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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

scavengers (Albert et al. 2010, Newton et al. 1990, Shore et al. 1999, Shore et al. 2006). 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

47 related to toxicity. Our principal objectives are: i) to determine SGAR liver
48 concentrations that may be associated with mortality in birds (ie- to quantify the "toxicity
49 threshold") and ii) using the threshold values, assess the extent and severity of exposure
50 in Canadian birds of prey.
51
52 **2. Methods**
53 **2.1. Toxicity Threshold**
54 2.1.1. Literature Search
55 Recently published (~ last 10 years) peer-reviewed publications as well as the United
56 States Environmental Protection Agency (EPA)'s Ecological Incident Information
57 System (EIIS) were surveyed in order to locate liver residue data sets for birds of prey.
58 The EIIS is the EPA's database managing information on incidents linked to the exposure
59 of non target plants and animals to pesticides. It is currently managed by the Office of
60 Pesticide Programs (Mastrota 2007). Data were retained for our assessment if they met a
61 set of pre-determined conditions. These conditions included:
62 i) SGAR detection limits in liver were under 0.02 mg/kg wet wt;
63 ii) post-mortem evaluations were conducted prior to liver extraction and analysis;
64 pathophysiological signs of rodenticide poisoning were included.
65 iii) post-mortem evaluations were conducted by a reputable professional such as a
66 doctor of veterinary medicine (DVM); and
67 iv) adequate sample sizes were available (n>15) for any given species (in order to
68 have greater statistical power).
69 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 (ie- 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 ($n \geq 15$). 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

93 2.2.1. Sample Collection

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

111 2.2.2. Chemical Analysis

112 Chemical analysis was conducted at the National Wildlife Research Center in Ottawa,
113 Ontario, Canada. Methods were similar to those reported by Albert et al. (2010). 50 mg of
114 liver was ground in a mortar with about 5 g anhydrous sodium sulphate (Fisher no. S420-
115 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/lL; transition 291.00>140.90) and flocoumafen (1 pg/lL; 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 BurrliOZ (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

161 Five sources of data matched our criteria and were used in the analysis. Data
162 published by Newton et al. (1990, 1998, 2000; n=45), Albert et al. (2010; n=164) as well
163 as data from the Ecological Incident Information System (EIIS; n=61). All but four of the
164 EIIS cases were submitted by the State of New York and several of the values were
165 published in Stone et al. (1999, 2003). Barn owl samples were collected from localized
166 areas across Canada and the United Kingdom (UK) with a few individuals from the
167 United States (USA). Barred owl samples were mostly collected in Canada with only one
168 from the USA while red-tailed hawk samples were obtained from the USA only. Great
169 horned owl samples were collected from across both Canada and the USA. Samples were
170 often collected from relatively developed areas or areas where the public was likely to
171 report and submit carcasses.
172 There were significant differences between species in liver SGAR concentrations
173 ($F_{(4,535)}$ =12.68, $p<0.0001$). Post hoc-tests (Tukey's Studentized Range test, $\alpha = 0.05$)
174 revealed that, on average, red-tailed hawks (n=32) were the species with the highest liver
175 concentrations of SGARs (Figure 1). All three owl species (great horned owl [n=86],
176 barred owl [n=26] and barn owl [n=126]) had SGAR liver residues that were comparable.
177 Logistic regression plots were calculated to predict the probability of a bird being
178 symptomatic as a function of SGAR liver residues (Figure 2). This was done for each
179 species separately and for all species combined (total of 270 individuals). Only the
180 predicted probability curve for the great horned owl (GHOW) was located inside the 95%
181 confidence limits for the pooled data and the estimated probability of becoming
182 symptomatic differed significantly between species ($F_{(1,4)} = 82.9$, $p<0.0001$). The curve
183 for the red-tailed hawk curve differed from those of the three owl species and the curves

184 for the great horned owl and the barn owl also differed from each other (Tukey's
185 Studentized Range post-hoc test, $P<0.05$).
186 Using the probability curves, we calculated the predicted SGAR liver residue levels
187 for different probability risk thresholds for different species (Table 1), although this was
188 not possible for red-tailed hawks, as the data for this species could not be significantly
189 modeled by a logistic regression. The majority of the calculated values are under the
190 >0.1-0.2 mg/kg threshold suggested by Newton et al. (1999) and all are below 0.2 mg/kg.
191 If the lower range of 0.1 mg/kg and 0.2 mg/kg from the potentially lethal range suggested
192 for barn owls is applied to the barn owl probability curve, they correspond to toxicity
193 probabilities of 11% and 22%, respectively. The higher 0.7 mg/kg level proposed by the
194 Rodenticide Registrants Task Force (Erickson and Urban 2004) corresponds to a 54%
195 probability of effect in barn owls.
196 Although the differences among the species curves indicate that probabilities of
197 toxicity should be considered on a species-by-species basis, that is not possible where
198 data for species are lacking. In such cases, it may be necessary to estimate toxicity
199 probabilities on the basis of pooled data for other species. The probability curve for the
200 pooled data in our study predicts that one in 20 birds with detectable residues would
201 become symptomatic with SGAR liver residues of 0.02 mg/kg and one in five when
202 residue levels reach 0.08mg/kg.
203 **3.2. The extent of SGAR exposure in Canada**
204 Of the two species sampled over a relatively broad area of Canada (great horned owl,
205 red-tailed hawk), great horned owls were most consistently exposed to SGARs (Figure
206 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

