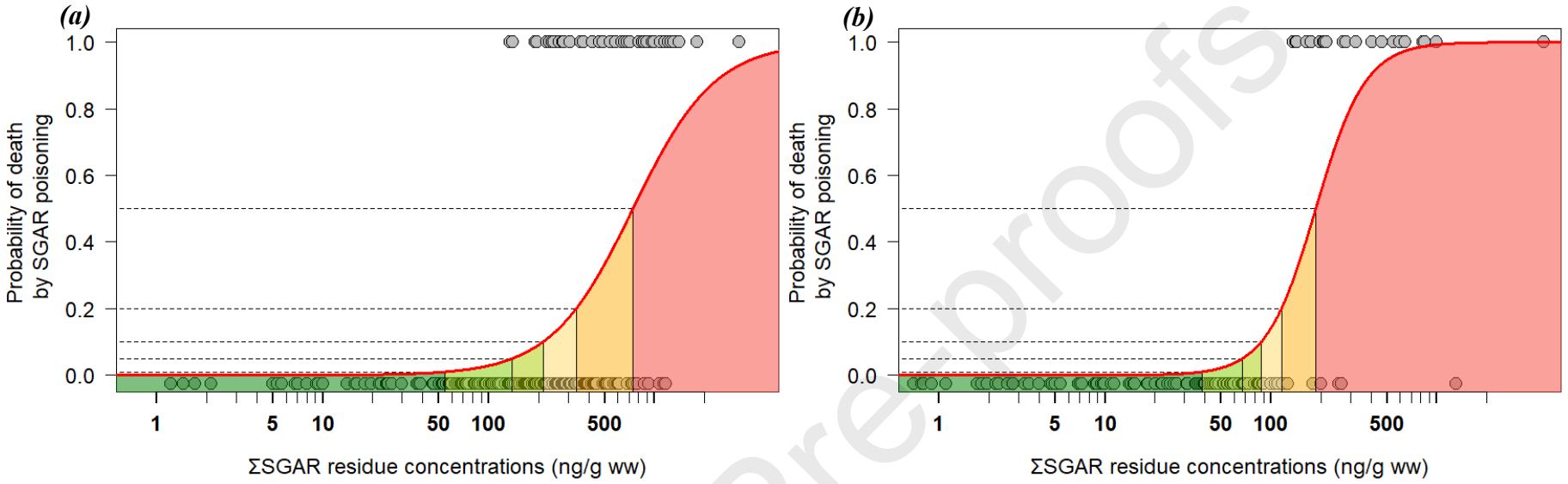


Figure 2: Model-based recursive partitioning (MOB) applied to the logistic regression model for the relationship between probability of lethal SGAR poisoning in predatory birds (red kites and buzzards collected in Great Britain) and ΣSGAR concentrations in the liver. Partitioning variables significantly modify the logistic regression model are listed with p-value.

