REPORT

Results from the Swedish National Screening Programme 2008

Subreport 3. Biocides: Difenacoum

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Report Summary

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Title and subtitle of the report

Results from the Swedish National Screening Programme 2008

Subreport 3. Biocider: Difenacoum

Summary

IVL Swedish Environmental Research Institute has performed a "Screening Study" of difenacoum and related compounds as an assignment from the Swedish Environmental Protection Agency. Difenacoum is used as a second generation anticoagulant rodenticides (SGAR). It is mainly used to control rodents around buildings and inside transport vehicles. Difenacoum is uptaken via ingestion and acts by disrupting the blood clotting process. In addition to difenacoum, the current study also includes another six substances with similar functions. The overall objectives of the study were to determine the concentrations of the selected substances in the Swedish environment. A sampling programme was developed and 60 samples were included in the study, representing surface water, sediment, fish, soil, in- and effluent water from sewage treatment plants, sludge, storm water, storm water and sludge, and eagle-owl tissues. All of the seven anticoagulant rodenticides were below detection limit in all abiotic samples and in fish. This shows that these substances are not widely distributed in the Swedish environment. However, difenacoum and three of the related compounds (coumatetralyl, bromadiolone and brodifacoum) were found in three of the eagle-owl individuals. Coumatetralyl and bromadiolone were found in highest levels followed by difenacoum and the liver sampled contained higher concentrations compared to muscle samples. This shows that secondary poisoning of animals feeding on rodents not can be excluded. Additional studies focusing on areas with known usage could reveal if other non-target organisms other than eagle-owls are likely to be exposed to rodenticides. The study also confirms that the liver is the main target for retention of these rodenticdes.

Keyword

difenacoum

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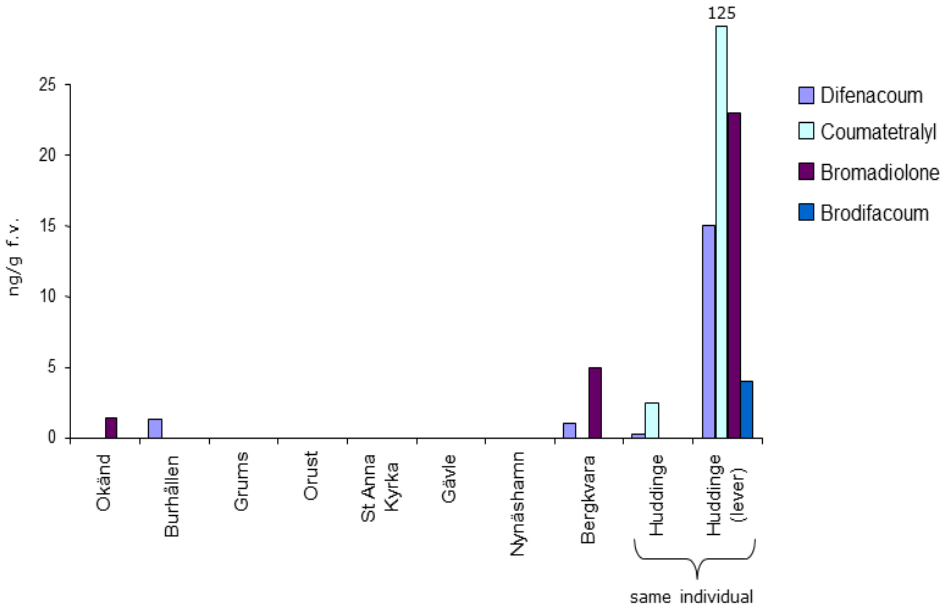
Sammanfattning

IVL Svenska Miljöinstitutet AB har på uppdrag av Naturvårdsverket genomfört en screening avseende difenakum och sex likartade föreningar med samma användningsområde.

Dessa föreningar är klassificerade som andra generationens antikoagulater och används som råttgift. De utvecklades på grund av resistans mot första generationens råttgift, tex Warfarin och används för kontroll av gnagare runt byggnader och i transportfordon. Difenakum tas upp via födan av råttor och möss och dess toxiska effekt är att den hämmar vitamin K cykeln i levern och förhindrar att blodet koagulerar och orsakar död genom förblödning. Difenakum lagras huvudsakligen i levern och elimineras långsamt. Difenakum och övriga likartade föreningar som ingår i studien har låg löslighet i vatten, är icke volatila och nedbrytningen i jord är långsam. Dess fysikaliska och kemiska egenskaper gör att spridningen i miljön troligen är begränsad.

