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Metals and organic contaminants in bank voles (*Myodes glareolus*) from northern, central and southern Sweden.

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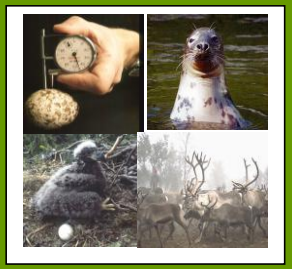

Content

Sammanfattning

Denna rapport är resultatet av ett uppdrag från Naturvårdsverket (Överenskommelse 222-0921). Syftet är att få en bild av hur halter av ett antal miljögifter såsom metaller, ett antal klorerade och bromerade substanser, fenolära substanser och perfluorerade substanser ser ut i svensk miljö i en matris, skogssork (*Myodes glareolus*) som finns relativt spridd över stora delar av landet. Skogssork finns i olika typer av biotoper från skogslandskap till subalpina områden över stora delar av landet. Sork har samlats in på olika lokaler i den nationella miljöövervakningen av smådäggdjur sedan 1970- och 80-talet. Insamlade sorkar har förvarats frysta i Miljöprovbanken och finns därigenom tillgängliga för retrospektiva analyser.
I denna rapport redovisas resultatet av analyser gjorda på skogssork insamlade hösten 2001 från fem lokaler i södra, mellersta och norra Sverige. Tre av lokalerna (N:a Kvill, Grimsö, Vindeln) representerar skogslandskap och två av lokalerna (Vålådalen, Ammarnäs) representerar en subalpin biotop. Från varje lokal har tre homogenat på lever respektive muskel preparerats med tio individer i varje homogenat.
De ämnen som har analyserats är metaller och grundelement som ingår i det svenska övervakningsprogrammet av miljögifter och som tidigare har analyserats på älg, ren och stare inom det terrestra övervakningsprogrammet. Vidare har ett antal klassiska miljögifter såsom DDT med metaboliter DDE och DDD, sju polyklorerade bifenyler (PCB), α-HCH, β-HCH, γ-HCH (lindan) och HCB analyserats. Av bromerade flamskyddsmedel har sex olika polybromerade difenyletrar (PBDEer) och HBCD analyserats. Vidare har fyra fenolära substanser (4-t-oktylfenol, 4-nonylfenol, pentaklorfenol, triclosan) analyserats. I gruppen perfluorerade substanser har 15 olika substanser (PFHxA, PFHpA, PFOA, PFNA, PFDcA, PFUnA, PFDoA, PFTriA, PFTeA, PFPeDA, PFBS, PFHxS, PFOS, PFDcS, PFOSA) analyserats.
Metaller och grundelement analyserades i lever. Ingen av de toxiska metallerna Pb, Cd och Hg förekom i höga halter hos sork från någon av lokalerna. Kadmium var den metall som förekom i högst halt och sorkar från Grimsö (0,25 µg/g färskvikt), Vindeln (0,20 µg/g färskvikt) och N:a Kvill (0,16 µg/g färskvikt) hade de högsta halterna. Den högsta kvicksilverhalten (0,09 µg/g färskvikt) fanns i sork från Vålådalen.
Klorerade, bromerade och fenolära substanser analyserades i muskel. Av de klorerade ämnena förekom HCB i alla prov som analyserades. Halterna var högst i N:a Kvill och Vålådalen. Även CB-153 fanns i kvantifierbara mängder i alla prov från N:a Kvill och Vålådalen. Likaså fanns BDE-153 i kvantifierbara mängder i alla prover från Vålådalen.

Triclosan hittades i kvantifierbara mängder i prov från alla lokalerna utom Vålådalen. I prov från Vålådalen fanns spår av triclosan. 4-nonylfenol hittades i kvantifierbara mängder i prov från N:a kvill och Vålådalen.
Perfluorerade ämnen analyserades i lever. PFUnA, PFHxS och PFOS förekom i kvantifierbara halter i samtliga analyserade prover. PFNA, PFDcA, PFDoA, PFTriA fanns också i kvantifierbara halter i alla prov från alla lokaler utom Ammarnäs och Grimsö. PFOS var den dominerande substansen på samtliga lokaler och den högsta halten återfanns i ett prov från Vindeln (17,5 ng/g färskvikt). Det högsta medelvärdet återfanns i proverna från Vålådalen (12,4 ng/g färskvikt) tätt följt av proverna från Vindeln (12,3 ng/g färskvikt). Skogssork från Ammarnäs uppvisade de lägsta halterna av PFOS (3,04 ng/g färskvikt).
Sammanfattningsvis kan man konstatera att halterna av samtliga analyserade ämnen var låga/mycket låga i skogssork. Ingen generell geografisk trend gick att urskilja i materialet men sorkarna från Vålådalen uppvisade förvånande höga halter av flera substanser jämfört med sork från övriga insamlingslokaler. Både HCB, CB-153, BDE-153 och ett flertal av de analyserade perfluorerade substanserna var högst i sork från Vålådalen. Anledningen till detta är okänd. En förklaring kan vara att sork i Vålådalen skulle i högre utsträckning kunna livnära sig på mossor och lavar och därigenom får i sig högre halter av luftburna föroreningar jämfört med sorkar på de övriga lokalerna.

Summary

The present study has been carried out on mandate of and in cooperation with the Swedish Environmental Protection Agency (SEPA) according to agreement 222-0921. The aim has been to obtain knowledge of the levels of certain environmental contaminants in Swedish terrestrial biota by using a matrix, bank vole (*Myodes glareolus*) that has not previously been examined.
Bank voles are herbivorous animals with a rather varied diet that are found all over the country in both forest and subalpine biotopes. Different species of voles have been collected since the 1970s and 80s at various sampling sites in the Programme on monitoring of small mammals.
The bank voles used in this study were collected in the autumn of 2001. Collected voles have been preserved frozen in the Swedish Environmental Specimen Bank (SESB).

In this study bank voles collected from five different sampling sites representing forest locations (N:a Kvill, Grimsö, Vindeln) and subalpine locations (Vålådalen, Ammarnäs) in south, central and northern Sweden have been analysed for different metal and elements, chlorinated and brominated compounds, phenolic compounds and perfluorinated compounds. Three homogenates with ten individual voles/homogenate have been prepared from each sampling site. Metals and perflurinated compounds were analysed in liver tissue. Chlorinated, brominated and phenolic compounds were analysed in muscle tissue.

All of the analysed contaminants were present in low or very low quantities. Cadmium was the most abundant of the toxic metals. The highest levels of Cd (0,16-0,25 µg/g ww) was found in voles from forest sampling sites. The highest level of mercury (0,09 µg/g ww) was found in voles from Vålådalen.

Of the chlorinated compounds, HCB was present in quantifiable amounts in all the analysed samples. The highest levels (11 ng/g lw) was found in voles from Vålådalen. DDT, DDE and DDD were found in detectable amounts in one sample from Grimsö and DDT was also found in one sample from N:a Kvill. This sample also contained detectable levels of CB-118, CB-153 and CB-138. CB-153 was present in detectable amounts in all samples from N:a Kvill and Vålådalen. Of the brominated compounds BDE-153 was present in detectable amounts in at least one sample from each sampling site except Ammarnäs. HBCD was found in one sample from Vindeln.

Detectable levels or traces of triclosan were found in at least one sample from all sampling sites. Traces or detectable levels of 4-nonylfenol was found at Ammarnäs, N:a Kvill and Vålådalen.

Three perfluorinated compounds, PFUnA, PFHxS and PFOS were found in detectable amounts in all the analysed samples. PFOS was the most abundant of the perfluorinated compounds.

PFNA, PFDcA, PFDoA, PFTriA were found in detectable amounts in all samples except in some from Ammarnäs and Grimsö. The highest levels of perfluorinated compounds were found in Vålådalen.

No geographical trends could be detected in the levels of analysed compounds in this study. Somewhat surprisingly, voles from Vålådalen had the highest levels of some of the analysed compounds (Hg, HCB, CB-153, BDE-153, PFCs). The reason for this is not known. One explanation could be that the diet of voles from Vålådalen might have a higher content of lichens and thus the voles from Vålådalen are more exposed to long range atmospheric transport of contaminants.

Aim

This work was carried out on request of and in cooperation with the Swedish Environmental Protection Agency (SEPA).
The aim is to investigate and compare geographically, the levels of environmental contaminants in a small herbivorous mammal, the bank vole that is found in a large part of Sweden both in forests and in subalpine areas. Bank voles are collected twice a year in the National Monitoring Programme on Small Mammals and stored in the Swedish Environmental Specimen Bank (SESB). The aim is also to evaluate voles as matrices in monitoring of contaminants in the terrestrial environment.

Organisation

The material analysed is collected by Birger Hörnfeldt (Swedish University of Agricultural Sciences SLU, Umeå) in the National Monitoring Programme on Small Mammals and preserved frozen in the Swedish Environmental Specimen Bank (SESB). Chemical analyses have been carried out by Vera Galgan and Lars Petersson, Department of Chemistry, National Veterinary Institute (Metals), Lillemor Asplund, ITM Department of Applied Environmental Science, Stockholm University, (chlorinated and brominated compounds), Margaretha Adolfsson-Erici, ITM Department of Applied Environmental Science, Stockholm University (phenolic substanses) and Urs Berger ITM Department of Applied Environmental Science, Stockholm University (Perfluorinated compounds).
Results have been evaluated and the report has been prepared by Ylva Lind and Tjelvar Odsjö at the Department of Contaminant Research, Swedish Museum of Natural History.
The study has been carried out in cooperation with Britta Hedlund, Jonas Rodhe and Axel Hullberg (SEPA) who also gave the financial support.

