<https://helda.helsinki.fi>

The prevalence and correlates of anticoagulant rodenticide exposure in non-target predators and scavengers in Finland

Koivisto, Elina

2018-11-15

Koivisto , E , Santangeli , A , Koivisto , P , Korkolainen , T , Vuorisalo , T , Hanski , I K ,
Loivamaa , I & Koivisto , S 2018 , ' The prevalence and correlates of anticoagulant
rodenticide exposure in non-target predators and scavengers in Finland ' , The Science of
the Total Environment , vol. 642 , pp. 701-707 . <https://doi.org/10.1016/j.scitotenv.2018.06.063>

<http://hdl.handle.net/10138/316142>

<https://doi.org/10.1016/j.scitotenv.2018.06.063>

cc_by_nc_nd

acceptedVersion

Downloaded from Helda, University of Helsinki institutional repository.

This is an electronic reprint of the original article.

This reprint may differ from the original in pagination and typographic detail.

Please cite the original version.

The prevalence and correlates of anticoagulant rodenticide exposure
in non-target predators and scavengers in Finland

Elina Koivisto^{1†*}, Andrea Santangeli², Pertti Koivisto³, Tapio Korkolainen⁴, Timo Vuorisalo¹,
Ilpo K. Hanski², Iida Loivamaa³, Sanna Koivisto⁴

¹University of Turku, Department of Biology, FI-20014 Turun yliopisto, Finland

²The Helsinki Lab of Ornithology, Finnish Museum of Natural History, University of
Helsinki, P.O. Box 17, FI-00014, Helsinki, Finland

³Finnish Food Safety Authority Evira, Mustialankatu 3, FI-00790 Helsinki, Finland

⁴Finnish Safety and Chemicals Agency Tukes, P.O. Box 66, FI-00251 Helsinki, Finland

[†]Current address: Finnish Environment Institute (SYKE), Joensuu, Oulu, and Helsinki
Offices, P.O. Box 140, FI-00251 Helsinki, Finland

* **Corresponding author:** Elina Koivisto, Tel: +358-41-4492300, fax: +358-29-4505050,
email: elanko@utu.fi

Highlights

- Anticoagulant rodenticides (ARs) were detected in 82% of the samples
- Bromadiolone was the most prevalent AR and was also found in highest concentrations
- Species group explained most variation in prevalence and concentrations
- Group represented by foxes and raccoon dogs had the highest values

<https://www.sciencedirect.com/science/article/pii/S004896971832134X>

Abstract

The most common rodent control method worldwide is anticoagulant rodenticides (ARs), which cause death by internal bleeding. ARs can transfer to non-target predators via secondary exposure, i.e. by consuming contaminated rodents. Here we quantify the prevalence of seven AR substances in the liver tissues of altogether 17 mammalian or avian predator or scavenger species in Finland. In addition, we identify the environmental and biological factors potentially linked to secondary AR poisoning. No previous AR screenings have been conducted in the country, despite the widespread use of ARs and their potential impacts on the high levels of the ecosystem food chain. ARs were detected ($\geq 0.3 \mu\text{g/kg}$) in 82% of the 131 samples. The most prevalent and the AR with highest concentrations was bromadiolone (65% of samples). In 77% of the positive samples more than one (2-5) different ARs were detected. Of the environmental variables, we only found a weakly positive relationship between the coumatetralyl concentration and the livestock farm density. Conversely, overall AR concentration and number, as well as the concentration of three separate ARs (coumatetralyl, difenacoum and bromadiolone) differed among the three species groups tested, with the group “other mammals” (largely represented by red fox and raccoon dog) having higher values than the groups presented by mustelids or by birds. ARs

are authorized only as biocides in Finland and a national strategy on risk management (e.g. for minimising secondary poisoning of non-target species) of ARs was adopted in 2011. Based on these results it appears that the risk mitigation measures (RMMs) either have not been followed or have not been effective in preventing wide scale secondary exposure. Continued monitoring of AR residues in non-target species is needed in order to evaluate the effectiveness of current RMMs and a need for new ones to reduce the risk of secondary poisoning.

Graphical abstract

Keywords: Non-target exposure, Residues monitoring, Secondary poisoning

1. Introduction

Under the impelling pressure to increase food production after World War II, the farming and food industry has progressively increased the use of chemical substances. Since 1950, the global pesticide use has risen more than 50-fold (Miller 2005). Substances such as herbicides and insecticides have become particularly widespread, and their presence in the environment has been often found to have detrimental secondary impacts on wildlife, and also on humans (Carson 1962; Pain and Pienkowski 1997). Rodents are among one of the most common, generalist and widespread pests, causing a number of damages to agriculture and public health, and are the target of widespread pest control campaigns (Singleton 2003). The most common method used for rodent control worldwide is anticoagulant rodenticides (ARs). All ARs have a similar chemical structure and the same mode of action: they act by blocking the vitamin K cycle, resulting in inability to produce essential blood-clotting factors, thereby leading to death by internal bleeding (Berny et al. 2014). These effects are gradual, typically developing over several days.

Anticoagulants can be divided into first and second generation substances. The first generation anticoagulant rodenticides (FGARs) are less toxic and are eliminated within days, thereby requiring multiple doses to be fatal. The second generation anticoagulant rodenticides (SGARs) were developed after rodents started to show resistance to first generation agents, and they are toxic at a much lower dose (IPCS 1995). SGARs are persistent or very persistent, bioaccumulative or very bioaccumulative and toxic (European Chemicals Agency, Biocidal Products Committee opinions on active substance approval, <http://dissemination.echa.europa.eu/Biocides/factsheet?id=0018-14>). Anticoagulants have been found to transfer to non-target animals either by direct consumption of baits (primary poisoning) or by consuming contaminated prey animals (secondary poisoning, Lambert et al.

