No. C 440
October 2019

Rodenticide screening 2016–2018 - Exposures in birds (raptors and gulls) and red foxes

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FILE NO.	NV-01571-8
CONTRACT NO.	NV-02782-15
PROGRAMME AREA	Miljögiftsamordning
SUBPROGRAMME	Screening

Rodenticide screening 2016–2018
Exposures in birds (raptors and gulls) and red foxes

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Report title and subtitle
Rodenticide screening 2016–2018
Exposures in birds (raptors and gulls) and red foxes

Purchaser
Swedish Environmental Protection Agency, Environmental Monitoring Unit
SE-106 48 Stockholm, Sweden

Funding
Specify, e.g. national or regional environmental monitoring

Keywords for location (specify in Swedish)
Sverige, Stockholm

Keywords for subject (specify in Swedish)
rodenticid, screening, rovfågel, fågel, räv, trut

Period in which underlying data were collected
2016-2018

Summary

Rodenticides are biocidal products that are used in order to control rats and mice. This screening study aims at investigating whether chemical substances belonging to the group anticoagulant rodenticides can be detected in Swedish non-target biota, and to investigate if the levels are different compared with the results from a previous study.

The levels of anticoagulant rodenticides detected in the present screening study are similar to those found in earlier studies in Sweden and elsewhere. The literature indicates that toxic effects can occur in birds at levels > 100 ng/g (liver) whereas the level > 200 ng/g has been proposed to be a threshold level in foxes. Some individuals of raptors (n =2) and several foxes (n = 7) exceed these levels in the present study. These data suggest that anticoagulant rodenticides that are transferred in the food web may cause secondary toxicity in non-target mammals and birds in Sweden. However, no pathology has been performed for the individuals of the present study that can confirm any concentration-effect relationship or reason for mortality.

Author: Jenny Aasa, Jasmin Sandberg, Tomas Viktor and Johan Fång
Funded by: Swedish Environmental Protection Agency, NV-01571-8
Photographer: Måns Söderberg
Report number: C 440
ISBN 978-91-7883-107-4
Edition Only available as PDF for individual printing

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This report has been reviewed and approved in accordance with IVL's audited and approved management system.

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Summary

Rodenticides are biocidal products that are used in order to control rats and mice. This screening study aims at investigating whether chemical substances belonging to the group anticoagulant rodenticides can be detected in Swedish non-target biota, and to investigate if the levels are different compared with the results from a previous study.

During 2012/2013 IVL Swedish Environmental Research Institute performed a screening study of anticoagulant rodenticides in raptors and red foxes. Anticoagulant rodenticides act by inhibiting an enzyme involved in the mechanism of blood coagulation, which leads to internal hemorrhage and death for the exposed rodents. As rodents are part of the diet for raptors and red foxes, the screening was performed in predators/scavangers with the aim to investigate levels of exposures and risk for secondary toxicity. In the present study, the occurrence of two so called first-generation anticoagulant rodenticides (FGARs, warfarin, coumatetralyl) and five so called second generation anticoagulant rodenticides (SGARs, bromadiolone, difenacoum, difethialon, brodifacoum, flocoumafen) were monitored in liver samples from red foxes (n = 12) and different avian species (n = 31), both raptors (owls, hawks and falcon) and omnivores (gulls). The aim of this follow-up study was to investigate whether regulatory restrictions that have been implemented in the time-frame between the two studies could be observed as changes in exposure levels and/or exposure patterns in non-target biota. Samples were selected to represent different parts of Sweden. A number of gull samples collected in the Stockholm area were included in the present study, in order to investigate if this group of omnivores / scavengers possibly had been exposed to rodenticides in the urban environment they inhabit.

The results show that 68 % of the analysed birds were exposed to at least one rodenticide, and 42 % to at least two. Bromadiolone was detected at the highest concentrations (< LOD – 220 ng/g) and was also the most frequently occurring rodenticide in bird samples. Warfarin was detected in one individual (0.56 ng/g). The levels of flocoumafen was below LOD in all individuals. The aggregated concentration of the different generations of rodenticides in the birds varied between <LOD–170 ng/g for the FGARs and between < LOD–220 ng/g for SGARs. The aggregated concentration of all rodenticides in the birds varied between < LOD–220 ng/g.

All red foxes (100 %) were exposed to at least one rodenticide and 92 % were exposed to at least three. Coumatetralyl and bromadiolone were the most frequently detected rodenticides, with levels between 0.4–260 ng/g (coumatetralyl) and < LOD–1300 ng/g (bromadiolone). Warfarin was detected in four individuals (0.43–2.9 ng/g). Flocoumafen was not detected in any individual (<LOD). The aggregated concentration of the different generations of rodenticides in the foxes varied between 0.5–260 ng/g for FGARs and between <LOD–1485 ng/g for SGARs. The aggregated concentration of all rodenticides in the foxes varied between 0.5–1700 ng/g.

The levels of rodenticides in raptors in the present study were in general similar to the levels found during the screening 2012/2013. For the foxes, the pattern was similar regarding exposure to SGARs, while the levels of FGARs, with one exception, were lower in the present study compared with the previous screening study. One individual fox, which was collected in central Stockholm, had been exposed to a high level of coumatetralyl (260 ng/g). It cannot be ruled out or confirmed that this fox could have been exposed to this substance from a temporary exempt of usage of a coumatetralyl powder formulation in the area where the fox was found. However, this individual had very high levels of other rodenticides as well, indicating a more general exposure to anticoagulant rodenticide baits or exposed rodents. Bromadiolone was the most commonly

occurring rodenticide among the foxes at both screening occasions. The mean levels of both brodifacoum and difenacoum have increased since the earlier screening, however with large variations.

The levels of anticoagulant rodenticides detected in the present screening study are similar to those found in earlier studies in Sweden and elsewhere. The literature indicates that toxic effects can occur in birds at levels > 100 ng/g (liver) whereas the level > 200 ng/g has been proposed to be a threshold level in foxes. Some individuals of raptors (n =2) and several foxes (n = 7) exceed these levels in the present study. These data suggest that anticoagulant rodenticides that are transferred in the food web may cause secondary toxicity in non-target mammals and birds in Sweden. However, no pathology has been performed for the individuals of the present study that can confirm any concentration-effect relationship or reason for mortality.

Sammanfattning

Rodenticider är biocidprodukter som används för att bekämpa råttor och möss. Denna screeningstudie syftar till att undersöka om kemiska substanser som tillhör gruppen antikoagulerande rodenticider kan detekteras i djur i Sverige som inte är avsedda målgrupper för rodenticider, samt att undersöka om nivåerna är annorlunda jämfört med resultat från en tidigare screening.

Under åren 2012/2013 utförde IVL Svenska Miljöinstitutet en screeningstudie med avseende på antikoagulerande rodenticider (råttgifter) i rovfåglar och rödrävar. Dessa rodenticider verkar genom att hämma ett enzym i den blodkoagulerande mekanismen, vilket leder till inre blödningar och död för de exponerade gnagarna. Eftersom gnagare utgör föda för rovfåglar och rödrävar gjordes screeningen i rovdjuren för att undersöka exponeringsgrad och risk för sekundär toxicitet. I denna studie har förekomsten av två s.k. första generationens antikoagulerande rodenticider (FGARs, warfarin, kumatetralyl) och fem s.k. andra generationens antikoagulerande rodenticider (SGARs, bromadiolon, difenakum, difetialon, brodifakum, flokumafen) undersökts i leverprover från rödräv (n = 12) och olika fågelarter (n =31), både rovfåglar (ugglor, hökar och falkar) och allätare (måsfåglar). Syftet med denna uppföljningsstudie har varit att undersöka om regulatoriska begränsningar, vilket har implementerats inom tidsramen mellan de två studierna, kan observeras som förändrade exponeringsnivåer och/eller exponeringsmönster i djur som inte är avsedda som målgrupp för rodenticider. Urvalet av prover gjordes med syfte att representera olika delar av Sverige. Ett antal prover av måsfåglar insamlade i Stockholmsområdet inkluderades i studien för att undersöka om denna grupp av allätare/asätare kunde ha blivit exponerade för rodenticider i sin hemmiljö (stadsmiljö).

Resultaten visar att av de analyserade fåglarna var 68 % exponerade för minst en rodenticid och 42 % för minst två. Bromadiolon detekterades i högst koncentration (upp till 220 ng/g) och var också den mest frekvent förekommande rodenticiden i fåglarna. Warfarin detekterades endast i en individ (0.56 ng/g). Flokumafenhalterna låg under LOD för alla individer. Den sammanlagda koncentrationen av de olika generationernas rodenticider i fåglarna varierade mellan < LOD – 170 ng/g för FGARs samt mellan < LOD – 220 ng/g för SGARs. Summan av alla AR i fåglar var < LOD–2202 ng/g.

Alla rödrävar (100 %) var exponerade för minst en rodenticid och 92 % var exponerade för minst tre. Kumatetralyl och bromadiolon var de mest frekvent detekterade rodenticiderna med halter mellan 0.4–260 ng/g för kumatetralyl och < LOD – 1300 ng/g för bromadiolon. Flokumafen detekterades inte i någon individ (< LOD). Den sammanlagda koncentrationen av de olika generationernas rodenticider i rävarna varierade mellan 0.5–260 ng/g för FGARs och mellan <LOD – 1485 ng/g för SGARs. Summan av alla AR i rödräv var 0.5–1700 ng/g.

Halterna av rodenticider hos rovfåglarna i denna studie var generellt i nivå med halterna i screeningen som gjordes 2012/2013. För rävarna var exponeringsnivåerna jämförbara för SGARs, medan nivåerna av FGARs, med ett undantag, var lägre i den nuvarande studien jämfört med i den tidigare. En individ av rävarna, vilken hittades i centrala Stockholm, hade exponerats för höga nivåer av kumatetralyl (260 ng/g). Det kan varken uteslutas eller bekräftas att denna räv har blivit exponerad för denna kemikalie till följd av ett tillfälligt tillstånd att använda puderpreparat innehållande kumatetralyl i det område där räven hittades. Denna individ uppvisade dock mycket höga halter även av andra rodenticider, vilket antyder en mer generell exponering för olika rodenticidbeten och/eller exponerade gnagare.

Bromadiolon var den vanligaste förekommande rodenticiden i rävarna vid båda screeningtillfällena. Medelhalterna av både brodifakum och difenakum har ökat sedan den tidigare screeningen, dock med stora spridningar.

