REPORT

Chemical and biological monitoring of sewage effluent water

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Report Summary

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Title and subtitle of the report

Chemical and biological monitoring of sewage effluent water

Summary

As an assignment from the Swedish Environmental Protection Agency, IVL has conducted a study with the overall aim to present basic data for decision-making regarding a monitoring program for STP effluents. A program for chemical and biological monitoring in effluent water from municipal sewage treatment plants is important for the understanding on spreading of chemicals in the environment, to identify changes in the use or substitution of chemicals, to identify possible emissions of "new" emerging substances to the environment, and also for the implementation and follow up of directives.

The study consisted of several parts; a chemical characterization of STP effluents consisting of both the analysis of specific compounds but also the identification of "unknown" compounds, measurements of estrogenic and androgenic activity with the bioassays YES and YAS, evaluation of seasonal variability in chemical composition, and chemical measurements in recipient water. Further, a literature survey of potential additional bioassays suitable for monitoring purposes was conducted.

Based on the results a recommendation for a monitoring program for municipal STP effluent is given.

Keywords

Chemical characterization**,** chemical identification, diffuse emissions, effect assays, emerging substances, monitoring, priority substances

Bibliographic data

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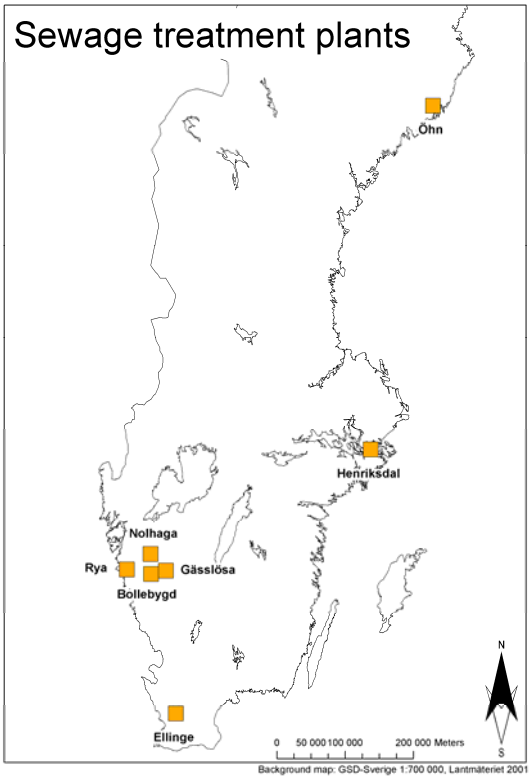
Sammanfattning

IVL har på uppdrag av Naturvårdsverket genomfört en studie med det övergripande syftet att generera ett underlag för ett program avseende kemisk och biologisk övervakning av utgående avloppsvatten.

Övervakning av utgående kommunalt avloppsvatten är av betydelse för förståelsen avseende diffus spridning av kemikalier i miljön, för att identifiera förändringar i användning och substitution av kemikalier, att identifiera ”nya” kemikalier som kan spridas via avloppsvatten, samt för arbetet med implementering och uppföljning av olika direktiv.

Studien har innehållit flera delar; en kemisk karakterisering bestående av både riktade analyser och identifiering av ”okända” substanser, mätningar av östrogena och androgena effekter med de jästbaserade testerna Yeast Estrogen Screen (YES) och Yeast Androgen Screen (YAS), utvärdering av säsongsvariation avseende halter i avloppsvatten, samt mätningar i en recipient. Vidare har en litteraturstudie genomförts med syfte att identifiera ytterligare tester för biologiska effekter lämpliga för övervakning. Sju reningsverk ingick i studien, se Figur nedan.



Resultat från den kemiska karakteriseringen av avloppsvatten summeras i tabellen nedan. För de ämnen som återfanns i halter över rapporteringsgränserna anges detektionsfrekvens samt om uppmätta halter var av samma storleksordning eller över toxicitets eller gränsvärden. Vidare anges om ämnet finns med bland de prioriterade ämnena (PS) eller är listat i Annex III i dotterdirektivet till EUs ramdirektiv för vatten (2008/105/EG), och/eller om ämnet är bland de som identifierats vara av särskild betydelse för Östersjön enligt Baltic Sea Action Plan.

Sammanfattning av ämnen funna över rapporteringsgränser, deras detektionsfrekvenser, resultat av jämförelser med toxicitets och gränsvärden, samt status avseende WFD och BSAP.