2007). Rodenticides are generally placed within bait boxes inaccessible to large animals. The target rodent that ingested the rodenticide will only die within a few days following the ingestion of lethal dose (Vandenbroucke et al. 2008). During this phase the rodent may be consumed by predators, causing secondary poisoning. Rodents dying aboveground can in turn represent an important risk of secondary poisoning for scavengers (Montaz et al. 2014).

Several species of mammalian and avian predators have been found to be exposed to ARs worldwide (see López-Perea and Mateo 2018 for a review), for example red foxes (*Vulpes vulpes*; Geduhn et al. 2015), mustelids (*Mustela* sp.; Elmeros et al. 2011), red kites (*Milvus milvus*; Berny and Gaillet 2008) and barn owls (*Tyto alba*; Newton et al. 1990). Besides in rodent-eating predators, ARs have also been found in non-target small mammals (e.g. Geduhn et al. 2014), and in insectivorous species, like European hedgehogs (*Erinaceus* sp., Dowding et al. 2010, López-Perea et al. 2015), shrews (*Sorex* sp., Geduhn et al. 2014) and passerine birds (Masuda et al. 2014). Anticoagulant poisoning is also a major issue for domestic animals, especially dogs (Berny et al. 2010 and references therein).

Quantifying the prevalence level of secondary AR poisoning in non-target wildlife is challenging, due to biases in the sample collection and to lab techniques to detect the prevalence of the substances. Moreover, the AR dosages acquired by consuming a poisoned rodent, for example, may be too low to have lethal consequences. However, in France intensive bromadiolone control following water vole (*Arvicola terrestris*) outbreaks has been reported to have caused considerable number of deaths of red kites and common buzzards (*Buteo buteo*; Coeurdassier et al. 2014). Wildlife AR poisoning and the unintentional wildlife deaths caused primarily or partially by ARs are systematically monitored only in few countries (e.g. SAGIR network in France, Millot et al. 2017), hence we still lack knowledge on the extension of problems caused by ARs to non-target wildlife from most countries. The potential sublethal and population level effects of secondary AR poisoning are even less

known or evaluated (but see Naim et al. 2010, Martínez-Padilla et al. 2017). However, repercussions could be important at the high levels of the food chain, because of the potentially increased dosage, and because the target species may be more sensitive to environmental change, e.g. pollution (as is the case for raptors; Newton 1998). There is thus a need to establish wider study schemes around the world both to assess the prevalence and associated risks caused by ARs, and to evaluate risk mitigation measures and eventually adjust them based on newly available information.

In Finland anticoagulant rodenticides are only authorized and predominantly used as biocides, and use for the crop protection is scarce. A national strategy on risk management of ARs was adopted in 2011. Rodenticides were regulated under pesticide acts until the end of 2006. They could be used for plant protection use (mainly to protect saplings) and for biocidal use. From beginning of 2007 rodenticides started to be regulated as biocides. The anticoagulant active substances found in this study have been on market in Finland at least since 1998. The most commonly used AR is bromadiolone. There has been no specific strategy or RMMs apart from the label claims. The use of ARs has not been studied in Finland, but it has most likely remained the same until 2011 and even after that despite of attempts of authorities to enlighten users of risk to non-target animals. There is still no surveillance or monitoring of use of ARs.

No AR screenings have ever been conducted in the country, despite the widespread use of ARs and their potential impacts on the high levels of the ecosystem food chain. Here we quantify the prevalence of anticoagulant rodenticide substances (FGARs chlorophacinone and coumatetralyl, SGARs brodifacoum, bromadiolone, difenacoum, difethialone, and flocoumafen) in the liver tissues of altogether 17 mammalian or avian predator or scavenger species (group “other mammals”: domestic cat, raccoon dog, brown rat, red fox; group “mustelids”: stoat, badger, least weasel, otter, pine marten; group “birds”: eagle owl,

goshawk, hooded crow, hen harrier, magpie, sparrow hawk, white-tailed sea eagle, tawny owl). In addition, we aim to identify the environmental (e.g. distance to industrial areas, which are typical areas for AR use) and biological factors (e.g. species group reflecting habitat use and diet preferences) that could be linked to secondary AR poisoning.

2. Material and methods

2.1 Sample collection

Liver samples were collected to determine the concentrations of the seven anticoagulant rodenticide substances (brodifacoum, bromadiolone, difenacoum, difethialone, flocoumafen, coumatetralyl, chlorophacinone) approved in Finland from altogether 131 individuals of different predator and scavenger species (group “other mammals”: domestic cat, raccoon dog, brown rat, red fox; group “mustelids”: stoat, badger, least weasel, otter, pine marten; group “birds”: eagle owl, goshawk, hooded crow, hen harrier, magpie, sparrow hawk, white-tailed sea eagle, tawny owl. Please see Table A.1 for more details). Animals were either found dead (e.g. road-kills) or were shot or trapped as part of predator removals from conservation areas (namely raccoon dog *Nyctereutes procyonoides*, which is an alien species in Finland). Samples were collected by the Zoological museums of the Universities of Turku and Helsinki, originally provided by museum personnel and private citizens. Most samples were collected in autumn-winter 2014 but some of the samples were of an older origin (mainly from years 2004-2013). The study species were selected because they are common species in Finland and because they are known to prey or scavenge on small animals. The species (listed in Table A.1 and Figure 2) included mainly rodent-eating predators, omnivores and scavengers, and as a reference also few carnivorous species that are not using rodents as their

main food source. In addition we had samples of a target species of ARs (brown rat *Rattus norvegicus*) from areas with known AR use. Samples were collected mostly from southwestern of Finland (Figure 1) and largely close to accessible areas (i.e. in the vicinity of human settlements) where exposure to the anticoagulants is most probable.

