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Prevalence of anticoagulant rodenticides in non-target predators and scavengers in Finland

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Summary

Residues of anticoagulant rodenticides (AR) in non-target animals were studied in Finland. ARs are acting by effective blocking of the vitamin K cycle, resulting in death by internal bleeding. These effects are gradual, developing over several days. ARs are the primary method for rodent control in Finland and they can be transferred to non-target animals feeding on poisoned rodents. In particular, second generation ARs (SGARs) have been found in non-target animals in many countries but no AR screening has been conducted in Finland earlier.

This study focused mainly on species which feed either on rodents or their carcasses and in which ARs have been found in other countries. Samples were collected mainly from human-populated areas on southern Finland. The animals were either found dead or were shot or trapped for other purposes. ARs approved in Finland, i.e. bromadiolone, difenacoum, brodifacoum, flocoumafen, chlorophacinone, difethialone and coumatetralyl were analysed in 136 liver samples by an UHPLC-triple quadrupole mass spectrometric method.

One or more ARs were detected in 87% of the samples. ARs were commonly found in eagle owls, tawny owls, raccoon dogs, red foxes and mustelids (pine martens, least weasels and stoats). The most prevalent AR was bromadiolone (found in 70% of the samples) which was also found in the highest concentrations. Bromadiolone has been the most frequently used AR in Finland since the beginning of 2000s. The second most common AR present in the livers was coumatetralyl (56%) followed by difenacoum (44%), brodifacoum (23%) and flocoumafen (15%). Overall, the prevalence of ARs corresponded well with the sales of these substances in Finland. A high variation of concentrations was found within and between the animals. Overall, concentrations measured in avian species were far lower than those in mammalian species. Highest concentrations were found in raccoon dogs and red foxes. In general, coumatetralyl was found in lower concentrations compared to SGARs.

The prevalence found in this study (87% in overall and 100% in half of the species studied) is high compared to several previous studies conducted in other countries. On the other hand, the majority of the concentrations found were quite low and thus probably not lethal for the animals. However, about 12.5% of the animals studied here were found with concentration above 200 µg/kg. This means that ARs could have influenced the blood clotting in these individuals. It may be concluded that biocidal use of these substances causes frequent exposure of non-target animals, since these ARs are authorized only as biocides in Finland and the use for the crop protection is insignificant. In Finland a national strategy on risk management of ARs was adopted in 2011. Based on these results it appears that the risk mitigation measures (RMMs) either have not been followed or have not been effective in preventing secondary exposure of the non-target animals. Current RMMs are discussed and new RMMs along with further study questions are suggested.

Tiivistelmä

Tutkimuksessa selvitettiin antikoagulanttijyrsijämyrkkyjen (AR) esiintymistä jyrsijöitä tai niiden raatoja syövissä eläimissä. AR:t siirtyvät myrkkyä syöneiden jyrsijöiden välityksellä niitä syöviin petoeläimiin. Muissa maissa jyrsijämyrkkyjä on löydetty etenkin jyrsijöitä ravintonaan käyttävistä nisäkäs- ja lintulajeista, mutta Suomessa asiaa ei ole aiemmin tutkittu.

Tutkitut eläimet olivat joko kuolleina löytyneitä tai ne ammuttiin tai pyydystettiin toista tarkoitusta varten. Tehoaineiden (bromadioloni, difenakumi, brodifakumi, flokumafeeni, kloorifasinoni, difetialoni ja kumatetralyyli) esiintyvyys ja pitoisuudet määritettiin yhteensä 136 maksanäytteestä nestekromatografi-kolmoiskvadrupolimassaspektrometrilla.

Näytteistä 87 prosentissa havaittiin joko yhtä tai useampaa määritetyistä tehoaineista. Jyrsijämyrkkyjä löydettiin yleisesti huuhkajista, lehtopöllöistä, ketuista, supikoirista ja näätäeläimistä (Taulukko 1). Useimmin havaittu tehoaine oli bromadioloni, jonka pitoisuudet olivat myös korkeimmat. Bromadioloni on ollut Suomessa eniten käytetty tehoaine 2000-luvun alusta. Seuraavaksi eniten havaittuja tehoaineita olivat alenevassa järjestyksessä kumatetralyyli, difenakumi, brodifakumi ja flokumafeeni. Havainnot vastasivat melko hyvin tehoaineiden myyntimääriä. Pitoisuuksissa oli paljon vaihtelua sekä eläinlajien välillä että niiden sisällä yksilöiden välillä. Kumatetralyylin pitoisuudet olivat alhaisempia toisen polven aineisiin verrattuna (Taulukko 2).

Myös kaikkein rajatuimmin käytettyjä tehoaineita eli niitä, joiden käyttö on sallittua vain ammattilaisille sisätiloissa, löytyi tutkituista eläinlajeista, joskin pienemmissä määrin kuin yleisempiä aineita. Kumatetralyylia löytyi näytteistä hämmästyttävän usein ottaen huomioon kumatetralyylivalmisteiden vähäisen käyttöasteen ja lyhyen puoliintumisajan. Toisaalta kumatetralyylin pitoisuudet syöteissä ovat useita kertoja muita tehoaineita

suurempia sen pienemmästä myrkyllisyydestä johtuen. Suurin osa havaituista pitoisuuksista oli pieniä, mutta noin 12,5 prosentissa tutkituista eläimistä pitoisuudet olivat niin suuria, että jyrsijämyrkyt ovat mahdollisesti vaikuttaneet veren hyytymiseen.

Taulukko 1. Lukumäärät ja prosenttiosuudet lajeittain niistä yksilöistä, joiden maksassa oli havaittu (pitoisuus ≥ 0,3 µg/kg) vähintään yhtä tehoainetta.

Laji	**N analysoitu**	**N havaittu**	**% havaittu**
Nisäkkäät			
Mäyrä	7	4	57
Kissa	4	4	100
Lumikko	9	9	100
Saukko	2	2	100
Näätä	7	7	100
Supikoira	41	40	98
Rotta	3	3	100
Kettu	12	12	100
Kärppä	12	8	67
Linnut			
Huuhkaja	12	12	100
Kanahaukka	2	2	100
Sinisuohaukka	1	0	0
Varis	6	3	50
Harakka	3	2	67
Lehtopöllö	13	11	85
Merikotka	1	0	0
Varpushaukka	1	0	0

Taulukko 2. Tehoaineiden keskimääräiset pitoisuudet analysoiduissa näytteissä. Mukana ovat ainoastaan kvantitiointirajan (≥ 1.0 µg/kg) ylittäneet havainnot.

Tehoaine	**Keskiarvo**	**Mediaani**	**Keskivirhe**	**Min**	**Maks**	***N***
Bromadioloni	116	32	21	1,0	920	*78*
Difenakumi	24	11	4,5	1,2	138	*54*
Brodifakumi	41	8,3	16	1,5	288	*26*
Flokumafeeni	2,4	1,7	0,9	1,1	7,6	*7*
Kumatetralyyli	6,4	4,1	0,9	1,0	20	*41*

Jyrsijämyrkyt on Suomessa hyväksytty vain biosidikäyttöön. Kasvinsuojelukäyttö on vähäistä, ainoastaan difenakumia sai poikkeusluvalla käyttää metsätaimitarhoilla vuonna 2014. Tulokset osoittavat, että pelkkä biosidikäyttö altistaa petoeläimiä jyrsijämyrkyille.

Kemikaalilaki velvoittaa käyttämään jyrsijämyrkkyjä niiden käyttöohjeiden mukaisesti. Jyrsijämyrkkyjen käyttöä Suomessa pyritään rajoittamaan siten, että yksityisille ihmisille on sallittu vain rajattu käyttö pääasiassa sisätiloissa, kun taas ammattimaisille tuholaistorjujille on sallittu laajempi tehoainevalikoima, kuten myös mahdollisuus käyttää jyrsijämyrkkyjä ulkotiloissa. Käytännössä käyttöohjeita noudatettaneen vaihtelevasti. Ammattimaisessa käytössä esimerkiksi rehu- ja elintarviketuotannossa ja maatiloilla on totuttu käyttämään jyrsijämyrkkyjä jatkuvasti ja tästä käytännöstä irtautuminen näyttää olevan hankalaa, ennen kaikkea kasvavien torjuntakustannusten takia. Suomessa jyrsijämyrkkyjen myyntiä ei säädellä erityisesti ja kaupoista saattaa löytyä vain ammattikäyttöön tarkoitettuja valmisteita. Kaupoissa saatetaan myös myydä jyrsijämyrkkyjä, mutta ei syöttilaatikoita. Osa jyrsijämyrkyistä on rajoitettu vain sisätiloissa käytettäväksi, mutta valvonnan puuttuessa Tukesilla ei ole tietoa, miten hyvin tämä rajoitus toteutuu.

Jyrsijämyrkkytehoaineet ovat uudelleen arvioitavana EU:ssa ja samalla mietitään miten niiden kertymistä muihin eläimiin voidaan vähentää.

Table of contents

1. Introduction

This report presents the results of the first screening for the prevalence of anticoagulant rodenticides in non-target animals, namely rodent-eating predators and scavengers in Finland. Rodenticides are biocidal products which are used for controlling of rodents, most commonly rats and mice, in apartments, storages, business premises and public places. The most common method used for rodent control in European countries is anticoagulant rodenticides (ARs), which cause a death by haemorrhage (Laakso et al. 2010). Although ARs are commonly used in bait boxes not accessible to larger animals than rodents, ARs can transfer to non-target animals via poisoned rodents eaten by predators.

1.1 Anticoagulant rodenticides

Anticoagulant rodenticides have a similar kind of a structural formula (Table 1) and the same mode of action: they are acting as effective blocking of the vitamin K cycle, resulting in inability to produce essential blood-clotting factors (Berny et al. 2014). In addition, anticoagulants cause damage to tiny blood vessels increasing their permeability causing diffuse internal bleeding. These effects are gradual, developing over several days. In the final phase of the intoxication, the exhausted rodent collapses due to haemorrhagic shock or severe anaemia.