Halterna av antikoagulerande rodenticider i denna studie är jämförbara med vad som har hittats i tidigare studier i både Sverige och på annat håll. Litteraturen indikerar att toxiska effekter kan påvisas i fåglar vid nivåer högre än 100 ng/g (lever), medan nivåer högre än 200 ng/g har diskuterats som en tröskeldos för rävar. Ett fåtal individer av fåglarna (n = 2) och flera av rävarna (n = 7) överskrider dessa halter i denna studie. Dessa data antyder att antikoagulerande rodenticider som sprids i näringskedjan eventuellt kan orsaka sekundär förgiftning. Ingen patologi har gjorts för de inkluderade individerna i denna studie som kan bekräfta några koncentrations-effektsamband eller dödsorsak.

1 Introduction

Anticoagulant rodenticides (AR) are biocidal products that are used globally in order to control rats, mice and other rodents. Anticoagulant rodenticides act by a common anti-vitamin K (AVK) mode of action, disrupting the normal blood clotting mechanisms, resulting in increased bleeding tendency and, eventually, profuse haemorrhage and death. Data show that this group of chemicals is highly toxic to non-target organisms.

Numerous studies have shown that predators feeding on contaminated preys are exposed to AR and consequently at risk for secondary poisoning (c.f. Fourel et al., 2018; Nakayama et al., 2019). IVL Swedish Environmental Research Institute (hereinafter denoted IVL) has in a previously study analysed AR in raptors and red foxes (*Vulpes vulpes*) in the Swedish environment (Norström et al., 2013). In that study, all foxes (n=10) and 65 % of the raptors (n=20) were shown to be exposed to at least one AR. IVL also did a screening of rodenticides in eagle-owls (*Bubo bubo*) during 2008, which indicated that ARs were distributed in non-target biota in Sweden (Norström et al., 2009).

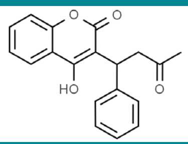
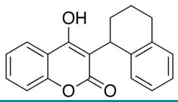
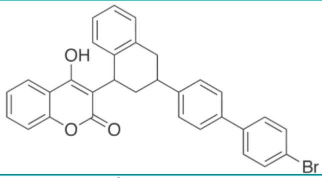
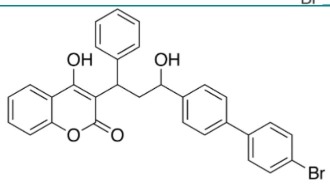
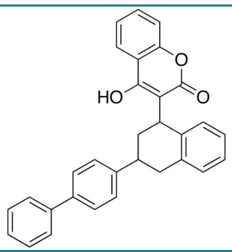
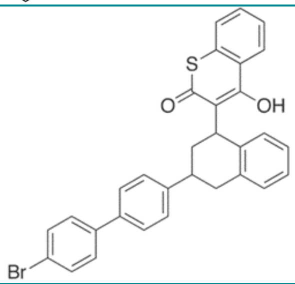
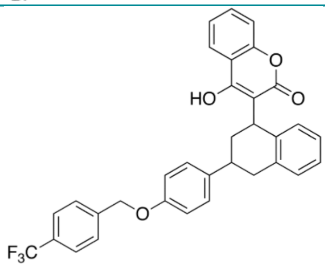
All rodenticides must be authorised by the Swedish Chemicals Agency before they can be sold and used in Sweden. Following from the implementation of Regulation (EU) No 528/2012 (EU Biocidal Products Regulation, BPR (EU, 2012), under which new data on the physico-chemical as well as (eco)-toxicological properties of the active substances were made available, the terms and conditions that apply for rodenticide products have become more restrictive, in Sweden as well as in other member states of the EU. This includes restrictions in e.g. how and by whom rodenticides may be used.

The present study is a follow-up on the previous study by IVL (Norström et al., 2013). The main objective of the present study is to investigate if the changes of the terms and conditions that apply for rodenticides have resulted in any effect of the exposure levels and pattern in non-target species (birds of prey, gulls and red foxes). Seven different AR (Table 1), from both the first-generation (FGARs) and the second-generation anticoagulant rodenticides (SGARs), have been quantified in the livers from birds and red foxes collected from different locations in the Swedish environment.

2 Chemical properties, fate and toxicity

The rodenticides included in the present screening study are presented in Table 1. All studied ARs belong to the same class of anticoagulants, i.e. 4-hydroxycoumarin derivatives.

Table 1. Anticoagulant rodenticides included in the screening.

Rodenticide	CAS	Chemical structure
Warfarin	81-81-2	
Coumatetralyl	5836-29-3	
Brodifacoum	56073-10-0	
Bromadiolone	28772-56-7	
Difenacoum	56073-07-5	
Difethialone	104653-34-1	
Flocoumafen	90035-08-8	

2.1 Properties and fate

The chemical and physical properties of the anticoagulant rodenticides included in the present study are shown in Table 2. The degradation rate in soil is relatively slow and varies depending on the type of soil. The K_{OC} values of the ARs indicate that they have low or no mobility in soil. Plant uptake is also believed to be limited, as residues in crops never have been detected in field studies (WHO, 1995a). The compounds are not expected to enter the atmosphere due to the low vapour pressure, but if released to the air they will exist mainly in the particulate phase. The water solubility and log K_{OW} varies between the ARs even though they have structural similarities and functional groups.

Table 2. Chemical and physical properties of the rodenticides of the present study (from Toxnet).

Rodenticide	M_w g/mol	Melting point °C	K_{oc}	Log K_{ow}	Solubility (aq.) at 20 °C mg/L	Vapor pressure mm Hg	pKa
Warfarin	307	161	16.8–261.3 (pH dependent)	2.70	17	1.1×10^{-8} (25 °C)	5.9
Coumatetralyl	292	172–176	3900	3.46	4.0[c]	6.4×10^{-11} (20 °C)	4.5–5
Brodifacoum	522	232	1.4×10^{5}	8.50[a]	0.24	1.1×10^{-18} (25 °C)	4.5[a]
Bromadiolone	526	198.3–199.8	1563–41600	3.8–4.1 (pH 6-7)	0.114 (pH 5); 2.48 (pH 7); 180 (pH 9)		4.5
Difenacoum	444	215–217	4.8×10^{6}	6.09–6.13	84 (pH 9.3); 2.5 (pH 7.3); 0.031 (pH 5.2)	5.0×10^{-11} (25 °C)	4.8
Difethialone	539	233–236	9.7×10^{6}	5.17	0.39 (25 °C)[c]	5.6×10^{-7} (25 °C)	
Flocoumafen	542	166–168[b]	4100	4.70	1.10	1.0×10^{-12} (25 °C)	

[a] estimated, [b] from PubChem, [c] from ChemIDPlus

2.2 Toxicity

The mode of action (MoA) for all AR of the present screening is inhibition of vitamin-K 2,3-epoxide reductase, which leads to disruption of the normal blood-clotting mechanisms and induction of damage to the capillaries (WHO, 1995b). The substances have an existing harmonized classification in accordance with the CLP Regulation (EC) No 1272/2008 (EC, 2008), including the hazard class Repr. 1A, with a specific concentration limits, $C \geq 0.003$ %. In addition, PBT assessment according to Annex XIII to Regulation (EC) No 1907/2006 (REACH Regulation, EC, 2006) show that all of these substances fall into the category T (Toxic), and all of the second generation anticoagulant rodenticides (SGARs, 5 of the 7 substances included in this study) are classified as persistent (P) or very persistent (vP), and bioaccumulative (B) or very bioaccumulative (vB) (Table 3).

Estimation of risk for an individual may be based on the sum of all concentrations of the different AR, i.e. the dose addition approach, as the MoA is the same for all studied AR (Meek et al., 2011).

The toxicokinetic properties differs between FGARs and SGARs, where the SGARs are more persistent in the blood and in body tissue (longer half-lives). Also, the SGARs have lower LD_{50} (more potent) in rats and in other mammals, e.g. in dogs, compared to FGARs (Table 3). The persistence of SGARs implies a higher risk for bioaccumulation and secondary toxicity to e.g. raptors and predatory mammals. For PBT assessment according to Annex XIII to Regulation (EC) No 1907/2006, see Table 3.

Table 3. Acute oral toxicity (LD_{50}) in rats of different strains and in dogs.

Rodenticide	LD_{50} rat (mg/kg)	LD_{50} dog (mg/kg)	Reference	PBT assessment[c]
First-generation				
Warfarin	112 (male) 5.6, 10.4 (female)[a]	20–50 200–300	U.S. EPA, 2004, ECHA, 2014a	T
Coumatetralyl	30 (male) 15 (female)	35	ECHA, 2011	T
Second-generation				
Brodifacoum	0.4–5 (female)	0.25–1	WHO, 1995b, ECHA, 2014b	P, vP, B, T
Bromadiolone	0.56–1.31	8.1	U.S. EPA, 2004	P, B, T
Difenacoum	7.33 (male) 6.0 (female)	0.01 (mg/kg/day, LOAEL[b])	U.S. EPA, 2007	P, vP, B, T
Difethialone	0.55 (male)	4	EC, 2007	P, vP, B, vB, T
Flocoumafen	0.13–0.5	0.075–0.25	Lund, 1988, EC, 2009	P, vP, B, vB, T

[a] From Wistar and Sprague-Dawley rats,, [b] LOAEL: lowest observed adverse effect level, [c] Data from BPC Opinions/ECHA.

3 Authorisation and use of rodenticides in Sweden

According to the EU Biocide Products Regulation (BPR), rodenticides require an authorisation before they can be placed on the market and used, and the active substances in those rodenticides must be previously approved. Due to the identified risk for environment and human health, and in particular the concern with respect to secondary poisoning of non-target organisms, anticoagulant rodenticides should normally not have been approved. However, evaluating authorities (in Sweden the Swedish Chemicals Agency) have found that not approving anticoagulant rodenticides for use would have a disproportionate negative impact on society when compared with the risk arising from the use of the product, thus fulfilling prerequisites in the BPR (Article 5.2 and 19.5). From this follows that the anticoagulant rodenticides have to be handled with great caution and all appropriate and available risk mitigation measures (RMMs) have to be applied. Such RMMs include e.g. the restriction to professional or trained professional users only, use in tamper-resistant bait boxes, or restrictions for usage indoors or in and around buildings. The approvals, including all terms and conditions, have to be re-evaluated every five years.

Rodenticide products containing six of the seven anticoagulant rodenticide substances included in the present screening study were authorised for use during the time period when the samples analysed in the present study were collected, 2016 – 2018 (The Swedish Pesticides Register, 2019). No products are currently authorised for usage directly into rats´ burrows outdoors. Since October 2018, no anticoagulant rodenticides are authorised to be used by the general public. For warfarin, no product was authorised to be used by any user category later than February 2015 (KIFS 2008:3).

Of all rodenticide substances, coumatetralyl was reported to be sold at the highest quantities for all years between 2015 and 2018, followed by bromadiolone and difenacoum. Very low sales quantities (<0.1 kg active substance) were reported for flocoumafen (Table 4) (data from the Products Register, C-H Eriksson, personal communication).

