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Anticoagulant Rodenticide Contamination of Terrestrial Birds of Prey from Western Canada: Patterns and Trends, 1988–2018

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Abstract: As the dominant means for control of pest rodent populations globally, anticoagulant rodenticides (ARs), particularly the second-generation compounds (SGARs), have widely contaminated nontarget organisms. We present data on hepatic residues of ARs in 741 raptorial birds found dead or brought into rehabilitation centers in British Columbia, Canada, over a 30-year period from 1988 to 2018. Exposure varied by species, by proximity to residential areas, and over time, with at least one SGAR residue detected in 74% of individuals and multiple residues in 50% of individuals. By comparison, we detected first-generation compounds in <5% of the raptors. Highest rates of exposure were in barred owls (*Strix varia*), 96%, and great horned owls (*Bubo virginianus*), 81%, species with diverse diets, including rats (*Rattus norvegicus* and *Rattus rattus*), and inhabiting suburban and intensive agricultural habitats. Barn owls (*Tyto alba*), mainly a vole (*Microtus*) eater, had a lower incidence of exposure of 65%. Putatively, bird-eating raptors also had a relatively high incidence of exposure, with 75% of Cooper's hawks (*Accipiter cooperii*) and 60% of sharp-shinned hawks (*Accipiter striatus*) exposed. Concentrations of SGARs varied greatly, for example, in barred owls, the geometric mean ∑SGAR = 0.13, ranging from <0.005 to 1.81 μg/g wet weight ($n = 208$). Barred owls had significantly higher ∑SGAR concentrations than all other species, driven by significantly higher bromadiolone concentrations, which was predicted by the proportion of residential land within their home ranges. Preliminary indications that risk mitigation measures implemented in 2013 are having an influence on exposure include a decrease in mean concentrations of brodifacoum and difethialone in barred and great horned owls and an increase in bromodialone around that inflection point. *Environ Toxicol Chem* 2022;00:1–15. © 2022 Her Majesty the Queen in Right of Canada. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC. Reproduced with the permission of the Minister of Environment and Climate Change Canada.

Keywords: Anticoagulant rodenticides; Raptors; Nontarget exposure; Secondary poisoning; Ecotoxicology

INTRODUCTION

With approximately 2550 extant species, rodents are the most diverse of the mammalian orders, having radiated into virtually all terrestrial and aquatic niches (Burgin et al., 2018). They constitute the primary group of vertebrate pests considered responsible for spreading disease and causing billions of dollars of damage annually to crops, food stores, and infrastructure worldwide (Jacob & Buckle, 2018). Many rodent species are important pests in agricultural situations, particularly in the tropics. However, a relatively small number are global commensal species, those responsible for domestic infestations and damage as well as to damage food stores and crops: the house mouse *Mus musculus*, the black rat *Rattus rattus*, and the Norway rat *Rattus norvegicus*. Controlling rodent infestations has proven to be challenging for human populations, likely since at least the neolithic age, when bulk storage of food became common practice (Reperant & Osterhaus, 2014).

For the past six decades, anticoagulant rodenticides (ARs) have provided an effective and cost-efficient tool for containing pest rodent infestations. In Canada and the United States, which attempt to harmonize pesticide regulation, three

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second-generation AR (SGAR) active ingredients are registered for use, brodifacoum, bromadiolone, and difethialone, and difenacoum in the United States only. From a regulatory perspective, SGARs are persistent, bioaccumulative, and toxic and would likely have been removed from the market in many jurisdictions; but they remain because of the demand for rodent control and lack of effective alternatives (Elliott et al., 2016; Jacob & Buckle, 2018). As with the first-generation compounds (FGARs), such as warfarin, chlorophacinone, and diphacinone, they function by inhibiting the hepatic pathways for regeneration of vitamin K and the synthesis of clotting factors. A relatively small dose of SGAR will cause fatal bleeding and death within a few days (Watt et al., 2005). Also, ARs exhibit other traits necessary for effective rodent control, being tasteless and odorless and causing delayed mortality, all of which mitigate against bait aversion. The acute toxicity of SGARs also extends to nontarget species of mammals, birds, and reptiles, which can be secondarily exposed by consuming targeted or nontarget rodents and other prey species that can enter bait stations (López-Perea & Mateo, 2018; Newton et al., 1990).

Evidence of the widespread SGAR exposure and poisoning of nontarget predators and scavengers has continued to increase (López-Perea & Mateo, 2018; Nakayama et al., 2019; Serieys et al., 2019; Okoniewski et al., 2021; Rial-Berriel et al., 2021; Thornton et al., 2022). Concern over their spread as contaminants has led to an intensified search for alternatives, as well as for regulatory and voluntary mitigation measures (Elliott et al., 2016; Witmer, 2018). We have updated previous reports on contamination of avian predators and scavengers (Albert et al., 2010; Hindmarch et al., 2019; Huang et al., 2016; Thomas et al., 2011) with an expanded sample, including putative bird-eating species. We have now accumulated data over a 30-year period on 741 specimens of mainly owl, hawk, and falcon species. Thus, in addition to species and spatial trends and patterns, we are able to examine the temporal nature of exposure and possible impact of regulatory changes implemented in 2013. We are conducting a separate assessment of anticoagulant poisoning from detailed postmortem examinations and the relationship to liver residue levels.

MATERIALS AND METHODS

Study area and sample collection

Between 1988 and 2018, we obtained 741 raptor carcasses fortuitously from locations in western Canada, mainly British Columbia, with one sample from the Red Deer Highway in Alberta and seven samples from the Yukon Territory (Figure 1; Supporting Information, Table S1). We received raptor carcasses from the British Columbia Ministry of Environment and Climate Change Strategy, the Yukon Ministry of Environment, the Canadian Wildlife Service, Monika's Wildlife Shelter, the Orphaned Wildlife Rehabilitation Society, the Wildlife Rescue Association, the Mountainaire Avian Rescue Society, Fur and Feather Taxidermy, the South Okanagan Rehabilitation Center for Owls, the North Island Wildlife Recovery Association, and the general public.

Necropsy

The majority of raptors were received dead on arrival to the rehabilitation centers and agencies, while others died in custody or were euthanized because of the severity of injuries. We did not

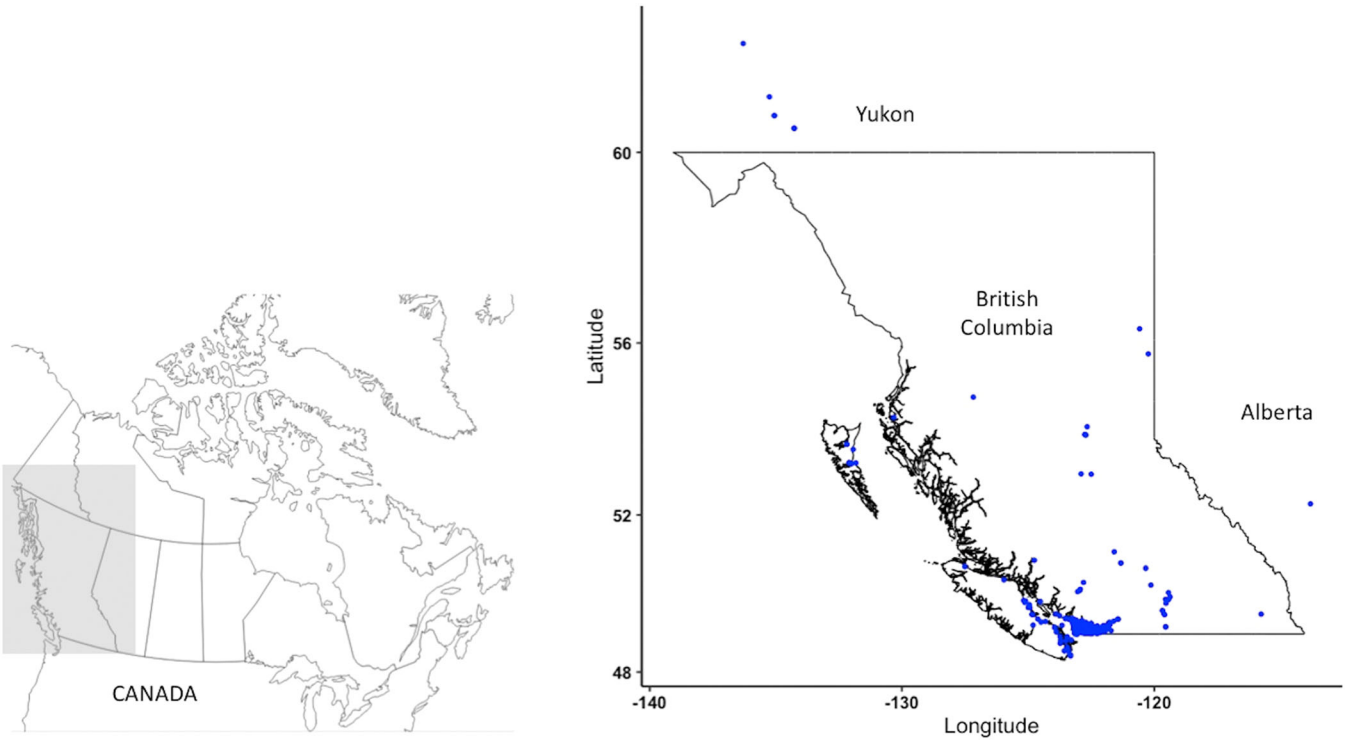

FIGURE 1: Collection locations of terrestrial raptor carcasses from British Columbia and surrounding areas, 1988–2018 ($n = 741$).

