

# Musk Compounds in the Nordic environment



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Betty Bügel Mogensen, Gunnar Pritzl, Suresh Rastogi, Ola Glesne, Britta Hedlund, Juha-Pekka Hirvi, Alf Lundgren & Albert Sigurdsson

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## **Musk Compounds in the Nordic environment**

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# Contents

Summary.....	7
Resumé 9	
<b>1 Introduction .....</b>	<b>11</b>
<b>2 Background .....</b>	<b>13</b>
<b>3 Musk findings in other studies .....</b>	<b>15</b>
3.1 <i>Musk compounds in the Nordic countries.....</i>	<i>17</i>
<b>4 Samples from the Nordic Countries.....</b>	<b>23</b>
4.1 <i>Choice of matrix.....</i>	<i>23</i>
4.2 <i>Sample collection.....</i>	<i>23</i>
4.3 <i>Methods of analysis .....</i>	<i>25</i>
4.3.1 <i>Principles.....</i>	<i>25</i>
4.3.2 <i>Extraction methods.....</i>	<i>25</i>
<b>5 Results.....</b>	<b>27</b>
<b>6 Discussion and conclusion.....</b>	<b>37</b>
<b>7 References .....</b>	<b>41</b>
<b>Appendix 1 .....</b>	<b>45</b>
<b>Appendix 2 .....</b>	<b>49</b>
<i>Information on sampling stations .....</i>	<i>49</i>
Denmark.....	49
Finland.....	52
Iceland.....	58
Norway.....	62
Sweden.....	67



# Summary

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A screening project of synthetic musk compounds in the environment of the Nordic countries was carried out in the second half of 2002. The project was initiated by the Nordic Terrestrial Monitoring Group, NTEM, with support from the Nordic Council of Ministers and from each of the Nordic Countries (Monitoring and Data Group and Chemicals group).

The project was carried out by the National Environmental Research Institute (NERI) in Denmark with the NTEM group serving as a steering group.

Each country was responsible for collection and transport of the samples according to a sampling manual.

23 samples of rain water, 27 samples of sludge from sewage treatment plants, 20 samples of blue mussels and 15 samples of liver from red fox, polar fox or racoon dog have been analysed for content of nine synthetic musk compounds i.e. six polycyclic musks: Cashmeran (DPMI), Celestolide (ADBI), Phantolide (AHDI), Traseolide (ATII), Galaxolide (HHCB) and Tonalide (AHTN) and three nitro-musk compounds: Musk xylene, musk keton and musk ambrette. These compounds represent the major part of synthetic musk compounds used in cosmetics, washing and cleaning agents etc.

The results show high concentrations of especially polycyclic musk compounds in sewage sludge, maximum concentration of Galaxolide being 26,500 µg/kg dry weight and of Tonalide 3,600 µg/kg dry weight. Polycyclic musks were detected in 11/20 samples of blue mussels. Celestolide is detected most frequently and at the highest concentration, 11,500 ng/g lipid.

Galaxolide was detected in 4/23 rainwater samples at concentrations in the range 12-29 ng/l. One sample additionally contained tonalide 35 ng/l.

Nitro musk compounds were only exceptionally detected in any samples

The environmental significance of the findings is difficult to assess, as there is a lack of data concerning effects of synthetic musk compounds.

To reduce the burden of the nordic environments with musk compounds following steps may be required: Specific regulations concerning emissions, updating sewage plants to efficiently degrade the musk compounds, and monitoring of musk compound in the environment, effluents and sewage sludge in particular. Monitoring of musk compounds in aquatic fauna is also relevant and should be focused on tissue with high lipid content. In rain water galaxolide and tonalide could serve as indicator compounds in monitoring programmes.





# Resumé

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Et screeningsprojekt der omfatter syntetiske musk stoffer i miljøet i de nordiske lande, blev gennemført i anden halvdel af 2002. Projektet blev igangsat af gruppen Nordic Terrestrial Environmental Monitoring group, NTEM, med støtte fra Nordisk Ministerråd, Monitoring og Data gruppen og Kemikaliegruppen.

Projektet blev udført af Danmarks Miljøundersøgelser (DMU) idet NTEM gruppen fungerede som styringsgruppe.

Hvert land havde ansvar for indsamling og forsendelse af prøverne i henhold til en rundsendt manual.

23 regnvandsprøver, 27 prøver af spildevandsslam, 20 prøver af blåmuslinger og 15 prøver af lever fra rød ræv, polarræv eller mårhund blev analyseret for indhold af 9 syntetiske musk stoffer. Disse består af 6 polycykliske musk stoffer: Cashmeran (DPMI), Celestolide (ADBI), Phantolide (AHDI), Traseolide (ATII), Galaxolide (HHCB) og Tonalide (AHTN) og tre nitro-musk forbindelser: Musk xylene, musk keton og musk ambrette. Disse stoffer repræsenterer størstedelen af de syntetiske musk stoffer, der finder anvendelse i kosmetik, rengørings- og rensmidler m.m.

Resultaterne viser høje koncentrationer af især de polycykliske musk stoffer i spildevandsslam. Koncentrationen af Galaxolide er op til 26.000 µg/kg tørstof og af Tonalide op til 3.600 µg/kg tørstof.

Polycykliske musk stoffer blev påvist i 11/20 prøver af blåmuslinger. Celestolide er det stof, der oftest er påvist og det findes i den højeste koncentration, 11.500 ng/g fedtstof. Galaxolide blev påvist i 4/23 regnvandsprøver i koncentrationen 12-29 ng/l. En prøve indeholdt desuden Tonalide i koncentrationen 35 ng/l.

Nitro musk forbindelser blev kun undtagelsesvis detekteret i nogen af prøverne.

Det er vanskeligt at vurdere den miljømæssige betydning af fundene af musk stoffer, idet der mangler data vedrørende effekten af syntetiske musk stoffer i miljøet.

For at nedsætte belastningen af miljøet i de nordiske lande med musk stoffer, kan det være nødvendigt at gennemføre nogle tiltag: Specifikke reguleringer af udledningen, forbedring af rensningsanlæggenes nedbrydning af musk stofferne og monitoring af musk forbindelser i miljøet. Monitoringen bør især rette sig mod spildevandsslam og udløbsvand fra rensningsanlæg. Monitoring af musk forbindelser i akvatisk fauna er også relevant og bør fokusere på væv med højt fedtindhold. I regnvand kan Galaxolide og Tonalide med fordel bruges som indikatorstoffer i monitoringsprogrammer.



# 1 Introduction

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The Nordic environmental collaboration (Nordiske miljøsamarbejde) 2001 – 2004 is a program directed by the Nordic Council of Ministers. It is meant to be a collaborative tool to guide and prioritise the activities and initiatives that must be started. Chemicals have high priority, and the aim is to ensure that the use of chemicals does not involve the risk of negative effects on human health and the environment. Furthermore the emissions of chemicals, that present a threat to health and the environment, must cease within a generation, defined as 25 years (Det Nordiske Miljøhåndlingsprogram 2001-2004).

To fulfil these objectives it is important to clarify and describe the state-of-the-art with respect to the occurrence of specific chemicals. The working group Nordic Terrestrial Environmental Monitoring group (NTEM) was supported by the Nordic Council of Ministers (Monitoring and Data group and Chemicals group) to carry out screening of potentially harmful chemicals in the environment. In 2001 musk compounds were selected for screening.

The NTEM group encompasses one member and one substitute from each of the Nordic Countries. The members constitute the steering group for the project. These are Ola Glesne, Norwegian Pollution Control Authority, Norway (chairman), Britta Hedlund, Swedish Environmental Protection Agency, Sweden, Juha-Pekka Hirvi, Finnish Environment Institute, Finland, Albert Sigurdsson, Environment and Food Agency, Iceland and Betty Bügel Mogensen, National Environmental Research Institute, Denmark and Alf Lundgren from National Chemicals Inspectorate in Sweden who represents The Nordic Chemicals group in the steering group. The steering group has designed the screening programme and prepared a tender invitation. The steering group has been responsible for collection and sending of all samples included in this project. The group wants to acknowledge all the national institutes, authorities and private people in the Nordic countries who have contributed to the sample collection.

The analysis programme and the reporting were carried out at NERI after a tender procedure, in which Erik Kirknel from the Danish Institute of Agricultural Science represented Denmark in the steering group. This report presents the results from this screening programme and discusses the results. The authors want to thank laboratory technicians Celestin Gnahore and Claus Jacobsen for their efforts with the chemical analyses.



## 2 Background

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Synthetic musks mainly encompass two groups of compounds: Nitro musks and polycyclic musks. The nitro musks include a group of five synthetic alkylated nitro benzenes: musk moskene, musk tibetene, musk xylene, musk ketone and musk ambrette. The polycyclic musks are chemically alkylated tetralin or indane systems. Main representatives are Galaxolide (HHCB), Tonalide (AHTN), Celestolide (ADBI), Phantolide (AHMI) and Traseolide (AITI) (Herren and Berset 2000). Cashmeran (DPMI) resembles the polycyclic musks and is considered to be a polycyclic musk by Rimkus 1998, but structure, physical and chemical properties and odour differ from those of regular polycyclic musks. A sixth polycyclic musk, Versalide, has not been produced since 1980 because of strong neurotoxic effects (Rimkus 1999). Galaxolide and Tonalide are the two most important polycyclic musks probably sharing 95% of the market for polycyclic musks.

The musk compounds are widely used as fragrance ingredients in washing and cleaning agents, fabric softeners, air fresheners, shampoos, perfumes and other cosmetic products, as food additives in fish baits and in cigarettes (Herren and Berset 2000, Kallenborn et al 1999). Human skin is exposed directly to musk compounds from many of these products. From the use in human household products synthetic musks are released to the atmosphere and to waste water. From wastewater treatment plants the compounds are released with effluents to the aquatic environment

The EU Commission has initiated a risk assessment of musk ketone and musk xylene. The authors have had access to draft reports on the environmental risk assessments, EU 2002 and EU 2003. Musk ketone and musk xylene shows no degradation in biodegradation tests. However, measurements of influent and effluent in sewage treatment plants indicate that 80-92% of musk ketone and 95-98% of musk xylene are removed by the treatment in the plant. It is suggested that apart from adsorption also a biotransformation take place during an anaerobic phase of the treatment reducing the nitro musks into the corresponding amino compounds. Musk compounds are only partly degraded in sewage treatment plants so they are introduced to the environment via effluent from sewage plants and are present in sewage sludge. The compounds are stable in the environment and may also bioaccumulate in the food chain (Suter-Eichenberg et al 1998). Bioconcentration factor of musk xylene is 1,600 l/kg (EU 2003) and that of musk ketone 1380 l/kg (EU 2002) whole fish wet weight.

The import of musk ketone and musk xylene in Europe in 2000 was 35 tonnes and 67 tonnes respectively (EU 2002 and EU 2003). The two polycyclic musks Galaxolide and Tonalide were used in amounts of 3285 t in 1992 and 2067 t in 1995 (Kallenborn et al 1999). Consumption of nitromusks in Norway in 2001 was 400 kg of musk ketone and 200 kg of musk xylene. The estimated maximum emission of musk xylene to the environment was 0.3 kg to the atmosphere and 338 kg to the aquatic environment with cosmetics and cleaning agents as primary source. (Glesne 2003).

Consumption in Denmark according to the Danish Product Register is: Galaxolide 333 kg/year mainly used in cosmetics and cleaning agents, Tonalide 33 kg/year and Traseolide 14 kg/year both mainly in cleaning agents, Cashmeran and Celestolide about 1 kg/year and Phantolide less than 1 kg/year. Musk xylene, 134 kg/year and musk ketone, 20 kg/year are mainly used in cosmetics. Information about use of fragrances

forwarded to the Product Register is given voluntarily. The consumption indicated above is therefore a minimum value.

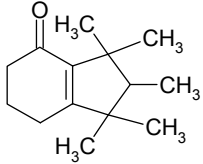
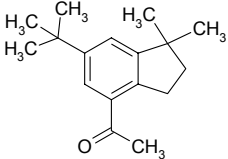
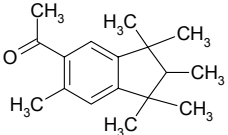
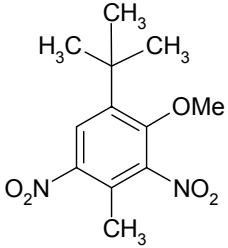
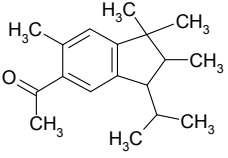
Musk compounds have been identified in the aquatic environment and in human milk. However, prior to this project only little was known about occurrence of musk compounds in the environment in the Nordic Countries. Because of the lack of Nordic data, the high production volume and the environmental properties mentioned above, the NTEM working group selected musk compounds as the first group of pollutants to be screened in a joint Nordic programme. The EU Commission's Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) has given opinions on safety evaluation of use of some musk fragrances in consumer products. However, the committee has not considered possible effects in the environment.

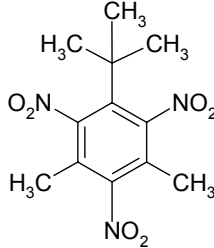
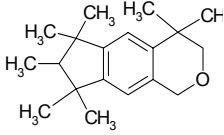
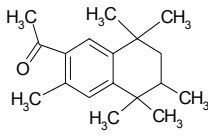
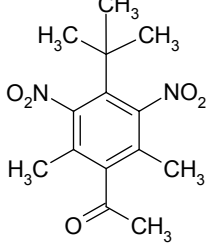
**Table 3.1** provides an overview of names and chemical structures of the musk compounds that are included in the current project. Table displays physical chemical data for some of the compounds.