Samples were taken from the liver, since anticoagulants accumulate in the liver and their concentrations are low in other tissues (Fournier-Chambrillon et al. 2004). In most cases, the liver as whole was taken from the carcass. The coordinates of the sampling site along with the species and sex of the animal were marked to the sample. Body mass was measured and ages defined whenever possible. Liver samples were stored in a freezer (-18 °C).

2.2 Analytics

The concentrations of seven rodenticides in liver samples were determined by a UHPLC - triple quadrupole mass spectrometric method. An external standard method was used for the quantification. Deuterated warfarin was used for confirming success of the sample preparation. The compound was not used as internal standard for calibration purposes, but only as an indicator of successful extractions.

2.2.1 Sample preparation

Defrosted liver samples were homogenised in a blender and pushed through a metal sieve. Thereafter water-acetonitrile (5 mL, 1:1) was added to the samples and they were vortexed 30 seconds and sonicated in ultrasonic path for 10 minutes. To the sonicated sample ca. 1 g of sodium chloride was added. Following this the samples were vortexed and re-incubated in a sonic bath (10 minutes) and centrifuged 5 minutes at 3000 rpm. The upper (organic layer)

phases were transferred to other test tubes. To the remaining tissue material tubes 2.5 mL of acetonitrile (ACN) was added and the sonication and centrifugation steps were repeated twice.

The ACN phases containing the analytes were transferred into new test tubes and evaporated to dryness under a stream of nitrogen (Turbovap evaporator). The dry residues were re-dissolved into ACN and sonicated after addition of an aliquot of purified water (25% ACN at final concentration). The samples were eluted through SPE columns and the columns were washed with 25% methanol (2 mL). Analytes were eluted from the column using 5 mL of ACN containing ammonium hydroxide (5%). Solvent was again evaporated to dryness under a stream of nitrogen. The dry samples were dissolved into methanol (90%, 0.5 mL) and filtered through 0.2 µm syringe filter to UHPLC-vials.

2.2.2 Chromatography

A UHPLC instrument equipped with C-18 1.7µm (2.1x100mm) column was applied. Eluents were 10 mM ammonium formate (pH 9.5) and methanol (MeOH). Linear gradient: from 20% MeOH to 70% MeOH in 5 or 6 minutes was used. Methanol was increased to 95% and kept 30 sec. at 95% and from 6.5 to 7 min descending gradient to 20% MeOH. Column temperature was kept at 35°C and eluent flow rate was 0.35mL/min. Injection volume was 5.0 µL and total run time was 10 minutes. Corresponding retention times were: brodifacoum c. 6.4 min, bromadiolone c. 6.1 and 6.2 mins (two isomers), difenacoum c. 6.2 min, difethialone c. 6.5 min, flocoumafen c. 6.35 min, chlorophacinone c. 5.5 min, coumatetralyl c. 3.6 min and warfarin D5 3.1 min.

2.2.3 Mass spectrometry

The determinations were carried out with Xevo TQ-ms triple quadrupole instrument. Negative electrospray ionisation was used for all compounds. MS transitions for quantitation were: brodifacoum 521.3 >135.0, bromadiolone 525.3 >250.0, difenacoum 443.5 >135.0, difethialone 537.3 >151.0, flocoumafen 541.4 >161.0, chlorophacinone 373.2 >201.0, coumatetralyl 291.3 >141.0.

2.2.4 Calibration range and linearity

Five standard samples were prepared for each analytical batch by spiking AR-free porcine liver with known amounts of the substances. The concentrations that were used were 1, 5, 20, 50 and 300 µg/kg. Correlation coefficients (R^2) for the calibration lines were typically 0.98 - 0.99.

2.2.5 Recovery

Recovery was tested by spiking porcine liver sample with 20 µg/kg of anticoagulant mixture compared to standards without purification step. Recoveries ($N = 3$) were for brodifacoum (17±30%), bromadiolone (35±21%), difenacoum (11±41%), difethialone (12±50%), flocoumafen (22±38%), coumatetralyl (59±26%), and chlorophacinone (20±52%). In comparison to pure standards a recovery of analysed anticoagulants was too low and as a result matrix added calibration of curves was made for each sample set. In this way sample analysis it is not necessary to make recovery correction.

2.2.6 Limit of quantification and limit of detection

The limit of quantification (LOQ) was set to 1.0 µg/kg wet tissue for brodifacoum, bromadiolone, difenacoum, difethialone, flocoumafen and coumatetralyl, however, for chlorophacinone it was 20 µg/kg. The limit of detection (LOD) for six of the substances was 0.3 µg/kg, except for chlorophacinone it was 5.0 µg/kg. Signal to noise value 10 was used as minimum allowable peak integration level.