Ämnesgrupp	**Ämne**	**Detektions-frekvens**	**WFD**	**BSAP**	**Konc. av samma storlek eller över toxicitets/gränsvärde**
Organiska tennföreningar	TBT, DBT, MBT	2, 7, & 3/7	PS	X	**Ja** (TBT)
	TPhT, MPhT	1 & 2/7		X	**Ja** (TPhT)
	DOT	1/7			Nej
Bromerade flamskydds-medel	BDEs (47, 99, 100)	6, 5 & 3/7	PS	X	**Ja** (∑BDE)
	HBCDD	7/7		X	Nej
Fenolära ämnen	4-Nonylfenol	4/7	PS	X	**Ja**
	4-t-Oktylfenol	2/7	PS	X	**Ja**
	Triclosan	7/7			**Ja**
	Bisfenol A	5/7	Annex III		**Ja**
Metaller	Hg	7/7	PS	X	Nej
	Cd	7/7	PS	X	Nej
	Pb	7/7	PS		Nej
	Ag	7/7			**Ja**
	As	7/7			Nej
	Cu	7/7			**Ja**
NSAIDs	Ibuprofen	7/7			**Ja**
	Naproxen	7/7			**Ja**
	Ketoprofen	7/7			Nej
	Diclofenac	7/7			**Ja**
Perfluorerade ämnen	PFOS	7/7	Annex III	X	Nej
	PFOA	7/7		X	Nej
	PFOSA	5/7			-
	PFHxA	7/7			-
	PFDcA	7/7			-
Ftalater	DEP	7/7			-
	DIBP	7/7			-
	DBP	7/7			Nej
	BBzP	4/7			-
	DEHP	6/7	PS		Nej
Organfosfat-estrar	TIBP	7/7			**Ja**
	TBP	7/7			Nej
	TCEP	7/7			Nej
	TDCP	6/7			Nej
	TBEP	7/7			**Ja**
	TPhP	7/7			**Ja**
	EHDPP	7/7			-
Volatila organiska ämnen	n-Hexan	6/7			-
	Bensen	6/7			Nej
	Toluen	7/7			Nej
	Etyl-bensen	5/7			Nej
	m+p-Xylen	7/7			-
	Styren	3/7			Nej
	o-Xylen	6/7			-
	n-Nonan	1/7			-
Volatila halogenerade ämnen	1,1,1-Trikloretan	3/7			-
	1,2-Diklorethan	7/7	PS		Nej
	Kloroform	7/7	PS		Nej
	Koltetraklorid	7/7	PS		Nej
	Tetrakloreten	7/7	PS		Nej
	Trikloreten	5/7	PS		Nej
Siloxaner	D6	2/7			-
	MM	1/7			-
	MDM	1/7			-
	MD2M	2/7			-
	MD3M	4/7			-

Uppmätta halter var generellt av samma storleksordning eller lägre jämfört med vad som rapporterats i andra studier. Halter av samma storleksordning, eller över, toxicitets eller gränsvärden, uppmättes för organiska tennföreningar (TBT och TPhT), bromerade flamskyddsmedel (∑BDEs), fenolära ämnen (samtliga), metaller (Ag och Cu), NSAIDs (ibuprofen, naproxen and diclofenac), samt organfosfatestrar (TIBP, TBEP and TPhP). För vissa ämnen överskreds toxicitets eller gränsvärden, men detektionsfrekvenserna var låga, t.ex. för organiska tennföreningar, bromerade flamskyddsmedel, 4-nonylfenol och 4-t-oktylfenol. För dessa ämnen bör arbete läggas på metodutveckling för att sänka rapporteringsgränser.

Följande ämnen förekom inte i halter över rapporteringsgränserna; de organiska tennföreningarna DPhT och MOT, BDE-kongenererna 85, 153, 154 och 209, ftalaterna DOP, DINP och DIDP, de volatila organiska ämnena 3-metyl-pentan, n-oktan och 1,3,5-TMB, det volatila halogenerade ämnet diklormetan, och siloxanerna D4 och D5. Vidare återfanns ingen av herbiciderna eller klorbensenerna som ingick i studien.

Multivariat utvärdering av data visade att det generellt var liten variation i kemisk sammansättning mellan proverna från de olika inkluderade reningsverken. Proverna från två av sju reningsverk skiljde sig dock från de övriga.

Utvärderingen av säsongsvariation visade inte på några tydliga skillnader i halter under året. Den generellt låga variationen i halter mellan prover tagna olika månader är i överensstämmelse med vad som kan förväntas för ämnen med ett diffust spridningsmönster. Det ska dock hållas i åtanke att proverna utgjordes av dygnsprover och alltså inte representerar hela provtagningsperioderna.