Syftet med föreliggande studie var att utreda om difenakum och de likartade substanserna förekommer i miljön och i vilka halter och matriser. En provtagningsstrategi utarbetades utifrån substansernas förutspådda fördelning i miljön. De utvalda provtagningsplatserna representerar punktkällor i urban miljö, diffusa källor samt bakgrundsområden. Totalt analyserades 60 prover. Dessa bestod av ytvatten, sediment, fisk, jord, ingående och utgående avloppsvatten, avloppsslam, dagvatten och dagvattenslam. Dessutom ingick prover från berguv för att studera sekundär exponering.



Halter (ng/ f.w.) av råttgift i berguv.

Difenakum och de övriga föreningarna som ingick i studien återfanns inte i något av vatten-, sediment,- jord-, eller fiskproven, vilket visar att de inte är allmänt spridda i miljön.

(Detektionsgränserna ges i Appendix, tabell 2.) Däremot återfanns difenakum samt tre av de andra föreningarna (coumatetralyl, bromadiolone, och brodifacoum) i tre av muskelproven från berguv. Coumatetralyl och bromadiolone återfanns i högst halter följt av difenakum, se figur ovan.

Från en individ analyserades förutom muskel även lever, vilken var den vävnad som innehöll högst halter och där coumatetralyl uppmättes till 120 ng/g färskvikt. Detta visar att sekundär förgiftning hos djur som äter t ex råttor och möss inte kan uteslutas. Studien på berguvar visar också att det är levern som är den vävnad som i eventuella framtida studier av biota borde analyseras.

Summary

As an assignment from the Swedish Environmental Protection Agency, IVL Swedish Environmental Research Institute has during 2008/2009 performed a "Screening Study" of difenacoum and related compounds.

Difenacoum is used as a second generation anticoagulant rodenticides (SGAR). It was developed because of the increase in resistance among rodents to older (first generation) anticoagulant rodenticides, such as warfarin. It is mainly used to control rodents around buildings and inside transport vehicles. Difenacoum is uptaken via ingestion and acts through inhibition of the vitamin K cycle in the liver, disrupts the blood clotting process and causes death by hemorrhage. It is stored mainly in the liver and is slowly eliminated, primarily via the faeces. In addition to difenacoum, the current study also includes another six substances with similar functions.

Difenacoum and the related compounds have low water solubility, low volatility and the degradation in soil is relatively slow. Difenacoum may be transferred via the food chain to higher organisms. However, as the use is restricted to very limited areas, the substances are not likely to be widely distributed in the environment. If released, the substances are most likely to be transported to waters close to application areas, e.g. in densely populated areas. The main area of concern is thus likely to be primary and secondary poisoning via ingestion of substance or contaminated prey.

The overall objectives of the study were to determine the concentrations of the selected substances in the Swedish environment and to assess the possibility of current emissions in Sweden. A sampling strategy was developed in order to determine concentrations of difenacoum and the related compounds in different matrices where the selection were based on the usage pattern of the substances. Selected sampling sites represent point sources in urban environments, diffuse sources and background areas. A total of 60 samples were included in the screening. The samples consisted of surface water, sediment, fish, soil, in- and effluent water from sewage treatment plants, sludge, storm water, storm water and sludge. Also were samples from eagle-owls were included to study secondary exposure, as they feed on mice and rats.

All of the seven anticoagulant rodenticides were below detection limit in all types of sample matrices: water, sediment, soil, sludge and the fish. This shows 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, difenacoum and three of the related compounds (coumatetralyl, bromadiolone and brodifacoum) were found in three of the eagle-owl individuals. Coumatetralyl and bromadiolone were found in highest levels followed by difenacoum. From one eagle-owl, both muscle and liver was analyzed and coumatetralyl was found in both tissues at levels of 2.5 and 124 ng/g f.w., respectively. The liver contained most number of anticougalants. This shows that secondary poisoning of animals feeding on rodents not can be excluded. Additional studies focusing on areas with known usage could reveal if other non-target organisms other than eagle-owls are likely to be exposed to rodenticides. The study also confirms that the liver is the main target for retention of these rodenticdes.

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1 Introduction

As an assignment from the Swedish Environmental Protection Agency, a screening study has been performed by IVL during 2008/2009. This screening includes biocides, unintentionally produced substances and fuel additives. These substances/substance groups are emitted and distributed in the environment via a variety of sources, e.g. different point sources and/or diffusive sources. Table 1 shows the major reason for their concern as well as the number of the report where individual results are presented.

Table 1. Substances / substance groups included in the screening.