### 3 Musk findings in other studies

A full review of musk compounds in the environment was not intended in this report. However, this chapter provides an overview of some recent findings. Only musk compounds that are analysed in the current screening project are referred. These compounds are listed in Table 3.1

**Table 3.1 Common name/trade name, chemical name, CAS no. and chemical structure of musk compounds included in the analysis programme**

Common name/trade name	Chemical name (Short name)	CAS no.	Structure
Cashmeran	6,7-Dihydro-1,1,2,3,3-pentamethyl-4(5H)indanone (DPMI)	33704-61-9	
Celestolide	4-Acetyl-1,1-dimethyl-6- <i>tert</i> -butyldihydroindene (ADBI)	13171-00-1	
Phantolide	6-Acetyl-1,1,2,3,3,5-hexamethyldihydroindene (AHDI)	15323-35-0	
Musk ambrette	1- <i>tert</i> -Butyl-2-methoxy-4-methyl-3,5-dinitrobenzene (MA)	83-66-9	
Traseolide	5-Acetyl-1,1,2,6-tetramethyl-3-isopropyl-dihydroindene (ATII)	68140-48-7	

Common name/trade name	Chemical name (Short name)	CAS no.	Structure
Musk xylene	1- <i>tert</i> -Butyl-3,5-dimethyl-2,4,6-trinitrobenzene (MX)	81-15-2	
Galaxolide	1,3,4,6,7,8-Hexahydro-4,6,6,7,8-hexamethylcyclopenta[g]-2-benzopyrane (HHCB)	1222-05-5	
Tonalide	7-Acetyl-1,1,3,4,4,6-hexamethyltetrahydro-naphtlene (AHTN)	1506-02-1	
Musk ketone	1- <i>tert</i> -Butyl-3,5-dimethyl-2,6-dinitrobenzene (MK)	81-14-1	

Physical chemical data for most of the compounds were available from Nylander 2001. (Table 3.2) Musk xylene and musk ketones are similar in properties and musk ambrette will possibly have similar properties as well. Cashmeran differs in structure from the polycyclic musk compounds by not having a benzene ring but the properties would probably resemble those of the polycyclic musks. All compounds show low water solubility and high log  $K_{ow}$  value indicating that the musk compounds are likely to accumulate in tissues with high lipid content.



**Table 3.2 Partition coefficients (octanol/water, log K<sub>ow</sub>) water solubility and molecular weight of musk compounds.**

Compounds	Log K <sub>ow</sub>	Water solubility (mg/l)	Molecular weight (g/mol)	Vapor pressure (Pa)
ADBI	5.9	0.22	244	
AHDI	5.85	0.25	244	
ATII	6.3	0.09	258	
MX	4.3	0.46	294.3	0.03x10 <sup>-3</sup>
HHCB	5.9	1.65	258.4	0.068
AHTN	5.7	1.22	258.4	0.073
MK	4.3	0.46	294.3	0.04x10 <sup>-3</sup>

Nylander 2001

### 3.1 Musk compounds in the Nordic countries

**Table 3.3** displays recent findings of synthetic musk compounds in the Nordic countries while Table 3.4 displays findings in other countries.

Emphasis has been put on samples similar to those in the current project from other European countries and a few data on sewage treatment plants in the USA.

EU 2002 and EU 2003 refer data on toxicity of musk ketone (MK) and musk xylene (MX) respectively. NOEC (No Observed Effects Concentration) is 0.088 mg/l (MK) and 0.56 mg/l (MX) for the algae *Selenastrum capricornutum*, 0.17 mg/ (MK) and 0.056 mg/l (MX) for the crustacea *Daphnia magna* reproduction test, 0.063 mg/l (MK) for rainbow trout (21-d flow through) and <0.1 mg/l (MX) for zebrafish (14 d semistatic). NOEC is the most sensitive toxicity end point.

**Table 3.3 Findings of musk compounds in the Nordic countries. Literature data**

Aquatic Environment													
<i>Sampling site</i>	<i>Sample type</i>	<i>Extractable lipid %</i>	<i>DPMI Ng/g lipid</i>	<i>HHCB ng/g lipid</i>	<i>AHTN ng/g lipid</i>	<i>ATII ng/g lipid</i>	<i>ADBI ng/g lipid</i>	<i>AHDI ng/g lipid</i>	<i>MX ng/g lipid</i>	<i>MK ng/g lipid</i>	<i>MA ng/g lipid</i>	<i>Reference</i>	
<b>Norway</b>													
Trondheim, inner harbour	Thornback ray filet	0.86	n.a	73	89	17	n.a	n.a	8	13		Kallenborn et al 2002	
do	Thornback ray, liver	39.6	n.a	21	3	2	n.a	n.a	1	1		do	
do	Haddock, filet	0.29-1.3	n.a	112-574	135-373	5-93	n.a	n.a	178	n.q.		do	
do	Haddock, liver	63.7-67.4	n.a	73-374	15-34	2-26	n.a	n.a	0.1	0.3		do	
Tromsø, inner harbour	Atlantic cod, filet	0.42-1.25	n.a	25-56	13-47	n.q.-11	n.a	n.a	3-4	1-3		do	
do	Atlantic cod, liver	15.4-28.6	n.a	29-43	6-10	2-3	n.a	n.a	2-4	n.q.-1		do	
do	Saithe, filet	2.3	n.a	225	93	13	n.a	n.a	n.a	n.a		do	
do	Saithe, liver	37.1	n.a	7	1	1	n.a	n.a	n.a	n.a.		do	
Oslo Fjord 6 sites	Atlantic cod, liver	28.5-42.8	n.a	132-1510	81-380	5-25	n.a	n.a	35-70	14-42		do	
Larvik, inner harbour	Atlantic cod liver	11.5	n.a	27	14	n.q.	n.a	n.a	4	2		do	
Grenland fjord 3 sites	Atlantic cod liver	26.3-45.2	n.a	9-31	3-13	n.q.-1	n.a	n.a	0.3-7	0.1-2		do	
<b>Sweden</b>													
Lake Hjärtsjön	Perch	0.40-0.79	n.a	89-367	30-125	n.d.	n.d.	n.d.	n.d.	n.d.	n.a	Hajslova 2002	
Lake Kväddöfjärden	Perch	0.61-0.95	n.a	97-254	<18-85	n.d.	n.d.	n.d.	n.d.	n.d.	n.a	do	
Lake Bysjön	Perch	0.26-0.50	n.a	<18-165	<18-41	n.d.	n.d.	n.d.	n.d.	n.d.	n.a	do	
River Viskan, 3 sites	Bream	0.95-3.11	n.a	<18-10272	<18-367	n.d.-38	n.d.-149	n.d.	n.d.-<18	n.d.	n.a	do	

<i>Sampling site</i>	<i>Sample type</i>	<i>Extractable lipid %</i>	<i>DPMI ng/g lipid</i>	<i>HHCB ng/g lipid</i>	<i>AHTN ng/g lipid</i>	<i>ATHI ng/g lipid</i>	<i>ADBI ng/g lipid</i>	<i>AHDI ng/g lipid</i>	<i>MX ng/g lipid</i>	<i>MK ng/g lipid</i>	<i>MA ng/g lipid</i>	<i>Reference</i>
Southern Vättern	Perch	0.69-1.15	n.a	254-655	155-301	n.d.	n.d.	n.d.	n.d.	n.d.	n.a	do
do	Arctic char	1.74-4.13	n.a	133-355	89-225	n.d.	n.d.	n.d.	n.d.	n.d.	n.a	do
Northern Vättern	Perch	0.58-1.15	n.a	198-254	124-154	n.d.	n.d.	n.d.	n.d.	n.d.	n.a	do
do	Arctic char	2.75-3.97	n.a	83-248	42-113	n.d.	n.d.	n.d.	n.d.	n.d.	n.a	do
<b>Denmark</b>												
Denmark	Rainbow trout	2.6-3.3	n.a	0.11-0.65	0.20-0.59	n.a	n.a	n.a	0.10-0.21	0.03-0.12	n.a	Gater-mann et al 1999
Fish ponds, Denmark	Trouts 50 samples	2.23-11.9	n.d-9523	n.d.-1065	5.70-252	n.d.-204	n.d.-242	n.d.-284	n.d.-26.8	n.d.-80.5	n.d.-30.2	Pedersen 2000
Fish ponds, Denmark	Trouts 50 samples	µg/kg fresh weight	n.a	n.d.-52.6	0.44-15.9	n.d.-15,6	n.d.-18.3	n.d.-21.5	n.d.-1.59	n.d.-5.16	n.d.-1.93	Pedersen 2000

#### Breast milk

<i>Sampling site</i>	<i>Sample type</i>	<i>Extractable lipid %</i>	<i>DPMI ng/g lipid</i>	<i>HHCB ng/g lipid</i>	<i>AHTN ng/g lipid</i>	<i>ATHI ng/g lipid</i>	<i>ADBI ng/g lipid</i>	<i>AHDI ng/g lipid</i>	<i>MX ng/g lipid</i>	<i>MK ng/g lipid</i>	<i>MA ng/g lipid</i>	<i>Reference</i>
Denmark	10 samples		n.d	38-422	5.58-37.9	n.d.-2.58	n.d.-11.2	n.d.-9.94	n.d.-46.4	n.d.-26.9	n.d.-46.4	Pedersen 2000
Sweden	30-44 samples		n.a.	14.5-370	<6-71.4	<3-6.7	<3-9.3	n.a.	<9-107	<9-52.9	n.a.	Eriksson et al 2003

#### Sewage water treatment plants

<i>Sampling site</i>	<i>Sample type</i>	<i>HHCB ng/g dry weight</i>	<i>AHTN ng/g dry weight</i>	<i>ATHI ng/g dry weight</i>	<i>ADBI ng/g dry weight</i>	<i>AHDI ng/g dry weight</i>	<i>MX ng/g dry weight</i>	<i>MK ng/g dry weight</i>	<i>MA ng/g dry weight</i>	<i>Reference</i>
Sweden										

Simsholmen, 2 dates nov. 2001	sediment	395-437	85-105	11	6.1-9.2	n.d.	2.2-5.2	n.d.	n.a.	Hajslova 2002
Huskvarna, 2 dates nov. 2001	sediment	453-833	106-194	13-22	8-23	n.d.	n.d.-4.5	n.d.	n.a.	do

**Table 3.4 Findings of musk compounds in the countries outside the Nordic countries with focus on the Northern part of Europe. Literature data**

Sampling site	Sample type	Extractable lipid %	HHCB ng/g lipid	AHTN ng/g lipid	ATI ng/g lipid	ADBI	AHDI	MX ng/g lipid	MK ng/g lipid	MA ng/g lipid	Reference
<b>Germany</b>											
River Ruhr	Breams	0.45-1.56	2.8-3.8	2.2-7.1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Gatermann et al 1999
Do	Perch	0.36-0.72	2.5-3.3	3.5-5.0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	do
Do	Eel	20.9-23.7	0.4-0.6	0.5-0.7	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	do
River Elbe	Eel	21.1-29.1	0.03-0.09	0.04-0.12	n.a.	n.a.	n.a.	0.01-0.07	0.01-0.03	n.a.	do
Do	Pike-perch	0.3-0.7	0.60-3.84	0.32-0.99	n.a.	n.a.	n.a.	<0.01-0.09	0.01-0.07	n.a.	do
River Stör	Brown trout	1.3-3.5	13.7-20.3	10.6-13.4	n.a.	n.a.	n.a.	0.20-0.24	1.00-1.15	n.a.	do
North Sea	Blue mussel	1.7-2.2	<0.03-0.11	<0.03-0.06	n.a.	n.a.	n.a.	<0.01-0.02	0.01-0.03	n.a.	do
Do	Shrimps	0.9-1.0	<0.04-0.37	<0.04-0.06	n.a.	n.a.	n.a.	<0.01-0.01	0.02-0.43	n.a.	do
Ponds at treatment plants in North Rhine Westfalia	Crucian carp	0.87-1.74	6.0-19.8	12.2-37.2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	do
Do	Chub	1.9-2.8	1.0-8.9	3.0-16.4	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	do
Pond at treatment plant in SchleswigHolstein	Eel	15.7-17.9	4.6-4.8	2.6-2.7	n.a.	n.a.	n.a.	0.21-0.27	0.35-0.45	n.a.	do
Do	Tench	0.5-1.4	147-159	30-42	n.a.	n.a.	n.a.	0.19-0.36	1.22-1.44	n.a.	do
Do	Rudd	0.7-0.9	6.2-7.5	5.0-6.1	n.a.	n.a.	n.a.	0.02-0.03	0.3-0.32	n.a.	do
Pond at treatment plant in SchleswigHolstein	Mussels	1.4	120	45	n.a.	n.a.	n.a.	0.12	1.28	n.a.	do
			µg/kg	µg/kg fresh		µg/kg		µg/kg fresh	µg/kg		

Sampling site	Sample type	Extractable lipid %	HHCB ng/g lipid	AHTN ng/g lipid	ATII ng/g lipid	ADBI	AHDI	MX ng/g lipid	MK ng/g lipid	MA ng/g lipid	Reference
			fresh weight	weight		fresh weight		weight	fresh weight		
Lakes, high input of sewage water	Eel muscle, 79 samples	mean value= 1364	mean value= 629	n.a.	n.a.	mean value= 4	n.a.	mean value= 39.4	mean value= 98.5	n.a.	Fromme et al 2001
Do	do	max value= 4131	max value= 1408	n.a.	n.a.	max value= 8	n.a.	max value= 170	max value= 380	n.a.	do
Lakes, low input of sewage water	Eel muscle 127 samples	mean value= 91	mean value= 35	n.a.	n.a.	n.d.	n.a.	mean value= 2.4	mean value= 3.8	n.a.	do
Do	do	max value= 306	max value= 176	n.a.	n.a.	n.d.	n.a.	max value= 74	max value= 120	n.a.	do

#### Breast milk

Sampling site	N	Extractable lipid %	DPMI µg/g lipid	HHCB µg/g lipid	AHTN µg/g lipid	ATII µg/g lipid	ADBI µg/g lipid	AHDI µg/g lipid	MX µg/g lipid	MK µg/g lipid	MA µg/g lipid	Reference
Germany, Schleswig-Holstein	5			0.016-0.108	0.011-0.058	n.a.	0.001-0.018	n.a.	0.01-0.03	0.005-0.015	n.a.	Rimkus 1998
Do	23		n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.02-0.19	0.01-0.09	n.a.	
Germany, Essen	2		n.a.	0.310-0.360	0.250-0.290	n.a.	0.020-0.024	n.a.	0.029-0.056	0.007-0.021	n.a.	