include individuals held in captivity >30 days before euthanization, to ensure that SGAR residues remained in their tissues at concentrations representative of initial exposure. This was a conservative estimate because the half-lives of SGARs in rat livers have been estimated at 318 days for bromadiolone (European Food Safety Authority, 2010), 28.5 days for difethialone (Horak et al., 2018), and 113.5–350 days for brodifacoum (Horak et al., 2018). Avian-specific half-lives for brodifacoum reportedly range from 49.5 to 297 days (Rattner et al., 2020). All carcasses were stored frozen at −20 °C at the Pacific Wildlife Research Centre, Delta, British Columbia, Canada, with some necropsied on-site; but most were transported to the British Columbia Animal Health Laboratory, Abbotsford, British Columbia, Canada for necropsy. Cause of death was determined during necropsy by the veterinary pathologist and information provided by wildlife rehabilitation personnel, such as circumstances at the time it was found and, in the case of birds still alive, on condition and behavior of the bird. Diagnosis of AR poisoning followed the principles and methods described by Murray (2018). We diagnosed AR poisoning as the final cause of death when necropsy indicated hemorrhage, bleed-out, or pallor in the absence of other potentially lethal traumatic injury, disease, emaciation, or pesticide or lead poisoning, with no evidence of blood clotting. For the present purposes, for final diagnosis, we also factored in the later detection of anticoagulant residues in the liver (Hindmarch et al., 2019; Thomas et al., 2011); but cause of death was not diagnosed based on AR residue concentration alone.

AR residue analysis

Livers were removed from carcasses and analyzed for FGARs and SGARs. Chemical analysis was conducted at the National Wildlife Research Centre (Ottawa, ON, Canada) following the methodology described previously (Albert et al., 2010; Hindmarch et al., 2019). Chemical analysis generally followed methods described in Albert et al. (2010), with some procedural and instrumentation changes, which are described. Liver samples were homogenized, accurately weighed, and transferred into a 15-ml polyethylene centrifuge tube, spiked with an internal standard solution, with 3 ml of acetonitrile added. Following further homogenization, an aliquot of pre-QuEChERS (quick, easy, cheap, effective, rugged, and safe) salt was added, followed by vortexing, shaking, and centrifuging with supernatant transferred into a UCT QuEChERS tube (ECQUUS1215CT; Chromatographic Specialties) to which 50 mg of SupelClean ENVI-18 had previously been added. Following further shaking and centrifuging, evaporation to dryness, and reconstitution in methanol, the final extract was filtered through a Life Sciences Acrodisc 13 mm, 0.45 μm Nylon filter using a 1-ml disposable Luer-Lok syringe directly into a 2-ml polytetrafluoroethylene/silicone septa autosampler vial prior to injection. Samples were then injected onto a liquid chromatography system (Agilent 1200 HPLC System; Agilent Technologies). Mass spectrometric analysis was done using an AB Sciex API 5000 Triple Quadropole Mass Spectrometer with the TurboSpray ion source in negative polarity using multiple reaction monitoring.

Quality assurance and control methods included the following. An aliquot of a clean double-crested cormorant (*Phalacrocorax auritus*) liver pool, prepared in-house and containing undetectable amounts of rodenticides, was spiked with internal standard solution and extracted with each set (nine samples) to monitor possible contamination from the procedure. Calibration accuracy was validated by determining the concentration of second source standards against our daily calibration curve; values had to be between 80% and 120% to be conformant. We measured method accuracy by spiking an aliquot of the clean cormorant liver pool with internal standard solution, warfarin (to represent first-generation rodenticides) and brodifacoum (for the second-generation rodenticides) and analyzed it with each set (nine samples). The acceptable range was 80%–120%. And finally, method precision was evaluated by extracting one random raptor liver sample per set (nine samples) in duplicate. The relative percent difference had to be <15%.

Statistical methods

Method reporting limits. Mass spectrometry analytical reporting limits (MRL) varied over the 30-year-study period for individual compounds, ranging from 0.0006 to 0.01 μg/g for FGARs (pindone, warfarin, chlorophacinone, and diphacinone) and from 0.0006 to 0.005 μg/g for SGARs (brodifacoum, bromadiolone, and difethialone; Supporting Information, Table S2). Those changes in MRL were factored into assessments of exposure. For assignment of a positive detection and, therefore, exposure to an AR in all figures, tables, and summary statistics, for any FGAR, the concentration was ≥0.01 μg/g wet weight and, for any SGAR, ≥0.005 μg/g wet weight. Those criteria are based on the least sensitive MRL for each residue type during the study period and allowed the comparison of the percentage of detections over time and across species. The proportions of raptors with reported trace detections were 8.1% for bromadiolone ($n = 60$, range 0.0001–0.004 μg/g wet wt), 6.7% for brodifacoum ($n = 50$, range 0.0005–0.0043 μg/g wet wt), and 6.1% for difethialone ($n = 45$, range 0.0004–0.0043 μg/g wet wt). Trace values were reported in <2% of raptors for all FGARs (see Supporting Information II, Table S4). Those trace values, along with nondetects, were assigned half the highest study period MRL.

For all statistical analyses and data summarization, nondetects were assigned half the MRL. We compared statistical results using the substitution method with linear models to censored regressions using the cenanova function in R package NADA2 (Julian & Helsel, 2021) and found no differences in results (see Supporting Information II). Throughout the present study, exposure is reported as the percentage of raptors with a positive AR detection, and hepatic concentrations are reported as the geometric mean and range or geometric mean ± 95% confidence interval because AR residue concentrations were inflated by low values.

To consider exposure to more than one SGAR compound, we have summed the concentrations to produce a ∑SGAR value. We opted for that simple approach as has been the convention in many previous reports (see López-Perea & Mateo, 2018). We do

recognize that the summing of concentrations does not take into account factors such as variation in molecular weight and in relative toxicity (Rattner & Harvey, 2021). However, despite the common mode of action, there are currently insufficient robust data on the relative toxicity of SGARs to produce and use a toxic equivalent approach, as has been developed for other contaminants such as dioxin-like compounds or cyclodiene insecticides (Elliott & Bishop, 2011; Van den Berg et al., 2006).

Our data are biased by the opportunistic nature of collections and unequal sample sizes by species and years. It is also important to emphasize that the majority of carcasses came from the southwestern corner (the Lower Mainland region) of British Columbia and, at least by Canadian standards, are representative of exposure in habitats which are relatively urbanized or under intensive agricultural use. Thus, they likely do not represent exposure to ARs over the broad expanse of forested or grassland habitats across Canadian ecosystems.

Species-level comparisons. Summary statistics were calculated and visualized in R (Ver 3.6.2; R Foundation for Statistical Computing, 2021). We assessed differences in residue ($\log_{10}$-transformed total SGAR, bromadiolone, brodifacoum, and difethialone) concentrations between species with $n > 30$ samples using linear models fit by maximum likelihood estimation (R Foundation for Statistical Computing, 2021). Post hoc (Tukey) pairwise comparisons of estimated marginal means of fitted models were performed in the R package "emmeans" (Lenth et al., 2021) to compare mean contaminant concentrations between species at a significance level of 0.05.

Land use and spatial analysis. We quantified the amount of commercial, industrial, residential, and agricultural land within a 1-km radius (3 km², 300 ha) for the following species: barn owl, great horned owl, barred owl, red-tailed hawk, and Cooper's hawk ($n = 393$) using geographic information system software (Environmental Systems Research Institute, 2019; Figure 2). We only included individuals with an exact collection location. We used a 1-km radius because this approximates the average home range for the great horned owl, barred owl, barn owl, and red-tailed hawk, which has been estimated at 3 km² (Houston et al., 1998; Mazur & James, 2021; Nicholls & Warner, 1972; Petersen, 1979; Smith et al., 2003; Taylor, 1994). The average 95% kernel density home range of Cooper's hawks in the Lower Mainland was estimated to have a radius of 1.23 km (Brogan et al., 2017), but we used a 1-km radius for consistency with the other species. Land use within the circular plots was derived from iMap BC, British Columbia's Provincial Map Repository (Province of British Columbia, 2021), and by creating land-use layers with Google Earth ortho photos (Google, 2021). We used Google Earth historical imagery to ensure that the surrounding land use was accurate for the year that the individual was found.