Introduction

The Swedish monitoring programmes on environmental pollution has been running since early 1980s. The purpose is to monitor long time changes as well as spatial variations in body burden of contaminants in chosen species. The species included and the sampling areas in the monitoring programmes have been chosen according to certain criteria (Odsjö and Olsson 1979; Odsjö and Olsson 1979; Odsjö and Olsson 1989). The purpose have been to monitor background/general pollutant levels in the environment i.e. pollutant levels not caused by local emissions but rather long range transports.

In the Swedish Environmental Monitoring Programmes, the population densities of small mammals have been included since 1979**.** Small mammals have been collected from different habitats in south, central and northern Sweden and preserved frozen in the Swedish Environmental Specimen Bank (SESB). Voles have not earlier been included in the monitoring programmes on environmental pollution that have been running since early 1980s. The present study was performed in order to investigate the levels of a number of metal and elements as well as organic compounds in bank voles (*Myodes glareolus*) collected at different localities representing different biotopes in southern, central and northern parts of Sweden. It was also to evaluate voles as a suitable organism for monitoring environmental pollutants. In the monitoring programme on contaminants in terrestrial environments fledglings of starling, moose and reindeer have been used since the 1980s. These species have been well documented and long-time series of contaminant levels in starlings, reindeer and moose are available. However, none of these species are available in different biotopes all over Sweden. Voles have the advantage of being present in a diversity of biotopes in large parts of the country. Voles are also important components in the diet of raptors, owls and predatory mammals and elevated level of contaminants in voles could have serious effects in food webs.

Material and methods

Bank voles (*Myodes glareolus*) are herbivorous animals found in both forest and subalpine biotopes. The diet of bank voles is rather varied and depends on the local biotope and season. Mushrooms and lichen is included in the diet, especially in northern biotopes. Bank voles can occasionally eat worms and insects. The voles in the present study are trapped in the autumn of 2001 and consist mainly of animals born during the same year but there are a few animals from each location born the previous year, i.e. they have lived through one winter. Voles of both sexes are included in this study. (Table 1)

Localities

The sampling localities for bank voles in the present study are shown in Figure 1.
Three of the sampling sites, N:a Kvill, Grimsö and Vindeln are considered as forest biotopes while the other two, Vålådalen and Ammarnäs are considered as subalpine biotopes. N:a Kvill is the southernmost location and Ammarnäs the northernmost.

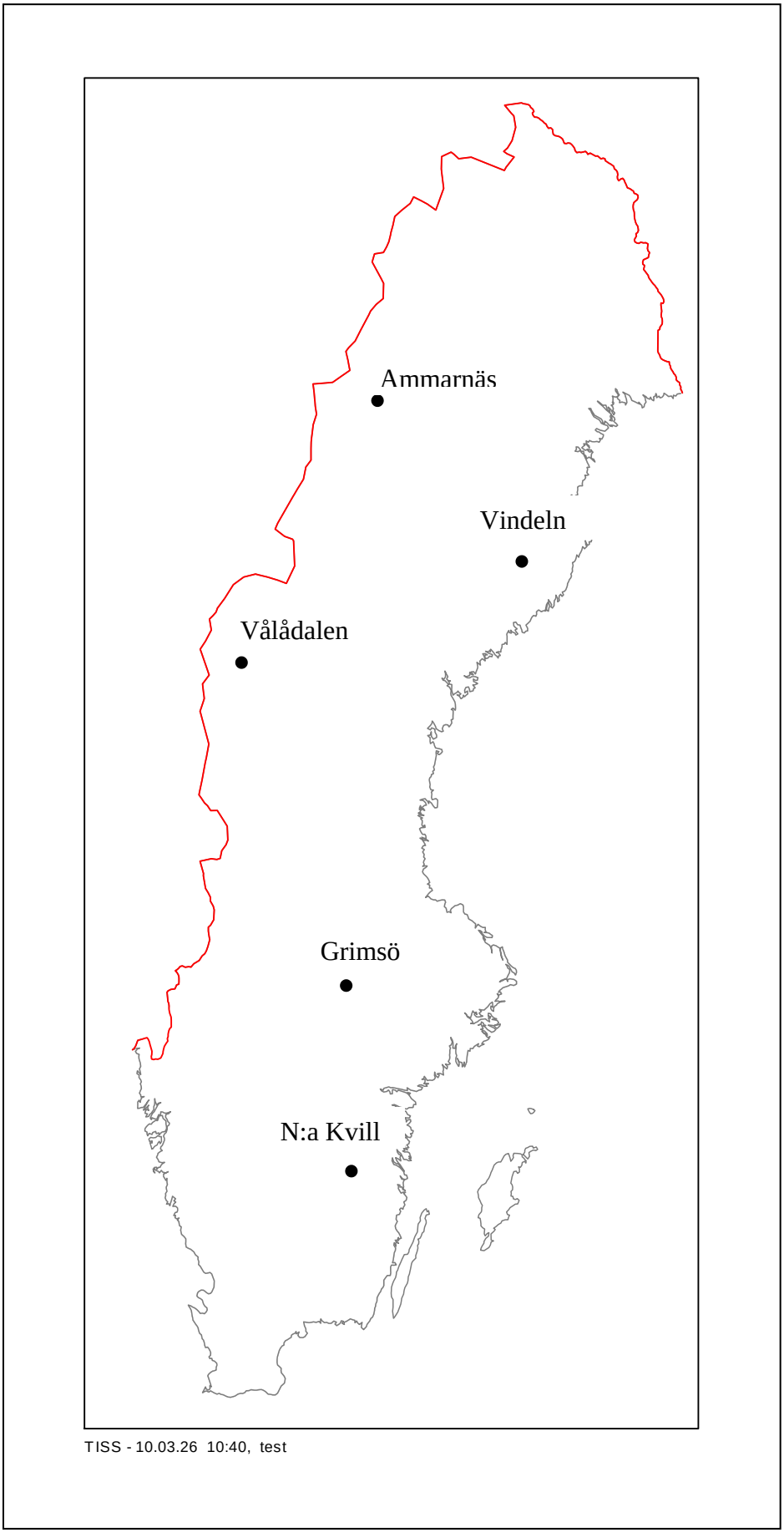

Figure 1. Sampling localities of bank voles (*Myodes glareolus)*

Sampling and preparation of tissue samples

The voles are collected by snap trapping and the sampling procedure has been described in (Hörnfeldt 1978; Hörnfeldt 1994; Hörnfeldt 2004). The voles were weighted, both gross body mass, as well as body mass without the intestines, liver, kidneys and spleen. The voles were aged from an upper molar according to the method described by (Viitala 1971). The liver and the carcass without head, fur and internal organs, was weighted to the nearest milligram and stored at approx. -20^{o}C, until analyzed.

Liver and muscle homogenates from bank voles collected from five different localities in the autumn of 2001 were prepared. From each locality, three homogenates consisting of aliquots from ten individuals were prepared. Sample composition according to age and sex distribution and time of sampling of the voles included in each sample are shown in Table 1.

Table 1. Age and sex distribution and week of sampling for the bank voles in the study. All voles were collected in 2001

Sampling site	week of sampling	sex ratio M/F	age Y/A[1]
Vindeln	40-41	4/6	7/3
	39-40	4/6	8/2
	39-40	5/5	8/2
Grimsö	38	3/7	9/1
	38	5/5	9/1
	38	5/5	9/1
N:a Kvill	39	6/4	9/1
	39	5/5	9/1
	39	4/6	9/1
Ammarnäs	34	4/6	8/2
	34	4/6	8/2
	34	6/4	8/2
Vålådalen	34	5/5	8/2
	34	7/3	9/1
	34	6/4	8/2

[1] Y-yearlings, A- voles born the previous year

Analysis of metals and elements

Metals and elements were analysed in liver tissue. The results are given in µg/g wet weight.

Pre-treatment of samples

Combustion of organs (5 g liver for multi-element determination using HNO_3, $HClO_4$ and H_2SO_4; about 3 g muscle for analysis of Hg using HNO_3 and $HClO_4$) was performed by automatic wet digestion according to a standard program (Frank 1976; Frank and Petersson 1983; Frank 1988; Frank et al. 1992). An electrically heated block of aluminium was used (Foss Tecator Digestion System, Model 40, Foss Tecator AB, Höganäs, Sweden).

Analysis

Analysis of 13 elements (Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pb, V and Zn) was performed using an inductively coupled plasma atomic emission spectrometer (ICP-AES, Jobin Yvon-Horiba SA, 91165 Longjumeau, France).

The determination of Hg was performed by using cold vapour (CV)- ICP-AES . (The methods are accredited according to SS-EN-ISO/IEC 17025).

Quality control was performed using appropriate reference materials (NCS ZC 71001 Beef Liver and DORM-3).

The chemical analyses on metals were carried out by the Department of Chemistry, National Veterinary Institute, Uppsala.

