

SWECO Environment
Screening Report

Screening of musk substances and metabolites

Client

Swedish Environmental Protection Agency

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Sammanfattning

Bakgrund och metoder

Myskämnen är benämningen på en rad artificiellt framställda ämnen med likartade doftegenskaper. Dessa används som viktiga komponenter i olika doftblandningar som används i en lång rad produkter:

- Tvättmedelsprodukter
- Mjukgörningsmedel för tyger
- Luftdoftare
- Schampo
- Parfymer
- Kosmetika
- Tobaksprodukter

År 2007 fick SWECO Environment i uppdrag från Naturvårdsverket att genomföra en screeningstudie av myskämnen. Provtagningen i denna studie fokuserade på en rad olika källor samt även provtagning i bakgrundslokaler. Viktiga slutsatser var att myskämnen är vanligt förekommande i avloppsreningsverk och deras recipienter samt att det inte går att utesluta att myskämnen utgör en reell miljörisk (SWECO 2008). De huvudsakliga rekommendationerna var att även metaboliter av myskämnen bör undersökas, att platser dit slam återförs (jordbruk och skog) bör undersökas med avseende på myskämnen samt att en framtida screening bör fokusera enbart på avloppsreningsverk och dess recipienter.

Med utgångspunkt från ovanstående rekommendationer har SWECO Environment på uppdrag av Naturvårdsverket genomfört en fortsatt screeningsstudie av myskämnen. I denna studie ingår myskämnen mysk xylene, mysk ketone, galaxolide, tonalide, celestolide, traesolide, phantolide, cashmeran, mysk ambrette, mysk moskene och mysk tibetene. Dessutom har metaboliter av galaxolide (galaxolide lactone), mysk xylene (2 och 4-amino mysk xylene) samt mysk ketone (2-amino mysk ketone) inkluderats i studien.

Syftet med screeningstudien var att belysa förekomsten av dessa ämnen i recipienter nedströms reningsverk samt i jordbruks- och skogsjordar till vilka det sker slamåterföring. Dessutom var ett syfte att summariskt bedöma om halterna utgör någon miljörisk.

Provtagningen genomfördes nationellt med provpunkter som valdes av Naturvårdsverket och SWECO samt regionalt med provpunkter som valdes av respektive läns-

styrelse. Resultaten från den regionala samt den nationella provtagningen samutvärderades.

En provtagningsstrategi togs fram omfattande provtagning av inkommande och utgående vatten samt slam vid avloppsreningsverk liksom provtagning av ytvatten, sediment och fisk i vattendrag som tar emot utgående vatten från avloppsreningsverk. Avloppsreningsverk valdes som provtagningsplatser eftersom den screeningen (SWECO 2008) visat att dessa är den klart dominerande källan till dessa ämnens förekomst i miljön.

Slutsatser och rekommendationer

Myskämmen har i den här studien frekvent påvisats i in- och utgående vatten samt slam från avloppsreningsverk. De har även frekvent påvisats i ytvatten, sediment och fisk i recipienter till avloppsreningsverk. De metaboliter som mättes var ofta mycket vanligt förekommande, ibland i nivåer som översteg halterna av ursprungssubstansen.

Myskämmen och metaboliter var också mycket vanligt förekommande i jordbruksjordar och skogsjordar till vilka slam förts. Dock kunde inga myskämmen påvisas i jordbruksjord ett år efter spridning av slam.

Myskämmen har påvisats i samtliga slamprover från avloppsreningsverk i den här studien. Koncentrationerna varierar kraftigt, vilket också har visats i tidigare studier. Däremot är det okänt vad denna höga variation beror på (t.ex. storlek och driftsparametrar i avloppsreningsverket samt avloppsvattnets ursprung).

För att avgöra om de halter som påvisats i recipienter till avloppsreningsverk har någon negativ påverkan på miljön har de uppmätta koncentrationerna jämförts med de lägsta halter som kan utgöra någon risk för miljön (PNEC, Predicted No Effect Concentration). Den högsta kvoten mellan påträffade koncentrationer och PNEC var 13 för galaxolide lactone i ytvatten och 17 för 4-amino mysk xylene i ytvatten samt 3 för galaxolide i jord. Dock baseras dessa riskkvoter på osäker (4-amino mysk xylene) och mycket osäker (galaxolide lactone) information om ämnenas ekotoxikologiska effekter. För galaxolide i jord är toxicitetsdata mindre osäkert, medan den uppmätta högsta koncentrationen i jord till viss del representerar ett slamprov snarare än ett jordprov. Några säkra slutsatser om eventuella risker i miljön kan därmed inte dras. Man kan inte heller utesluta negativa effekter uppkomna som ett resultat av den sammantagna effekten av flera samtidigt förekommande myskämmen.

De huvudsakliga slutsatserna från denna studie var att:

- Myskämmen och metaboliter är vanliga i inkommande vatten, slam och utgående vatten från avloppsreningsverk samt i ytvatten, sediment och fisk i nedströms recipienter.
- Myskämmen och metaboliter är vanligt förekommande i jord där slam från reningsverk sprids.
- I vilken grad de uppmätta koncentrationerna utgör någon miljörisk är i nuläget osäkert, även om kvoter mellan uppmätta koncentrationer och lågefektnivåer i flera fall översteg 1.
- Vid en jämförelse med tidigare screeningstudier förefaller det inte som om halter av myskämmen i miljön minskar.

Några rekommendationer är:

- Vidare spridning och upptag av myskämmen i miljön efter slamspridning vore av intresse att undersöka
- En utförlig utvärdering om behovet att låta myskämmen ingå i den ordinarie miljöövervakningen rekommenderas
- Det finns ett behov av studier om ekotoxikologiska effekter av myskämmen, även om sådana studier ligger utanför ramen för screeningprogrammet. Även additiva effekter av screeningämnen behöver mätas/utvärderas.
- En massbalansstudie kan vara av intresse där mängden myskämmen som når avloppsreningsverk, mängden som lagras i slam och mängden som når ytvatten kvantifieras. Dessa mängder skulle kunna jämföras med mängder i produktregistret.
- En studie av hur driftsparametrar i avloppsreningsverken samt källor till inkommande vatten påverkar mängden myskämmen i slam och utgående vatten vore av intresse, för att bättre förstå vad som styr förekomsten av myskämmen i recipienter.

Summary

Background and methods

År 2007 fick SWECO Environment i uppdrag från Naturvårdsverket att genomföra en screeningstudie av muskämnen. Provtagningen i denna studie fokuserade på en rad olika källor samt även provtagning i bakgrundslokaler. Viktiga slutsatser var att muskämnen är vanligt förekommande i avloppsreningsverk och deras recipienter samt att det inte går att utesluta att muskämnen utgör en reell miljörisk (SWECO 2008). De huvudsakliga rekommendationerna var att även metaboliter av muskämnen bör undersökas, att platser dit slam återförs (jordbruk och skog) bör undersökas med avseende på muskämnen samt att en framtida screening bör fokusera enbart på avloppsreningsverk och dess recipienter.

In 2007 SWECO Environment performed a screening study of musk substances in a number of matrices and also at background localities with the Swedish Environmental Protection Agency as the client. The main conclusions were that musk substances commonly occurs in waste water treatment plants and in their receiving surface waters, and that environmental risks with the level seen in the environment can not be excluded. The main recommendations were that musk metabolites should also be studied, that sludge amendment sites (agricultural fields and forests) should be included in future screening studies, and that screening studies of musk substances should only focus on waste water treatment plants and receiving surface waters.

Based on these recommendations, SWECO Environment has had the assignment from the Swedish Environmental Protection Agency to perform a new screening study of the musk substances Musk Xylene, Musk Ketone, Galaxolide, Tonalide, Celestolide, Traesolide, Phantolide, Cashmeran, Musk Ambrette, Musk Moskene, and Musk Tibetene. Also included were the metabolites of Galaxolide (Galaxolide Lactone), musk Xylene (2 and 4-amino musk Xylene) and musk ketone (2-amino musk ketone).

The objectives of the project were to elucidate the levels of these substances in STPs, downstream limnic environments and in soils receiving sludge amendments. The study also aimed at briefly assessing whether the levels constitute an environmental problem.

A national sampling strategy was devised which included sampling of incoming water, effluents and sludge at sewage treatment plants as well as sampling of surface waters, sediments and fish in streams receiving effluents from sewage treatment plants. Sewage treatment plants were chosen as sampling localities because earlier screening studies demonstrate that these are the major sources of these substances to the environment. Also included were agricultural soils and forest soils receiving sludge amendment.

Conclusions and recommendations

The main conclusions from this investigation were:

- Musk compounds and metabolites are common in incoming water, sludge and effluents at sewage treatment plants.
- Musk compounds and metabolites are common in surface waters, sediments and fish in downstream limnic environment.
- Musk compounds and metabolites are common in soils to which sludge has been amended
- It is uncertain whether the measured levels of musk substances and metabolites
- It is uncertain to what degree the levels of musk substances in the environment constitute any environmental problem despite the fact that ratios between measured concentrations and low risk levels sometimes exceeded one.
- In comparison with results from earlier screening studies, it can be concluded that the levels of musk substances in the environment is not decreasing.

Some recommendation for future studies are:

- The fate of musk substances that has been amended to soils needs to be further assessed
- Given that musk substances are very frequent in most environmental compartments, there is a need for a comprehensive assessment on the need for including these substances in the regular monitoring programs.
- More detailed studies on the effects and some musk substances and metabolites in surface water, soils and sediments are needed. Additive effects between different musk substances also need to be considered when assessing the risk and possible effects.
- A mass balance study of the amounts of different musk substances reaching waste water treatment plants, the amounts stored in sludge and the amounts reaching surface waters would be of interest.
- A study of how operating parameters in the sewage treatment plants, as well as sources of incoming water, affect the levels of musk substances in sludge and outgoing water could also be of interest.

1 Introduction

1.1 Background

At present there is a lack of knowledge regarding the emission, distribution and exposure for many of the chemicals emitted to the environment. The aim of the screening program financed by the Swedish Environmental Protection Agency is to alleviate this lack of knowledge by estimating the occurrence of different chemicals in the environment in relevant matrices (soil, water etc.).

To maximize the information gained from the screening program measurements are made in many matrices at many sites, but with few samples per site. The Swedish EPA is responsible for the screening at the national level and selects the chemicals that are to be included. The regional county boards have the option to complement and extend the sampling program by choosing additional sampling point that are of regional interest.

Within the screening program of 2007, SWECO Environment performed a screening study of musk substances in various matrices (SWECO 2008). Some main conclusions from this study were:

- Musk compounds are common in incoming water, sludge and effluents at sewage treatment plants
- Musk compounds occur in surface waters and sediments downstream of sewage treatment plants. The study indicates that sewage treatment plants are the most likely source of musk substances to the environment.
- The MEC/PNEC quotient was consistently below one. However, it was still high enough (0.26 for Galaxolide in surface waters) that risk to the aquatic environment could not be excluded.

Some of the main recommendations in the earlier screening study were:

- A screening of musk substance metabolites could be of interest given that metabolites of Musk Xylene and Musk Ketone have been found in sludge and effluent from sewage treatment plants in USA and Germany. Such a screening could focus on sewage treatment plants and their downstream surface water recipients.
- Screening of musk substances could be performed in soil where sludge has been applied to agricultural soils, since musk substances has been found in earth worms from agricultural fields

Based on the above recommendations, SWECO has had the assignment from the Swedish Environmental Protection Agency to measure the occurrence of musk substances and their metabolites with a focus on waste water treatment plants and their recipients.

The following musk substances were included:

Ämnen	Kommentar
Musk amberette (MA)	Musk substance
Musk xylene (MX)	Musk substance
Musk moskene	Musk substance
Musk tibetene	Musk substance
Musk ketone (MK)	Musk substance
Cashmeran (DPMI)	Musk substance
Celestolide (ADBI)	Musk substance
Phantolide (AHDI)	Musk substance
Traseolide ATII)	Musk substance
Galaxolide(HHCB)	Musk substance
Tonalide (AHTN)	Musk substance
4-amino musk xylene	Metabolite originating from Musk xylene
2-amino musk xylene	Metabolite originating from Musk xylene
2-amino musk ketone	Metabolite originating from ketone
Galaxolide lactone	Metabolite originating from Galaxolide

Note that the metabolite 2-amino musk moskene was originally intended to be included in the study. However, no analytical standards could be obtained for this compound, and consequently it was not possible identify or analyze.

1.2 Objectives

The objectives of the project were to:

- Elucidate whether musk metabolites are occurring both in the waste water treatment plants, and in receiving surface waters
- Elucidate whether musk substances and metabolites occur in agricultural fields and forests where sludge is applied
- If the substances are found, assess whether the levels constitutes an environmental problem

1.3 Substance information

1.3.1 Usage

Musk is the name originally given to a substance with a penetrating odor obtained from a gland of the male musk deer and the substance has been used as a perfume since ancient times. The name “musk” has later on come to encompass a wide variety of substances with somewhat similar odors although many of them are quite different in their chemical structures (Wikipedia, Merriam Websters Dictionary, Summer 2004). This includes artificial substances with similar odors which at present are the ones that are used almost exclusively. Artificial musk substances are also the focus of this screening study.