Log transformation of the heavily zero-inflated dependent and independent variables did not reduce skew, so we conducted nonparametric tests. We tested for collinearity, and agricultural was highly correlated with the other land-use variables and excluded from further analyses. Total SGAR, bromadiolone, and brodifacoum exposures were transformed into binary variables; and raptors were grouped into ≥0.1 μg/g concentration and <0.1 μg/g concentration. We used this threshold because 0.1–0.2 μg/g SGARs in the liver has been identified as the "potentially lethal range" for free-ranging barn owls in the United Kingdom (Newton et al., 1990, 1999). Similarly, in captive eastern screech owls, coagulopathy was associated with liver concentrations >0.1 μg/g (Rattner et al., 2014), and 0.1 μg/g has been used as a threshold value determined in other AR exposure studies (Badry et al., 2021; Thomas et al., 2011).

Logistic regressions were used to assess how the proportion of residential and industrial/commercial lands within the approximated home ranges influenced SGAR residue concentrations found in red-tailed and Cooper's hawks separately and the three owl species combined (barred, barn, and great

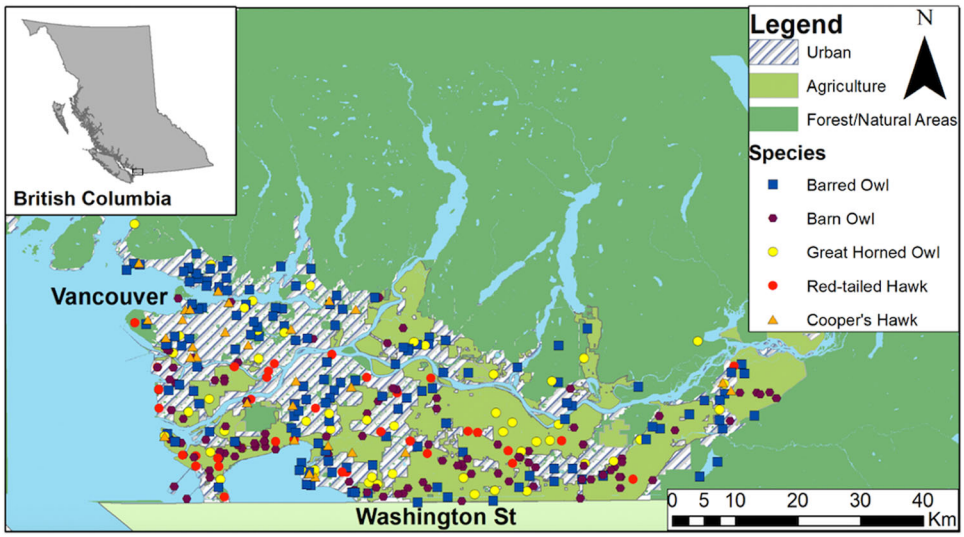

FIGURE 2: Spatial distribution of raptor species ($n = 393$) collected across different land-use types in the Lower Mainland region of British Columbia, 1988–2018.

horned owls). We did not evaluate difethialone independently because only 7% of raptors had difethialone concentrations ≥0.1 μg/g. We used IBM SPSS (Ver 24; IBM, 2016) and R (Ver 3.6.2; R Foundation for Statistical Computing, 2021).

Temporal trends in exposure. For the three owl species that were consistently sampled throughout the study period (barred, barn, and great horned owls), mean residue concentrations before and after 2013 were compared using a nonparametric Mann-Whitney *U* test. We also compared the proportion of raptors exposed to individual residues and total SGAR before and after 2013 using a two-proportions *z* test. Further statistical evaluation of temporal trends was restricted by unequal sample sizes among years and species.

RESULTS

Species-level differences in AR exposure

At least one SGAR residue was detected in 74% of all raptor livers ($n = 550/741$), with a mean ∑SGAR concentration of 0.037 μg/g, ranging from below detection (<0.005) to 1.81 μg/g (Supporting Information, Table S3). Brodifacoum was detected in 58% ($n = 430/741$) of raptor livers, with a mean detected concentration of 0.012 μg/g (range <0.005–1.15 μg/g). Similarly, the overall exposure rate to bromadiolone was 54% ($n = 398/741$), with a mean concentration of 0.013 μg/g (range <0.005–1.74 μg/g). Difethialone had a lower rate of detection (34%, $n = 252/741$) and mean concentration (0.005, range <0.005–0.86 μg/g) than the other SGARs (Supporting Information, Table S3). Multiple SGAR residues were detected in 50% of all raptor livers ($n = 368/741$). Barred owls ($n = 208$) had the highest incidence of multiple exposure events, with 73% of individuals exposed to two or more SGARs. By comparison, only 4.4% of raptors were exposed to an FGAR ($n = 33/741$), of which 19 individuals were exposed to chlorophacinone, 12 individuals were exposed to diphacinone, two individuals were exposed to pindone, and only one individual was exposed to warfarin (Supporting Information, Table S4).

For the well-sampled species ($n \geq 30$), exposure rates to at least one SGAR were highest in barred owls (96%, $n = 208$), followed by great horned owls (81%, $n = 129$), red-tailed hawks (78%, $n = 50$), Cooper's hawks (75%, $n = 36$), barn owls (65%, $n = 211$), and bald eagles (62%, $n = 34$; Figure 3; Supporting Information, Tables S3). Barred owls had a higher ∑SGAR (0.13, range <0.005–1.81 μg/g) than great horned owls ($\beta = 0.39 \pm 0.08$, $z = 4.578$, $p < 0.001$), red-tailed hawks ($\beta = 0.55 \pm 0.12$, $z = 4.693$, $p < 0.001$), Cooper's hawks ($\beta = 0.60 \pm 0.14$, $z = 4.411$, $p < 0.001$), barn owls ($\beta = 0.75 \pm 0.07$, $z = 10.345$, $p < 0.001$), and bald eagles ($\beta = 0.83 \pm 0.14$, $z = 6.081$, $p < 0.001$; Figures 4 and 5; Supporting Information, Tables S3 and S5). Barred owls also had higher bromadiolone (0.05, range <0.005–1.74 μg/g) residues than all other species ($p < 0.001$; Figures 4 and 5; Supporting Information, Tables S3 and S5). Great horned owls (0.02, range <0.005–0.63 μg/g) had higher bromadiolone than red-tailed hawks ($\beta = 0.46 \pm 0.11$, $z = 3.952$, $p = 0.001$), Cooper's hawks ($\beta = 0.57 \pm 0.13$, $z = 4.358$, $p < 0.001$), barn owls ($\beta = 0.50 \pm 0.08$,

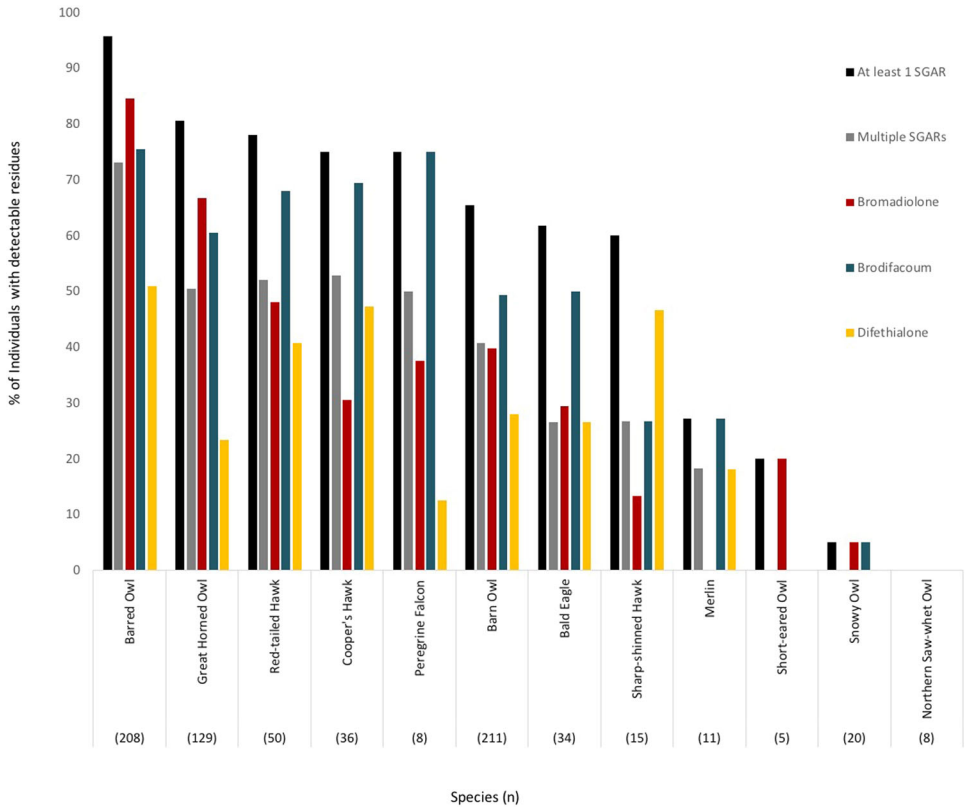

FIGURE 3: Species-level comparison of the frequency of exposure of terrestrial raptors to second-generation anticoagulant rodenticides (SGARs) in western Canada, 1988–2018. Sample sizes are indicated in parentheses. Any SGAR was counted as detected above 0.005 μg/g wet weight.