Table 4. Sold quantities (kg active substance) in Sweden of the anticoagulant rodenticides included in the present study, from year 2015 to 2018. Quantities reported <0.1 kg active substance are presented as 0. (data from the Products Register, C-H Eriksson, personal communication).

Substance	2015	2016	2017	2018
Warfarin[1]				
Coumatetralyl	10.5	4.4	4.4	5.9
Brodifacoum	0	0	0	0.1
Bromadiolone	1.0	1.6	2.6	1.6
Difenacoum	1.6	1.6	2.3	1.2
Difethialone	0	0	0.1	0.1
Flocoumafen	0	0	0	*no data*

[1] No rodenticide with warfarin as active substance was authorised to be sold in 2015- 2018.

4 Sampling

The same sampling strategy was applied as in the previous study by IVL (Norström et al., 2013), with the difference that seven, instead of six, anticoagulant rodenticides were analysed, and also that gulls (scavangers) in addition to predators (fox and raptors) were included. Predators and scavengers may be exposed to anticoagulant rodenticides when feeding on contaminated prey or rodenticide bait. The sampling program was focused on individuals that may be at risk for exposure, such as foxes and different species of raptors but also omnivores/scavangers represented by different species of gull, as specified in Table 5. The foxes were collected from January 2017 to April 2018, the raptors from January 2016 to June 2018, and the gulls from February to June 2018. For all individuals included in the study, samples from livers were used.

The majority of liver samples from the different species of birds were provided by the specimen bank at the Swedish Museum of National History, the remaining were provided by Victor Persson at Stockholm Vildfågel Rehab (SVR). The liver samples from the foxes were provided by the National Veterinary Institute.

Table 5. Sampling program for monitoring of anticoagulant rodenticides in liver samples.

Species	Latin name	Number of individuals	Samples provided by
Tawny owl	*Strix aluco*	14	Swedish Museum of National History
Eagle owl	*Bubo bubo*	8	Swedish Museum of National History
Long-eared owl	*Asio otus*	1	Swedish Museum of National History
Great black-backed gull	*Larus marinus*	1	Stockholm Vildfågel Rehab
Lesser black-backed gull	*Larus fuscus*	1	Stockholm Vildfågel Rehab
Herring gull	*Larus argentatus*	2	Stockholm Vildfågel Rehab
Goshawk	*Accipiter gentilis*	3	Stockholm Vildfågel Rehab
Eurasian hobby	*Falco subbuteo*	1	Stockholm Vildfågel Rehab
Red fox	*Vulpes vulpes*	12	National Veterinary Institute

5 Methods

5.1 Sample preparation

Liver samples (0.5 g) from avians and red foxes were homogenized and spiked (100 µL) with the internal standard coumachlor, 1000 ng/L (CAS 81-82-3) in plastic test tubes. The samples were extracted twice with acetonitrile (5 mL), vortexed for 30 seconds and put in an ultrasonic bath. After 15 min, the samples were rotated for 1 hour and centrifuged for 10 min (3500 rpm). After each extraction cycle the organic phases were pooled in a new test tube. To the combined organic solvent phases, hexane (2 mL) was added, and the samples were rocked carefully for 5 min before centrifugation for 10 min (3500 rpm). The acetonitrile fraction (ca 2 mL) was removed and evaporated to dryness. The samples were dissolved in methanol (1 ml) and transferred to Eppendorf test tubes, to which the injection standard ibuprofen-d3 (1000 ng/L) was added. After storage at -20 °C overnight another centrifugation followed for 10 min (10 000 rpm), and the extracts were transferred to vials for LC-MS analysis.

5.2 Analysis

5.2.1 Instrumentation

The samples were analysed using a high-performance liquid chromatography system consisting of a Prominence UFLC system (Shimadzu) with two pumps (LC 20AD), a degasser (DGU-20A5), an auto sampler (SIL-20ACHT) and a column oven (CTO-20AC). For analysis, 10 µl sample extract in methanol was injected onto the analytical column (Thermo HyPurity C8 50 mm x 3 mm, particle size 5 µm, from Dalco Chromtech). The column temperature was set to 35 ˚C.

The mobile phases consisted of 10 mM acetic acid in water (phase A) and methanol (phase B) running at a flow rate of 0.4 mL/min. A gradient elution was performed: 0–8 min 40 % B, 8–15 min linear increase to 95 % B, 15–16 min isocratic 95 % B. Equilibration time (4 min) when B reached 40 % again.

The effluent was directed to an API 4000 triple quadrupole mass spectrometer (Applied Biosystems), using electrospray ionisation (ESI) with negative ion mode and multiple reaction monitoring (MRM). The identification and quantification were performed by comparison to retention times of authentic reference compounds at known concentrations and the MRM transitions in Table 6.

Table 6. Molecular masses (*m/z*) used for quantification of the anticoagulant rodenticides.

Rodenticide	Precursor ion [M-H] *m/z*	Product ion (quantification) *m/z*	Product ion (qualification) *m/z*
Warfarin	307.0	250.0	161.0
Coumatetralyl	291.1	142.9	140.9
Brodifacoum	521.3	134.9	142.9
Bromadiolone	525.3	249.9	180.8
Difenacoum	443.4	293.1	135.0
Difethialone	537.2	150.8	371.0
Flocoumafen	541.3	382.0	160.9

5.2.2 Quality controls

- To ensure the quality of the identification of the target compounds, two MRM transitions were used for each compound, see Table 6. Also, the retention time should match those of the authentic standard compounds within ± 0.2 min.
- For each series of ten samples, two solvent method blanks were prepared in parallel with the samples to assess possible interferences and contamination from the background.
- Coumachlor was used as internal standard in all samples.
- The background contamination in the blank samples was subtracted from the measured sample values and the limit of detection (LOD) was defined as three times the standard deviation of the blank samples noise.

6 Results

All results for all individuals, including the LOD of each AR, are presented in Appendix A (birds) and B (red foxes). The detection frequencies and mean concentrations of the ARs for each species are presented in Table 7.

6.1 Birds

The concentration of the studied ARs in the present screening of different birds are presented in Figure 1 and in Table 7 (all data is included in Appendix A).

The birds were found to be exposed to at least one AR in 68 % of the samples, and 42 % were exposed to at least two ARs. Higher levels of SGARs were detected compared to FGARs (Figure 2a and Figure 3). The most frequently detected AR was bromadiolone (Table 7). Warfarin was detected in one individual and flocoumafen was not detected in any species.

Table 7. Number of individuals of each analysed species with detectable rodenticides and mean (range, if more than one individual) of each rodenticide concentration (ng/g). The sum of the mean values, as well as the sum of the minimum and maximum, of the total rodenticide exposure are also presented.

Species	Total number of individuals	Warfarin	Coumatetralyl	Brodifacoum	Bromadiolone	Difenacoum	Difethialone	Flocoumafen	Sum (min-max)
Tawny owl	14	0 N/A	6 38 (0.4–170)	2 15 (10–19)	5 7 (5–12)	0 N/A	5 0.4 (0.3–0.7)	0 N/A	60 (16–202)
Eagle owl	8	0 N/A	1 3	1 17	5 68 (1–220)	3 7 (4–10)	3 1 (0.5–2)	0 N/A	95 (25–251)
Long-eared owl	1	0 N/A	0 N/A	0 N/A	0 N/A	0 N/A	0 N/A	0 N/A	0
Great black-backed gull	1	0 N/A	0 N/A	0 N/A	0 N/A	0 N/A	0 N/A	0 N/A	0
Lesser black-backed gul	1	0 N/A	0 N/A	0 N/A	1 2	0 N/A	1 3	0 N/A	5.0
Herring gull	2	0 N/A	0 N/A	0 N/A	0 N/A	0 N/A	0 N/A	0 N/A	0
Goshawk	3	1 1	2 9 (7–11)	1 24	2 30 (15–45)	1 2	1 1	0 N/A	66 (48–83)
Eurasian hobby	1	0 N/A	0 N/A	0 N/A	1	0 N/A	0 N/A	0 N/A	0
Red fox	12	4 1 (0.4–3)	12 27 (0.4–260)	6 39 (2–180)	10 403 (50–1300)	6 23 (2–85)	2 1 (0.6–2)	0 N/A	495 (54–1830)

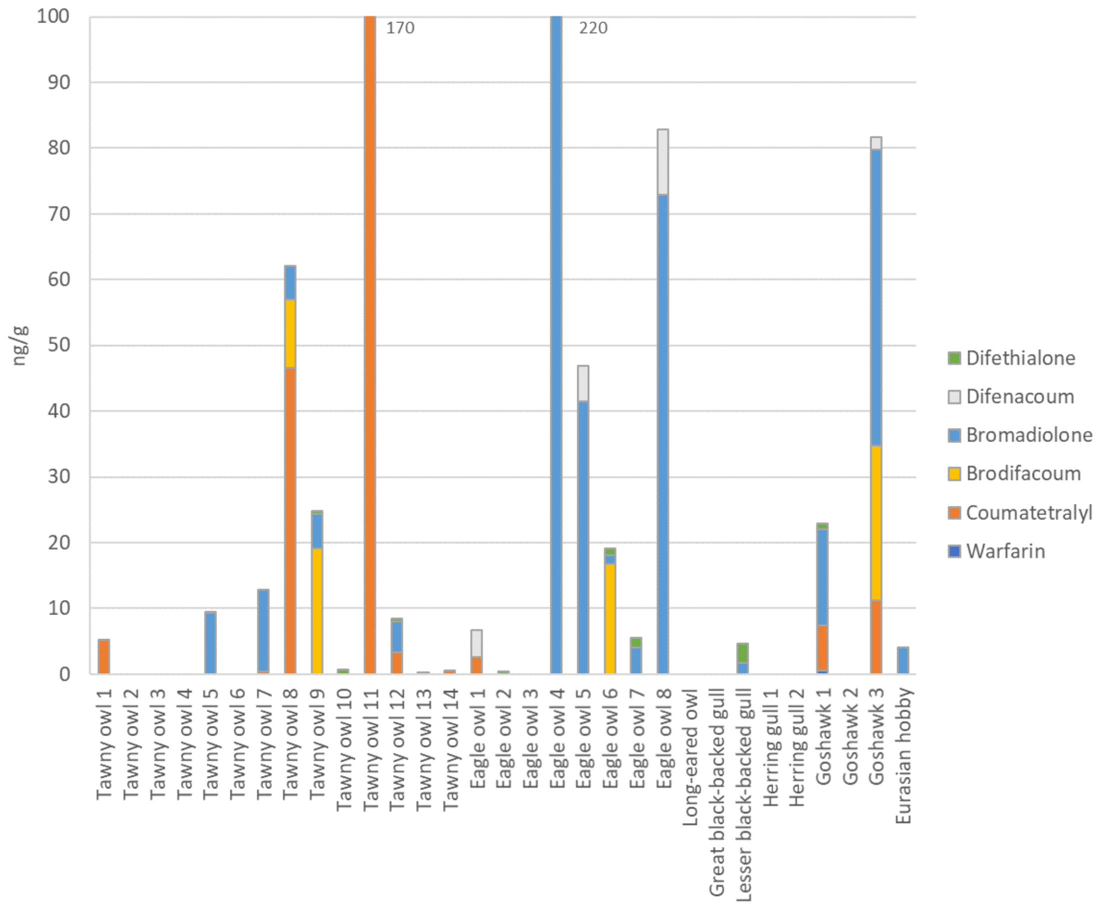


Figure 1. Concentration (ng/g) of rodenticides detected in individual birds. Flocoumafen was not detected in any individual (< LOD). [a] Coumatetralyl, [b] Bromadiolone. All sample information is included in Appendix A.

