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Title: Second generation anticoagulant rodenticides in predatory birds: probabilistic characterisation of toxic liver concentrations and implications for predatory bird populations in Canada.

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Abstract:
Second-generation anticoagulant rodenticides (SGARs) are widely used to control rodent pests but exposure and poisonings occur in non-target species, such as birds of prey. Liver residues are often analyzed to detect exposure in birds found dead but their use to assess toxicity of SGARs is problematic. We analyzed published data on hepatic rodenticide residues and associated symptoms of anticoagulant poisoning from 270 birds of prey using logistic regression to estimate the probability of toxicosis associated with different liver SGAR residues. We also evaluated exposure to SGARs on a national level in Canada by analyzing 196 livers from great horned owls (Bubo virginianus) and red-tailed hawks (Buteo jamaicensis) found dead at locations across the country. Analysis of a broader sample of raptor species from Quebec also helped define the taxonomic breadth of contamination. Calculated probability curves suggest significant species differences in sensitivity to SGARs and significant likelihood of toxicosis below previously suggested concentrations of concern (<0.1 mg/kg). Analysis of birds from Quebec showed that a broad range of raptor species are exposed to SGARs, indicating that generalized terrestrial food chains could be contaminated in the vicinity of the sampled areas. Of the two species for which we had samples from across Canada, great horned owls are exposed to SGARs to a greater extent than red-tailed hawks and liver residue levels were also higher. Using our probability estimates of effect, we estimate that a minimum of 11% of the sampled great horned owl population is at risk of being directly killed by SGARs. This is the first time the potential mortality impact of SGARs on a raptor population has been estimated.

Keywords: rodent, exposure, liver residues, toxicity threshold, anticoagulant rodenticide

SGAR – second generation anticoagulant rodenticide
GHOW – great horned owl
RTHA – red-tailed hawk
BAOW – barred owl
BNOW – barn owl
ALL – all bird species pooled
EIIS – ecological incident information system
EPA – environmental protection agency
1. Introduction

Introduced in the 1970s, second-generation anticoagulant rodenticides (SGARs) were developed to combat the reported development of rodent resistance to first-generation compounds (Buckle et al. 1994). These newer anticoagulant poisons differ from their first-generation counterparts in that they are more acutely toxic at lower doses (often allowing a lethal dose to be obtained in a single feeding), and are more persistent in vertebrate livers (Parmar et al. 1987, Stone et al. 1999, Newton et al. 1999, Erickson and Urban 2004). Greater acute toxicity increases the potential for primary poisoning amongst non-target species while the longer tissue half-lives of SGARs enhance the potential for bioaccumulation in non-target predators in particular, and so may increase the risk of secondary poisoning. Furthermore, rodents survive for several days after consuming a lethal dose of SGARs and often will continue feeding on the bait (Cox and Smith 1992). That increases the likelihood that the body burden in poisoned rodents may significantly exceed the LD50 or even LD100 dose, and poisoned animals may remain active and available for capture by predators for some period after ingestion of the rodenticide. Additionally, poisoned rodents exhibit an altered state of behaviour, such as spending more time in open areas in a lethargic state, and this may further predispose them to predation (Cox and Smith 1992).

SGARs bind and inhibit vitamin K epoxide reductase and persist for at least six months in organs and tissues containing this enzyme such as the liver (Stone et al. 1999, Eason et al. 2002). In an attempt to monitor exposure in non-target wildlife, the presence of detectable SGAR residues as well as the magnitude of concentrations has been measured in the livers of some Canadian, American and European predatory birds and

There was a common trend among those studies for most SGARs, namely brodifacoum, bromadiolone, difenacoum and difethialone being detected at an increasing frequency in numerous predators and scavengers. Species most commonly monitored in North America are great horned owls (*Bubo virginianus*) and red-tailed hawks (*Buteo jamaicensis*) (Albert et al. 2010, Erickson and Urban 2004).