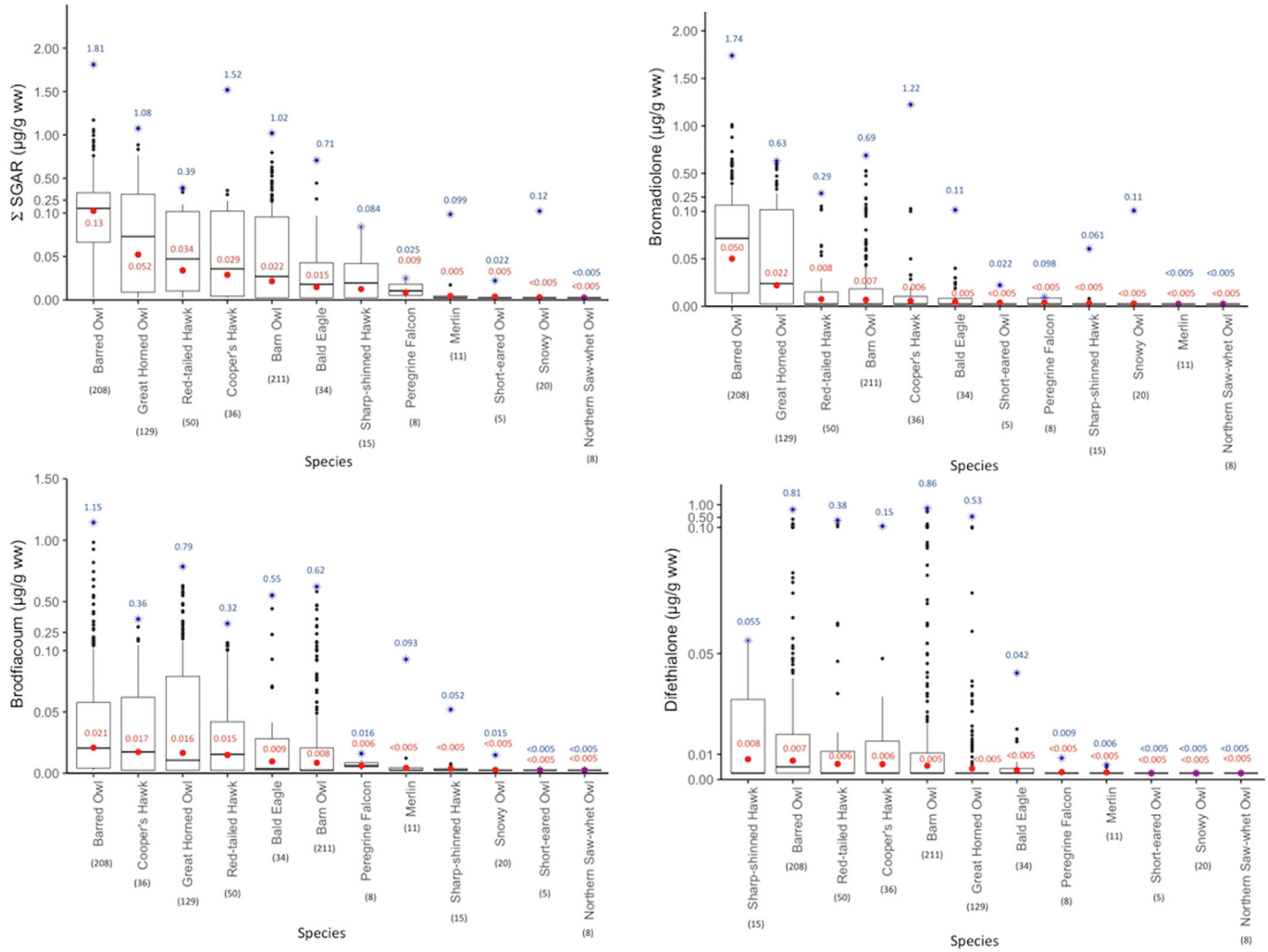

FIGURE 4: Species-level comparison of hepatic sum of second-generation anticoagulant rodenticides (∑SGAR), bromadiolone, brodifacoum, and difethialone residue concentrations in terrestrial raptors sampled in western Canada, 1988–2018. Horizontal box lines represent the median, and lower and upper hinges correspond to the 25th and 75th percentiles. The geometric mean (micrograms per gram wet wt) is indicated in red and the maximum observed value in blue. For ∑SGAR, bromadiolone, and brodifacoum, values <0.1 µg/g were scaled; *10 on the *y*-axis; and values >0.1 µg/g were divided by 10 to highlight small values. For difethialone, the scaling factor was 100. Sample sizes are indicated in parentheses. ww = wet weight.

$z = 6.456$, $p < 0.001$), and bald eagles ($\beta = 0.67 \pm 0.13$, $z = 5.06$, $p < 0.001$) and higher ∑SGAR than barn owl ($\beta = 0.37 \pm 0.08$, $z = 4.455$, $p < 0.001$) and bald eagle ($\beta = 0.45 \pm 0.14$, $z = 3.147$, $p = 0.02$; Figure 5; Supporting Information, Tables S3 and S5). Brodifacoum concentrations were higher in barred owl ($\beta = 0.39 \pm 0.07$, $z = 5.454$, $p < 0.001$) and great horned owl ($\beta = 0.29 \pm 0.08$, $z = 3.556$, $p = 0.005$) compared with barn owls (Figures 4 and 5; Supporting Information, Tables S3 and S5). Difethialone concentrations were higher in barred owls compared to great horned owls ($\beta = 0.23 \pm 0.06$, $z = 3.818$, $p = 0.002$) and bald eagles ($\beta = 0.30 \pm 0.10$, $z = 3.042$, $p = 0.03$; Figures 4 and 5; Supporting Information, Tables S3 and S5).

The exposure rate to FGARs was consistently low for all raptor species, with the highest exposure rate recorded in sharp-shinned hawks (13%, $n = 15$), of which chlorophacinone was the only detected FGAR residue (Supporting Information, Table S4).

Spatial variation in AR exposure

Quantifying land use in home range. The proportion of each land-use type within theorized home ranges was calculated for $n = 393$ raptors which had exact collection locations (Table 1).

Cooper's and red-tailed hawks. For Cooper's hawks ($n = 29$), we examined the relationship between brodifacoum and total SGAR exposure and the proportion of residential and of commercial/industrial within theorized home ranges. Proportion agriculture was negatively and significantly correlated with proportion residential ($r = -0.59$, 95% confidence interval [CI] −0.79 to −0.28, $p < 0.01$) and was not included in the analysis. We did not examine bromadiolone independently because only three individuals had bromadiolone concentration levels >0.1 µg/g. Neither proportion