Substance / Substance group		Banned/ restricted	HPV[a]	Indications of toxicity	B/P[b]	Sub-report #
Biocides	3-Iodo-2-proponyl butyl carbamate (IPCB) 2,2-Dibromo-2-cyanoacetamide (DBNPA)			x		1
	Glutaraldehyde		x	x		2
	Difenacoum	**x**		**x**	**x**	**3**
Unintentionally produced substances	Nitro-PAH 3-Nitrobezantron Oxy-PAH Heterocyclics Brominated dioxins and aromatics			x	x	4
Fuel additives	Methyl *tert*-butyl ether (MTBE) Ethyl *tert*-butyl ether (ETBE)		x	x	x	5

[a] High Production Volume
[b] Bioaccumulation/Persistence

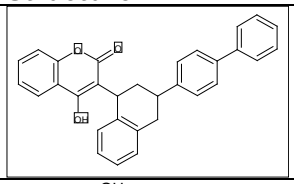
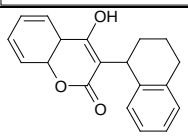
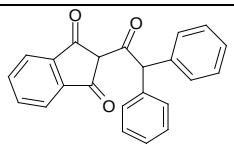
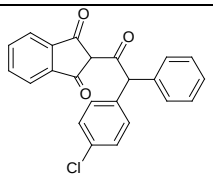
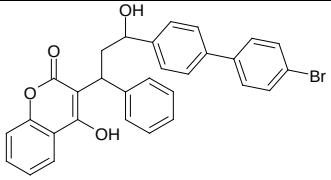
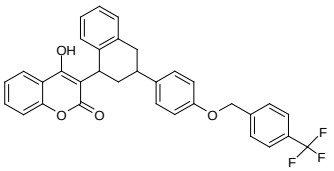
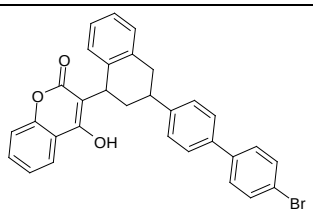
The overall objectives of the screening studies are to determine the concentrations of the selected substances in a variety of media in the Swedish environment, to highlight important transport pathways, and to assess the possibility of current emissions in Sweden.

This sub-report concerns the screening of **difenacoum**. Results for the other chemicals are presented in subreport 1,2,4 and 5. In the case of difenacoum, a particular objective has been to investigate the potential and extent of secondary poisoning by higher animals feeding on possible contaminated prey.

2 Chemical properties, fate and toxicity

Difenacoum belongs to the 4-hydroxycoumarin class of anticoagulants and is used as a second generation anticoagulant rodenticides (SGAR). In addition to difenacoum, the current study also includes another six substances with similar functions; names, CAS-numbers and structures given in Table 2. As evident from the table, 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 2. Rodenticides included in this screening study.

Name	CAS	Structure
Difenacoum	56073-07-5	
Coumatetralyl	5836-29-3	
Diphenadione	82-66-6	
Chlorophacione	3691-35-8	
Bromadiolone	28772-56-7	
Flocoumafen	90035-08-8	
Brodifacoum	56073-10-0	

2.1 Properties and fate

The chemical and physical data of the different rodenticides included in the current study were gathered from ChemIDplus and are shown in Table 3. Difenacoum, which is the main target substance for this study, occurs in room temperature as an off-white powder, but is sold in the form of blue-green pellets, usually containing 0.005% of the substance (WHO, 1995) and aimed to be ingested by rats and house mice, where it acts by disrupting the blood-clotting process. Its water solubility is very low and the half-life in aerobic soil has been reported to be 429 days (US EPA, 2007).

Table 3. Chemical and physical data of the anticoagulants included in this study.

Name	MW (g/mol)	Melting Point (°C)	Log Kow	Water Solubility (mg/L)	Vapor Pressure (mm Hg)	pKa
Difenacoum	***444***	***216***	***7.62***	***0.031 at 20 °C***	***1.2×10^{-6} at 20 °C***	
Coumatetralyl	292	172		4 at 20 °C	6.4×10^{-11} at 20 °C	4.75
Diphenadione	340	146.5	4.85	0.3	1.0×10^{-10} at 25 °C	
Chlorophacione	374	140	5.50	100 at 20 °C	7.5×10^{-10} at 25 °C	3.4
Bromadiolone	526	205	7.02	19 at 20 °C	1.5×10^{-8} at 20 °C	4.04
Flocoumafen	542	170		1.1	1.0×10^{-12} at 25 °C	
Brodifacoum	522	230	8.50	0.0038 at 20°C		

As evident from the table, difenacoum has low water solubility and low volatility. It is not expected to enter the atmosphere, due to the low vapour pressure. Its form of application implies that the main emission matrix will be onto soil of some kind in small restricted areas. The low water solubility results in an expected partitioning to soil solids, and transport in pore water is expected to be negligible. 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). Difenacoum will be ingested by rodents or other smaller insectovores and may be transferred via the food chain to higher organisms, and may be excreted as metabolites via urine and feces. Dowding et al. (2009) found residues of various rodenticides (e.g. difenacoum) in 58 % of the 120 studied British hedgehogs, an example of a non-target organisms, indicating the potential for the substance to be ingested also by other organisms.