The artificial musk compounds (hereafter named only musk compounds) are used as ingredients in fragrance compositions which are complex mixtures prepared by blending many fragrance ingredients in varying concentrations. The musk containing fragrance compositions are used in a large number of products. This includes (European Union 2005, Kallenborn et al 1999):

- washing and cleaning agents
- fabric softeners
- air fresheners
- shampoos
- perfumes
- cosmetic products
- food additives in fish baits
- cigarettes

There is no production of nitro musk compounds in the European Union. Instead, producers in China are now the most important source for the European imports (European Union 2005a ,2005b). Galaxolide and Tonalide are produced at one inland plant in the EU for each substance (OSPAR 2004). Musk ketone, Musk Xylene, Galaxolide and Tonalide represented about 95 percent of the European market 2004.

Table 1.1 shows the usage/import of the most common musk compounds by fragrance compounding facilities in Europe. The amounts in table 1.1 supposedly account for approximately 90% of the total use in Europe as 32 of the major fragrance companies involved in fragrance compounding responded to the survey (OSPAR 2004). In the Swedish product registry there is only registered data on Galaxolide (Figure 1.1). Other musk substances either lack data or there are only zero tonnes registered yearly. The reason is most likely that these substances are imported in products at low concentrations in which case they will not be reported to the registry.

Consumption of Musk Ketone and Musk Xylene in Norway in 2001 was 400 kg and 200 kg respectively (TemaNord 2004).

Consumption in Denmark according to the Danish product register is given in table 1.2 below. Information about use of fragrances forwarded to the Danish product register is given voluntarily and the consumption indicated in table 1.2 is therefore a minimum value.

Table 1.1 Usage/import (ton) of the most common musk compounds by fragrance compounding facilities in Europe 2001. Source: (OSPAR 2004).

Year	Musk ketone	Musk xylene	Galaxolide	Tonalide	Celestolide	Musk moskene	Musk tibetene
1992	124	174	2400	885			
1995	61	110	1482	585	34	5	0.8
1996	54	105					
1998	40	86	1473	385	18		
2000	35	67	1427	358	15		

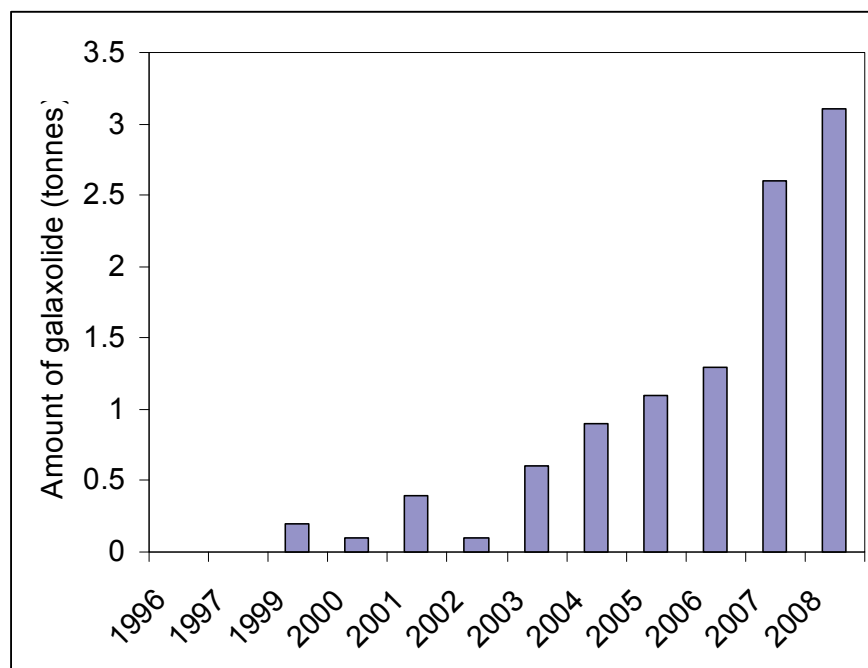


Figure 1.1 Registered amounts of Galaxolide in Sweden from the year 1996 – 2008.

Table 1.2 Consumption and usage of musk substances in Denmark (year unknown). Source: TemaNord (2004).

substance	amount per year (kg)	main use
Musk ketone	20	cosmetics
Musk xylene	134	cosmetics
Galaxolide	333	cosmetics and cleaning agents
Tonalide	33	cleaning agents
Traesolide	14	cleaning agents
Cashmeran	1	
Celestolide	< 1	
Phantolide	< 1	

1.3.2 Metabolites

Musk metabolites are mostly the result of biotic or abiotic degradation of the parent compound, both in waste water treatments plants (Dietrich and Hitzfield 2004) in water and sediments of receiving surfaces waters (Biselli et al. 2004) and in organisms (Biselli et al. 2004).

Metabolites may also be an impurity originating from the fragrance product. This is well known for galaxolide lactone where the concentrations in fragrance products may be up to 5% of the galaxolide mother compound (Einer and Kannan 2006). This is also why incoming wastewater contain up to 480 µg/l galaxolide lactone (Bester 2005).

Nitro musk compounds have been shown to be transformed via reduction of the 4-nitro group or the 2-nitro group producing 2- and 4-amino musk xylene (Biselli et al. 2004). Of these two, 4-amino musk xylene is probably much more common because of sterical hindrance to the degradation of the 2-nitro group. Additional polar transformation products of the nitro musks are probable since higher organisms and possibly microorganisms are capable of hydroxylation of aniline derivatives. Only one main transformation product of musk ketone has been identified, namely 2-amino musk ketone.

The transformation of Galaxolide to Galaxolide lactone has been investigated in a number of studies that have elucidated the different degradation steps (Biselli et al. 2004). Initially, HHCB is activated through the formation of hydroxy radicals which is followed by the addition of molecular oxygen. Peroxide moieties of the molecule are then formed which is followed by protonation and removal of a water molecule. There are good indications that several stable intermediates are formed during this

process, but the exact identity of these has not been established. It is also possible that HHCB-lactone is hydroxylated to form the corresponding hydroxy acids, but these remain to be identified. Experimental studies show that little or no mineralization of Galaxolide takes place. Consequently, the degradation products still contain the same amount of organic carbon and only a small fraction of the theoretical oxygen demand has been incorporated (EU 2008). The implication is that Galaxolide lactone may be a relatively stable end product of Galaxolide metabolism.

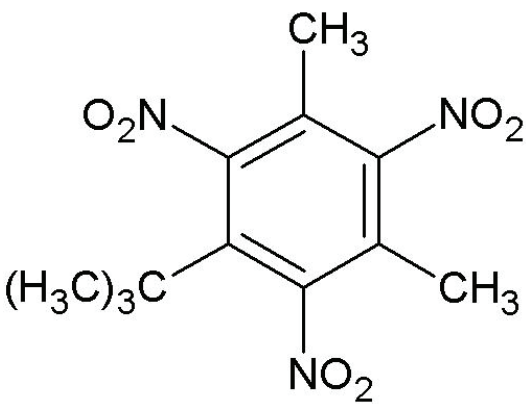
It is also interesting that gas-chromatographic analyses of commercial fragrance mixtures and sludge has revealed several identical signals that may tentatively be attributed to additional unknown musk compounds that are presumably formed during the technical syntheses of musk compounds. Several of these signals has also been confirmed in SPMD extracts from the river Elbe (Biselli et al. 2004).

Both laboratory biodegradation tests as well as measurements of metabolites in sewage water, sludge, surface waters and sediments have demonstrated that the musk compounds are very amenable to (bio)degradation processes; Both the nitro musk metabolites as well as the polycyclic musk metabolites have been found at levels above their mother compounds in a number of environmental matrices (Biselli et al. 2004). The laboratory based half-life of Galaxolide in river water has been determined to be around 100 days while the half life in river sediments is approximately 150 – 200 days.

1.3.3 Properties

Table 1.3 to table 1.17 presents physical and chemical properties as well as toxicological and ecotoxicological information for the substances included in this study. For most musk substances there is only scant available information. The exception is Musk Xylene and Musk Ketone which have been the focus of risk assessments within the European Union (European Union 2005a ,2005b).

Table 1.3 Physiochemical and (Eco)toxicological properties of Musk Xylene. Source European Union, 2005b) except for PNEC_{freshwater}

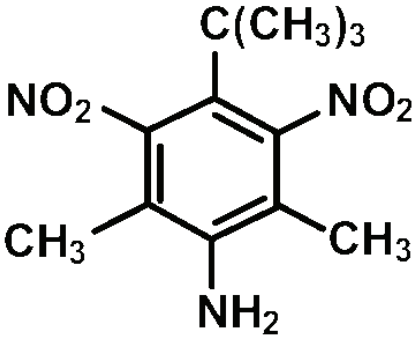
Common name	Musk xylene (MX)			
				
Name	5-tert-butyl-2,4,6-trinitro-m-xylene			
CAS #	81-15-2			
Labelling	E; Xn; N R: 2-40-50/53 S: (2-)36/37-46-60-61			
		Min	Max	Unit
Physico-chemical properties	Water solubility	0.15 ¹	0.46 ²	mg/l
	Log K _{ow}	3.4 ¹	4.9 ¹	
	Henry's law constant	0.0595		Pa*m ³ /mol
	Vapour pressure	3*10 ⁻⁵		Pa
Ecotoxicology aquatic	EC ₅₀ and LC ₅₀	0.12	3.75	mg/l
	NOEC and LOEC	0.056		mg/l
	PNEC _{freshwater}	0.00056 ³		mg/l
	PNEC _{sed}	0.3		mg/kg
Ecotoxicology terrestrial	EC ₅₀ and LC ₅₀			mg/kg soil
	NOEC and LOEC	>50		mg/kg soil
	PNEC	0.26		mg/kg soil
Toxicology	Oral LD ₅₀ >2000 = mg/kg; dermal LD ₅₀ >2000 = mg/kg			
Persistence, Bioaccumulation, Toxicity (PBT)	Musk xylene is considered to be a PBT candidate substance.			
Theoretical removal in STP	98.7 %			

¹ Measured

² Calculated

³ EU dossier on Musk Xylene prepared as background for setting environmental quality standards for musk xylene within the water framework directive

Table 1.4 Physiochemical and (Eco)toxicological properties of 4-amino musk xylene.

Common name	4-amino musk xylene			
				
Name	4-amino-1-tert.-butyl-3,5-dimethyl-2,6-dinitrobenzene			
CAS #	107342-55-2			
		Min	Max	Unit
Physico-chemical properties	Water solubility	4.1 ¹	4.1 ¹	mg/l
	Log K _{ow}	3.6 ²	4.3 ²	
	Henry's law constant	3.8E-11 ¹	9.3E-10 ¹	Pa*m ³ /mol
	Vapour pressure	5E-5 ¹	5E-5 ¹	Pa
Ecotoxicology aquatic	EC ₅₀ and LC ₅₀	0.25 ³	500 ⁴	µg/l
	NOEC and LOEC			mg/l
	PNEC _{water}			mg/l
	PNEC _{sed}			mg/kg
Ecotoxicology terrestrial	EC ₅₀ and LC ₅₀			mg/kg soil
	NOEC and LOEC			mg/kg soil
	PNEC			mg/kg soil
Toxicology				
Persistence, Bioaccumulation, Toxicity (PBT)	<i>P b T</i> ⁵			
Theoretical removal in STP	63 % ¹			

¹ Calculated in Epiwin using SMILES representation of the molecule

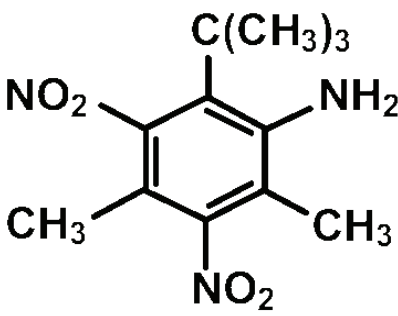
² Dietrich and Hitzfeld (2004)

³ Behechti et al. (1998)

⁴ Gidding et al. (2000)

⁵ Calculated using the PBT profiler. Capital letters (Italicized or underlined) indicate that the US EPA criteria for persistence, bioaccumulation and/or toxicity have been exceeded. The criteria are explained here: (<http://www.pbtprofiler.net/criteria.asp>). 4-amino musk xylene is considered to be persistent and highly toxic.

Table 1.5 Physiochemical and (Eco)toxicological properties of 2-amino musk xylene.

Common name	2-amino musk xylene			
				
Name	2-amino-1-tert.-butyl-3,5-dimethyl-4,6- dinitrobenzene			
CAS #	107342-67-6			
		Min	Max	Unit
Physico-chemical properties	Water solubility	4.1 ¹	4.1 ¹	mg/l
	Log K _{ow}	2.7 ²	4.3 ²	
	Henry's law constant	3.8E-11 ¹	9.3E-10 ¹	Pa*m ³ /mol
	Vapour pressure	4.9E-5 ¹	4.9E-5 ¹	Pa
Ecotoxicology aquatic	EC ₅₀ and LC ₅₀	1.1 ³	500 ⁶	µg/l
	NOEC and LOEC			mg/l
	PNEC _{water}			mg/l
	PNEC _{sed}			mg/kg
Ecotoxicology terrestrial	EC ₅₀ and LC ₅₀			mg/kg soil
	NOEC and LOEC			mg/kg soil
	PNEC			mg/kg soil
Toxicology				
Persistence, Bioaccumulation, Toxicity (PBT)	<i>P b T</i> ⁵			
Theoretical removal in STP	63 % ¹			

¹ Calculated in Epiwin using SMILES representation of the molecule

² Dietrich and Hitzfeld (2004)

³ Behechti et al. (1998)

⁴ Gidding et al. (2000)

⁵ Calculated using the PBT profiler. Capital letters (Italicized or underlined) indicate that the US EPA criteria for persistence, bioaccumulation and or toxicity have been exceeded. The criteria are explained here: (<http://www.pbtprofiler.net/criteria.asp>). 2-amino musk xylene is considered to be persistent and highly toxic.