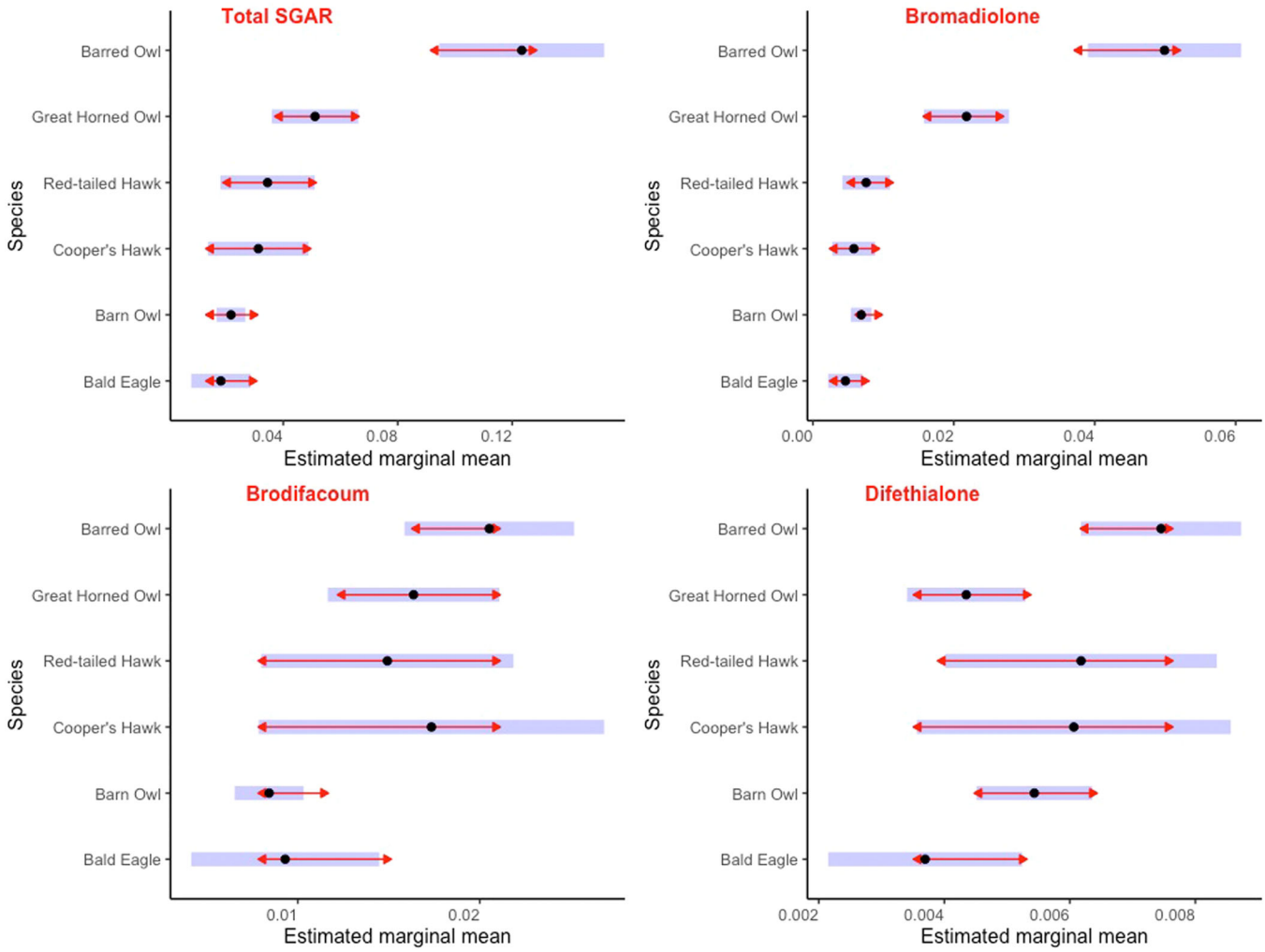

FIGURE 5: Species-level pairwise comparison of estimated marginal mean second-generation anticoagulant rodenticide residue concentrations (micrograms per gram wet wt) in terrestrial raptors sampled in western Canada, 1988–2018. Blue bars represent 95% confidence intervals for the emmeans, and red arrows indicate the comparisons among emmeans. If an arrow from one mean overlaps an arrow from another group, the difference is not "significant" at $\alpha = 0.05$. SGAR = second-generation anticoagulant rodenticide.

industrial/commercial (Exp B = 2.40, 95% CI 0.02–239.45, $p = 0.71$) nor proportion residential land (Exp B = 0.04, 95% CI 0.00–1.25, $p = 0.07$) was a significant predictor of ∑SGAR ($\chi^2 = 4.33$, $p = 0.12$) or brodifacoum exposure ($\chi^2 = 0.15$, $p = 0.93$, residential Exp B = 0.81, 95% CI 0.04–16.97, $p = 0.89$), industrial/commercial (Exp B = 2.12, 95% CI 0.20–219.75, $p = 0.75$) in Cooper's hawks.

Similarly, for red-tailed hawks ($n = 36$), proportional agricultural land was excluded from analyses because it was correlated with proportional residential and industrial/commercial ($r = -0.70$, 95% CI −0.84 to −0.49, and −0.55, 95% CI −0.74 to −0.27, $p < 0.01$, respectively). We only analyzed ∑SGAR compounds because few individuals had >0.1 μg/g for the individual compounds. Neither the proportion of industrial/commercial nor that of residential land predicted the total exposure of SGARs in red-tailed hawks ($\chi^2 = 0.67$, $p = 0.71$, residential Exp B = 3.18, 95% CI 0.18–57.35], $p = 0.43$; industrial/commercial Exp B = 0.51, 95% CI 0.01–42.79, $p = 0.76$).

TABLE 1: Proportion of each land-use type in theorized home ranges (3 km^2) for select raptor species across western Canada, 1988 to 2018

		Land use area			
Species	*n*	Residential	Industrial	Commercial	Agriculture
Barred owl	148	0.37 ± 0.27	0.037 ± 0.10	0.05 ± 0.11	0.22 ± 0.33
Barn owl	126	0.09 ± 0.16	0.042 ± 0.13	0.05 ± 0.17	0.63 ± 0.38
Great horned owl	54	0.26 ± 0.29	0.02 ± 0.05	0.03 ± 0.07	0.40 ± 0.41
Red-tailed hawk	36	0.19 ± 0.24	0.05 ± 0.12	0.05 ± 0.12	0.48 ± 0.43
Cooper's hawk	29	0.49 ± 0.26	0.05 ± 0.12	0.10 ± 0.15	0.10 ± 0.25

Values represent mean ± standard deviation proportion land-use type.

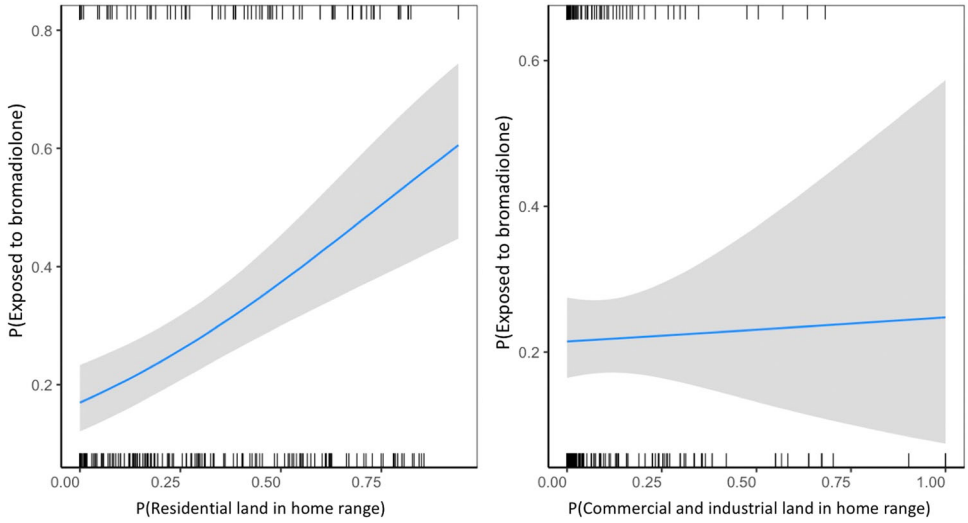

FIGURE 6: Effects plots for residential and industrial/commercial development showing that owls living in areas with proportionally more residential development within theorized home ranges are more likely to have bromadiolone concentrations >0.1 μg/g ($\chi^2 = 21.96$, $p < 0.001$, Nagelkerke $R^2 = 0.09$).

Barred, barn, and great horned owl species. We pooled the owls ($n = 328$; barred, barn, and great horned) because they are year-round residents in the study area and performed logistic regressions on the binary-transformed residues (bromadiolone, brodifacoum, and ∑SGARs). Proportion agriculture was highly correlated with both proportional residential and industrial/commercial and was excluded from the analyses ($r = -0.70$, 95% CI −0.75 to −0.64, and $r = -0.37$, 95% CI −0.46 to −0.27, $p < 0.01$). Proportion of residential development was a predictor of bromadiolone concentrations ($\chi^2 = 21.96$, Exp B = 7.95, 95% CI 3.23–19.54, $p < 0.001$, Nagelkerke $R^2 = 0.09$; Figure 6) and ∑SGARs ($\chi^2 = 15.80$, Exp B = 5.39, 95% CI 2.28–12.72, $p < 0.01$, Nagelkerke $R^2 = 0.06$) but not for brodifacoum exposure ($\chi^2 = 1.97$, Exp B = 1.95, 95% CI 0.73–5.24, $p = 0.37$). The proportion of industrial/commercial land within the owls' home range was not a significant predictor for bromadiolone ($p = 0.81$, Exp B = 1.23, 95% CI 0.27–5.54; Figure 6), brodifacoum ($p = 0.66$, Exp B = 0.68, 95% CI 0.12–3.95) or ∑SGARs ($p = 0.71$, Exp B = 1.27, 95% CI 0.36–4.53; Figure 6). Given that neither land-use variable predicted brodifacoum exposure in the owls, the significant relationship between ∑SGARs and proportion residential development is likely driven by the effect of residential development on bromadiolone exposure.