2.3 Environmental drivers and statistical analyses

We then assessed the relationship between AR substances and relevant environmental covariates. The sample unit for these analyses was the single observation (the AR value measured in the tissue of each sample) yielding a total of 131 samples where ARs were measured from the specimen and where the coordinates of the collection place were known, thus allowing the calculation of landscape variables (see below). These landscape variables included the density of livestock farms, the density of cereal farms with no livestock, as well as the distance to the closest industrial settlement. These three variables were extracted in GIS from original layers depicting the location of farms and industrial settings, and the values for the variables were calculated from the point where the sample was collected. The presence of farms (particularly livestock farms) has been linked to the prevalence of ARs in red fox tissues (Geduhn et al. 2015). Distance to the closest industrial setting was considered here as ARs are commonly used in such places to control or prevent rodent infestations. This variable is however strongly correlated with many other similar variables depicting distance from other infrastructures such as houses of different types, schools and the like. Furthermore, we also considered a categorical variable depicting three groups of species (birds, mustelids, other mammals), and a categorical variable depicting how the sample was

found (shot or trapped, road-kill, other or unknown). Because the number of analysed samples was low (e.g. < 10 ; see supporting Table A.1.) for many of the species, it was not possible to correlate species-level traits (such as diet and habitat preference) to the AR substances found in the specimens, hence the pooling of species into three broad categories as detailed above.

We built five separate full models including all the five above mentioned predictors. Four models had as response variable in turn the total concentration of all ARs together, as well as concentration of coumatetralyl, difenacoum and bromadiolone (using linear regression with Gaussian distribution and identity link). A fifth model included the number of different AR substances found in each sample (using a Poisson distribution with log link). For each of the five separate models, we started with the full model (including all five predictors), and proceeded with model comparison and multimodel inference using Akaike Information Criterion (AIC) following the approach of Burnham and Anderson (2002). We run all model combinations with the five variables in each case and then derived model averaged coefficients and statistics from the set of best ranked models (i.e. with $AIC < 4$) using the package MuMin (Bartoń 2011) in R version 3.0.3 (R Core Team 2015). Prior to analyses, we scaled and centred all the three continuous predictors to aid convergence, and we log transformed the four response variables depicting concentrations of AR to reduce the spread of these data and the occurrence of outliers. Collinearity among predictor variables was checked by means of variance inflation factor (VIF) analysis. All variables had a VIF value < 3 , suggesting low collinearity, thus their inclusion in the same model is appropriate (Zuur et al. 2009). It was not possible to run the above models using brodifacoum and flocoumafen concentrations as responses because most of the competing sub-models for these failed to converge.

3. Results

3.1 Prevalence of anticoagulant rodenticides

Anticoagulant rodenticide residues were detected ($\geq 0.3 \mu\text{g/kg}$) in 82% of the 131 samples analysed (Figure 2a). Of the analysed ARs, chlorophacinone and difethialone were not found in any of the samples. Of the detected ARs, the most common substance was bromadiolone (found in 65% of the analysed samples), followed in decreasing order of commonness by coumatetralyl (48%), difenacoum (42%), brodifacoum (23%) and lastly flocoumafen (12%). In 77% of the positive samples more than one (2-5) different ARs were detected (Figure 2b).

ARs were commonly detected e.g. in foxes, raccoon dogs, mustelids and owls (Figure 2a). Of these, foxes and raccoon dogs had the highest observed concentrations (Figure 3). Concentrations of bromadiolone were the highest among all ARs analysed, while brodifacoum and difenacoum showed moderate concentrations, and coumatetralyl (the only first generation substance) and flocoumafen were very scarce (Table A.1, Figure 3).

3.2 Environmental drivers

Overall, we found no effect of environmental variables, such as the density of farms with or without livestock, and the distance to the closest industrial setting, on any of the five response variables considered (Table 1). The only exception is represented by a weakly positive relationship between the concentration of coumatetralyl in the samples and the density of livestock farms (Table 1).

Conversely, overall AR concentration and number, as well as the concentration of each of the three ARs separately differed among the three species groups tested, with other mammals (largely represented by red fox and raccoon dog samples) having higher values than

292 mustelids and birds (see Table A.2, Figure 2b, Figure 3). In the case of difenacoum, the
293 concentration was also significantly higher for samples that were shot or trapped (LS mean =
294 1.31, SE = 0.18) as compared to the other samples (road-kill: LS mean = 0.56, SE = 0.29,
295 other or unknown: LS mean = 0.45, SE = 0.26).

4. Discussion

Here we quantified the prevalence of anticoagulant rodenticide (AR) substances in liver samples of non-target mammalian or avian predator or scavenger species susceptible for secondary AR exposure for the first time in Finland. Residues of AR substances were found in most of the samples analysed (in 82% of 131 samples from 17 species). Apart from chlorophacinone and difethialone which have not been on the market in Finland so far despite of the authorization, all the other five analysed substances (coumatetralyl, brodifacoum, bromadiolone, difenacoum and flocoumafen) were detected. Of these, bromadiolone was the most commonly detected one and it also had the highest observed concentrations. In 77% of the positive samples more than one (2-5) different ARs were detected. In addition, we identified the environmental and biological factors potentially linked to secondary AR poisoning. Overall, we found no effect of the studied environmental variables (e.g. distance to the closest industrial setting), except for a weakly positive relationship between the concentration of coumatetralyl and the density of livestock farms. Conversely, overall AR concentration and number, as well as the concentration of three of the ARs (coumatetralyl, difenacoum and bromadiolone) separately differed among the three species groups tested, with the group “other mammals” (largely represented by fox and raccoon dog samples) having higher values than groups “mustelids” (e.g. least weasels and stoats) and “birds” (e.g. eagle owls and hooded crows).