253 below the level of quantification using older analytical methodology were assigned an
254 inflated limit value (Taylor et al. 2009). Species also vary markedly in their sensitivity to
255 SGARs. This is known for laboratory mammals (World Health Organisation 1995) but
256 almost nothing is known about the relative sensitivity of different avian species (Walker
257 et al. 2008a). Our risk probability curves strongly suggest significant differences exist
258 among raptor species.

259 To date, the only residue toxicity threshold for SGARs in raptors that has been
260 suggested is the >0.1-0.2 mg/kg "potentially lethal range" for barn owls (Newton et al.
261 1998, 1999). At best, that provides a range of concern for potential toxicity, and gives no
262 indication of likelihood of effects. The approach described in the current study offers a
263 major advance in our ability to assess risk from SGAR residues in that it proposes
264 quantitative toxicity thresholds for different probability levels of dying from SGAR
265 intoxication for three species, including the barn owl. If sufficient data were available, it
266 should be possible to extend this approach to other species. That, in turn, would help to
267 identify raptor species that may be more sensitive to SGAR toxicity. Overall, on the basis
268 of the probability curves defined so far, it would seem that the >0.1-0.2 mg/kg level for
269 barn owls already carries considerable risk of acute intoxication (> 10-20% of barn owls
270 with this residue being likely to suffer mortality). Clearly, the probability of acute
271 poisoning associated with the 0.7 mg/kg residue level proposed by the Rodenticide
272 Registrants Task Force (Erickson and Urban 2004) is worse still.

273 The probabilistic methods described here are, as with all predictive methods, subject
274 to biases and uncertainties. Of these, perhaps two of the most important are likely to be
275 underestimation of non-lethal residues, because birds characterised as "zeros" 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

299 difference could be explained by the lower dietary diversity of owls than hawks. Marti
300 and Kochert (1995) showed that, on a finer scale, food-niche breadth became narrower
301 along an eastward transect from the west coast of North America. This may reflect
302 greater diversity of available prey in the west that could permit local populations of those
303 two raptors to increase their diet segregation in western regions (Marti and Kochert
304 1995). Houston et al. (1998) lists the main prey of great horned owls as including rabbits
305 and hares, coots and other waterfowl and mice. While snowshoe hares (*Lepus*
306 *americanus*), black-tailed jackrabbits (*Lepus californicus*), and ground squirrels
307 (*Spermophilus spp.*) dominate the hawk's diet in western and northern parts of North
308 America (Preston and Beane 2009). The bulk of their diet in eastern and midwestern
309 North America includes voles (*Microtus*), mice (*Peromyscus spp., Reithrodontomys spp.,*
310 *Mus musculus*), rats (*Sigmodon hispidus, Oryzomys palustris*), and cottontails (*Sylvilagus*
311 *spp*.) (Preston and Beane, 2009). Thus, it may be that in eastern areas that are more
312 agricultural and urban (and subject to a higher degree of SGAR use), red-tailed hawks are
313 exposed more frequently to SGARs through their increased feeding on rodents and
314 reduced predation on other prey.
315 To obtain a more reliable estimate on actual exposure in Canada, we examined the
316 livers of birds found dead from all causes. Our data indicate that, despite a smaller human
317 population and the harsher climate in Canada (albeit some south-western regions of the
318 country are characterised by milder weather), both of which should limit the need for
319 rodenticides, the scale of exposure reported in our study are comparable to those in
320 Europe. In the French Department of Loire Atlantique, 73% of a sample consisting of
321 common kestrels (*Falco tinnunculus*), common buzzards (*Buteo buteo*), barn owls and