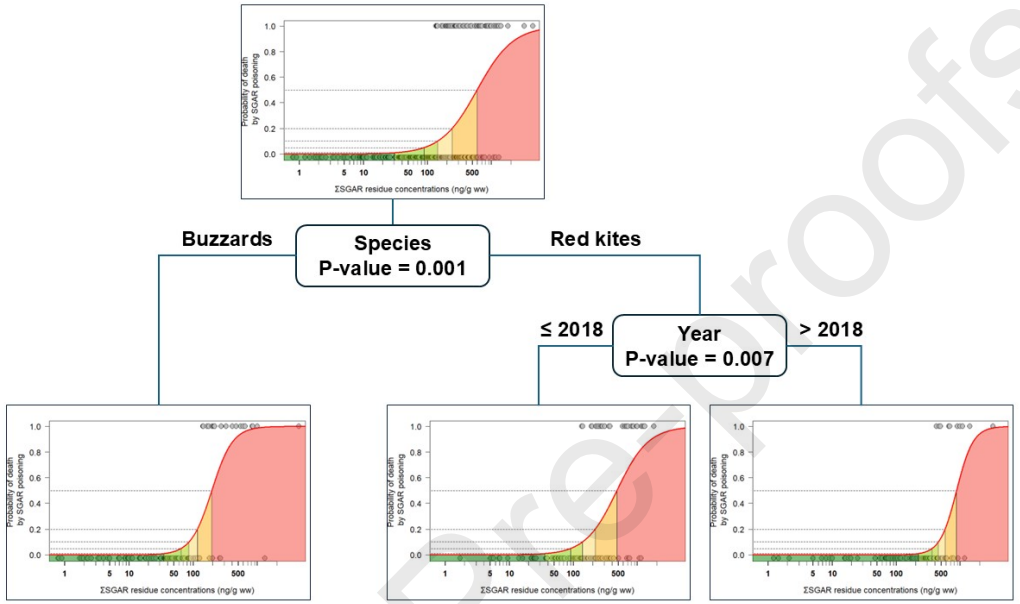


Table 1. Statistical summary showing the detection rate and the minimum, median, and maximum concentrations of each SGAR active substance (ng/g wet weight) and ΣSGARs in red kites (2015 – 2022) and buzzards (2009 – 2021) collected across Great Britain.

		Bromadiolone	Difenacoum	Brodifacoum	Difethialone	Flocoumafen	ΣSGARs
Red kites	**Detected**	196	230	229	43	2	248
	Non-detected	67	33	34	220	261	15
	Detection rate	74.5%	87.5%	87.1%	16.3%	0.8%	94.3%
	Minimum	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ
	Median	7.3	21.0	43.9	<LoQ	<LoQ	194.5
	Maximum	650.0	833.0	3200.0	1042.0	4.1	3223.7
Buzzards	**Detected**	104	116	97	6	5	141
	Non-detected	55	43	62	153	154	18

	Detection rate	65.4%	73.0%	61.0%	3.8%	3.1%	88.7%
	Minimum	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ
	Median	1.3	4.3	1.7	<LoQ	<LoQ	35.7
	Maximum	1300.0	440.0	4400.0	340.0	67.0	4433.0

Table 2. Toxicity threshold values (ng/g wet weight) in the liver for 1, 5, 10, 20 and 50% of lethal SGAR poisoning for red kites (2015 – 2022) and buzzards (2009 – 2021) collected in Great Britain.

Probability	**Red kites** (ΣSGAR ng/g ww)	**Buzzards** (ΣSGAR ng/g ww)
N	236	139
0.01	54.2	38.2
0.05	138.5	67.4
0.10	211.9	87.2
0.20	335.9	115.3
0.50	738.7	185.8

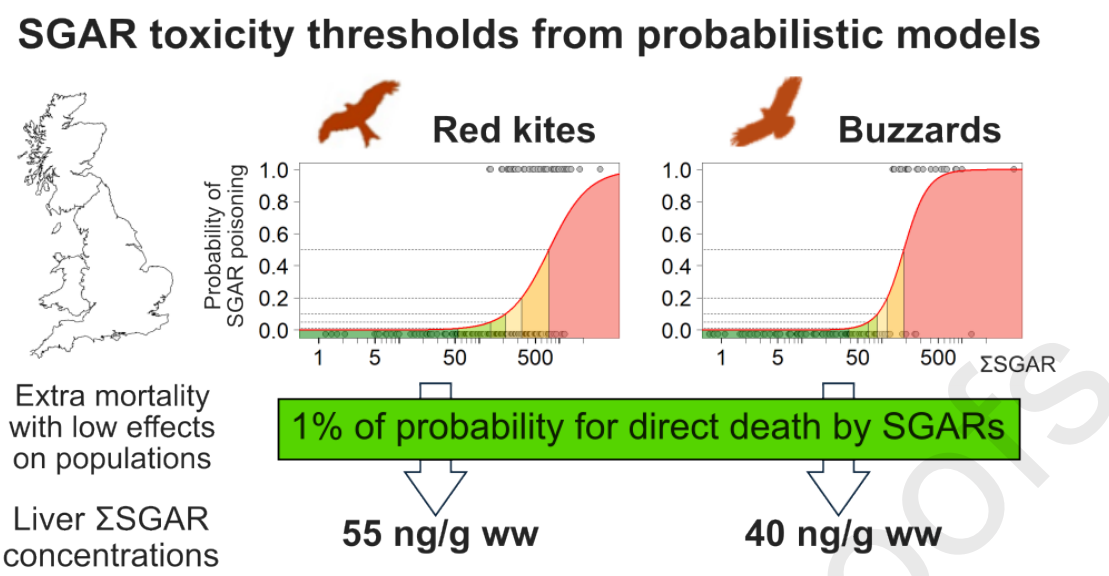
Table 3. Toxicity threshold values (ng/g wet weight) in the liver for 1, 5, 10, 20 and 50% of lethal SGAR poisoning for the three sub-sets of the data for red kites and buzzards collected in Great Britain, determined by MOB. Samples with unknown collection date and location were excluded.