The use of difenacoum is restricted to very limited areas and the substance is not likely to be widely distributed in the environment. If released, the substance is most likely to be transported to waters near application areas, e.g. in densely populated areas. The main area of concern is likely to be primary and secondary poisoning via ingestion of substance or contaminated prey.

2.2 Toxicity

As difenacoum is produced in order to act as a rodenticide, its toxic effects are intended and obvious. It is uptaken via ingestion and acts through inhibition of the vitamin K cycle in the liver, disrupts the blood clotting process and causes death by hemorrhage. It is stored mainly in the liver and is slowly eliminated, primarily via the faeces. The distribution of difenacoum is probably governed by binding to specific sites in tissues. The elimination process from the liver is slow, with a half-life of ca 120 days, and in the pancreas even slower, with a half-life of 182 days (WHO 1995). The metabolism in humans has not been studied. Table 4 lists acute toxicity data for difenacoum.

No long-term studies have been carried out to conclude its potential for mutagenic or carcinogenic effects. If consumed, difenacoum will exert similar effects on humans as on rodents. Incidents of intentional and unintentional human poisoning have been reported (WHO, 1995). The U.S. EPA has concluded that predatory birds will be affected by anticoagulant properties of difenacoum if they feed on contaminated animals (US EPA, 2007). Similar types of feeding studies with mammals are not available.

Table 4. Acute toxicity data for difenacoum (WHO, 1995)

Species	Endpoint	Concentration (mg/kg body weight)	Exposure route	Effects
Rat	LD_{50}	1.8	Oral	Anticoagulant
Rabbit	LD_{50}	2.0	Oral	Anticoagulant
Mammals	LD_{50}	50-100	Oral	Anticoagulant
Rat, rabbit	LD_{50}	>50	Dermal	Anticoagulant

3 Production, use and regulation

The second generation anticoagulant rodenticides (SGAR), to which difenacoum belongs, were developed because of the increasing development of resistance among rodents to older (first generation) anticoagulant rodenticides, such as warfarin (Walker et al 2008). It is mainly used to control rodents around buildings and inside transport vehicles. In Sweden, difenacoum is used as a rodenticide and occurred in four preparations in 2006 and 2007 (SPIN, 2009). It is reported to have been sold to the agricultural, industrial and household sectors within Sweden since 1992 but amounts reported as 0 tonnes, i.e. <100 kg. The total amounts of sold active substance of rodenticides (of which difenacoum is only one) was reported to be 0.1-0.2 tonnes/year between 1986 and 2007 (KEMI, 2009), i.e. the amounts that are annually exposed to the environment are very low, and the restriction of use ensures a controlled distribution of the substance. Difenacoum is regulated under the European directive on biocides EG 2032/2003. All usage of the substance has to be reported to national agencies. As a result of the restricted use, the emissions of difenacoum are likely to be limited.

4 Previous measurements in the environment

Raptors and rodents are exposed to SGAR by eating the substance directly or by consuming contaminated prey. Also other organisms may be exposed to SGAR. Bromadiolone, difenacoum and brodifacoum has been detected in British tawny owl livers (Walker et al 2008). The geometric mean concentrations for these three substances were 0.2, 0.03, and 0.13 µg/g wet weight, for bromadiolone, difenacoum and brodifacoum respectively. Dowding et al. (2009) found SGAR residues in a large number of British hedgehogs, illustrating that non-target organisms may also be exposed to the substance. In British polecat livers, bromadiolone and difenacoum were detected at concentrations of 0.05-0.15 µg/g w.w. and 0.1-0.35 µg/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 concentration of approximately 0.25 µg/g w.w. (Lambert et al. 2007). Also coumafen and coumatetralyl could be detected in a few samples.

5 Sampling strategy and study sites

5.1 Screening program

A sampling strategy was developed in order to determine concentrations of difenacoum and the related compounds in different matrices in the Swedish environment. The sampling programme was focused on matrices, where the substance may possible be detected based mainly on the use pattern. Background areas were also included. The sampling programme is summarized in Table 5.

Due to the physico-chemical properties of the substances, the high log Kow and the functional groups on the coumarine unites, the compounds are expected to absorb to solid particles. The usage pattern indicates that soil from city parks is a matrix that is of interest to sample as rodenticides may be used here in order to eliminate rats and rabbits from the public landscape. Urban storm water, and sewage treatment plants that handle large quantities of storm water are sample locations of interest, as well as their recipients in order to judge if the substances have a potential to reach the aquatic environment, possibly attached to particles. It is also of interest to study if use of these second generation anticoagulants may result in exposure of aquatic organisms.