4.1 Prevalence and concentrations of anticoagulant rodenticides

The most common substance found was bromadiolone, which is also the most commonly used AR in Finland. Concentrations of bromadiolone were also the highest among all ARs analysed. In general, the prevalence of the ARs in non-target species corresponded well with

the sales of the substances in Finland. The sale volumes are collected yearly by the Finnish Safety and Chemicals Agency (Tukes), but the information is confidential, hence the sales of separate products cannot be disclosed. The only first generation AR (FGAR), coumatetralyl, was found in 48% of the samples. This is a high prevalence compared to some other studies, e.g. prevalence of only 5.7% in German red foxes found by Geduhn et al. (2015). Coumatetralyl may not be commonly used in many countries, due to prevalence of FGAR resistance (Geduhn et al. 2015). In Finland products containing coumatetralyl are not used frequently, but the concentration in the products is 7.5-81 times higher compared to second generation ARs (SGARs). Although coumatetralyl was found commonly in the samples, the concentrations were low compared to the SGARs. This is most probably due to the much shorter elimination half-life of coumatetralyl (1.8-4 days) compared to the SGARs (118-350 days). The half-lives of 118 days (difenacoum) and 350 days (brodifacoum) are given in the List of Endpoints (Chapter 3. Impact on Human Health, rate and extent of excretion) of the Assessment Reports for these substances. (<http://dissemination.echa.europa.eu/Biocides/factsheet?id=0026-14>, <http://dissemination.echa.europa.eu/Biocides/factsheet?id=0011-14>). The half-lives of coumatetralyl (1.8-4 days) are reported in the Document III-A, Data_001.pdf, Section 6.2 Toxicokinetics. <http://dissemination.echa.europa.eu/Biocides/factsheet?id=0018-14>).

In general, ARs were most often found in species which have a diet with a high percentage of rodents (e.g. owls, mustelids, red fox) as expected (López-Perea and Mateo 2018). The prevalence found in this study (82% over all samples) is high compared to some previous studies (see López-Perea and Mateo 2018 for a compilation table). For comparison, in the Netherlands the prevalence found in the predatory birds studied was 50% (van den Brink 2014; LOD = 10 µg/kg), in Spain 38.7% in all the studied animals and 49.5% in nocturnal raptors and carnivorous mammals (Sánchez-Barbudo 2012; LODs = 1-6 µg/kg), and in

France in raptors 73% (Lambert et al. 2007; LOD = 80 µg/kg). On the other hand, an equally high prevalence was found e.g. in Denmark (84-100% in all the avian and mustelid species studied; Christensen et al. 2010; LODs = 2-3 µg/kg) and USA (96% in four species of birds of prey; Murray 2017).

The high prevalence found could be due to several factors. First, the limits of detection (LOD) for the ARs in the present study were quite low (≥ 0.3 µg/kg) compared to some of the other studies (see above). Second, sample sizes for many species were quite low, increasing the possibility of chance affecting the results. In addition, the samples were on purpose collected mostly from accessible areas with dense human population and thus high potential for AR exposure. Geduhn et al. (2015), for example, found local livestock density and the percentage of urban area to be good indicators for AR residue occurrence in red foxes. Higher exposure to ARs in predators from urban areas has also been observed in other studies (e.g. Riley et al. 2007, López-Perea et al. 2015). The prevalence of AR exposed animals is also known to decrease with increasing distance to baiting boxes (Geduhn et al. 2014, Miljøministeriet 2015). In densely human populated areas the patches where no baiting is conducted are probably scarce, and considering the large hunting areas of the mobile predators (Hunter 2011), their likelihood of AR exposure in urban areas is probably very high (see Hindmarch and Elliott 2018 for review).

Although the prevalence was high, the concentrations found in this study were rather low. The potentially lethal level for SGAR residues in barn owls has been described as > 100 µg/kg (Newton et al. 1999a) and > 200 µg/kg (Newton et al. 1999b). Overall, the majority of the concentrations found in this study were below these ranges and thus probably not lethal for the animals. However, the highest concentration found was 920 µg/kg of bromadiolone in a fox. Besides direct lethal effects, ARs can have negative effects on non-target animals also in other more subtle ways such as impairing reproduction or body condition. For example,

common kestrel (*Falco tinnunculus*) fledglings with bromadiolone in their blood, regardless of the concentration, have been found to have a lower body mass than those without detectable bromadiolone (Martínez-Padilla et al. 2015). AR residues have also been hypothesized to predispose for example bobcats (*Lynx rufus*) to other medical conditions such as an inability to mount strong, anti-mite immunity (Riley et al. 2007, Serieys et al. 2013), and recent findings indeed suggest that sublethal toxicant exposure may indirectly cause mortality in bobcats by severely weakening their immune system (Serieys et al. 2018).

4.2 Environmental drivers

As a result of the study no remarkable effect of the environmental variables considered was found. The lack of effect found could be due to the aggregated sample collection near residential areas, thereby reducing the variance in the environmental covariates and masking any possible impact. Alternatively, as AR prevalence and concentration differences were largely driven by species group, the lack of effect of environmental drivers could reflect a dominance of the species group “other mammals” in the samples. Raccoon dogs represented almost a third of all the samples. Compared to other species studied, raccoon dogs and red foxes had a high prevalence and highest concentrations found in the livers. Both species are well-adapted to urban and semi-urban environments and have thus high probability to be exposed to ARs, both directly and indirectly (Harris and Baker 2001, Kauhala and Ihalainen 2014).