⁶ Calculated in ECOSAR using SMILES representation of the molecule. Note that a calculated toxicity value should only be used in a weight of evidence approach.

Table 1.6 Physiochemical and (Eco)toxicological properties of Musk Ketone. Source European Union, 2005a)

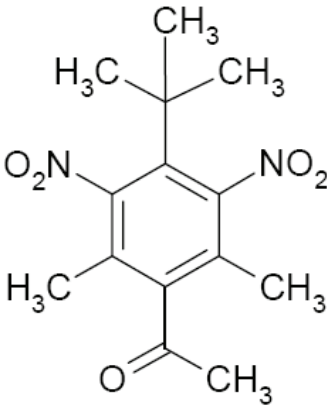
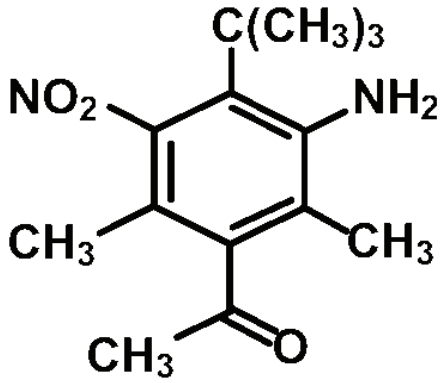
Common name	Musk ketone (MK)			
				
Name	1-tert-Butyl-3,5-dimethyl-2,6-dinitrobenzene			
CAS #	81-14-1			
Labelling	Xn; N R40-50/53 S(2)-36/37-46-60-61			
		Min	Max	Unit
Physico-chemical properties	Water solubility	0.46	1.9	mg/l
	Log K _{ow}	3.2	4.3	
	Henry's law constant	0.0256		Pa*m ³ /mol
	Vapor pressure	4*10 ⁻⁵		Pa
Ecotoxicology aquatic	EC ₅₀ and LC ₅₀	0.17	2.5	mg/l
	NOEC and LOEC	0.010	0.100	mg/l
	PNEC	0.001	0.37	mg/l
Ecotoxicology terrestrial	EC ₅₀ and LC ₅₀		1	mg/kg soil
	NOEC and LOEC	32	316	mg/kg soil
	PNEC	0.22		mg/kg soil
Toxicology	Oral LD ₅₀ >2000 = mg/kg; dermal LD ₅₀ >2000 = mg/kg			
Persistence, Bioaccumulation, Toxicity (PBT)	Musk ketone is considered not to be a PBT candidate substance.			
Theoretical removal in STP	92 %			

Table 1.7 Physicochemical and (Eco)toxicological properties of 2-amino musk ketone.

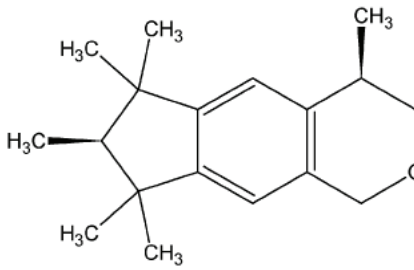
Common name	2-amino musk ketone			
				
Name	2-amino-1-tert.-butyl-3,5-dimethyl- 6-nitro-4-acetyl-benzene			
CAS #	107342-67-6			
		Min	Max	Unit
Physico-chemical properties	Water solubility	17 ¹	17 ¹	mg/l
	Log K _{ow}	4.1 ¹	4.1 ¹	
	Henry's law constant	4.3E-11 ¹	9.3E-11 ¹	Pa*m ³ /mol
	Vapour pressure	8.8E-5 ¹	8.8E-5 ¹	Pa
Ecotoxicology aquatic	EC ₅₀ and LC ₅₀	500 ⁶	1800 ⁶	µg/l
	NOEC and LOEC			mg/l
	PNEC _{water}			mg/l
	PNEC _{sed}			mg/kg
Ecotoxicology terrestrial	EC ₅₀ and LC ₅₀			mg/kg soil
	NOEC and LOEC			mg/kg soil
	PNEC			mg/kg soil
Toxicology				
Persistence, Bioaccumulation, Toxicity (PBT)	<i>P b T</i> ⁵			
Theoretical removal in STP	55 % ¹			

¹ Calculated in Epiwin using SMILES representation of the molecule

⁵ Calculated using the PBT profiler. Capital letters (Italicized or underlined) indicate that the US EPA criteria for persistence, bioaccumulation and or toxicity have been exceeded. The criteria are explained here: (<http://www.pbtprofiler.net/criteria.asp>). 2-amino musk xylene is considered to be persistent and highly toxic.

⁶ Calculated in ECOSAR using SMILES representation of the molecule. Note that a calculated toxicity value should only be used in a weight of evidence approach.

Table 1.8 Physiochemical and (Eco)toxicological properties of Galaxolide.

Common name	Galaxolide (HHCB)			
				
Name	1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[g]-2-benzopyrane			
CAS #	1222-05-5			
Labelling	Xi N - Irritant, Dangerous for the environment. R 38 50/53 ¹			
Physico-chemical properties		Min	Max	Unit
	Water solubility	1.65 ²	1.75 ¹	mg/l
	Log K _{ow}	5.9 ²	6.6 ¹	
	Henry's law constant	1.1*10 ⁻⁴³		atm*m ³ /mol
	Vapour pressure	0.068 ²	0.0727 ³	Pa
Ecotoxicology aquatic	EC ₅₀ and LC ₅₀	0.24 ⁴	0.29 ⁴	mg/l
	NOEC and LOEC		0.14 ⁴	mg/l
	PNECaqua ⁶		4.4 ⁶	µg/l
	PNECsediment ⁶		2 ⁶	mg/kg
Ecotoxicology terrestrial	EC ₅₀ and LC ₅₀	105 ⁴		mg/kg soil
	PNECsoil		300 ⁶	mg/kg soil
Toxicology	Oral LD ₅₀ >3000 mg/kg; dermal LD ₅₀ >6500 mg/kg ³			
Persistence, Bioaccumulation, Toxicity (PBT)	Not PBT (Pbt) ⁵			
Theoretical removal in STP	118 % av daglig belastning ⁵			

¹ <http://www.thegoodscentscompany.com/data/rw1007751.html>

² <http://www.norden.org/pub/miljo/miljo/sk/rw1007751.pdf>

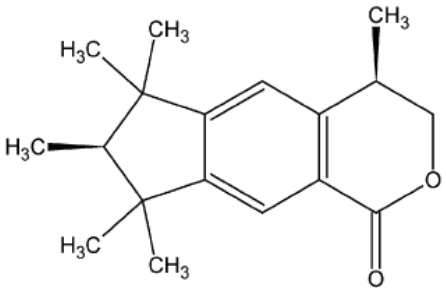
³ HERA 2004b

⁴ [http://jhs.pharm.or.jp/52\(3\)/52_276.pdf](http://jhs.pharm.or.jp/52(3)/52_276.pdf)

⁵ <http://www.heraproject.com/files/28-E-36551E10-F8EF-E807-E4199B9BB0076A9F.pdf>

⁶ EU risk assessment HHCB - http://ecb.jrc.ec.europa.eu/documents/Existing-Chemicals/RISK_ASSESSMENT/REPORT/hhcbreport414.pdf

Table 1.9 Physicochemical and (Eco)toxicological properties of Galaxolide lactone

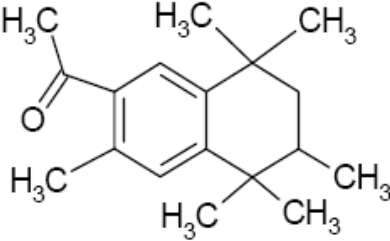
Common name	Galaxolide lactone			
				
Name	1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[g]-2-benzopyran-1-one			
CAS #				
		Min	Max	Unit
Physico-chemical properties	Water solubility	17 ¹	17 ¹	mg/l
	Log K _{ow}	5.3 ¹	5.3 ¹	
	Henry's law constant	2.1E-6 ¹	6.6E-5 ¹	Pa*m ³ /mol
	Vapour pressure	0.000271 ¹	0.000271 ¹	Pa
Ecotoxicology aquatic	EC ₅₀ and LC ₅₀	100 ⁶	600 ⁶	µg/l
	NOEC and LOEC			mg/l
	PNEC _{water}			mg/l
	PNEC _{sed}			mg/kg
Ecotoxicology terrestrial	EC ₅₀ and LC ₅₀			mg/kg soil
	NOEC and LOEC			mg/kg soil
	PNEC			mg/kg soil
Toxicology				
Persistence, Bioaccumulation, Toxicity (PBT)	<i>PBT</i> ⁵			
Theoretical removal in STP	93 % ¹			

¹ Calculated in Epiwin using SMILES representation of the molecule

⁵ Calculated using the PBT profiler. Capital letters (Italicized or underlined) indicate that the US EPA criteria for persistence, bioaccumulation and or toxicity have been exceeded. The criteria are explained here: (<http://www.pbtprofiler.net/criteria.asp>). Galaxolide lactone is calculated to be persistent, bioaccumulative and toxic.

⁶ Calculated in ECOSAR using SMILES representation of the molecule. Note that a calculated toxicity value should only be used in a weight of evidence approach.

Table 1.10 Physiochemical and (Eco)toxicological properties of Tonalide.

Common name	Tonalide (AHTN)			
				
Name	7-Acetyl-1,1,3,4,4,6-hexamethyltetrahydro-naphthalene			
CAS #	1506-02-1			
Labelling ¹	Xn N R 22 50/53			
Physico-chemical properties		Min	Max	Unit
	Water solubility	0.015 ¹	0.22 ²	mg/l
	Log K _{ow}	5.7 ²	6.6 ¹	
	Henry's law constant			
	Vapour pressure	0.068 ³		Pa
Ecotoxicology aquatic	EC ₅₀ and LC ₅₀	0.28 ³	255.2 ³	mg/l
	NOEC and LOEC		0.14 ³	mg/l
	PNECaqua ⁶		0.00035 ⁶	mg/l
	PNECsediment ⁶		0.2 ⁶	mg/kg
Ecotoxicology terrestrial	EC ₅₀ and LC ₅₀	0.28 ³		mg/kg soil
	NOEC and LOEC	105 ³	250 ³	mg/kg soil
Toxicology	Oral LD ₅₀ 570 mg/kg; dermal LD ₅₀ >5000 mg/kg ⁴			
Persistence, Bioaccumulation, Toxicity (PBT)	Not PBT (Pbt) ⁶			
Theoretical removal in STP	82 % of daily load ⁵			

¹ <http://www.thegoodscentscompany.com/msds/md102487.html>

² <http://www.norden.org/pub/miljo/miljo/sk/TN2004503.pdf>

³ [http://jhs.pharm.or.jp/52\(3\)/52_276.pdf](http://jhs.pharm.or.jp/52(3)/52_276.pdf)

⁴ HERA 2004c

⁵ <http://www.heraproject.com/files/28-E-36551E10-F8EF-E807-E4199B9BB0076A9F.pdf>

⁶ Marine environment. OSPAR 2004.

Table 1.11 Physiochemical and (Eco)toxicological properties of Celestolide.

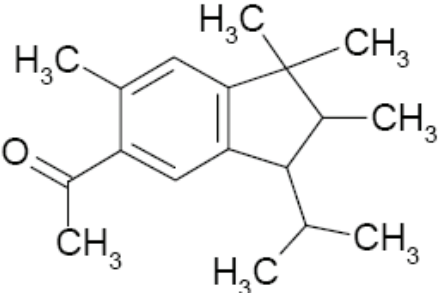
Common name	Celestolide (ADBI)			
Name	4-Acetyl-1,1-dimethyl-6-tertbutyldihydroindene			
CAS #	13171-00-1			
Labelling	Xi ¹			
		Min	Max	Unit
Physico-chemical properties	Water solubility	0.015 ¹	0.22 ²	mg/l
	Log K _{ow}	5.9 ²	6.6 ¹	
	Henry's law constant			
	Organic carbon-water partition coefficient (K _{oc})	4.47 ³	4.47 ³	
Ecotoxicology aquatic	EC ₅₀ and LC ₅₀			mg/l
	NOEC and LOEC			mg/l
Ecotoxicology terrestrial	EC ₅₀ and LC ₅₀			mg/kg soil
	NOEC and LOEC			mg/kg soil
Toxicology				
Persistence, Bioaccumulation, Toxicity (PBT)				
Degradation	No mineralisation under standard test conditions: not inherently biodegradable MITI II, <1 % biodegradation after 28 days ³			

¹ <http://www.thegoodscentcompany.com/data/rw1007691.html>

² <http://www.norden.org/pub/miljo/miljo/sk/TN2004503.pdf>

³ http://www.sea.eawag.ch/inhalt/sites/stoffe/pdf/PMV_e.pdf

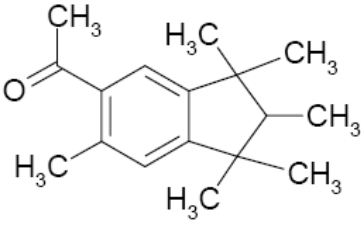
Table 1.12 Physiochemical and (Eco)toxicological properties of traseolide.

