REPORT

Screening 2012, Rodenticides

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Report Summary

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Screening 2012, Rodenticides

Summary

As an assignment from the Swedish Environmental Protection Agency, a screening study of rodenticides has been performed by IVL. Warfarin, difenacoum, coumatetralyl, brodifacoum and flocoumafen are anticoagulant rodenticides and are all being used in Sweden. These chemicals are persistent and bioaccumulative and are emitted and distributed in the environment due to its usage. These substances are produced to act as rodenticides and are taken up via ingestion. They act through inhibition of the vitamin K cycle in the liver, disrupt the blood clotting process and causes death by haemorrhage. The overall objective of the present study was to determine the concentrations of rodenticide anticoagulants in livers of red foxes and different raptors in the Swedish environment and to investigate if there is a possible risk for secondary poisoning of these compounds. Ten livers from red foxes and twenty livers from four species of raptors (tawny owl, long-eared owl, Eurasian eagle owl and common kestrel) were analysed. Bromadiolone was detected in highest concentration and most frequently. Warfarin was only found in the foxes while flocoumafen could not be detected in any of the samples. In the foxes, the summed concentration of the anticoagulant rodenticides ranged between 2 and 1 100 ng/g w.w and the raptors contained < LOQ to 870 ng/g w.w. All foxes contained at least one of the compounds while 65% of the raptors contained at least one. The levels found in this study may be compared with the level for toxic effects of SGARs; >100 to 200 ng/g, which has been suggested as a "potential lethal range". At least one of the raptor individuals contained higher concentrations and poisoning cannot be ruled out. This study shows that the anticoagulant rodenticides included are found in non-target organisms that feed on rodents in concentrations such that secondary poisoning cannot be ruled out.

Keyword

Rodenticides, avian raptors, red fox, warfarin, difenacoum

Bibliographic data

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Sammanfattning

IVL Svenska Miljöinstitutet har på uppdrag av Naturvårdsverket genomfört en screening avseende rodenticider som används som råttgift. Warfarin, difenacoum, coumatetralyl, bromadiolone, flocoumafen och brodifacoum har bestämts i leverprover från rovfåglar och rävar. Warfarin är ett första generationens antikoagulat och difenacoum, coumatetralyl, brodifacoum och flocoumafen är andra generationens antikoagulat vilka utvecklades på grund av en ökad resistens mot warfarin bland gnagare. Dessa kemikalier kan bioackumuleras och sprids till miljön genom sitt sätt att användas. Exponeringen hos råttor och möss sker genom kontaminerad föda och dess toxiska effekt är att de hämmar vitamin K cykeln i levern och förhindrar att blodet koagulerar och orsakar död genom förblödning. Alla de ämnen som ingår i studien används i Sverige idag och difenacoum, bromadiolone, coumatetralyl och warfarin är de ämnen som förekommer i flest antal produkter (SPIDER 2013).

IVL har i en tidigare screeningstudie analyserat råttgifter (Norström et al. 2009). Studien visade att dessa substanser inte är allmänt spridda i miljön men att sekundär förgiftning hos djur som livnärar sig på bland annat råttor och möss var av intresse att studera vidare då ett flertal av antikoagulaten hittades i ett leverprov från en uggla som hade dött på en avfallsdeponi.

Syftet med denna studie var att bestämma antikoagulat, vilka är vanligt förekommande i råttgifter, i leverprover från rävar och olika arter av rovfåglar för att se om det finns en eventuell risk för sekundär förgiftning.

Prov från tio rävar analyserades och samtliga innehöll minst en av substanserna men mönstret varierade mellan individerna. Bromadiolone detekterades i högst koncentration (< LOQ – 1 100 ng/g v.v) och var också det ämne som hade högst detektionsfrekvens följt av coumatetralyl (< LOQ – 520 ng/g v.v). Warfarin hittades i hälften av individerna (< LOQ – 170 ng/g v.v). Flocoumafen och brodifacoum var under LOQ i samtliga prover. Den sammanlagda koncentrationen av antikoagulanterna varierade mellan 2 och 1 100 ng/g v.v.

20 leverprov från sammanlagt fyra olika arter av rovfåglar analyserades (berguv, kattuggla, tornfalk och hornuggla) och 65% av individerna innehöll rodenticider. Bromadiolone detekterades i högst koncentration (< LOQ – 870 ng/g v.v.) med en detektionsfrekvens på 50 %. I flera av individerna var bromadiolone det enda ämne som kunde hittas. Difenacoum, coumatetralyl och brodifacoum hittades i 15% av fåglarna medan flocoumafen och warfarin var under LOQ i samtliga prover. 65% av rovfåglarna innehöll minst ett av ämnena.