Anticoagulants can be divided into first and second generation substances. The first generation rodenticides (FGARs) were introduced for pest control already in the 1940s and some of them are still in use. First generation rodenticides are less toxic and are eliminated within days and require thus multiple doses in order to be fatal. Because chemical control of rodents relies almost exclusively on ARs, many distinct resistant strains of the brown rat (*Rattus norvegicus*) and the house mouse (*Mus musculus*) have been identified (Pelz et al. 2005, Berny et al. 2014). These resistant strains have developed specific genetic traits through a modification of the VKORC1 enzyme involved in the catalytic recycling of vitamin K (Li et al. 2004, Rost et al. 2004).

The second generation anticoagulant rodenticides (SGARs) were developed after rodents started to show resistance to first generation agents, and they are toxic at a much lower dose (IPCS 1995, Table 1). The SGAR group includes brodifacoum, bromadiolone, difenacoum, difethialone and flocoumafen. SGARs are potential PBT substances (meaning that they are persistent, bioaccumulative and toxic (Commission Regulation (EU) No 253/2011). The persistent nature of SGARs is reflected in the long elimination half-lives of these substances (Table 1). SGARs fulfil the exclusion criteria of the Biocidal Products Regulation (528/2012), but they have been approved as biocides exceptionally for the sake of public health and hygiene but for a limited period of five years only. The renewal evaluation of anticoagulant rodenticides is in progress in the EU.

1.2 Control of commensal rodents in Finland

In Finland the most common commensal rodent species is the yellow-necked field mouse (*Apodemus flavicollis*), which is estimated to be the target rodent in more than half of the commercial rodent control cases. The next common rodent is the brown rat estimated to be the target rodent in about 30% of cases and the least common rodent pest is the house mouse being a target only in few cases. These percentages are based on the questionnaire made by Kaseli[1] to Finnish professional pest control operators (PCOs) in 2014. The questionnaire was estimated to cover about 75% of the professional pest control technicians operating in Finland. The numbers are at best rough estimates but no other statistics are available.

The yellow-necked field mouse is the rodent pest that private citizens need to control most often. Sometimes also bank voles (*Myodes glareolus*) enter residential buildings in particular in autumns. Yellow-necked field mice are common in particularly in the food and feed industry whereas rats are commonly controlled in residential buildings in urban and semi-urban areas. The house mouse can be met in buildings where cereals are processed or stored, or in feed mills. Sometimes house mice are also found in cellars of old buildings. There are PCOs who have not seen a house mouse for several years. Brown rats are common e.g. in cities and in farms.

Historically, urban rat populations have been controlled in Finland by specific rat control projects since the early 1900s (Vuorisalo et al. 2001). For instance, the first rat war of Helsinki in 1902 resulted in 37 908 killed animals. Currently, the most commonly used method for rodent control in Finland is anticoagulant rodenticides. From the FGAR group

[1] Kaseli is a unit in the Plant Protection Society in Finland. Kaseli aims to develop pest control in apartments and in food and feed branch.

chlorophacinone and coumatetralyl are allowed for rodent control in Finland. Of the SGAR substances brodifacoum, bromadiolone, difenacoum, difethialone, and flocoumafen were registered as biocidal products in Finland in 2014. Carbon dioxide was also registered as a rodenticide in Finland, but it is used less compared to ARs.

Rodent control is required as a part of own control in food and feed branch. The most common way has been proactive permanent baiting with 4-6 control visits per year. Considering the long intervals between revisits it is likely that the amounts have been loaded in the bait stations that exceed the maximum amount advised in the labels. Sometimes different products may have been filled in the bait boxes in order to maximise the effectiveness of the treatment. Baits may not always be removed when the service contract with the client has ended. The typical use violates the use instructions of rodenticides in many ways. There seems also to be sometimes reluctance among the clients in applying preventive measures. It is further assumed that the general public may not always follow the use instructions of rodenticides. On the other hand the use of mechanical and electronic traps and other non-chemical methods has increased among the PCOs.

1.3 Exposure of non-target animals

Rodenticides are used generally within bait boxes inaccessible to larger animals. Anticoagulants have been found to transfer in non-target animals either by direct consumption of baits (primary poisoning) or more commonly by consuming contaminated rodents (secondary poisoning, Lambert et al. 2007, Figure 1). As anticoagulants cause death to rodents within a few days (Laakso et al. 2010), during that time delay rodents can be preyed upon by predators, exposing them to rodenticides via eaten prey. In other European countries, like in United Kingdom and France, anticoagulants have been found for example in owls, raptors, foxes and mustelids (Berny and Gaillet 2008, Laakso et al. 2010). In the other Nordic countries (Denmark, Norway and Sweden) ARs have been found in e.g. eagle owls (*Bubo bubo*), common buzzards (*Buteo buteo*), tawny owls (*Strix aluco*), European kestrels (*Falco tinnunculus*) and mustelids (Norström et al. 2009, Christensen et al. 2010, NIVA 2012).

Besides in rodent-eating predators, ARs have also been found in non-target small mammals (e.g. Geduhn et al. 2014), and in insectivorous species, like European hedgehogs (*Erinaceus* sp., Dowding et al. 2010, Lopez-Perea et al. 2015), shrews (*Sorex* sp., Geduhn et al. 2014) and passerine birds (Masuda et al. 2014). Songbirds, for example house sparrows (*Passer domesticus*) have been reported to feed on AR baits (Elliott et al. 2014). Although insectivorous, shrews have been also observed to consume baits (e.g. Brakes and Smith 2005) but are more likely to be exposed via invertebrates known to feed on AR baits (e.g. Elliott et al. 2014). The same route of exposure has also been suggested for European hedgehogs (Dowding et al. 2010).

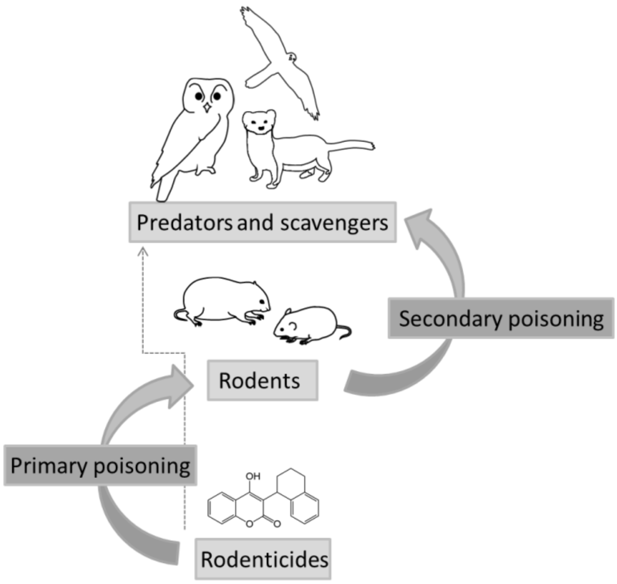


Figure 1. Main routes of exposure of target and non-target animals to rodenticides. In some cases predators may also feed on toxic baits directly.

1.4 Aims of the study

The prevalence of anticoagulants in wildlife has not previously been studied in Finland. Here, we aim for the first time to see the prevalence of anticoagulants in the rodent-eating predators and scavengers. This study will give important first-hand information about the situation in comparison to other countries. The results can further be used to outline the risk mitigation measures in connection to reapproval of the ARs.

2. Material and methods

2.1 Sample collection

Liver samples were collected from altogether 136 individuals of several predator and scavenger species (see Table 4) to determine the concentrations of seven anticoagulant rodenticide substances approved in Finland (Table 1). Animals were either found dead (e.g. road kills) or were shot or trapped as part of predator removals from conservation areas (namely raccoon dog, which is an alien species in Finland). Samples were collected by the Zoological museums of the Universities of Turku and Helsinki, originally provided by museum personnel and private citizens. Most samples were collected in autumn-winter 2014 but some of the samples were of an older origin (mainly from years 2004-2013). The study species were selected mainly due to their availability, taking into account their ecology, namely whether they are likely to be exposed to anticoagulants due to their diet and habitat preferences. The species (listed in Table 4) included 1) rodent-eating predators (mammals: red fox, domestic cat, least weasel, stoat, pine marten; birds: eagle owl, tawny owl, hen harrier), 2) omnivores and scavengers (mammals: badger, raccoon dog; birds: magpie, hooded crow) and as a reference 3) carnivorous species that are not using rodents as their main food source (mammals: otter; birds: goshawk, sparrow hawk, white-tailed sea eagle). In addition we had samples of 4) the target species of ARs (brown rat and yellow-necked field mouse) from areas with known AR use. The analysis of mice failed and hence they are excluded from the results. Sample collection was targeted to contain as many species as possible but with a sufficient sample size per species. Samples were collected mostly from south-western of Finland in the vicinity of human settlements (houses, farms etc.), i.e. in places where exposure to the anticoagulants is most probable (Figure 2).

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

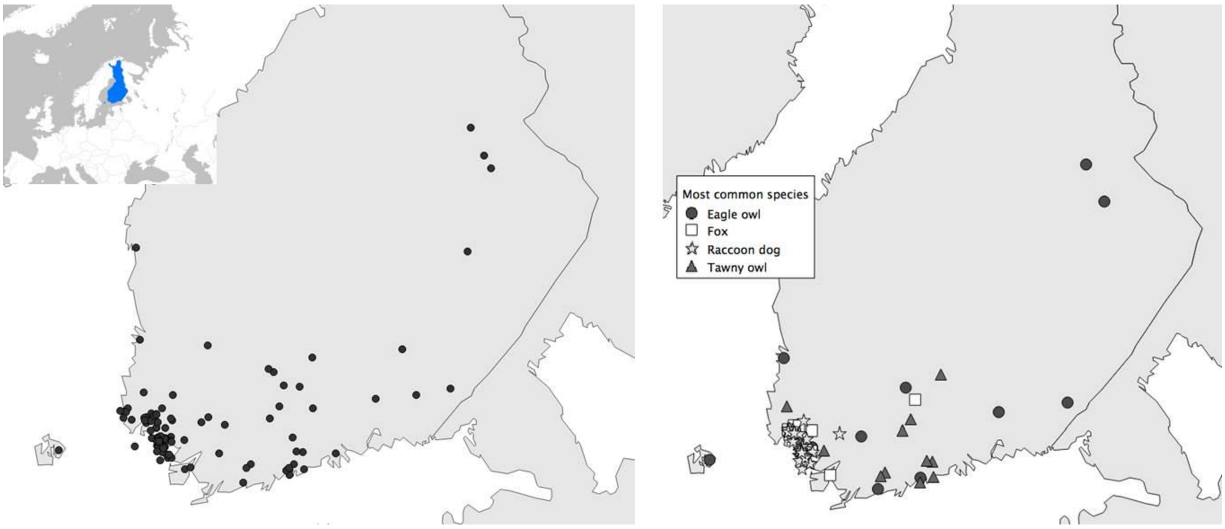
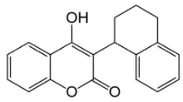
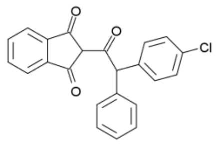
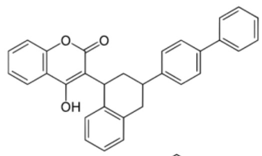
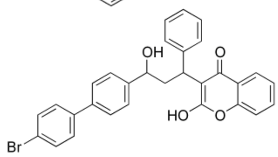
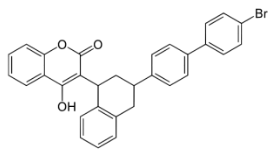
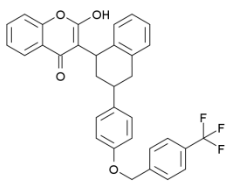
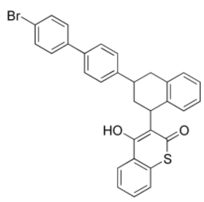


Figure 2. Maps showing the locations of sample collection. Map on the left: all the samples (species pooled), map on the right: locations of the four most abundant species (eagle owl, fox, raccoon dog and tawny owl) samples.