Sewage water treatment plants

Sampling site	Sample type	HHCB ng/L	AHTN	ATH	ADBI	AHDI	MX	MK	MA	Reference
USA	Waste water, influent	13700	10700	n.a.	n.a.	n.a.	376	569	n.a.	Simonich et al 2000
Do		7760	6990	n.a.	n.a.	n.a.	230	332	n.a.	do
Do	Waste water, final effluent	1170	1180	n.a.	n.a.	n.a.	5	99	n.a.	do

Sampling site	Sample type	DPMI µg/kg dry weight	HHCB µg/kg dry weight	AHTN	ATH	ADBI	AHDI	MX	MK	MA	Reference
Switzerland, sewage treatment plants	A. sludge, mainly domestic	47.2-332	2347-12157	973-4161	n.q.	61.6-245	103-843	n.d.	n.d.-6.9	n.d.	Herren and Berset 2000
Do	B. sludge, domestic +storm water runoff+some industrial	38.4-93.3	2293-4074	741-1418	n.q.	41-330	64.9-198	n.d.-32.5	n.d.-7.0	n.d.	do
Do	C. like B but more industrial waste water	64.1-147	2977-3229	784-1290	n.q.	96.6-113	180-266	n.d	n.d.	n.d.	do

## 4 Samples from the Nordic Countries

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### 4.1 Choice of matrix

Musk compounds are introduced into the environment from sewage water. They are partly retained and degraded in sewage treatment plants. During treatment in the plants musks may be released to the air. Effluents from treatment plants are a major source of synthetic musk compounds to the aquatic environment and can be accumulated in aquatic organisms. Musk compounds are further released directly to air from cosmetics and household products where musk compounds are used as fragrances. Rainwater may wash musk compounds from the atmosphere. Humans are exposed to musk compounds directly from cosmetics and washed fabrics and the compounds may end up in fat tissues and in breast milk. Musk compounds may enter the terrestrial food chain from the above mentioned sources and via household waste. Foxes in urban areas feed partly on garbage from households. They are also predators and may accumulate musk compounds via the food chain.

Based upon the above considerations the NTEM working group decided to focus on the following matrices for screening of musk compounds: Sludge from waste water treatment plants, rain water, blue mussels and fox liver. Sampling sites should preferably represent urban areas as well as more pristine areas. Breast milk was also considered an important matrix. However, the available budget and the cost of analysis made it necessary to prioritise the choice of matrix. In the final sampling programme breast milk was therefore not included in the monitoring programme.

### 4.2 Sample collection

A sampling manual was distributed to all members of the steering group prior to the sample collection took place. The sampling manual is attached as annex 1. Each of the Nordic Countries was responsible for collecting samples and sending the samples to NERI for analysis.

It has not been possible to collect all samples from all countries. Summer 2002 was very dry in Scandinavia making it difficult to collect rainwater. Further, it turned out to be difficult to get the proposed number of fox liver samples.

**Figure 4.1** provides an overview of the sampling sites and Annex 2 contains the information provided from each country.



Figure 4.1 All sampling sites from the Nordic countries. Each sampling station is indicated by a red circle.



## 4.3 Methods of analysis

### 4.3.1 Principles

The analytical method consists of an extraction and cleanup step and a chromatographic analysis. Extraction and cleanup differs according to sample type. Water samples were liquid-liquid extracted, sludge samples were extracted by shaking the samples with solvent and biota samples were extracted by soxhlet extraction. All detection was carried out by GC-MS.  $^{14}\text{C}$  marked Tonalide and Musk xylene were used as recovery standard in all samples. Alfa-amyl cinnamic aldehyde was used as an internal standard in some diluted sludge samples and biota samples.

### 4.3.2 Extraction methods

#### *Rain water*

Water samples: 250  $\mu\text{l}$  of recovery standard (200 ng/ml) were added to 1000 ml of rainwater. The water was shaken manually with dichloromethane (100+100+50 ml). The extracts were transferred to a round bottom flask through anhydrous sodium sulphate. The combined extracts were reduced by rotary evaporation (30°C) to 4-5 ml and transferred to 10 ml volumetric centrifuge tubes. Volume was reduced to 500  $\mu\text{l}$  under nitrogen at room temperature using toluene as a keeper. Volume was adjusted to 1 ml with toluene.

#### *Sewage sludge*

10 g of sewage sludge was extracted with 150 ml of dichloromethane:methanol (80:20) by initial sonification for 10 minutes followed by 2 hours of shaking on a shaking table. Before extraction, 250  $\mu\text{l}$  of recovery standard (200 ng/ml) was added to the sludge. The extract was decanted through anhydrous sodium sulphate into a round bottom flask. The volume was reduced by rotary evaporation to 500  $\mu\text{l}$  using toluene as a keeper. The extract was cleaned up by solid phase extraction on silica cartridges from International Sorbent Technology, 2 g of silica gel in glass tubes. The columns were conditioned with dichloromethane and pentane. After application of the sample, the column was rinsed with 5 ml of pentane and eluted with 10 ml of dichloromethane:methanol (80:20). The volume was reduced under nitrogen at room temperature to 500  $\mu\text{l}$  using toluene as a keeper. Volume was adjusted to 1 ml.

Some of the extracts showed high concentrations of some of the musk compounds. They were diluted and reanalysed.

#### *Biota*

All blue mussels from each sampling site were homogenised using ultraturrax homogenizer. Likewise each whole liver sample was homogenised. The homogenised samples were kept frozen (-18°C) until extraction. 10 g of blue mussel or 2 g of fox liver was soxhlet extracted with 300 ml of dichloromethane:methanol (80:20) for 16 hours. Before extraction, 200  $\mu\text{l}$  of recovery standard 200 ng/ml were added to the sample. The extract was filtered through anhydrous sodium sulphate into a round bottom flask and the volume was reduced by rotary evaporation to about 50 ml. The extract was transferred to a measuring flask and the volume was adjusted to 100 ml. 10 ml of the extract was used for determination of the fat content. The remaining 90 ml were further reduced to 2 ml. An aliquot of the extract, 1000 or 250  $\mu\text{l}$  of respectively

mussel and liver extract were cleaned up to remove the fat from the extract using a Phenogel GPC (Gel Permeation Chromatography) column 300x21.2 mm packed with a styrene-divinylbenzene polymer, 10 $\mu$  particle size, 100 Å pore size (Phenomenex). The HPLC system and fraction collector was from Gilson encompassing a 233 XL injection and collection system, a 322 pump, a 402 syringe pump and a 155 UV/VIS detector. Eluent was dichloromethane:methanol (98:2) at a flow of 4 ml/min. The fraction containing the musk compounds was selected after running a 10  $\mu$ g/ml standard solution and detecting the retention time with UV spectrometry. The 20 ml fraction was reduced to 500  $\mu$ l by nitrogen using toluene as a keeper and volume adjusted to 1 ml.

### Detection

Detection was carried out with GC-MS. The equipment was a Perkin Elmer Autosystem XL gas chromatograph equipped with a Perkin Elmer Turbomass quadrupole mass spectrometer. A Chrompack WCOT fused silica 50 m long, diameter 0.32 mm coated with CP-SIL 5 CB low bleed material, film density 1.20  $\mu$ m was used to separate the analytes. Calibration was carried out using a cubic curve fit.

### GC conditions

Splitless injection of 2  $\mu$ l. Injector temperature 250°C. Oven programme: Initial temperature 40°C, 40 to 220°C at 40°C/min, 220 to 280°C at 2°C/min hold for 3 minutes. Helium at a flow of 2.00 ml/min. was used as carrier gas. The capillary column was coupled to the ion source 250°C, transfer line temperature 280°C

### MS conditions

The following MS parameters were used (Table ): EI 70 eV, selected ion mode.

**Table 4.1 Retention times and ions used for identification and quantification of musk compounds**

Compound	Ret. Time min.	Qualifier ions [m/z]	Mass used for quantification [m/z]
Cashmeran (DPMI)	10.93	163, 206	191
Celestolide (ADBI)	14.47	173, 244	229
Phantolide (AHDI)	15.68	187,244	229
Musk ambrette (MA)	17.60	145, 268	253
Musk xylene*	18.04	136, 312	294
Traseolide (ATII)	18.28	173, 258	215
Musk xylene (MX)	18.50	128, 297	282
Galaxolide (HHCB)	18.65	213, 258	243
Tonalide*	18.73	190, 261	246
Tonalide (AHTN)	18.87	187, 258	243
Musk ketone (MK)	22.30	128, 294	279
Internal standard	Ret. time	Qualifier ions [m/z]	Mass used for quantification [m/z]
Alpha-amyl cinnamic aldehyde	12.79	145, 202	115

## 5 Results

Recovery and limits of detection for each matrix are indicated in **Table 5.1**. Recovery is determined with samples spiked with a mixture of musk compounds in a matrix similar to the natural sample. The samples were spiked at levels below 5 times the limit of detection. Limit of detection (LOD) is calculated as three times signal to noise in blanks of each matrix taking into account sample amount and recovery. LOQ in rainwater is calculated as three times standard deviation for 6 recovery samples spiked with 50 ng/l of all musk compounds. In sewage sludge and biological samples LOQ is calculated as three times LOD.

**Table 5.1 Detection and recovery data**

Matrix	Compound	Recovery %	Uncertainty %	LOQ	LOD	
<b>Rain water ng/l</b>	Cashmeran (DPMI)	40	25	8	5	
	Celestolide (ADBI)	47	24	14	5	
	Phantolide (AHDI)	50	24	16	5	
	Musk ambrette (MA)	53	19	11	10	
	Traseolide (ATII)	50	26	18	5	
	Musk xylene (MX)				10	
	Galaxolide (HHCB)	46	25	20	5	
	Tonalide (AHTN)	58	25	20	10	
	Musk ketone (MK)	56	20	25	25	
	<b>Sludge µg/kg wet weight</b>	Cashmeran (DPMI)		17	9	3
		Celestolide (ADBI)		22	9	3
Phantolide (AHDI)			22	9	3	
Musk ambrette (MA)			35	30	10	
Traseolide (ATII)			24	9	3	
Musk xylene (MX)			69	30	10	
Galaxolide (HHCB)			26	9	3	
Tonalide (AHTN)			25	15	5	
Musk ketone (MK)			39	45	15	
<b>Blue mussels ng/g lipid</b> Average lipid content 1.3 %		Cashmeran (DPMI)	23		260	85
	Celestolide (ADBI)	75		260	85	
	Phantolide (AHDI)	27		260	85	
	Musk ambrette (MA)	31		525	175	
	Traseolide (ATII)	73		260	85	
	Musk xylene (MX)	31		525	175	
	Galaxolide (HHCB)	35		260	85	
	Tonalide (AHTN)	21		525	175	
	Musk ketone (MK)	37		1310	435	

**Table 5.1 Continued**

<b>Fox liver ng/g lipid</b>	Cashmeran DPMI)	85		1155	385
Average lipid content 7.2 %	Celestolide ADBI)	110		1155	385
	Phantolide (AHDI)	45		1155	385
	Musk ambrette (MA)	30		2315	770
	Traseolide (ATII)	250		1155	385
	Musk xylene (MX)	35		2315	770
	Galaxolide (HHCB)	70		1155	385
	Tonalide (AHTN)	30		2315	770
	Musk ketone (MK)	35		5785	1930

Recovery of Traseolide above 200% is due to matrix interference which has a high impact at the very low concentration in the spiked samples.

Measured concentrations of musk compounds in the various matrices are displayed in **Table 5.2** to **Table 5.5**.