De fyra ämnen som detekterades i rovfåglar i denna studie hittades även i leverprovet från berguv i den tidigare screeningstudien (Norström et al. 2009). Warfarin som är vanligt förekommande i råttgift kunde enbart detekteras i rävar och inte i rovfåglarna.

Halterna som hittades i rovfåglar i denna studie kan jämföras med de nivåer som har rapporterats kunna ge toxiska effekter, >100 – 200 ng/g (refererat i Langford et al. 2012). Det har även diskuterats att nivån då toxiska effekter kan uppstå kan vara <100 ng/g men att det är signifikanta skillnader i känslighet mellan arter. Därför är det svårt att göra någon riskbedömning men om 100 ng/g antas vara en tröskelnivå för toxiska effekter så kan inte sekundär förgiftning uteslutas för åtminstone en av fåglarna i denna studie.

Summary

As an assignment from the Swedish Environmental Protection Agency, a screening study of rodenticides has been performed by IVL during 2012/2013.

Warfarin is a first generation anticoagulant rodenticide (FGAR) and difenacoum, coumatetralyl, brodifacoum and flocoumafen are second generation anticoagulant rodenticides (SGAR) and were developed because of an increased resistance towards warfarin among rodents. These chemicals are persistent and bioaccumulative and are emitted and distributed in the environment due to its usage. As these substances are produced to act as rodenticides, their toxic effects are intended and obvious. They are taken up via ingestion and act through inhibition of the vitamin K cycle in the liver, disrupt the blood clotting process and causes death by haemorrhage. All rodenticides included in this study are being used in Sweden.

IVL has in a previous screening study analysed rodenticides (Norström et al 2009). The previous study showed that these substances are not widely distributed in the Swedish environment, and are not likely to be of major concern from a general environmental perspective. However, secondary poisoning of animals feeding on contaminated prey is of particular interest because several of the compounds could be detected in the liver of an Eurasian eagle-owl.

The overall objective of the present screening study was to determine the concentrations of rodenticide anticoagulants in livers of red foxes and different raptors in the Swedish environment and to investigate if there is a possible risk for secondary poisoning of these compounds.

Ten red foxes were analysed and all of them contained at least one of the rodenticides but the pattern varied between the individuals. Bromadiolone was detected in highest concentration (< LOQ – 1 100 ng/g w.w) and was also the compound most frequently detected followed by coumatetralyl (< LOQ – 520 ng/g w.w.). Warfarin was detected in half of the individuals (< LOQ – 170 ng/g w.w.). Flocoumafen and brodifacoum was below LOQ in all samples. The summed concentration of the included rodenticide anticoagulants ranged between 2 and 1 100 ng/g w.w.

Difenacoum, bromadiolone, coumatetralyl and warfarin occur in highest frequency in the preparations use in Sweden (SPIDER 2013). However, the register does not include information about the amount of each preparation that has been sold. Twenty livers from four species of raptors were analysed (tawny owl, long-eared owl, Eurasian eagle owl and common kestrel) and 65% contained rodenticides. Bromadiolone was detected in highest concentration (< LOQ – 870 ng/g w.w.) and was also the compound most frequently detected (50%). In many individuals bromadiolone was the only compound found. Difenacoum, coumatetralyl and brodifacoum were found in 15% of the individuals. Flocoumafen and warfarin were below LOQ in all samples. 65% of the raptors contained at least one of the compounds.

In the previous screening study by IVL (Norström et al 2009), the same four rodenticides were found in the liver of one individual of Eurasian eagle-owl, found dead at a landfill. It is notable that warfarin, which is commonly used in Sweden, not could be found in raptors, it was only found in foxes. Environmental concentrations of Warfarin are not commonly reported in the literature.

The levels found in this study may be compared with the level for toxic effects of SGARs; >100 to 200 ng/g, which has been suggested as a "potential lethal range". A probabilistic characterization of toxic liver concentration in different species of raptors have suggested that the likelihood of poisoning is below the suggested concentration 100 ng/g and that there is significant differences between species sensitivities. The differences between species sensitivities make it difficult to relate to the raptor species in this study and therefor any risk assessment cannot be amde. But if 100 ng/g is assumed to be the level for a toxicological effect, at least one of the raptor individuals contains higher concentrations of rodenticides so poisoning cannot be ruled out. This screening study shows that the anticoagulant rodenticides difenacoum, bromadiolone, coumatetraly, brodifacoum and warfarin are found in non-target organisms that feed on rodents in concentrations such that secondary poisoning cannot be ruled out.

Contents

1 Introduction

As an assignment from the Swedish Environmental Protection Agency, a screening study of anticoagulant rodenticides has been performed by IVL during 2012/2013. These chemicals are persistent and bioaccumulative and are emitted and distributed in the environment via distribution due its usage as rodenticides.