It is still uncertain what SGAR liver concentration is diagnostic of a potentially lethal dose and, indeed Erickson and Urban (2004) have questioned whether such a cause-effect relationship is appropriate. A sometimes cited –toxicity threshold is given as –greater than 0.1 – 0.2 mg/kg wet weight (Newton et al. 1998, Newton et al. 1999). This was, in fact, described as a –potentially lethal range and was derived for a single species, the barn owl (*Tyto alba*); it stems from two sets of observations (Shore et al. 2001). Firstly, barn owls diagnosed post-mortem as having died from rodenticides had liver concentrations > 0.1 mg/kg. Those owls exhibited classical toxicosis signs such as haemorrhaging from organs such as the heart, lungs, liver, brain and/or subcutaneous areas (Newton et al. 1998). Secondly, owls that were experimentally poisoned had liver residues in the range of 0.2 – 1.72 mg/kg (Newton et al. 1999). However, it is uncertain whether these barn owl criteria would apply to other species. Liver residues associated with SGAR poisonings in various species typically range over two orders of magnitude and were reported to be as low as 0.01 mg/kg wet wt in one great horned owl that was examined (Stone et al. 1999). Thus, liver SGAR concentrations associated with toxicity vary markedly among both individuals and species. This suggests a probabilistic approach; which we adopt to review the evidence pertaining to how liver residues are
related to toxicity. Our principal objectives are: i) to determine SGAR liver
concentrations that may be associated with mortality in birds (i.e. to quantify the toxicity
threshold) and ii) using the threshold values, assess the extent and severity of exposure
in Canadian birds of prey.

2. Methods

2.1. Toxicity Threshold

2.1.1. Literature Search

Recently published (~ last 10 years) peer-reviewed publications as well as the United
States Environmental Protection Agency (EPA)’s Ecological Incident Information
System (EIIS) were surveyed in order to locate liver residue data sets for birds of prey.
The EIIS is the EPA’s database managing information on incidents linked to the exposure
of non target plants and animals to pesticides. It is currently managed by the Office of
Pesticide Programs (Mastrota 2007). Data were retained for our assessment if they met a
set of pre-determined conditions. These conditions included:

i) SGAR detection limits in liver were under 0.02 mg/kg wet wt;

ii) post-mortem evaluations were conducted prior to liver extraction and analysis;

iii) pathophysiological signs of rodenticide poisoning were included.

iv) post-mortem evaluations were conducted by a reputable professional such as a
doctor of veterinary medicine (DVM); and

iv) adequate sample sizes were available (n>15) for any given species (in order to
have greater statistical power).

2.1.2. Data Analysis
Raptor necropsies with attending SGAR liver analyses were collected and compiled in database software, and each case was given a binary code as positive (1) or negative (0) for pathophysiological signs of poisoning. A positive coding meant that, after a detailed post-mortem evaluation, an anticoagulant was diagnosed as being the cause of death or a significant contributory factor (i.e., when necropsies showed hemorrhage or anemia in the absence of traumatic injury or infectious or parasitic diseases and an anticoagulant residue was detected in the liver). A negative coding represented cases where the cause of death was deemed to be natural or accidental (for example incidental take, hunting, motor vehicle collisions, starvation).

The binary dataset was imported into SAS/STAT (version 9.2 TS2M0). Residue concentrations of all SGAR compounds were summed for the logistic regression. Concentrations were log transformed to meet the assumption of normality and re-tested. The PROC LOGISTIC macro was invoked to determine how liver residues affected presence or absence of poisoning symptoms. An effects plot was generated to illustrate the relationship and equations were built for every species with sufficient data (n≥15).

Using these equations, liver residue levels (in mg/kg wet weight (ww)) were determined for probabilities of 5%, 10%, 15% and 20% of exhibiting pathologies consistent with rodenticides exposure. Species comparisons were completed using analysis of variance (ANOVA) in conjunction with Tukey’s Studentized Range test. Because all birds were found dead or moribund, there was a logical inference that those pathologies (haemorrhaging of the heart, lungs, liver, brain and/or subcutaneous areas) were responsible for, or strongly contributed to, the mortality of the individual.

2.2. Exposure extent in Canada
2.2.1. Sample Collection

To obtain a cross-Canada survey of residue levels, liver samples of birds were selected, irrespective of the cause of death, from British Columbia, the prairie provinces, Ontario and Quebec. The birds were collected near agricultural and urban areas of the country where SGAR use was thought to be common. They were typically submitted to rehabilitation or veterinary centres either dead or in a moribund state. Initial diagnosis frequently involved car strike or other obvious ‘mishap’. They were not chosen because they showed signs of anticoagulant poisoning, but rather reflect the population of reported birds of prey dying from a multitude of causes. The subsequent liver samples were harvested initially as part of previous investigations of exposure to heavy metals or other toxicants, and then rodenticides residues were determined in later years. Three main collections were sampled. These included an Ontario/prairie sample of red-tailed hawks and great-horned owls, two common species known to scavenge; a broader phylogenetic collection from Quebec and a collection of three owl species from British Columbia (barn owl, barred owl [Strix varia] and great-horned owl). Those owl species are less mobile than most of the hawk species and were chosen to help identify geographical patterns of contamination and hence, potential sources of rodenticides residues. Results from the latter have already been reported (Albert et al. 2010).