Only a weakly positive relationship between the concentration of coumatetralyl in the samples and the density of livestock farms could be estimated. Coumatetralyl is the only active substance that has been available as a contact poison, which are not eaten by rodents,

but groomed from the fur. Such poisons are favoured by farmers because the effectiveness of contact poisons is not hampered by the excess of food easily available on livestock farms.

4.3 Conclusions

Based on the high AR prevalence found in this study, it seems that the Risk Mitigation Measures (RMMs) to reduce collateral effects of ARs either have not been followed or have not been effective enough in preventing wide scale secondary exposure of the non-target animals in Finland. While the advice and guidance are seen as the major measures to reduce harmful effects of the ARs, there would clearly be a need to increase surveillance and monitoring, which at current are completely lacking. Continued monitoring of AR residues in non-target species is needed in order to evaluate the effectiveness of current RMMs and adjust them accordingly.

Exposure of non-target wildlife is an unwanted, unnecessary, and unethical ramification of AR use. Full prevention of secondary poisoning is not possible as long as ARs are used. However, given the lack of knowledge of their potential effects on wildlife, it is very important to apply a precautionary principle whereby RMMs like restriction to indoor use or restriction to professional use only are designed and enforced to minimise the collateral impacts of AR use on non-target species. The implications of this study span far beyond the study system considered here. ARs are widely used across most countries in the world (www.fao.org), including those in the global South where the most imperilled biodiversity locates. Predators and scavengers play crucial ecosystem roles but are also at high risk of extinction due to anthropogenic drivers (O'Bryan et al. 2018). The added threat due to secondary poisoning of predators and scavengers from ARs is largely unknown in many areas yet could have ecosystem level repercussions. As such, we urge more studies investigating

the prevalence of AR spilling across the different levels of the food chain. Only the evidence gathered by these studies could drive the designation and implementation of effective mitigation measures, such as risk management measures. These would ultimately allow for rodent pest control while minimising spill-over impacts that represent a threat, to other non-target species as well as humans.

5. Acknowledgements

This study was financially supported by the Finnish Ministry of Environment. We want to thank all the people who have participated in the sample collection, especially Ari Karhilahti, the staff of the zoological museums of the Universities of Helsinki and Turku, Anticimex Oy and all the volunteers. Ville Vähttilä is thanked for lab work and Niko Turunen for assisting in analyses. Tiina Tuusa, Mikko Heini and Jouni Siltala are acknowledged for the comments on the manuscript and Mari S. Lyly for animal drawings.

6. References

- Bartoń K 2011. Package ‘MuMIn’ <<http://cran.r-project.org/web/packages/MuMIn/index.html>>. (Last accessed 25.01.15).
- Berny PJ, Gaillet JR 2008. Acute poisoning of red kites (*Milvus milvus*) in France: data from the SAGIR network. *Journal of Wildlife Diseases* 44: 417–426.
- Berny PJ, Caloni F, Croubels S, Sachana M, Vandenbroucke V, Davanzo F, Guitart R 2010. Animal poisoning in Europe. Part 2: Companion animals. *The Veterinary Journal* 183: 255–259.

441

442 Berny PJ, Esther A, Jacob J, Prescott C 2014. Risk mitigation measures for anticoagulant rodenticides as
 443 biocidal products. Final report. European Commission, Luxembourg.

444

445 Burnham KP, Anderson DR 2002. Model selection and multimodel inference: a practical information-theoretic
 446 approach, 2nd edn. Springer, New York.

447

448 Carson R 1962. Silent spring. Houghton Mifflin Company.

449

450 Christensen TK, Elmeros M, Lassen P 2010. Forekomst af antikoagulante rodenticider i danske rovfugle, ugler
 451 og små rovpattedyr. Faglig rapport fra DMU nr. 788.

452

453 Coeurdassier M, Riols R, Decors A, Mionnet A, David F, Quintaine T, Truchetet D, Scheifler R, Giraudoux P
 454 2014. Unintentional wildlife poisoning and proposals for sustainable management of rodents. Conservation
 455 Biology 28: 315-21.

456

457 Dowding CV, Shore RF, Worgan A, Baker PJ, Harris S 2010. Accumulation of anticoagulant rodenticides in a
 458 non-target insectivore, the European hedgehog (*Erinaceus europaeus*). Environmental Pollution 158: 161–166.

459

460 Elmeros M, Christensen TK, Lassen P 2011. Concentrations of anticoagulant rodenticides in stoats *Mustela*
 461 *erminea* and weasels *Mustela nivalis* from Denmark. Science of the Total Environment 409: 2373–2378.

462

463 Fournier-Chambrillon C, Berny JP, Coiffier O, Barbedienne P, Dassé B, Delas G, Galineau H, Mazet A,
 464 Pouzenc P, Rosoux R, Fournier P 2004. Field evidence of secondary poisoning of free-ranging riparian
 465 mustelids by anticoagulant rodenticides in France: Implications for the conservation of the European mink
 466 (*Mustela lutreola*). Journal of Wildlife Diseases 40: 688–695.

467

468 Geduhn A, Esther A, Schenke D, Mattes H, Jacob J 2014. Spatial and temporal exposure patterns in non-target
 469 small mammals during brodifacoum rat control. Science of the Total Environment 496: 328– 338.