Analysis of chlorinated compounds

Chlorinated substances were analysed in muscle tissue. Results are given in ng/g lipid weight. The samples for the analysis of the chlorinated substances were extracted and cleaned-up in the same way as the brominated substances but analysed by a gas chromatograph equipped with an EC-detector.Two fused capillary columns of 60 m (0.25 mm i.d, 0.25 µm film thickness) were used in parallell, one DB-5 and one DB-1701. Argon/Methane was used as make-up gas and CB-53 as internal standard (Eriksson et al. 1997).

The analyses were carried out by the Department of Applied Environmental Science (ITM), Stockholm University

Table 2. Chlorinated compounds analysed in muscle tissue of bank voles.

HCB	Hexachlorobenzene
AHCH	alfa-HCH
BHCH	beta-HCH
LINDAN	gamma-HCH
DDE	p,p'-DDE,
DDD	p,p´-DDD
DDT	p,p´-DDT,
CB-28	2,4,4'–trichlorobiphenyl (1 -orto)
CB-52	2,5,2',5' –tetrachlorobiphenyl (2 -orto)
CB-101	2,4,5,2',5' –pentachlorobiphenyl (2 -orto)
CB-118	2,4,5,3',4' –Pentachlorobiphenyl (1 -orto)
CB-153	2,4,5,2',4',5' –hexachlorobiphenyl (2 -orto)
CB-138	Σ CB-138 (2,3,4,2',4',5' –hexachlorobiphenyl l (2 -orto)) and CB-163 (2,3,3',4',5,6-hexachlorobiphenyl)
CB-180	2,3,4,5,2',4',5' –Heptachlorobiphenyl (2 -orto)

Analysis of brominated flame retardants

Brominated flame retardants (BFR) were analysed in muscle tissue. Results are given in ng/g lipid weight.

The samples of 10 g muscle tissue were extracted with a mixture of acetone/*n*-hexane and *n*-hexane/diethyl ether. The organic phase was liquid/liquid partitioned with a solution of sodium chloride/phosphoric acid. The aqueous phase was reextracted with *n*-hexane and the combined organic phases were evaporated to dryness. The lipid content was determined gravimetrically. After treatment of the dissolved lipid extract with concentrated sulphuric acid (Jensen *et al*., 1983), the samples were analysed by gas chromatograph/mass spectrometry (GC-MS) in electron capture ionization (ECNI) mode. A 30 m DB-5 MS fused silica column (0.25 mm i.d., 0.25 μm film thickness) was used for the lower brominated analytes while a 15 m DB-5 MS fused silica column (0.25 mm i.d., 0.10 μm film thickness) was used for BDE 209. Ammonia was used as the reaction gas. The mass fragments monitored were *m/z* 79 and 81 for all brominated compounds and *m/z* 237 and 239 for dechlorane, used as internal standard (Sellström et al. 2003).

The analyses were carried out by the Department of Applied Environmental Science (ITM), Stockholm University

Table 3. Brominated flame retardants analysed in muscle tissue of bank voles.

BDE-47	2,2´,4,4´-tetrabromodiphenyl ether (TeBDE)
BDE-99	2,2´,4,4´,5-pentabromodiphenyl ether (Pe2BDE)
BDE-100	2,2´,4,4´,6-pentabromodiphenyl ether (Pe1BDE)
BDE-153	2,2´,4,4´,5,5´-hexabromodiphenyl ether
BDE-154	2,2´,4,4´,5,6´-hexabromodiphenyl ether
HBCD	hexabromcyclododecane

Analysis of phenolic compounds

Phenolic compounds were analysed in muscle tissue. Results are given in ng/g lipid weight for 4-t octylphenol, pentachlorfenol and triclosan and in µg/g lipid weight for nonylphenol. The sample, 3 g of muscle tissue, was homogenized with hexane/acetone twice, and the combined organic phases were treated with sodium chloride / phosphoric acid. The aqueous phase was reextracted with hexane and the combined organic phases were evaporated to dryness. The lipid content was determined, and the residue was redissolved in hexane/MTBE. The phenols were extracted into KOH/ethanol, and neutral compounds were removed by extracting the aqueous phase twice with hexane. After acidification of the aqueous phase, the phenolic compounds were extracted into hexane, and converted into their pentafluorobenzoyl esters. For clean-up, the derivatives were treated with sulphuric acid monohydrate. The determination was done by GC/ECNI/MS (Allmyr et al. 2006). The following surrogate standards were added to the muscle homogenate: ^{13}C-6-pentachlorophenol, ^{13}C-12-triclosan and 4-n-nonylphenol. The analyses on phenolic compounds were carried out by the Department of Applied Environmental Science (ITM), Stockholm University.

Table 4. Phenolic compounds analysed in muscle tissue of bank voles. (LOD for 4-nonylphenol is in µg/g lw)

		LOD **(**ng/g lw)
4-t-oktylfenol	4-(1,1,3,3-Tetramethylbutyl)phenol	5
4-nonylfenol	p-nonylphenol	**3** (µg/g lw)
Pentaklorfenol (PCP)	2,3,4,5,6-Pentachlorophenol	20
triclosan	2,4,4'-triklor-2'-hydroxidifenyleter	0,2

Analysis of perfluorinated compounds

Perfluorinated compounds were analysed in liver tissue. Results are given in ng/g fresh weight.

Sample extraction and clean-up was based on the method by (Powley et al. 2005) with modifications for biota samples described by (Verreault et al. 2007). In short, 1 g of the homogenized liver was spiked with the mass-labeled internal standards. Extraction was performed twice with 5 mL acetonitrile in an ultrasonic bath. The combined extracts were concentrated to 1 mL and subjected to dispersive clean-up on graphitized carbon. The cleaned-up extract was added to aqueous ammonium acetate. Precipitation occurred and the extract was centrifuged before instrumental analysis of the clear supernatant. Aliquots of the final extracts were injected automatically on a high performance liquid chromatography system coupled to a tandem mass spectrometer. Compound separation was achieved on an C18 reversed phase column with a binary gradient of buffered (ammonium acetate) methanol and water. The mass spectrometer was operated in negative electrospray ionization mode. Quantification was performed in selected reaction monitoring chromatograms using the internal standard method. The analyses on phenolic compounds were carried out by the Department of Applied Environmental Science (ITM), Stockholm University.

Table 5. Perfluorinated compounds analysed in liver tissue of bank voles.

		LOD (ng/g ww)
PFHxA	perfluorohexanoate	0,3
PFHpA	perfluoroheptanoate	0,3
PFOA	perfluorooctanate	0,4
PFNA	perfluorononanonate	0,3
PFDcA	perfluorodecanoate	0,5
PFUnA	perfluoroundecanoate	
PFDoA	perfluorododecanoate	0,2
PFTriA	perfluorotridecanoiate	0,2
PFTeA	perfluorotetradecanoate	0,3
PFPeDA	perfluoropentadecanoate	0,5
PFBS	perfluorobutane sulfonate	0,5
PFHxS	perfluorohexane sulfonate	
PFOS	perfluorooctane sulfonate	
PFDcS	perfluorodecane sulfonate	0,3
PFOSA	perfluorooctane sulphonamide	0,3

Limit of detection (LOD) and limit of quantification (LOQ)

For the analyses on chlorinated and brominated compounds the lab results have been given in values above and below limit of quantification (LOQ). Values below LOQ have been assigned with a minus in the lab reports. The LOQ denotes the lowest level of a substance where a reasonably accurate quantification is possible. For lipid soluble substances that are determined on lipid weight basis, LOQ is dependent on the lipid content of the sample. Thus,

the LOQ for the analysis differs between samples. The expression “below LOQ” is used in the text to denote these cases but no actual numbers are given. Values below LOQ have not been included in the figures but are given in the tables in Appendix. For metals and elements, phenolic compounds and perfluorinated compounds the limit of detection (LOD) for the analysis has been given in the lab reports and these values have either been assigned with a minus (metals and phenolic compounds) or with < (perfluorinated compounds). In the text these cases are referred to as “below LOD” and the LOD for the analysis is given in brackets. They are not included in the figures. In the report on analysis of phenolic compounds the term “traces” is used to denote that the compound is present in the sample but not possible to quantify.

Results

Lipid content

The lipid content of the muscle samples is shown in Table 6. Chlorinated and brominated substances are analysed on the same subsample while phenolic substances are analysed on a different subsample. The same individuals are included in both subsamples.

Table 6. Lipid content (%)

	Cl+Br	Phenolic
Vindeln	4,96	3,24
Vindeln	4,64	3,09
Vindeln	3,64	3,12
Grimsö	4,09	4,55
Grimsö	3,49	4,06
Grimsö	4,38	5,09
Ammarnäs	3,87	4,18
Ammarnäs	2,96	3,68
Ammarnäs	3,55	4,73
N.a. Kvill	2,49	2,45
N.a. Kvill	3,50	2,48
N.a. Kvill	3,86	2,27
Vålådalen	3,47	3,64
Vålådalen	3,27	5,55
Vålådalen	3,24	4,79

Metals and elements

Fourteen metals and elements (Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pb, V, Zn, Hg) were analysed in liver tissue. Of these fourteen elements, Ca, Co, Cu, Fe, Mg, Mo and Zn are essential. Vanadium is believed to be essential to mammals but no known function have yet

been found. Cd, Cr, Hg, Ni and Pb are toxic elements with no known function in mammals. Levels of essential elements in the body are generally homeostatically regulated within certain physiological ranges but they also have a potential for toxicity. If environmental levels are too elevated, the homeostatic regulation of essential elements in the body might be put out of control. Furthermore, uptake of toxic metals could be influenced by levels of essential elements as they often share the same mechanisms for uptake.
The metal levels found in bank voles at the different sampling sites are shown in Figure 2 and Figure 3 (Appendix, Table 1).