2.2.2. Chemical Analysis

Chemical analysis was conducted at the National Wildlife Research Center in Ottawa, Ontario, Canada. Methods were similar to those reported by Albert et al. (2010). 50 mg of liver was ground in a mortar with about 5 g anhydrous sodium sulphate (Fisher no. S420-3). The resulting mixture was transferred to an amber glass septum bottle and acetonitrile
(EMD Omnisolv, AX0142-1, HPLC grade; 1 x 7 mL and 2 x 5 mL) was used for extraction. The extract was shaken for 2 minutes by hand and 15 minutes mechanically. After centrifuging for 15 minutes at 1000 rpm, the supernatant was removed and transferred into a 40 mL conical tube. The supernatant of the two subsequent extractions were combined with the first supernatant. The total product was evaporated to 10 mL under a stream of nitrogen in a water bath kept at 40°C.

In order to clean up liver extract, a 2 mL portion was transferred into a test tube and heated to dryness. The sample was reconstituted in acetonitrile and cleaned by solid-phase extraction. After the introduction of the sample into the SPE cartridge, the tube containing the sample was rinsed with acetonitrile and added to the SPE cartridge solution. The eluate was then evaporated to dryness and reconstituted in MeOH and filtered through an Acrodisk® syringe filter with a polyvinylidene fluoride (PVDF) membrane. A volume of 10 μL of the diluted filtered extract was analyzed by liquid chromatography-mass spectrometry (LC-MSMS). Some of the owl samples analysed (mainly from British Columbia) were not cleaned using an SPE cartridge. However, limits of detection were calculated for the procedure with and without an SPE sample cleaning phase and were found to be identical. For this reason, both SPE-cleaned data and non-SPE data were pooled for our analysis.

Brodifacoum, bromadiolone and difethialone were detected with a triple quadrupole mass Quatro- Ultima (Waters) with negative electrospray ionization (ESI) in multiple reaction monitoring scanning mode (MRM). LC-MSMS, MRM parameters and triple quadrupole settings were identical as the ones reported in Albert et al. 2010.
The method’s detection limit was 0.005 mg/kg for difethialone and 0.002 mg/kg for brodifacoum and bromadiolone. The standards were all analytical grade (>98% purity). A calibration curve was built with five levels of concentrations ranging from 2.5 to 80 pg with an $r^2$>0.99. Samples were diluted in order to fit within the limits of the calibration curve. Recoveries at low and high level were >70% for all compounds. Known amounts of coumatetralyl (5 pg/IL; transition 291.00>140.90) and flocoumafen (1 pg/IL; transition 541.40>382.00) were added to each sample prior to the injection allowing ion suppression monitoring. Methanol was injected between each sample to monitor any possible contamination.

2.2.3. Statistical Analysis

Since great horned owls and red-tailed hawks represented the two species consistently found across Canada (no red-tailed hawk samples were submitted from British Columbia, however) and for which we had a large enough sample size to warrant a meaningful analysis, cumulative frequency distribution graphs were constructed for these species. The graphs were generated through a bootstrapping procedure (501 samples) using BurriOZ (version 1.0.14, © Commonwealth Scientific and Industrial Research Organisation, Australia 2000). Using the values identified in our toxicity threshold analysis, it was possible to identify the percentage of the sampled population exposed to SGARs belonging to a certain risk category (5%, 10%, 15% and 20% risk of becoming symptomatic).

