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The Relative Importance of Different Trophic Pathways for Secondary Exposure to Anticoagulant Rodenticides

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ABSTRACT: Secondary exposure of predators to anticoagulant rodenticides, and in particular second generation anticoagulant rodenticides (SGARs), is a global phenomenon. The widespread and large-scale nature of this exposure has attracted considerable concern, although the consequences in terms of likelihood of poisoning of individuals and resultant impacts on populations are not well characterised. Secondary exposure of predators may as rise from once or more of: (i) eating contaminated commensal rodents subject to control (target species are typically rats and house mice); (ii) consumption of contaminated non-target small mammals (such as *Peromyscus*, *Microtus*, and *Apodemus* species) that encounter and feed on what are rodent-attractive baits; (iii) consumption of non-rodent vertebrate and invertebrate prey that may also incidentally encounter and eat baits. We hypothesised that predators feeding primarily on target species may be most at risk of exposure to SGARs while those predominantly taking non-mammalian prey may be at least risk. We tested this hypothesis by comparing exposure, determined from the presence and magnitude of SGAR liver residues, in red kites, which feed extensively on rats; in barn owls, kestrels, and tawny owls that feed widely on non-target small mammals; and in sparrowhawks that feed predominantly on small birds. We found that the scale and magnitude of exposure was broadly consistent with our hypothesis, and that controlling for age in the analysis could be important as older birds can accumulate residues with age. However, exposure in kestrels was typically greater than that in barn owls and tawny owls, despite what is thought to be a general similarity among the species in their diets. We discuss the relative importance of trophic pathways relative to other factors that may drive secondary exposure in predators, and confirm that species that feed on rats or other target species may be at most risk of exposure and poisoning.

KEY WORDS: *Accipiter nisus*, exposure, *Falco tinnunculus,* kestrel, liver residue, *Milvus milvus*, nontarget risk, red kite, second-generation anticoagulant rodenticide, secondary hazard, sparrowhawk, *Strix aluco,* tawny owl, *Tyto alba*

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The use of anticoagulant rodenticides (ARs) to control rodent populations is designed to reduce the risk of disease transmission (to people and agricultural livestock) and limit what can be billions of dollars of damage to infrastructure, field, and stored crops and forestry (Battersby 2004, 2015; Lund 2015, Meyer and Kaukeinen 2015, Shore 2018). ARs have also been used extensively in conservation to eradicate introduced rodents from islands where their predation of eggs and chicks threatens seabird colonies (Howald et al. 2015). However, the extensive use of ARs, particularly second-generation anticoagulant rodenticides (SGARs), has led to unintentional widespread primary exposure of non-target vertebrate and invertebrate wildlife (Tosh et al. 2012, Elliott et al. 2014, Geduhn et al. 2014, Shore and Coeurdassier 2018). This in turn has led to global secondary exposure of predators that feed on contaminated target and non-target species, as recently reviewed by López-Perea and Mateo (2018). Environmental risk assements indicate that SGARs pose a significant secondary poisoning risk and this has been borne out reports of poisonings (Rattner et al. 2014, Murray 2017, 2018), although the ecological significance for most species remains unclear (but see Nogeire et al. 2015). It is clear, however, that there is extensive secondary exposure across a very diverse range of mammalian and avian predators and scavengers (see review by López-Perea and Mateo 2018 and studies by Geduhn et al. 2015, 2016; Huang et al. 2016, Ruiz-Suárez et al. 2016, Salim et al. 2016, Herring et al. 2017, Hindmarsh et al. 2017, Justice-Allen and Loyd 2017, Thomas et al. 2017, Vyas 2017, Shore et al. 2017, Walker et al. 2017, Elmeros et al. 2018, Sainsbury et al. 2018). Given the many studies of secondary exposure that have been conducted, it might be expected that it should be possible to identify which trophic transfer pathways are most important for SGARs and which species may therefore be most at risk. Such knowledge and understanding would enhance the targeting of mitigation measures towards the potentially most vulnerable (in terms of exposure) species. However, direct comparison of different studies is hampered by the fact that such studies are typically not contemporaneous, have been conducted in different geographical areas, and have used analytical methodologies that differ in their sensitivity. Studies from the UK alone have shown that accumulation of residues by predators varies geograph-