Table 5.2 Concentration of musk compounds in rainwater

Sampling site	Sample id.	DPMI ng/l	HHCB ng/l	AHTN ng/l	ATII ng/l	ADBI ng/l	AHDI ng/l	MX ng/l	MK ng/l	MA ng/l
<b>Denmark</b>										
Ulfborg	2-1315-mk	n.d	29	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Ulfborg	2-1829-mk	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Ulfborg	2-1830-mk	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Ulfborg	2-1831-mk	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Roskilde	2-1520-mk	n.d	13	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Roskilde.	2-1660-mk	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
<b>Finland</b>										
City-area of Helsinki	2-1258-mk	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Helsinki (industrial and main city,)	2-1556-mk	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Helsinki (industrial /urban)	2-1587-mk	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Helsinki (industrial /urban)	2-1588-mk	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Helsinki (industrial /urban)	2-1621-mk	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Pallas (Sweden) Pristine	2-1690-mk	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
<b>Iceland</b>										
Rjúpnahæð	2-1667-mk	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Hveravellir	2-1668-mk	n.d	12	35	n.d	n.d	n.d	n.d	n.d	n.d
Rjúpnahæð	2-1669-mk	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Rjúpnahæð	2-1670-mk	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Reykjavik	2-1671-mk	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d

**Table 5.2. Continued**

<b>Norway</b>													
Hurdal 1	2-1646-mk	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Hurdal 2	2-1656-mk	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Lista fyr 1	2-1657-mk	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Lista fyr 3	2-1658-mk	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Lista fyr 2	2-1659-mk	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
<b>Sweden</b>													
Vandprøve, Råö,	2-1689-mk	n.d.	16	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Blank values of HHCb (6 ng/ml) and AHTN (5 ng/l) are subtracted from the findings.

**Table 5.3 Concentration of musk compounds in sewage sludge**

Sampling site	Sample id.	Dry matter %	DPMI µg/kg dry weight	HHCb µg/kg dry weight	AHTN µg/kg dry weight	ATI µg/kg dry weight	ADBI µg/kg dry weight	AHDI µg/kg dry weight	MX µg/kg dry weight	MK µg/kg dry weight	MA µg/kg dry weight
<b>Denmark</b>											
Bjergmarken sewage plant (after drying) Roskilde	2-1498-mk	26.4	54	26500	3610	242	84	12	n.d.	n.d.	n.d.
Stige sewage plant (Odense)	2-1886-mk	15.0	25	13700	2020	891	178	32	n.d.	n.d.	83
Skævinge sewage plant	2-1887-mk	14.3	36	14600	2180	*	294	24	n.d.	n.d.	72
Hasselø sewage plant	2-1888-mk	15.8	n.d.	14100	1840	394	63	<12	n.d.	n.d.	35
Avedøre sewage plant	2-1889-mk	15.1	35	11400	1130	*	115	23	n.d.	n.d.	52
<b>Finland</b>											
Industrial/urban area of Porvoo	2-1254-mk	20.1	12	5650	2270	218	n.d.	22	n.d.	n.d.	n.d.
Rural area of Pornainen	2-1255-mk	16.0	59	8750	2120	136	n.d.	45	n.d.	n.d.	n.d.
Industrial/main city area of Helsinki	2-1256-mk	33.0	29	6060	1070	46	66	14	n.d.	n.d.	n.d.

**Table 5.3 Continued**

Sampling site	Sample id.	Dry matter %	DPMI µg/kg dry weight	HHCB µg/kg dry weight	AHTN µg/kg dry weight	ATII µg/kg dry weight	ADBI µg/kg dry weight	AHDI µg/kg dry weight	MX µg/kg dryweight	MK µg/kg dry weight	MA µg/kg dry weight
Industrial/ main city area of Espoo	2-1257-mk	17.0	<12	495	97	n.d.	12	<12	n.d.	n.d.	n.d.
(>10.000 pers. ) Helsinki)	2-1557-mk	29,1	41	6000	875	150	100	55	n.d.	n.d.	n.d.
<b>Iceland</b>											
"Klettagardar" sewage plant i Reykjavik	2-1474-mk	19.9	n.d.	2160	553	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
"Klettagardar" sewage plant i Reykjavik	2-1475-mk	22.5	n.d.	595	146	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
"Ananaust" sewage plant i Reykjavik	2-1476-mk	25.9	n.d.	408	98	n.d.	175	n.d.	n.d.	n.d.	n.d.
"Ananaust" sewage plant i Reykjavik	2-1477-mk	23.8	n.d.	437	118	n.d.	167	n.d.	n.d.	n.d.	n.d.
<b>Norway</b>											
Strømmen, AS sentralrenseanlægget - RA-2.	2-0975-mk	22.4	n.d.	7690	2100	99	n.d.	12	n.d.	n.d.	n.d.
Bekkelaget Sewage Treatment Plant, Oslo	2-0976-mk	30.4	n.d.	22400	3500	601	170	64	n.d.	n.d.	n.d.
Bekkelaget Sewage Treatment Plant, Oslo	2-0977-mk	34.4	128	15400	2280	232	139	n.d.	n.d.	n.d.	n.d.
Vinterbro, Nordre Follo Sewage Treatment Plant.	2-0978-mk	23.1	100	790	177	112	50	46	n.d.	n.d.	n.d.
Rygge , Fuglevik Sewage Treatment Plant.	2-0979-mk	27.2	n.d.	254	68	n.d.	148	n.d.	n.d.	n.d.	n.d.

Table 5.3 Continued

Sampling site	Sample id.	Dry matter %	DPMI µg/kg dry weight	HHCB µg/kg dry weight	AHTN µg/kg dry weight	ATII µg/kg dry weight	ADBI µg/kg dry weight	AHDI µg/kg dry weight	MX µg/kg dry weight	MK µg/kg dry weight	MA µg/kg dry weight
<b>Sweden</b>											
Vimmerby Treatment plant	2-1661-mk	19.0	<12	16200	2070	108	86	13	n.d.	n.d.	n.d.
Slam fra Vimmeby Treatment plant	2-1662-mk	19.2	17	24000	3350	252	153	33	n.d.	n.d.	n.d.
Slam fra Bromma Treatment plant	2-1663-mk	26.0	<12	10600	1020	65	52	<12	n.d.	n.d.	n.d.
Slam fra Bromma Treatment plant	2-1664-mk	25.8	<12	19200	1910	200	116	16	n.d.	n.d.	n.d.
Henriksdals Treatment plant (2b)	2-1665-mk	24.5	12	7820	1240	*	63	n.d.	n.d.	n.d.	n.d.
Vimmerby rensningsanlæg (2a)	2-1666-mk	25.5	n.d.	10600	952	38	53	12	n.d.	n.d.	23
Stråvliden	2-1771-mk	14.7	<12	11500	2070	241	83	34	n.d.	n.d.	n.d.
Slam fra Stenungsund	2-1772-mk	22.6	n.d.	17000	1110	126	52	n.d.	n.d.	n.d.	n.d.

\*) Traseolide (ATII) is present in these samples but could not be quantified.

Concentrations are not corrected for recovery. Spiked concentrations of <sup>14</sup>C-marked musk compounds could not be quantified.



Table 5.4 Concentration of musk compounds in blue mussels

Sampling site	Sample id.	Extractable lipid %	DPMI ng/g lipid	HHCB ng/g lipid	AHTN ng/g lipid	ATI ng/g lipid	ADBI ng/g lipid	AHDI ng/g lipid	MX ng/g lipid	MK ng/g lipid	MA ng/g lipid
<b>Denmark</b>											
Odense Fjord st. M2	2-1528-mk	0.85	n.d	n.d	<285	n.d	n.d	<285	n.d	n.d	n.d
Nyborg Fjord	2-1532-mk	1.4	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Lynetten	2-1536-mk	1.2	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Århus Bugt ydermole	2-1595-mk	0.53	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Roskilde Fjord, st. 65	2-1604-mk	1.4	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Halen	2-1736-mk	0.74	n.d	n.d	< 330	n.d	n.d	n.d	n.d	n.d	n.d
<b>Finland</b>											
Espoo city. (tæt ved udløb) Helsinki	2-1558-mk	1.3	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Espoo city. (just outside sea area)	2-1559-mk	1.8	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Helsinki coast	2-1560-mk	2.0	n.d	n.d	n.d	n.d	1135	n.d	n.d	n.d	n.d
Tvärminne (reference) at Hanko (southern Finland)	2-1586-mk	1.5	n.d	n.d	n.d	n.d	< 160				
<b>Iceland</b>											
"Hvassahraun" ( Reykjanes, near Reykjavik)	2-1478-mk	0.00	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
"Straumur" ( Reykjanes, near Reykjavik)	2-1479-mk	2.2	n.d	n.d	n.d	n.d	220	n.d	n.d	n.d	n.d
"Eyri" (Hvalfjörour, near Reykjavik)	2-1480-mk	1.9	n.d	n.d	n.d	n.d	720	n.d	n.d	n.d	n.d
"Hvítanes" (Hvalfjörour, Reykjavik)	2-1481-mk	2.2	n.d	n.d	<115	125	11500	n.d	n.d	n.d	n.d

**Table 5.4 Continued**

Sampling site	Sample id.	Extractable lipid %	DPMI ng/g lipid	HHCB ng/g lipid	AHTN ng/g lipid	ATII ng/g lipid	ADBI ng/g lipid	AHDI ng/g lipid	MX ng/g lipid	MK ng/g lipid	MA ng/g lipid
<b>Norway</b>											
Gressholmen (30 A)	2-1773-mk	0.82	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Ramton (307)	2-1774-mk	0.74	n.d	n.d	n.d	n.d	320	n.d	n.d	n.d	n.d
Gåsøya (304)	2-1775-mk	0.72	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Færder (36 A)	2-1776-mk	0.87	n.d	n.d	< 280	n.d	n.d	n.d	n.d	n.d	n.d
<b>Sweden</b>											
Bohus-Malmön	2-1507-mk	0.65	1140	n.d	570	n.d	n.d	n.d	n.d	n.d	n.d
Bohus-Malmön	2-1508-mk	0.91	960	n.d	< 265	n.d	n.d	n.d	n.d	n.d	n.d

**Table 5.5 Concentration of musk compounds in fox liver. The livers are from red fox unless otherwise indicated.**

Sampling site	Sample id.	Extractable lipid %	DPMI ng/g lipid	HHCB ng/g lipid	AHTN ng/g lipid	ATII ng/g lipid	ADBI ng/g lipid	AHDI ng/g lipid	MX ng/g lipid	MK ng/g lipid	MA ng/g lipid
<b>Denmark</b>											
(Vejen/Rødding (Southern Jutland)	2-1584-mk		<55	n.d	n.d	n.d	<30	<30	n.d	n.d	n.d
Nykøbing F. (Falster)	2-1632-mk	5.31	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
<b>Finland</b>											
Lahti (southern Finland).	2-1561-mk	8.89	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Raccoon dog (FIN 1)	2-1563-mk	6.31	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Near Evo (North of Helsinki)	2-1565-mk	8.75	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Raccoon dog, (Code su 5815) (FIN 2)	2-1566-mk	8.86	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d

**Table 5.5 Continued**

Sampling site	Sample id.	Extractable lipid %	DPMI ng/g lipid	HHCB ng/g lipid	AHTN ng/g lipid	ATTI ng/g lipid	ADBI ng/g lipid	AHDI ng/g lipid	MX ng/g lipid	MK ng/g lipid	MA ng/g lipid
<b>Iceland</b>											
7023 Miðnesheidi, við Norðurko	2-1672-mk	6.52	n.d	n.d	n.d	n.d	30	n.d	47	n.d	n.d
7024 Sandgerdi, við Tjörmina	2-1673-mk	5.85	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
7033 Kirkjuhvammshreppur, Illugastadalur	2-1674-mk	10.48	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
7035 Þverárhreppur, N-Krossanesfjell	2-1675-mk	7.37	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
7038 Strandahreppur, Grjótdalur	2-1676-mk	7.43	n.d	n.d	n.d	325	n.d	<20	n.d	n.d	n.d
7039 Strandahreppur, Grjótdalur	2-1677-mk	5.91	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
<b>Norway</b>											
Bærum kommune	2-1781-mk	7.37	n.d	n.d	n.d	n.d	46	n.d	n.d	n.d	n.d
Oslo Kommune	2-1783-mk	5.88	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Svalbard (polar fox)	2-1796-mk	6.96	n.d	n.d	n.d	n.d	224	n.d	n.d	n.d	n.d

The results reported in **Tables 5.2 – 5.5** are not corrected for recovery. It is not possible to give exact recovery percent for the musk compounds from mussels and liver samples as 5 out of six experiments (liver and mussels) to determine recovery did not show any recovery. The possible reason for this may be that the samples were spiked at a too low level to estimate recovery. Nominal concentration in the final extract was 5 ng/ml for fox liver and 20 ng/ml for blue mussels. Further, the GPC method used for clean up might have been unstable. Due to time pressure the experiments could not be repeated. Thus, the contents of musk compounds reported in Table 5.4 - Table 5.5 should be considered as the minimum concentrations in the analysed samples. Sediment samples were spiked with deuterium marked musk xylene and Tonalide but the compounds could not be quantified in the extracts. However, Peak areas in the chromatograms show high variation. Especially, the 4 samples from Iceland, the sample from the industrial area of Espo in Finland and from Strømmen sentralrensningsanlæg in Norway show low recovery.

## 6 Discussion and conclusion

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In 2002 the Nordic Environmental Monitoring group (NTEM) initiated a screening project supported by the NMR to generate information about the occurrence and distribution of musk compounds in the Nordic environment. The NTEM group was responsible for collection of samples that were transported to NERI in Denmark and analysed for content of 9 synthetic musk compounds.

Sample types included rain water, sewage sludge, blue mussels and fox liver.

Information from literature on findings in other studies are summarized in a separate chapter.