Cadmium
Of the toxic metals, cadmium was the most abundant in voles from all localities except Vålådalen where mercury levels were higher compared to cadmium levels. The highest cadmium levels was found in bank voles from Grimsö (0,08-0,35 µg/g ww) and Vinden (0,19-0,21 µg/g ww) and the lowest levels were found in bank voles from Ammarnäs (0,03-0,05 µg/g ww).

Lead
The largest variation between the homogenates was found in bank voles from N:a Kvill (0,03-0,08 µg/g ww). Voles from Vindeln (0,06-0,07 µg/g ww) and Grimsö (0,04-0,07 µg/g ww) had approximately the same lead levels. Lead levels were lowest in bank voles from Ammarnäs (0,01-0,03 µg/g ww).

Mercury.
The bank voles from Vålådalen had higher mercury levels (0,06-0,14 µg/g ww) compared to voles from the other localities.

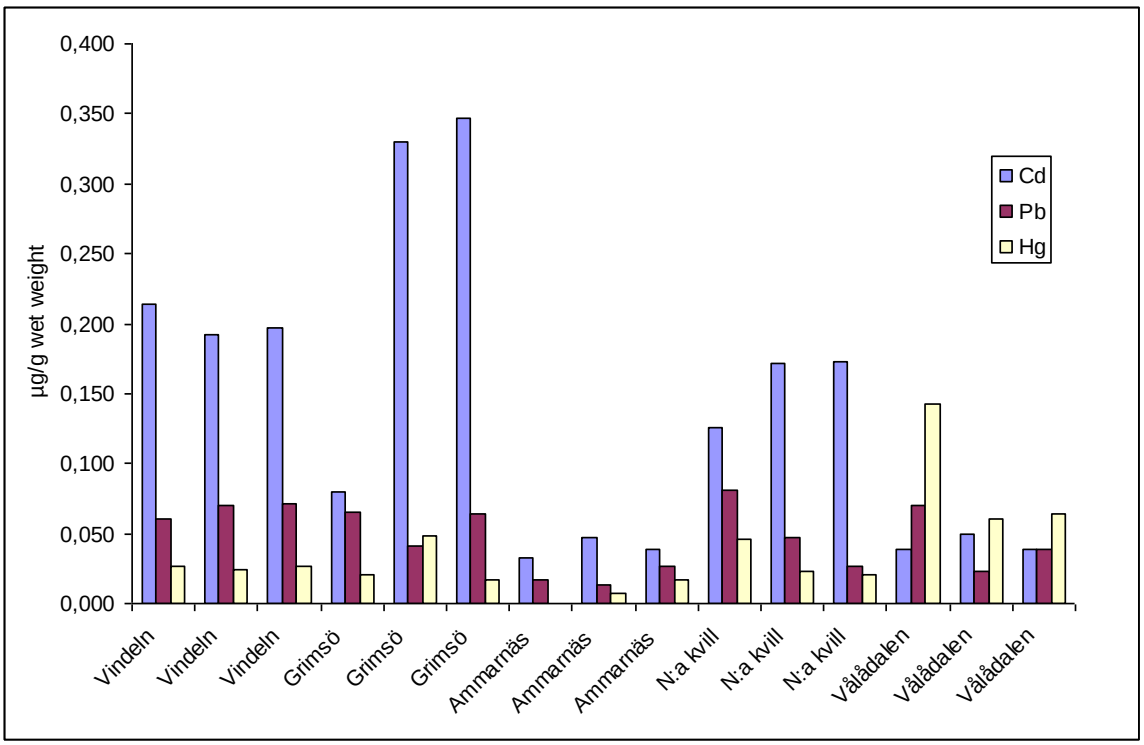

Figure 2. Cadmium, lead and mercury (µg/g ww) in liver tissue of bank voles from five different localities in Sweden.

Chromium

Chromium was found in detectable levels in two samples from Vålådalen (0,024-0,026 µg/ g ww). In samples from the other sampling sites it was found below LOD (0,02 µg/g ww).

Nickel

Nickel was found in detectable levels in the samples from Ammarnäs (0,024-0,028 µg/g ww) and in one sample from Vålådalen (0,022 µg/g ww). At the other localities Ni levels was below LOD (0,018 µg/g ww).

Essential metals and elements

For essential elements the variation between different localities was less than for the toxic elements (Fig 3). Calcium levels that was highest in bank voles from Vålådalen (85,9-305 µg/g ww). The samples from Vålådalen also displayed the largest variation between the three homogenates.

Summary metals

Cadmium, lead and mercury were found in low but detectable levels in liver tissue of bank voles from all sampling sites. Cadmium was the most abundant of the toxic metals on all sites except Vålådalen where mercury was the most abundant. No geographical trend could be

detected but voles from the subalpine sampling sites had lower cadmium levels compared to voles from forest sampling sites. Mercury was 2-3 times higher in voles from Vålådalen compared to voles from the other sampling sites. The levels of Cd, Pb and Hg in bank voles from Ammarnäs in the present study were comparable to what was found in bank voles from Ammarnäs in the middle of 1990s (unpublished). The bank voles in the present study had higher liver cadmium levels but lower lead levels compared to starling fledglings sampled in 2006 from south and central Sweden (Odsjö et al. 2008).

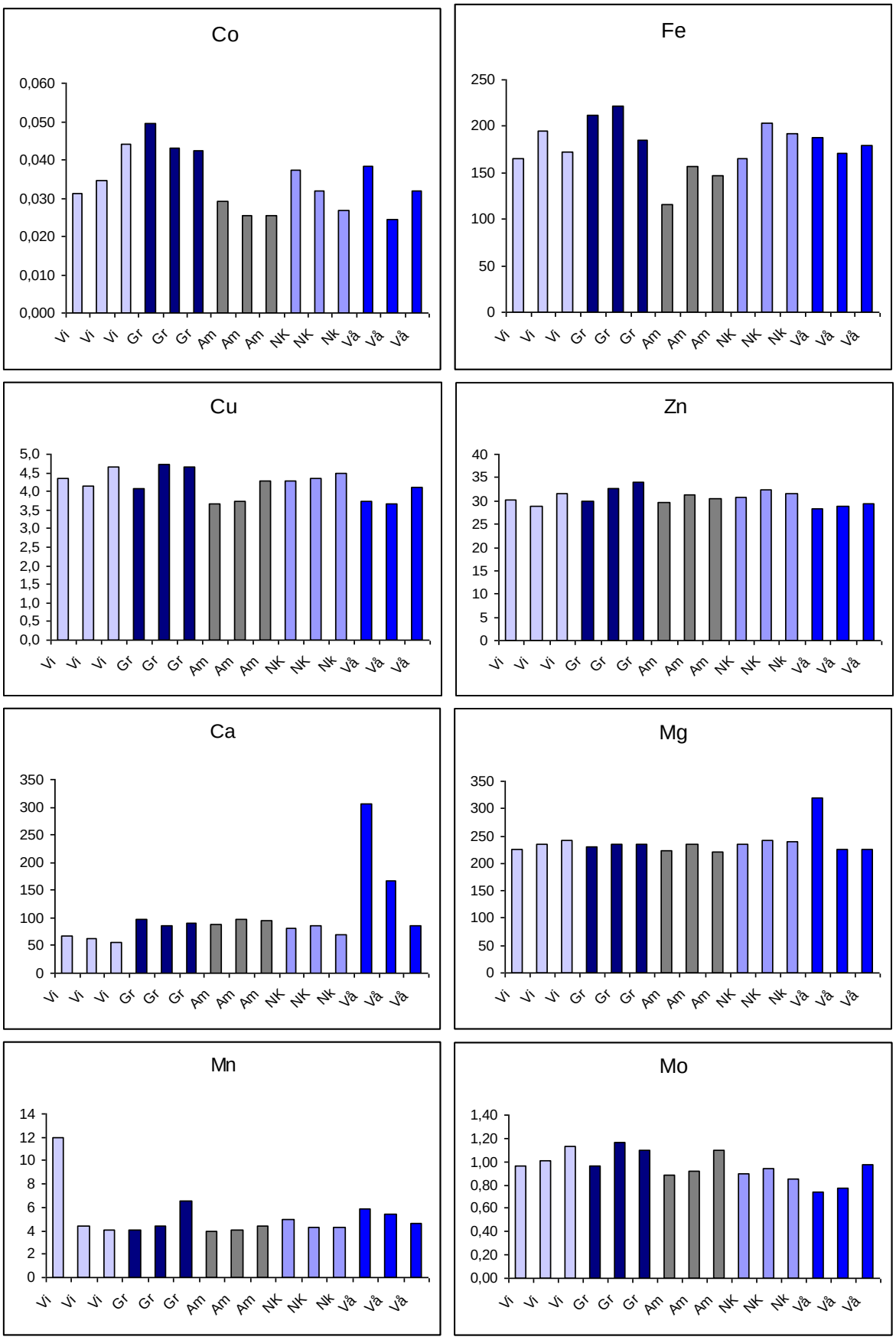

Figure 3. Essential metals and elements (µg/g ww) in liver tissue of bank voles from five different localities in Sweden.