2.2 Analytical method for determination of the anticoagulant rodenticides

The concentrations of seven anticoagulant rodenticides (see Table 1) in liver samples were determined by an UHPLC - triple quadrupole mass spectrometric method. External standard method was used for the quantification. Deuterated warfarin was used for confirming success of the sample preparation.

Table 1. List and properties of different anticoagulant rodenticides analysed (ECHA, European Chemicals Agency, http://echa.europa.eu/web/guest/information-on-chemicals/biocidal-active-substances).

CAS number	Substance	LD50 (mg/kg body wt) for rat	Half-life (day) in rat	Structural formula
5836-29-3	Coumatetralyl	15	1.8-4	
3691-35-8	Chlorophacinone	3.2	7	
56073-07-5	Difenacoum	1.8	118	
28772-56-7	Bromadiolone	1.3	318	
56073-10-0	Brodifacoum	0.4	282-350	
90035-08-8	Flocoumafen	0.3	215	
104653-34-1	Difethialone	0.6	126	

Preliminary treatment

Defrosted liver samples (weight 10-100 g) were homogenised in a blender and thereafter pushed through a metal sieve. The samples were cleaned-up by adding them in water-acetonitrile (1:1). For 1 g of tissue 5 ml of this mixture was used. The samples were mixed and incubated in an ultrasonic path. Thereafter sodium chloride was added to the samples. After further incubation in a sonic bath, the samples were centrifuged and the upper phases were removed to another test tube. An aliquot of 2.5 ml of acetonitrile (ACN) was added to each of these tubes and the sonication was repeated.

The ACN phases of these two treatments were transferred into new test tubes and evaporated to dryness under a stream of nitrogen. The dry residue was re-dissolved into ACN and sonicated after addition of an aliquot of purified water (30% ACN). After this step the samples were eluted through SPE columns with ACN containing 5% of ammonium hydroxide and the eluents were evaporated to dryness under a stream of nitrogen.

The dry samples were dissolved into methanol (90%) and analysed by UHPLC-MSMS system.

Chromatography

Waters UPLC instrument equipped with Acquity UPLC BEH C-18 1.7µm (2.1x100mm) column and precolumn (Acquity UPLC BEH 2.1x5mm). Mobile phase gradient was: 10 mM ammonium formate pH 9.5 / Methanol (MeOH). Linear gradient: from 20% MeOH to 70% MeOH in 5 or 6 minutes. Methanol was increased to 95% and kept 30 sec. at 95% and from 6.5 to 7 min descending gradient to 20% MeOH. Column temperature was kept at 35°C and eluent flow rate was 0.35 mL/min. Injection volume was 5.0 µL and total run time was 10 minutes.

Retention times for anticoagulants were: brodifacoum c. 6.4 min, bromadiolone c. 6.1 and 6.2 mins (two isomers), difenacoum c. 6.2 min, difethialone c. 6.5 min, flocoumafen c. 6.35 min, chlorophacinone c. 5.5 min, coumatetralyl c. 3.6 min and warfarin D5 3.1 min.

Mass spectrometry

Mass spectrometer was Xevo TQ-ms triple quadrupole instrument. Negative ionisation was used for all compounds. Source temperature was 150°C was and 500 °C for capillary gas temperature (nitrogen gas flow 900L/h). Collision gas was argon (flow 0.15mL/min) and instruments automatic sample tuning was used for collision energy and for cone voltages for the substances under study.

MS transitions for quantitation: brodifacoum 521.3 >135.0, bromadiolone 525.3 >250.0, difenacoum 443.5 >135.0, difethialone 537.3 >151.0, flocoumafen 541.4 >161.0, chlorophacinone 373.2 >201.0, coumatetralyl 291.3 >141.0.

Calibration range and linearity

Five standard samples were prepared for each analytical batch by spiking AR-free porcine liver with known amounts of the substances. The concentrations were 1, 5, 20, 50, 300 µg/kg. External standard method was used for quantitation. Correlation coefficients (R2) for the calibration lines were typically 0.98 - 0.99. Purity of used standards: brodifacoum 99%, bromadiolone 94%, difenacoum 99%, difethialone 98%, flocoumafen 99%, chlorophacinone 99%, coumatetralyl 99% and deuterated warfarin 98%.

Limit of quantification and limit of detection

The limit of quantitation was set to 1.0 µg/kg wet tissue for each substance, except for chlorophacinone it was 20 µg/kg. The limit of detection (LOD) for each of the substances was 0.3 µg/kg, except for chlorophacinone it was 5.0 µg/kg.

3. Results

3.1 Residues of anticoagulant rodenticides

ARs were detected (≥ 0.3 µg/kg) in 87% of the 136 samples analysed. Chlorophacinone and difethialone were not found in any of the samples analysed. Despite of registration, products containing these substances have not been in sale in Finland. All the other substances were found at least in some of the samples. The most common substance was bromadiolone, which was found in 70% of the analysed samples (Figure 3). Bromadiolone was followed in decreasing order of commonness by coumatetralyl (56%), difenacoum (44%), brodifacoum (23%) and lastly flocoumafen (15%). In 65% of samples more than one (2-5) different ARs were detected (Figure 4).

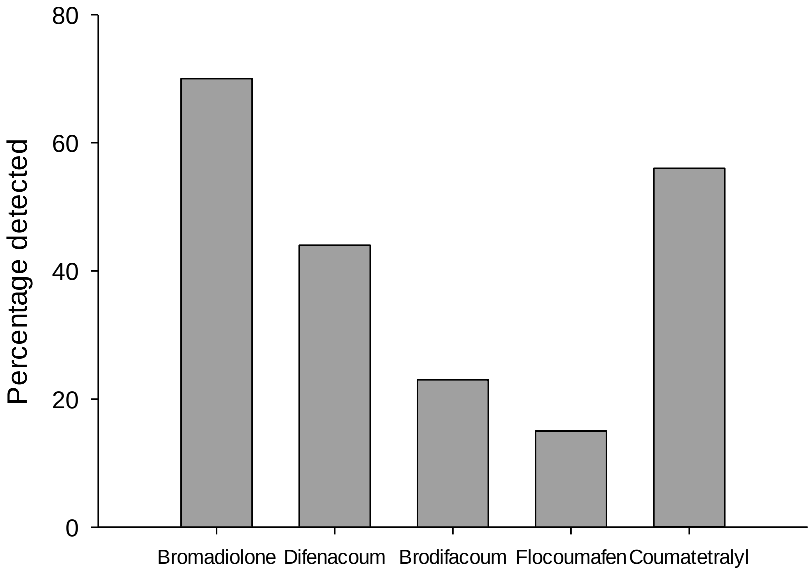


Figure 3. Percentage of samples with anticoagulant rodenticides detected (≥ 0.3 µg/kg) in the liver samples. Total number of samples analysed was 136.

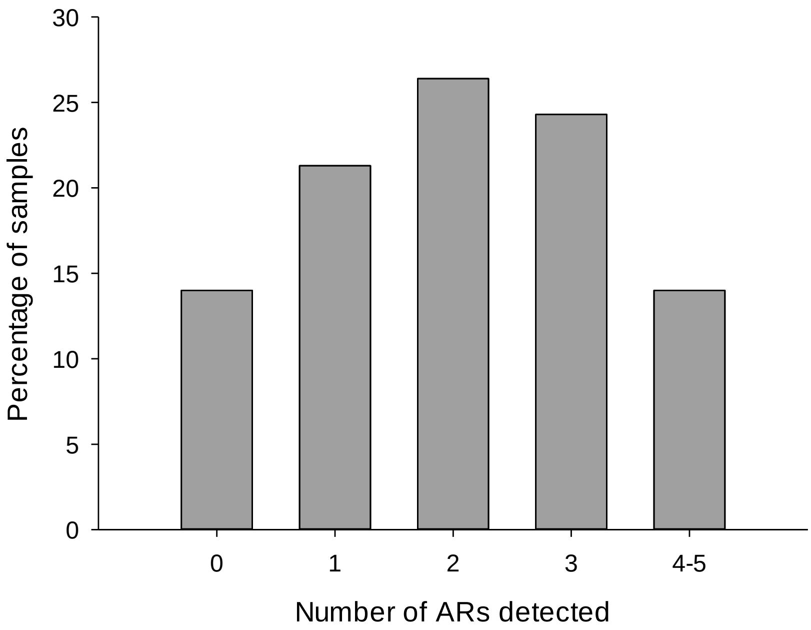


Figure 4. Percentage of individuals (all species) with residues of one, two, three or four-five anticoagulant rodenticides detected (≥ 0.3 µg/kg).

Concentrations of bromadiolone were the highest observed (mean value 116 µg/kg, Table 2, Figure 5), while brodifacoum and difenacoum showed moderate concentrations (means 41 µg/kg and 24 µg/kg, respectively). Concentrations of coumatetralyl (the only FGAR) and flocoumafen were quite low (means 6.4 µg/kg and 2.4 µg/kg, respectively). Of these, flocoumafen was found in levels above quantification only in seven samples.