3. Results

3.1. Toxicity Threshold
Five sources of data matched our criteria and were used in the analysis. Data published by Newton et al. (1990, 1998, 2000; n=45), Albert et al. (2010; n=164) as well as data from the Ecological Incident Information System (EIIS; n=61). All but four of the EIIS cases were submitted by the State of New York and several of the values were published in Stone et al. (1999, 2003). Barn owl samples were collected from localized areas across Canada and the United Kingdom (UK) with a few individuals from the United States (USA). Barred owl samples were mostly collected in Canada with only one from the USA while red-tailed hawk samples were obtained from the USA only. Great horned owl samples were collected from across both Canada and the USA. Samples were often collected from relatively developed areas or areas where the public was likely to report and submit carcasses.

There were significant differences between species in liver SGAR concentrations (F(4,535)=12.68, p<0.0001). Post hoc-tests (Tukey’s Studentized Range test, α = 0.05) revealed that, on average, red-tailed hawks (n=32) were the species with the highest liver concentrations of SGARs (Figure 1). All three owl species (great horned owl [n=86], barred owl [n=26] and barn owl [n=126]) had SGAR liver residues that were comparable. Logistic regression plots were calculated to predict the probability of a bird being symptomatic as a function of SGAR liver residues (Figure 2). This was done for each species separately and for all species combined (total of 270 individuals). Only the predicted probability curve for the great horned owl (GHOW) was located inside the 95% confidence limits for the pooled data and the estimated probability of becoming symptomatic differed significantly between species (F(1,4) = 82.9, p<0.0001). The curve for the red-tailed hawk curve differed from those of the three owl species and the curves
for the great horned owl and the barn owl also differed from each other (Tukey’s Studentized Range post-hoc test, P<0.05).

Using the probability curves, we calculated the predicted SGAR liver residue levels for different probability risk thresholds for different species (Table 1), although this was not possible for red-tailed hawks, as the data for this species could not be significantly modeled by a logistic regression. The majority of the calculated values are under the >0.1-0.2 mg/kg threshold suggested by Newton et al. (1999) and all are below 0.2 mg/kg. If the lower range of 0.1 mg/kg and 0.2 mg/kg from the potentially lethal range suggested for barn owls is applied to the barn owl probability curve, they correspond to toxicity probabilities of 11% and 22%, respectively. The higher 0.7 mg/kg level proposed by the Rodenticide Registrants Task Force (Erickson and Urban 2004) corresponds to a 54% probability of effect in barn owls.

Although the differences among the species curves indicate that probabilities of toxicity should be considered on a species-by-species basis, that is not possible where data for species are lacking. In such cases, it may be necessary to estimate toxicity probabilities on the basis of pooled data for other species. The probability curve for the pooled data in our study predicts that one in 20 birds with detectable residues would become symptomatic with SGAR liver residues of 0.02 mg/kg and one in five when residue levels reach 0.08mg/kg.

3.2. The extent of SGAR exposure in Canada

Of the two species sampled over a relatively broad area of Canada (great horned owl, red-tailed hawk), great horned owls were most consistently exposed to SGARs (Figure 3). Roughly 65% of great horned owls across Canada had detectable levels of SGARs in
their liver (detection limit of 0.005 mg/kg ww). Frequency of exposure in red-tailed hawks seemed to increase eastward from the Prairie Provinces to Ontario and Quebec. The frequency of exposed birds was the lowest (~20%) in the Prairie and Northern provinces (and territories), increased to ~70% in Ontario and reached the highest in Quebec (~90% of red-tailed hawks found with detectable SGAR liver residues), although the sample size in Quebec was smaller than in the other regions. However, as sampling was fortuitous and sampling effort was not uniform, these spatial comparisons must be considered preliminary.

Great horned owls and red-tailed hawks were exposed to a number of SGARs (Figure 4). The majority of great-horned owls had multiple compounds in the liver; it was the only species with detectable levels of all three registered compounds. Sixty percent of red-tailed hawks had detectable liver residues of one or two compounds (Figure 4). Although the proportion of great horned owls with detectable residues was greater than for red-tailed hawks, this difference was not significant when data were compared for those provinces from which carcasses of both species were collected (Prairie Provinces, Ontario and Quebec; (paired t-test, t(2)= - 0.78, p = 0.26; Figure 4). Brodifacoum and bromadiolone were both detected in great horned owls and red-tailed hawks. Difethialone was only ever detected in great horned owls (Table 2) but has only been registered in Canada relatively recently.