Chlorinated compounds

The chlorinated compounds hexachlorobenzene (HCB), α-, β-, γ-hexachlorocyclohexan (α-HCH, β-HCH, lindan), DDT DDE, DDD, CB-28, CB-52, CB-101, CB-118, CB-153, CB-138 and CB-180 were analysed in muscle tissue.

Levels above LOQ found in bank vole muscle tissues are shown in Figure 4 (Appendix, Table 2).

Hexachlorobenzene (HCB) was the only compound that was found above LOQ in all samples from all localities. CB-153 was above LOQ in all homogenates from N:a Kvill and Vålådalen. DDT, DDE and DDD were present above LOQ in one of the homogenates from Grimsö. CB-118, CB-153, CB-138 and CB-180 were present above LOQ in one of the homogenates from N:a Kvill. This homogenate also contained the highest level found of HCB (and of CB-153. It was also the only one that contained quantifiable amounts of CB-118 and CB-180. The voles from N.a Kvill and Vålådalen had the highest mean levels of HCB and CB-153. α-HCH, β-HCH, lindan were not detected above LOQ in any of the homogenates. Nor were CB-28, CB-58 or CB-101 found above LOQ.

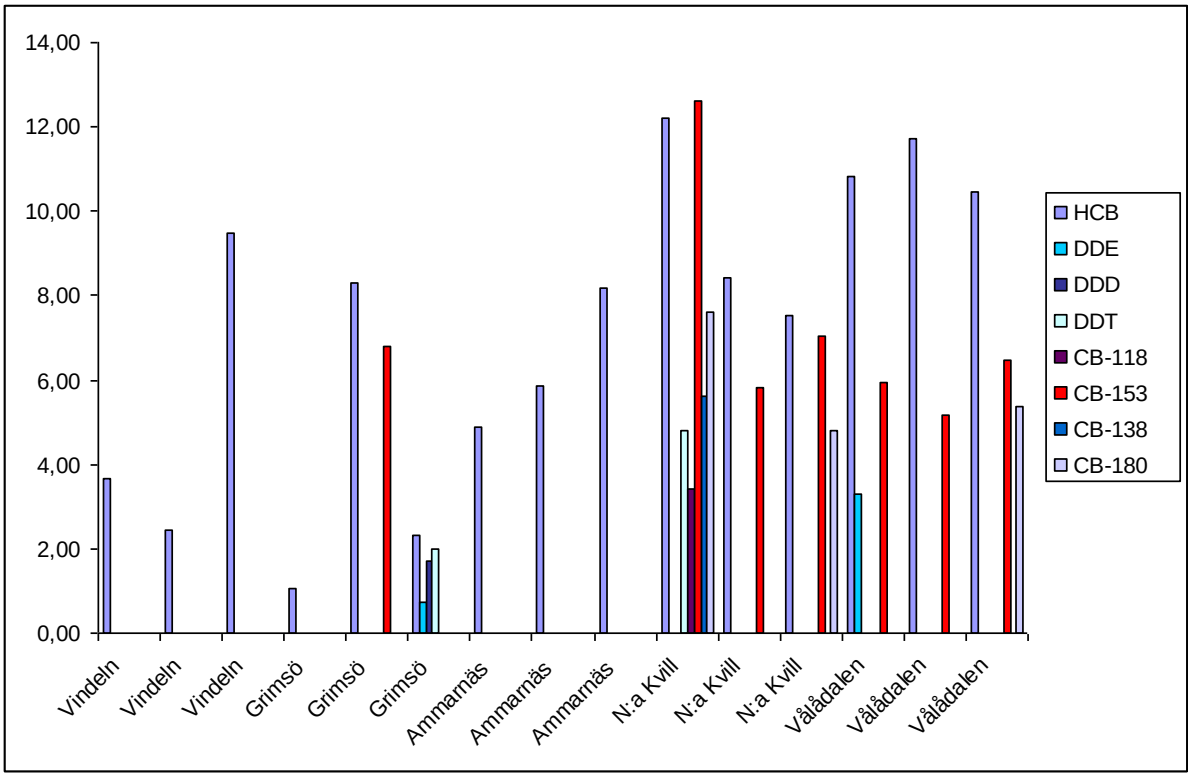

Figure 4. Chlorinated compounds (ng/g lw) in muscle tissue of bank voles from five different localities in Sweden.

Summary chlorinated compounds

Hexachlorbenzene (HCB) was the only chlorinated compound analysed that was found, although in low levels, in all of the analysed samples. No geographical trend could be detected and the levels were highest in voles from Vålådalen, a subalpine northern area and N:a Kvill, a forest area in southern Sweden. HCB has been used as fungicide and seed dressing agent but the use of HCB in Sweden has been banned since the 1980. It is also known to be produced at incomplete combustion conditions. HCB is very persistent and is known to biomagnificate (Niiemi 1987). Time trends of HCB in moose (1986-2005) and reindeer (1987-2006) have previously been analysed and the levels have decreased significantly by 6,9 % yearly in moose (Danielsson et al. 2008). In reindeer, the data are more difficult to interpret as the levels are high in certain years. The overall mean (1986-2005) of HCB in muscle tissue of moose collected at Grimsö was 23,5 ng/g lw. In muscle tissue of reindeer collected at Abisko in the northernmost part of Sweden the overall mean (1987-2006) was 45 ng/g lw (Danielsson et al. 2008). A large part of the autumn diet of reindeer consists of lichen which is known for its ability to accumulate contaminants from air and this should explain the higher levels of HCB in reindeer compared to moose. This could also be an explanation to the relatively high levels of HCB found in voles from Vålådalen and Ammarnäs as the diet of voles in subalpine areas to a larger part includes lichens.

Of the PCB congeners analysed, CB-153 was the most abundant and it was found above LOQ in all the samples from N:a Kvill and Vålådalen. The levels found in bank vole muscle tissue were somewhat higher compared to levels found in moose and reindeer (Danielsson et al. 2008).

Brominated flame retardants

Six different congeners of BRFs and hexabromocyclododecane (HBCD) were analysed. Levels above LOQ found in bank vole muscle tissue are shown in Figure 5 (Appendix, Table 3). BDE-99, BDE-100 and BDE-153 were found above LOQ in one muscle homogenate from Vindeln. Further, BDE-153 was found in two homogenates from Grimsö, in one homogenate from N:a Kvill and in all of the homogenates from Vålådalen. The homogenates from Vålådalen also contained the highest concentrations of CB-153. HBCD was found in one of the homogenates from Vindeln. BDE-47, BDE-154 and BDE-209 were not detected above LOQ in any of the homogenates analysed. In one homogenate from Vindeln 4,4 µg/g lw of HBCD was found. HBCD was not detected above LOQ in any of the other samples analysed.

None of the analysed compounds were found above LOQ in voles from Ammarnäs.

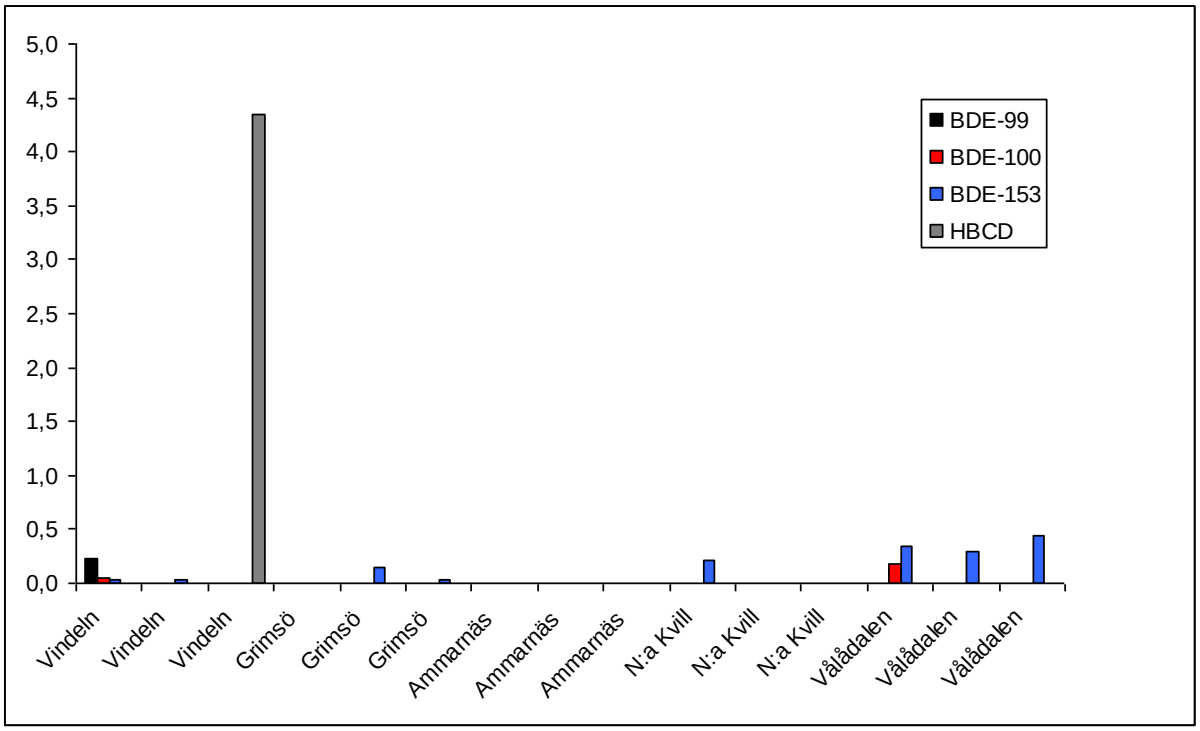

Figure 5. Levels of brominated flame retardants (ng/g lw) in muscle tissue of bank voles from five different localities in Sweden.