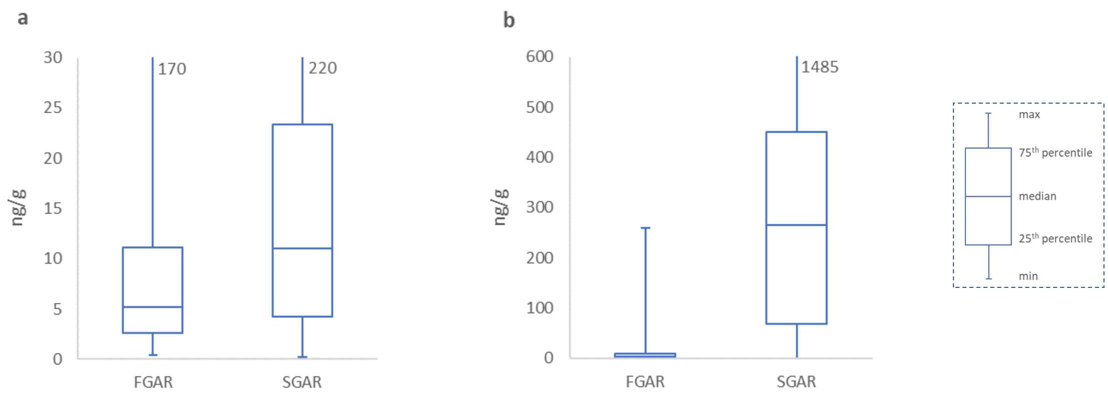


Figure 2. Concentrations (ng/g) of first-generation anticoagulant rodenticides (FGARs) and second-generation anticoagulant rodenticides (SGARs) in a) birds and b) red foxes. FGARs includes warfarin and coumatetralyl, and SGARs includes brodifacoum, bromadiolone, difenacoum and difethialone (no flocoumafen was detected, < LOD).

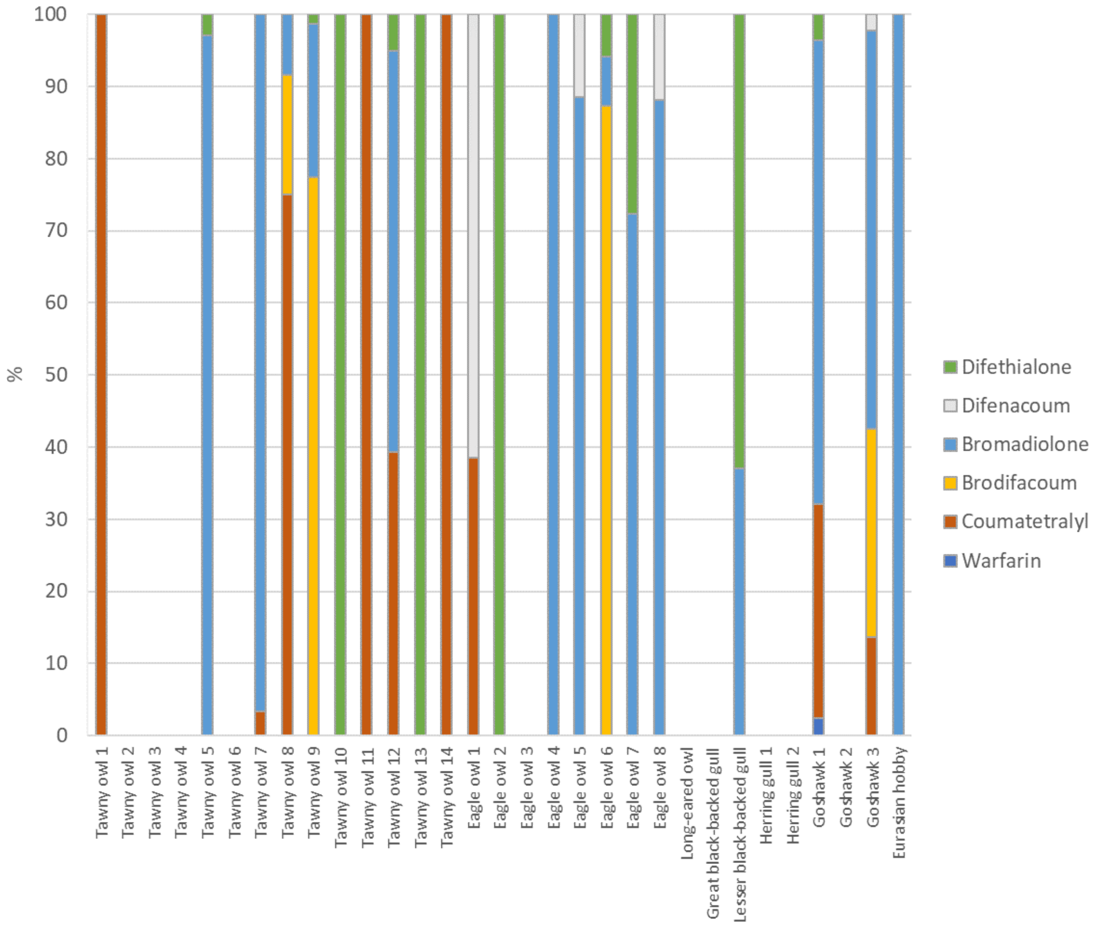


Figure 3. Frequency (concentration per total concentration) of the rodenticides detected in individuals of raptors and gulls (no flocoumafen was detected, < LOD).

6.2 Red fox (*Vulpes vulpes*)

The levels of the studied ARs in foxes in the present screening are presented in Figure 4 and in Table 7 (all individual data is included in Appendix B).

All analysed foxes in the present study were exposed to at least one AR, and 92 % were exposed to at least three ARs. The levels of SGARs were higher compared to FGARs (Figure 2b and Figure 5), Coumatetralyl and bromadiolone were the dominant rodenticides, with 100 % and 83 % of the foxes being exposed –bromadiolone constitutes the majority of the total AR exposure in 10 out of 12 individuals (Table 7 and Figure 5). Difethialone and warfarin were detected at the lowest frequency, with 17 % and 33 % of the foxes being exposed, respectively (Table 7). Flocoumafen was not detected in any individual (< LOD).

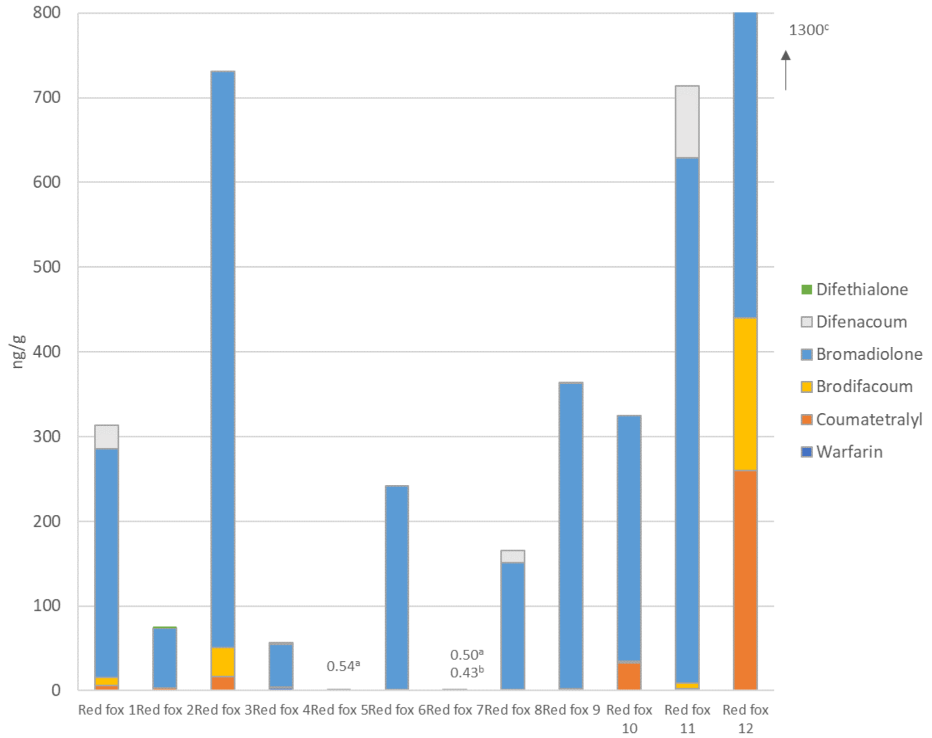


Figure 4. Concentration of rodenticides detected in individuals of red fox. Flocoumafen was not detected in any individual (< LOD). [a] Coumatetralyl, [b] Warfarin, [c] Bromadiolone. All sample information is included in Appendix B.

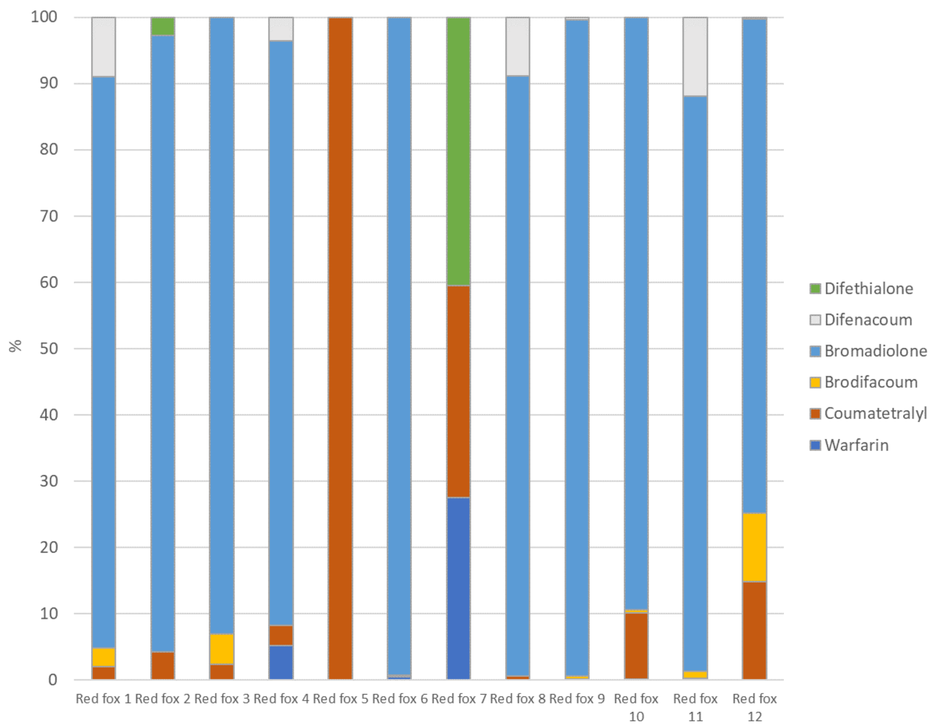


Figure 5. Composition (concentration per total concentration) of the rodenticides detected in individuals of red fox (no flocoumafen was detected, < LOD).