IVL has in a previous screening study analysed anticoagulant rodenticides (Norström et al 2009). None of the seven substances included in the study were detected in water, sediment, soil, sludge or fish. In three individuals of eagle-owls, difenacoum, coumatetralyl, bromadiolone and brodifacoum were found. The concentrations were higher in liver than in muscle. Liver also contained the highest number of substances.

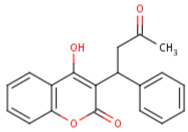
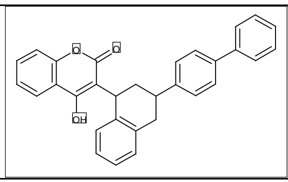
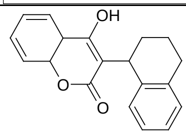
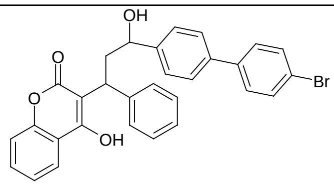
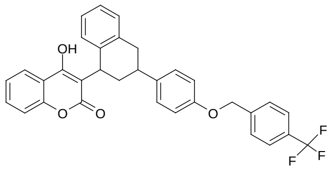
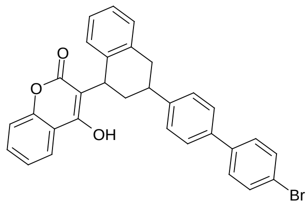
The study by Norström et al. 2009 showed that the measured rodenticides are not widely distributed in the Swedish environment, and are not likely to be of major concern from a general environmental perspective. However, secondary poisoning of animals feeding on contaminated prey is of particular interest.

The overall objective of the present screening study is to determine the concentrations of rodenticide anticouagulants in livers of red foxes and different raptors in the Swedish environment and to investigate if there is a possible risk for secondary poisoning of these compounds.

2 Chemical properties, fate and toxicity

Table 1 presents the rodenticides that are included in this study. These substances all belong to the 4-hydroxycoumarin class of anticoagulants. Warfarin is a first generation anticoagulant rodenticide (FGAR) and was first introduced as a rodenticide in 1984 against mice and rats. Difenacoum, coumatetralyl, brodifacoum and flocoumafen are second generation anticoagulant rodenticides (SGAR) and were developed because of an increased resistance towards warfarin among rodents. They all have similar functions, their names, CAS-numbers and structures are given in Table 1. As evident from the structures, all substances included are fairly large and complex molecules with two or more aromatic rings and one or more functional groups attached to the coumarine units.

Table 1. Rodenticides included in this screening study.

Name	CAS	Structure
Warfarin	81-81-2	
Difenacoum	56073-07-5	
Coumatetralyl	5836-29-3	
Bromadiolone	28772-56-7	
Flocoumafen	90035-08-8	
Brodifacoum	56073-10-0	

2.1 Properties and fate

The chemical and physical data of the anticoagulant rodenticides included in the current study are shown in Table 2. The application of these chemicals will result in direct release to the environment. All compounds are expected not to enter the atmosphere due to the low vapour pressure but if released to the air, they will exist mainly in the particulate phase. Their K_{OC} values indicate that they have low or no mobility in soil. Even though these anticoagulants have similar functions, they have a wide range of water solubility and log K_{ow}. Its form of application implies that the main emission matrix will be onto soil of some kind in small restricted areas. The degradation rate in soil is relatively slow and varies depending on soil type. Plant uptake is also believed to be limited, as residues in crops have never been detected in field studies (WHO, 1995).

As these substances are produced in order to act as rodenticides, their toxic effects are intended and obvious. They are taken up in rodents via ingestion and act through inhibition of the vitamin K cycle in the liver, disrupt the blood clotting process and causes death by haemorrhage. They are stored mainly in the liver and are slowly eliminated, primarily via the faeces.

The rodenticides ingested by rodents or other smaller insectivores may be transferred via the food chain to higher organisms, and may be excreted as metabolites via urine and faeces. The main area of concern is likely to be primary and secondary poisoning via ingestion of the substances or contaminated prey.

Table 2. Chemical and physical data of the anticoagulants included in this study (ChemIDplus).