322 tawny owls (*Strix aluco*) had detectable SGAR liver residues (Lambert et al. 2007). In the
323 UK, between 40% and 74% of barn owls, kestrels, and avian scavengers such as buzzards
324 and red kites (*Milvus milvus*) found dead from various causes had detectable liver SGAR
325 residues (Newton et al. 1999; Shore et al., 1999, 2006; Walker 2008b). However, it
326 should be noted that the sampled areas of Canada were those with higher population
327 densities and where landscape features are not greatly dissimilar from Europe. That may
328 at least in part account for the apparent similarity in the frequency of contamination.
329 The widespread exposure in Canada in part most likely reflects the increase in sales
330 and use of SGARs in the last few decades (Albert et al. 2010), and the use of persistent
331 compounds that remain detectable in the liver long after the exposure event (Laas et al.
332 1985). However, it is also clear from our data that multiple exposures, as detected by the
333 presence of multiple compounds in the liver, are common. Although SGARs cannot be
334 used legally on crops or orchards in Canada and are labelled for 'indoor uses' only,
335 'indoor' is defined to include use of baits outside farms and food establishments. This is
336 likely to increase the exposure of non-target organisms. SGARs in Canada are currently
337 labelled for domestic use although this is likely to change soon. Proposed regulatory
338 actions relating to exposure risks for wildlife includes (amongst others), prohibiting use
339 of SGAR compounds in residential settings or outdoor areas where wildlife may be
340 exposed. In the case of commercial applications, bait stations would be required where
341 wildlife could be exposed. Furthermore, labels of commercial class products would be
342 amended to state that those products could be used only by certified operators, farmers
343 and persons authorized in government-approved pest control programs (Pest Management

344 Regulatory Agency 2009). Those risk mitigation measures should have an overall
345 positive impact on reducing unnecessary exposure risks to wildlife.
346 Regarding the impact of SGARs, we must be cautious in extrapolating from our data
347 to predict likely mortality. However, if the probability of mortality is applied to each
348 residue value in our dataset for great horned owls, this equates to an estimated predicted
349 mortality of 11% (calculated by multiplying the probability of being exposed to SGARs
350 [65% in GHOW] by the mean probability of exhibiting signs of intoxication [17% in
351 GHOW]). This is the first time that the scale of potential mortality from SGARs has been
352 estimated for any wild raptor population. That estimate may well be too low, as some
353 proportion of poisoned birds likely die out of sight (Shore et al. 2005) and so be under-
354 represented in our sample. Furthermore, our estimates of the scale of mortality do not
355 account for any indirect effects that SGARs may have. Sub-lethal exposures may
356 indirectly increase mortality associated with natural or accidental events. For instance,
357 SGARs may hinder the recovery of birds from non-fatal collisions or accidents. They
358 may also impair hunting ability through behavioural changes such as lethargy, thus
359 increasing the probability of starvation. Intoxication with rodenticides has been shown to
360 alter behaviour in rodents (Cox and Smith 1992) but there is no evidence to date of
361 indirect effects in free-ranging raptors (Shore et al. 2005).
362 The lack of a probability plot for red-tailed hawks means that a comparable estimate
363 for SGAR-induced mortality in Canada cannot be made for this species. The available
364 data suggest that red-tailed hawks may be more sensitive to SGARs than great horned
365 owls (Figure 2) but red-tailed hawks generally had lower liver SGAR concentrations in
366 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.

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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(-(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 (≥ 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.

Probability	BAOW n=26 0=22 1=4 p=0.008	BNOW n=126 0=114 1=12 p=<0.0001	GHOW n=86 0=62 1=24 p=<0.0001	RTHA n=32 0=3 1=29 p=0.37	ALL n=270 0=201 1=69 p=<0.0001
0.05	0.06	0.05	0.02	---	0.02
0.10	0.09	0.09	0.03	---	0.04
0.15	0.13	0.13	0.05	---	0.06
0.20	0.16	0.18	0.07	---	0.08

--- - 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.

	PYR		PNR		Ontario		Quebec		Pooled – all provinces	
	GHOW	**RTHA**	**GHOW**	**RTHA**	**GHOW**	**RTHA**	**GHOW**	**RTHA**	**GHOW**	**RTHA**
Brodifacoum	0.04 (0.003-0.61) n=28	N/A	0.008 (0.001-0.016) n=6	0.004 (0.001-0.02) n=3	0.007 (0.001-0.05) n=17	0.006 (0.001-0.17) n=18	0.013 (0.003-0.08) n=7	0.01 (0.008-0.04) n=5	0.017 (0.001-0.61) n=58	0.006 (0.001-0.17) n=26
Bromadiolone	0.03 (0.005-0.57) n=33	N/A	0.007 (0.001-0.07) n=7	0.004 (0.001-0.008) n=3	0.01 (0.001-0.07) n=15	0.004 (0.001-0.06) n=25	0.01 (0.003-0.14) n=6	0.003 (0.002-0.006) n=4	0.018 (0.001-0.57) n=61	0.004 (0.001-0.064) n=32
Difethialone	0.02 (0.013-0.03) n=3	N/A	ND	ND	0.003 (0.003-0.003) n=1	ND	ND	ND	0.013 (0.003-0.03) n=4	0
Pooled - all compounds	0.03 (0.003-0.609) n=64	N/A	0.007 (0.001-0.07) n=13	0.004 (0.001-0.017) n=6	0.008 (0.001-0.07) n=33	0.005 (0.001-0.17) n=43	0.012 (0.003-0.14) n=13	0.006 (0.002-0.04) n=9	0.016 (0.001 - 0.61) n=123	0.005 (0.001 - 0.064) n=58