Table 2. The mean concentrations of the anticoagulant rodenticides in samples analysed. Only results ≥ 1.0 µg/kg are included (total number of samples analysed quantitatively = 104).

Substance	**Mean**	**Median**	**SE**	**Min**	**Max**	***N***
Bromadiolone	116	32	21	1.0	920	*78*
Difenacoum	24	11	4.5	1.2	138	*54*
Brodifacoum	41	8.3	16	1.5	288	*26*
Flocoumafen	2.4	1.7	0.9	1.1	7.6	*7*
Coumatetralyl	6.4	4.1	0.9	1.0	20	*41*

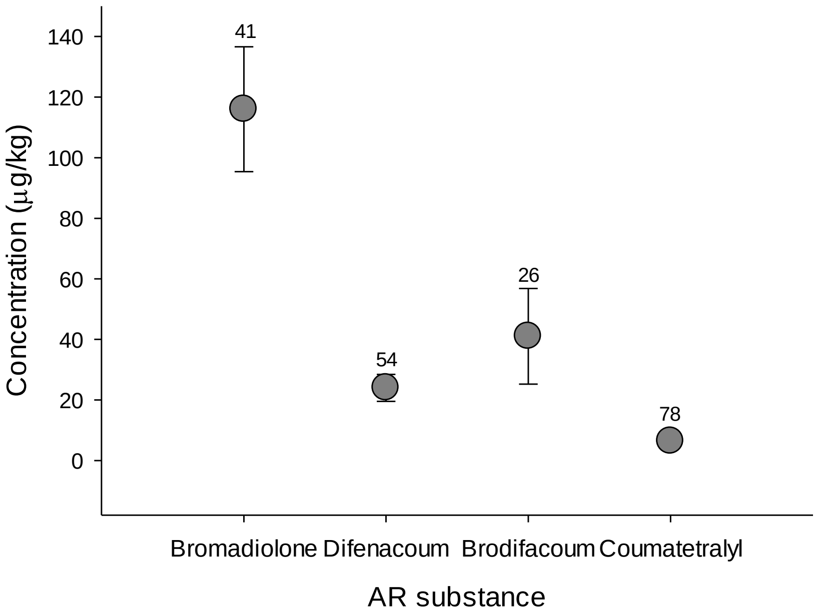


Figure 5. Concentrations (mean ± SE) of the four most commonly detected anticoagulant rodenticides (in all the species). Only results ≥ 1.0 µg/kg are included. Substance-specific sample size shown above each symbol.

3.2 Mammals and birds

ARs were detected very commonly (57-100%, Table 3) in all of the mammalian samples analysed. One or more substances were found in all of the samples of the domestic cat, the least weasel, the otter (only two individuals analysed), the brown rat and the red fox. Also almost all of the raccoon dogs (98%) had at least one of the substances detected. A bit more than half of the badgers and stoats had ARs detected (57% and 67%, respectively). Bromadiolone and coumatetralyl were detected most often in the species with highest number of samples (Figure 6).

Highest concentrations of brodifacoum were found in raccoon dogs, followed by badgers (Table 4). Red foxes and raccoon dogs had highest concentrations of bromadiolone (mean values 209 µg/kg and 189 µg/kg, respectively; Figure 7, Table 4). Cats (mean 85 µg/kg) and pine martens (mean 76 µg/kg) had moderately high concentrations of bromadiolone. Rats had rather high concentrations of difenacoum, but they were collected in an area where rodents were poisoned with difenacoum. Flocoumafen was quantified only in raccoon dogs and red foxes.

ARs were detected also commonly in most of the studied avian species (50-100%, Table 3), most commonly in eagle owls (100%), goshawks (100% but only two individuals analysed) and tawny owls (85%). No substances were found in the hen harrier, white-tailed sea eagle or sparrow hawk, tough only one individual of each species was analysed.

Overall, concentrations measured in avian species were far lower than those in mammalian species (Figure 7, Table 4). Eagle owls and goshawks (only two individuals analysed for the latter) had the highest bromadiolone concentrations. Coumatetralyl was found above the limit of quantification only in the two owl species, the eagle owl and the tawny owl (Figure 7, Table 4).

Table 3. Number and percentage of samples (by species) with detected levels (≥ 0.3 µg/kg) of at least one anticoagulant rodenticide in the liver.

Species	N analysed	N detected	% detected
Mammals			
Badger	7	4	57
Cat	4	4	100
Least weasel	9	9	100
Otter	2	2	100
Pine marten	7	7	100
Raccoon dog	41	40	98
Rat	3	3	100
Red fox	12	12	100
Stoat	12	8	67
Birds			
Eagle owl	12	12	100
Goshawk	2	2	100
Hen harrier	1	0	0
Hooded crow	6	3	50
Magpie	3	2	67
Tawny owl	13	11	85
Sea eagle	1	0	0
Sparrow hawk	1	0	0

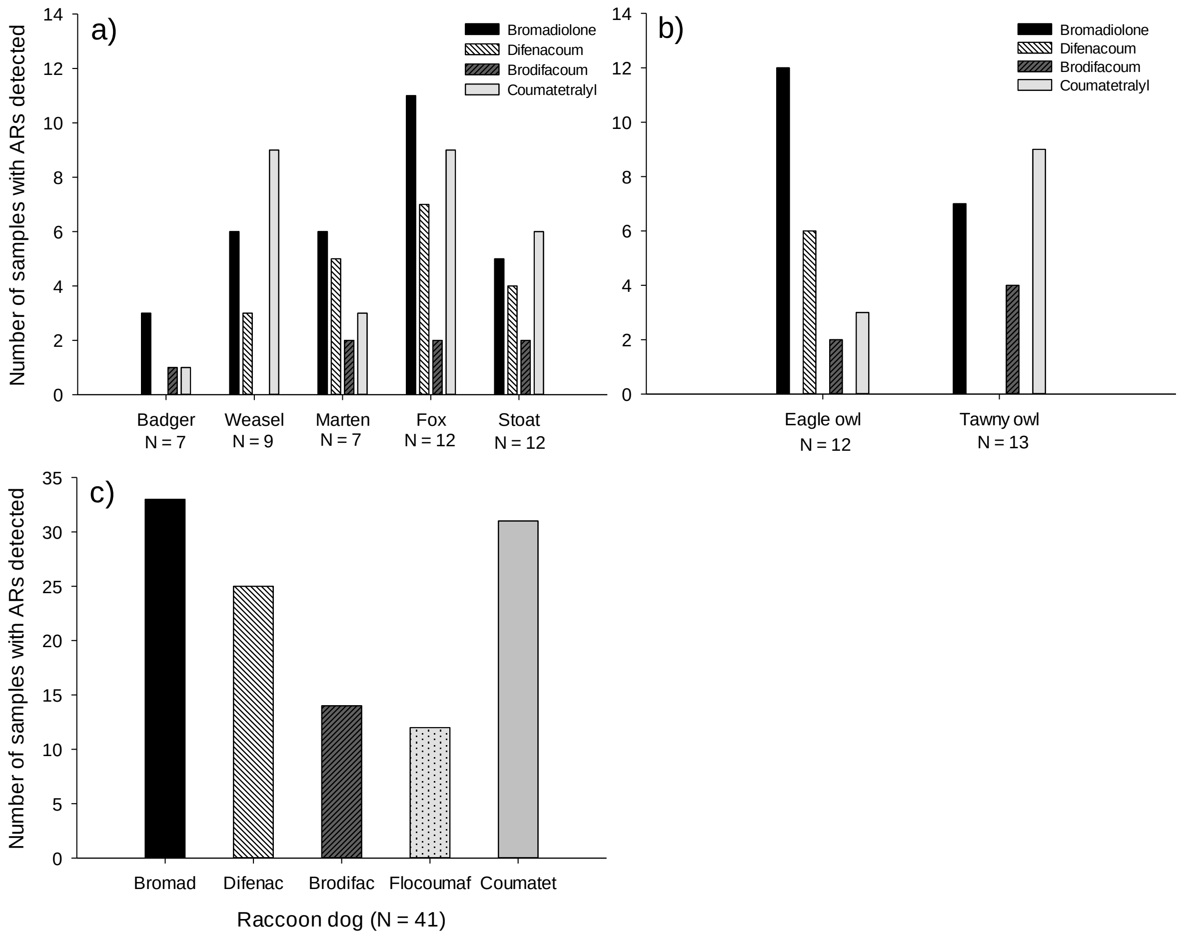


Figure 6. Number of anticoagulant rodenticides detected (≥ 0.3 µg/kg) in a) mammals with highest number of samples, b) owls and c) raccoon dogs. Please note the different scale of y-axis in figure c. Number of samples for each species is shown below the x-axis.

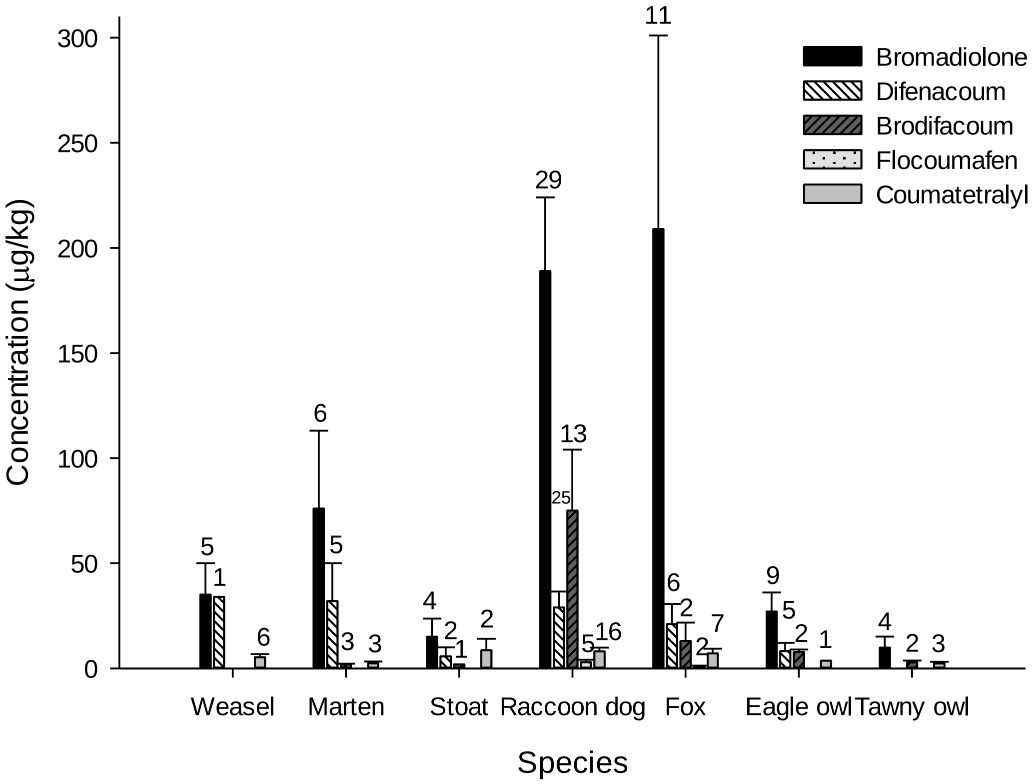


Figure 7. Concentrations (mean ± SE) of different anticoagulant rodenticides in species with highest number of individuals analysed (substance- and species-specific sample numbers are shown above the bars). Only values ≥ 1µg/kg are included.