470

Geduhn A, Jacob J, Schenke D, Keller B, Kleinschmidt S, Esther A 2015. Relation between intensity of biocide practice and residues of anticoagulant rodenticides in red foxes (*Vulpes vulpes*). PLoS ONE 10(9): e0139191. doi:10.1371/journal.pone.0139191

Harris S, Baker P 2001. Urban foxes. British Natural History Series. Whittet Books, Suffolk.

Hindmarch S, Elliott JE 2018. Ecological Factors Driving Uptake of Anticoagulant Rodenticides in Predators. In: van den Brink N, Elliott J, Shore R, Rattner B (eds) Anticoagulant Rodenticides and Wildlife. Emerging Topics in Ecotoxicology (Principles, Approaches and Perspectives), vol 5. Springer, Cham.

Hunter LT 2011. Carnivores of the world. Princeton University Press.

IPCS 1995. *Environmental Health Criteria* 175. World Health Organization. ISBN 92 4 157175 6, ISSN 0250–863X.

Kauhala K, Ihalainen A 2014. Impact of landscape and habitat diversity on the diversity of diets of two omnivorous carnivores. *Acta Theriologica* 59: 1–12.

Lambert O, Pouliquen H, Larhantec M, Thorin C, L’Hostis M 2007. Exposure of raptors and waterbirds to anticoagulant rodenticides (difenacoum, bromadiolone, coumatetralyl, coumaten, brodifacoum): Epidemiological survey in Loire Atlantique (France). *Bulletin of Environmental Contamination and Toxicology* 79: 91–94.

López-Perea JJ, Mateo R 2018. Secondary Exposure to Anticoagulant Rodenticides and Effects on Predators. In: van den Brink N, Elliott J, Shore R, Rattner B (eds) Anticoagulant Rodenticides and Wildlife. Emerging Topics in Ecotoxicology (Principles, Approaches and Perspectives), vol 5. Springer, Cham.

López-Perea JJ, Camarero PR, Molina-López RA, Parpal L, Obón E, Sola J, Mateo R 2015. Interspecific and geographical differences in anticoagulant rodenticide residues of predatory wildlife from the Mediterranean region of Spain. *Science of the Total Environment* 511: 259–267.

501

502 Martínez-Padilla J, López-Idiáquez D, López-Perea JJ, Mateo R, Paz A, Viñuela J 2017. A negative association

503 between bromadiolone exposure and nestling body condition in common kestrels: management implications for

504 vole outbreaks. *Pest Management Science* 73: 364–370.

505

506 Masuda BM, Fisher P, Jamieson IG 2014. Anticoagulant rodenticide brodifacoum detected in dead nestlings of

507 an insectivorous passerine. *New Zealand Journal of Ecology* 38: 110–115.

508

509 Murray M 2017. Anticoagulant rodenticide exposure and toxicosis in four species of birds of prey in

510 Massachusetts, USA, 2012–2016, in relation to use of rodenticides by pest management professionals.

511 *Ecotoxicology* 26: 1041-1050.

512

513 Miller GT 2005. *Living in the Environment. Principles, Connections, Solutions*. 14th ed. Brooks/Cole-Thomson

514 Learning, Pacific Grove.

515

516 Millot F, Decors A, Mastain O, Quintaine T, Berny P, Vey D, Lasseur R, Bro E 2017. Field evidence of bird

517 poisonings by imidacloprid-treated seeds: a review of incidents reported by the French SAGIR network from

518 1995 to 2014. *Environmental and Pollution Research* 24: 5469-5485.

519

520 Miljøministeriet 2015. Spredning af antikoagulante rodenticider med mus og eksponeringsrisiko for rovdyr.

521 Bekæmpelsesmiddelforskning nr. 159. (In Danish with English summary)

522

523 Montaz J, Jacquot M, Coeurdassier M 2014. Scavenging of rodent carcasses following simulated mortality due

524 to field applications of anticoagulant rodenticide. *Ecotoxicology* 23: 1671-1680.

525

526 Naim M, Hafidzi MN, Azhar K, Jalila A 2010. Growth performance of nestling barn owls, *Tyto Alba javanica*,

527 in rat baiting area in Malaysia. *ARPN Journal of Agricultural and Biological Science* 5:1–13.

528

529 Newton, I. 1998. *Population limitation in birds*. Academic Press, San Diego, California.

530

Newton I, Wyllie I, Freestone P 1990. Rodenticides in British Barn Owls. *Environmental Pollution* 68: 101-117.

Newton I, Dale L, Finnie JK, Freestone P, Wright J, Wyatt C, Wyllie I 1999a. *Wildlife and Pollution: 1997/98 Annual Report*, JNCC Report, No. 285.

Newton I, Shore RF, Wyllie I, Birks JDS, Dale L 1999b. Empirical evidence of side-effects of rodenticides on some predatory birds and mammals. In: Cowan DP, Feare CJ (eds). *Advances in vertebrate pest management*. pp. 347–67. Filander Verlag, Fürth, Germany.

O’Bryan CJ, Brackowski AR, Beyer HL, Carter NH, Watson JEM, McDonald-Madden E 2018. The contribution of predators and scavengers to human well-being. *Nature Ecology & Evolution* 2: 229-236.

Pain DJ, Pienkowski MW 1997. *Farming and birds in Europe. The common agricultural policy and its implications for bird conservation*. Academic Press, London.

R Core Team 2015. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. Available from <http://www.R-project.org/>

Riley S, Bromley C, Poppenga R 2007. Anticoagulant exposure and notoedric mange in bobcats and mountain lions in urban southern California. *Journal of Wildlife Management* 71: 1874–1884.