Temporal variation in AR exposure

Concentrations of SGARs varied greatly throughout the study period, within and between years (Figure 7; Supporting Information, Table S6). Plotting the geometric means over time shows an apparent trend of increasing bromadiolone concentrations and decreasing brodifacoum after 2013 in both barred and great horned owls (Figure 7). However, because of the variability in concentrations, bromadiolone concentrations did not change in barred owls ($U = 5.12$, $p = 0.32$, $r = 0.07$, $n = 208$) or barn owls ($U = 3.31$, $p = 0.063$, $r = 0.13$, $n = 211$), but they did increase in great horned owls post-2013 ($U = 1.39$, $p = 0.006$, $r = 0.24$, $n = 129$; Table 2; Supporting Information, Tables S7–S9). Brodifacoum decreased post-2013 in barred owls ($U = 3.62$, $p = 0.006$, $r = -0.19$, $n = 208$) and increased post-2013 in barn owls ($U = 3.42$, $p = 0.031$, $r = 0.15$, $n = 211$) but did not change in great horned owls ($U = 1.00$, $p = 0.96$, $r = 0$, $n = 129$; Table 2; Supporting Information, Tables S7–S9). Difethialone increased post-2013 in barn owls ($U = 3.63$, $p = 0.001$, $r = 0.23$, $n = 211$) and great horned owls ($U = 1.22$, $p = 0.04$, $r = 0.18$, $n = 129$) but did not change in barred owls ($U = 4.92$, $p = 0.6$, $r = 0.04$; Table 2; Supporting Information, Tables S7–S9). We also examined variation over time in the incidence of exposure of both individual compounds and summed SGAR concentrations. Exposure did not change before 2013 and thereafter in barred or great horned owls (Table 3), but barn owls experienced statistically higher exposure to all three residues and summed SGAR post-2013 (see Table 3; Supporting Information, Figures S2–S6).

DISCUSSION

Species and exposure patterns

Raptor species were selected for our study based on risk factors such as diet and habitat use. Spatial focus was determined by the sources of fortuitously obtained carcasses, in turn determined by human landscapes with intensive agriculture or urban land use and sufficient green spaces to support raptor populations. Exposure to SGARs was widespread and virtually pervasive in at-risk species over the sampled landscapes. Both incidence of exposure and mean hepatic concentrations of SGARs were greatest in larger generalist owls and hawks with more varied diets, particularly the barred owl, genus *Strix*, which has adapted to urban and suburban environments across North America (Hindmarch & Elliott, 2015a; Livezey, 2009). Similarly, the great horned owl, genus *Bubo* (Hindmarch & Elliott, 2018), and the red-tailed hawk, genus *Buteo*, both of which have adapted well to

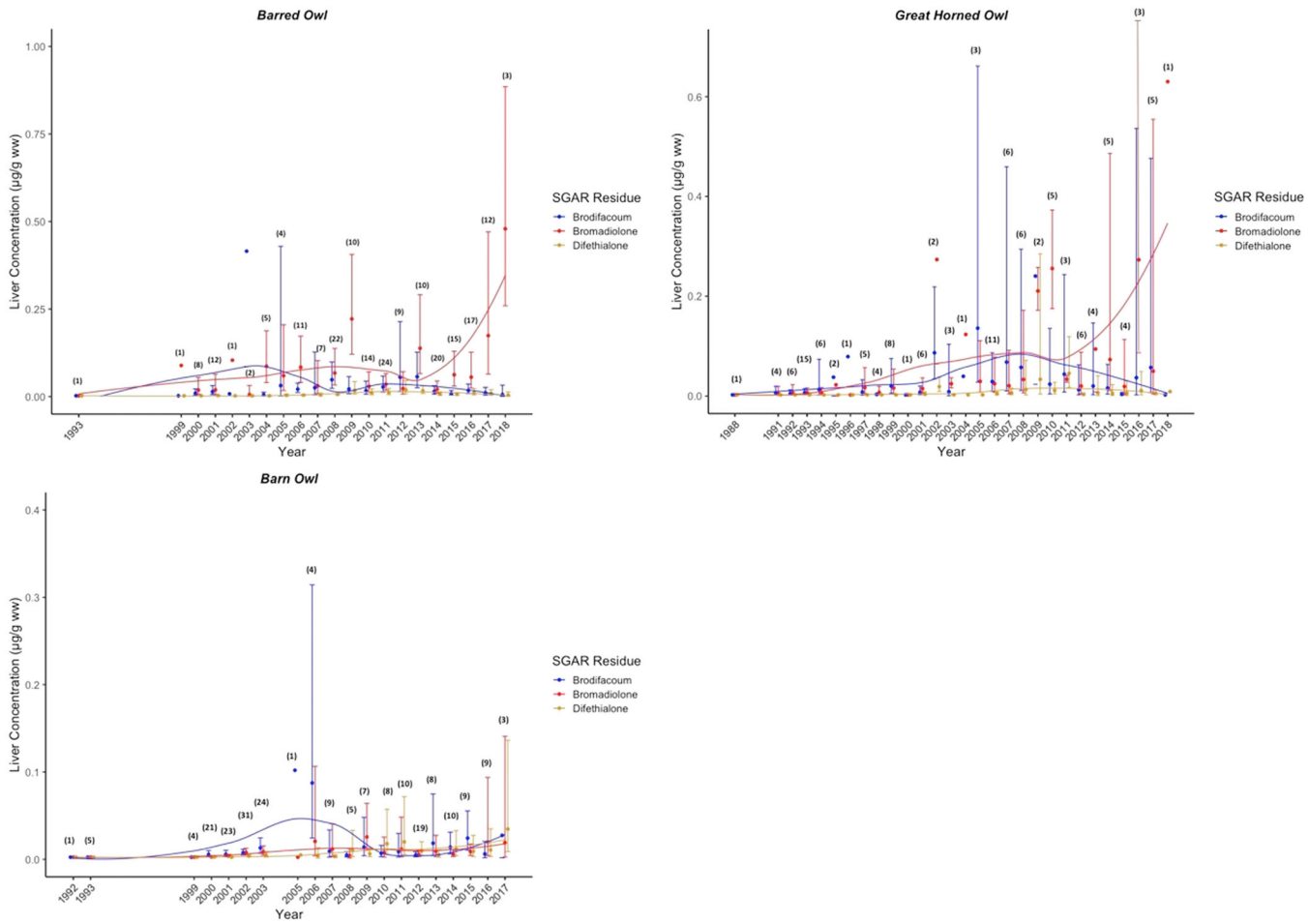

FIGURE 7: Temporal trends in second-generation anticoagulant rodenticide (SGAR) residue concentrations (geometric mean ± 95% confidence interval) in owl species sampled in western Canada, 1988–2018. Sample sizes are indicated in parentheses. Total SGAR concentrations increased significantly post-2013 in barn owls ($U = 3.87$, $p = 0.001$, $r = 0.24$) and great horned owls ($U = 1.41$, $p = 0.005$, $r = 0.25$) but not in barred owls ($U = 4.78$, $p = 0.89$, $r = 0.01$). Bromadiolone increased significantly post-2013 in great horned owls ($U = 1.39$, $p = 0.006$, $r = 0.24$) but not in the other species. Brodifacoum decreased significantly post-2013 in barred owls ($U = 3.62$, $p = 0.006$, $r = -0.19$) and increased significantly post-2013 in barn owls ($U = 3.42$, $p = 0.031$, $r = 0.15$). Difethialone increased significantly post-2013 in barn owls ($U = 3.63$, $p = 0.001$, $r = 0.23$) and great horned owls ($U = 1.22$, $p = 0.04$, $r = 0.18$).

agriculture-dominated ecosystems, had consistently high rates of exposure. Species such as the barn owl (*Tyto alba*) that feed preferentially on microtine voles, exhibited consistent but somewhat lower rates of exposure. Hawks and falcons considered to be mainly predators of other birds also had high rates of AR exposure, particularly Cooper's hawk, genus *Accipiter*, another species that has colonized urban and suburban environments across North America (Stout & Rosenfield, 2010). Studies from elsewhere have reported that rodent-eating and scavenging species are generally the most exposed, particularly nocturnal owls and some *Buteo* species (Albert et al., 2010; Christensen et al., 2012; Hong et al., 2019; Hughes et al., 2013; Lambert et al., 2007; Lohr, 2018; Okoniewski et al., 2021; Rial-Berriel et al., 2021). However, greatest exposures have been reported elsewhere in species considered to have other diet preferences, such as the putative snake-eating crested serpent eagle *Spilornis cheela* in Taiwan (Hong et al., 2019), the bird-eating goshawk *Accipiter gentilis* in Germany (Badry et al., 2021), and Eurasian kestrels on Gran Canary Island (Ruiz-Suárez et al., 2014).