When the liver SGAR concentrations in great horned owls measured in the present study were plotted as a cumulative frequency graph (Figure 5; birds with detectable residues only), it was apparent that approximately 25% had liver SGARs that exceeded the 20% probability level for effect (0.07mg/kg; Table 1). The lack of a probability curve
for red-tailed hawks precludes making a similar calculation for that species, but it is
evident that liver residue levels were much lower than for great-horned owls (Figure 5
and 6). For-example, 50% of great horned owls with detectable residues had liver
concentrations greater than 0.05 mg/kg ww compared with only 10% of red-tailed hawks.
Comparison of liver concentrations in the two species in which birds were matched by
province confirmed that liver residues were significantly higher in the owls than in the
hawks (paired t-test; \(t_{(2)} = -4.0, p=0.03\)). This finding is in contrast to the previously
published literature (Figure 1) where liver residues were higher in red-tailed hawks than
in great-horned owls.

Of the small number of individuals from 13 other species analyzed from Quebec,
eight of those had at least one individual with detectable liver SGAR residues (Figure 7).
That indicates that a wide breadth of species is probably also exposed to these
compounds elsewhere in Canada.

4. Discussion

4.1. Toxicity Threshold

Critical SGAR liver concentrations associated with adverse effects and/or mortality
have not been defined for most raptor species (Walker et al. 2008a), and establishing liver
-toxicity thresholds for SGARs is problematic (Stone et al. 2003). This is partly
because there are a number of factors that contribute uncertainty. For instance, the limit
of quantification used to measure the liver SGAR residues can vary widely with the
analytical method. That can lead to underestimates of the extent of contamination but,
conversely, inflation of residue magnitude if residues which were detected but were
below the level of quantification using older analytical methodology were assigned an
inflated limit value (Taylor et al. 2009). Species also vary markedly in their sensitivity to
SGARs. This is known for laboratory mammals (World Health Organisation 1995) but
almost nothing is known about the relative sensitivity of different avian species (Walker
et al. 2008a). Our risk probability curves strongly suggest significant differences exist
among raptor species.

To date, the only residue toxicity threshold for SGARs in raptors that has been
suggested is the >0.1-0.2 mg/kg -potentially lethal range- for barn owls (Newton et al.
1998, 1999). At best, that provides a range of concern for potential toxicity, and gives no
indication of likelihood of effects. The approach described in the current study offers a
major advance in our ability to assess risk from SGAR residues in that it proposes
quantitative toxicity thresholds for different probability levels of dying from SGAR
intoxication for three species, including the barn owl. If sufficient data were available, it
should be possible to extend this approach to other species. That, in turn, would help to
identify raptor species that may be more sensitive to SGAR toxicity. Overall, on the basis
of the probability curves defined so far, it would seem that the >0.1-0.2 mg/kg level for
barn owls already carries considerable risk of acute intoxication (> 10-20% of barn owls
with this residue being likely to suffer mortality). Clearly, the probability of acute
poisoning associated with the 0.7 mg/kg residue level proposed by the Rodenticide
Registrants Task Force (Erickson and Urban 2004) is worse still.

The probabilistic methods described here are, as with all predictive methods, subject
to biases and uncertainties. Of these, perhaps two of the most important are likely to be
underestimation of non-lethal residues, because birds characterised as -zerosl in the
probabilistic plot may have metabolised some of their non-lethal SGAR residues before
dying [from non-SGAR related causes], and over-estimation of residues associated with
mortality because birds ingest more than a lethal dose before they die; animals typically
die some 5-7 days after ingestion of a lethal dose (Meehan 1984). Both biases would
have the effect of flattening the probability curve.

4.2. Exposure extent in Canada

4.2.1. Spatial extent

Stone et al. (2003) stated that, at the time, SGARs appeared to be present in the
majority of great horned owls and in roughly half of the red-tailed hawks from the
sampled areas of the State of New York. That conclusion can be directly applied to our
situation in Canada. Furthermore, a substantial fraction of a number of other raptors in
Quebec (from the western half of the province including areas surrounding Gatineau,
Montreal, Sherbrooke, Quebec and as far north as Obedjiwan) were also exposed to
SGARs (43% – or 13 of 30 birds tested), supporting the notion that other avian species
are also being impacted by SGAR use. This wider exposure in Quebec suggests a broad
contamination of terrestrial food chains as Accipiters, such as the Cooper's hawk, as well
as other species such as the merlin and the American kestrel, feed predominantly on small
birds and occasionally on insects (Ehrlich et al. 1988). Small birds, if the source of
rodenticides, are most likely being exposed to SGARs from insects or other invertebrates,
and possibly through direct uptake of grain-based baits.