7 Discussion

7.1 Spatial and intraspecific comparison

The majority (20 out of 31) of the avian samples were acquired from less densely populated areas but raptors and gulls, found in Stockholm City (SthlmC) were also included in this study (Figure 6, Figure 7). See Appendix A for detailed information about sampling sites.

Of the individual bird samples from the highly populated areas, 29 % were exposed to FGARs and 57 % to SGARs. A higher frequency of the individuals in the SthlmC area was exposed to FGARs (57 %) and SGARs (71 %). Only one gull was exposed to relatively low levels of bromadiolone and difethialone. The limited number of gulls (n=4) included in the study hinder conclusions from comparisons regarding exposures differences and similarities between raptors and omnivores. However, this study shows that non-target species (scavengers) can be unintentionally exposed when rodenticides are employed as pest control.

The spatial distribution of rodenticides in both fox and bird samples indicates that exposure of ARs to non-target animals cannot be considered to be located to any specific region of Sweden, as seen in Figure 7. It is noteworthy that FGARs can be found to a higher degree in the red fox samples than in the bird samples. One conclusion that can be drawn for the spatial exposure pattern (exposure pattern of extremes, either high or low exposure) is that the individuals were exposed from point-sources or hot-spots rather than a more general contamination of the environment. However, the point-sources seem to be distributed across Sweden.

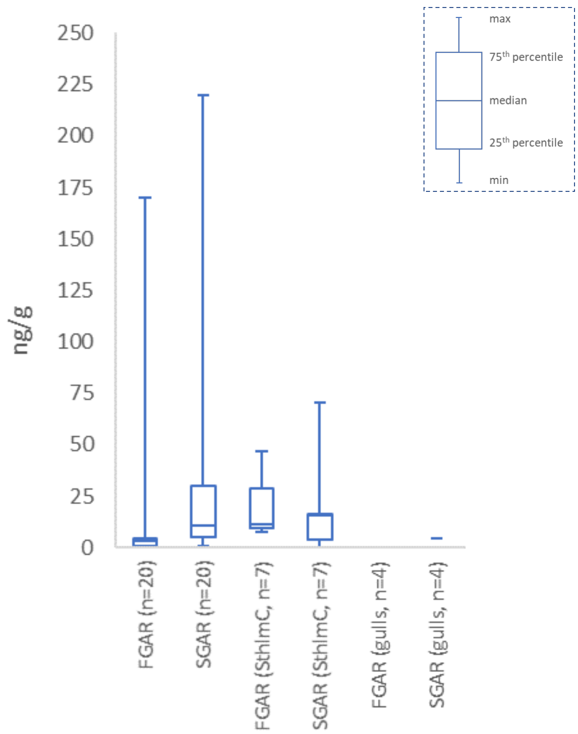


Figure 6. Concentrations (ng/g) of first-generation anticoagulant rodenticides (FGARs) and second-generation anticoagulant rodenticides (SGARs) in raptors (first four boxes) and gulls (two boxes) collected at different locations, less densely populated or highly populated (SthlmC) areas (gulls were only collected in the SthlmC area). FGARs includes warfarin and coumatetralyl, and SGARs includes brodifacoum, bromadiolone, difenacoum and difethialone (no flocoumafen was detected, < LOD).

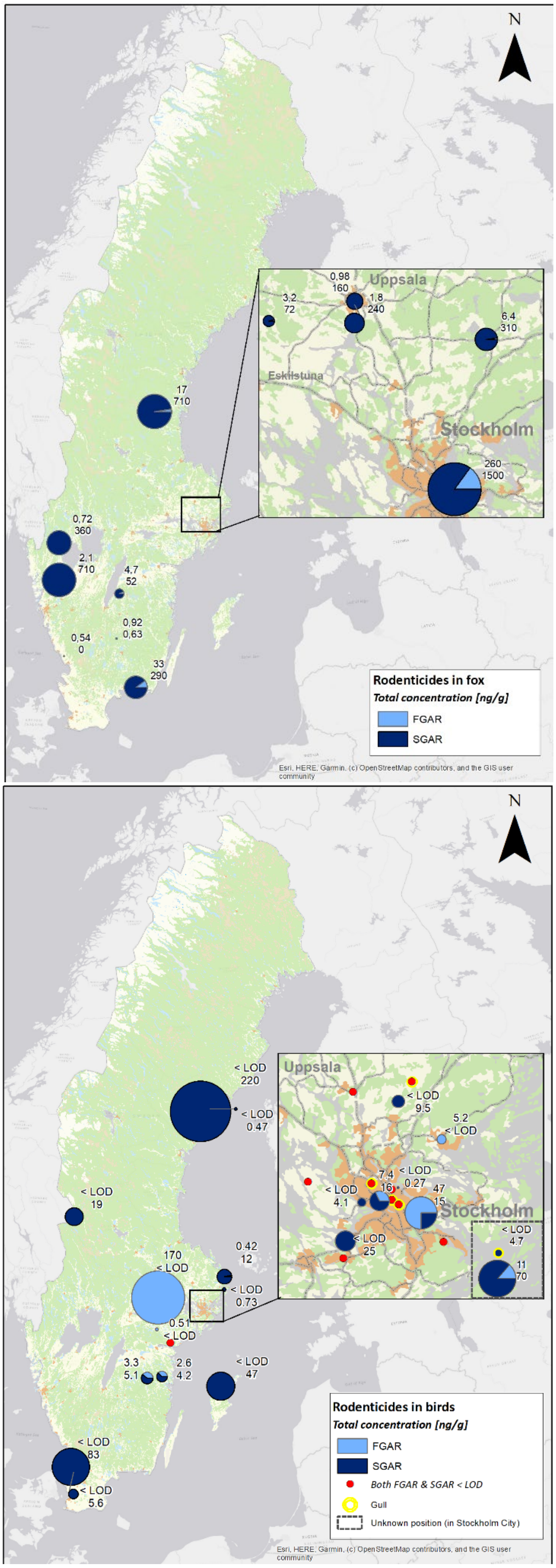


Figure 7. Spatial distribution of FGARs and SGARs in red fox (top) and birds (bottom) in Sweden in the present study. The circles' size represents the concentrations in each sample (note that the circles are only proportional for the respective map).

7.2 Toxicity of rodenticides in birds

Birds are thought to be sensitive to ARs due to limited ability to detoxify ARs compared to mammals. Longer elimination half-lives have been observed for the structurally similar FGAR diphacinone in the liver of screech-owls (*Megascops asio*) compared to mammals (reviewed by Nakayama et al., 2019). Furthermore, the cytochrome P450 dependent metabolism of warfarin in owls is very low compared to rats and other avian species (Watanabe et al., 2010).

A defined critical threshold concentration for ARs associated with toxicity is difficult to obtain. The only published "toxicity threshold" for SGARs in avians, referred to as a "potentially lethal range" (> 100 – 200 ng/g) has been reported for barn owls (*Tyto alba*), diagnosed post-mortem in two different studies (reviewed by Thomas et al., 2011). The owls were exhibiting toxic signs typical for AR exposure. However, the range (> 100–200 ng/g) only indicates potential toxicity and no likelihood of effects is presented in the study. It is also uncertain if the levels apply to other species, due to species differences in sensitivity for SGARs and/or metabolic capacity. In the study by Thomas et al. (2011) the probability for poisoning due to SGARs exposure was characterized in different avian species, based on liver concentrations. The study implies significant differences in sensitiveness between the studied species.

If 100 ng/g is assumed as the level of toxicity for all ARs, two avian individuals in the present study were found to exceed that concentration. The Eurasian eagle-owl (*Bubo bubo*) no. 4 and the tawny owl (*Strix aluco*) no. 11 had higher concentrations of the SGAR bromadiolone (220 ng/g) and the FGAR coumatetralyl (170 ng/g), respectively. Thus, poisoning cannot be ruled out. To assess the risk for toxicity the concentrations of each AR in each individual should be added for a total exposure assessment (see Appendix A for summarized levels in each individual). However, no other individuals than the Eurasian eagle-owl no. 4 and the tawny owl no. 11 exceeded the level of 100 ng/g (Figure 1). No pathology was performed for the animals that can confirm or reject any correlation between levels of ARs and toxic signs/death.

7.3 Toxicity of rodenticides in red foxes

High variability of exposure data of ARs occurs in the literature, and a true toxic threshold concentration is difficult to obtain. In one published study, captive red foxes were exposed to bromadiolone via spiked water voles for two or five days (Sage et al., 2010). The concentrations of bromadiolone in the voles were similar to that found in the field. The levels of retained bromadiolone in the livers of the treated foxes were found to be about 2 mg/kg. Bromadiolone could not be detected in plasma 24–26 days after the exposure had ceased. All foxes demonstrated toxic findings of different severity. In a field study of red foxes by Berny et al. (1997) it is discussed that a liver concentration of 200 ng/g can be used as a threshold for secondary toxicity. The levels of bromadiolone in the livers of the studied foxes in that study ranged between 0.8–6.9 µg/g. In a study by Geduhn et al. (2015), eight different ARs were monitored in liver samples from 331 red foxes. The predominant ARs were bromadiolone and brodifacoum, at median levels of 0.061 µg/g (min–max: 0.004–1.574) and 0.091 µg/g (min–max: 0.010–2.433), respectively, in individuals with toxic signs (27.8 % and 45.6 % respectively).