ically, because of differences in use (Shore et al. 2015), and can change over time (Sainsbury et al. 2018). Furthermore, most laboratories have adopted Liquid Chromatography Tandem Mass Spectrometry (LCMS MS) analysis at some stage over the last 15 years and this has reduced detection limits for SGAR residues in tissues by approximately an order of magnitude. Data from studies using older analytical techniques (such as High Performance Liquid Chromatography coupled with fluorescence detection; HPLC/FD) are not directly comparable with studies that have used LCMSMS unless common limits of quantification (LoQs) are adopted (Dowding et al. 2010).

In the UK, long-term monitoring of liver SGAR residues has been conducted in the barn owl (*Tyto alba*) as part of the Predatory Bird Monitoring Scheme (PBMS; http://pbms.ceh.ac.uk/). We have used spatial and temporal variation in liver concentrations to indicate change in exposure. The barn owl feeds predominantly on small mammals and the field vole (*Microtus agrestis*) is the preferred prey species, although extensive numbers of wood mice (*Apodemus sylvaticus*) and bank voles (*Myodes glareolus*) are also taken (Love et al. 2000). We have also conducted other studies on a variety of other raptors and owls over varying periods alongside the long-term monitoring of barn owls. These additional studies provide an opportunity to compare the prevalence and magnitude of residues in various species with that in the barn owl, all comparisons using birds from the same time period and geographical area and analysed using the same techniques. The results from these comparisons would allow us to draw general inferences about the relative risk from exposure through different trophic pathways.

The aim of the current study was to compare the liver SGAR concentrations in barn owls with those in the kestrel (*Falco tinnunculus*), tawny owl (*Strix aluco*), sparrowhawk (*Accipiter nisus*), and red kite (*Milvus milvus*). There is considerable dietary overlap between the barn owl, kestrel, and tawny owl, all three predominantly feeding on non-target voles and mice, and we hypothesised that exposure in the three species would be broadly similar. Sparrowhawks predominately take avian prey (Newton 1986). Although small birds can enter boxes and feed on bait and can also be exposed secondarily through eating contaminated insects (Vyas 2017, Shore and Coeurdassier 2018), we hypothesised that this may be a less important transfer pathway than that involving small mammals. Therefore, we hypothesised that residues would be less prevalent and/or lower in sparrowhawks than in barn owls. Red kites are scavengers and although this in itself may not necessarily predispose them to higher exposure to SGARs (Shore and Coeurdassier 2018), they eat rats, which are a main target for control using SGARs. Thus, we anticipated SGAR contamination in red kites would be significantly greater than in barn owls, and of the five species examined overall, kites would be at most risk from secondary poisoning.

METHODS

The data for each of the four comparative analyses were taken from the long-term monitoring of barn owls conducted by the PBMS (Walker et al. 2014, Shore et al. 2017) and from shorter-term specific PBMS studies of residues in tawny owls (Walker et al. 2008b), kestrels (Walker et al. 2007), sparrowhawks (Walker et al. 2015) and red kites (Walker et al. 2016). In all cases, livers were taken from the carcasses of birds found dead by members of the public and had died from various causes, particularly starvation and collisions (Walker et al. 2008a). The periods over which data included in the present analysis were collected were 1997-2005 for the kestrel vs barn owls analysis, 2003-2005 for tawny owl vs barn owl, and 2010-2013 for the comparison between sparrowhawk and barn owl and red kite and barn owl. The analytical methodology used to determine liver SGAR residues are given in the various papers and reports and involved analysis by HPLC-FD prior to 2006 and LCMSMS subsequently.

We only included data in our analysis for birds known to have died in England as the prevalence and magnitude of SGAR residues in barn owls varies between England, Scotland, and Wales, reflecting lower use in those countries (Shore et al. 2015). Data were also further restricted to birds of known age class; first-year birds were classed as individuals that hatched in the current or previous year to that in which they were found dead. Age class was taken into account for the analyses as liver SGAR residues can increase with age (e.g., Ruiz-Suárez et al. 2016).