Common name	Traseolide (ATII)			
				
Name	5-Acetyl-1,1,2,6-tetramethyl-3-isopropyl-dihydroindene			
CAS #	68140-48-7			
Labelling	Xi – Irritant1			
		Min	Max	Unit
Physico-chemical properties	Water solubility	0.085 ¹	0.09 ²	mg/l
	Log K _{ow}	6.14 ¹	6.3 ²	
	Henry's law constant			
	Vapour pressure			Pa
Ecotoxicology aquatic	EC ₅₀ and LC ₅₀			mg/l
	NOEC and LOEC			mg/l
Ecotoxicology terrestrial	EC ₅₀ and LC ₅₀			mg/kg soil
	NOEC and LOEC			mg/kg soil
Toxicology				
Persistence, Bioaccumulation, Toxicity (PBT)				
Theoretical removal in STP				

¹ <http://www.thegoodscentscompany.com/data/rw1023771.html>

² <http://www.norden.org/pub/miljo/miljo/sk/TN2004503.pdf>

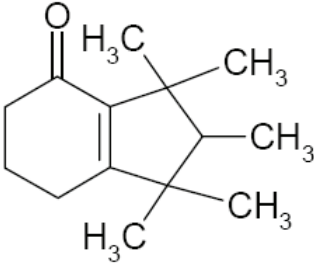
Table 1.13 Physicochemical and (Eco)toxicological properties of Phantolide.

Common name	Phantolide (AHDl)			
				
Name	6-Acetyl-1,1,2,3,3,5-hexamethyldihydroindene			
CAS #	15323-35-0			
Labelling	Xi ¹			
		Min	Max	Unit
Physico-chemical properties	Water solubility	0.027 ¹		mg/l
	Log K _{ow}	5.8 ¹	5.85 ²	
	Henry's law constant			
	Vapour pressure			Pa
Ecotoxicology aquatic	EC ₅₀ and LC ₅₀	0.33 ²		mg/l
	NOEC and LOEC	0.044 ²	0.90 ²	mg/l
Ecotoxicology terrestrial	EC ₅₀ and LC ₅₀			mg/kg soil
	NOEC and LOEC			mg/kg soil
Toxicology				
Persistence, Bioaccumulation, Toxicity (PBT)				
Degradation	No mineralization under standard test conditions (low mineralisation, ISO 10708) ²			

¹ <http://www.thegoodscentcompany.com/data/rw1007691.html>

² http://www.sea.eawag.ch/inhalt/sites/stoffe/pdf/PMV_e.pdf

Table 1.14 Physiochemical and (Eco)toxicological properties of Cashmeran..

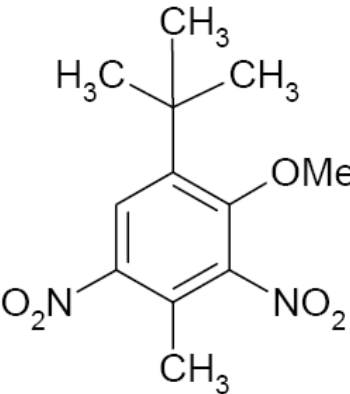
Common name	Cashmeran (DPMI)			
				
Name	6,7-Dihydro-1,1,2,3,3-pentamethyl-4(5H)indanone			
CAS #	33704-61-9			
Labelling	Xi, R 36/37/38, S 26, S 36 ³			
		Min	Max	Unit
Physico-chemical properties	Water solubility	0.17 ¹		mg/l
	Log K _{ow}	4.62 ¹	4.9 ²	
	Henry's law constant			
	Vapour pressure	5.2 ²		Pa
Ecotoxicology aquatic	EC ₅₀ and LC ₅₀			mg/l
	NOEC and LOEC			mg/l
Ecotoxicology terrestrial	EC ₅₀ and LC ₅₀			mg/kg soil
	NOEC and LOEC	-	-	mg/kg soil
Toxicology	Oral LD ₅₀ = 2900 mg/kg ¹ .			
Persistence, Bioaccumulation, Toxicity (PBT)				
Theoretical removal in STP				

¹ <http://www.thegoodscentscompany.com/data/rw1024091.html>

² Chen et al. (2007)

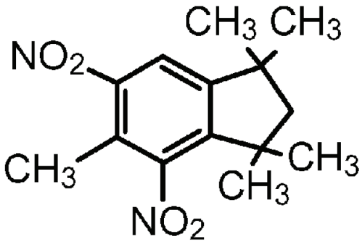
³ <http://www.thegoodscentscompany.com/msds/md103295.html>

Table 1.15 Physiochemical and (Eco)toxicological properties of Musk Ambrette.

Common name	Musk ambrette (MA)		
			
Name	1-tert-Butyl-2-methoxy-4-methyl-3,5-dinitrobenzene		
CAS #	83-66-9		
Labelling			
		Min	Max
Physico-chemical properties	Water solubility		mg/l
	Log K _{ow}	3.71 ¹	
	Henry's law constant		
	Vapour pressure		Pa
Ecotoxicology aquatic	EC ₅₀ and LC ₅₀		mg/l
	NOEC and LOEC		mg/l
Ecotoxicology terrestrial	EC ₅₀ and LC ₅₀		mg/kg soil
	NOEC and LOEC		mg/kg soil
Toxicology			
Persistence, Bioaccumulation, Toxicity (PBT)			
Theoretical removal in STP			

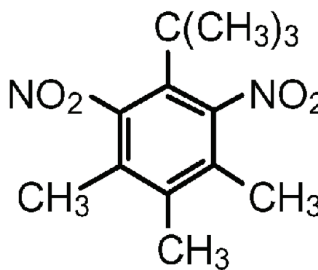
¹ <http://www.thegoodscentscompany.com/data/rw1008671..html>

Table 1.16 Physiochemical and (Eco)toxicological properties of Musk Moskene.

Common name	Musk moskene (MM)			
				
Name	4,6-dinitro-1,1,3,3, 5-pentamethylindane			
CAS #	116-66-5			
Labelling				
		Min	Max	Unit
Physico-chemical properties	Water solubility	0.046 ¹	0.046 ¹	mg/l
	Log K _{ow}	5.3 ¹	5.3 ¹	
	Henry's law constant			
	Vapour pressure			Pa
Ecotoxicology aquatic	EC ₅₀ and LC ₅₀			mg/l
	NOEC and LOEC			mg/l
Ecotoxicology terrestrial	EC ₅₀ and LC ₅₀			mg/kg soil
	NOEC and LOEC			mg/kg soil
Toxicology				
Persistence, Bioaccumulation, Toxicity (PBT)				
Theoretical removal in STP				

¹ OSPAR 2004

Table 1.17 Physiochemical and (Eco)toxicological properties of musk tibetene.

Common name	Musk tibetene (MM)			
				
Name	1-tert-butyl-2,6-dinitro-3,4,5-trimethylbenzene			
CAS #	145-39-1			
Labelling				
		Min	Max	Unit
Physico-chemical properties	Water solubility	0.052 ¹	0.052 ¹	mg/l
	Log K _{ow}	5.0 ¹	5.0 ¹	
	Henry's law constant			
	Vapour pressure			Pa
Ecotoxicology aquatic	EC ₅₀ and LC ₅₀			mg/l
	NOEC and LOEC			mg/l
Ecotoxicology terrestrial	EC ₅₀ and LC ₅₀			mg/kg soil
	NOEC and LOEC			mg/kg soil
Toxicology				
Persistence, Bioaccumulation, Toxicity (PBT)				
Theoretical removal in STP				

¹ OSPAR 2004

2 Methods

2.1 Sampling Strategy

A sampling strategy was devised based on two objectives:

- Elucidate the levels of mother compounds and metabolites within sewage treatment plants (STPs) and in receiving surface water recipients
- Elucidate the levels of mother compounds and metabolites in soils receiving sludge

Because the earlier screening study had determined STPs as the main sources of musk substances, no sampling effort was made at other possible sources. Two types of sludge application sites were included:

- Agricultural fields within the same local region where the same farmer had applied sludge 2008 and 2009. The same crops were grown in the fields.
- Two forest sites where sludge had been applied to improve forest growth.

At two of the STPs (in the county of Jämtland), sampling of incoming water, outgoing water and receiving surface water was performed on two occasions to roughly assess temporal variation.

The different matrices chosen and types of sampling points is presented in table 2.1. Detailed result tables in appendix 1 shows the types of samples taken at the different locations.

2.2 Sampling methods

Sampling instructions were given to all sampling personnel. The instructions included sampling procedures and handling of samples during transport.

2.2.1 Soil

Soil was sampled from the topmost layer after the removal of dead and living plant parts. Also, stones and larger objects were avoided. Soil samples were collected into diffusion proof clean sampling plastic bags or plastic containers and sent to the laboratory within a day of sampling. Samples were kept cold until analysis. For the agricultural soil sampling,, at least 5 samples was taken from each field to get as representative samples as possible. More details on the samples from the agricultural fields are given in Figure 3.6.

Table 2.1 Sampling matrices and the types of samples investigated for musk compounds and metabolites. The first value denotes samples taken within the national screening program. The second value (after the slash) denotes samples financed by the regional screening program.

Sampling matrices musk substances							
Sources	Incoming water	sludge	Outgoing water	Surface water	Sediment	Fish	Soil
Waste water treatment plants	6/4	4/6	8/4				
Receiving surface waters				10/4	8/6	6/1	
Sludge application sites							8/5
Landfills			0/1				
Total	<u>6/4</u>	<u>4/6</u>	<u>8/5</u>	<u>10/4</u>	<u>8/6</u>	<u>6/1</u>	<u>8/5</u>
Total	<u>50/31</u>						

2.2.2 Sediment

Sediment samples were collected by means of a core sampler. All sediment samples were transferred to pre-burned and dark glass jars and sent to laboratory within one or two days of collection. They were stored cold until analysis.

2.2.3 Sewage Treatment Plant (STP) sludge and water

The staff at the sewage treatment plants collected the sludge samples and water samples in acid rinsed pre burned dark glass bottles. All STP samples were sent to the laboratory within one or two days of collection. They were stored cold until analysis.

2.2.4 Fish

Samples from Lake Mälaren (perch, *Perca fluviatilis*) and Lake Abiskojaure (arctic char) were supplied from the Environmental Specimen Bank at the Museum of Natural history (A. Bignert and colleagues). All other fish were collected using fishing net. All fish samples were stored frozen until analysis.

2.2.5 Surface water

Unfiltrated water was collected in clean in acid rinsed pre-burned dark glass bottles. Water samples were stored cold until analysis.

2.3 Analytical methods

2.3.1 Water samples:

1000 ml of sample was extracted by shaking repeatedly with 30 mL Cyclohexane after addition of internal standards AHTN-D3 + musk- xylene D15 to the sample.

After extraction, Nonan was added as keeper and the extract was concentrated before fractionation on a silica-gel column. 1 µl of the extract was injected on a Agilent GC/MS 6890 fitted with a DB-5 column (30m x 0.25 mm x 0.25 µm). Detection technique for all compounds was GC-MS with EI mode. Native compounds for all analytes were used as external standards.

2.3.2 Soil and biota samples:

10 grams of the original sample was extracted repeatedly with a mixture of acetone/hexane using ultrasonic extraction for 1 hour each cycle, after addition of internal standards AHTN-D3 + musk- xylene D15 to the sample. After extraction, Nonane was added as a keeper and the extract was concentrated before fractionation on a silica-gel column. Soil, sediment and sludge samples were cleaned from interfering compounds using solid phase extraction (GPC). 1 µl of the extract was injected on a Agilent GC/MS 6890 fitted with a DB-5 column (30m x 0.25 mm x 0.25 µm). Detection technique for all compounds was GC-MS with EI mode. Native compounds for all analytes were used as external standards.

3 Results

The results are summarized in Table 3.1 and Figure 3.1 - Figure 3.2 where the statistical distribution for the mother compounds and the degradation products are presented. In Figure 3.3- Figure 3.5, the levels of Galaxolide, Musk Xylene and Musk Ketone and their metabolites are presented in incoming water, effluent water and sludge. The levels of musk compounds and metabolites in agricultural soils and forest soils receiving sludge applications are presented in Figure 3.6 - Figure 3.7. - Figure 3.8.

Table 3.1 Number of samples above LOQ (n), median concentration, 25th and 75th percentile of musk substances in different matrices. Note that only concentrations above LOQ were included in the statistical calculations.