Name	*MW (g/mol)*	*Melting Point (°C)*	*K_{OC}*	*Log Kow*	*Water Solubility (mg/L)*	*Vapor Pressure (mm Hg)*	*pKa*
Warfarin	307	161	701-918	2.6	17 (20°C)	1.2×10^{-7} (21 °C)	
Difenacoum	444	216		7.62	0.031 (20°C)	1.2×10^{-6} (20 °C)	
Coumatetralyl	292	172	1800		4 (20 °C)	6.4×10^{-11} (20 °C)	4.75
Bromadiolone	526	205	2.1×10^{5}	7.02	19 (20 °C)	1.5×10^{-8} (20 °C)	4.04
Flocoumafen	542	170	4100		1.1	1.0×10^{-12} (25 °C)	
Brodifacoum	522	230	7.5×10^{6}	8.50	0.0038 (20°C)		

2.2 Toxicity

The level for toxic effects of SGARs varies a lot for different species. The only toxicity data that have been reported for SGARs in raptor livers is a "potential lethal range" for barn owls (>100 to 200 ng/g) (referred to in Thomas et al. 2011). This value only provides a range of concern for potential toxicity, and gives no indication of likelihood of effects. Thomas et al 2011 made a probabilistic characterization of toxic liver concentrations in different species of raptors and suggested that likelihood of poisoning is below 100 ng/g and that there is significant differences between species sensitivities. Owls that were diagnosed to have died from anticoagulant rodenticide poisoning contained significantly higher levels of FGARs and SGARs compared with owls, whose death was caused by other reasons (Courtney et al 2010). The mean liver rodenticide residues concentrations in these owls were 400 ng/g wet weight.

In a report from Langford et al. 2012 there is a summary of LD50 values from the literature which has been copied to this report, see Table 3.

Table 3. LD50 values for different second generation anticoagulant rodenticides for non-target species (Langford et al. 2012).

Species	*Anticoagulant*	*LD50 (ng/g)*
Mink (*Mustela lutreola*)	Brodifacoum	9 200
Australasian harrier (*Circus approximant*)	Brodifacoum	10 000
Raven (*Corvus corax*)	Brodifacoum	560
Canada goos *(Branta Canadensis*)	Brodifacoum	<750
Mallard (*Anas platyrhynchos*)	Brodifacoum	260
Nothern bobwhite (*Colinus virginianus*)	Difethialone	260
Nothern bobwhite (*Colinus virginianus*)	Brodifacoum	138 000

3 Production and use

The second generation anticoagulant rodenticides (SGAR) were developed because of the increasing development of resistance among rodents to older (first generation) anticoagulant rodenticides, such as warfarin (Walker et al. 2008). SGARs are mainly used to control rodents around buildings and inside transport vehicles.

According to the Swedish Pesticides Register (SPIDER) all compounds that are included in this study are used in Sweden. The number of preparations for each substance is given in brackets; warfarin (6), coumatetralyl (4), difenacoum (12), bromadiolone (8), flocoumafen (1) and brodifacoum (2) (SPIDER 2013).

4 Previous measurements in the environment

Table 4 summarizes concentrations of warfarin and SGARs in different species of owls and red fox from the literature published the last few years. Brodifacoum and bromadiolone were the most frequently detected SGARs in a screening study performed by Langford et al. (2012). There were no clear relationship with respect to the location where the birds were collected and the content of SGARs in livers. However, the majority of the raptors analysed were from the more heavily populated areas of Norway.

Sánchez-Barbudo et al. (2012) reported SGARs in red foxes from Spain. 31 red foxes were analysed for SGARs and 39% contained three of the substances, see Table 4.

There are several examples of other non-target organisms exposed to these chemicals. In British tawny owl livers, the geometric mean concentrations for bromadiolone, difenacoum and brodifacoum were 200, 30, and 130 ng/g wet weight, respectively (Walker et al 2008). Dowding et al. (2010) found SGAR residues in a large number of British hedgehogs, illustrating additional non-target organisms also exposed to the substances. In British polecat livers, bromadiolone and difenacoum were detected at concentrations of 50-150 ng/g w.w. and 100-350 ng/g w.w, respectively (Shore et al 2003). In different water birds and raptors in France, bromadiolone and difenacoum were detected in the livers at a concentration of approximately 250 ng/g w.w. (Lambert et al. 2007). Also coumafen and coumatetralyl could be detected in a few samples.

Table 4. Concentrations of anticoagulant rodenticides (ng/g wet weight) in avian raptors and red fox. The data from Christensen 2012 are mean concentrations with maximum concentrations in brackets.