Summary brominated flame retardants

The BFRs analysed was below LOQ in most samples. BDE-153 was the only BFR that was found above LOQ in at least one sample from four out of five sampling sites in the present study. It was present in quantifiable amounts in all of the samples from Vålådalen. Hexabromocyclododecane (HBCD) was present (4,4 ng/g lw) in one sample from Vindeln but could not be found in quantifiable amounts in a any of the other samples. Brominated flame retardants was not present in detectable amounts in moose and reindeer muscle (Danielsson et al. 2008). The levels found in muscle tissues of voles in the present study was comparable to the levels found in starling muscle tissue but the dominant congener was BDE-153 in voles and BDE-99 in starlings (Odsjö et al. 2008) No geographical trends could be detected. BDE-153 was found in detectable amounts in muscle tissue of bank voles from Vindeln, Grimsö, N:a Kvill and Vålådalen. It was not found in detectable amounts in samples from Ammarnäs. On the other hand was BDE 153 found in detectable amounts in all the samples from Vålådalen.

Phenolic compounds

Levels above LOD of 4-nonylphenol (LOD – 3 µg/g lw) and triclosan (LOD – 0,2 ng/g lw) was found in bank vole muscle tissue (Figure 6 and Appendix, table 4). Triclosan was found in levels above LOD at all sampling sites except Vålådalen. In one of the samples from Vålådalen traces of triclosan was found. The highest level of triclosan (12 ng/g lw) was found in one homogenate from Ammarnäs. Levels above LOD of 4-nonylphenol was found at N:a Kvill and Vålådalen. The highest level (14 µg/g lw) was found in one homogenate from N:a Kvill. 4-tert-octylphenol was not found in levels above LOD (5 ng/g lw) in any of the samples analysed but traces was found in samples from Ammarnäs and N:a Kvill. Pentachlorphenol was not found above LOD (20 ng/g lw) nor was any trace of pentachlorphenol found in any of the analysed homogenates.

Summary phenolic compounds

No geographical trends could be detected in the distribution of phenolic compounds analysed in this study. Traces of or detectable levels of triclosan and/or 4-nonylphenol was found in samples from all sampling sites and traces of 4-t-octyl-phenol was found in samples from Ammarnäs and N:a Kvill. The highest level of triclosan (12 ng/g lw) was found in one sample from Ammarnäs, corresponding to approximately 0,4 ng/g ww. This is somewhat higher compared to the highest level found in starling (0,25 ng/g ww) found at one sampling sites in south west of Sweden (Odsjö et al. 2008).

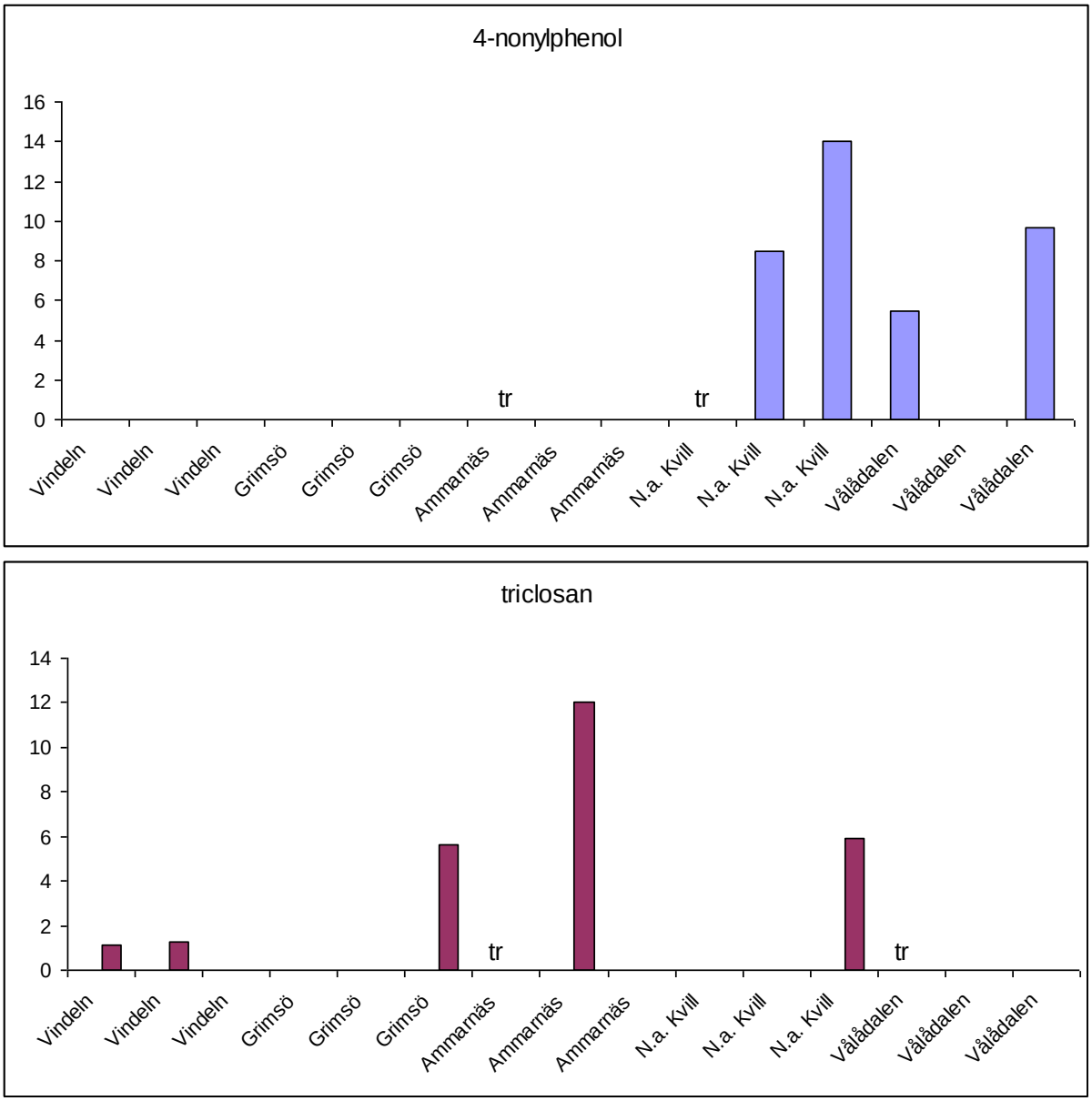

Figure 6. 4-nonylphenol (µg/g lw) and triclosan (ng/g lw) in muscle tissue of voles from five different localities in Sweden.(Tr- traces <LOD)

Perfluorinated compounds (PFCs)

Fifteen PFCs were analysed in liver homogenates. Levels above LOD found in liver tissue of bank voles are shown in Figure 7 (Appendix, Table 5)

PFUnA, PFHxS and PFOS were detected in all samples. PFHxA, PFHpA, PFOA , PFTeA, PFPeDA, PFBS, PFDcS and PFOSA on the other hand was not found above LOD in any of the analysed homogenates.

PFOS was the dominating compound at all localities and the highest levels were found in Vålådalen (12,4 ng/g ww) and in Vindeln (12,3 ng/g ww). Voles from Vålådalen also had the

highest levels of PFUnA (1,8 ng/g ww), PFHxS (1,4 ng/g ww), PFDoA (0,72 ng/g ww) and PFTriA (0,50 ng/g ww).