One individual of the foxes in the present screening (Red fox 12) had markedly higher exposure of bromadiolone, at 1300 ng/g. The same individual also had the highest concentrations of brodifacoum (180 ng/g) and coumatetralyl (260 ng/g) compared to the other individuals (Figure 4,

Appendix B). The cause of mortality (not confirmed) for this individual may be due to the high observed levels of bromadiolone and coumatetralyl. Using the sum of the concentrations of the different ARs (i.e. dose addition) for estimation of the risk of toxicity implies that individuals exposed to several ARs may be at risk even though the concentration of each AR is below the suggested threshold value (see Appendix B for summarized levels in each individual). As observed in Figure 4, several foxes were exposed to levels that exceeded 200 ng/g, used as a suggested toxic threshold value (see above). As for the birds no pathology was performed for the foxes, and accordingly no confirmation of typical toxic signs connected to the levels of ARs can be performed. Poisoning (and mortality) due to ARs exposure can neither be excluded nor confirmed.

7.4 Comparison with other studies on rodenticides in the environment

There are many published studies where the exposure to ARs in non-target species have been studied, where a few have been briefly summarized in Table 8. In Norström et al (2013), concentrations of six different ARs were monitored in raptors and red foxes in the Swedish environment. The second-generation AR bromadiolone was detected most frequently and at the highest concentrations. Warfarin was only detected in foxes, while flocoumafen was not detected at all. The findings of that screening are discussed more in detail and in relation to the results of the present study in section 7.4.1.

Bromadiolone was also the most frequently (81 %) detected AR in red foxes recently monitored in three different areas of France (Fourel et al., 2018). The mean and maximum concentrations were 355 ng/g and 2060 ng/g, respectively. The concentrations for all monitored ARs of that study is included in Table 8.

In a review by Nakayama et al. (2019) non-target animals exposed to ARs have been analysed on a global level. They concluded from the literature between 1998–2015 that the exposure rate of ARs for 17 avian species was between 62 % to 100 %. Ten of the seventeen species had exposures that exceeded more than 100 ng/g, where three of these species (kestrel, barn owl and tawny owl) were found in Denmark. Brodifacoum was the most frequently detected AR, followed by bromadiolone. A comprehensive compilation of the studied ARs is presented in the paper (Nakayama et al., 2019) Another recent study of rodenticide exposure in raptors was published by Murray (2017). In 96 birds from four different species of raptors in Massachusetts, USA, the dominant ARs were brodifacoum, bromadiolone and difethialone.

Table 8. Concentration ranges of anticoagulants (ng/g) monitored in raptors (several species) and red fox, published in peer-reviewed papers.

Rodenticide	Red fox (n=48)[a]	Raptors[b]
Warfarin	7.1 (no range available)	2.5–720
Coumatetralyl	2.4–3.3	2.3–9.3
Chlorophacinone	2.5–61.4	n/a
Brodifacoum	< LOQ	1–957
Bromadiolone	1.5–2060	1–1012
Difenacoum	2–33.2	<2–450
Difethialone	4–37.6	n/a
Flocoumafen	< LOD	0–117

[a] Fourel et al., 2018, [b] Collected from Norström et al., 2013.

7.4.1 Comparison with previous screening

The mean concentrations of the studied ARs in the present screening compared to those in the previous screening by IVL (Norström et al., 2013) are presented in Table 9 (birds) and Table 10 (red foxes), and illustrated in Figure 8 (birds) and Figure 9 (red foxes). Difethialone was only included in the present screening.

Birds

In the previous screening, four ARs in total were detected in the analysed raptor livers. In the present screening, all ARs except flocoumafen were detected in the raptor samples. No amounts of flocoumafen were reported to be sold during the sampling campaign (Table 4), which could explain the lack of flocoumafen exposed individuals. Bromadiolone was the most frequently detected AR during both screening campaigns. No statistically significant difference in the levels of the rodenticides between the screenings was observed (t-test, $p < 0.05$).

The pattern observed in the previous screening, that Tawny owls (*Strix aluco*) and Eurasian Eagle owls (*Bubo bubo*) were exposed to ARs and may be at risk of secondary poisoning, was confirmed in the present study. The total concentration of AR was higher in several of the individual birds analysed in the present study than observed in the previous screening.

In the present study, several ARs were found in two of the three analysed Goshawks (*Accipiter gentilis*) collected in the Stockholm area. Goshawks living in urban environments are known to feed on rodents, especially rats, and these results clearly show that they are exposed to ARs and may be at risk for secondary poisoning. However, the third Goshawk, a juvenile, had no detectable levels of ARs.

In the previous study, 3 of 7 individuals of the Common kestrel (*Falco tinnunculus*) were exposed to one or more AR. Individuals of this species could be expected to be at risk for secondary exposure, based on their preferred diet including small rodents. Worth noting is that the only falcon included in the present study, one Eurasian hobby (*Falco subbuteo*), had been exposed to bromadiolone. Since this species is feeding on large flying insects and birds (BirdLife International, 2019), the AR exposure route is not obvious. The Eurasian hobby being a migratory bird, it may have been exposed at its wintering quarters in Africa or southern Asia, or along the migratory route. Outside of the EU, it is possible that bromadiolone rodenticides in other formulations than baits are being used, thus, birds coming in contact with e.g. powder or gel formulations might be exposed through preening of the feathers. However, the present study cannot give any indications of the exposure route.

A similar exposure route might contribute to the AR exposure of the likewise migratory Lesser black-backed gull (*Larus fuscus*), that in the present study was found to be exposed to bromadiolone and difethialone. Although much more likely than the Eurasian hobby to feed on dead or dying rodents, as well as rodenticide bait, preening of the feathers potentially exposed to contact formulated ARs could be one contributing exposure route.

No Goshawks or gulls were included in the previous study.

Worth noting is that warfarin was found in one of the analysed bird samples, a Goshawk, despite this substance not being allowed to be used in Sweden since the beginning of year 2015. Young Goshawks have a short-distance migratory behaviour pattern, and it is therefore possible that this individual had been exposed to warfarin in another European country. It could also be a signal that rodenticide products containing warfarin have been used in Sweden long after their approvals expired. Warfarin was not detected in any bird sample in the previous study (Norström et al., 2013).

Red foxes (*Vulpes vulpes)*

ARs were found in all individual samples of fox in the present study as well as in the previous screening by IVL (Norström et al., 2013). The levels of SGARs were higher compared to FGARs (Figure 2b and Figure 5), consistent with studied foxes in the previous study and also with e.g. a study in Germany (Geduhn et al., 2015).

In the screening 2012/2013, warfarin was detected at relatively high concentrations (up to170 ng/g) in 50 % of the foxes. In the present screening, warfarin was detected in 33 % of the foxes, but at much lower levels (up to 2.9 ng/g). As mentioned above, it was not expected to detect warfarin in any sample, as no product containing warfarin has been allowed to be used after February 2015, i.e. long before the samples analysed in the presented study were collected. Although finding warfarin in these samples could reflect that the substance remains bioavailable in the environment, unauthorized usage cannot be excluded.

Coumatetralyl was detected in all foxes in the present study, albeit at lower levels compared to the earlier screening. Since the previous sampling, the authorisation of one powder formulation with coumatetralyl expired, and that product was not authorised to be used after July 2014. This contact formulation was previously authorised for usage directly into rats´ burrows, thus likely leading to direct exposure to the environment, to target as well as non-target organisms, long after the application. The lower levels of coumatetralyl in the present study could be a reflection of this specific rodenticide product not being available on the market.

However, in 2016 the Swedish Chemicals Agency issued a temporary exempt that allowed some controlled, specific usage of the same powder formulation for 180 days, in certain well-defined areas in Stockholm, in order to control rat infestations. The Red fox no. 12 (found in the City of Stockholm area) had particularly high levels of coumatetralyl, which could possibly be explained by this exempt. However, this individual fox had very high levels of three different SGARs as well, thus suggesting that it had been feeding on rodents or other prey that had been exposed to several different anticoagulant rodenticides, and/or that the fox had been feeding on rodenticide bait products.

Brodifacoum and difenacoum were detected at higher mean levels in the foxes in the present study compared to earlier. Bromadiolone is still the most commonly detected AR, with similar levels in both screenings.

Table 9. Levels of rodenticides (ng/g) in avian samples measured during 2012/2013 (raptors only) (Norström et al., 2013) and in the present study (2019 (27 raptors, 4 gulls)).

	Warfarin	Coumatetralyl	Brodifacoum	Bromadiolone	Difenacoum	Difethialone	Flocoumafen
2013 (n=20)							
Mean	0	7.1	3.9	99.7	4.3	n/a	0
SD	n/a	7.3	n/a	271	5.0	n/a	n/a
Min	n/a	0.7	3.9	1.1	0.9	n/a	n/a
Max	n/a	15	3.9	870	10	n/a	n/a
Rate (%)[a]	0	15	5	50	15	n/a	0
2019 (n=31)							
Mean	0.56	27.4	17.4	31.6	5.3	0.9	0
SD	n/a	55.4	5.5	58.2	3.4	0.8	n/a
Min	0.6	0.4	10.3	1.30	1.9	0.3	n/a
Max	0.6	170	23.5	220	9.9	2.9	n/a
Rate (%)[a]	3	29	13	45	13	32	0

[a] Percentage of individuals with detected levels.

Table 10. Levels of rodenticides (ng/g) in red foxes measured during 2012/2013 (Norström et al., 2013) and in the present study (2019).

	Warfarin	Coumatetralyl	Brodifacoum	Bromadiolone	Difenacoum	Difethialone	Flocoumafen
2013 (n=10)							
Mean	47.0	120	3.0	356	3.2	n/a	0
SD	69.6	188	0.2	390	1.6	n/a	n/a
Min	3.3	0.9	2.8	0.9	1.7	n/a	n/a
Max	170	520	3.1	1100	4.8	n/a	n/a
Rate (%)[a a]	50	70	20	80	30	n/a	0
2019 (n=12)							
Mean	1.3	27.2	38.8	403.0	22.7	1.3	0
SD	1.1	73.9	70.2	378.1	32.1	1.0	n/a
Min	0.4	0.4	1.5	49.8	1.6	0.6	n/a
Max	2.9	260	180	1300.0	84.9	2.1	n/a
Rate (%)[a]	33	100	50	83	50	17	0

[a] Percentage of individuals with detected levels.