	Incoming water (ng/l)			Effluent (ng/l)			Sludge (mg/kg)			Surface water (ng/l)			Sediment (µg/kg)			Fish (µg/kg)			Soil (µg/kg)											
	n	median	75	n	median	75	n	median	75	n	median	75	n	median	75	n	median	75	n	median	75									
Musk Xylene	2	260	220	300	3	1.4	1.4	1.8	n.d.	4	6.0	4.4	7.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.									
Musk Ketone	2	2	9	11	8	5.4	4.3	4.3	n.d.	1	3.7			n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.									
Musk Ambrette	3	46	31	53.5	n.d.				4	33	18	51	n.d.	3	21	1	11	n.d.	7	7.6	2.5	19								
Gal-axolide	10	1650	842	2150	12	320	228	423	8	1700	1270	1975	8	114	62	218	4	72	35	99	19	8	35	9	11	4.0	15			
Moskene	n.d.				n.d.				n.d.				n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3	16	9.3	16	
Tonalide	9	200	92	280	12	22	17	42	8	190	158	228	4	14	11	19	3	15	11	22	3.6	2.5	4.1	6	6.1	4.7	22			
Ce-lestolide	5	7.9	5.8	8.6	6	2.6	2.3	3.1	3	7.7	6.6	12.9	2	1.4	1.3	1.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1	5.1			
Phantolide	n.d.				n.d.				n.d.				n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Traesolide	n.d.				2	9.4	8.1	10.7	n.d.				1	5.5			n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cash-meran	10	57	45.25	108	12	23.5	17.5	33.3	7	68	41	75	6	14	7	20	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2	5.4	5.2	5.5		

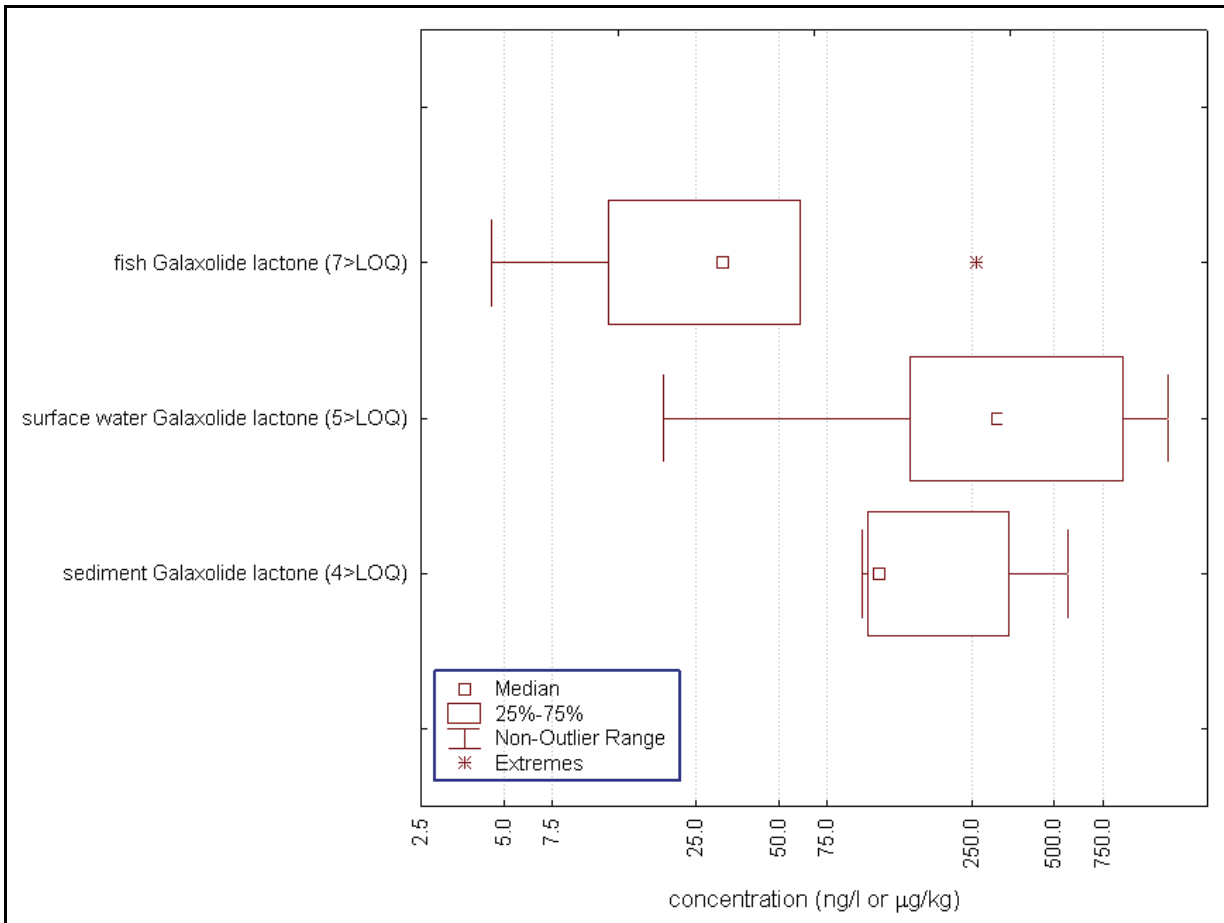


Figure 3.1 Distribution of musk metabolite concentrations in surface water matrixes.

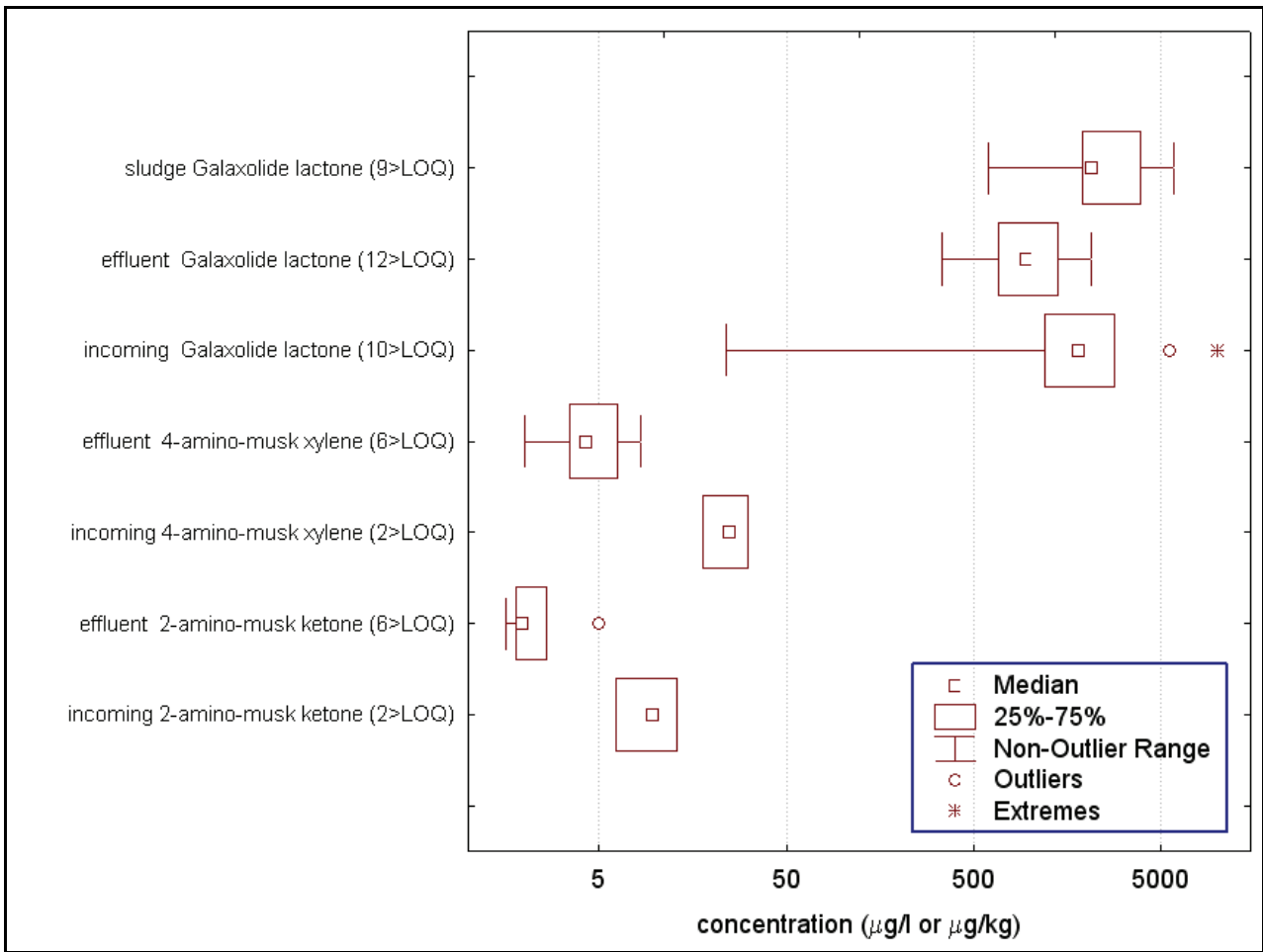


Figure 3.2 Distribution of musk metabolite concentrations in STP matrixes

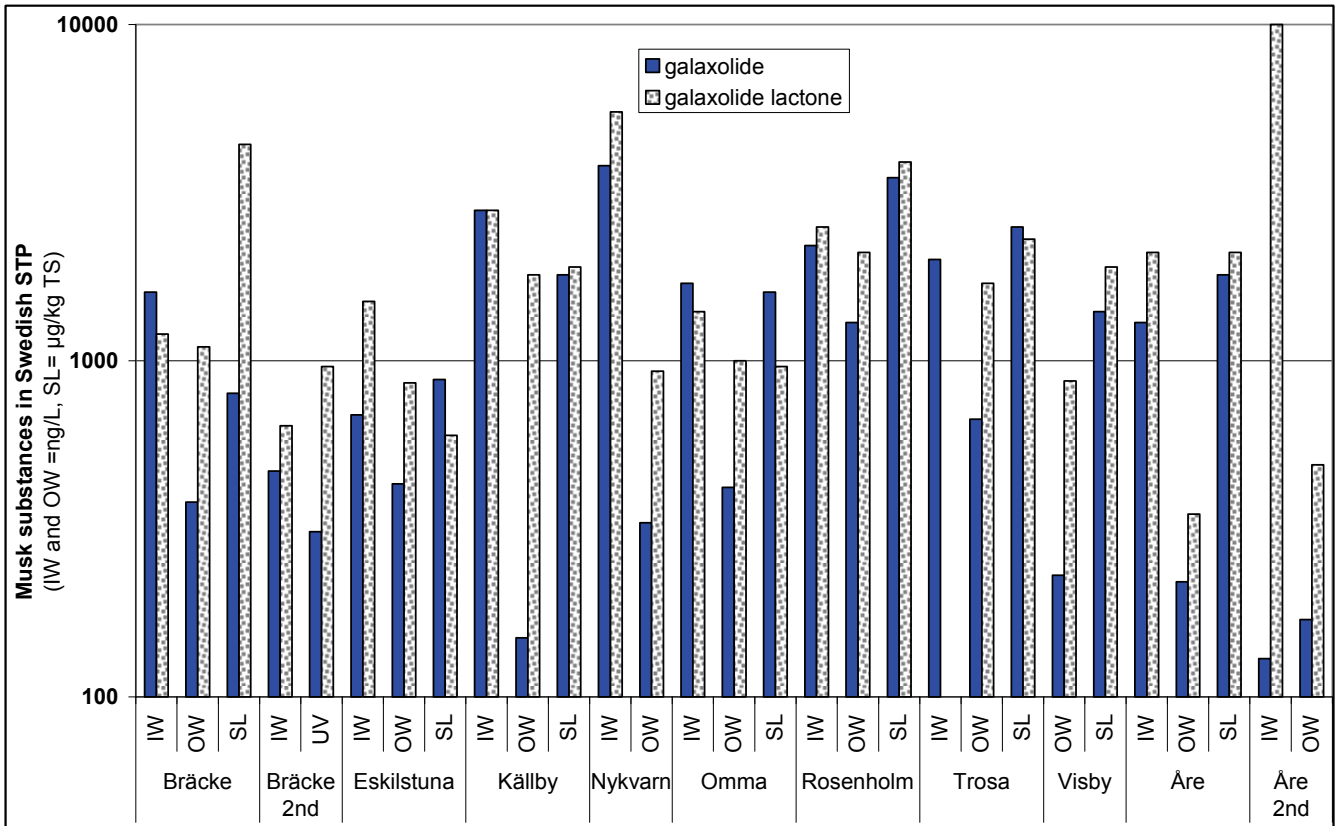


Figure 3.3 Levels of Galaxolide and the degradation product Galaxolide lactone in incoming water, effluent and sludge of STPs. IW=incoming water, OW=outgoing water, SL=sludge.