In our study, great horned owls were consistently exposed to SGARs across the
country. In apparent contrast, their daytime ecological counterpart, the red-tailed hawk,
showed an increasing frequency of exposure eastward from the Prairie Provinces. This
difference could be explained by the lower dietary diversity of owls than hawks. Marti and Kochert (1995) showed that, on a finer scale, food-niche breadth became narrower along an eastward transect from the west coast of North America. This may reflect greater diversity of available prey in the west that could permit local populations of those two raptors to increase their diet segregation in western regions (Marti and Kochert 1995). Houston et al. (1998) lists the main prey of great horned owls as including rabbits and hares, coots and other waterfowl and mice. While snowshoe hares (*Lepus americanus*), black-tailed jackrabbits (*Lepus californicus*), and ground squirrels (*Spermophilus spp.* ) dominate the hawk’s diet in western and northern parts of North America (Preston and Beane 2009). The bulk of their diet in eastern and midwestern North America includes voles (*Microtus*), mice (*Peromyscus spp.*, *Reithrodontomys spp.*, *Mus musculus*), rats (*Sigmodon hispidus, Oryzomys palustris*), and cottontails (*Sylvilagus spp.* ) (Preston and Beane, 2009). Thus, it may be that in eastern areas that are more agricultural and urban (and subject to a higher degree of SGAR use), red-tailed hawks are exposed more frequently to SGARs through their increased feeding on rodents and reduced predation on other prey.

To obtain a more reliable estimate on actual exposure in Canada, we examined the livers of birds found dead from all causes. Our data indicate that, despite a smaller human population and the harsher climate in Canada (albeit some south-western regions of the country are characterised by milder weather), both of which should limit the need for rodenticides, the scale of exposure reported in our study are comparable to those in Europe. In the French Department of Loire Atlantique, 73% of a sample consisting of common kestrels (*Falco tinnunculus*), common buzzards (*Buteo buteo*), barn owls and
Tawny owls (*Strix aluco*) had detectable SGAR liver residues (Lambert et al. 2007). In the UK, between 40% and 74% of barn owls, kestrels, and avian scavengers such as buzzards and red kites (*Milvus milvus*) found dead from various causes had detectable liver SGAR residues (Newton et al. 1999; Shore et al., 1999, 2006; Walker 2008b). However, it should be noted that the sampled areas of Canada were those with higher population densities and where landscape features are not greatly dissimilar from Europe. That may at least in part account for the apparent similarity in the frequency of contamination.

The widespread exposure in Canada in part most likely reflects the increase in sales and use of SGARs in the last few decades (Albert et al. 2010), and the use of persistent compounds that remain detectable in the liver long after the exposure event (Laas et al. 1985). However, it is also clear from our data that multiple exposures, as detected by the presence of multiple compounds in the liver, are common. Although SGARs cannot be used legally on crops or orchards in Canada and are labelled for ‘indoor uses’ only, ‘indoor’ is defined to include use of baits outside farms and food establishments. This is likely to increase the exposure of non-target organisms. SGARs in Canada are currently labelled for domestic use although this is likely to change soon. Proposed regulatory actions relating to exposure risks for wildlife includes (amongst others), prohibiting use of SGAR compounds in residential settings or outdoor areas where wildlife may be exposed. In the case of commercial applications, bait stations would be required where wildlife could be exposed. Furthermore, labels of commercial class products would be amended to state that those products could be used only by certified operators, farmers and persons authorized in government-approved pest control programs (Pest Management
Regulatory Agency 2009). Those risk mitigation measures should have an overall positive impact on reducing unnecessary exposure risks to wildlife.