In an attempt to understand exposure pathways, we previously examined diets of the more common owl species along an urban to rural landscape gradient in relation to ARs (Hindmarch & Elliott, 2018). Both *R. norvegicus* and *R. rattus* were important prey items for barred and great horned owls inhabiting urban parks and other green spaces (Hindmarch & Elliott, 2014, 2015a). Rats increased in the diet of barn owls as the surrounding habitat became more urbanized and the open green space decreased (Huang et al., 2016; Hindmarch & Elliott, 2015b; Hindmarch & Elliott, 2018). Our attempt to identify exposure pathways in two locations of the Fraser Valley, but with different farming practices, indicated Norway rats as likely the primary vector, although exposure of nontargets such as microtine voles was also documented and showed that they would enter and feed in bait stations (Elliott et al., 2014). Extensive use of bait stations by nontarget small mammals and contamination by brodifacoum were documented in a study of an agricultural landscape in Germany (Walther, Ennen, et al., 2021). An associated study of the behavior of Norway rats exposed to brodifacoum showed that poisoned rats sought

TABLE 2: Mean concentrations of second-generation anticoagulant rodenticide from before and after implementation of Canadian federal risk mitigation measures in 2013

Species	SGAR	Mean ± SD (median) 1988–2013	Geo-mean 1988–2013	Mean ± SD (median) 2014–2018	Geo-mean 2014–2018	U	p	r (effect size)
Barred owl (*Strix varia*)	∑SGAR	0.25 ± 0.28 (0.15) n = 141	0.12	0.24 ± 023 (0.19) n = 67	0.14	4.78	0.89	0.01
	Bromadiolone	0.13 ± 0.21 (0.07) n = 141	0.05	0.18 ± 0.23 (0.08) n = 67	0.06	5.12	0.32	0.07
	Brodifacoum	0.10 ± 0.20 (0.03) n = 141	0.03	0.04 ± 0.08 (0.01) n = 67	0.01	3.62	0.006*	−0.19
	Difethialone	0.02 ± 0.08 (0.00) n = 141	0.007	0.03 ± 0.06 (0.01) n = 67	0.008	4.92	0.6	0.04
Barn owl (*Tyto alba*)	∑SGAR	0.08 ± 0.15 (0.02) n = 180	0.02	0.14 ± 0.17 (0.07) n = 31	0.06	3.87	0.001*	0.24
	Bromadiolone	0.03 ± 0.08 (0.00) n = 180	0.006	0.05 ± 0.13 (0.01) n = 31	0.01	3.31	0.063	0.13
	Brodifacoum	0.03 ± 0.09 (0.00) n = 180	0.008	0.05 ± 0.12 (0.01) n = 31	0.01	3.42	0.031*	0.15
	Difethialone	0.02 ± 0.09 (0.00) n = 180	0.005	0.04 ± 0.05 (0.01) n = 31	0.01	3.63	0.001*	0.23
Great horned owl (*Bubo virginianus*)	∑SGAR	0.17 ± 23 (0.05) n = 111	0.04	0.34 ± 0.25 (0.34) n = 18	0.16	1.41	0.005*	0.25
	Bromadiolone	0.07 ± 0.12 (0.02) n = 111	0.02	0.23 ± 0.23 (0.19) n = 18	0.07	1.39	0.006*	0.24
	Brodifacoum	0.09 ± 0.15 (0.01) n = 111	0.02	0.10 ± 0.19 (0.01) n = 18	0.02	1.00	0.96	0.00
	Difethialone	0.01 ± 0.05 (0.00) n = 111	0.004	0.02 ± 0.03 (0.00) n = 18	0.006	1.22	0.04*	0.18

SGAR = second-generation anticoagulant rodenticide; SD = standard deviation; Geo-mean = geometric mean.
*$p < 0.05$.

shelter and that very few were available for larger scavengers and predators (Walther, Geduhn, et al., 2021). It should be noted that the latter report contrasts with an earlier finding that rodents suffering from AR toxicity may exhibit behavioral changes because they tend to move about farther in the open and during daytime, thus increasing the likelihood of being predated or dying in the open and being more easily scavenged (Cox & Smith, 1992). Regardless, Walther, Ennen, et al. (2021) concluded that, based on their recent work and previous work (Geduhn et al., 2014), the exposure pathway to predators and scavengers in agricultural settings is likely via nontarget small mammals. However, our previous data suggest that in more urbanized or interface habitats, targeted rats appear to provide an important pathway of exposure in owls. Regardless of whether the pathway is via target or nontarget small mammals, AR deployment in bait stations, often permanent, is contaminating birds of prey throughout the sampled areas of western Canada and in many other parts of the world (López-Perea & Mateo, 2018; Okoniewski et al., 2021; Rial-Berriel et al., 2021; Serieys et al., 2019; Thornton et al., 2022). Whether target or nontarget rodents are the primary pathway is perhaps not an important question unless it is possible to design bait stations to allow targeted rats to enter but exclude nontarget small mammals. Of relevance is whether widespread exposure of raptors is via sanctioned legal use, rather than, for example, illegal deployment outside of bait stations for control of species considered pests, such as wild canids or corvid birds. Such illegal use of ARs has been documented in some jurisdictions (Sánchez-Barbudo et al., 2012) but is not widely recognized as a problem in North America.

In the case of the raptors considered to prey mainly on birds, the falcons and *Accipiter* hawks, the pathway is also not clear. Potential routes include primary exposure of songbird prey from use of bait stations, secondary exposure of songbird prey by feeding on primary-exposed invertebrates, or feeding on nontarget or target small mammals, including rats. We found some limited evidence of songbird use of bait stations (Elliott et al., 2014). A recent and more thorough investigation from Germany, however, reported that 30% of passerine birds collected in snap traps during a baiting program with brodifacoum had hepatic residues (Walther, Ennen, et al., 2021). That is the first detailed study of potential exposure of small birds and identifies primary-exposed small birds as a potential pathway to raptors. The bait was deployed according to registered best practices in a rolled oat formulation of a type no longer permitted in North America. Given the obvious appeal of such a formulation to songbirds, the results may not be directly applicable to North America. Although consumption of songbirds does provide a potential AR exposure pathway, consumption of small mammals, especially targeted rats, must be considered, given that Cooper's hawks (Bielefeldt et al., 1992) in particular but also sharp-shinned hawks (Joy et al., 1994), will take mammalian prey. We found some evidence of AR contamination of invertebrates, as have some other studies (Alomar et al., 2018; Howald et al., 1999); but we think that the invertebrate to songbird pathway is likely to be less important, at least in the landscapes and AR-use scenarios

TABLE 3: Exposure (% detections) of terrestrial raptors to second generation anticoagulant rodenticide concentrations from before and after implementation of Canadian federal risk mitigation measures in 2013

Species	*n* (pre-2013)	*n* (post-2013)	SGAR	% Exposed pre-2013	% Exposed post-2013	*Chi-square*	*df*	*p*
Barred owl (*Strix varia*)	141	67	∑SGAR	95.0	97.0	0.08	1	0.77
			Bromadiolone	83.0	88.1	0.55	1	0.46
			Brodifacoum	78.7	68.7	1.97	1	0.16
			Difethialone	51.1	52.2	1.00E-05	1	0.99
Barn owl (*Tyto alba*)	180	31	∑SGAR	61.7	87.1	6.48	1	**0.01***
			Bromadiolone	36.7	58.1	4.19	1	**0.04***
			Brodifacoum	46.1	67.7	4.12	1	**0.04***
			Difethialone	23.9	51.6	8.76	1	**0.003***
Great horned owl (*Bubo virginianus*)	111	18	∑SGAR	79.3	88.9	0.40	1	0.52
			Bromadiolone	64.9	77.8	0.65	1	0.42
			Brodifacoum	60.4	61.1	6.60E-31	1	1
			Difethialone	20.7	38.9	1.94	1	0.16

SGAR = second-generation anticoagulant rodenticide; *df* = degrees of freedom.
*$p < 0.05$.

that dominate our study sites (Elliott et al., 2014). Most of these bird-eating raptor carcasses were collected from urbanized or intensive agricultural landscapes. For example, of the eight Cooper's hawks with ∑SGARs >0.1 μg/g, all came from such locations. Studies elsewhere have found generally lower exposure rates and concentrations in accipiters and falcons (Hughes et al., 2013; Walker et al., 2015). A recent study from the Canadian province of Ontario reported similar results to those of the present study, with zero or low incidence of SGARs in sharp-shinned hawks and falcons but higher incidence in Cooper's hawks (Thornton et al., 2022). However, northern goshawks collected from Berlin, Germany, had the greatest SGAR incidence and concentrations among collected species; and the authors of that study speculated that the pathway was likely via passerine birds entering bait stations (Badry et al., 2021), as reported in Walther et al. (2020).