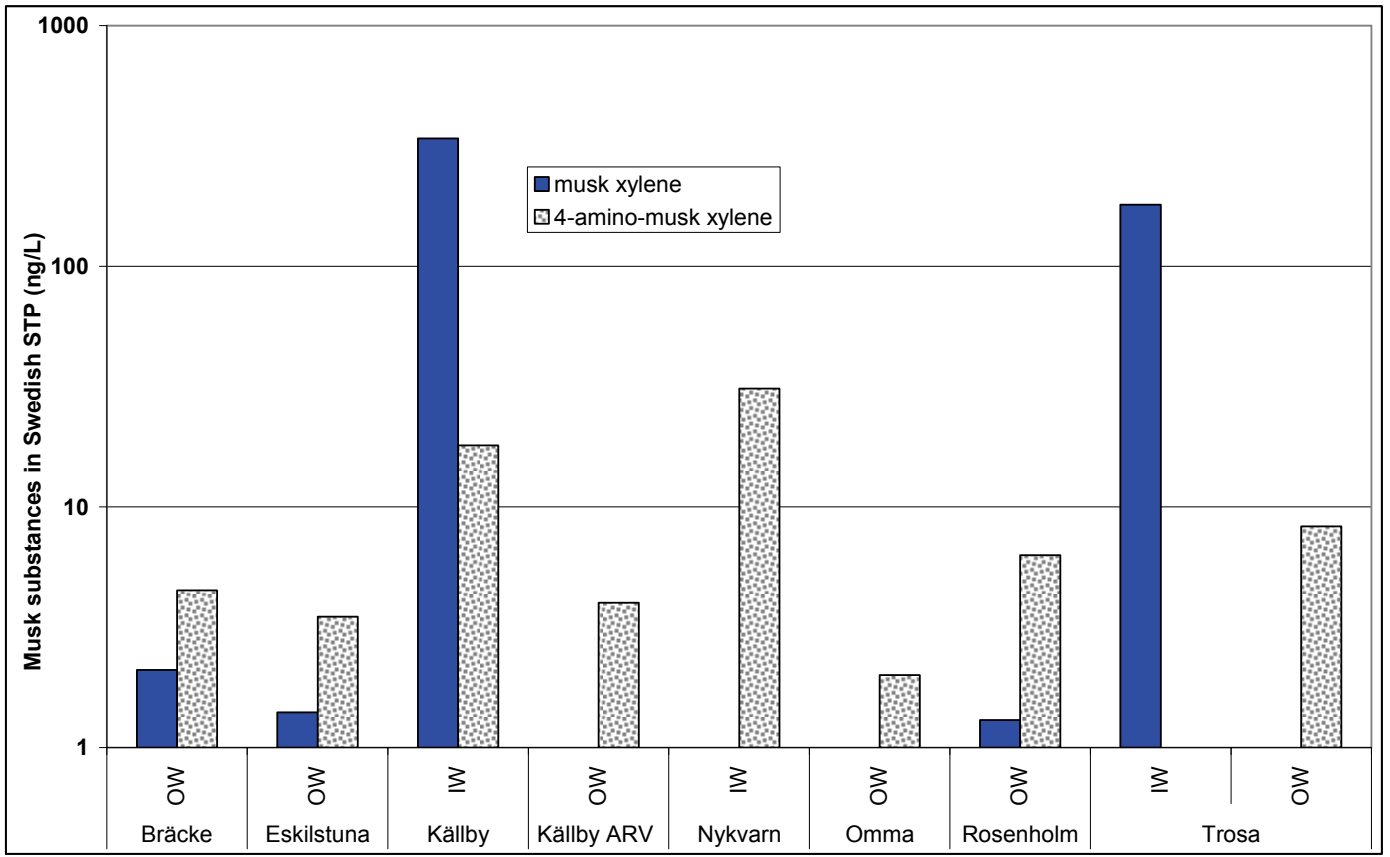


Figure 3.4 Levels of musk xylene and the degradation product 4-amino musk xylene in incoming water and outgoing water (effluent) of STPs. IW=incoming water, OW=outgoing water.

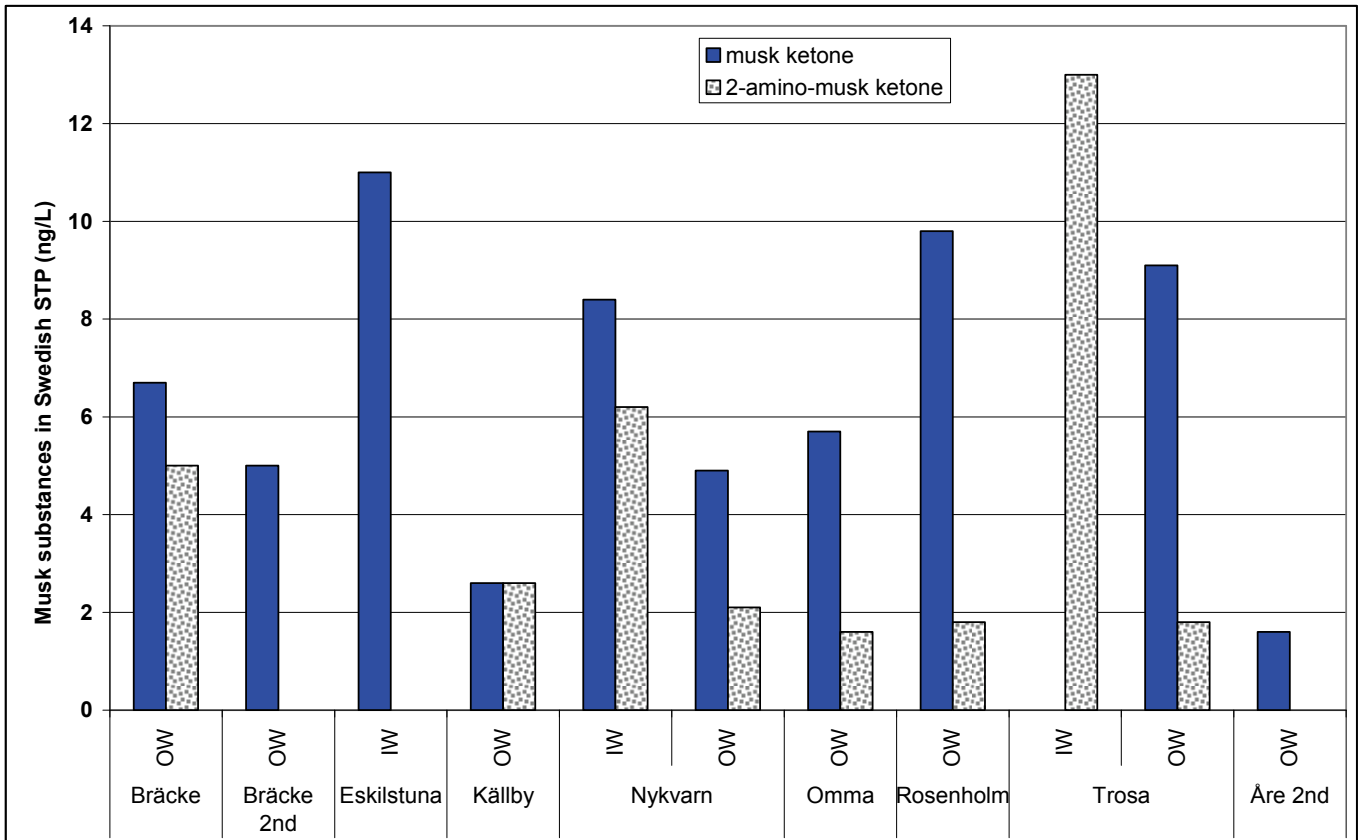


Figure 3.5 Levels of musk ketone and the degradation product 2-amino musk ketone in incoming water and outgoing water (effluent) of STPs. IW=incoming water, OW=outgoing water.

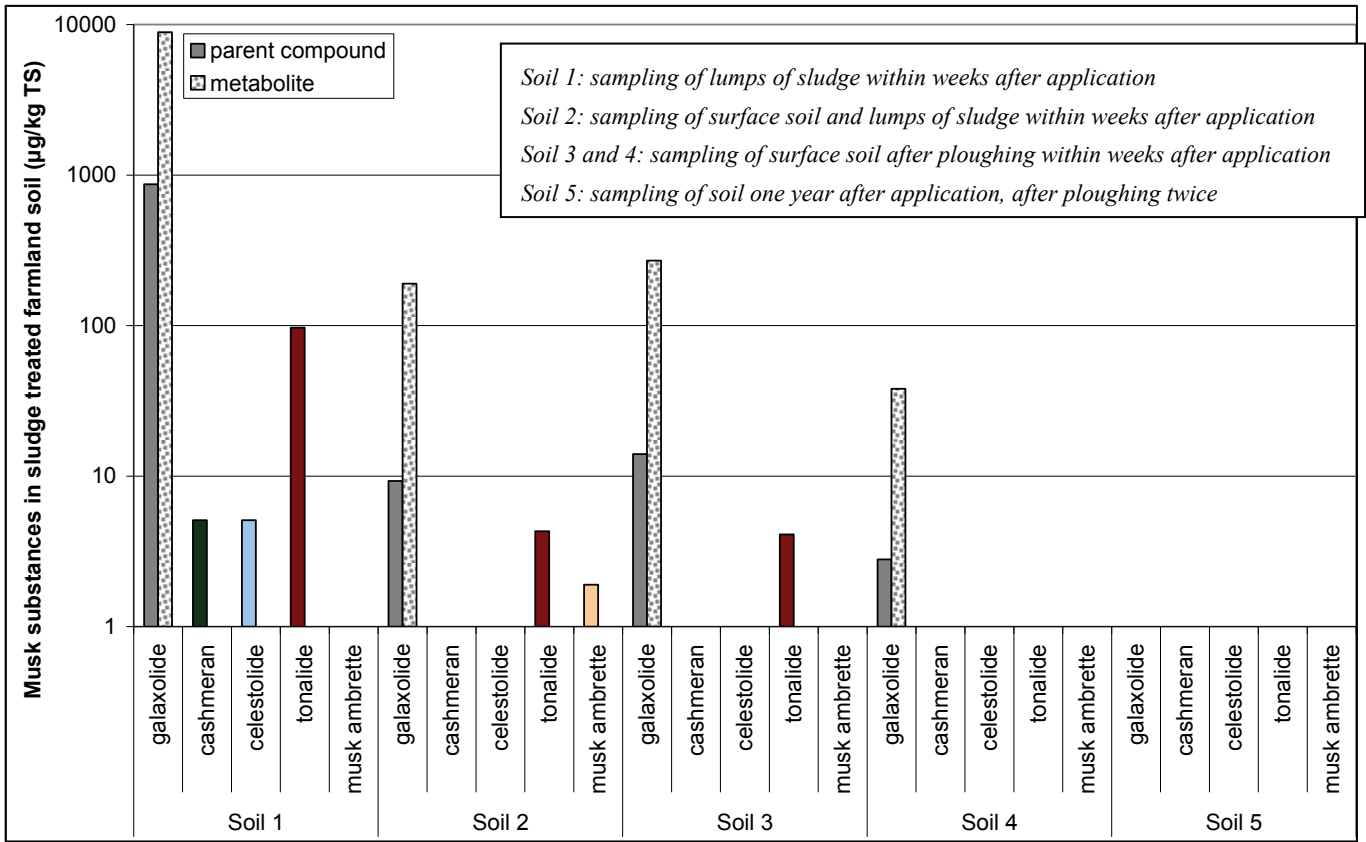


Figure 3.6 Levels of musk substances (and the metabolite Galaxolide lactone) in agricultural soils.

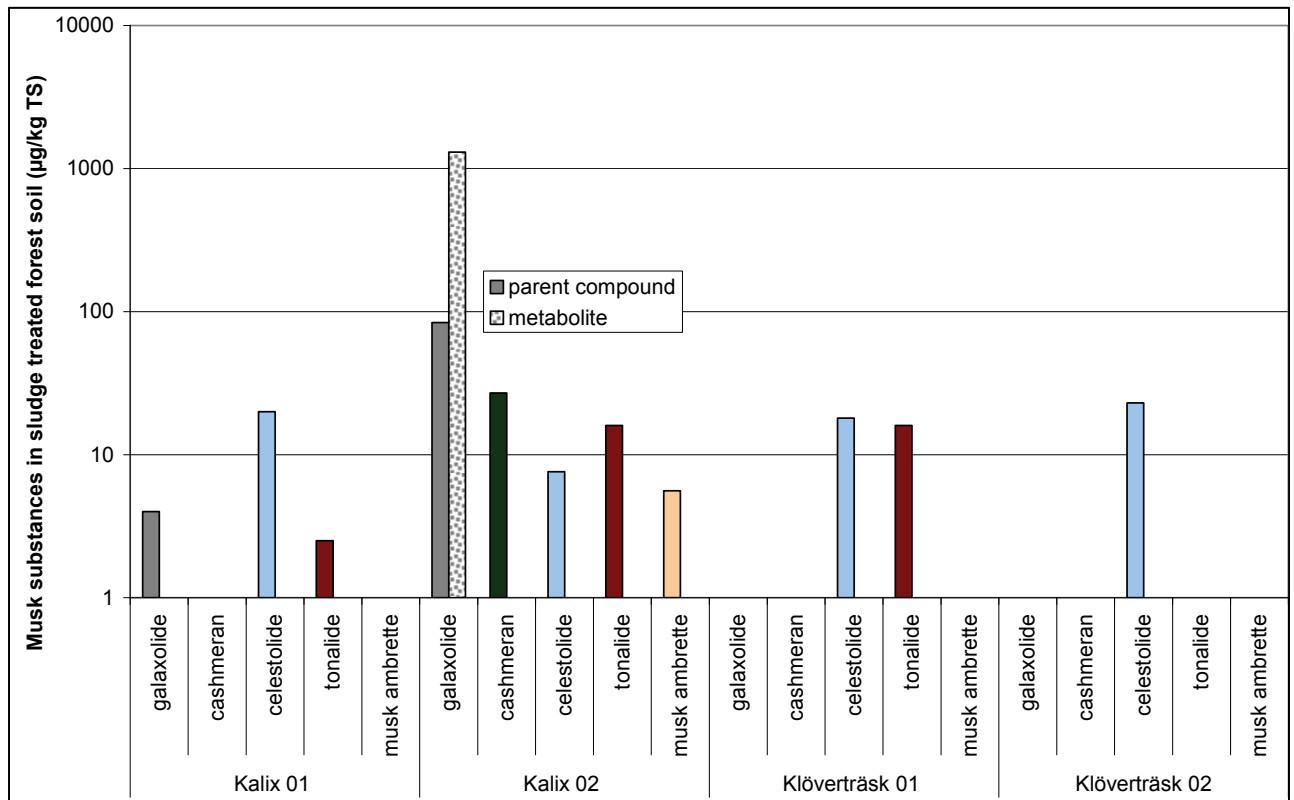


Figure 3.7 Levels of musk substances (and the metabolite Galaxolide lactone) in forest soils to which STP sludge has been applied.