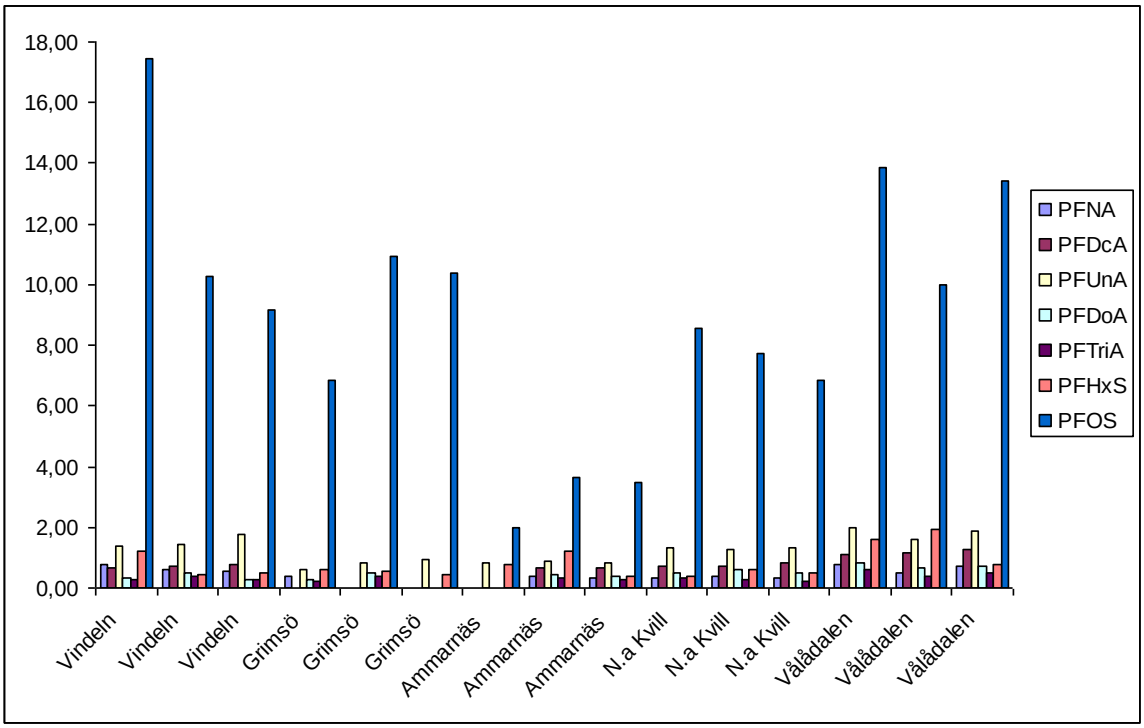

Figure 7. Levels of fluorinated compounds (ng/g ww) in liver tissue of bank voles from five localities in Sweden.

Summary PFCs

PFUnA, PFHxS and PFOS were found in low but detectable levels in all of the analysed samples with PFOS being the dominating compound. The highest level of PFCs were found in one sample from Vindeln (22,2 ng/g ww). The highest mean levels of PFCs (18,7 ng/g ww) was found in samples from Vålådalen while the samples from Ammarnäs had the lowest mean levels (5,8 ng/g ww). The samples from Ammarnäs differed in that the percentage of PFOS was lower (47 %) compared to the samples from the other sites (66-79 %). No geographical trends could be detected in levels of PFCs in bank voles. The levels found in bank voles in the present study are somewhat higher to what was found in starlings (Odsjö et al. 2008)

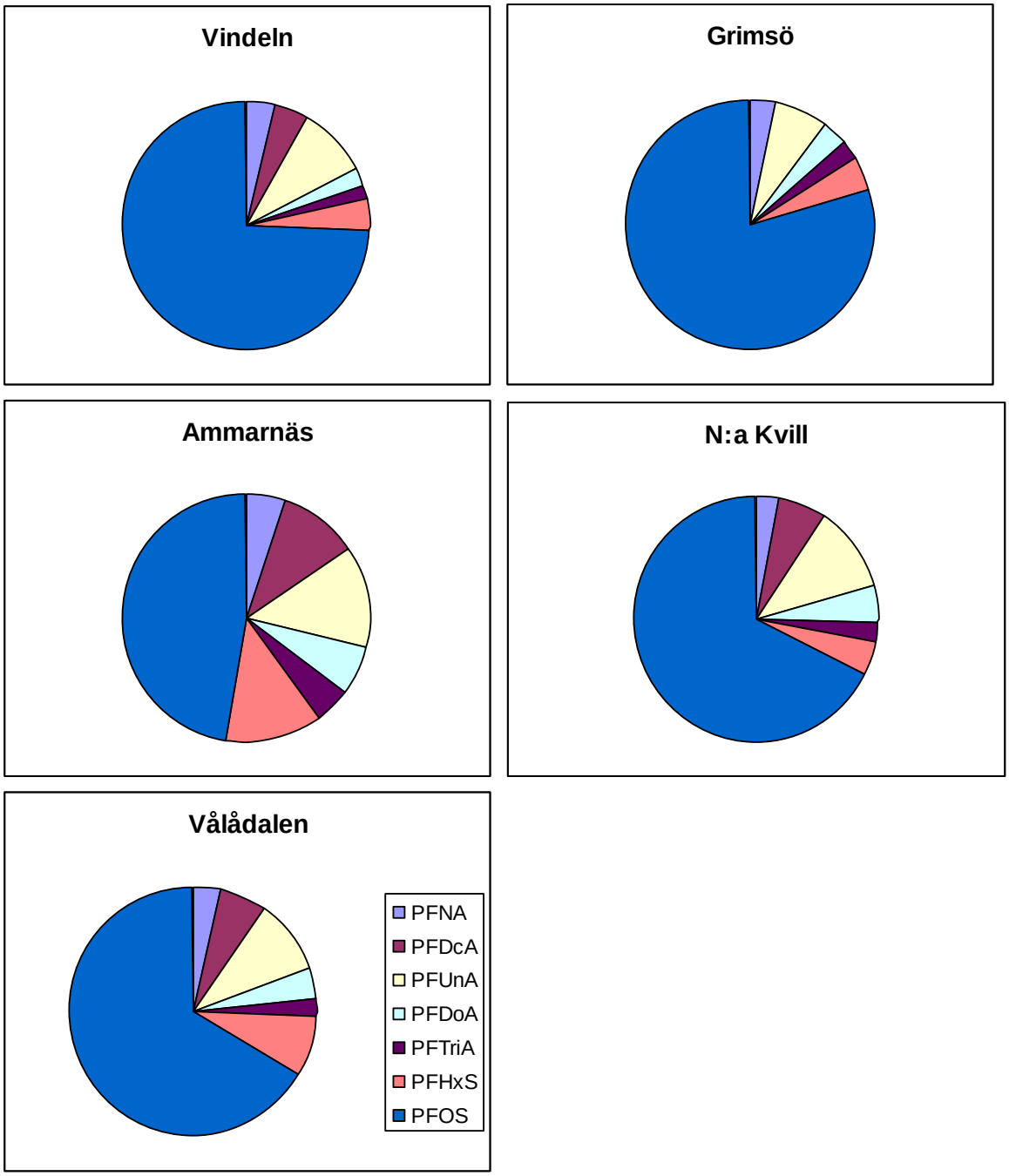

Figure 8. The distribution (%) of different perfluorinated compounds in liver tissue of bank voles.

Conclusions

There were generally low or very low levels of all the analysed compounds in voles from all the sampling sites. No geographical trend could be detected but somewhat surprisingly, bank voles collected at Vålådalen had the highest levels of mercury, HCB, CB-153 and also of some of the perfluorinated compounds. There were also detectable levels of 4-nonylfenol and

traces of triclosan in homogenates from Vålådalen. Diets of bank voles could be rather varied and is depending on the biotope. In northern, subalpine areas the proportions of lichen and mushrooms in bank vole diet are generally higher compared to forest populations of bank voles (Löfgren 1995). One explanation to the higher levels found in bank voles from Vålådalen could be a higher proportion of lichen in their diet. Lichens are known to accumulate atmospheric depositions of contaminants and the higher levels of mercury, HCB, CB-153 and perfluorinated compounds in these voles would thus reflect a long range atmospheric transport of these very stable compounds. A diffuse contamination of these compounds can be detected in this study on bank voles and if this contamination is increasing or decreasing ought to be evaluated.

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Appendix

Table 1. Metal and elements (µg/g wet weight)in liver tissue of bank voles. LOD – limit of detection.

	Ca	Cd	Co	Cr[1]	Cu	Fe	Mg	Mn	Mo	Ni[2]	Pb	V[3]	Zn	Hg
Vindeln	67,1	0,214	0,031	LOD	4,36	165	226	12,0	0,967	LOD	0,060	LOD	30,2	0,027
Vindeln	63,5	0,192	0,035	LOD	4,15	195	234	4,42	1,00	0,018	0,071	LOD	28,9	0,024
Vindeln	55,2	0,197	0,044	LOD	4,66	173	241	4,03	1,14	LOD	0,071	LOD	31,5	0,026
Grimsö	97,8	0,079	0,050	LOD	4,07	212	230	4,11	0,962	LOD	0,066	0,006	29,9	0,021
Grimsö	85,0	0,330	0,043	LOD	4,74	222	236	4,42	1,16	LOD	0,041	0,002	32,6	0,049
Grimsö	89,4	0,346	0,042	LOD	4,65	185	236	6,53	1,10	LOD	0,064	LOD	34,1	0,017
Ammarnäs	88,7	0,033	0,029	LOD	3,66	115	224	3,90	0,882	0,024	0,017	LOD	29,7	-
Ammarnäs	96,3	0,047	0,026	LOD	3,72	157	236	4,07	0,924	0,026	0,013	LOD	31,4	0,007
Ammarnäs	95,0	0,039	0,025	LOD	4,27	146	221	4,41	1,10	0,028	0,027	LOD	30,5	0,017
N:a Kvill	81,7	0,126	0,037	LOD	4,29	165	234	4,97	0,900	LOD	0,081	LOD	30,6	0,046
N:a Kvill	85,2	0,172	0,032	LOD	4,36	203	241	4,28	0,942	LOD	0,047	LOD	32,5	0,023
N:a Kvill	68,8	0,173	0,027	0,020	4,48	192	239	4,24	0,853	LOD	0,027	LOD	31,6	0,021
Vålådalen	305,0	0,039	0,038	LOD	3,72	188	320	5,86	0,736	0,022	0,070	0,002	28,4	0,142
Vålådalen	165,9	0,049	0,024	0,024	3,65	171	225	5,42	0,775	LOD	0,023	LOD	28,9	0,061
Vålådalen	85,9	0,039	0,032	0,026	4,12	179	226	4,60	0,973	LOD	0,039	0,002	29,4	0,064

[1] LOD – 0,040 µg/g wet weight
[2] LOD – 0,050 µg/g wet weight
[3] LOD – 0,010 µg/g wet weight

Table 2. Chlorinated compounds (ng/g lw) in muscle tissue of bank vole. Minus (-) denotes values below LOQ. -99.99 denoted values below LOD.