Regarding the impact of SGARs, we must be cautious in extrapolating from our data to predict likely mortality. However, if the probability of mortality is applied to each residue value in our dataset for great horned owls, this equates to an estimated predicted mortality of 11% (calculated by multiplying the probability of being exposed to SGARs [65% in GHOW]) by the mean probability of exhibiting signs of intoxication [17% in GHOW]). This is the first time that the scale of potential mortality from SGARs has been estimated for any wild raptor population. That estimate may well be too low, as some proportion of poisoned birds likely die out of sight (Shore et al. 2005) and so be under-represented in our sample. Furthermore, our estimates of the scale of mortality do not account for any indirect effects that SGARs may have. Sub-lethal exposures may indirectly increase mortality associated with natural or accidental events. For instance, SGARs may hinder the recovery of birds from non-fatal collisions or accidents. They may also impair hunting ability through behavioural changes such as lethargy, thus increasing the probability of starvation. Intoxication with rodenticides has been shown to alter behaviour in rodents (Cox and Smith 1992) but there is no evidence to date of indirect effects in free-ranging raptors (Shore et al. 2005).

The lack of a probability plot for red-tailed hawks means that a comparable estimate for SGAR-induced mortality in Canada cannot be made for this species. The available data suggest that red-tailed hawks may be more sensitive to SGARs than great horned owls (Figure 2) but red-tailed hawks generally had lower liver SGAR concentrations in Canada, and, it is notable that in New York, great horned owls are poisoned more
frequently than red-tailed hawks (Stone et al. 1999, 2003). Additional studies and monitoring of red-tailed hawk SGAR residues would strengthen our ability to estimate the risk of toxicosis following exposure to SGARs.

4.2.2. Future directions

Most studies that investigate exposure of non-target species to SGARs have focused on the uptake of poisoned rodents by various predators (Newton et al. 1990, 1999; Berny et al. 1997; McDonald et al. 1998; Howald et al. 1999; Shore et al. 1999, 2003). The finding that falcons and accipiters were also exposed in Quebec suggests that terrestrial food chains are broadly contaminated by SGARs despite their very restricted use. Invertebrates represent another route of exposure, especially in insectivorous avian species (Dowding et al. 2006). Some potential routes of exposure to aerial insectivores include the consumption of invertebrates that previously fed on rodent faeces or carcasses and even the consumption of ground-dwelling earthworms and beetles that ingested residues or actual rodent bait (Spurr and Drew 1999; Dunlevy et al. 2000). Clearly, given the fact that many ecosystems contain a larger proportion of insectivorous vertebrates relative to higher trophic predators, exposure could even be greater in those taxa (Dowding et al. 2010). Developing probability curves or even metabolism studies for a wider range of species would provide us with insight into the relative sensitivities and risks to other species (Watanabe et al. 2010). Finally, researching further indirect effects of SGARs on survival would refine current risk assessments of direct and indirect mortalities in wildlife.

4.3. Conclusion
Our results continue to support recommendations that persistent SGARs such as brodifacoum, bromadiolone and difethialone should be used with caution (or not at all in some circumstances) given that it appears difficult to eliminate the risk of exposure to non-target wildlife. The results presented will hopefully aid policy-makers in refining risk-assessments of SGARs on non-target wildlife.

Our results can also help regulatory agencies worldwide provide guidance on both commercial and residential use of SGARs and enforce appropriate risk mitigation as needed. In this context, the extent of non-target exposure to SGARs may not always depend on the amount of bait used, but also on the way it is used (Shore et al. 2006).

Focusing on improving application methods, such as baiting in areas of high rat activity only, conducting periodic and frequent searches for dead or dying rodents, enclosing the bait in a fashion that reduces invertebrate uptake may help reduce exposure of SGARs to predatory birds and other non-target species. Whether or not rodenticide resistance is common, an Integrated Pest Management (IPM) approach, that seeks to combine mechanical, biological and chemical controls, should be favoured as opposed to relying on a purely chemical mode of control.
Acknowledgements

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References:


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http://bna.birds.cornell.edu/bna/species/372


Mastrota, N.F. 2007. EIIS Version 2.0 – User Manual. EFED Information and Support Branch, Environmental Fate and Effects Division, Office of Pesticide Programs, USA.


Figure captions:

Figure 1: Published liver SGAR residues (combined concentrations of bromadiolone, brodifacoum and difethialone) in barred owl (BAOW), barn owl (BNOW), great horned owl (GHOW) and red-tailed hawk (RTHA). Total number of birds = 270 and do not include birds with non-detected residues. Diamond in the center of the box represents average, line is the median, box is the upper and lower quartiles and the whiskers are the standard deviation. Sources of the data are: Newton et al. 1990, 1998, 2000; Stone et al. 1999, 2003; Albert et al. 2010; EIIS 2010 download.