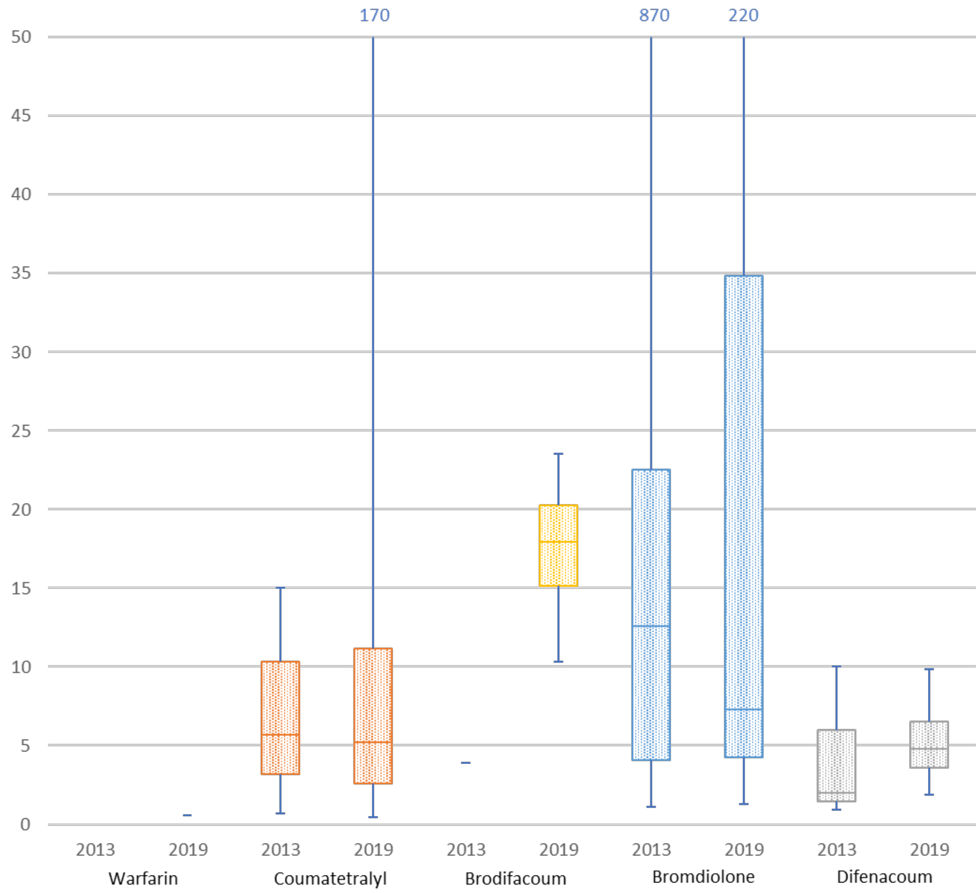


Figure 8. Mean concentrations (ng/g) of rodenticides measured in liver samples from birds during 2012/2013 (Norström et al., 2013) and in the present study (2019). The boxes represent the 25- and 75-percentile of each analyte in respective study, and the horizontal line within the box defines the mean concentration. Minimum and maximum concentrations are shown by the bars outside of the boxes.

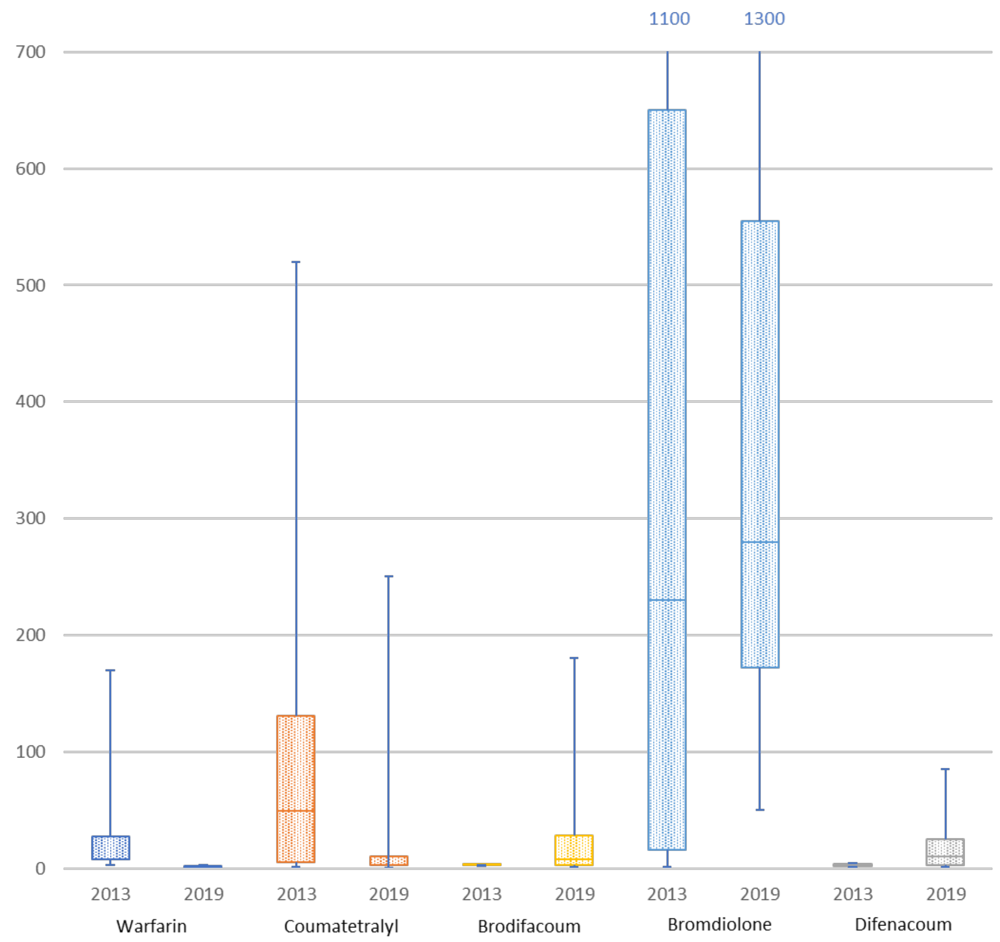


Figure 9. Mean concentrations (ng/g) of rodenticides in liver samples from red foxes during 2012/2013 (Norström et al., 2013) and in the present study (2019). The boxes represent the 25- and 75-percentile of each analyte in respective study, and the horizontal line within the box defines the mean concentration. Minimum and maximum concentrations are shown by the bars outside of the boxes.

8 Conclusions

From this screening it can be concluded that various raptors and predatory mammals (red foxes), which feed on rodents, were exposed to anticoagulant rodenticides. The SGARs were more frequently detected in all species compared to FGARs, although FGARs could be found in individuals of birds as well as in red foxes.

All red foxes were exposed to at least one rodenticide, and as much as 92 % of the foxes were exposed to at least three rodenticides. For the avian samples, 68 % were exposed to at least one rodenticide and 42 % were exposed to at least two. In total, bromadiolone was the most frequently detected rodenticide, with 45 % of the birds and 83 % of the red foxes being exposed. Coumatetralyl was detected in all individuals of the foxes and in 29 % of all birds. Flocoumafen was not detected in any species.

One of the individuals of the foxes, found in the central parts of Stockholm, was exposed to very high levels of bromadiolone, brodifacoum and coumatetralyl compared to the other foxes. One eagle owl and one tawny owl were exposed to high levels of bromadiolone and coumatetralyl, respectively, compared to the other avians. The suggested threshold for anticoagulant rodenticide toxicity is exceeded in these individuals and secondary poisoning cannot be excluded. The sum concentration of all rodenticides in each individual (dose addition) in this screening study results in a total rodenticide level that exceeds the threshold for toxicity in additionally five foxes, but no additional avians. Potential secondary poisoning can thus have occurred for several individuals of foxes and raptors in this study.

Acknowledgements

Ylva Lind and Anna Roos at the Swedish Museum of National History are acknowledged for providing the liver samples from birds, as well as useful information. Henrik Uhlhorn at the National Veterinary Institute is acknowledged for providing the liver samples from red foxes. Victor Persson at Stockholm Vildfågel Rehab is acknowledged for providing birds.. Carl-Henrik Eriksson and Kerstin Gustafsson at the Swedish Chemicals Agency are acknowledged for providing rodenticide sales statistics and regulatory input, respectively.

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Appendices

Appendix A. Sample information and levels (ng/g) of anticoagulant rodenticides in birds (liver samples)

Sample information:

Sample ID	SVA ID	Species	Latin name	Gender	Age (years)	Weight (g)	Lenght (cm)	Cause of death / injury	Location	County
MR8892	Kattuggla 1 lever 8g	Tawny owl 1	*Strix aluco*	?	?	-	-	fracture at wing, killed	RV 276 N Åkersberga	Uppland
MR8897	Kattuggla 2 lever 4g	Tawny owl 2	*Strix aluco*	?	?	-	-	emaciated, fed, dewormed, dead at 2018-03-18	Tyresö	
MR8901	Kattuggla, unge	Tawny owl 3	*Strix aluco*	?	young	-	-	blind	Botkyrka	Södermanland
MR8903	A2016/06002	Long-eared owl	*Asio otus*	male	?	306	34.1	train	Ca 1 km N Frösundastation, Vallentuna. N 59 37.866 / E 18 10.836	Uppland
MR8906	A2016/06374	Tawny owl 4	*Strix aluco*	male	2	-	-	traffic/found at road	lv 263/lb 273 ihm Arlanda stad	Uppland
MR8907	A2016/06905	Tawny owl 5	*Strix aluco*	male	2	434	-	?	Lindholmen N 59 35.000 E 18 6.500	Uppland
MR8908	A2016/06908	Tawny owl 6	*Strix aluco*	male	3	362	39.6	killed	Färningsö, Ekerö N 59 23.400 E 17 38.660	Uppland
MR8912	A2017/06011	Tawny owl 7	*Strix aluco*	female	3	640	40.9	killed	Nysättra, Norrtälje	Uppland
MR8913	A2017/06028	Tawny owl 8	*Strix aluco*	female	2	512	41	train	T-centralen, tunnelbanan, Stockholm	Uppland
MR8914	A2017/06029	Tawny owl 9	*Strix aluco*	female	2	504	41	car	E4 mellan Södertälje och Stockholm	Södermanland
MR8915	A2017/06052	Tawny owl 10	*Strix aluco*	male	2	437	36.6	chimney	Stubboda 18, Norrtälje	Uppland
MR8916	A2017/06053	Tawny owl 11	*Strix aluco*	male	4	584	40.3	possibly traffic	Strömsholm	Västmanland
MR8917	A2017/06092	Tawny owl 12	*Strix aluco*	female	1.5	506	-	traffic	Väg 35 norr om Överum N 57 60.000 E 16 17.000	Småland
MR8920	A2017/06271	Tawny owl 13	*Strix aluco*	female	?	456	-	found dead at ground	Lilla Skuggan Stockholm, Norra Djurgården N 59 21.974 E 18 5.200	Uppland
MR8921	A2017/06402	Tawny owl 14	*Strix aluco*	male	?	584	39	traffic	Snacketorpsvägen (lv551) Djulökvarn Katrineholm	Södermanland
MR8904	A2016/06149	Eagle owl 1	*Bubo bubo*	male	7	2088	64.2	found dead	Kocketorp, Bildstena, Överum. N 58 1.831 / E 16 21.575	Småland
MR8905	A2016/06217	Eagle owl 2	*Bubo bubo*	female	?	3660	68	killed	Örnsköldsvikstrakten	Ångermanland
MR8909	A2016/06909	Eagle owl 3	*Bubo bubo*	female	?	2517	64.2	building/barb wire, killed	Jonslund-Källmossen, Lundaskog, Jönåker, Nyköping N 58 41.235 E 16 43.091	Södermanland
MR8910	A2016/06964	Eagle owl 4	*Bubo bubo*	female	?	2530	66.7	burned at substation	Ett par 100 m från Orrvik 144. Örnsköldsvik	Ångermanland
MR8911	A2016/07014	Eagle owl 5	*Bubo bubo*	male	2	1494	58.7	disease, inflammation	Gotland, Stenkyrka	Gotland
MR8918	A2017/06113	Eagle owl 6	*Bubo bubo*	female	4	2083	69	electric wire?	Närsjön, Sälen N 6785409 E 386327 N 61 11.195 E 12 53.137	Dalarna
MR8919	A2017/06162	Eagle owl 7	*Bubo bubo*	male	?	1158	62	?	Svenska pappersbruket AB, Klippan N 56 7.269 E 13 8.725	Skåne
MR8922	A2017/06141	Eagle owl 8	*Bubo bubo*	male	2	-	60.4	traffic or electric wire	Ebbarpsvägen efter korsningen vid Tyrbyttevägen intill	Skåne
MR8894	Havstrut	Great black-backed gull	*Larus marinus*	?	adult	-	-	bad general condition, vomits, killed 2018-03-12	Norra Bantorget	Uppland
MR8899	Silltrut level 7 g	Lesser black-backed gull	*Larus fuscus*	?	old	-	-	?	Storstockholm (ej soptipp)	?
MR8893	Gråtrut 5 lever 17 g	Herring gull 1	*Larus argentatus*	?	adult	-	-	open fracture, killed immediately	Kastellholmen	Uppland
MR8895	Gråtrut 4 lever 23 g	Herring gull 2	*Larus argentatus*	?	2	-	-	swollen feet, malnourished, killed	Hornbach Sundbyberg	Uppland
MR8896	Duvhök 6 lever 15 g	Goshawk 1	*Accipiter gentilis*	?	2	-	-	broken neck	Traneberg	Uppland
MR8898	Duvhök, unge	Goshawk 2	*Accipiter gentilis*	?	young	-	-	?	Universitetsavfarten	Uppland
MR8900	Duvhök, level 13 g	Goshawk 3	*Accipiter gentilis*	?	old	-	-	injured wing	Storstockholm (ej soptipp)	?
MR8902	Lärkfalk 2 g	Eurasian hobby	*Falco subbuteo*	?	?	-	-	missing wing	Nockebyhov	Uppland