	HCB	AHCH	BHCH	LINDAN	DDE	DDD	DDT	CB-28	CB-52	CB-101	CB-118	CB-153	CB-138	CB-180
Vindeln	3,65	-0,8	-0,8	-1,0	-0,6	-1,4	-1,2	-0,8	-0,8	-0,8	-0,8	-1,2	-1,0	-1,0
Vindeln	2,43	-0,9	-0,9	-1,1	-0,6	-1,5	-1,3	-0,9	-0,9	-0,9	-0,9	-1,3	-1,1	-1,1
Vindeln	9,49	-3,8	-5,2	-4,9	-3,8	-8,0	-6,9	-3,8	-3,8	-4,7	-4,4	-6,3	-5,2	-4,9
Grimsö	1,08	-1,0	-1,0	-1,2	-0,7	-1,7	-1,5	-1,0	-1,0	-1,0	-1,0	-1,5	-1,2	-1,2
Grimsö	8,31	-4,0	-5,4	-5,2	-4,0	-8,3	-7,2	-4,0	-4,0	-4,9	-4,6	6,79	-5,4	-5,2
Grimsö	2,33	-99.99	-0,9	-1,1	0,730	1,71	1,99	-0,9	-0,9	-0,9	-0,9	-1,4	-1,1	-1,1
Ammarnäs	4,89	-3,6	-4,9	-4,7	-3,6	-7,5	-6,5	-3,6	-3,6	-4,4	-4,1	-5,9	-4,9	-4,7
Ammarnäs	5,85	-3,7	-4,7	-4,7	-3,4	-7,4	-6,4	-3,7	-3,7	-4,4	-4,1	-5,7	-4,7	-4,7
Ammarnäs	8,19	-3,1	-3,9	-3,9	-2,8	-6,2	-5,4	-3,1	-3,1	-3,7	-3,4	-4,8	-3,9	-3,9
N:a Kvill	12,2	-2,8	-4,0	-3,6	-2,8	-5,6	4,8	-2,8	-2,8	-2,8	3,41	12,6	5,61	7,62
N:a Kvill	8,43	-99.99	-4,0	-4,0	-2,9	-6,3	-5,4	-3,1	-3,1	-3,7	-3,4	5,80	-4,0	-4,0
N:a Kvill	7,51	-3,6	-4,9	-4,7	-3,6	-7,5	-6,5	-3,6	-3,6	-4,4	-4,1	7,02	-4,9	4,82
Vålådalen	10,8	-3,2	-4,0	-4,0	3,28	-6,3	-5,5	-3,2	-3,2	-3,7	-3,5	5,93	-4,0	-4,0
Vålådalen	11,7	-3,4	-4,3	-4,3	-3,1	-6,7	-5,8	-3,4	-3,4	-4,0	-3,7	5,17	-4,3	-4,3
Vålådalen	10,4	-3,4	-4,3	-4,3	-3,1	-6,8	-5,9	-3,4	-3,4	-4,0	-3,7	6,46	-4,3	5,38

Table 3. Brominated flame retardants (ng/g lw) in muscle tissue of bank voles.
Minus (-) denotes values below LOQ

	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	HBCD	BDE-209
Vindeln	-0,2	0,2	0,04	0,04	-0,02	-0,4	-1,0
Vindeln	-0,2	-0,1	-0,04	0,03	-0,03	-0,4	-1,1
Vindeln	-0,9	-0,6	-0,2	-0,1	-0,1	4,4	-5,6
Grimsö	-0,2	-0,1	-0,1	-0,03	-0,03	-0,5	-1,3
Grimsö	-1,0	-0,6	-0,2	0,1	-0,1	-2,3	-5,7
Grimsö	-0,2	-0,1	-0,05	0,02	-0,03	-0,5	-1,1
Ammarnäs	-0,9	-0,5	-0,2	-0,1	-0,1	-2,1	-5,1
Ammarnäs	-0,8	-0,5	-0,2	-0,1	-0,1	-2,0	-5,0
Ammarnäs	-1,0	-1,3	-0,2	-0,2	-0,1	-1,7	-4,2
N:a Kvill	-0,9	-1,2	-0,2	0,2	-0,1	-1,6	-3,9
N:a Kvill	-1,0	-1,3	-0,2	-0,2	-0,1	-1,7	-4,3
N:a Kvill	-1,2	-1,6	-0,2	-0,3	-0,1	-2,1	-5,2
Vålådalen	-1,0	-1,3	0,2	0,4	-0,1	-1,7	-4,3
Vålådalen	-1,1	-1,4	-0,2	0,3	-0,1	-1,8	-4,6
Vålådalen	-1,1	-1,4	-0,2	0,4	-0,1	-1,8	-4,6

Table 4. Phenolic compounds (ng/g lw) in muscle tissue of bank voles. Minus (-) denotes valus below LOD.

	4-t-oktylfenol	4-nonylfenol	pentaklorfenol	triclosan
Vindeln	-5	-3	-20	1,1
Vindeln	-5	-3	-20	1,3
Vindeln	-5	-3	-20	-0,2
Grimsö	-5	-3	-20	-0,2
Grimsö	-5	-3	-20	-0,2
Grimsö	-5	-3	-20	5,6
Ammarnäs	traces	traces	-20	traces
Ammarnäs	traces	-3	-20	12
Ammarnäs	-5	-3	-20	-0,2
N.a. Kvill	traces	traces	-20	-0,2
N.a. Kvill	traces	8,5	-20	-0,2
N.a. Kvill	traces	14	-20	5,9
Vålådalen	-5	5,5	-20	traces
Vålådalen	-5	-3	-20	-0,2
Vålådalen	-5	9,7	-20	-0,2

[1] µg/g lw

Table 5. PFCs (ng/g ww) in liver tissue of bank voles. < denotes values below LOD.

	PFHxA	PFHpA	PFOA	PFNA	PFDcA	PFUnA	PFDoA	PFTriA	PFTeA	PFPeDA	PFBS	PFHxS	PFOS	PFDcS	PFOSA
Vindeln	<0,3	<0,3	<0,4	0,79	0,67	1,40	0,31	0,30	<0,3	<0,5	<0,5	1,24	17,5	<0,3	<0,3
Vindeln	<0,3	<0,3	<0,4	0,59	0,74	1,42	0,50	0,41	<0,3	<0,5	<0,5	0,43	10,3	<0,3	<0,3
Vindeln	<0,3	<0,3	<0,4	0,56	0,77	1,77	0,27	0,28	<0,3	<0,5	<0,5	0,47	9,14	<0,3	<0,3
Grimsö	<0,3	<0,3	<0,4	0,40	<0,5	0,63	0,29	0,22	<0,3	<0,5	<0,5	0,63	6,82	<0,3	<0,3
Grimsö	<0,3	<0,3	<0,4	<0,3	<0,5	0,85	0,51	0,37	<0,3	<0,5	<0,5	0,54	10,9	<0,3	<0,3
Grimsö	<0,3	<0,3	<0,4	<0,3	<0,5	0,95	<0,2	<0,2	<0,3	<0,5	<0,5	0,42	10,4	<0,3	<0,3
Ammarnäs	<0,3	<0,3	<0,4	<0,3	<0,5	0,84	<0,2	<0,2	<0,3	<0,5	<0,5	0,77	2,01	<0,3	<0,3
Ammarnäs	<0,3	<0,3	<0,4	0,36	0,64	0,89	0,44	0,33	<0,3	<0,5	<0,5	1,23	3,63	<0,3	<0,3
Ammarnäs	<0,3	<0,3	<0,4	0,31	0,66	0,85	0,39	0,26	<0,3	<0,5	<0,5	0,40	3,49	<0,3	<0,3
N.a Kvill	<0,3	<0,3	<0,4	0,30	0,70	1,34	0,48	0,34	<0,3	<0,5	<0,5	0,40	8,57	<0,3	<0,3
N.a Kvill	<0,3	<0,3	<0,4	0,40	0,70	1,29	0,62	0,28	<0,3	<0,5	<0,5	0,61	7,76	<0,3	<0,3
N.a Kvill	<0,3	<0,3	<0,4	0,31	0,84	1,31	0,51	0,24	<0,3	<0,5	<0,5	0,50	6,83	<0,3	<0,3
Vålådalen	<0,3	<0,3	<0,4	0,75	1,09	1,96	0,82	0,61	<0,3	<0,5	<0,5	1,60	13,9	<0,3	<0,3
Vålådalen	<0,3	<0,3	<0,4	0,49	1,18	1,58	0,65	0,37	<0,3	<0,5	<0,5	1,95	9,97	<0,3	<0,3
Vålådalen	<0,3	<0,3	<0,4	0,71	1,26	1,86	0,69	0,50	<0,3	<0,5	<0,5	0,76	13,4	<0,3	<0,3