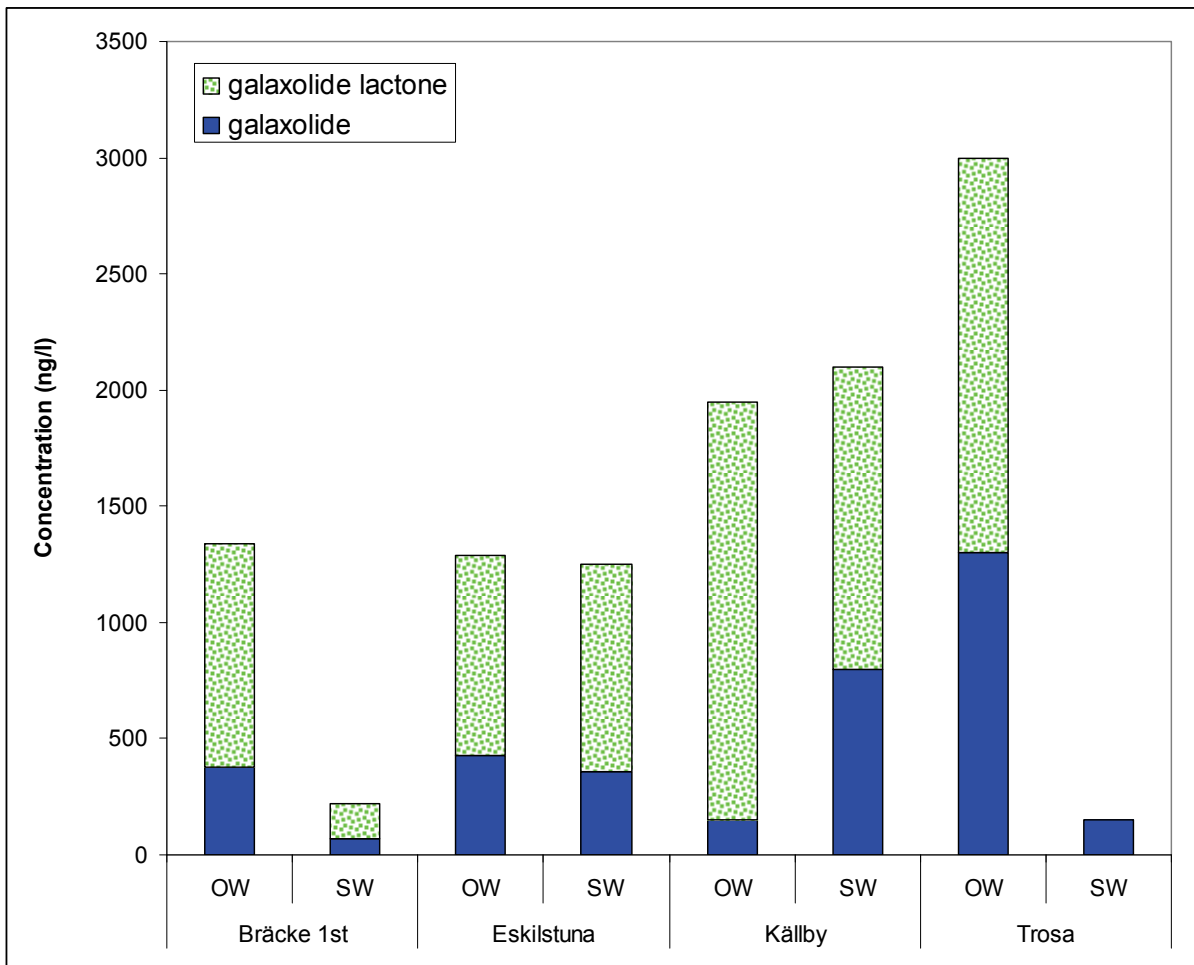


Figure 3.8 Levels of Galaxolide and Galaxolide lactone in STP effluent and surface waters down stream of STPs

4 Discussion

4.1 Levels of musk compounds and metabolites

Both galaxolide and galaxolide lactone was found in arctic char from the remote national background lake Abiskojaure at levels of 13 and 12 µg/kg. In the previous screening study from 2007, no musk substances were found in the sediment from this lake or soil surrounding it. Also, no musk substances were found in fish from other background localities in the 2007 screening study. In a previous Nordic screening study from 2003, Galaxolide was detected in 4/23 rainwater samples at concentrations in the range 12-29 ng/l (Tema Nord 2004). Some intermittent long range transport of Galaxolide may thus occur, and the substance is apparently stable/persistent enough for this to be reflected in fish in remote areas. Generally elevated levels of musk substances is also evidenced by the detection of different musk substances (galaxolide, galaxolide lactone, musk ambrette and tonalide) in surface water, sediment and fish *upstream* of STPs (Omma ARV, Nykvarn ARV and Trosa ARV). The detection of galaxolide, galaxolide lactone and tonalide in perch from lake Mälaren also indicate that musk substances are generally widespread. There are other studies demonstrating that musk substances are present in large water bodies in Nordic countries. In studies performed 1990 – 1995, Musk Ketone, Musk Xylene, Tonalide and Galaxolide was found in water sampled from a depth of 7 - 10 m in the North Sea at concentrations of 0.08 ng/l, 0.17 ng/l, 0.26 ng/l and 0.2 ng /l (no other depths were sampled) (TemaNord 2004).

As in the screening study from 2007, musk substances were found abundantly both in incoming water, sludge and effluent. The levels were in general comparable to the previous screening study (Table 4.1). However, more substances were detected 2009 at generally higher levels. This may indicate an increasing usage of these substances as evidenced by data from the product registry for galaxolide (Figure 1.1). It may also be result of a sampling program with more than double the amount of samples at STPs and their recipients which increase the likelihood of detecting high levels. It remains unknown whether the levels in Sweden are increasing, an issue that may need to be resolved given the prevalence of these compounds.

A comparison between the levels of musk metabolites in Sweden (present study) and other countries show that more metabolites were found at higher levels in Sweden. In some cases, the metabolites have never been previously analyzed in the matrix that was included in the present study. It seems unlikely that metabolite levels should be higher in Sweden compared to other countries, and the results are probably a result of a more encompassing sampling and analysis program compared to previous studies from other countries.

However, it is also clear that musk metabolites are as important to monitor as the mother compounds since several of the metabolites generally occur at levels similar to, or higher than their mother compounds in both STPs and limnic environments.

Table 4.1 Concentrations of different musk compounds in the present study compared to the screening study from 2007.

	Incoming water (ng/l)		Effluent (ng/l)		Sludge (µg/kg)		Surface water (ng/l)		Sediment (µg/kg)		Fish (µg/kg)	
	2007	2009	2007	2009	2007	2009	2007	2009	2007	2009	2007	2009
Musk Xylene (MX)	n.d.	180 - 340	n.d.	1.3-2.1	n.d.	n.d.	n.d.	1.2-8.7	n.d.	n.d.	n.d.	n.d.
Musk Ketone (MK)	0.5 - 42	8.4-11	0.5-9.1	1.6-9.8	2-845	n.d.	n.d.	3.7	n.d.	n.d.	n.d.	n.d.
Musk Ambrette (MA)	n.d.	16-61	n.d.	n.d.	n.d.	13-65	n.d.	n.d.	n.d.	6.2-49	n.d.	n.d.
Galaxolide (HHCB)	890-3300	130-3800	0.4-1040	340-2100	1430-29400	800-3500	0.4-180	2.9-800	0.4-33	7.1-100	6-7-48	3.4-44
Tonalide (AHTN)	62-202	18-380	0.5-74	6.6-62	88-2600	110-410	n.d.	5-32	0.5-4	6.2-28	n.d.	1-4.7
Celestolide (ADBI)	n.d.	5-17	n.d.	1.9-4	0.3-105	5.5-18	n.d.	1.2-1.5	n.d.	n.d.	n.d.	n.d.
Phantolide (AHDl)	n.d.	n.d.	n.d.	1.3	2.9-30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Traesolide (ATII)	n.d.	n.d.	n.d.	6.8-12	4-99	2-4	n.d.	5.5	n.d.	n.d.	n.d.	n.d.
Cashmeran (DPMI)	n.d.	17-250	n.d.	11-56	2.8-3510	20-98	n.d.	1.8-26	n.d.	n.d.	n.d.	n.d.

Table 4.2 Concentrations of different musk metabolites in the present study compared to studies from other countries.

Matrix	unit	present study	other studies	Countries	Reference
<u>Incoming water</u>					
4-amino-musk xylene	ng/l	<4 - 31	(0 in 7 out)	Germany, Canada	a, b
2-amino-musk ketone	ng/l	<4 - 13			
galaxolide lactone	ng/l	24 – 10 000	230 – 438 430 230 170-270	Germany Switzerland Germany Germany (STP influent)	a f b b
<u>Sludge</u>					
galaxolide lactone	µg/kg	600 – 5 800	55	Germany	c, d
<u>Outgoing water</u>					
4-amino-musk xylene	ng/l	<1 – 8,3	(0 in 7 out)	Germany	b
2-amino-musk ketone	ng/l	<1 - 5			
galaxolide lactone	ng/l	340 – 2 100	30 – 300 900 370, 335-420	Germany (STP effluent) Switzerland Germany (STP effluent) Germany (STP effluent)	a f, b b
<u>Surface water</u>					
4-amino-musk xylene	ng/l	<1 – 4,3			
2-amino-musk ketone	ng/l	<1 – 3,6			
galaxolide lactone	ng/l	<1 – 1 300	<10 – 300	Germany (Ruhr) Germany (Ruhr, Rhine and Lenne)	a b
<u>Sediment</u>					
4-amino-musk xylene	µg/kg	<5	0,5-0,8	Germany	a
galaxolide lactone	µg/kg	<2 - 560			
<u>Leachate</u>					
galaxolide lactone	ng/l	37			
<u>Agricultural soil</u>					
galaxolide lactone	µg/kg	<2 – 8 900			
<u>Forest soil</u>					
galaxolide lactone	µg/kg	<2 – 1 300			
<u>Fish</u>					
4-amino-musk xylene	µg/kg	<5	16- 3600*	Germany	c, d

Matrix	unit	present study	other studies	Countries	Reference
2-amino-musk xylene	µg/kg	<5	<1-100*	Germany	c, d
2-amino-musk ketone	µg/kg	<5	<1-180*	Germany	c, d
galaxolide lactone	µg/kg	<10 - 260	3,9-34* (9-337 in cod liver) 4-33*	Northern Germany (Norway) Germany, pond of a sewage treatment plant	c, d c, d d, e

* Concentrations in $n\ g\ g^{-1}$ lipid weight

^a Bester (2005)

^b Bester (2007)

^c Biselli et al. (2004)

^d Rimkus (200)

^e Hühnerfuss et al. (2004)

^f Ternes and Joss (2006)

4.2 Sludge application

A few studies have investigated the levels of musk substances in agricultural soil after the application of STP sludge. In an agricultural field amended with biosolids from the Peterborough STP (Ontario, Canada) Galaxolide and Tonalide were detected in soil immediately after application at mean concentrations of 1.0 and 1.3 µg/kg, respectively, but concentrations declined relatively rapidly over the next 6 weeks, post-application (Yang et al. 2006).

Kinney et al (2008) found both Galaxolide and Tonalide at levels of ca. 100 – 3000 µg/kg in agricultural soils that were relatively unaffected and soils that were heavily influenced by biosolid (sludge) amendments. They also found that the musk concentrations in earthworms living in the agricultural soils were equal to or higher than the levels in the soil. It should be noted that more (25 in total) pharmaceutical and personal care product related compounds were found in earthworms and at higher concentrations compared to soil. This shows the potential appropriateness of earthworms for biomonitoring of screening substances.

In the present study, many musk substances were present in the agricultural soils a few weeks after sludge application before and after plowing, but they could not be detected one year after application (Figure 3.6). This may partly be a sampling related result, because it is difficult to find sludge related material in a soil that was amended one year ago and plowed twice. It may also be a result of biodegradation, which is supported by an increasing ratio between galaxolide lactone and galaxolide with increasing mixing of soils and sludge (Figure 3.6). However, Xijuan, et al. (2009) found no degradation of Galaxolide or Tonalide and no increase in the levels

of Galaxolide lactone after 13 months of experimental incubation in a reed bed sludge treatment system. On the other hand, field measurements on sludge amended soil indicated that Galaxolide disappeared to a very large degree from soil within one year. The half-life based on unfrozen conditions in sludge amended soil studies was around 140 - 145 days (DiFrancesco et al. 2004)

A lack of musk substances after one year may also be a result of leaching from soil to groundwater, although Ternes et al. (2007) found very limited leaching of galaxolide and tonalide in agricultural soils to which biosolids had been applied. The latter results were explained by a high sorption to the soil.