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. Therefore, eagle owls were included in the study, as they feed on mice and rats. Eagle owls frequently live on landfills where rodenticides may be used.

In order to determine background levels, samples of soil, sediment, surface water and fish were analysed from the area around three background lakes, classified as reference lakes by the Swedish Museum of National History.

Table 5. Sampling programme for biocide measurements

Site	**Water**				**Sludge**	**Sedi-ment**	**Soil**	**Fish**	**Eagle -owl**	**Total**
	In	Out	Storm	Surface						
Background										
Lakes				3		2	1	3		9
Diffuse sources										
Urban area			5	3	3	3		3	10	17
Municipal STPs	5	5			7					4
Point sources										
City Parks							7			7
Total	5	5	5	6	10	5	8	6	10	60

6 Methods

6.1 Sampling

De-watered **sludg**e samples were collected from the anaerobic chambers by staff at the different sewage treatment plants. The sludge was transferred into glass jars and stored in a freezer (-20 ºC) until analysed. **Influent** and **effluent** waters were sampled in 1 or 2 L glass bottles.

Surface water samples from background lakes and from the city of Stockholm were sampled in glass bottles and the pH was adjusted to pH 3 with H_3PO_4 and stored at 6 ºC.

Storm water samples and **storm water sludge** samples were provided by the Swedish Road Administration (Vägverket) and "Gatukontoret" in Göteborg.

The upper 2-3 cm of surface **soil** was collected in glass jars.

Surface **sediment** (0-2 cm) samples were collected by means of a Kajak sampler. The sediment was transferred into muffled (400 ºC) glass jars and stored in a freezer (-20 ºC) until analysed. One sediment sample from the background lake Tärnan was provided from the specimen bank at the Swedish Museum of Natural History.

F**ish** were collected by means of fishing nets. The net fishing was approved by the fishery authorities in Stockholm and the ethical board for animal testing in northern Stockholm (D. no. 527/07). The herring muscle from Väderöarna and the perch muscle from Kvädöfjärden were provided from the specimen bank at the Swedish Museum of Natural History. Both of the fish muscle samples consisted each of a homogenate of 10 individuals. All fish samples were stored at -18 ºC in pre-cleaned glass jars.

Eagle owl *(bubo bubo)* muscle and liver samples were provided by the Swedish Museum of Natural History.

All samples are listed in Appendix A.

6.2 Analysis

6.2.1 Sample preparation

Biota samples, i.e. fish muscle (1-2 g), eagle-owl (1-2 g) were ground with Na_2SO_4 and spiked with internal standard. The samples were then extracted once with 30 mL methanol by one hour rotation and the volume were reduced to 10 mL before HPLC-MS/MS analysis. Sludge (0.5 g), sediment (1.5-2 g) and soil (1.5-2 g) were ground with Na_2SO_4 and extracted twice with methanol in the same manner as the biota samples.

A SPE C18 column (500 mg, 6 mL) (NIST) was activated with MeOH and acidic water. The water samples were spiked with internal standard and sucked through the column. The analytes were then eluted with 5 ml MeOH. Volumes used: surface water: 300-400 ml, storm water 100-300 mL,

influent water from sewage treatment plant: 100 ml, and effluent water from sewage treatment plant: 200 ml.

6.2.2 Instrumentation

The samples were analyzed applying a high performance liquid chromatography system consisting of a Prominence UFLC system (Shimadzu) with two pumps LC 20AD, degasser DGU-20A5, autosampler 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 30 ºC. The mobile phase A was a solution of 5 mM ammonium formate in water (adjusted to pH 9 using ammonia in water), and solvent B was 5 mM ammonium formate in methanol, adopted from Vandenbroucke et al. 2008. The flow rate of the mobile phase was 0.4 ml/min. A gradient elution was performed: 0-4 min 15% B, 4-10 min linear increase to 90% B, 10-14 min isocratic 90% B, 14-14.5 linear increase to 95% B, 14.5-19 min isocratic 95% B, 19-20 min linear decrease to 15% B. Equilibration time. 6 min.