Table 4. Concentrations of the five anticoagulant rodenticides (mean, min, max) by species. Only values ≥ 1μg/kg (limit of quantification) are included. N_{tot} is showing the total number of samples analysed, whereas N shows a substance-specific number of positive observations. Percentage (%) refers to a substance-specific portion of positive observations including also the values between the limit of detection (≥ 0.3μg/kg) and the limit of quantification.

Common name	***Sci name***		**Coumatetralyl**					**Difenacoum**					**Brodifacoum**					**Bromadiolone**					**Flocoumafen**				
		N tot	**%**	**Mean**	**Min**	**Max**	***N***	**%**	**Mean**	**Min**	**Max**	***N***	**%**	**Mean**	**Min**	**Max**	***N***	**%**	**Mean**	**Min**	**Max**	***N***	**%**	**Mean**	**Min**	**Max**	***N***
<u>*Mammals*</u>																											
Badger	*Meles meles*	*7*	**14**	1.8	1.8	1.8	*1*	**0**					**14**	34	34	34	*1*	**43**	45	31	71	*3*	**0**				
Domestic cat	*Felis catus*	*4*	**25**					**50**	2	1.7	2.3	*2*	**25**	1.6	1.6	1.6	*1*	**50**	85	85	85	*1*	**0**				
Least weasel	*Mustela nivalis*	*9*	**100**	5.3	2.2	12	*6*	**33**	34	34	34	*1*	**0**					**67**	35	8.9	94	*5*	**0**				
Otter	*Lutra lutra*	*2*	**100**	7.6	5.8	9.4	*2*	**50**	2.5	2.5	2.5	*1*	**0**					**100**	8.6	6.2	11	*2*	**50**				
Pine marten	*Martes martes*	*7*	**43**	2.4	1.1	4.1	*3*	**71**	32	1.2	99	*5*	**29**	1.9	1.5	2.1	*2*	**86**	76	6.1	238	*6*	**0**				
Raccoon dog	*Nyctereutes procyonoides*	*41*	**76**	8.1	1.2	20	*16*	**61**	29	1.3	138	*25*	**34**	75	2.3	288	*13*	**81**	189	2.8	640	*29*	**30**	2.9	1.1	7.6	*5*
Brown rat	*Rattu norvegicus*	*3*	**33**					**100**	57	3.5	105	*3*	**33**	12	12	12	*1*	**33**	1.2	1.2	1.2	*1*	**0**				
Red fox	*Vulpes vulpes*	*12*	**75**	7.1	1.0	17	*7*	**58**	21	3.6	62	*6*	**17**	13	4.7	22	*2*	**92**	209	1.7	920	*11*	**17**	1.2	1.1	1.3	*2*
Stoat	*Mustela erminea*	*12*	**50**	8.6	3.2	14	*2*	**33**	5.7	1.3	10	*2*	**17**	1.9	1.9	1.9	*1*	**42**	15	1.2	40	*4*	**0**				
<u>*Birds*</u>																											
Eagle owl	*Bubo bubo*	*12*	**25**	3.6	3.6	3.6	*1*	**50**	8.2	1.2	20	*5*	**50**	7.7	6.4	9	*2*	**100**	27	3.3	87	*9*	**25**				
Goshawk	*Accipiter gentilis*	*2*	**50**					**50**	2.8	2.8	2.8	*1*	**50**					**100**	36	36	36	*1*	**50**				
Hooded crow	*Corvus corone*	*6*	**0**					**33**	13	1.8	24	*2*	**0**					**50**	2.3	1.0	3.5	*2*	**0**				
Hen harrier	*Circus cyaneus*	*1*	**0**					**0**					**0**					**0**					**0**				
Magpie	*Pica pica*	*3*	**0**					**33**	5.4	5.4	5.4	*1*	**33**	1.6	1.6	1.6	*1*	**67**					**0**				
Sparrow hawk	*Accipiter nisus*	*1*	**0**					**0**					**0**					**0**					**0**				
White-tailed sea eagle	*Haliaeetus albicilla*	*1*	**0**					**0**					**0**					**0**					**0**				
Tawny owl	*Strix aluco*	*13*	**69**	2.3	1.4	3.9	*3*	**0**					**31**	2.8	1.9	3.7	*2*	**54**	9.8	1.0	24	*4*	**15**				

4. Discussion

Residues of AR substances were found in most of the species studied (in 87% of 136 individuals). Apart from chlorophacinone and difethialone which were not sold in Finland, all the other analysed substances were found in 15-70% of the samples. Of mammalian species 57-100% had one or more ARs at detectable levels, whereas for avian species the percentages varied between zero and one hundred species dependently. The results show that ARs used as biocides causes frequent exposure of non-target animals that prey on rodents or their carcasses. ARs are not registered for plant protection purposes in Finland, but there was a derogation granted for some forest nurseries to use difenacoum in 2014. The map on forest nurseries can be found from the web pages of The Natural Resources Institute Finland (http://www.metla.fi/metinfo/taimitieto/index.htm).

4.1 Residues of anticoagulant rodenticides in relation to their use in Finland

The most prevalent AR was bromadiolone (found in 70% of the samples) which is also the most commonly used AR in Finland. The second prevalent AR was coumatetralyl followed in decreasing order by difenacoum, brodifacoum and flocoumafen. In general, the prevalence of the ARs in non-target species corresponded well with the sales of the substances in Finland. The most commonly used substances in Finland based on sales in decreasing order are: 1) bromadiolone, 2) difenacoum, 3) coumatetralyl, 4) brodifacoum and 5) flocoumafen. Coumatetralyl is sold least when considering sales in products, but third most when sales are recalculated to amount of the active substances. This is due to the higher concentration of coumatetralyl in the products compared to SGARs. The sale volumes are collected yearly by the Finnish Safety and Chemicals Agency Tukes, but since the information is confidential, sales of separate products cannot be presented here. The total amount of anticoagulant rodenticides sold in Finland in 2014 was approximately 250 000 kg and there has been a slight but steady increase during the last three years.

At the time of this study bromadiolone was used only in one product, which is a loose bait of rolled oats (see Table 5). This product has been available to both PCOs and the private consumers. Based on the questionnaire made by Kaseli[2] to PCOs on the use of rodenticides, bromadiolone was used in two thirds of the control cases. Most used formulation type was loose bait (flakes, see Figure 8a). The results of questionnaire were presented in the yearly lecture day for PCOs on 27 March 2015 (see Figure 8b). The total amount of rodenticides used by PCOs was about 12 000 kg which is only a minor proportion of the total sale of ARs in 2014.

[2] See footnote [1] on p. 10.

Table 5. The number and formulation types of anticoagulant rodenticide products registered and sold as biocide products in Finland in 2014 (http://biosidit.tukes.fi/).

	Loose bait[1]	Paste	Block	Foam	Total
Brodifacoum[2]			2		**2**
Bromadiolone[2]	1				**1**
Coumatetralyl[3]		2		1	**3**
Difenacoum[2]	1	3	4		**8**
Flocoumafen[2]	1		1		**2**
Total	**3**	**5**	**7**	**1**	**16**

[1] Loose baits include grain and pellet formulations
[2] Concentration in the products 50 mg/kg
[3] Concentration in the paste and foam 375 mg/kg and 4 062 mg/kg, respectively

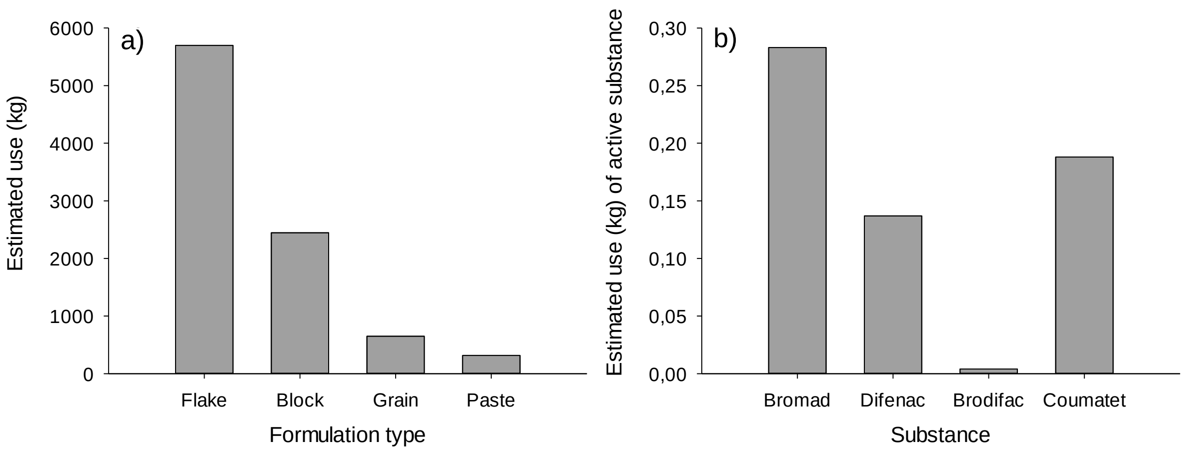


Figure 8. Estimated use of the anticoagulant rodenticides by the professional pest control operators in Finland in 2014 based on the questionnaire made by Kaseli (redrawn with the courtesy of Kaseli). a) Use by formulation type, b) Use of the four most common anticoagulant rodenticides. Estimated use of products is recalculated to active substance by using the active substance concentration of0.005% for SGARs and 0.22% for coumatetralyl. For coumatetralyl it is assumed that half of the active substance is used in the pasta formulation (375 mg/kg) and half in the foam formulation (4062 mg/kg).