Polycyclic musks are detected more frequently and at higher concentrations than the nitro musk compounds. This is in good accordance with the use pattern of these compounds with polycyclic musks replacing nitro musks as there has been some concern about possible adverse effects of these compounds to the consumer.

The analytical methods are more sensitive for the polycyclic musks with higher recovery and lower detection limit. This may be another reason for the more frequent findings of polycyclic musks.

Musk compounds are generally not detected in rainwater. Two samples from Denmark, one from Sweden and one from Iceland contain Galaxolide (HCCB) and the Icelandic sample contained Tonalide (AHTN) as well. No pattern in the occurrence can be concluded from these findings.

Sewage sludge shows the highest frequency of findings. All sewage sludge samples contain polycyclic musk compounds and usually a mixture of all 6 compounds. This confirms the suggestion by EU 2002 and EU 2003 that musk compounds are removed from sewage water primarily by adsorption to sludge particles. Galaxolide and Tonalide are present at much higher concentrations than the other compounds. Galaxolide concentrations range within 250 - 26500  $\mu\text{g}/\text{kg}$  dry weight compared to 70 – 3600  $\mu\text{g}/\text{kg}$  dry weight of Tonalide. There is high variation, more than 1 order of magnitude, of recovery between sludge extracts. It is therefore not meaningful to compare concentrations at different sites. The big variation in concentration of different musk compounds in the extracts made it necessary to dilute the extracts several times to measure all the polycyclic musk compounds. This has further contributed to the overall uncertainty of the results. The results should be considered as minimum values.

Polycyclic musks are used in many consumer products like cosmetics and cleaning products and are therefore likely to end up in the sewer system. Even though most of the musk compounds may be adsorbed to sludge, effluents from sewage treatment plants are an important source of musk compounds in the aquatic environment. This is reflected in Gaterman et al 1999 who found high concentrations of musk compounds in eels from lakes with high input of effluents from sewage treatment plants compared to those in lakes with low input of effluents (Gaterman et al 1999). If sludge from sewage treatment plants are spread on agricultural land soil organisms will become exposed to musk compounds at a mg/kg level.

Gaterman et al 2002 have demonstrated that the bioaccumulation of musk compounds in freshwater fish and mussels is dependent on the fat content of the animal tissue as well as on the metabolism of these compounds by various species. An additional obvious factor is also the concentration of the musk compounds in the water. Thus, the animals

including mussels in pond water receiving effluents from sewage plants have been found to contain significant amounts of musk compounds, while the content of these compounds in animals in sea water has been found to be none to relatively low (Fromme et al 2001, Gaterman et al. 1999). In the present study polycyclic musks have been detected in 11 mussel samples out of 20 distributed all over the Nordic countries. Cashmeran was detected in two samples, Tonalide in 6 samples, Phantolide in one sample at concentration levels up to 960 ng/g extractable liquid. Celestolide was detected in 6 samples showing the highest concentrations, one sample from Iceland containing 11500 ng/g extractable liquid. The low content of lipid in mussels results in high uncertainty in the results. Galaxolide, the most frequently found musk compounds in the sewage sludge, was not detected in any of the samples. Galaxolide was found in mussels from ponds receiving effluents from sewage plants (Gaterman 1999). The absence of galaxolide in samples from the present study may be explained on the basis of the factors (discussed above), which influence bioaccumulation of musk compounds in mussels having less fat content. However, it can not be ruled out that the absence of galaxolide in any of the mussel samples may be associated with a discrepancy of the GPC method used for clean up.

Five out of the 15 investigated fox livers were found to contain musk compounds. The type and amount of musk compounds present in these samples were very different. Celestolide was detected in four samples with the highest concentration, 224 ng/g extractable liquid in a sample from the arctic area of Svalbard. Traseolide was detected in one sample, Phantolide in two samples and musk xylene in one sample. The variation may be expected as the native places and thus the food available to these animals also varies. There are no data available in the literature to compare our findings with any similar study. It should be noted that galaxolide was not found in any of the liver samples.

The absence or low frequency of musk xylene, musk ketone and musk ambrette in the environment may be explained by a limited use of these compounds in the consumer products. Musk ambrette is not permitted in cosmetic products due to its phototoxic or neurotoxic properties (EU Cosmetic Directive ). The Cosmetic Directive has recently regulated the use of musk xylene and musk ketone (maximum 1.0 % MX and 1.4% MK in fine perfumes) in cosmetic products (26th approximation of the Cosmetic Directive, 15 April 2002). Safety evaluation of MX and MK in EU (by ECB) is not yet complete.

The results of the present study are in agreement with the data in literature, which show that sewage sludge contain significant amounts of polycyclic musks. Although the toxic effects of these compounds at the levels found in the environment have not yet been demonstrated, these compounds may bioaccumulate. In the recently adopted opinions, EU Scientific Committee on Cosmetic Products and the Non-Food Products (SCCNFP) has found the use of 2,4% and 0,96% of galaxolide and tonalide, respectively, -in eau de toilette safe for consumers. However, the SCCNFP did not consider the environmental impact of these compounds.

Most of the studies, so far, assessing environmental burden of musk compounds have concentrated on nitromusks, galaxolide and tonalide. In the present study, all of the important industrial musk compounds were included. The study has revealed that celestolide is also very frequently found in the environment, and the concentration of this compound in the environment is comparable to those of tonalide and galaxolide. To reduce the burden of the nordic environments with musk compounds following steps may be required: Specific regulations concerning emissions, updating sewage plants to

efficiently degrade the musk compounds, and monitoring of musk compound in the environment, effluents and sewage sludge in particular. Monitoring of musk compounds in aquatic fauna is also relevant and should be focused on tissue with high lipid content. In rain water galaxolide and tonalide should serve as indicator compounds in monitoring programmes





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# Appendix 1

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Manual for sample handling and protocol of sampling for the project: “Analysis of Musk compounds in a Nordic cooperation on screening”.

## Sample types:

The project includes analysis of musk compounds in the following sample types (matrices):

A: Precipitation (rain) – to be taken during July – September

B: Municipal sewage sludge – to be taken as soon as possible (July –August)

C: Blue Mussel (*Mytilus edulis*) – to be taken October

D: Fox liver – to be taken as soon as possible (Latest end of September)

## General remarks:

Musk compounds are widely used in cosmetics (incl. soaps) and a row of technical products, so great care has to be taken to avoid contamination of the samples.

When handling the samples gloves are mandatory, and measures should be taken to exclude that the personnel involved are using cosmetics (especially soap, perfume and deodorants) containing musk compounds in the sample-handling period. Even cleaned laboratory air can contain vapors of musk compounds; so prolonged direct contact with the laboratory air should be avoided.

Furthermore all utensils coming in contact with the samples should be solvent rinsed with 3 times acetone and 3 times n-pentane following the normal cleaning. Glass and metal utensils should finally be heated for 2 hours at 450 °C; Teflon utensils should be heated for 12 hours at 200 °C.

Contact with polymer utensils should be kept at a minimum, and restricted to utensils made of Teflon and Nylon, the latter only in form of special sample bags as Rilsan® bags.

All samples of one kind from each country should be sent together to NERI as soon as possible after the sampling. NERI should be notified about the arrival of samples in order to ensure that the samples are received and handled properly.

### **A: Precipitation**

Five rain water samples should be collected from at least three stations representing different levels of expected emissions of musk substances, e.g. industrial area (especially if containing industries fabricating or using musk substances) or city-areas, suburban area, rural area.

The sampling should preferably take place during a period of warm weather over a period of max one month, covering precipitation of at least 20 mm. The sampling should preferably take place during periods of heavy rain.

The samples should preferable be taken with a cooled wet-only sampler (e.g. of a type like NSA 181/KE, G. K. Walter Eigenbrodt Environmental Measuring Systems, Königsmoor, Germany), with a funnel area of about 500 cm<sup>2</sup>. Otherwise the sampling laboratory has to insure that the sample temperature does not exceed 4 °C, that the sample is kept protected against UV-light and that no dry deposition takes place.

The final sampling size shall be 2 - 3 liter, corresponding to about 40-60 mm of precipitation, and shall be transported to the analytical laboratory preferable within 24 hours kept at a temperature between 0 °C and 4 °C together with the following information:

Information to be gathered and delivered together with the sample:

Position: Geographical coordinates, height above ground level, description of immediate surroundings, e.g. pine forest, street, open land etc.

Sampling area type: Industrial, city, suburban area, rural area.

Possible emitters of musk substances in the area

Sampling period

Weather condition during the sampling and the week before

Sampling method including description of the sampling device.

Other remarks

### **B: Municipal sewage sludge**

Five samples of municipal sewage sludge should be collected from at least three stations representing different levels of expected emissions of musk substances, e.g. industrial area (especially if containing industries fabricating or using musk substances and emitting waste water to the sewage plant) or city-areas, suburban area, rural area.

The sludge collected has to be fresh from the sewage plant, taken within one hour from final dewatering/stabilization, following a period of normal weather conditions.

Approximately 500 gram of sludge shall be collected in Rilsan bags or rinsed glass jars, and placed at 4 °C in the dark immediately.

The sample shall be transported to the analytical laboratory preferably within 24 hours at a temperature between 0°C and 4°C together with the following information:

Information to be gathered and delivered together with the sample:

Type of wastewater catchment area: Industrial, municipal or mixed wastewater

Possible emitters of musk substances in the area

Sampling date

Information about the plant (type, size, running conditions at the sampling time etc, any abnormalities in the operation of the plant during the week before sampling.)

Weather condition during the sampling and the week before

Other remarks

### **C: Blue Mussels**

Four samples of Blue Mussels (*Mytilus edulis*) should be collected from at least three stations representing different levels of expected emissions of musk substances, e.g. industrial area (especially if containing industries fabricating or using musk) or city-area, suburban area, rural area.

The mussel samples are collected as 30 – 40 preferably bottom-dwelling individuals at 40 – 60 mm lengths, collected by hand or trawl after the spawning season (in October).

The living mussels are rinsed for sand etc. at the shells with water from the sampling place, and depurated for 24 hours in the same type of water before dissection. After opening and passive dewatering for 10 seconds, all the soft tissue (incl. the adductor muscle) is removed and weighted for each individual and the length of the shell measured.

The soft tissue (incl. the adductor muscle) from all the mussels are pooled in a glass jar, and frozen at - 20 °C.

The sample shall be transported to the analytical laboratory preferably within 24 hours at a temperature between - 5 °C and - 20 °C together with the following information:  
Information to be gathered and delivered together with the sample:  
Position: Geographical coordinates, description of sampling area including water depth and type of bottom.  
Possible emitters of musk substances in the area  
Sampling date and method  
General information concerns the conditions of the mussels (fouling, physical state etc.).  
Other remarks

**D: Fox (liver)**

Four samples of Fox (*Vulpes vulpes*) liver should be collected from stations representing different levels of expected emissions of musk substances, e.g. industrial area (especially if containing industries fabricating or using musk) or city-area, suburban area, rural agriculture area and intended pristine nature area.

The fox liver samples shall consist of whole livers and lower jaws from two males of at least two years of age collected before the beginning of the autumn shedding to winter fur, preferable before the end of September.

The foxes are to be newly killed and dissected within 24 hours after the killing. The whole livers are placed in double Rilsan or Teflon bags, while the bags for the lower jaws can be of ordinary plastic.

The samples from the two individuals shall be kept separate, and frozen at - 20 °C within 4 hours from dissection.

The sample shall be transported to the analytical laboratory as soon as possible at a temperature between - 5 °C and - 20 °C together with the following information:

Information to be gathered and delivered together with the sample:

Position: Geographical coordinates, description of sampling area in general terms including a possible quote of the feeding habits of the actual foxes

Possible emitters of musk substances in the area

Sampling date and method

General information concerns the conditions of the foxes (sex, nutritional and physical state, a rough estimate of age etc.).

Other remarks





# Appendix 2

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## Information on sampling stations

### Denmark

The location of the Danish sampling sites and geographical coordinates and classification of the sites in relation to pollution can be found in table 1. The number of specimen collected from different sites is summarized in table 2. Even those sites, where collection did not succeed, are listed in table 2. Figure 1 displays the Danish sampling sites.



Figure 1. Danish sampling sites.

**TABLE 1. Coordinates of the sampling sites in Demark year 2002. Joint Nordic screening project on musk substances. National Environment Research Institute (NERI).**

Roskilde	55.65283	12.04097	Urban + industrial
Roskilde (marine area)	55.70043	12.06707	
Copenhagen	55.36291	12.27023	Urban + industrial
Copenhagen (marine area)	55.42572	12.36518	
Halen	55.26251	08.26334	Pristine
Skaevinge	55.54265	12.07465	Rural
Nykoebing F.	54.46264	11.52552	Suburban/rural
Skoerringe	54.50365	11.57401	Forest
Odense	55.23561	10.25167	Urban + industrial
Odense (marine area)	55.28458	10.29574	
Nyborg	55.16566	10.48277	Urban + industrial
Koebenhoved	55.25197	09.03546	Forest
Ulfborg	56.17380	08.25205	Forest
Aarhus	56.09326	10.14044	Urban + industrial

**TABLE 2. Summary of the samples collected at the sampling sites in Denmark 2002. Joint Nordic screening project on musk substances. National Environment Research Institute (NERI).**

Specimen	Total number of samples	Urban+Industrial sites (n)	Forest+rural sites (n)	Pristine sites (n)
Sludge	5	Copenhagen (1) Nykoebing F. (1) Roskilde (1) Odense (1)	Skaevinge (1)	
Rain water	5	Roskilde (2) Copenhagen (0)	Ulfborg (3)	
Blue mussel	6	Copenhagen (1) Odense (1) Aarhus (1)	Roskilde (1) Nyborg (1)	Halen (1)
Red fox	2	Copenhagen (0)	Skoerringe (1) Koebenhoved (1)	Ulfborg (0)

### *Sludge sampling*

Sampling was performed according to the method description of NERI Appendix 1. The samples were collected by NERI. All single samples are summarized in table 3.