	Difenacoum	Coumatetralyl	Bromadiolone	Flocoumafen	Brodifacoum	Warfarin	area	ref
golden eagle	<2		<5-154	<2-117	<5-110		Norway	Langford et al. 2013
eagle owl	<2-39		<5	<2-13	<5-158		Norway	Langford et al. 2013
golden eagle			11-110	15-117	22-154		Norway	NIVA 2012
eagle owl	39-181		74-158				Norway	NIVA 2012
horned owl			1-570		1-610		Canada	Thomas et al. 2011
red-tailed hawks			1-64		1-170		Canada	Thomas et al. 2011
barn-owl			8		28		Spain	Sanches-Barbudo et al. 2012
eurasian eagle-owl			4	3-32	10-830		Spain	Sanches-Barbudo et al. 2012
little owl	56						Spain	Sanches-Barbudo et al. 2012
red fox	78		5-12300		5-4500		Spain	Sanches-Barbudo et al. 2012
great horned owls			5-571		1-609	2.5-720	Canada	Courtney et al. 2010
barred owls			2-1012		1-927	2.5-5	Canada	Courtney et al. 2010
barn owls			5-720		10-470	2.5-8	Canada	Courtney et al. 2010
common Kestrel (n=66)	18.8 (450)	6.1 (64)	33.5 (679)	5.2 (20)	7.7 (298)		Denmark	Christensen et al . 2010
common buzzard (n= ca 140)	17.1 (170)	9.3 (435)	22.8 (282)	3.4 (115)	10.3 (613)		Denmark	Christensen et al . 2010
rough-legged Buz (n=31)	17.6 (105)	2.3 (3)	30.2 (130)	0	6.5 (34)		Denmark	Christensen et al . 2010
tawny Owl (n=43)	10.8 (90)	6.3 (39)	35.1 (496)	10 (42)	9 (220)		Denmark	Christensen et al . 2010
barn owl (n=ca 80)	17.1 (233)	4.7 (18)	33.5 (252)	4.6 (34)	12.9 (957)		Denmark	Christensen et al . 2010
eurasian Eagle Owl (n=10)	14.5 (233)	6.9 (16)	105.5 (308)	2.8 (8)	16.6 (142)		Denmark	Christensen et al . 2010
lLong-eared Owl (n=38)	9.9 (52)	6.1 (29)	9.9 (33)	1.6 (2)	5.4 (40)		Denmark	Christensen et al . 2010

5 Sampling

5.1 Screening program

A sampling strategy was developed in order to determine concentrations of the six rodenticides in different predators in the Swedish environment. An important aspect of the SGAR usage is the risk for secondary poisoning of predators feeding on rodents and other small animals which may be contaminated. The sampling program was focused on foxes and different species of raptors and is shown in Table 5. For all individuals included in the study, samples of liver were used.

All liver samples from the different species of raptors were provided by the specimen bank at the Swedish Museum of National History. The liver samples from the foxes were provided by the National Veterinary Institute, SVA.

For more details, see Appendix A.

Table 5. Sampling program.

Species	*Latin name*	*No.*	*comment*
Red fox	*Vulpes vulpes*	10	
Eurasian eagle-owl	*Bubo bubo*	8	Omnivore, stationary
Tawny owl	*Strix aluco*	3	Omnivore, stationary
Long-eared owl	*Asio otus*	2	Feed on rodents, migrant
Common kestrel	*Falco tinnunculus*	7	Feed on rodents, migrant

6 Methods

6.1 Analysis

6.1.2 Sample preparation

Biota samples, raptor liver (0.5 g) and fox liver (0.5 g) were ground with Na_2SO_4 and spiked with internal standard. The extraction method was adopted from Vandenbroucke et al. 2008. The samples were extracted with acetone (5 mL) in an ultrasonic bath (30 min) followed by rotation (30 min). The samples were re-extracted with acetone (4 mL). To the combined organic solvent phase, diethyl ether (1 ml) was added and the sample was centrifuged (10 min, 3 500 rpm). The sample was evaporated to dryness and dissolved in methanol (1 ml) and the extract was transferred to an Eppendorf test tube. Finally, the samples were centrifuged (10 min, 10 000 rpm) before being transferred to vial for HPLC analysis.

6.1.3 Instrumentation

The samples were analysed applying a high performance liquid chromatography system consisting of a Prominence UFLC system (Shimadzu) with two pumps LC 20AD, degasser DGU-20A5, auto sampler SIL-20ACHT and column oven CTO-20AC. The analytical column was a Thermo HyPurity C8 50 mm x 3 mm, particle size 5 µm (Dalco Chromtech). The column temperature was 35 °C. The mobile phase A was a solution of 10 mM acetic acid in water and solvent B was methanol. The flow rate of the mobile phase was 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). For analysis, 10 µl sample extract in methanol was injected. ESI in negative ion mode and multiple reaction monitoring (MRM) were used. The masses used for quantification are shown in Table 6. Identification was done by retention time and quantification was done using authentic reference compounds.

Table 6. Quantification masses (m/z) for determination of the compounds of interest.

Compounds	*Precursor ion [M-H] m/z*	*Product ion used for quantification, m/z*	*Product ion used as qualifier, m/z*
Warfarin	307	250	161
Difenacoum	443.4	293.1	135
Coumatetralyl	291.1	140.9	142.9
Bromadiolone	525.3	249.9	180.8
Flocoumafen	541.3	382	160.9
Brodifacoum	521.3	134.9	142.9

6.1.4 Quality control

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 (CAS# 81-82-3) 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 quantification (LOQ) was defined as three times the standard deviation of the blank samples noise.