N/A = no samples obtained; ND = no detectable residue in any livers; n= number of birds with detectable residues.

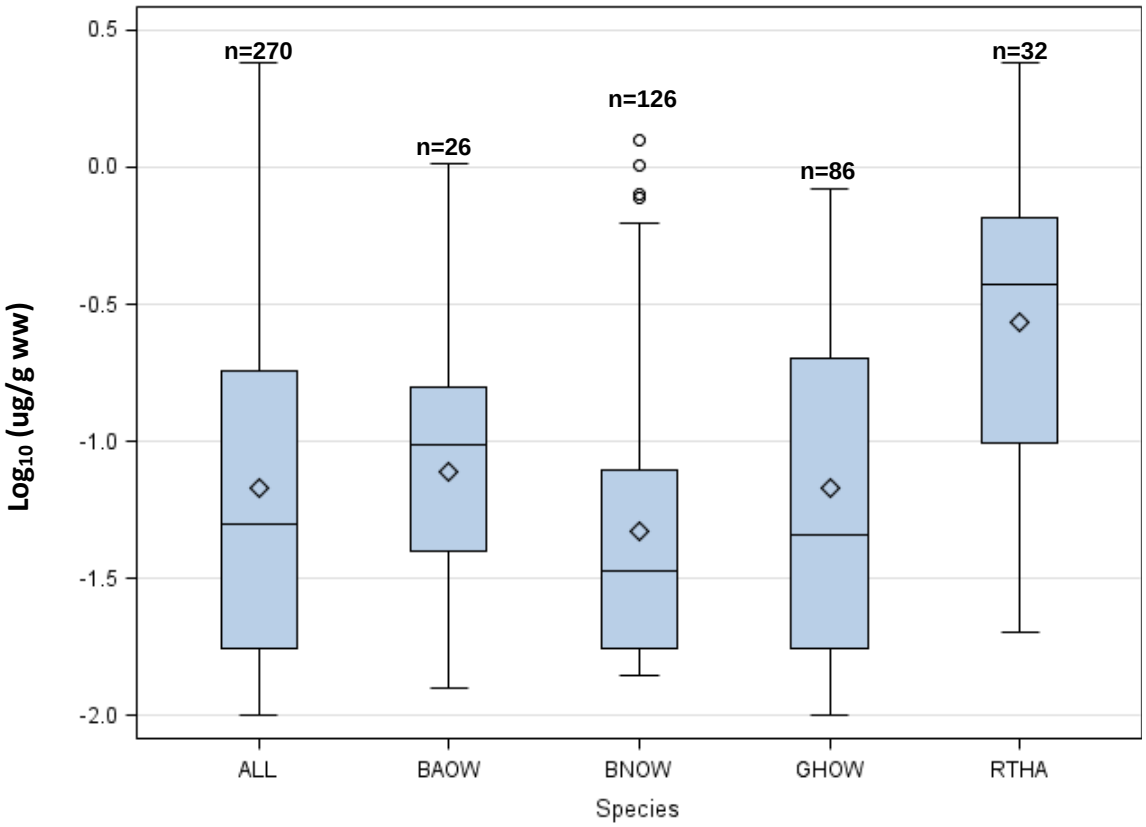
n=270
n=26
n=126
n=86
n=32
0.5
0.0
-0.5
-1.0
-1.5
-2.0
Log10 (ug/g ww)
ALL
BAOW
BNOW
GHOW
RTHA
Species