Determining the exposure pathway for falcon species is also problematic. Although sample sizes are low, $n = 8$ for peregrine falcons and 11 for merlins, incidence of exposure to at least one SGAR was 75% for the former and 27% for the latter. Opportunistic preying on rats or nontarget rodents seems possible for peregrines because they will take mammalian prey (Bradley & Oliphant, 1991). With the exception of bats, merlins are reported to prey almost exclusively on birds. The report that urban merlins take large numbers of house sparrows (Sodhi & Oliphant, 1993) may suggest a pathway, given our earlier finding that house sparrows will readily enter bait stations and peck at baits and the recent findings from Germany (Elliott et al., 2014; Walther, Ennen, et al., 2021).

Spatial patterns and trends

The landscape that we have sampled most intensively through the fortuitous collection of carcasses is the Lower Mainland region of British Columbia, which includes Metro Vancouver and the Fraser Valley. It is the broad delta of the Fraser River and has largely been dyked and cleared for agricultural use and increasingly for urban development. For the examination of spatial factors, we estimated habitat breakdown for 393 raptors with known locations. For barred owls and Cooper's hawks, residential land use dominated the habitat where the bird was found, followed by agricultural use. For great horned owls and red-tailed hawks, that pattern was reversed, with agricultural land dominating home ranges. Degree of exposure to bromadiolone was significantly related to the proportion of residential land within the home ranges of the three most common owl species, barred, barn, and great horned owls. That is consistent with the decision to limit outdoor use of SGARs solely to bromadialone and only by licensed operators. Retail sales are restricted to FGARs along with point-of-sale formulation, volume, and packaging limits. The regulatory changes have led to widespread deployment of bromadialone, rather than previously available brodifacoum and difethialone, by pest control operators in and around multi-family residential buildings, as well as food production, storage, and transport facilities. Increased use of bromadiolone and decreased use of brodifacoum are evident from provincial sales figures (British Columbia Ministry of Environment and Climate Change Strategy, Victoria, BC, Canada, unpublished data; Supporting Information, Table S10). Thus, it does appear that the risk mitigation measures are having some effect on exposure of raptors to SGARs.

Previously, we examined the influence of land use on exposure of barn owls to ARs in an area of the Lower Mainland with a typical mix of intensive agricultural and increasing urban land use (Hindmarch et al., 2017). Barn owls fed preferentially along grassy roadside verges, and the relative risk of consuming rodenticide-exposed small mammal prey was also highest in such roadside grassy habitats. Thus, similar to what we have found in the present study, the exposure pathway appears to be primarily from deployment in bait stations around residential and commercial buildings in suburban areas and likely via feeding on both target and nontarget rodents, as discussed above, and from a recent California study (Hofstadter et al., 2021). In a study of exposure of furbearing mammals to ARs in the oil sands region of Canada, hepatic SGAR concentrations were related to the extent of industrial activity on the landscape (Thomas et al., 2017). Other studies of mammalian meso-carnivores in California have

reported positive associations between AR concentrations and urban development (Beier et al., 2010; Gehrt & Riley, 2010). A study of caracals (*Caracal caracal*) in South Africa found an association with vineyards, though they thought that the actual AR source may still have been in adjacent urban areas (Serieys et al., 2019). Exposure to ARs also increased with proximity to developed habitats in an Australian predatory bird, the southern boobook owl (*Ninox boobook*; Lohr, 2018).

Temporal trends

The temporal data show a tendency for increasing bromadiolone concentrations and decreasing brodifacoum after 2013 in both barred and great horned owls. Attempts to regulate ARs were initiated in 2008 by both the US Environmental Protection Agency (USEPA) and the Canadian Pest Management Regulatory Agency. At least in British Columbia, notices of coming restrictions on outdoor use of brodifacoum and difethialone were addressed by pest control operators and sales began to decrease as they switched to use of bromadiolone, for which sales increased (Elliott et al., 2014; Supporting Information I, Table S10). The full implementation of the risk mitigation measures to limit retail purchase of ARs by volume and the requirement for a sealed bait station and restriction to FGARs only began to be implemented in Canada in 2011. Following our study reporting widespread noncompliance with AR regulations in part of British Columbia (Hindmarch et al., 2018), both federal and provincial enforcement efforts were increased and probably reduced the amount of noncompliant outdoor use of SGARs. Thus, we have chosen 2013 as the date at which the measures were likely to be in effect in Canada. The most obvious change associated with that time point is the apparent increase in mean hepatic bromadiolone concentrations in the two large owl species, although the variation in the data decreases the confidence in a statistically significant change.

We recognize that other variables may be affecting the application of ARs, including changes in land use over the time period. The landscape in British Columbia has seen limited conversion of agricultural to urban land use because of restrictions under the Agricultural Land Reserve regulations (https://www.alc.gov.bc.ca/alc/content/alr-maps), although there has been an increase in loss of agricultural land not protected under the Agricultural Land Reserve provisions. At the same time, ongoing conversion of large residential lots to apartment and commercial buildings has increased the densification of many communities, though that would likely result in an overall increase in rodenticide usage (currently still dominated by ARs), not in the relative amounts of each compound being used. An increase in bromadialone and decreases in brodifacoum and difethialone would still be expected, as is evident from the commercial sales data (Supporting Information I, Table S10). In barn owls, we did, however, determine an increase in the percentage exposed to all three individual SGAR compounds and to ∑SGARs. Barn owls in particular have been impacted by increased densification of land use and loss of grassland habitat (Hindmarch et al., 2017), as discussed above, which has led to their designation as endangered (Environment and Climate Change Canada, 2021). Those changes appear to have led to an overall increase in SGAR exposure of barn owls.

In the United States, a major retailer refused to comply with the measures and chose to litigate, which delayed full implementation until 2014 (USEPA, 2014). The one relevant report for the United States did not find any change in exposure of red-tailed hawks pre– and post–risk mitigation measures in Massachusetts (Murray, 2020). Other reports from the United States made the same conclusion (Quinn, 2019) and may be partly the result of online availability of SGARs (e.g., https://www.amazon.com/bromadiolone/s?k=bromadiolone). Data from Ontario, Canada, 2017–2019 and thus subsequent to risk mitigation measures, reported that SGAR incidence in a broad suite of raptors was similar to studies taken before that period elsewhere in North America (Thornton et al., 2022). They questioned the effectiveness of the measures.

The decision to prohibit outdoor use of brodifacoum and difethialone but allow continued use of bromodialone (by licensed operators) was presumably intended to reduce poisoning of nontarget wildlife while still enabling effective rodent control (Bradbury, 2008). Retention of an SGAR, along with alternatives to anticoagulants, for outdoor use was also possibly an attempt to reduce the development of pest resistance, which is particularly associated with use of FGARs. However, pest resistance to SGARs has been reported for some time, particularly where testing has been widely undertaken and likely elsewhere (Berny et al., 2018). The regulatory decision was based originally on a USEPA review of the available data on the relative avian toxicity of SGARs (Erickson & Urban, 2004). Among the SGARs examined in that report, bromadiolone appears unusual in being very toxic to mammals but less so to birds. Our in-progress analysis of AR exposure and toxicity using a North America–wide database should provide insights into the relative toxicity of SGARs to birds of prey, while providing revised criteria to interpret liver residue data for birds of prey. Initial indications are that bromadialone is at least as toxic to birds as brodifacoum or difethialone, and thus the mitigation measures may be changing exposure patterns but not the poisoning of nontarget wildlife (V. Silverthorn et al., unpublished manuscript).

SUMMARY AND CONCLUSIONS

Anticoagulant rodenticides have become pervasive contaminants of terrestrial birds of prey. All species of bird of prey sampled in the present study exhibited some degree of exposure to SGARs. Highest rates of exposure were in the barred and great horned owls, species with diverse diets that can include targeted rats; and these owls inhabit both suburban and intensive agricultural habitats. Barn owls, which are mainly field vole eaters, had a lower incidence of exposure. Concentrations of SGARs were highly variable; as with incidence of exposure, barred owls had highest levels measured in liver with a geometric mean ∑SGAR = 0.13 and a range <0.005–1.81 μg/g wet weight ($n = 208$). Putatively bird-eating raptors such as Cooper's hawks, sharp-shinned hawks, and peregrine falcons also had relatively high exposure incidence rates; mean concentrations were, however, lower in

those hawks and falcons than in the large owls. Analysis of the spatial trends and patterns of hepatic SGAR residues revealed that exposure of owls was greater in landscapes with an increased degree of residential land use. There are preliminary indications that risk mitigation measures implemented circa 2013 are having an influence on exposure; mean concentrations of brodifacoum and difethialone decreased in barred and great horned owls, while bromadiolone increased around that inflection point. Whether that apparent change in exposure patterns is also reducing the rates of poisoning is not yet known.

Supporting Information—The Supporting Information is available on the Wiley Online Library at https://doi.org/10.1002/etc.5361.

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