The only FGAR, coumatetralyl, was found in 56% of the studied animals. This is a high prevalence compared to some other studies, e.g. to only 5.7% in German red foxes found by Geduhn et al. 2015. The limit of detection (LOD) used was higher in Geduhn et al. 2015 compared to this study (1.0 µg/kg versus 0.3 µg/kg) but this deviation does not yet explain the difference in coumatetralyl prevalence. A more likely explanation could be resistance. Coumatetralyl may not be commonly used in many European countries, due to prevalence of FGAR resistance, as also speculated by Geduhn et al. 2015 to be the reason for the low prevalence of FGARs found in their study. However, the use volumes of rodenticide active substances were not specified in the article. In Finland products containing coumatetralyl are not used frequently, but its concentration in the products is 7.5-81 times higher compared to SGARs. Although coumatetralyl was found commonly in the samples, the concentrations of it were low compared to the SGARs. This is most probably due to the much shorter elimination half-life of coumatetralyl (1.8-4 days, Table 1) compared to the SGARs (118-350 days).

Difenacoum was found in 44% of the samples. Brodifacoum was less prevalent and was detected in 23% of the animals studied. Brodifacoum is used much less than difenacoum (Figure 8b) but the concentrations of brodifacoum are on average higher than those of difenacoum. Also the elimination half-life of brodifacoum is longer (282-350 days) compared to difenacoum (118 days). Flocoumafen seemed to be less prevalent and it was found only in 15% of the samples. Also the concentrations were quite low. In Finland flocoumafen is used least of the studied substances.

4.2 Comparison to residues of anticoagulant rodenticides in other countries

A wide variation of concentrations was found within and between species. The lowest quantified concentrations were close to the limit of quantification (1.0 µg/kg) while the highest concentrations exceeded 200 µg/kg, which is considered as a trigger for biological effects. The highest concentrations were found for bromadiolone in red foxes and in raccoon dogs.

In general, the prevalence found in this study (87% in overall and 100% in half of the species studied) is high compared to several previous studies conducted in other European countries. For example, the prevalence found in Germany in red foxes was 59.8% (Geduhn et al. 2015), in Netherlands in the predatory birds studied 50% (van den Brink 2014), in Spain 38.7% in all the studied animals and 49.5% in nocturnal raptors and carnivorous mammals (Sánchez-Barbudo 2012), in Norway 60% in golden eagles (*Aquila chrysaetos*) and eagle owls (NIVA 2012), and in France in raptors 73% (Lambert et al. 2007). On the other hand, an equally high prevalence was found in Denmark: 84-100% in all the avian and mustelid species studied (Christensen et al. 2010) and 96.5% in mustelids (stone martens *Martes foina* and polecats *Mustela putorius*, Miljøministeriet 2015).

The high prevalence found could be due to several factors. First, the limits of detection for the ARs in this study were quite low (≥ 0.3 µg/kg) compared to some of the other studies. Second, samples were on purpose collected mostly from urban areas with high levels of human population and thus also high potential for AR exposure. Geduhn et al. 2015, for example, found local livestock density and the percentage of urban area to be good indicators for AR residue occurrence in red foxes. ARs are also known to decrease with increasing distance to baiting boxes (Geduhn et al. 2014, Miljøministeriet 2015). In densely human populated areas the patches where no baiting is conducted are probably scarce, and considering the large hunting areas of the mobile predators, their likelihood of AR exposure in urban areas is probably very high.

There is not much information available about the lethal concentrations in livers in different species, not to mention the potential sublethal effects. The LD50 values (Table 1) cannot be used for direct comparison but they can be used as a help for careful assessments. Geduhn et al. 2014 estimated based on AR liver concentrations of laboratory rats and mice given a dose of bromadiolone that small mammal individuals containing residues > 1 000 µg/kg (1 724 µg/kg when corrected by recovery rate) to be potentially moribund. Thomas et al. 2011, on the other hand, suggests that > 100-200 µg/kg level for barn owls (*Tyto alba*) already carries a considerable risk of acute intoxication. The potentially lethal range for SGAR residues in barn owls has variously been described as > 100 µg/kg (Newton et al. 1999a) and > 200 µg/kg (Newton et al. 1999b). Overall, the majority of the concentrations found in this study were quite low and thus probably not lethal for the animals. However, about 12.5% of the animals studied here were found with concentrations above 200 µg/kg, making it possible that ARs could have influenced the blood clotting in in these individuals. Further studies are obviously needed to clarify the relationship between effects and liver concentrations.

In the other Nordic countries similar combinations of ARs have also been found in non-target animals. In Sweden, difenacoum, coumatetralyl, bromadiolone and brodifacoum were found in three eagle owl individuals: difenacoum in all three, bromadiolone in two of the individuals and coumatetralyl and brodifacoum in one (Norström et al. 2009, LOD 1.0 µg/kg). Only one of the individuals was analysed by a liver sample and the other two by muscle samples only. The concentrations in liver were considerably higher than those in muscle, leading probably to an underestimation of both prevalence and concentrations. Brodifacoum, for example, was found in the liver sample only (Norström et al. 2009). In Norway, brodifacoum, bromadiolone, difenacoum and flocoumafen were detected in the livers of the golden eagle and the eagle owl (NIVA 2012, LODs 2.0-5.0 µg/kg). Brodifacoum was determined in 7 out of 16 golden eagle livers (~ 44%) with concentrations between 22 and 154 µg/kg. Bromadiolone was also found in ~ 44% of the golden eagles and in 50% of the eagle owls with measureable concentrations of between 11 and 110 µg/kg and 74 and 158 µg/kg, respectively. Difenacoum was detected only in two of the eagle owls (25%) at level of 39 and 181 µg/kg. Flocoumafen was detected in two (12.5%) of the golden eagles (15 and 117 µg/kg) and in one eagle owl (12.5%). In Danish screening (Christensen et al. 2010) anticoagulant rodenticides were detected in 84-100% of all avian and mustelid specimens examined (LODs: 1.0 µg/kg for flocoumafen, 2.0 µg/kg for coumatetralyl, brodifacoum and difenacoum and 3.0 µg/kg for bromadiolone). ARs difenacoum, bromadiolone and brodifacoum were most commonly present, and coumatetralyl and flocoumafen occurred in fewer individuals. Bromadiolone was the most sold AR in kilos in Denmark in 1999-2008, followed by coumatetralyl. Sale volumes of other ARs were considerably lower during the time period. Between 5.7% and 22% of the examined kestrels, common buzzards, tawny owls, barn owls, little owls (*Athene noctua*), stoats and weasels had concentrations higher than 200 µg/kg. Amongst the red kite (*Milvus milvus*) and the eagle owl the proportion of individuals with more than 200 µg/kg liver were 66% and 70%, respectively, while there were no critically high concentrations recorded in the rough-legged buzzard (*Buteo lagopus*), the marsh harrier (*Circus aeruginosus*), the long-eared owl (*Asio otus*) and the short-eared owl (*Asio flammeus*), although small sample size for the last species prevented firm conclusions.

4.2.1 Mammals

Overall, the mammalian species studied had both higher prevalence and concentration of ARs than did the avian species. This could be caused by the higher sample size in mammalian than in avian species. Alternatively, especially smaller mammalian predators might get more easily exposed to poisoned prey due to their capability of entering burrows and spaces under buildings. Highest concentrations of ARs in all the studied animals were found in raccoon dogs and foxes, which is probably due to their habitat and diet preferences. Both species occur regularly in urban environments and can take an advantage of feeding from garbage bins when given the chance, making also direct exposure via bait eating a possibility especially for these species.

Foxes are generalist predators, which typically have a high percentage of rodents in their diet, especially when voles are abundant (Dell'Arte et al. 2007). Foxes are also well-adapted in living in urban and semi-urban environments (Harris and Baker 2001, Vuorisalo et al. 2014), which together with their diet preferences makes them vulnerable for secondary AR exposure. AR residues have been found in foxes also for example in France (Berny et al. 1997) and Spain (Sanchez-Barbudo et al. 2012). Geduhn et al. 2015 found in Germany that 198 out of 331 liver samples (59.8%) from red foxes contained residues of at least one AR, and 38.7% contained more than one active substance. Median value of residue concentrations of AR positive samples varied between 10 µg/kg for warfarin (LOD 2.0 µg/kg) and 91 µg/kg for brodifacoum (LOD 3.0 µg/kg) and was 61 µg/kg for bromadiolone (LOD 3.0 µg/kg). Highest concentration was found for brodifacoum (2 433 µg/kg), and a high maximum concentration was also observed for bromadiolone (1 574 µg/kg). These concentrations are much higher than what was found here. In the samples of the present study the highest concentration of all the samples was found in a red fox (920 µg/kg of brodifacoum). That particular individual was found in a garage and was observed to suffer from sarcoptic mange. AR residues have been hypothesized to predispose for example bobcats (*Lynx rufus*) to other medical conditions such as an inability to mount strong, anti-mite immunity (Riley et al. 2007, Serieys et al. 2013) but the link between mange and AR exposure is still unclear.

Raccoon dogs are omnivores, which consume for example bird eggs, earthworms and plant material (Kauhala and Ihalainen 2014). However, Kauhala and Ihalainen 2014 found that in the Ruissalo Island near the City of Turku, an area from which some of our raccoon dog samples were the percentage of rodents in the diet of raccoon dogs was as high as 60%. The raccoon dog is an alien species in Finland and they are thus removed from the nature conservation area of Ruissalo. The Ruissalo Island is sparsely populated, so the AR residues found from the raccoon dogs from Ruissalo are unlikely caused by private AR use. There are however, a botanical garden and farms in the island, in which rodenticides are most likely used. Badgers, which are also omnivorous (Siivonen 1974), were found in the same study to have less than 20% of rodents in their diet (Kauhala and Ihalainen 2014). Badgers did also have a considerably lower prevalence of ARs in this study than did raccoon dogs (57% in badgers, 98% in raccoon dogs).