Figure 1

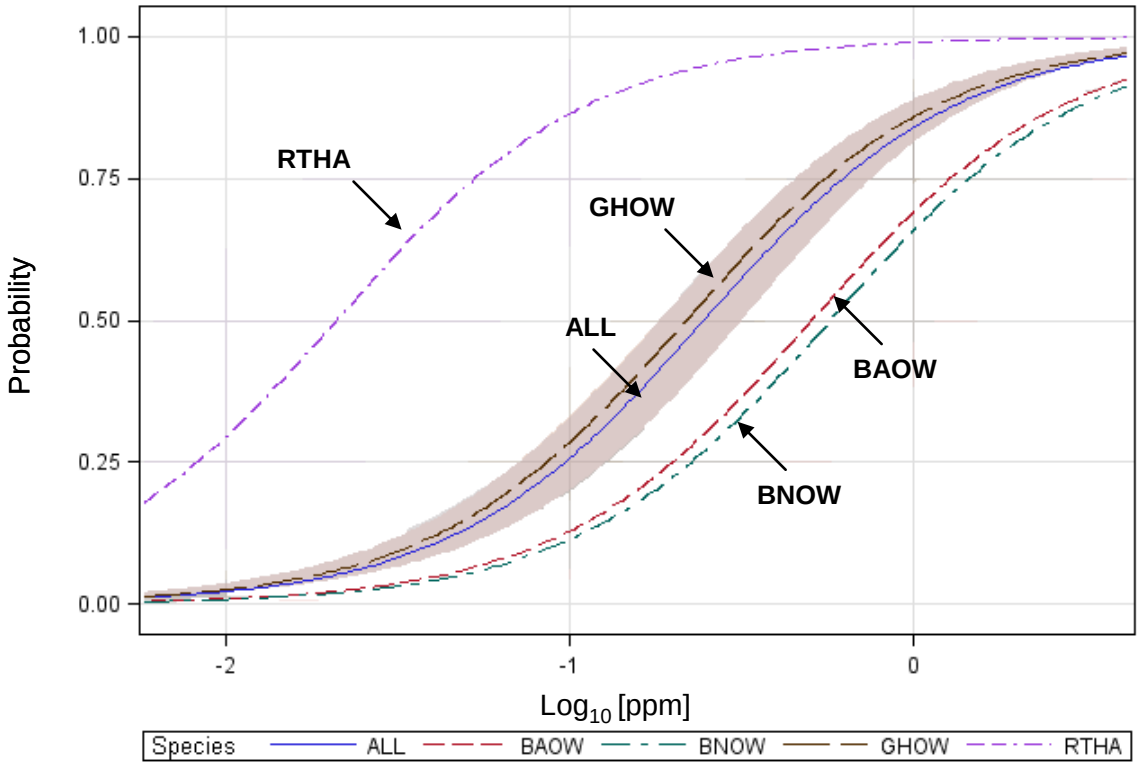
1.00
0.75
0.50
0.25
0.00
Probability
RTHA
GHOW
ALL
BAOW
BNOW
-2
-1
0
Log10 [ppm]
Species
ALL
BAOW
BNOW
GHOW
RTHA

Figure 2

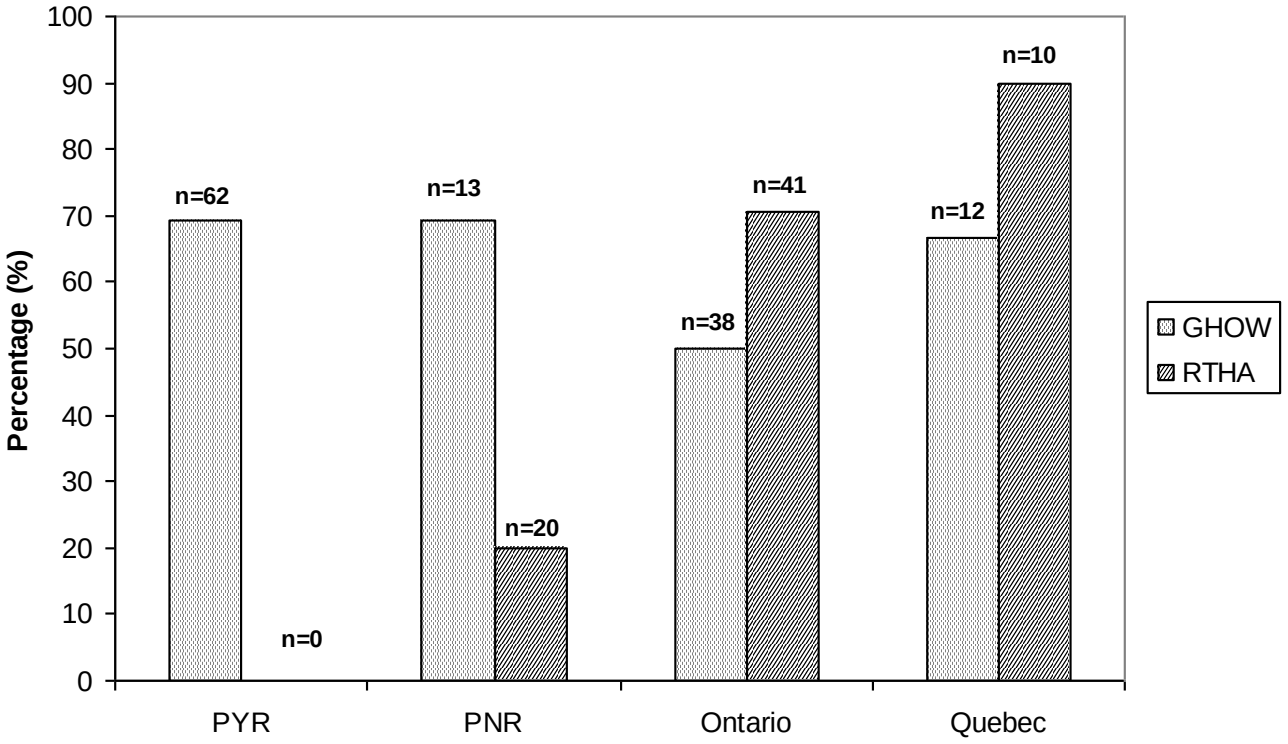
100
90
80
70
60
50
40
30
20
10
0
Percentage (%)
n=62
n=0
n=13
n=20
n=38
n=41
n=12
n=10
PYR
PNR
Ontario
Quebec
GHOW
RTHA