Level (ng/g):

Sample ID	SVA ID	Species	Warfarin	Coumatetralyl	Brodifacoum	Bromadiolone	Difenacoum	Difethialone	Flocoumafen	Total conc.
MR8892	Kattuggla 1 lever 8g	Tawny owl 1	< LOD	5.2	< LOD	< LOD	< LOD	< LOD	< LOD	5.2
MR8897	Kattuggla 2 lever 4g	Tawny owl 2	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.0
MR8901	Kattuggla, unge	Tawny owl 3	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.0
MR8906	A2016/06374	Tawny owl 4	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.0
MR8907	A2016/06905	Tawny owl 5	< LOD	< LOD	< LOD	9.3	< LOD	0.3	< LOD	9.5
MR8908	A2016/06908	Tawny owl 6	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.0
MR8912	A2017/06011	Tawny owl 7	< LOD	0.4	< LOD	12.4	< LOD	< LOD	< LOD	12.9
MR8913	A2017/06028	Tawny owl 8	< LOD	46.6	10.3	5.2	< LOD	< LOD	< LOD	62.1
MR8914	A2017/06029	Tawny owl 9	< LOD	< LOD	19.1	5.3	< LOD	0.3	< LOD	24.7
MR8915	A2017/06052	Tawny owl 10	< LOD	< LOD	< LOD	< LOD	< LOD	0.7	< LOD	0.7
MR8916	A2017/06053	Tawny owl 11	< LOD	170.0	< LOD	< LOD	< LOD	< LOD	< LOD	170.0
MR8917	A2017/06092	Tawny owl 12	< LOD	3.3	< LOD	4.7	< LOD	0.4	< LOD	8.4
MR8920	A2017/06271	Tawny owl 13	< LOD	< LOD	< LOD	< LOD	< LOD	0.3	< LOD	0.3
MR8921	A2017/06402	Tawny owl 14	< LOD	0.5	< LOD	< LOD	< LOD	< LOD	< LOD	0.5
MR8904	A2016/06149	Eagle owl 1	< LOD	2.6	< LOD	< LOD	4.1	< LOD	< LOD	6.8
MR8905	A2016/06217	Eagle owl 2	< LOD	< LOD	< LOD	< LOD	< LOD	0.5	< LOD	0.5
MR8909	A2016/06909	Eagle owl 3	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.0
MR8910	A2016/06964	Eagle owl 4	< LOD	< LOD	< LOD	220.0	< LOD	< LOD	< LOD	220.0
MR8911	A2016/07014	Eagle owl 5	< LOD	< LOD	< LOD	41.5	5.4	< LOD	< LOD	46.9
MR8918	A2017/06113	Eagle owl 6	< LOD	< LOD	16.7	1.3	< LOD	1.1	< LOD	19.2
MR8919	A2017/06162	Eagle owl 7	< LOD	< LOD	< LOD	4.0	< LOD	1.5	< LOD	5.6
MR8922	A2017/06141	Eagle owl 8	< LOD	< LOD	< LOD	72.9	9.9	< LOD	< LOD	82.8
MR8903	A2016/06002	Long-eared owl	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.0
MR8894	Havstrut	Great black-backed gull	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.0
MR8899	Silltrut Ad 7g lever	Lesser black-backed gull	< LOD	< LOD	< LOD	1.7	< LOD	2.9	< LOD	4.7
MR8893	Gråtrut 5 lever 17	Herring gull 1	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.0
MR8895	Gråtrut 4 lever 23g	Herring gull 2	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.0
MR8896	Duvhök 6 lever 15g	Goshawk 1	0.6	6.8	< LOD	14.8	< LOD	0.8	< LOD	22.9
MR8898	Duvhök, unge	Goshawk 2	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.0
MR8900	Duvhök, gammal 13g lever	Goshawk 3	< LOD	11.2	23.5	45.1	1.8	< LOD	< LOD	81.7
MR8902	Lärkfalk 2g	Eurasian hobby	< LOD	< LOD	< LOD	4.1	< LOD	< LOD	< LOD	4.1
Limit of detection			0.03	0.1	1.6	1.0	0.4	0.2	1.7	

Appendix B. Sample information and levels (ng/g) of anticoagulant rodenticides in red foxes (liver samples)

Sample information:

ID	SVA ID	Species	Latin name	Gender	Age	GIS x/y	Location	Municipality	Date
MR7555	V1258/17	Red fox 1	*Vulpes vulpes*	male	adult	6627000/1643000	Rimbo	Norrtälje	2017-03-25
MR7556	V198/18	Red fox 2	*Vulpes vulpes*	male	adult	6633412/1576875	Nysätra, Alstomta	Enköping	2018-02-06
MR7557	V902/18	Red fox 3	*Vulpes vulpes*	male	adult	6839036/1516235	Järvsö	Ljusdal	2018-04-21
MR7558	V1787/17	Red fox 4	*Vulpes vulpes*	female	adult	6447000/1431000	Klämmestorp	Ödeshög	2017-06-12
MR7559	V345/18	Red fox 5	*Vulpes vulpes*	female	adult	6302196/1310296	Bårarp stenbrott	Halmstad	2018-02-27
MR7560	V2335/17	Red fox 6	*Vulpes vulpes*	male	< 1 year	6638000/1603000	Bergsbrunnagatan. Datum är ankomstdatum till SVA	Uppsala	2017-09-08
MR7561	V274/17	Red fox 7	*Vulpes vulpes*	male	1 year	6339171/1426407	Ugglevägen 6, Lammhult	Växjö	2017-01-25
MR7562	V932/17	Red fox 8	*Vulpes vulpes*	male	adult	6639108/1603116	Karsvreta träsk	Österåker	2017-02-12
MR7563	V1200/17	Red fox 9	*Vulpes vulpes*	male	adult	6552000/1302000	Bengtsfors, Gröven Kaserna Gård	Bengtsfors	2017-03-17
MR7564	V1305/17	Red fox 10	*Vulpes vulpes*	female	adult		Gårdsplan utanför Ronneby, exakt läge ej angivet	Ronneby	2017-04-06 (arrival to SVA)
MR7565	V2419/17	Red fox 11	*Vulpes vulpes*	female	adult	6470230/1300741	Västra Tunhem	Vänersborg	2017-09-11
MR7566	V2434/17	Red fox 12	*Vulpes vulpes*	male	adult	6580283/1632749	Södra Djurgården, Manilla	Stockholm	2017-08-29

Level (ng/g):

ID	SVA ID	Species	Warfarin	Coumatetralyl	Brodifacoum	Bromadiolone	Difenacoum	Difethialone	Flocoumafen	Total conc.
MR7555	V1258/17	Red fox 1	< LOD	6.4	8.9	270	28	< LOD	< LOD	313.4
MR7556	V198/18	Red fox 2	< LOD	3.2	< LOD	70	< LOD	2.1	< LOD	75.5
MR7557	V902/18	Red fox 3	< LOD	17	34	680	< LOD	< LOD	< LOD	731.2
MR7558	V1787/17	Red fox 4	2.9	1.8	< LOD	50	2.1	< LOD	< LOD	56.6
MR7559	V345/18	Red fox 5	< LOD	0.54	< LOD	< LOD	< LOD	< LOD	< LOD	0.5
MR7560	V2335/17	Red fox 6	1.4	0.41	< LOD	240	< LOD	< LOD	< LOD	241.8
MR7561	V274/17	Red fox 7	0.43	0.50	< LOD	< LOD	< LOD	0.63	< LOD	1.6
MR7562	V932/17	Red fox 8	< LOD	1.0	< LOD	150	15	< LOD	< LOD	165.6
MR7563	V1200/17	Red fox 9	< LOD	0.72	1.5	360	1.6	< LOD	< LOD	364.1
MR7564	V1305/17	Red fox 10	0.62	32	1.5	290	< LOD	< LOD	< LOD	324.4
MR7565	V2419/17	Red fox 11	< LOD	2.1	7.0	620	85	< LOD	< LOD	714.0
MR7566	V2434/17	Red fox 12	< LOD	260	180	1300	5.2	< LOD	< LOD	1745.2
Limit of detection			0.03	0.1	1.6	1.0	0.4	0.2	1.7	

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