Figure 2: Effect plot of the probability of becoming symptomatic (0,1) as a function of log$_{10}$ [mg/kg]. ALL represents pooled data (n=270), BAOW represents barred owls (n=26), BNOW represents barn owls (n=126), GHOW represents great horned owls (n=86) and RTHA represents red-tailed hawks (n=32). Shading represents 95% confidence limits for ALL birds. Curves were drawn using the formula $y$(probability)=$1/(1+\exp(-(\text{int} + b*x))$ where int is the intercept and b is the parameter estimate for X (concentration).

Figure 3: Percentage of great horned owls (GHOW) and red-tailed hawks (RTHA) across Canada sampled in our study that had detectable ($\geq$ 0.005 mg/kg ww) liver SGAR residues. No RTHA samples were collected from PYR. PYR stands for the Pacific and Yukon region of Canada and PNR is the Prairie and Northern Region.

Figure 4: Percentage of great horned owls (GHOW) and red-tailed hawks (RTHA) with 0, 1, 2 and 3 different SGARs detected in the liver. Tested compounds were brodifacoum, bromadiolone and difethialone.

Figure 5: Cumulative frequency graph for liver SGAR residues in 79 great horned owls. Red line represents the 20% probability level for effect (0.07 mg/kg; Table 1).

Figure 6: Cumulative frequency graph for liver SGAR residues in 42 red-tailed hawks.

Figure 7: Numbers of birds of prey from Québec that contained detectable and non-detectable liver SGAR residues (13/30 samples tested positive or 43%).
Table 1: Toxicity threshold values (mg/kg ww) for 5%, 10%, 15% and 20% probability risk levels. For example, in barred owls (BAOW), an owl with 0.06mg/kg SGAR residues in the liver would have a 5% chance of showing signs of toxicosis. Sample sizes (n) as well as the number of positive (1) and negative (0) cases are presented. P value representing binary logit model fit is also showed. BNOW stands for barn owl, GHOW is the great horned owl, RTHA the red-tailed hawk and ALL represents the pooled data for all birds.

<table>
<thead>
<tr>
<th>Probability</th>
<th>BAOW</th>
<th>BNOW</th>
<th>GHOW</th>
<th>RTHA</th>
<th>ALL</th>
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<tbody>
<tr>
<td>n=26</td>
<td>n=126</td>
<td>n=86</td>
<td>n=32</td>
<td>n=270</td>
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<tr>
<td>0=22</td>
<td>0=114</td>
<td>0=62</td>
<td>0=3</td>
<td>0=201</td>
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<td>1=4</td>
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<td>1=24</td>
<td>1=29</td>
<td>1=69</td>
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<td>p=0.008</td>
<td>p=&lt;0.0001</td>
<td>p=&lt;0.0001</td>
<td>p=0.37</td>
<td>p=&lt;0.0001</td>
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</table>

--- values not presented if binary logit model fit was not statistically significant
Table 2: Geometric mean (range) liver SGAR concentrations [mg/kg ww] for great horned owls (GHOW) and red-tailed hawks (RTHA) from the Pacific and Yukon region of Canada (PYR), the prairie and northern region (PNR), Ontario and Quebec.

<table>
<thead>
<tr>
<th></th>
<th>PYR</th>
<th>PNR</th>
<th>Ontario</th>
<th>Quebec</th>
<th>Pooled – all provinces</th>
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<tr>
<td></td>
<td>GHOW</td>
<td>RTHA</td>
<td>GHOW</td>
<td>RTHA</td>
<td>GHOW</td>
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<tr>
<td>Brodifacoum</td>
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<td>N/A</td>
<td>0.008</td>
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<td></td>
<td>(0.003-)</td>
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<td>(0.001-)</td>
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<tr>
<td></td>
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<td></td>
<td>0.016</td>
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<td>6</td>
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<td>17</td>
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<tr>
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<td>0.004</td>
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<tr>
<td></td>
<td>(0.005-)</td>
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<tr>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>(0.013-)</td>
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<td>(ND)</td>
<td>(ND)</td>
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<tr>
<td></td>
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<td>0.07</td>
<td>0.017</td>
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<td>n</td>
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<td></td>
<td>13</td>
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<td>33</td>
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</tbody>
</table>

N/A = no samples obtained; ND = no detectable residue in any livers; n= number of birds with detectable residues.
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5
Figure 6
Figure 7