A common LoQ was used for all individual SGAR residues in each comparative study and was 25 ng/g wet weight (ww) for analyses conducted by HPLC-FD and 2.3 ng/g ww for comparisons involving only LCMSMS analysis. Only data for summed (Σ) SGARS are presented in the current study here and were calculated by summing the concentrations of each individual SGAR detected; non-detected values were treated as zeros.

Comparisons between species of prevalence of detected residues were conducted using Fisher's exact tests; adult and first-year birds were treated as separate groups. Differences between species and age classes in the magnitude of detected residues (non-detected values not included) were by Kruskal-Wallis non-parametric ANOVA coupled with *post-hoc* Dunn's multiple comparison tests; parametric tests were not used as the underlying assumptions of the tests were violated.

RESULTS

The proportion of barn owls with detected residues between 1997-2005 was between 30% and 40% (Figure 1) reflecting that the analysis was by HPLC-FD with an associated high LoQ; measures in later studies indicate a prevalence of 80-100% (Figure 3, Figure 4) with most of the additional birds containing low residues that are undetectable by HPLC-FD (Walker et al. 2010). When we compared the prevalence of residues in barn owls with that in kestrels (also measured by HPLC-FD), that had also been measured using HPLC-FD, we found that the proportion of both adult and first-year birds that contained detectable residues of at least one SGAR was significantly higher in kestrels (Figure 1). In addition, there was an age difference in kestrels; the proportion of

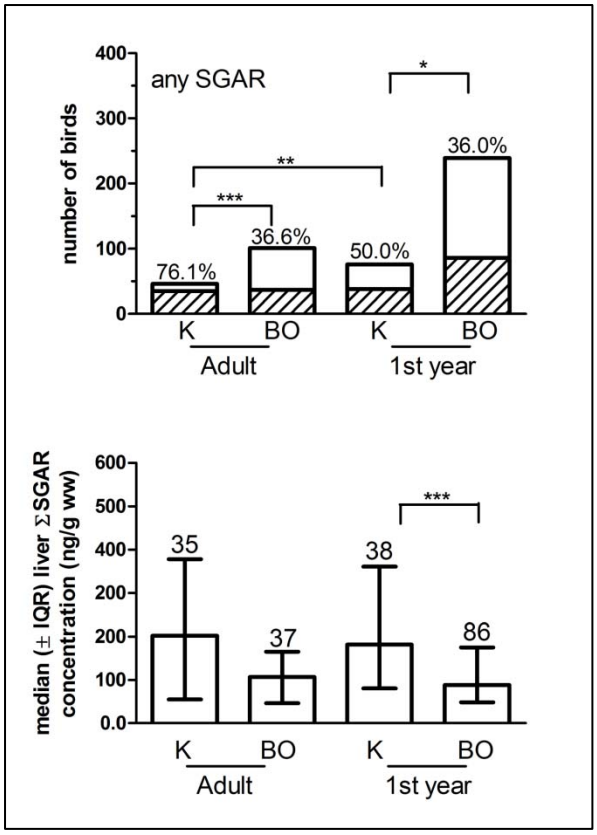

Figure 1. Prevalence and magnitude of liver SGAR residues in adult and 1st-year kestrels (K) and barn owls (BO) from England in the period 1997-2005 (HPLC-FD detection method).

***Top graph*: Numbers of birds with at least one detected liver SGAR residue (hatched part of bar) or no detected residue (clear part of bar); the % of the sample with at least one detectable liver SGAR residue is indicated above the bar.**

***Bottom graph*: Median (and interquartile range) ΣSGAR concentration for detected residues. Values above the bars indicate the number of birds in the sample. In both graphs, significant differences between bars (see methods for details of statistical tests) are indicated as: * P < 0.05, **P < 0.01, ***P < 0.001)**