7 Results and discussion

All results are presented in detail in Appendix A. The limit of quantification (LOQ) is defined for each of the anticoagulant rodenticides in Appendix A. The sex (raptors only) and the area where each individual were found are included. Table 7 shows a survey of the detection frequencies of the rodenticides for each species analysed.

Table 7. Detection frequency for each rodenticide in the red foxes and in the different species of raptors that was included in the present screening study.

		Detection frequency					
no.	species	Difena-coum	Couma-tetralyl	Broma-diolone	Flocou-mafen	Brodi-facoum	Warfarin
10	Red fox	30%	70%	90%	0%	0%	50%
3	Tawny Owl	0%	33%	33%	0%	67%	0%
2	Long-eared Owl	0%	0%	50%	0%	0%	0%
8	Eurasian Eagle Owl	25%	13%	63%	0%	13%	0%
7	Common Kestrel	14%	14%	43%	0%	0%	0%

7.1 Red Fox

The concentrations of the different anticoagulant rodenticides in livers of the 10 red foxes are shown in Figure 1 as well as the sum of the six substances in each individual. Bromadiolone was detected in highest concentration (0.90-1100 ng/g w.w.) and was also the compound most frequently detected (90%). Flocoumafen and brodifacoum were not detected in any of the samples (<LOQ). The total concentration of the sum of the detected rodenticides ranged between 2 and 1100 ng/g w.w.

All foxes analysed contained at least one of the rodenticides but the pattern varied between the individuals. This may be a reflection of the usage of the product in the specific area where the exposure has taken place. Accordning to SPIDER difenacoum, bromadiolone, coumatetralyl and warfarin occur in highest frequency in the preparations (SPIDER 2013). However, this register does not include information about the amount of each preparation that has been sold.

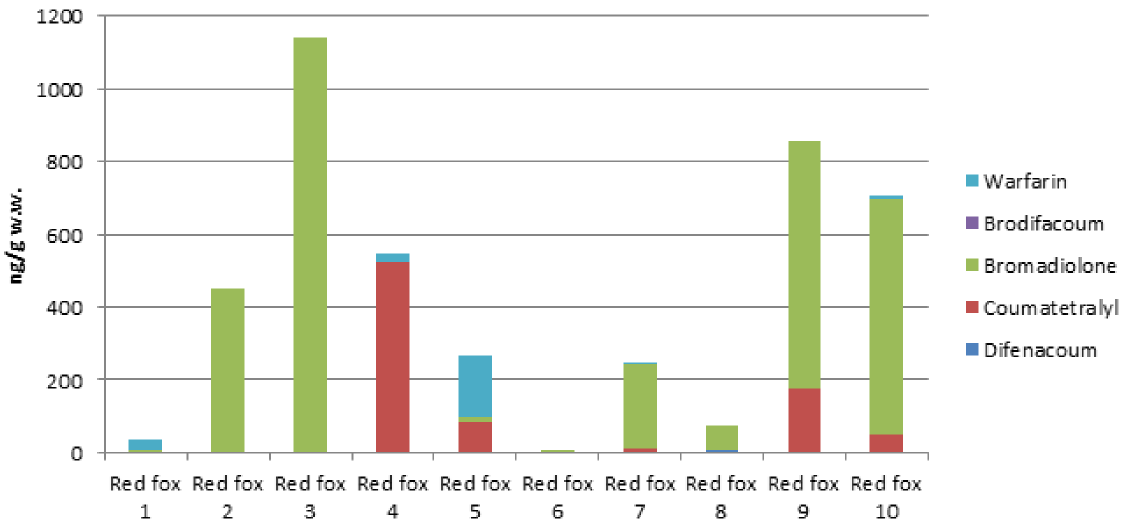


Figure 1. Rodenticide concentrations (ng/g w.w.) in red foxes.

Figure 2 shows how many percent each anticoagulant rodenticide represented of the total concentration. In most individuals bromadiolone was the dominating compound followed by coumatetralyl and warfarin. However, a few individuals had a different pattern where coumatetralyl and warfarin were the dominating compounds. In Appendix A the place where each individual were found is shown but no conclusions about concentrations connected to area can be made.

A previous study from Spain shows anticouagulant rodenticides in red foxes (Sanches-Barbudo et al. 2012). 31 individuals were analysed and 39% contained rodenticides. Coumateralyl, bromadiolone and brodifacoum were found, see Table 4. As in this study bromadiolone was the compound found in highest concentration and the concentration range was 5-12000 ng/g w.w. The corresponding range for bromadiolone in the present study is 0.90-1100 ng/g w.w. General for all compounds in the present study as well as in previous studies is that the concentration difference between individuals is large.