Also pine martens were found to have a high prevalence (100%) and a quite high concentration of ARs. Pine marten diet consists of rodents, hares and squirrels and is complemented by birds, such as grouse, but there seems to be strong variation within and between years due to the availability of prey (Storch et al.1990, Pulliainen and Ollinmäki 1996). Pine martens also feed on carcasses (Siivonen 1974). Diet of martens living in urban or semi-urban habitats might differ though from that what has been observed in the woodlands.

Mustelids in general have been found to have a high prevalence of ARs. In Denmark AR residues were detected in 99% of the stone martens, in 94% of the polecats (Miljøministeriet 2015), in 97% of stoats and in 95% of weasels (Elmeros et al. 2011). Difenacoum had the highest prevalence (82% in stoats and 88% in weasels) but bromadiolone was detected in the highest concentrations in both stoat (median 44 µg/kg, maximum 1 290 µg/kg ww) and weasel (median 55 µg/kg, maximum 1 610 µg/kg ww, Elmeros et al. 2011). In the present study AR residues were detected in all of the least weasel samples but only in 67% of the stoat samples. Concentrations were much lower than those measured in Denmark: highest concentrations for both species were found for bromadiolone (in least weasels mean 39 µg/kg, maximum 94 µg/kg) and in stoats mean 15 µg/kg, maximum 40 µg/kg). Of the mammals probably the most surprising result here was the high prevalence (100%, although with only two individuals analysed) found in otters, which are using fish as their main source of food. Although otters are fish specialists, they also forage on crayfish, frogs, muskrats, water voles and birds (Siivonen 1974).

Several predatory species rarely eat rats due to their large size, implicating non-target small mammals as a major route of exposure (Laakso et al. 2010, see also Brakes and Smith 2005). In Danish AR screening (Miljøministeriet 2015) bromadiolone residues were found in 5-21% of the analyzed small mammals, and were detected in all the examined species (voles, mice and shrews). Likewise, Geduhn et al. 2014 found bromadiolone residues in liver samples of 23% of non-target small mammals.

The only analysed individuals of a target species, the brown rat, were trapped from an area where rats were poisoned with difenacoum and the livers contained difenacoum with concentrations of 3.5, 62 and 105 µg/kg. The concentrations in two rats were higher than in most other animals for difenacoum.

4.2.2 Birds

Of the avian species studied here, ARs were most often observed in eagle owls (100%), tawny owls (85%) and goshawks (100% but only two individuals analysed). Owls in general have been found to have a high prevalence of ARs is many countries. For example in Spain nocturnal raptors were the secondary consumers with highest prevalence (62%) of AR exposure, especially to second generation ARs (Sanchez-Barbudo et al. 2012). In Sweden, difenacoum, coumatetralyl, bromadiolone and brodifacoum found in eagle owls (coumatetralyl concentration in liver 124 µg/kg, Norström et al. 2009, in the present study mean concentration for coumatetralyl in eagle owls was 3.6 µg/kg). In Norway, brodifacoum, bromadiolone, difenacoum and flocoumafen were detected in golden eagle and eagle owl livers at a total SGAR concentration from 11 to 255 µg/kg, in approximately 70% of the golden eagles and 50% of the eagle owls examined in the study (NIVA 2012). In Denmark, anticoagulant rodenticides were detected in 84-100% of all avian and mustelid specimens examined (Christensen et al. 2010). Difenacoum, bromadiolone and brodifacoum were most commonly present, whereas coumatetralyl and flocoumafen occurred in fewer individuals. For the eagle owl, the average cumulative concentration of all anticoagulants in the liver was 162 µg/kg (Christensen et al. 2010). In the present study the mean concentration of the AR with highest concentration, bromadiolone, was 27 µg/kg).

Eagle owls and tawny owls prey for rodents for a considerable part of their diet (von Haartman et al. 1963-1972), making both species likely targets for secondary exposure of

ARs. The diet of eagle owls mainly consists of small to medium-sized mammals (sometimes including young foxes) and birds, including corvids (von Haartman et al. 1963-1972). The tawny owl, on the other hand, has a more varied diet than other owl species, although small mammals nearly always dominate. Its prey species include voles and mice, birds, frogs, shrews and rats. Birds residing close to human habitation, such as house sparrows, are most common avian prey species (von Haartman et al. 1963-1972).

In Netherlands the prevalence of SGARs was studied in several rodent-eating avian predators (van den Brink 2014). SGARs were found in 50% of the liver samples (total sample size was 30). Most prevalent SGAR was brodifacoum (LOD 10 µg/kg), and highest concentrations were found in kestrels and eagle owls.

In Scotland ARs are regularly screened in predatory birds under the Predatory Bird Monitoring Scheme (PBMS, https://wiki.ceh.ac.uk/display/pbms/Home) and incidents of suspected poisoning of animals by pesticides are investigated under the Wildlife Incident Investigation Scheme (WIIS, https://www.sasa.gov.uk/wildlife-environment/wildlife-incident-investigation-scheme-wiis). In WIIS in 2012, residues were detected and identified in the livers of 51 specimens, i.e. in 36% of the total number of samples tested (SASA 2012). In the same year, six incidents were attributed to anticoagulant rodenticide poisoning. In PBMS in 2010 SGARs were found in 84% of barn owls, 100% of kestrels and 94% of red kites (Walker et al. 2013). Most prevalent AR was bromadiolone (LOD 1.4 µg/kg), which was found in 69% of barn owls, 83% in kestrels and in all of the red kites. Also difenacoum (LOD 1.2 µg/kg) was commonly detected in all the species (53% in barn owls, 90% in kestrels and 83% in red kites). Brodifacoum (LOD 1.4 µg/kg) was found quite often in red kites (78%) and in kestrels (55%) but less often in barn owls (33%). Flocoumafen (LOD 1.1 µg/kg) and difethialone (LOD 1.0 µg/kg) were found only in some samples. Both the substance- and species-specific prevalence seems to vary from year to year, showing the importance of regular monitoring.

A quite surprising result was found here for the goshawk. Although the sample size was very low (only two), the high prevalence found (100%) suggests that goshawks are exposed to ARs in Finland, although their diet includes a fairly small proportion of rodents. In the province of Tavastia, Finland, in the years 1995-1998 79.5% of goshawk prey was birds in spring and 90% in summer (Sulkava 1999). Most commonly preyed birds in summer were the corvids (39% of all prey species, all birds and mammals included). In recent years, the goshawk has started to urbanize in Finland, most clearly in the Helsinki metropolitan area (Solonen 2014). One of the reasons for this is the greater abundance of food compared to adjacent rural areas. Corvids, thrushes and pigeons comprise the bulk of the breeding season diet of urban goshawks in Finland (Solonen 2014). Similarly, Hughes et al. 2013 in Scotland found unexpectedly, that sparrow hawks, which prey almost exclusively on birds, had similar exposure rates to species which prey on rodents. The exposure for goshawks could thus come e.g. via corvids, as they were found also in this study to contain AR residues.

4.3 Risk mitigation measures set for anticoagulant rodenticides in Finland

ARs have been approved in the EU by including them in the Annex I of the Biocide Product Directive (98/8/EC). In the inclusion directives specific provisions were set on SGARs due to their potential PBT status and risk of exposure to children and non-target animals. The risk mitigation measures (RMMs) included among others restriction to professional users and the use of tamper resistant and secured bait boxes.

In Finland a national strategy on risk management of ARs was adopted in 2011. The strategy was published on the homepage of Finnish Safety and Chemicals Agency Tukes (see the link below) in order to inform both applicants and users of ARs. Users of rodenticides were divided in three user categories: 1) PCOs, 2) other professional users like farmers and 3) private users. The principle was that the PCOs have the widest use and the private users have the most restricted use.

http://www.tukes.fi/en/Branches/Chemicals-biocides-plant-protection-products/Biocides/Restrictions-on-the-use-of-biocidal-products/Rodenticides/

When comparing the results of the present study to the RMMs in place it appears that they have not been very effective in preventing secondary exposure of the non-target animals. The great majority of the studied animals carried residues of ARs, often two or three different substances. On the other hand, it is unclear how well the label claims and use instructions have been followed in practice.

There is no survey available on how ARs are actually used by the different user categories. Instructions of use may not always be followed. Sometimes poisonings or even deaths of dogs have been reported to Tukes. The best knowledge on the rodent control in Finland is in the possession of PCOs, although the quality of skills as well as the willingness to follow label claims may vary also among them. The PCOs have also told that some of the clients are not willing to pay for the rodent control performed according to the label claims. On the other hand the PCOs seem to recognize quite well the risks of the secondary exposure of non-target animals and clearly the use of non-chemical methods is increasing. With the training and obligatory certificate from the beginning of 2017 the risk management skills are expected to develop further among the PCOs (Chemicals Act 599/2013).

The renewal evaluation of ARs is in progress in the EU with the focus on the risk management. In Finland the RMMs should be reconsidered with respect to the results of this study. Since the private users are assumed to be the biggest user group, further restrictions concerning them seem necessary. The FGARs would have been a better group of ARs for private consumers because they are less toxic than SGARs. In Finland the only FGAR in use, i.e. coumatetralyl, was indeed found here in lower concentrations compared to SGARs but the classification as toxic for reproduction will prevent the use of FGARs by the private users. The same classification has been assigned to all ARs, but the use of SGARs by private users may continue due to possibility to lower the concentration of these substances below the classification limit.

In the report on the risk mitigation measures for anticoagulant rodenticides as biocidal products (Berny et al. 2014) several risk mitigation measures have been thoroughly reflected. The RMM that should be in particular considered for private users in Finland is the use of prefilled bait boxes. In addition, it should be seriously considered if loose baits can be used safely by the non-trained private users. Separate products for private users would provide information on the amount of products used predominantly by the private persons. The prohibition of selling on self-service basis as well as guidance in the connection of selling are assumed to be effective measures to decrease careless use of ARs by private users. Currently the sale is not regulated at all and it seems to be common that only the rodenticide products are on sale, but the bait boxes are not.

The education is assumed to increase and maintain the awareness of PCOs on the correct use of rodenticides as well as risks associated with their use. The focus should be on the compliance with label claims and use instructions, and in particular in the avoidance of permanent baiting which still seems to be a common practice in Finland. It may be difficult to reintroduce further RMMs without jeopardizing effective rodent control. On the contrast there appears to be a need to use all ARs outdoors, i.e. around buildings. It should however be seriously considered how necessary the use of ARs in open areas and waste dumps is and if alternative methods could be used. The effectiveness and economic feasibility of the non-chemical methods should be investigated.