**TABLE 3. Sludge samples collected by Denmark 2002. Joint Nordic screening project on musk substances. National Environmental Research Institute (NERI).**

Sample	Site /sewage plant	Person equivalent (peq)	Classification
Sample 1.	Skaevinge	5000 - 6000	rural municipal
Sample 2.	Roskilde	50000	mainly municipal less industry
Sample 3.	Avedøre	400000	heavy industrial+municipal
Sample 4.	Nykøbing F.	40 000	industrial+municipal mixed
Sample 5.	Odense NE	80 000	municipal + industrial mixed

### *Rain water sampling*

Three samples of rainwater were collected in the urban area of Roskilde (at mid-Zealand) at the ground of a sewage plant (sample 3 + 4 at one location, sample 5 100 m apart), and two samples were collected from a coniferous forest of pristine nature in western Jutland (Ulfborg). A planned sampling in the city of Copenhagen was cancelled for logistic reasons. The samplings were performed according to the method description of NERI Appendix 1 during september-november 2002. All samples are listed in table 4.

**TABLE 4. Samples of rainwater collected in Denmark 2002. Joint Nordic screening project on musk substances. National Environmental Research Institute (NERI).**

Sample	Site	Sampling period	Volume (L)
Sample 1.	Ulfborg	13.9.2002 - 20.10.2002	2 liter
Sample 2.	Ulfborg	20.10.2002 - 15.11.2002	2 liter
Sample 3.	Roskilde	26.9.2002 - 7.10.2002	2 liter
Sample 4.	Roskilde	7.10.2002 - 1.11.2002	2 liter
Sample 5.	Roskilde	2.11.2002 - 15.11.2002	2 liter

### *Blue mussel sampling*

Sampling of mussels was performed by the personnel from the different counties in connection with the sampling for the National Monitoring Programme at stations at Zealand, Funen and Jutland in October 2002. The mussels were dissected according to the method description given by NERI (ref: NERI-DK 28.6.2002). The soft tissue from at least 20 mussel individuals was kept frozen in brown glass jars until analysis. All samples are summarised in table 5.

**TABLE 5. Blue mussel samples collected in the coastal sea area of Denmark 2002. Joint Nordic screening project on musk substances. National Environmental Research Institute (NERI).**

Sample	Date	Site (name, depth in m)	Size class (mm); mean	Remarks
Sample 1.	October, 2002	Copenhagen (Lynetten, 5)	40-60 ; 48	In vicinity of effluent outlet
Sample 2.	October, 2002	Roskilde (Roskilde Fjord, 2)	40-60 ; 42	Rather unpolluted area
Sample 3.	October, 2002	Nyborg (Nyborg Fjord, 3)	40-60 ; 54	Industrial polluted
Sample 4.	October, 2002	Wadden Sea (Halen, 1)	40-60 ; 54	Reference sea area
Sample 5.	October, 2002	Odense (Odense Fjord, 4)	40-60 ; 49	In vicinity of effluent outlet
Sample 6.	October, 2002	Aarhus (Aarhus Harbour, 6)	40-60 ; 52	Polluted industrial harbour area

### *Fox samples*

The samples of liver tissue were collected from red fox (*Vulpes vulpes*). Only two animals could be sampled during the autumn 2002, and the planned collection of fox samples from urban/industrial location did not succeed. The summary of the samples with background information is presented in table 6.

**TABLE 6. The samples of red fox and raccoon dog collected in Denmark 2002. Joint Nordic screening project on musk substances. National Environmental Research Institute (NERI).**

Sample	Animal	Sex	Hunting date	Age (year)	Site and classification
Sample 1.	Red fox	F	25.10.2002	< 2	Koebenhoved Forest in rural area
Sample 2.	Red fox	F	10.11.2002	> 2	Skoerringe Forest in suburban area

### Finland

The location of the Finnish sampling sites is presented in the figure 2 and geographical coordinates and classification of the sites in relation to pollution can be found in table 7. Number of specimen collected from different sites is summarized in table 8.

Even those sites, where collection and hunting of animals didn't result any success, are pointed out in table 7 and figure 2.

**TABLE 7 Coordinates of the sampling sites in Finland year 2002. Joint Nordic screening project on musk substances. Finnish Environment Institute (SYKE).**

Sampling site	North	East	Site classification
Helsinki	60,2795	24,8735	Urban + industrial
Helsinki (sea area)	60,1072	25,0326	
Espoo	60,1158	24,7484	Urban + industrial
Porvoo	60,3774	25,6142	Urban + industrial
Pornainen	60,4608	25,3989	Rural / pristine
Jokela	60,5525	24,9543	Rural
Evo / Lahti	61,1954	25,1568	Suburban
Evo	61,1954	25,1568	Forest / pristine /South
Pallas	67,9980	24,1724	Forest / pristine /North
Kevo	69,6594	27,0519	Pristine / subarctic
Tvärminne	59,8376	23,2175	Pristine / marine

**TABLE 8 Summary of the samples collected in the sampling sites in Finland 2002. Joint Nordic screening project on musk substances. Finnish Environment Institute (SYKE).**

Specimen	Total number of samples	Urban+Industrial sites (n)	Forest+rural sites (n)	Pristine sites (n)
Sludge	6	Helsinki (2) Espoo (1) Porvoo (1)	Jokela (1) Pornainen (1)	
Rain water	5	Helsinki (5)	Evo (0)	Pallas (sampling by IVL Sweden)
Blue mussel	4	Helsinki (3)		Tvärminne (1)
Red fox Red fox Raccoon dog	4	Helsinki (0)	Evo (1) Evo/Lahti (1) Evo (2)	Kevo (0)

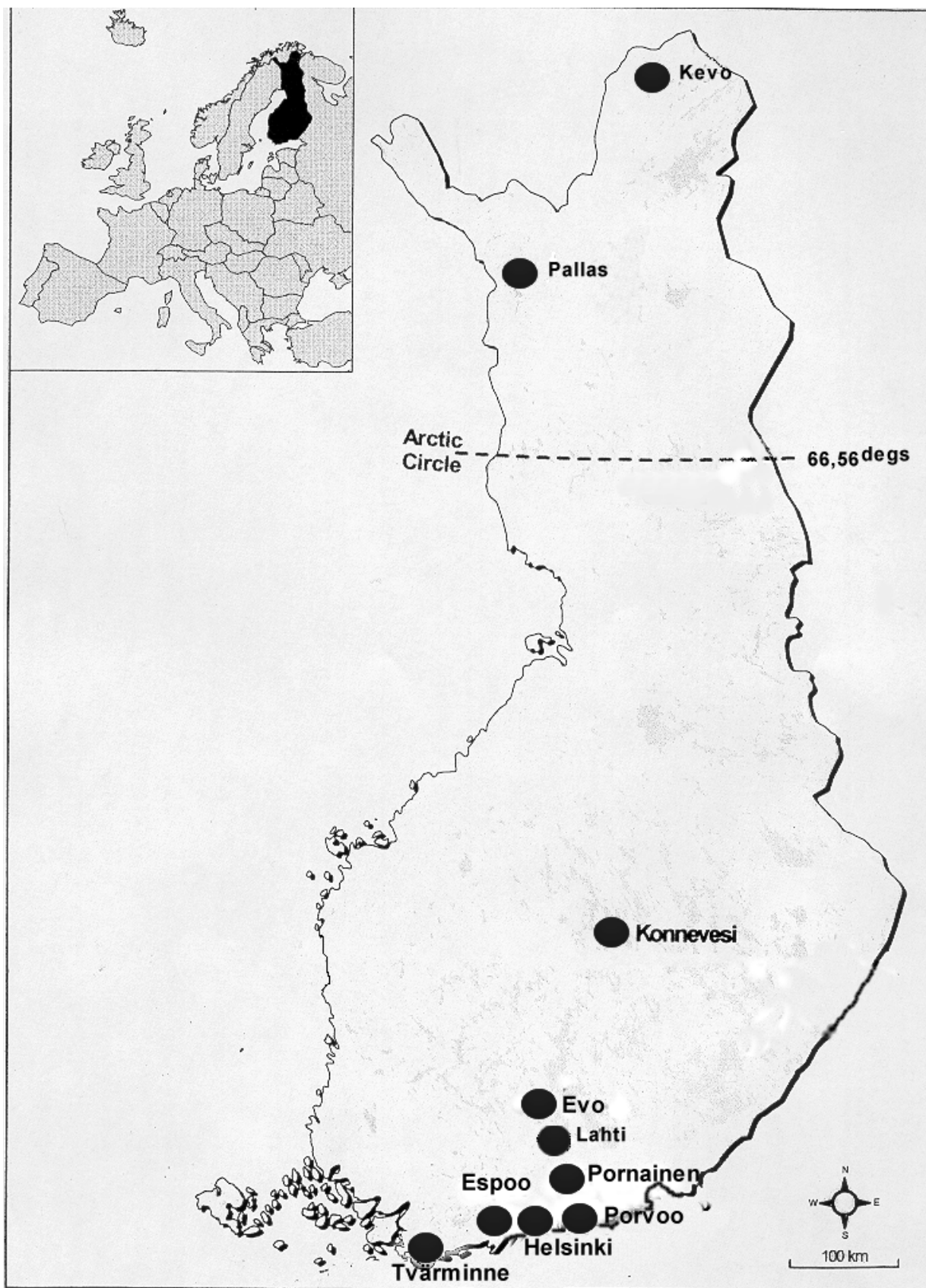


FIGURE 2. The sampling sites in Finland 2002. Joint Nordic screening project on musk substances. Finnish environment institute (SYKE).

### *Sludge sampling*

Sampling was performed according to the method description of NERI Appendix 1. The samples were delivered in glass jars to NERI. All single samples are summarised in table 9.

**TABLE 9 Sludge samples delivered by Finland 2002. Joint Nordic screening project on musk substances. Finnish Environment Institute (SYKE).**

Sample code /date	Site /sewage plant	Person equivalent (peq)	Classification	Remarks
Sample 1. FIN 3.9.2002	Jokela	5000 - 6000	rural municipal	Old system not dewatered not accepted
Sample 2. FIN 3.9.2002	Porvoo	> 10 000	industrial+municipal mixed	New system
Sample 3. FIN 3.9.2002	Pornainen	< 2 000	rural municipal	Old system
Sample 4. FIN 4.9.2002	Helsinki	> 10 000	industrial+municipal mixed	Largest treatment plant in the North
Sample 5. FIN 4.9.2002	Espoo	> 10 000	industrial+municipal mixed	New system, industrial emitter in the area
Sample 6. FIN 17.10.2002	Helsinki	> 10 000	industrial+municipal mixed	Replacing sample no 1 (Jokela)

### *Rain water sampling*

Five samples of rainwater were collected in the industrial urban area of Helsinki. In the original plan also samples from the Evo region (southern pristine area) and the Pallas station (northern pristine area) were intended, but because of very dry summer and autumn, the samples could be collected only from the Helsinki city area. The sampler was placed in the open wood area in the vicinity of SYKE's laboratory, which is locating a little bit outside of Helsinki city. The sampler was easy to serve daily if needed. The sampling apparatus requested by NERI in their manual couldn't be used and was not available. (Cleaning of sampler, tools and bottles was made according the NERI's manual). We used a self-manufactured sampler of stainless steel plate for intensive sampling of a single rain shower (Figure 3). Sampler's size was 1 m<sup>2</sup> and 1 mm rain gave 1 liter water. Sampling was started manually by opening the cover and putting 5 liters' glass bottle on its place whenever the rain began. After the rain shower, the glass bottle with rain was moved back to the cooling room (+4°C) in the laboratory. That was the only way to get rainwater collected because of very dry weather during the period from 1 July to 15 October, 2002. All samples are listed in table 10

**TABLE 10. Samples of rainwater collected in Finland 2002. Joint Nordic screening project on musk substances. Finnish Environment Institute (SYKE).**

Sample code	Site	Sampling date/period	Volume (L)	Remarks
Sample 1. FIN	Helsinki	30.8.2002	2 liter	Single heavy rain 5 mm/h after very long warm period
Sample 2. FIN 2.	Helsinki	16.9. – 15.10.2002	3 liter	Few single rain spouts – very dry
Sample 3. FIN 3.	Helsinki	26.-27.10.2002	5 liter	Heavy rain 3 mm/h
Sample 4. FIN 4.	Helsinki	31.10.-1.11.2002	5 liter	Heavy rain 2 mm/h
Sample 5. FIN 5.	Helsinki	9. – 10.11.2002	3 - 5 liter	Wet snow after stormy weather



**FIGURE 3. Pieces and tools for sampling of rain water in Southern Finland. Photo taken 14<sup>th</sup> November 2002. Joint Nordic screening project on musk substances. Finnish Environment Institute (SYKE).**

Figure 3. Demonstration of the sampler in use for intensive sampling of rainwater in the urban area of Helsinki, Finland. Photo taken 14<sup>th</sup> November 2002. Joint Nordic screening project on musk substances. Finnish Environment Institute (SYKE).