Figure 3

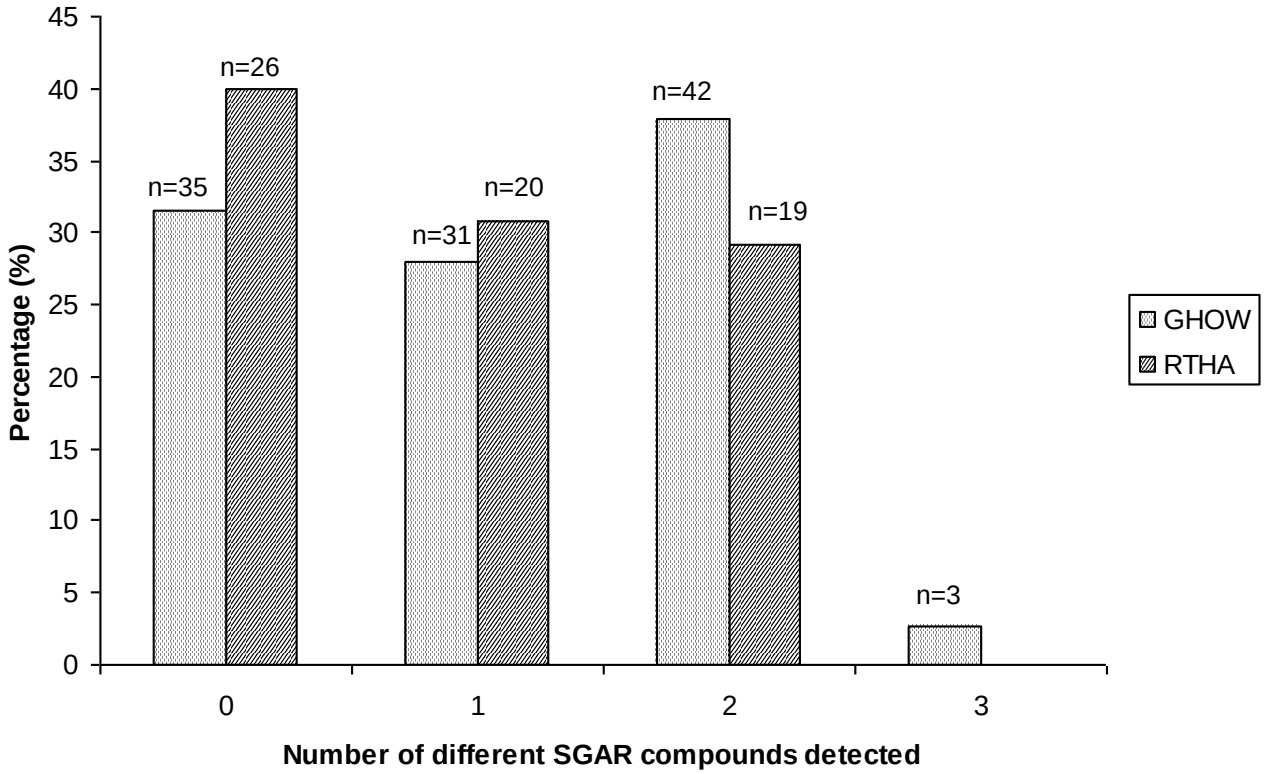
45
40
35
30
25
20
15
10
5
0
Percentage (%)
n=35
n=26
n=31
n=20
n=42
n=19
n=3
0
1
2
3
Number of different SGAR compounds detected
GHOW
RTHA