There has been no training of farmers and other professionals than PCOs on the rodent control and the use of rodenticides. From the beginning of 2017 farmers are allowed to use those ARs restricted to PCOs on their own farms only if they have completed the training and certificate for the plant protection products (PPP). Currently rodent control or rodenticides are not included in the PPP training. The introduction of pest control module in the PPP education would increase the understanding of farmers on the risks of rodenticides. The category of “other professionals” should be removed and the users in this category should either use only the products aimed for the general public or carry out the obligatory qualification for PCOs and use the products aimed for PCOs.

There is a specific decree on the integrated pest management (IPM) concerning the plant protection products (7/2012) and the principle is assumed to be well known among farmers using plant protection products in Finland. IPM seems to be less known among the PCOs and among their clients in the food and feed industry. The sustainable use and IPM are essential principles also in the Biocide Product Regulation (528/2012) and a regulation by a national law would certainly improve the understanding of IPM among professional users of rodenticides.

Another element in use for the PPPs is the control of selling. The introduction of a certificate for the buying of ARs as it is done for the plant protection products would most likely prevent use of products restricted to PCOs by non-qualified users.

4.4. Recommendations for further actions and studies

There is no research institute in Finland that would study rats and mice as urban and rural pest organisms and neither is there any study on the control of these organisms. The competence of rodent control should be increased among the authorities and scientists so that objective information free of economic constraints would become available in Finland. Authorities and scientists should also start a survey on the potential AR resistance situation in Finland. Resistant rodents may carry higher concentrations of ARs compared to non-resistant rodents. There is also an urgent need to create a code of conduct or best practice guidelines on the rodent control for farmers and PCOs, but there is no obvious organization that could contribute in the preparation of such guidance. While the advice and guidance are seen as the major measures to reduce harmful effects of ARs, there would clearly be a need to increase surveillance and monitoring of the use too.

The purpose of this study was to be the first screening of AR prevalence in Finland and thus the species collected here were for a large part a result of a random sampling of available individuals. Some of the species studied here had a low sample size but a high prevalence (e.g. goshawk, otter), making them likely candidate species for further studies. Also non-target species having a potential for AR exposure but lacking from this study should be considered in further studies. These species include for example the European herring gull (*Larus argentatus*), the Eurasian red squirrel (*Sciurus vulgaris*), the European hedgehog, passerine birds, predatory birds not included in this study (e.g. the European kestrel, the common buzzard, the golden eagle and other owl species not studied here, like the Eurasian pygmy owl, *Glaucidium passerinum*), the common European adder (*Vipera berus*), shrews and other non-target small mammals. Samples should be collected also elsewhere of Finland and from less densely populated and uninhabited parts of the country as a reference material. Prevalence should preferably be monitored regularly, so the feasibility of the RMMs could be estimated in the long run.

4.5 Conclusions

ARs were found in majority (87%) of all the studied animals and 100% of studied eagle owls, red foxes, pine martens and least weasels carried ARs. Residues were commonly found also in tawny owls, raccoon dogs and stoats. Biocidal use of ARs causes thus frequent exposure of non-target animals that prey on rodents or their carcasses. In most animals concentrations were assumed to be sublethal but in 12.5% of animals the concentrations exceeded 200 µg/kg, which is considered as a trigger for biological effects. Bromadiolone was the most commonly detected substance followed by coumatetralyl and difenacoum. The most commonly used ARs in Finland are bromadiolone, difenacoum and coumatetralyl. Bromadiolone also was found in higher concentrations than other ARs. These results show that the current RMMs used in Finland are not effective enough to prevent AR exposure of non-target predators and scavengers. Further studies and additional RMMs are thus needed.

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7. References

Berny PJ, Gaillet JR 2008. Acute poisoning of red kites (*Milvus milvus*) in France: data from the SAGIR network. – *Journal of Wildlife Diseases* 44: 417–426.

Berny PJ, Buronfosse T, Buronfosse F, Lamarque F, Lorgue G 1997. Field evidence of secondary poisoning of foxes (*Vulpes vulpes*) and buzzards (*Buteo buteo*) by bromadiolone, a 4-year survey. – *Chemosphere* 35: 1817–1829.

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

Brakes CR, Smith RH 2005. Exposure of non-target small mammals to rodenticides: short-term effects, recovery and implications for secondary poisoning. – *Journal of Applied Ecology* 42: 118–28.

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

Commission Regulation (EU) No 253/2011 of 15 March 2011 amending Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards Annex XIII.
Dell'Arte GL, Laaksonen T, Norrdahl K, Korpimäki E 2007. Variation in the diet composition of a generalist predator, the red fox, in relation to season and density of main prey. – *Acta Oecologica* 31: 276–281.

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

Elliott J, Hindmarch S, Albert C, Emery J, Mineau P, Maisonneuve F 2014. Exposure pathways of anticoagulant rodenticides to nontarget wildlife. – *Environmental Monitoring and Assessment* 186: 895–906.

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

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

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

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

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

Hughes J, Sharp E, Taylor MJ, Melton L, Hartley G 2013. Monitoring agricultural rodenticide use and secondary exposure of raptors in Scotland. – *Ecotoxicology* 22: 974–984.

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

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

Laakso S, Suomalainen K, Koivisto S 2010. Literature review on residues of anticoagulant rodenticides in non-target animals. – *TemaNord* 2010: 541.

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

Li T, Chang C-Y, Jin D-Y, Lin P-J, Khvorova A, Stafford DW 2004. Identification of the gene for vitamin K epoxide reductase. – *Nature* 427: 541–544.

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

Masuda BM, Fisher P, Jamieson IG 2014. Anticoagulant rodenticide brodifacoum detected in dead nestlings of an insectivorous passerine. – *New Zealand Journal of Ecology* 38: 110–115.

Miljøministeriet 2015. Spredning af antikoagulante rodenticider med mus og eksponeringsrisiko for rovdyr. Bekæmpelsesmiddelforskning nr. 159. (In Danish with English summary)

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

Newton I, Shore RF, Wyllie I, Birks JDS, Dale L 1999b. Empirical evidence of side-effects of rodenticides on some predatory birds and mammals. In: Cowan DP, Feare CJ, editors. Advances in vertebrate pest management. Furth: Filander Verlag, pp. 347–67.NIVA 2012. Screening of selected alkylphenol compunds, biocides, rodenticides and current use pesticides. Statilig program for forureningsovervåkning Rapportnr. 1116/1012.

NIVA 2012. Screening of selected alkylphenol compunds, biocides, rodenticides and current use pesticides. Statilig program for forureningsovervåkning Rapportnr. 1116/1012

Norström K, Remberger M, Lennart K, Palm Cousins A, Brorström-Lundén E 2009. *Subreport 3*. Results from the Swedish National Screening Programme 2008. Subreport 3. Biocides: Difenacoum. IVL Swedish Environmental Research Institute.

Pelz H-J, Rost S, Hünerberg M, Fregin A, Heiberg A-C, Baert K, MacNicoll AD, Prescott CV, Walker A-S, Oldenburg J, Müller CR 2005. The genetic basis of resistance to anticoagulants in rodents. – *Genetics* 170: 1839–1847.

Pulliainen E, Ollinmäki P 1996. A long-term study of the winter food niche of the pine marten *Martes martes* in northern boreal Finland. – *Acta Theriologica* 41: 337–352.

Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products.

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

Rost S, Fregin A, Ivankevicius V, Conzelmann E, Hörtnagel K, Pelz H-J, Lappegard K, Seifred E, Scharrer I, Tuddenham EG, Müller CR, Strom TM, Oldenburg J 2004. Mutations in VKORC1 cause warfarin resistance in multiple coagulation factor deficiency type 2. – *Nature* 427: 537– 541.

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

SASA 2012. Pesticide Poisoning of Animals in 2012: A Report of Investigations in Scotland. ISBN: 978-1-78412-111-2. www.scotland.gov.uk.

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

Siivonen L 1974. Pohjolan nisäkkäät (Mammals of Northern Europe). Otava, Helsinki. (*In Finnish*)

Solonen T 2014 Kanahaukan kaupunkilaistuminen Suomessa. Linnut-vuosikirja 2014, pp. 126–131. (*In Finnish*)

Storch I, Lindström E, de Jounge J 1990. Diet and habitat selection of the pine marten in relation to competition with the red fox. – *Acta Theriologica* 35: 311–320.

Sulkava S 1999. Luita, sulkia, karvoja – rengastajien keräämät saalistähteet kertovat petolintujen ravinnosta. Linnut-vuosikirja 1999, pp.148–151. (*In Finnish*)

Thomas PJ, Mineau P, Shore RF, Champoux L, Martin PA, Wilson LK, Fitzgerald G, Elliott JE 2011. Second generation anticoagulant rodenticides in predatory birds: Probabilistic characterisation of toxic liver concentrations and implications for predatory bird populations in Canada. – *Environmental International* 37: 914– 920.

van den Brink N 2014. Risico's van anticoagulantia rodenticides voor niet-doelsoorten en predatoren. Een scan van beschikbare kennis in Europa en analyses in roofvogels uit Nederland. Alterra-rapport 2589| ISSN 1566-7197. (In Dutch with English summary)

von Haartman L, Hildén O, Linkola P, Suomalainen P, Tenovuo R 1963-1972. Pohjolan linnut värikuvin. Otava, Helsinki. (*In Finnish*)

Vuorisalo T, Lahtinen R, Laaksonen H 2001. Urban biodiversity in local newspapers: a historical perspective. – *Biodiversity and Conservation* 10: 1739–1756.

Vuorisalo T, Talvitie K, Kauhala K, Bläuer A, Lahtinen R 2014. Urban red foxes (*Vulpes vulpes* L.) in Finland: a historical perspective. – *Landscape and Urban Planning* 124: 109–117.

Walker LA, Chaplow JS, Llewellyn NR, Pereira MG, Potter ED, Sainsbury AW, Shore RF 2013. Anticoagulant rodenticides in predatory birds 2011: a Predatory Bird Monitoring Scheme (PBMS) report. Centre for Ecology & Hydrology, Lancaster, UK. 29pp.

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