### *Blue mussel sampling*

Sampling of mussels was performed by the divers from SYKE (Helsinki and Espoo) and from the University of Helsinki (Tvärminne, research station). The mussels were dissected according to the method description given by NERI Appendix 1. The soft tissue from more than 60 mussel individuals was removed and weighted until to 20 g of total sample weight reached. The samples were frozen in Rilsan bags inserted in glass jars and sent cooled to the NERI. Observe that size class requested (preferred) in the NERI's manual was as high as 40 – 60 mm lengths. Such big individuals of blue mussel cannot be found in the northern Baltic Sea. All samples are summarised in table 11

**Table 11. Blue mussel samples collected in the southern coastal sea area of Finland 2002. Joint Nordic screening project on musk substances. Finnish Environment Institute (SYKE).**

Sample code	Date	Site* (depth, m)	Size class (mm) (mean $\pm$ SD; N)	Remarks
Sample 1. FIN 1.	16.10.2002	Espoo I (5m)	21 $\pm$ 4; 68	In vicinity of effluent outlet from Espoo
Sample 2. FIN 2.	16.10.2002	Espoo II (5m)	20 $\pm$ 3,5; 72	In vicinity of effluent outlet from Espoo
Sample 3. FIN 3.	16.10.2002	Helsinki (6m)	17,5 $\pm$ 3; 104	In vicinity of effluent outlet from Helsinki
Sample 4. FIN 4.	October, 2002	Tvärminne (8m)	25,5 $\pm$ 3; 79	Reference sea area
<b>Coastal sea sites* Espoo 1 = Longgrundet SW. Espoo 2 = Knapperskäret SW. Helsinki = Mustakupu SW, Santahamina E. Tvärminne = Brännskär (4 - 8 m).</b>				

### *Fox and racoon dog samples*

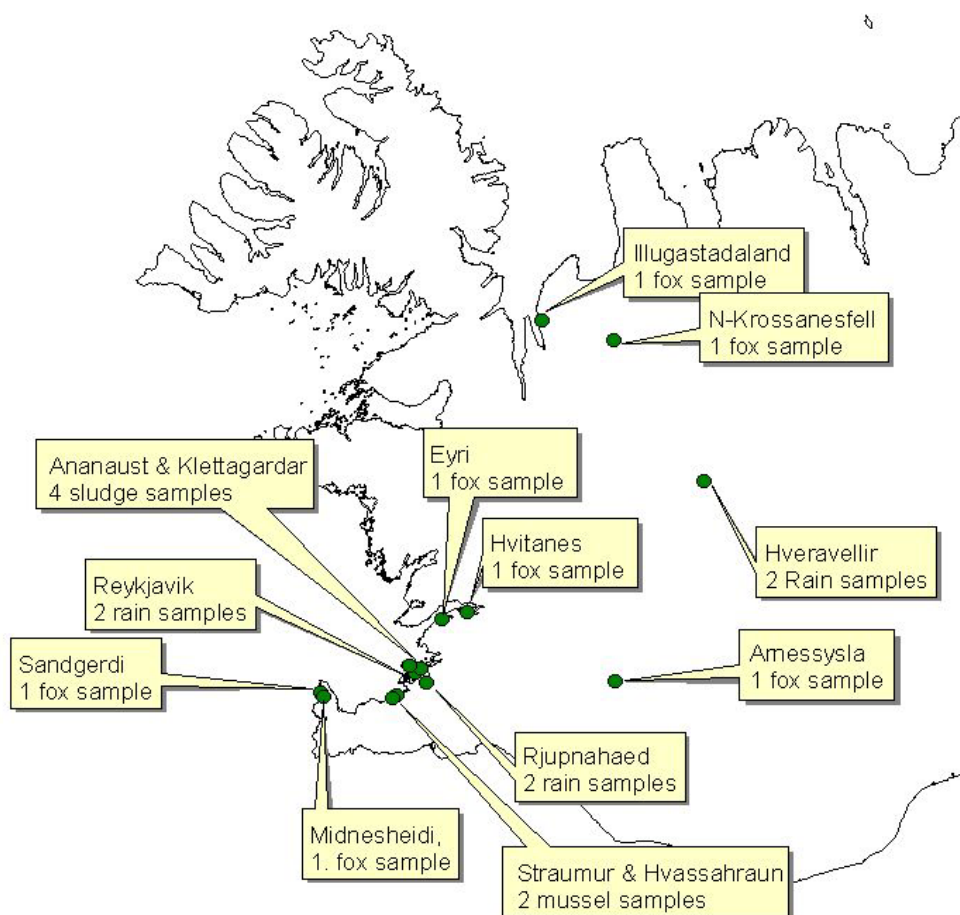
The samples of liver tissue were collected from red fox (*Vulpes vulpes*) and racoon dog (*Nyctereutes procyonoides*, Gray). Two animals could be hunted during the autumn 2002 (fresh samples), and other two were taken from the SYKE's environment specimen bank (banked samples). The summary of the samples with background information is presented in table 12

**Table 12. The samples of red fox and racoon dog collected in Finland 2002. Joint Nordic screening project on musk substances. Finnish Environment Institute (SYKE).**

Sample code	Animal	Sex	Hunting date	Specimen to NERI	Age (y)	Site and classification
Sample 1. FIN 1.	Red fox	F	5.10.2002	Liver + lower jaw	> 2	Evo/Lahti Suburban
Sample 2. FIN 2.	Racoon dog	F	10.8.2002	Liver + lower jaw	> 2	Evo pristine
Sample 3. FIN 3.	Red fox	M	1.12.2000 ESB code ke 603	Liver	2	Evo pristine
Sample 4. FIN 4.	Racoon dog	M	21.10.2000 ESB code su 5815	Liver	2	Evo pristine

## Iceland

The location of the Icelandic sampling sites is presented in the figure 4.



**FIGURE 4. The sampling sites in Iceland 2002. Joint Nordic screening project on musk substances. Environment & Food Agency of Iceland (UST).**

### *Sludge sampling*

Sampling was performed according to the method description of NERI Appendix 1. The samples were delivered in teflon bags (Rilsan) to NERI. See summary in table 13.

**TABLE 13. Sludge samples delivered by Iceland 2002. Joint Nordic screening project on musk substances. Environment & Food Agency of Iceland (UST)**

Sample code /date	Site /sewage plant	Person equivalent (peq)	Classification	Coordinate
KL1. / 30.9.2002	Klettagardar, Reykjavik	> 10 000	industrial+municipal mixed	64°9,122'N, 21° 51,994'V
KL2. / 30.9.2002	Klettagardar, Reykjavik	> 10 000	industrial+municipal mixed	64°9,122'N, 21° 51,994'V
AN1. / 30.9.2002	Ananaust, Reykjavik	> 10 000	municipal	64°9,540'N, - 21° 56,285'V
AN2. / 30.9.2002	Ananaust, Reykjavik	> 10 000	municipal	64°9,540'N, 21° 56,285'V

### Remarks

Coordinate system we used is ISN93-WGS84

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### *Rain water sampling*

Five samples of rainwater were collected 1) from municipal part of Reykjavik, 2) from a suburban part of Reykjavik, and 3) from Hveravellir located in central Iceland (rural background/northern pristine area).

Sampler 1) was placed in the open area in the vicinity of the Icelandic Meteorological Institute laboratory, which is locating a within Reykjavik city.

Sampler 2) was placed in the open area on a hilltop outside of Reykjavik where the Icelandic Meteorological Institute laboratory has a full weather station.

Sampler 3) was placed in the open area in Hveravellir, central Icelandic highlands. The Icelandic Meteorological Institute also has a manned weather station in this location.

All stations are served daily. The sampling apparatus requested by NERI in their manual was not available. (Cleaning of sampler, tools and bottles was made according the NERI's manual). We used a existing sampler of stainless steel plate. Sampler's size was 30 cm<sup>2</sup>. Sampling was continued until bottle was full. When the bottle was full of rain it was moved to a refrigerator (+4°C) in the Meteorological Institute. All samples are listed in table 14

**TABLE 14 Samples of rainwater collected in Iceland 2002. Joint Nordic screening project on musk substances. Environment & Food Agency of Iceland (UST).**

Sample code	Site	Sampling period	Volume (L)	Remarks
Reykjavik, Aug. 02	Reykjavik	Aug. 2002	2 liter	52 m above sea level. Flat grassy hilltop, 100 m from housing
Reykjavik, Sept. 02	Reykjavik	Sept. 2002	2 liter	52 m above sea level. Flat grassy hilltop, 100 m from housing
Hveravellir, Aug. 02	Hveravellir	Aug. 2002	2 liter	641 m above sea level. Flat, grass/lava, uninhabited rural highlands
Rjúpnahæð, Aug. 02	Rjúpnahæð	Aug. 2002	2 liter	120 m above sea level. Flat grassy hilltop, near Reykjavik, Suburban
Rjúpnahæð, Sept. 02	Rjúpnahæð	Sept. 2002	2 liter	120 m above sea level. Flat grassy hilltop, near Reykjavik, Suburban

**TABLE 15 Coordinates of rainwater sampling sites in Iceland**

Reykjavik	64°07.646'N, 21°54.146'V, (64.13, 21.90)
Hveravellir	64°52.001'N, 19°33.723'V, (64.87, 19.56)
Rjúpnahæð	64°05'N, 21°51'V, (64.08, 21.85)

### *Blue mussel sampling*

Sampling of mussels was performed by the Marine Research Institute of Iceland. The mussels were dissected according to the method description given by NERI (ref: NERI-DK 28.6.2002). The soft tissue from more than 30 mussel individuals was removed and weighted. The samples were frozen in Rilsan bags, and later sent cooled to the NERI.

All samples are summarised in table 15

**TABLE 16 Blue mussel samples collected in the southwestern coastal sea area of Iceland 2002. Joint Nordic screening project on musk substances. Environment & Food Agency (UST).**

Sample code = Place	Sample date	Depth	Coordinates (degrees)	Size class (mm) (mean ± SD; N)	Remarks Type of bottom
Hvasshraun	23.9.2002	2 meters	640200 - 220790	45 ± 7; 30	Sand and gravel - tidal beach*
Straumur	23.09.02	2 meters	640264 - 220275	48 ± 8; 31	Sand and gravel - tidal beach*
Eyri	23.09.02	2 meters	642034 - 214367	48 ± 8; 34	Sand and gravel - tidal beach*
Hvítanes	23.09.02	2 meters	642177 - 212971	46 ± 7; 31	Sand and gravel - tidal beach*

\* Samples were collected into a bucket (w. water holes) by hand in low tide. Sampling site is on average 1,5-2 meters below sea level except on low tides, every other week. This means that on low tide it is possible to wear boots and pick up the mussels by hand. After picking the mussels were dipped into a barrel of sea water for 24 hours.

Samples were then brought to a laboratory where each individual was dissected. Dissection took ca. 1,5 hours for each sampling site, other individuals were meanwhile kept in a cooler; dissected samples were also put in cooler. After all dissection was done the samples were brought to a 20 degree cold freezer until being mailed to NERI.

**TABLE 17 Possible emitters of Musk around the blue mussel sampling sites**

Hvassahraun	This site is on Reykjanes close to Reykjavik. There is an aluminum smelter & industrial activity in the area
Straumur	This site is on Reykjanes close to Reykjavik. There is an aluminum smelter & industrial activity in the area
Eyri	This site is in Hvalfjörður close to Reykjavik. There is an aluminum smelter in the area
Hvítanes	This site is in Hvalfjörður close to Reykjavik. There is an aluminum smelter in the area

***Fox sampling***

The samples of liver tissue were collected from arctic. Several animals were hunted during the summer and autumn 2002 (frozen samples). The summary of the samples with background information is presented in table 16

**TABLE 18 The samples of arctic fox collected in Iceland 2002. Joint Nordic screening project on musk substances. Environment & Food Agency (UST)**

Code	Hunting date	Site (all rural)	Sex	Weight	Stomach contents	Age guess
7023	25.10.2002	Miðnesheidi, við Norðurkot	male	5,2 kg	80% fish & 20% seabird	> 1 years
7024	23.10.2002	Sandgerdi, við Tjörnina	male	4,3 kg	90% sea bird & 10% mouse	> 1 years
7033	18.5.2002	Kirkjuhvammshreppur, Illugastadal	female	3,5 kg	Small bird	> 1 years
7035	16.6.2002	Þverárhreppur, N-Krossanesfell	female	3,5 kg	Meat, egg & small bird	> 1 years
7038	22.6.2002	Strandahreppur, Grjótdalur	male	4,0 kg	Large bird, small bird, sea weed	> 1 years
7039	22.6.2002	Strandahreppur, Grjótdalur	female	3,7 kg	Small bird	> 1 years

Fox teeth were sent to NERI in January for detailed age determination.