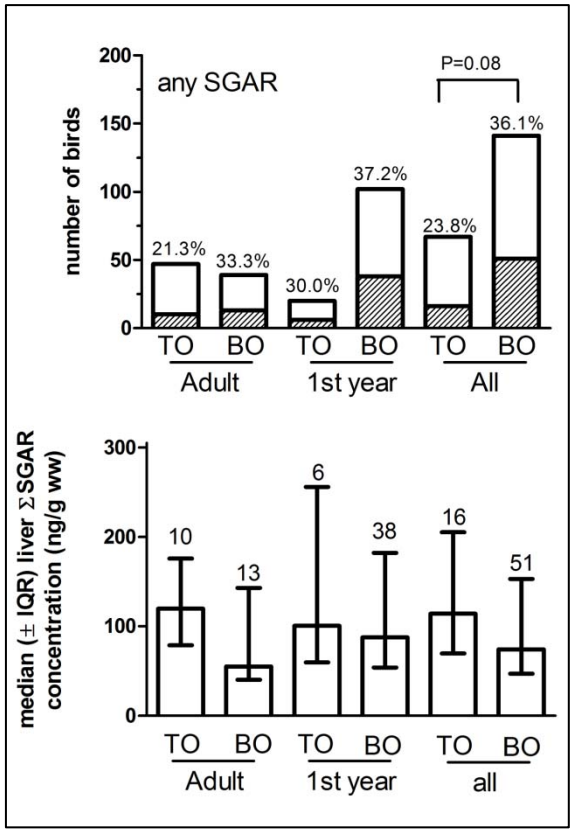

Figure 2. Prevalence and magnitude of liver SGAR residues in adult and 1st-year tawny owls (TO) and barn owls (BO) from England in the period 2003-2005 (HPLC-FD detection method).

***Top graph*: Numbers of birds with at least one detected liver SGAR residue (hatched part of bar) or no detected residue (clear part of bar); the % of the sample with at least one detectable liver SGAR residue is indicated above the bar.**

***Bottom graph*: Median (and interquartile range) ΣSGAR concentration for detected residues. Values above the bars indicate the number of birds in the sample**.

birds with a detectable residue was higher in adults than first-years; this age difference was not apparent in barn owls (Figure 1). The magnitude of those residues that were detected was also higher in kestrels than barn owls by a factor of approximately two. This was true for both age classes but the difference was only statistically significant in first-year birds, perhaps reflecting the greater power of the analysis afforded by the large sample size of first-year barn owls.

The comparison between barn owls and tawny owls was also based on residues measured by HPLC-FD. However, in contrast to the differences between kestrels and barn owls, we found no significant difference between tawny owls and barn owls in either the prevalence of detected residues or in residue magnitude (Figure 2). There were likewise no significant differences between age classes. When data for the two age classes were pooled, the prevalence of residues was found to be marginally higher in barn owls than tawny owls, although this difference just failed to make statistical significance (P = 0.08).

Comparative data for liver ΣSGARs in sparrowhawk and barn owl were generated using LCMSMS. This was reflected by the overall detection rate of liver SGAR residues (Figure 3). In fact, all the adult birds of both species had at least one detectable liver SGAR residue. There was also no species difference for the proportion of first-year birds with residues but for both species, fewer first-years had a detectable residue compared with adults. The magnitude of the detected residues was approximately 2-fold greater in barn owls than sparrowhawks for both age classes, but the difference was only statis-