Figure 4

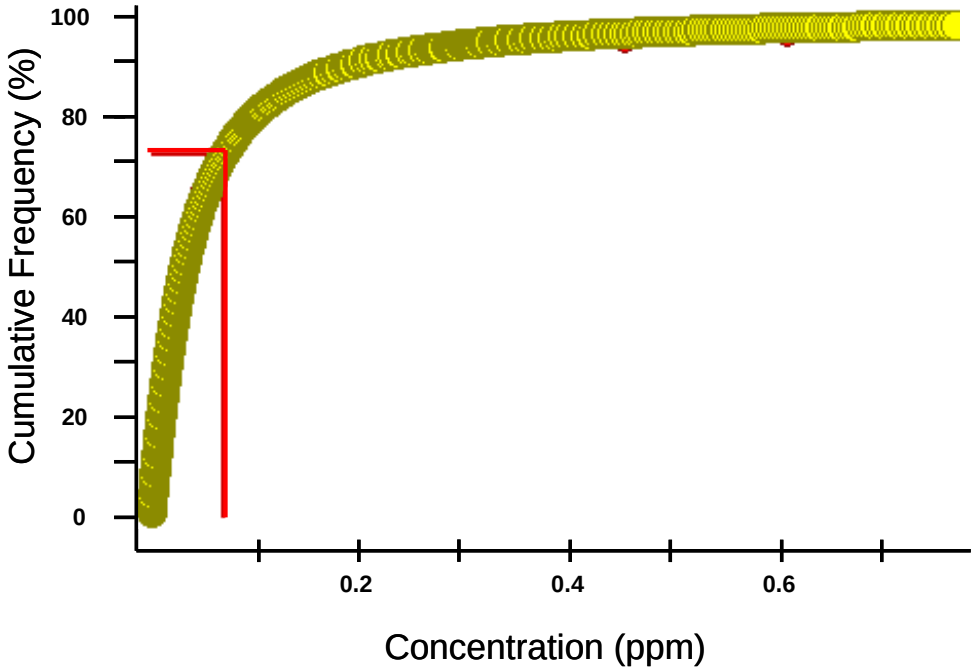
Cumulative Frequency (%)
100
80
60
40
20
0
0.2
0.4
0.6
Concentration (ppm)

Figure 5

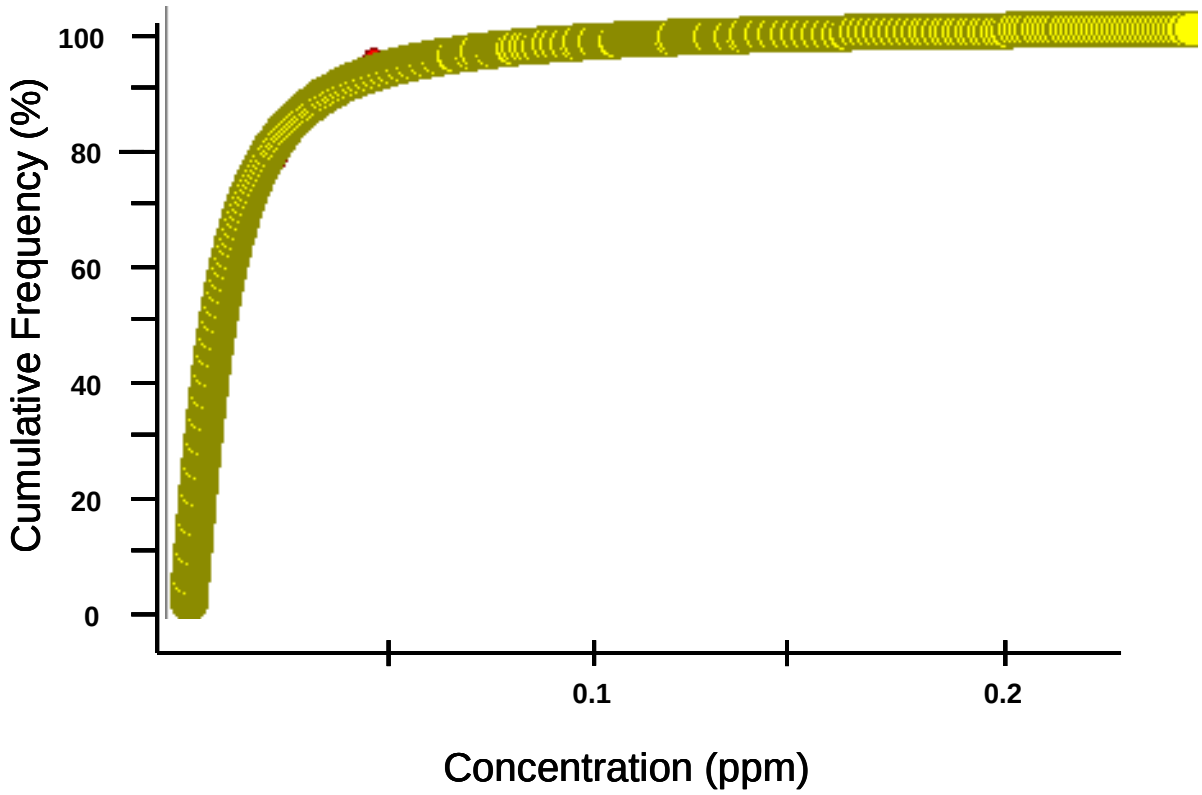
Cumulative Frequency (%)
100
80
60
40
20
0
0.1
0.2
Concentration (ppm)