**TABLE 19 Coordinates of rainwater sampling sites in Iceland**

Site	Coordinates
Miðnesheidi (7023)	Lambert 2 Parallels Northing:394813 Easting: 319663
Sandgerdi (7024)	64°2,292'N, -22° 42,173'V (ISN93 - WGS84)
Húnavatnssýsla, Illugastadal (7033)	Lambert 2 Parallels Northing:569138 Easting: 413209
16.6.2002	
Árnessýsla, Grjótdalur (7038)	Lambert 2 Parallels Northing: 367577 Easting: 435527
Árnessýsla, Grjótdalur (7039)	Lambert 2 Parallels Northing: 367577 Easting: 435527

## Norway

The Norwegian samples were (if no other is stated) collected and prepared according to the manual given by NERI (see appendix 1). The location of the Norwegian sampling sites and geographical coordinates and classification of the sites in relation to pollution can be found in table 20 Number of specimen collected from different sites is summarized in table 21

**TABLE 20 Coordinates of the sampling sites in Norway year 2002. Joint Nordic screening project on musk substances.**

Sampling site	North	East	Site classification
Gressholmen (Oslofiord, Station 30 A).	59.88	10.71	Urban (marine)
Gåsøya (Oslofiord, Station 304)	59.85	10.59	Suburban (marine)
Ramton (Oslofiord, Station 307)	59.74	10.52	Suburban (marine)
Færder (Oslofiord, Station 36A)	59.03	10.53	Pristine (marine)
Strømmen (AS Sentral-renseanlegget RA-2)	59.95	11.04	Suburban
Oslo (Bekkelaget Sewage Treatment Plant)	59.88	10.77	Urban
Vinterbro (Nordre Follo Sewage Treatment Plant)	59.75	10.78	Suburban
Rygge (Fuglevik Sewage Treatment Plant)	59.38	10.66	Urban to suburban
Lista (Rain water)	58.10	06.57	Rural (coastal)
Hurdal (Rain water)	60.37	11.07	Rural (forest)
Bærum (Fox)	59.90	10.58	Suburban
Oslo (Fox)	59.93	10.68	Suburban
Spitsbergen (Fox)	78.30	17	Pristine (arctic)

**TABLE 21 Summary of the samples collected at the sampling sites in Norway 2002. Joint Nordic screening project on musk substances.**

Specimen	Total number of samples	Urban + suburban sites (n)	Forest+rural sites (n)	Pristine sites (n)
Sludge	5	Strømmen (1) Oslo (2) Vinterbro (1) Rygge (1)		
Rain water	4		Lista (3) Hurdal (1)	
Blue mussel	4	Gresholmen (1) Gåsøya (1) Ramton (1)		Færder (1)
Red fox	2	Oslo (1) Bærum (1)		
Arctic fox	1			Spitsbergen (1)

### *Sludge sampling*

The samples were collected by Svein Stene Johansen, Norwegian Institute of Water Research (NIVA). All single samples are summarized in table 22

**TABLE 22 Sludge samples collected by Norway 200 for joint Nordic screening project on musk substances. Sampling by Svein Stene Johansen, Norwegian Institute of Water Research (NIVA).**

Sample	Site /sewage plant	Person equivalent (peq)	Classification
Sample 1.	Strømmen, AS Sentralrenseanlegget RA-2 (27.08.02 at 08.30 hrs.) Chemical treatment	103.000	Suburban (percentage industrial waste water not known)
Sample 2.	Oslo, Bekkelaget Sewage Treatment Plant (27.08.02 at 09.45 hrs.) Chemical and biological treatment with nitrogen removal	280.000	Urban (with ca. 30% industrial waste water)
Sample 3.	Oslo, Bekkelaget Sewage Treatment Plant (27.08.02 at 09.45 hrs.) Same as above	Same as above	Same as above
Sample 4.	Vinterbro, Nordre Follo Sewage Treatment Plant (27.08.02 at 11.15 hrs.) Chemical and biological treatment	70.000	Suburban (with an estimation of 30% industrial waste water, including a factory that produces cosmetics etc.)
Sample 5.	Rygge, Fuglevik Sewage Treatment Plant (27.08.02 at 12.15 hrs.) Chemical treatment	50.000	Urban to suburban

### *Rain water sampling*

Sampling of rain water was lead by Martin Schlabach, Norwegian Institute of Air Research (NILU). Three samples of rain water were collected at Lista and two were collected in Hurdal. All samples are listed in table 23

Description of the sampling equipment: Bulk sampling, glass funnel with 285 mm diameter directly connected to a 2 liter glass bottle. Funnel is permanently open (a wet only system is not used). Height above ground level: 2 m.

**TABLE 23** Samples of rainwater collected in Norway 2002. Joint Nordic screening project on musk substances. Sampling led by Martin Schlabach, Norwegian Institute of Air Research (NILU).

Sample	Site	Sampling period	Volume (L)	Remarks
Sample Lista 1	Lista	07.10.02 – 24.10.02	2 liter	Rural. Immediate surroundings : Grass 95%, forest 5%. Ca. 1 km from Elkem (industry) 13 m a.s.l.
Sample Lista 2	Lista	25.10.02 – 06.11.02	2 liter	Same as above
Sample Lista 3	Lista	06.11.02 – 07.11.02	2 liter	Same as above
Sample Hurdal 1	Hurdal	16.10.02 – 28.10.02		Immediate surroundings: Forest with clearing (about 3000 m <sup>2</sup> ). About 700 m from nearest farm and road. 300 m a.s.l.
Sample Hurdal 2	Hurdal	28.10.02 – 01.11.02	2 liter	Same as above

#### *Blue mussel sampling*

Sampling of mussels was performed by Norwegian Institute of Water Research (NIVA) The living mussels were rinsed according to the manual and depurated for 12 hours in water from the sampling site for 12 hours in a refrigerated room. All samples are summarized in table 24

**Table 24** Blue mussel samples collected in different parts of the Oslofiord. Joint Nordic screening project on musk substances. Sampling led by Norman Greene, Norwegian institute of Water Research (NIVA).

Sample	Date	Site (name, depth in m)	Size class (mm); mean	Remarks
Sample St. 30A	01.10.02	Oslofiord, Gressholmen (Station 30 A). 0,5 – 1 m.	45,2±2,6 (40 individuals)	Close to Oslo harbour in the Oslofiord, 16 km from Oslo's main sewage treatment plant at Slemmestad
Sample St 304	01.10.02	Oslofiord, Gåsøya (Station 304) 1 m.	45,3±2,8 (40 individuals)	Inner part of Oslofiord, 9 km from Oslo's main sewage treatment plant at Slemmestad
Sample St 307	01.10.02	Oslofiord, Ramton (Station 307) 0,5 m	44,5±2,4 (40 individuals)	Inner part of Oslofjord, 4 km from Oslo's main sewage treatment plant at Slemmestad
Sample St 36A	11.10.02	Oslofiord, Færder (Station 36A) 0,5 – 1m.	47,6±5,7 (30 individuals)	At the outmost part Oslofiord. Can probably be seen as a reference condition station for northern Skagerrak and inner part of Oslofjord.



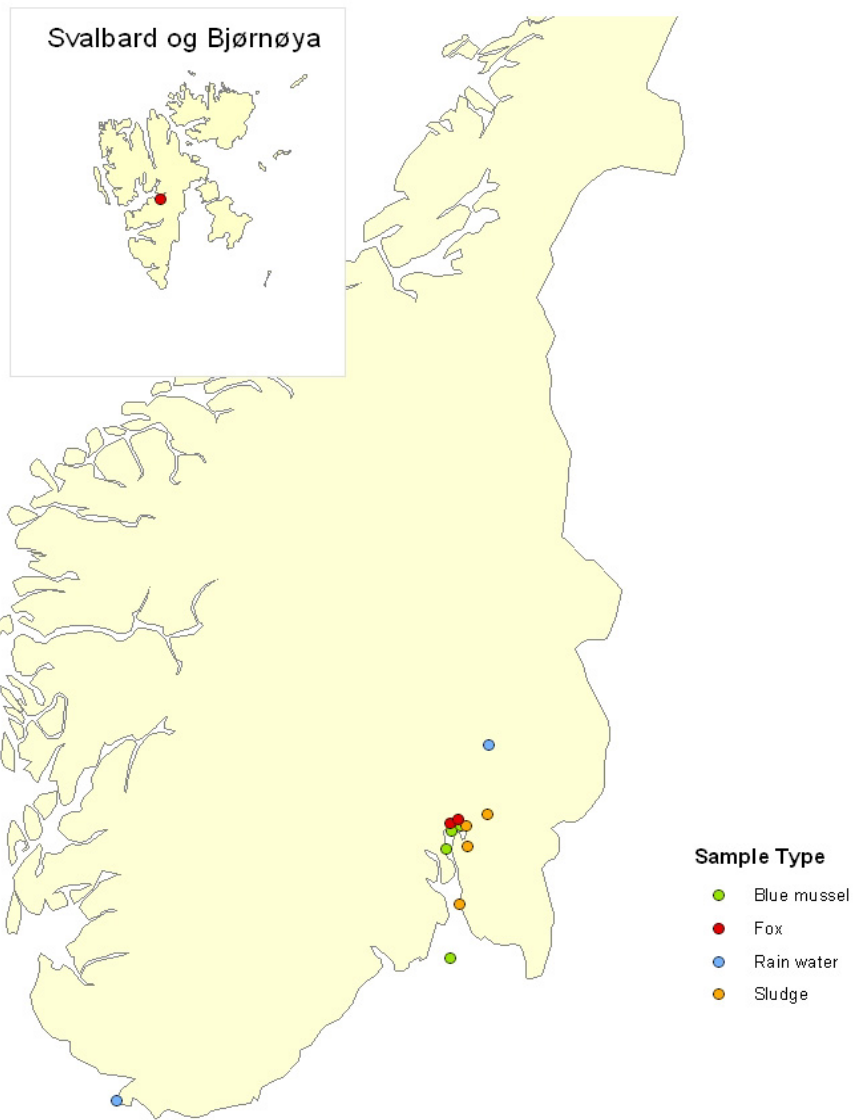
### **Fox samples**

Only two samples of red fox (*Vulpes vulpes*) could be collected from the Oslo area during the autumn of 2002. In addition we have delivered one sample of Arctic fox (*Alopex lagopus*) from Spitsbergen. The arctic foxes on Svalbard feed on both the terrestrial and marine food web. The samples of liver tissue from red fox were collected by Anders Gimse (Oslo municipality) and Paul Berger Hansen (Bærum municipality) and prepared and described by Kjell Handeland, National Veterinary Institute. The samples of liver tissue from arctic fox were collected, prepared and described by Eva Fuglei, Norwegian Polar Institute.

The summary of the samples with background information is presented in table 25

**TABLE 25 The samples of red fox and arctic fox collected in Norway 2002. Joint Nordic screening project on musk substances. Sampled by Eva Fuglei (Norwegian Polar Institute), Anders Gimse (Oslo municipality) and Paul Berger Hansen (Bærum municipality).**

<b>Sample</b>	<b>Animal</b>	<b>Sex</b>	<b>Hunting date</b>	<b>Age (year)</b>	<b>Site and classification</b>
Sample, Spitsbergen	Arctic fox	male	February 2002	8	Spitsbergen, pristine (arctic) 32 km from Longyearbyen
Sample, Oslo	Red fox	male	05.11.2002	Young animal	Ullern in Oslo municipality, suburban
Sample, Bærum	Red fox	female	14.10.2002	Adult	Høvik in Bærum municipality, suburban



*Figure 5 Sample sites in Norway.*

## Sweden

### *Sludge sampling*

Sampling was performed according to the method description of NERI Appendix 1.

**TABLE 26 Sludge samples taken**

Site /sewage plant	Person equivalent (peq)	Sampling date	Classification	Coordinate
Vimmerby	> 10 000	2002-11-18	municipal	57.67° N, 15.85° E
Stenungsund	> 20 000	2002-11-20	Industrial+municipal	58.07° N, 11.82° E
Stockholm:Henriksdal	> 400 000	2002-11-18	Municipal (big city)	59.32° N, 18.07° E

Notes: There are possible emitters of musk substances in the area, either industrial or households No known musk sources; in Stenungsund, however, large chemical industry nearby, e.g. vinyl flooring industry  
Weather condition during the sampling and the week before:

**Vimmerby:** overcast, occasional rain, +5 degrees C

**Stenungsund:** Overcast, some rain, a few plus degrees Celsius

**Bromma:** Occasional rain, a few +-degrees Celsius

### *Rain water sampling*

The sampling apparatus requested by NERI in their manual couldn't be used and was not available. The rain samples were collected using a glass funnel with a diameter of 20 cm connected to a glass bottle. The sampler was wrapped in aluminum foil during the sampling. Two samplers were used in parallel at each sampling site.

**TABLE 27: Rain water samples taken**

Station	Classification	Sampling date	Volume	Coordinate
Pallas	Background station, forest area			75.65° N, 18.47° E
Råö	Background station, Forest area	2002-09-09 – 2002-10-16		63.70° N 12.66° E

Notes: Weather conditions during the sampling period

### *Blue mussel sampling*

Samplings of Blue mussels were carried out according to Nordic Environmental Specimen Banking - methods in use in ESB. Manual for the Nordic countries. (TemaNord 1995:543).

Blue mussels were collected by hand from the inter tidal zone. It is recommended that sampling should be done as near to the same depth and exposure (in respect to sunlight and wave action) as possible. Late autumn is recommended as sampling time because mussels are in a less unstable physiological state. The blue mussels were pooled in size groups according to shell length, each group consisting of 20 individuals.

**TABLE 28. Blue mussel samples collected.**

<b>Sample code</b>	<b>Date</b>	<b>Site* (depth, m)</b>	<b>Size class (mm) (mean <math>\pm</math> SD)</b>	<b>Coordinate</b>
Kolerakyrkogården Bohus-Malmön	2002-09-01	1-1.5 m	60+-10	58.21° N 11.19° E

Remarks: Possible industrial source of musk substances in the area.

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