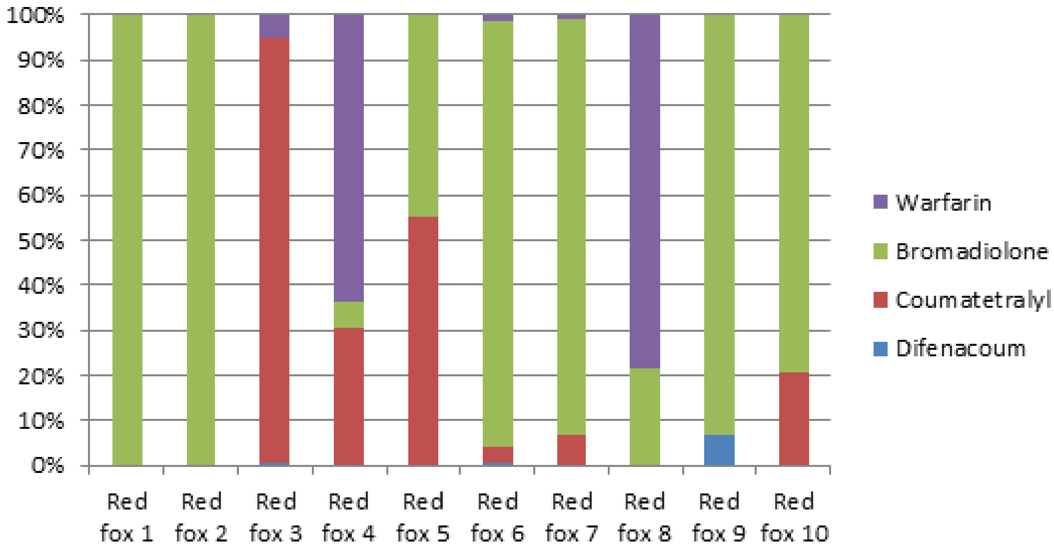


Figure 2. Percent of the total concentration for each anticoagulant rodenticide detected in each individual of red fox.

7.2 Raptors

The concentrations of the different anticoagulant rodenticides in livers of raptors analysed in this screening study are shown in Figure 3 as well as the sum of the six substances in each individual. Bromadiolone was detected in highest concentration and was also the compound most frequently detected (50%, <LOQ-870 ng/g w.w.) Flocoumafen and warfarin was below LOQ in all samples. 65% of the raptors analysed contained at least one of the compounds. Figure 4 shows how many percent each anticoagulant rodenticide represents of the total concentration. In most individuals bromadiolone is the dominating compound and in many cases, the only compound found. In Figure 4 the area where each raptor where found is shown but no conclusions can be made regarding concentrations and the local area.

In the previous screening study by Norström et al 2009, the same four rodenticides were found in the liver of one individual of Eurasian eagle-owl, found dead at a landfill. It is notable that warfarin, which is commonly used in rodenticides in Sweden, not could be found in raptors. Warfarin is not commonly reported in the literature but one study from Canada reports warfarin in generally low levels in different species of owls (Courtney et al. 2010). The results in this study is consistent with other studies, see Table 4, where bromadiolone also is the compound detected in highest concentrations and with highest detection frequency. General in all studies is that the concentration range is wide.

The levels found in this study can be compared with the level for toxic effects of SGARs (>100 to 200 ng/g) which has been suggested as a "potential lethal range" for barn owls as reffered to in Langford et al. 2012. Thomas et al 2011 made a probabilistic characterization of toxic liver concentrations in different species of raptors and suggested that likelihood of poisoning is below the suggested concentration 100 ng/g and that there are significant differences between species sensitivities. The differences between species sensitivities make it difficult to relate to the raptor species in this study and therefor any risk assessment is difficult. But if 100 ng/g is assumed to be the level for a toxicological effect, at least one of the raptor individuals contains higher concentrations of rodenticides so poisoning cannot be ruled out.