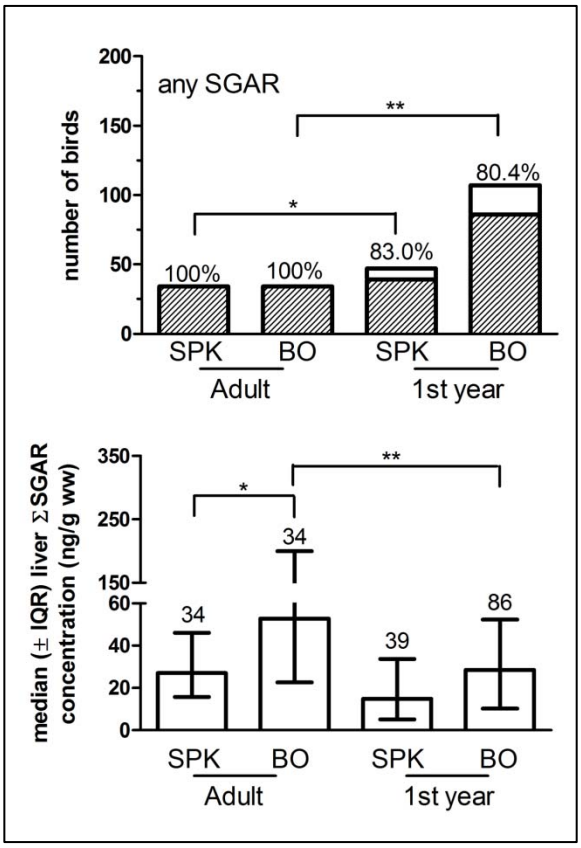

Figure 3. Prevalence and magnitude of liver SGAR residues in adult and 1st-year sparrowhawks (SPK) and barn owls (BO) from England in the period 2010-2013 (LCMSMS detection method).

***Top graph*: Numbers of birds with at least one detected liver SGAR residue (hatched part of bar) or no detected residue (clear part of bar); the % of the sample with at least one detectable liver SGAR residue is indicated above the bar.**

***Bottom graph*: Median (and interquartile range) ΣSGAR concentration for detected residues. Values above the bars indicate the number of birds in the sample. In both graphs, significant differences between bars (see methods for details of statistical tests) are indicated as: * P < 0.05, **P < 0.01).**

tically significant in adults (Figure 3).

The data for the red kite vs barn owl comparison were also from the same period as that for the sparrowhawk/barn owl comparison. All except 1 out of 36 adults and 1 out of 9 first-year red kites had detectable liver SGAR residues, as compared with 100% of adult barn owls but 80% of first-year owls (Figure 4). The magnitude of detected residues was some 4-fold higher in red kites than barn owls, both for adults and first-years, but the difference was only statistically significant in adult birds. The lack of significance for what was a similar magnitude difference between first-year birds may have been due to the low number of first-year kites and resultant low power of the statistical analysis.

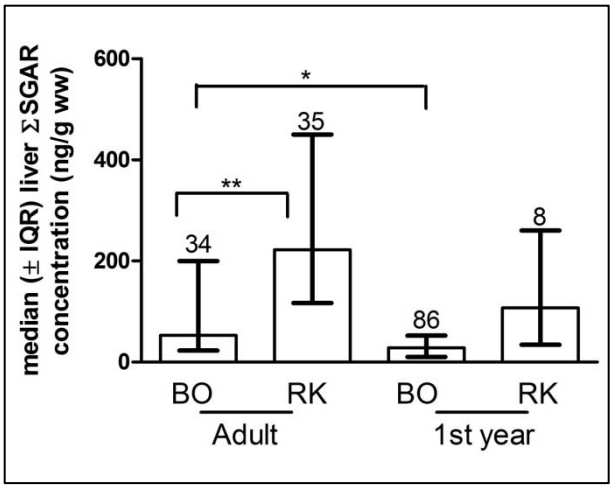

Figure 4. Median (and interquartile range) ΣSGAR concentration for detected liver SGAR residues in adult and 1st-year barn owls (BO) and red kites (RK) from England in the period 2010-2013 (LCMSMS detection method). Values above the bars indicate the number of birds in the sample. Significant differences between bars (see methods for details of statistical tests) are indicated as: * P < 0.05, **P < 0.01).

DISCUSSION

Our use of the barn owl as a focal point for comparisons has allowed us to examine differences in exposure in species that utilise the same and different trophic pathways, although it is recognised that any differences in residue prevalence and magnitude may also partly be due to variation in liver binding capacity and accumulation. Our first hypothesis, that species (barn owl, kestrel, tawny owl) utilising the same (small mammal prey) trophic pathway would have similar exposure to SGARs, was only partly supported. We found that exposure appeared broadly similar in tawny and barn owls, despite previously concluding it may be lower in tawny owls; this was based on an analysis that had not controlled for age class or within-UK provenance (Walker et al. 2008b). However, inter-year, within-country, and more local spatial differences may exist between the species. For example, tawny owls prey more on wood mice and bank voles compared with barn owls which often hunt field voles, and it is wood mice and bank voles that are typically more likely to encounter and eat bait than field voles (Brakes and Smith 2005). Indeed, tawny owls hunting mice and voles in woods used for rearing gamebirds may be at particular risk of exposure, as gamekeepers use ARs to control rats around rearing pens (McDonald and Harris 2000). However, barn owls also take high numbers of wood mice and bank voles in years and habitats with low field vole numbers (Love et al. 2000) and may hunt closer to farms where non-target prey are more likely to encounter bait and feed on bait (Tosh et al. 2012, Geduhn et al. 2014). These conflicting exposure factors may effectively cancel each other out