Sludge is also added to forest soils to increase the yield. Despite this, organic pollutants such as pharmaceuticals and/or household related chemicals have rarely been measured in amended forest soils. Musk substances, for instance, has never been analyzed in sludge amended forest soils. The sites that were sampled in this study are situated in the north of Sweden and have been amended since 2001 with sludge granules from an STP in the Stockholm region. The levels of musk substances seen in the forest soils (Figure 3.7) may thus reflect a long term increase. Given the lower temperature and the lower microbial activity in a forest soil compared to an agricultural soil it is not surprising that organic pollutants can persist for longer time periods in such soils.

4.3 STPs and receiving surface waters

4.3.1 Musk compounds

As found in earlier studies, the concentrations of musk compounds were highly variable in the sludge samples (Table 3.1). The reason for the high variability is unknown although a number of studies suggest that the musk substance “profile” is highly specific to each STP (Bester 2007).

Galaxolide and Tonalide occurred in incoming water (waste water), sludge and effluents. They were also common in surface water and sediments downstream of the sewage treatment plants (Table 3.1). This demonstrates the importance of STPs as sources of these substances to the environment as shown in other studies (TemaNord 2004, OSPAR 2004, Kanda et al. 2003, HERA 2004b-c).

The reason that Galaxolide and Tonalide occurs in compartments downstream of sewage treatment plants is a combination of a high usage and possibly a suboptimal removal in sewage treatment plants (Table 4.4). Simonich et al. (2002) related the degree of removal of Galaxolide and Tonalide in wastewater treatment mainly to the degree of removal of total suspended solids which correlates well with the high levels of these substances found in sludge.

Table 4.3 Degree of removal of musk substances in sewage treatment plants in Europe and USA. Source: Simonich et al. (2002), Osemwengie and Gerstenbergerb (2004). Certain types of sewage treatment plants were excluded since they are only used in USA.

substance	Mimimum removal	Maximum removal
Galaxolide	39%	94%
Tonalide	57%	96%
Musk Xylene	89%	98%
Musk Ketone	85%	91%

The polycyclic musk substances found at the lowest concentrations in sludge (Celestolide, Traesolide, Cashmeran) were also detected in surface waters and sediments (Table 3.1). This is in contrast to the earlier screening studies where these substances were found in sludge but not in surface waters or sediments. This may indicate increasing environmental levels, but may also be a result of a more comprehensive sampling program. These substances have been found in sludge before, but only rarely in outgoing water (Kanda et al. 2003, OSPAR 2004), and the hypothesis has been that they most likely are removed to a large degree in the sewage treatment works, either through incorporation into the sludge or through biodegradation.

There was a fairly good correspondence between the number of occurrences above LOQ for incoming water and sludge (Table 3.1). This is also in contrast to the earlier 2007 screening study where a number of musk substances were found in sludge but not in incoming water. These results refute the hypothesis from the screening study of 2007 that sludge was the main source of musk compounds to the environment because of a discontinued usage. Instead, continued usage may very well be one important reason for the continuing occurrence in the environment.

4.3.2 Metabolites

Results from STPs show that the musk metabolites are prevalent both in incoming water, sludge and effluent (Figure 3.3 - Figure 3.6); Galaxolide lactone concentrations are at par with Galaxolide concentrations, 4-amino musk xylene concentrations are often higher than the concentrations of the mother compound, while 2-amino musk ketone concentrations are lower than the mother compound. The conclusion that musk metabolites are almost as common as the mother compounds in STPs is at par with results from previous studies from Germany (Bester et al. 2005 Bester et al. 2007).

There is no consistent pattern in the ratio between metabolite and mother compound in STPs. Some observations that can be made are:

- That musk metabolites are often present in incoming water to the STPs (Figure 3.3 - Figure 3.6). This may partly be a result of impurities in the fragrance products, but also indicates that biodegradation has occurred prior to the STPs, Since it is known that musk substances are common in human adipose tissue, breast milk and blood (Brunn et al. 2004), it is likely that biodegradation products in incoming water originates in the human body
- That the ratio of Galaxolide lactone to Galaxolide is often higher in effluent compared to incoming water (Figure 3.3). This indicates that biodegradation of Galaxolide occurs in the STP. However, the ratio varies between STPs which is in accordance with a German study of 9 STPs where the ratio was highly specific to each STP and not related to known operating parameters (Bester 2007).
- That the ratio between Galaxolide lactone and Galaxolide was often lower in sludge compared to outgoing water. The same results has been noted elsewhere (Bester 2007) and may be possibly be attributed to a higher water solubility of Galaxolide lactone compared to Galaxolide (see Table 1.8 and Table 1.9)
- That musk ketone and musk xylene metabolites were more common in effluent compared to incoming water, and they were not found in sludge at all (Figure 3.4 - Figure 3.5). One possible explanation may be that the levels in sludge were below the LOQ but still high enough so that the sludge acts as a source to the effluents. This is supported by the fact that the usage of musk xylene and ketone has decreased to a very large degree during the last ten years since these substances have been associated with risks to consumers (Bester 2007, OSPAR 2004, HERA 2004a) which would indicate that sludge may be the main source of these substances at present.

Both Galaxolide and Galaxolide lactone occurred in surface water downstream of STPs (Figure 3.8). Downstream of the Eskilstuna STP and Källby STP the concentrations in surface waters were at par with concentrations in effluents which is not excepted since both recipients are relatively large which should cause significant dilution. This may be a sampling related result because spot sampling may “catch” temporarily high or low concentrations in the surface waters and effluents. Nevertheless, the results show that, as a conservative estimate, the concentrations in surface waters can sometimes be equal to concentrations in effluents. This is important when evaluating the effects of STP on the limnic environment.

Galaxolide lactone as well as Galaxolide frequently occurred in both fish and sediments (Table 3.1, Figure 3.1) although the metabolite occurred at generally higher concentrations. This shows that the calculated *PBT* designation for Galaxolide lactone (Table 1.9) is consistent with field measurements, and that the designation as

Pbt for Galaxolide (Table 1.8) may underestimate the tendency for biota accumulation.

Metabolites of musk Xylene and musk Ketone were only detected (4.3 and 3.6 ng/l) in surface water in the stream Høije å (downstream of the Källby STP situated in the city of Lund). The levels were relatively low, and their detection at this site correlates with high levels of several Musk substances as well as Galaxolide lactone in the stream.

4.4 Risk assessment

The question remains whether the concentrations in recipient surface waters and sediments are of any environmental concern.

A risk comparison between the maximum measured concentration in surface water, sediment and soil, and the predicted no effect concentration (PNEC) of these substances, results in a maximum risk ratio of 13 for Galaxolide lactone in surface water (Table 4.4). This ratio is however based on very uncertain data including a high assessment factor and a calculated LC50 value using ECOSAR. On the other hand, Galaxolide lactone belongs to a substance groups (esters) with a relatively good correlation between chemical structure and ecotoxicity and the ECOSAR model predicts lower *chronic* toxicity values compared to acute. Any conclusion on the risk of galaxolide lactone to the surface water environment has to wait until there is experimental data to base the conclusion on.

The risk ratio was 17 for 4-amino musk xylene. The PNEC value is based on a measured EC50 (*Daphnia magna*, immobilization) of 0.25 µg/l which, using an assessment factor of 1000, gives a PNEC of 0.25 ng/l. This is a very low PNEC value, and the low EC50 has been contested in further studies which gave an EC50 of 500 µg/l (Giddings et al. 2000). Until further studies confirm or refute the low toxicity value of 4-amino musk xylene, it can not be concluded whether the substance actually causes any risks to the aquatic environment.

No PNEC value was exceeded in the sediments. A comprehensive study from 2009 on the effects of Galaxolide on juveniles and adults of the snail *Potamopyrgus antipodarum* demonstrates effects on reproduction and feeding rate at 10 – 30 mg/kg (Pedersen et al. 2009). There are also indications (not statistically significant) of effects at concentrations of 1 mg / kg which is still well above the concentrations found in this study. The much higher levels of Galaxolide lactone in sediments demonstrates the need for ecotoxicity testing of this substance.

The PNEC value for galaxolide was exceeded by a factor of three in agricultural soil a few weeks after sludge application and before the soil had been plowed. Note that the sampled soil consisted to a large part of sludge lumps together with soil, and the

levels seen in the soil/sludge samples are probably mostly a reflection of sludge levels. In forest soil the maximum galaxolide concentration was approximately half the PNEC value. The PNEC value for soil is somewhat robust (based on two tests of chronic toxicity) and an exceedance probably indicates true risks. Galaxolide lactone levels were often higher than the mother compound in soil, and the highest metabolite concentration in both agricultural and forest soil exceeded the highest galaxolide concentration. Given that galaxolide and its lactone metabolite are structurally similar there is a possibility that the metabolite have similar ecotoxicological properties. Consequently, there may be an environmental risk with the levels of galaxolide lactone found in soils.

In general, although the risk ratios may not give rise to any immediate concern, they are high enough that the environmental effects of some musk substances can not be totally ruled out.

Table 4.4 Risk assessment for detected musk substances in surface waters, sediments and soils. If no other information is given, PNEC values are taken from table 1.3 – 1.17.

substance	Maximum measured concentration (MEC) in this study	PNEC	MEC/PNEC ratio
Surface water (ng/l):			
Galaxolide	800	4400	0.2
Galaxolide lactone	1300	100 ¹	13
Musk tibetene	640	No data	
Tonalide	32	350 ²	0.01
Cashmeran	26	No data	
Musk xylene	8.7	560	0.01
Traseolide	5.5	No data	
4-amino-musk xylene	4.3	0.25 ³	17.2
Musk ketone	3.7	1000	0.004
2-amino-musk ketone	3.6	50000	0.0001
Celestolide	1.5	No data	
Sediment (µg/kg):			
Galaxolide	100	2000	0.05
Galaxolide lactone	560	No data	
Musk ambrette	49	No data	
Tonalide	28	200 ²	0.01

substance	Maximum measured concentration (MEC) in this study	PNEC	MEC/PNEC ratio
Soil (µg/kg):			
Galaxolide lactone	8900	No data	
Galaxolide	870	310	2.8
Tonalide	97	2100 ⁵	0.05
Musk ambrette	23	No data	
Musk moskene	16	No data	
Cashmeran	5.6	No data	
Celestolide	5.1	No data	

¹ EC50 (table 1.9) divided by 1000 according to EU TGD

² Marine environment and consequently uncertain for limnic environment

³ EC50 value (table 1.6) divided by 1000 according to EU TGD

⁴ New data from 2009, LOEC value for snail larvae according to Pedersen et al. (2009)

⁵For Tonalide two long term tests are available (Table 1.10) , allowing an assessment factor of 50 to be applied to the lowest NOEC. Therefore PNECsoil 2.1 mg/kg dwt.

4.5 Temporal variability

Temporal variability was briefly assessed by sampling on two occasions (ca. 2 months apart) in incoming water, effluent and outgoing water from two STPs (Åre och Bräcke) in the county of Jämtland. The concentrations were often within 100 – 200% of each other showing surprisingly little temporal variability. Brändli (2002) found that musk concentrations in waste water varied on different days of the week. For most of the compounds peak concentrations were measured on Mondays which was hypothesized to be a result of personal care behavior.

5 Conclusions and recommendations

This and other studies show that musk compounds frequently occur in sewage treatment plants and in receiving surface waters. Also, they frequently occur in soils receiving sludge amendment. There are indications that risk levels in surface waters may be exceeded, although the toxicity data is too uncertain to draw any conclusions.

When the results from this study are compared with results from the 2007 screening study, it seems that the levels of musk substances in the environment are not decreasing. The levels of some substances (Galaxolide, Tonalide and their metabolites) may even be increasing which correlates with increasing usage.

Musk substance metabolites frequently occurred in most matrixes, sometimes at levels above the mother compounds. The risk that these metabolites pose to the environment is highly uncertain given limited (eco)toxicity data, but they need to be considered when assessing the risks of musk substances to the environment.

The following recommendations are put forward.

- The fate of musk substances that has been amended to soils needs to be further assessed since there is a risk of both uptake in plant and animals from these soils as well as transport to surface water and groundwater.
- Given that musk substances are very frequent in most environmental compartments, there is a need for a comprehensive assessment on the need for including these substances in the regular monitoring programs.
- More detailed studies on the effects of musk substances and metabolites in surface water, soils and sediments are needed. This is generally outside the scope of the screening program of the Swedish EPA.
- There are indications of additive effects between different musk substances (Behechti et al. 1998, Bester 2007) which needs to be considered if a refined risk assessment is performed.
- A mass balance study of the amounts of different musk substances reaching waste water treatment plants, the amounts stored in sludge and the amounts reaching surface waters would be of interest to elucidate the magnitude of the environmental problems with musk substances.
- A study of how operating parameters in the sewage treatment plants, as well as sources of incoming water, affect the levels of musk substances in

sludge and outgoing water could also be of interest. The purpose would be to better understand why these substances occur in the downstream recipients.

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