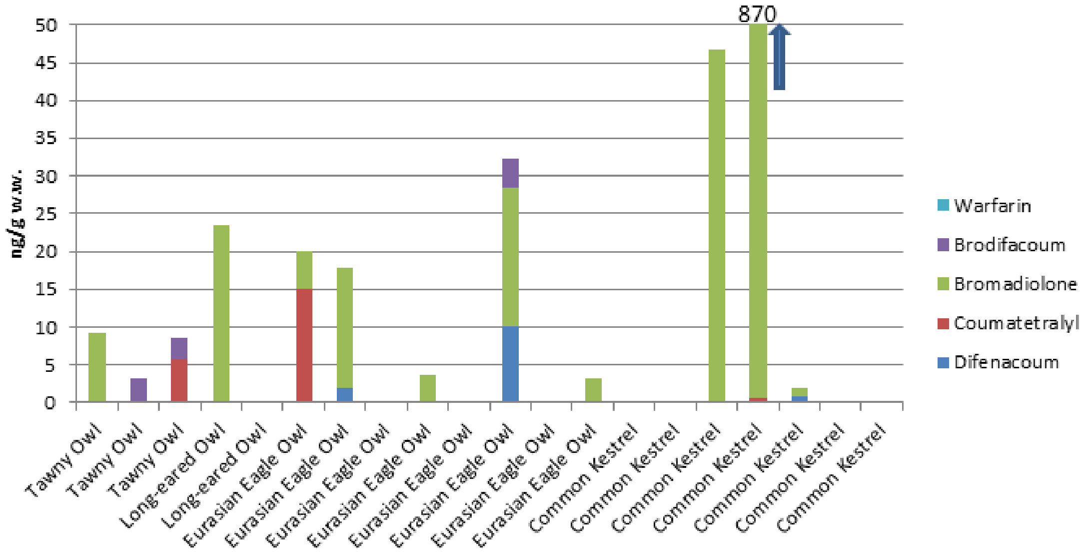


Figure 3. Rodenticide concentration (ng/g w.w.) in different species of avian rptors.

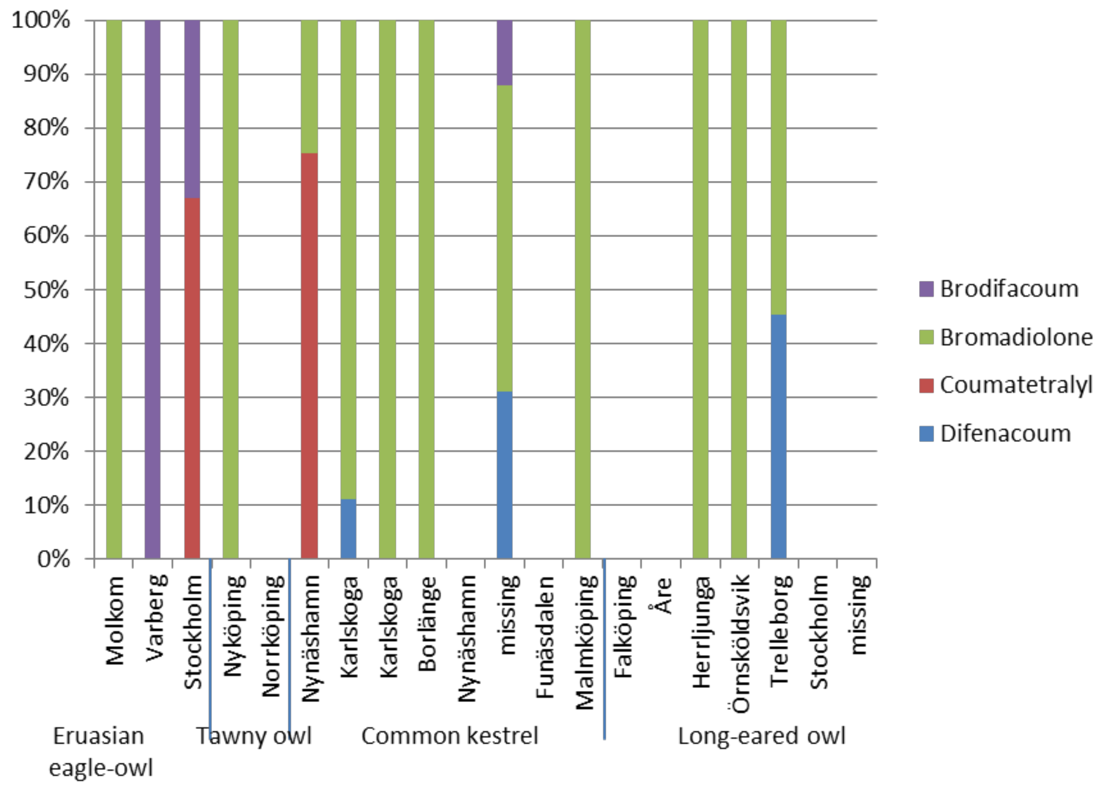


Figure 4. Percent of the total concentration for each anticoagulant rodenticide detected in each individual of the raptors.

8 Conclusions

This screening study shows that the anticoagulant rodenticides difenacoum, bromadiolone, coumatetraly, brodifacoum and warfarin are found in non-target organisms that feed on rodents.

All red foxes (n=10) contained rodenticides. At least two of the compounds could be detected in each individual. The foxes contained in general higher concentrations than the raptors.

Warfarin was found in 50% of the foxes but was below LOQ in all species of raptors.

At least one of the raptor individuals contained anticoagulant levels above the suggested threshold level for poisoning for owls (100 ng/g w.w.).

Finally, secondary poisoning cannot be excluded for predators feeding on rodents that are exposed to rodenticides.

9 Acknowledgements

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