Figure 6

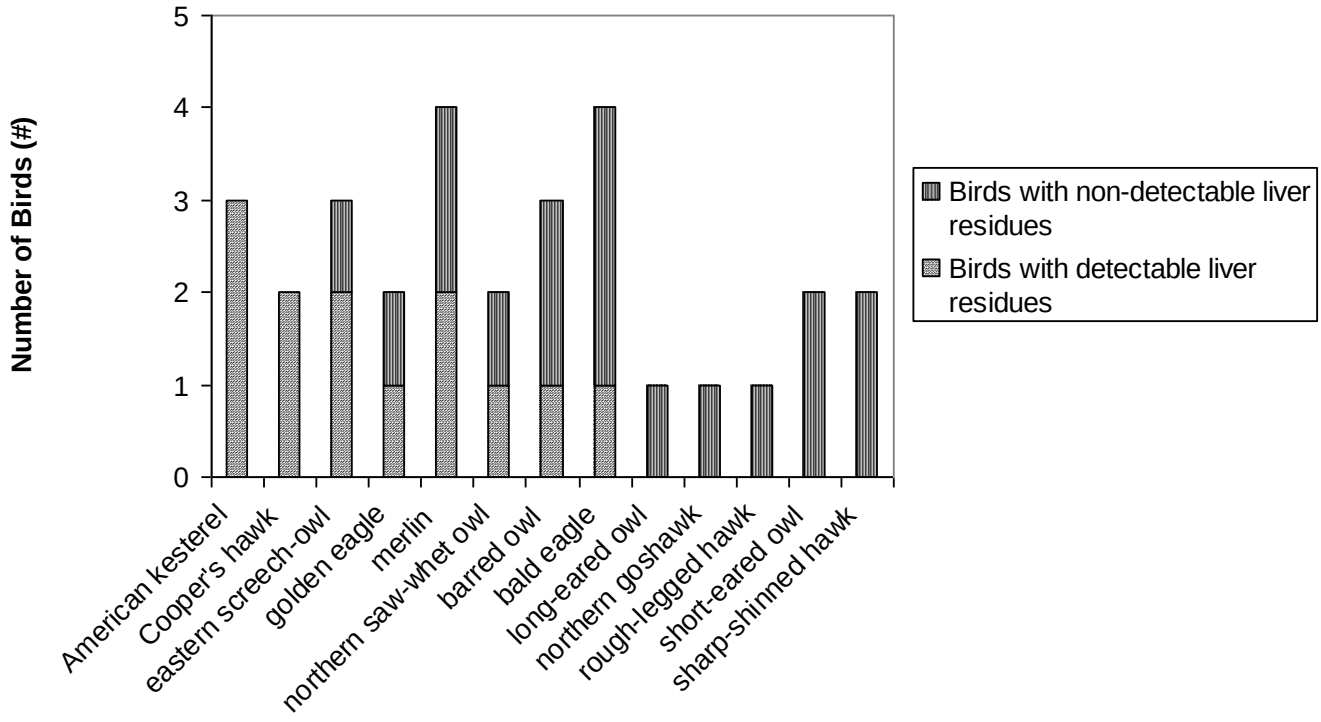
Number of Birds (#)
0
1
2
3
4
5
American kestrel
Cooper's hawk
eastern screech-owl
golden eagle
merlin
northern saw-whet owl
barred owl
bald eagle
long-eared owl
northern goshawk
rough-legged hawk
short-eared owl
sharp-shinned hawk
Birds with non-detectable liver residues
Birds with detectable liver residues

Figure 7

916
917
918
919
920