The effluent was directed to and API 4000 triple quadrupole mass spectrometer (Applied Biosystems). For analysis, 10 µl sample extract in methanol were 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 m/z	Qualifier ion m/z
Difenacoum	443.4	293.1	135
Coumatetralyl	291.1	140.9	142.9
Diphenadione	339.2	167.2	177.2
Chlorophacione	373.1	201.1	144.9
Bromadiolone	525.3	249.9	180.8
Flocoumafen	541.3	382	160.9
Brodifacoum	521.3	134.9	142.9

6.2.3 Quality control

To ensure the quality of the identification of the target compounds, two MRM transitions were used for each compounds, one precursor ion, one product ion which was used for quantification and one qualifier ion, See Table 6. Also, the retention time should match those of the authentic standard compounds within ± 0.2 min.

For each matrix, two solvent method blanks were prepared in parallel with the samples to assess possible interferences and contamination from the background.

Coumafuryl (CAS# 117-52-2) was used as internal standard in all samples. The recoveries of the internal standard in the different matrices were 70% in eagle-owl, 73% in fish, 57% in soil, sludge and sediment, and 97% in water. All reported values are recovery-corrected.

The background contamination in the blank samples was subtracted from the measured sample values and the limit of detection (LOD) was defined as three times the standard deviation of the blank samples noise.

7 Results and discussion

The concentrations of the seven anticoagulant rodenticides were all below LOD in all types of water samples, sediment, soil, sludge and in the fish samples. The LOD for the different types of samples are presented in Table 7.

Table 7. Limit of detection (LOD) in ng/L and ng/g fresh weight in different matrices for the rodenticide anticoagulates.

	Water	Sediment	Soil	Sludge	Fish
Difenacoum	< 5 ng/L	< 1 ng/g	< 1 ng/g	< 1 ng/g	< 1 ng/g
Coumatetralyl	< 5 ng/L	< 1 ng/g	< 1 ng/g	< 1 ng/g	< 1 ng/g
Diphenadione	< 5 ng/L	< 1 ng/g	< 1 ng/g	< 1 ng/g	< 1 ng/g
Chlorophacione	< 5 ng/L	< 1 ng/g	< 1 ng/g	< 1 ng/g	< 1 ng/g
Bromadiolone	< 5 ng/L	< 1 ng/g	< 1 ng/g	< 1 ng/g	< 1 ng/g
Flocoumafen	< 5 ng/L	< 1 ng/g	< 1 ng/g	< 1 ng/g	< 1 ng/g
Brodifacoum	< 5 ng/L	< 1 ng/g	< 1 ng/g	< 1 ng/g	< 1 ng/g

7.1 Eagle-owl

Difenacoum was found in three of the eagle-owl individuals, see Figure 1 and Table A2 in the Appendix. Bromadiolone was found in two of the individuals and coumatetralyl in one. From one eagle-owl, from Huddinge, both muscle and liver were analyzed. The difenacoum and coumatetralyl concentrations in the liver was 8 and 50 times higher in the liver compared to the muscle. The bromadiolone concentration in the liver was higher than in any of the muscle samples. Brodifacoum was found solely in the liver. The LOD in the eagle-owls was < 1 ng/g f.w.

The eagle-owl from Huddinge was found dead on a landfill. Despite its apparent exposure to rodenticides, it has not been stated that this exposure was the cause of death. Rodenticides are known to accumulate in the liver (Vandenbroucke et al. 2008) which is shown in this individual where the concentrations are higher in the liver compared to the muscle.

According to the Swedish Pesticides Register (SPIDER), difenacoum, bromadiolone, coumatetralyl, flocoumafen and brodifacoum are all used in rodenticides in Sweden (SPIDER, 2012). Difenacoum and bromadiolone are the rodenticides most frequently found in birds in previous studies from other countries (Walker et al. 2008, Shore et al. 2003, Lambert et al. 2007). In this study, the substance that was detected at the highest levels, in the eagle owl from Huddinge, was

coumatetralyl. It should be noted that warfarin, which is commonly used in Sweden in rodenticide products, is not included in this study