Sánchez-Barbudo IS, Camarero PR, Mateo R 2012. Primary and secondary poisoning by anticoagulant rodenticides of non-target animals in Spain. *Science of the Total Environment* 420: 280–288.

Serieys LEK, Foley J, Owens S, Woods S, Boydston EE, Lyren LM, Poppenga RH, Clifford DL, Stephenson N, Rudd J, Riley SPD 2013. Serum chemistry, hematologic, and post-mortem findings in free-ranging bobcats (*Lynx rufus*) with notoedric mange. *Journal of Parasitology* 99: 989– 996.

559 Serieys LEK, Lea AJ, Epeldegui M, Armenta TC, Moriarty J, VandeWoude S, Carver S, Foley J, Wayne RK,
 560 Riley SPD, Uittenbogaart CH 2018. Urbanization and anticoagulant poisons promote immune dysfunction in
 561 bobcats. *Proceedings of the Royal Society B* 285: 20172533.
 562
 563 Singleton G 2003. *Rats, Mice and People: Rodent Biology and Management*. Australian Centre for International
 564 Agricultural Research.
 565
 566 van den Brink N 2014. Risico's van anticoagulantia rodenticides voor niet-doelsoorten en predatoren. Een scan
 567 van beschikbare kennis in Europa en analyses in roofvogels uit Nederland. Alterra-rapport 2589| ISSN 1566-
 568 7197. (In Dutch with English summary)
 569
 570 Vandenbroucke V, Bousquet-Melou A, De Backer P, Croubels S 2008. Pharmacokinetics of eight anticoagulant
 571 rodenticides in mice after single oral administration. *Journal of Veterinary Pharmacology and Therapeutics* 31:
 572 437-45.
 573
 574 Zuur A, Ieno EN, Walker N, Saveliev AA, Smith GM 2009. *Mixed effects models and extensions in ecology*
 575 *with R*. Springer, New York.
 576
 577

Figure 1. The location of the 131 samples (white triangles) available for this study in southern and central Finland. The pixels with a black and white colour gradient depict the human accessibility of the area (data retrieved from Verburg PH, Ellis EC and Letourneau A 2011. A global assessment of market accessibility and market influence for global environmental change studies. *Environmental Research Letters* 6:034019.), from highly accessible (black) to least accessible (white). The inset on the top left of the figure shows the location of Finland (grey) within Europe.

Figure 2. Number of samples a) in all the analysed species with and without detected levels ($\geq 0.3 \mu\text{g/kg}$) of residues of at least one anticoagulant rodenticide in the liver, and b) with the number of detected ARs (1, 2-3 or 4-5) in species with detected residues. Please note the different scaling of axes.

592

593

594 *Figure 3. Concentrations (mean $\pm$ SE) of the five detected anticoagulant rodenticides in species with highest*
595 *number of samples analysed. Only values $\geq 1\mu\text{g/kg}$ were included (number of samples shown below). Fox = red*
596 *fox, marten = pine marten, weasel = least weasel.*

Variables:	AR total concentration				AR total count				Coumatetralyl				Difenacoum				Bromadiolone			
	Coeff	SE	z	p	Coeff	SE	z	p	Coeff	SE	z	p	Coeff	SE	z	p	Coeff	SE	z	p
(Intercept)	1.29	0.35	3.69	<0.001	0.34	0.14	2.42	0.015	0.22	0.16	1.34	0.180	0.22	0.26	0.84	0.401	1.04	0.34	3.05	0.002
Closest industrial	-0.14	0.17	0.81	0.416	-0.08	0.07	1.08	0.282	0.04	0.07	0.51	0.609	-0.14	0.12	1.17	0.241	-0.04	0.18	0.23	0.816
Farms with no livestock	0.13	0.20	0.66	0.512	0.09	0.08	1.00	0.317	0.12	0.12	0.99	0.321	0.15	0.21	0.71	0.475	0.10	0.19	0.51	0.607
Farms with livestock	0.01	0.19	0.06	0.953	0.03	0.08	0.38	0.703	0.20	0.08	2.36	0.018	-0.19	0.18	1.06	0.289	0.09	0.18	0.47	0.638
How – Road-kill	0.32	0.53	0.61	0.542	0.14	0.22	0.63	0.532	-0.25	0.23	1.07	0.287	0.19	0.40	0.48	0.628	0.48	0.58	0.83	0.409
How – Shot / Trap	0.83	0.46	1.78	0.075	0.01	0.20	0.07	0.943	-0.40	0.20	1.97	0.049	1.01	0.36	2.74	0.006	0.47	0.51	0.91	0.364
Species – Other mammals	2.13	0.46	4.60	<0.001	0.56	0.16	3.47	0.001	0.76	0.21	3.63	<0.001	0.66	0.33	1.98	0.047	1.84	0.43	4.26	<0.001
Species – Mustelids	0.99	0.47	2.07	0.038	0.03	0.21	0.12	0.903	0.46	0.21	2.22	0.026	0.03	0.34	0.08	0.937	0.73	0.50	1.45	0.146

Table 1. The results of the effects of environmental variables (distance to the closest industrial settlement, density of farms with or without livestock), species group (birds, mustelids, other mammals) and how the sample was found (shot or trapped, road-kill, other or unknown) on the total concentration of AR, total count of ARs and on the concentration of coumatetralyl, difenacoum and bromadiolone. The reference category for species was set to be "birds", and for "How" it was set to be "other or unknown". Significant p-values (<0.05) are marked with italics.