with the result that liver SGAR residues are similar overall in the two owl species. However, our results give a clear indication of significantly greater exposure/ accumulation of SGARs in kestrels than in barn owls, and by inference, than in tawny owls. Both the proportion of birds exposed and the median magnitude of detected residues were higher in kestrels; this was evident in first-year birds and maintained thereafter. It is uncertain whether the reasons for the species differences are ecological, physiological, or both. The relatively high median ΣSGAR concentrations in adult and first-year kestrels is a cause for concern as it is similar to those for red kites (Figures 2 and 4), which are known to be vulnerable to secondary poisoning. Unlike with kites, the PBMS has rarely found evidence of kestrels poisoned by SGARs, but populations have declined in the UK. The causes of this decline in numbers may be multiple, but probably include agricultural intensification (British Trust for Ornithology 2017a). The role of SGARs, if any, is under investigation.

The results from the current analyses also indicate there are significant differences in exposure between species utilising different trophic pathways. Our expectation that exposure in sparrowhawks would be lower than in barn owls was only partly supported. The scale of exposure (proportion of individuals with detected residues) was equally high in sparrowhawks and barn owls but the rate of accumulation appeared lower in sparrowhawks, perhaps because the rate of encounter with contaminated prey is lower, although physiological differences between the species again cannot be ruled out. Nevertheless, it is evident that exposure in raptors that feed on small birds is widespread and common (see also Hughes et al. 2013), suggesting that the avian trophic pathway is widely contaminated (Vyas 2017) and may be almost as an important transfer pathway as that of small mammal prey.

Our analyses also indicate that red kites, which feed on target species (rats), do indeed have relatively high liver SGAR residues and almost all (first-year and adult) birds, particularly in England, are exposed to SGARs. This is consistent with the concept that feeding/ scavenging on target prey confers a high risk of exposure. High levels of exposure would normally be expected to increase the risk of poisoning. In 2015, necropsy and residue data for red kites that died were collated for the first time from across the various exposure monitoring and poisoning investigation laboratories in the UK. This yielded information for 26 red kites from England and Wales and 8 kites from Scotland (Walker et al. 2017). SGARs were judged a likely cause of death if there was necropsy evidence of haemorrhaging (unrelated to trauma) and liver SGAR residues were detected. SGARs were implicated in the deaths of 9 (35%) of the birds from England and Wales and 2 (25%) of the red kites from Scotland. Despite this apparent high poisoning pressure, red kite populations are still increasing in the UK following their reintroductions in several regions (British Trust for Ornithology 2017b).

The scale of secondary exposure in birds of prey and other wildlife has led to the introduction of stewardship regime for SGARs in the UK (Buckle et al. 2017). A major aim of this regime is to reduce non-target primary and secondary exposure by improving best practice in usage, enhancing effective control of resistance populations, and reducing the extent of open area baiting. This has also involved relaxation of the regulations that limited the use (to indoors only) of the most acutely toxic compounds (brodifacoum, flocoumafen, and difethialone). These compounds are now also available for use in and around buildings and are expected to be deployed against rat populations that are resistant to other SGARs. While the intention is that stewardship will reduce primary exposure of non-target small mammals and the predators that feed on them, it is less clear to what extent it will reduce any risk to predators that feed on target prey, given those targets may be more likely in future to be contaminated by the most acutely toxic SGARs. The future exposure of red kites and other species that feed on commensal rodents, such as the polecat (Shore 2003, Sainsbury 2018), needs to be monitored as it may change in terms of which SGARs animals are exposed to and the magnitude of that exposure.

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