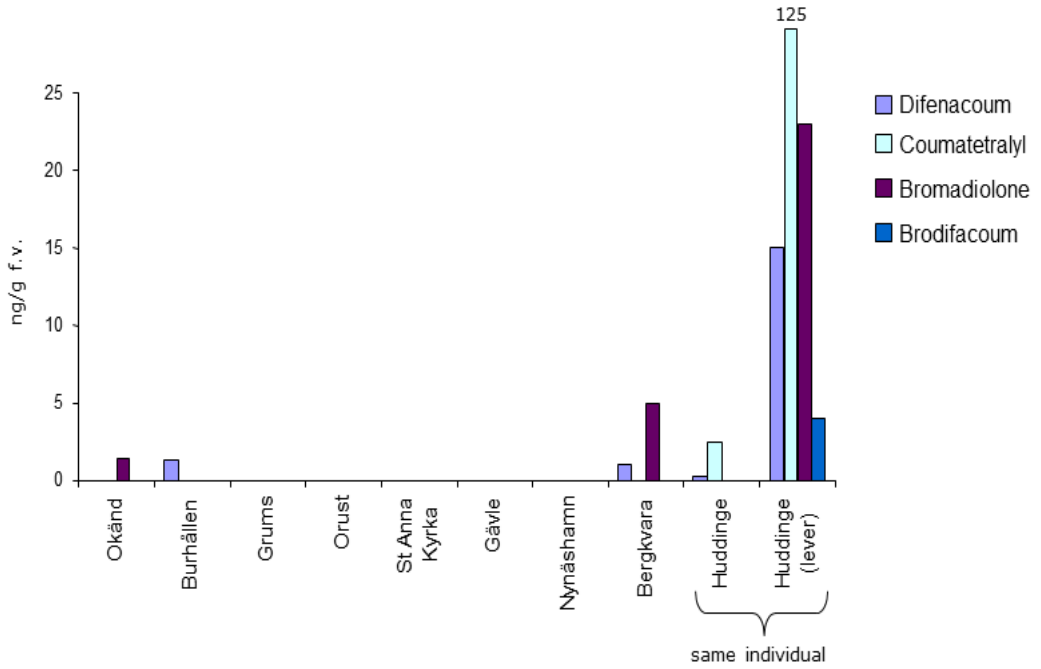


Figure 1. Concentrations (ng/ f.w.) of four rodenticide anticoagulants in eagle-owl muscle and liver.

8 Conclusions

The following conclusions can be drawn from the current screening study:

- The rodenticides difenacoum, coumatetralyl, diphenadione, chlorophacione, bromadiolone, flocoumafen and brodifacoum are not widely distributed in the Swedish environment, and are not likely to be of major concern from a general environmental perspective.

- Secondary poisoning of animals feeding on rodents cannot be excluded. Additional studies focusing on restricted areas with known usage, e.g. landfills or specific urban areas could reveal if other non-target organisms such as e.g. hedgehogs, cats or birds other than eagle owls are likely to be exposed to toxic levels of rodenticides.

- This study confirms that liver is the main organ for accumulation of rodenticide anticoagulants, thus this is the tissue of interest if further analysis of biota will be performed in the future.

9 Acknowledgement

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Appendix A.

Table A1: Sample information from the National Screening programme 2008.

Category	Sample ID	Matrix	Site	Sampling date	DW (%)	Notes
Background	7678	Soil	Gårdsjön	2008-11-20	21.8	
Background	7673	Sediment	Gårdsjön	2008-11-20	7.9	
Background	7708	Sediment	Tärnan	2008-09-23	9.8	
Background	7679	Surface water	Gårdsjön	2008-11-20		
Background	7615	Surface water	Tärnan	2008-11-30		
Background	7616	Surface water	Largen	2008-11-31		
Background	7682	Fish muscle	Gårdsjön	2008-11-20		Perch
Background	7706	Fish muscle	Kvädöfjärden	2008-08-12		Perch
Background	7707	Fish muscle	Väderöarna	2008-08-27		Herring
Diffuse, STP	7766	STP sluge	Stockholm, Henriksdals STP	2009-01-30	26.7	
Diffuse, STP	7767	STP sluge	Stockholm, Henriksdals STP	2009-02-05	26	
Diffuse, STP	7726	STP sluge	Stockholm, Käppalaverket STP	2008-12-15	17.4	
Diffuse, STP	7727	STP sluge	Stockholm, Käppalaverket STP	2008-12-17	12.2	
Diffuse, STP	7841	STP sluge	Helsingborg, STP	2009-04-20	25.1	
Diffuse, STP	7782	STP sluge	Malmö, Sjölunda, STP	2009-02-23	23.4	
Diffuse, STP	7760	STP sluge	Göteborg,Ryaverken, STP	2009-01-29	32.4	
Diffuse, STP	7722	Influent water	Stockholm, Henriksdals STP	2008-12-16		
Diffuse, STP	7724	Influent water	Stockholm, Käppalaverket STP	2008-12-16		
Diffuse, STP	7839	Influent water	Helsingborg, STP	2009-04-21		
Diffuse, STP	7779	Influent water	Malmö, Sjölunda, STP	2009-02-23		
Diffuse, STP	7761	Influent water	Göteborg,Ryaverken, STP	2009-01-29		
Diffuse, STP	7723	Effluent water	Stockholm, Henriksdals STP	2008-12-16		
Diffuse, STP	7725	Effluent water	Stockholm, Käppalaverket STP	2008-12-16		
Diffuse, STP	7840	Effluent water	Helsingborg, STP	2009-04-21		
Diffuse, STP	7784	Effluent water	Malmö, Sjölunda, STP	2009-02-23		
Diffuse, STP	7762	Effluent water	Göteborg,Ryaverken, STP	2009-01-29		
Diffuse, urban	7585	Sediment	Stockholm, St Essingen	2008-11-08		