Probability	**Buzzards (2009 – 2022)** (ΣSGAR ng/g ww)	**Red kites (2015 – 2018)** (ΣSGAR ng/g ww)	**Red kites (2019 – 2021)** (ΣSGAR ng/g ww)
N	107	119	93
0.01	34.9	35.5	220.6
0.05	64.6	90.3	361.3
0.10	85.6	137.7	451.7
0.20	115.6	217.6	575.5
0.50	194.0	475.9	871.0

Table 4. SGAR toxicity threshold values (ng/g wet weight in the liver) from the literature

References	**Threshold value** *(wet weight)*	**Species**	**Note**
Newton et al. (2000, 1999a, 1999b, 1994, 1990)	100 ng/g	Barn owl	(i) Fatally AR-poisoned birds had a liver residue concentration of >100 ng/g ww; (ii) Fatally poisoned owls in laboratory experiments had residues of 200 ng/g ww (Shore et al., 2005, 2001).
	200 ng/g	Barn owl	
Kaukeinen et al. (2000)	700 ng/g	Barn owl	Proposed by the Rodenticide Registrants Task Force. All barn owls with a liver residue exceeding this value died in previous field- and laboratory-based studies.
Thomas et al. (2011)	70 ng/g	Great horned owl	Expected ΣSGARs (brodifacoum, bromadiolone, difethialone) from probabilistic modelling for the probability of 20% of lethal poisoning
	160 ng/g	Barred owl	
	180 ng/g	Barn owl	
Elliott et al. (2024)	8.2 ng/g	Accipitridae	Expected ΣSGARs (brodifacoum, bromadiolone, difethialone) from probabilistic modelling for the probability of 20% of lethal poisoning
	7.9 ng/g	Falconidae	
	14.5 ng/g	Strigidae	
	0.32 ng/g	Tytonidae	

Graphical Abstract



Highlights

- Threshold values for SGAR effects were estimated by probabilistic modelling.

- Liver SGAR residues linked to the probability of lethal SGAR poisoning were assessed.

- Residues associated with 1% probability were 55 & 40 ng/g for red kites and buzzards.

- The thresholds did not significantly differ among the sex, age, or British country.

- Threshold values for red kites, but not buzzards, increased over years.

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Shinji Ozaki reports financial support was provided by Natural England. Shinji Ozaki reports financial support was provided by UK Research and Innovation Natural Environment Research Council. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

S. Ozaki: Formal analysis, Visualisation, Writing – Original draft preparation. **E.A. Barnett**: Data curation, Writing – Review & Editing. **H. Carter**: Investigation, Writing – Review & Editing. **J.S. Chaplow**: Writing – Review & Editing. **S. Charman**: Investigation, Data curation, Writing – Review & Editing. **M. Galloway**: Data curation, Writing – Review & Editing. **M.G. Pereira**: Investigation, Methodology, Writing – Review & Editing. **E. Potter**: Data Curation, Investigation, Writing – Review & Editing. **A.W. Sainsbury**: Resources, Writing – Review & Editing. **T. Shadbolt**: Resources, Writing – Review & Editing. **E.A. Sharp**: Investigation, Data curation, Writing – Review & Editing. **G. Shaw**: Conceptualisation, Supervision, Writing – Review & Editing. **D. Sleep**: Investigation, Writing – Review & Editing. **L.A. Walker**: Conceptualisation, Funding acquisition, Project administration, Supervision, Writing – Review & Editing. **S.M. Qassim**: Conceptualisation, Funding acquisition, Project administration, Writing – Review & Editing.