Category	Sample ID	Matrix	Site	Sampling date	DW (%)	Notes
Diffuse, urban	7583	Sediment	Stockholm, Årstaviken	2008-11-08	15.7	
Diffuse, urban	7584	Sediment	Stockholm, Riddarfjärden	2008-11-08	15.6	
Diffuse, urban	7582	Surface water	Stockholm, St Essingen	2008-11-08		
Diffuse, urban	7578	Surface water	Stockholm, Årstaviken	2008-11-10		
Diffuse, urban	7581	Surface water	Stockholm, Riddarfjärden	2008-11-08		
Diffuse, urban	7845	Storm water	Stockholm, Årstafältet	2009-04-27		
Diffuse, urban	7846	Storm water	Stockholm, Huddinge	2009-04-27		
Diffuse, urban	7771	Storm water	Göteborg, Odingsplatsen	2009-02-23		
Diffuse, urban	7773	Storm water	Göteborg, Gårda	2009-02-23		
Diffuse, urban	7775	Storm water	Göteborg, Korsvägen	2009-02-23		
Diffuse, urban	7772	Storm water sludge	Göteborg, Odingsplatsen	2009-02-23	21.6	
Diffuse, urban	7774	Storm water sludge	Göteborg, Gårda	2009-02-23	19.3	
Diffuse, urban	7776	Storm water sludge	Göteborg, Korsvägen	2009-02-23	40.9	
Diffuse, urban	7768	Fish muscle	Stockholm, Årstaviken	2009-02-13		Perch
Diffuse, urban	7769	Fish muscle	Stockholm, Riddarfjärden	2009-02-13		Perch
Diffuse, urban	7770	Fish muscle	Stockholm, St Essinge	2009-02-16		Perch
Point source, urban	7669	Soil	Stockholm, Vårbergstoppen	2008-12-29	28.2	
Point source, urban	7670	Soil	Stockholm, Årstafältet	2008-12-29	67.9	
Point source, urban	7719	Soil	Stockholm, Humlegården	2009-01-13	55.7	
Point source, urban	7720	Soil	Stockholm, Kungsträdgården	2009-01-14	45.2	
Point source, urban	7721	Soil	Stockholm, Observatorielunden	2009-01-14	67.7	
Point source, urban	7759	Soil	Göteborg, Vasaparken	2009-01-27	64.5	
Point source, urban	7758	Soil	Göteborg, Allen	2009-01-30	64.9	
	7709	Eagle-owl muscle	?			
	7710	Eagle-owl muscle	In cage			
Point source, urban	7711	Eagle-owl muscle	Huddinge			
	7712	Eagle-owl muscle	Grums			
	7713	Eagle-owl muscle	Orust			
	7714	Eagle-owl muscle	St Anna Kyrka			

Category	Sample ID	Matrix	Site	Sampling date	DW (%)	Notes
	7715	Eagle-owl muscle	Gävle			
	7716	Eagle-owl muscle	Nynäshamn			
	7717	Eagle-owl muscle	Bergkvara			
Point source, urban	7718	Eagle-owl liver	Huddinge			

Table A2. Concentration (ng/g f.w.) in eagle-owls.

Category	Sample ID	Matrix	Site	Difenacoum	Coumatetralyl	Diphenadione	Chlorophacione	Bromadiolone	Flocoumafen	Brodifacoum
	7709	Muscle	?	< 1	< 1	< 1	< 1	1.4	< 1	< 1
	7710	Muscle	In cage	1.4	< 1	< 1	< 1	< 1	< 1	< 1
Point source, urban	7711	Muscle	Huddinge	1.8	2.5	< 1	< 1	< 1	< 1	< 1
	7712	Muscle	Grums	< 1	< 1	< 1	< 1	< 1	< 1	< 1
	7713	Muscle	Orust	< 1	< 1	< 1	< 1	< 1	< 1	< 1
	7714	Muscle	St Anna Kyrka	< 1	< 1	< 1	< 1	< 1	< 1	< 1
	7715	Muscle	Gävle	< 1	< 1	< 1	< 1	< 1	< 1	< 1
	7716	Muscle	Nynäshamn	< 1	< 1	< 1	< 1	< 1	< 1	< 1
	7717	Muscle	Bergkvara	0.9	< 1	< 1	< 1	5.0	< 1	< 1
Point source, urban	7718	Liver	Huddinge	15	124	< 1	< 1	22	< 1	4.2