Mätningarna i avloppsvatten från ett av reningsverken, Gässlösa, samt i ytvatten från recipienten Viskan visade att andra källor är av betydelse för belastningen på denna vattenförekomst. För de fenolära ämnena indikerade resultaten att belastning via avloppsverket är av betydelse, men för andra ämnena, särskilt metallerna, var halterna i recipienten av samma storleksordning eller högre jämfört med i avloppsvattnet.

Metodiken använd för identifiering av "okända" ämnen, i vilken fraktioner framtagna för riktade analyser utnyttjades, var framgångsrik. Ett stort antal ämnen identifierades, varav flera i halter av storleksordningen µg/l. Vidare identifierades ett antal metaboliter och nedbrytningsprodukter vilka är viktiga för att studera spridning av deras respektive modersubstanser.

Det faktum att halterna av flera ämnen var av samma storleksordning eller över toxicitets/gränsvärden, samt att ett stort antal "okända" ämnen identifierades och återfanns i relativt höga halter, visar på behovet av att utvärdera toxiciteten för komplexa blandningar. Ett sätt att göra detta är att använda effekttester. Lämpliga tester bör vara av relevans för utvärdering av potentiell kronisk toxicitet, vara snabba, kostnadseffektiva, samt undvika användande av försöksdjur. I denna studie uppmättes viss östrogen effekt, och indikationer på anti-androgen effekt kunde ses, och litteraturstudien om effekttester identifierade ett antal ytterligare effekter av relevans för övervakning.

Utifrån resultaten från den kemiska och biologiska karakteriseringen av avloppsvatten, utvärderingen av säsongsvariation, mätningarna på prov från recipienten, samt litteraturstudien om effekttester, presenteras ett förslag på ett övervakningsprogram för utgående avloppsvatten.

Summary

As an assignment from the Swedish Environmental Protection Agency, IVL has conducted a study with the overall aim to present basic data for decision-making regarding a monitoring program for sewage treatment plant (STP) effluents. A program for chemical and biological monitoring in effluent water from municipal sewage treatment plants is important for the understanding on spreading of chemicals in the environment, for the implementation of directives, to identify changes in the use or substitution of chemicals and to identify possible emissions of "new" emerging substances to the environment.

The study consisted of several parts; a chemical characterization of STP effluents consisting of both the analysis of specific compounds but also the identification of "unknown" compounds, measurements of estrogenic and androgenic activity with the bioassays Yeast Estrogen Screen (YES) and Yeast Androgen Screen (YAS), evaluation of seasonal variability in chemical composition, and chemical measurements in recipient water. Further, a literature survey of potential additional bioassays suitable for monitoring purposes was conducted. Seven STPs were included in the study.

The concentrations of the substances found in effluent waters are presented in the Table below. The measured concentrations were in general in the same range or lower compared to what have been reported in other studies. The concentrations were in the same range or above toxicity or limit values for organic tin compounds (TBT and TPhT), brominated flame retardants (∑BDEs), phenolic compounds (all), metals (Ag and Cu), NSAIDs (ibuprofen, naproxen and diclofenac) and organophosphorus esters (TIBP, TBEP and TPhP).

For some of the substances the concentrations exceeded or were in the same range as toxicity or limit values, but the detection frequencies were low, e.g. for the organic tin compounds, the brominated diphenyl ethers, 4-nonylphenol and 4-t-octylphenol. For these substances there should be a focus on method development in order to lower the reporting limits.

Substances not found were the organic tin compounds DPhT and MOT, the brominated flame retardants BDE congeners 85, 153, 154 and 209, the phthalates DOP, DINP and DIDP, the volatile organic carbons 3-methyl-pentane, n-octan and 1,3,5-TMB, the volatile halogenated substance dichloromethane, and the siloxanes D4 and D5. Further, none of the herbicides or the chlorobenzenes included in the study were found.

Multivariate data analysis showed that, in general, there is relatively low variability in chemical composition in municipal STP effluents. However, differences between Ellinge and Bollebygd compared to the other STPs were found.

Summary of substances found above the reporting limits, their detection frequencies, results on comparisons with toxicity/limit values, and information regarding WFD and BSAP status.

Substance group	Substance	Detection frequency	WFD	BSAP	Conc. in the same range or above toxicity / limit value
Organic tin compounds	TBT, DBT, MBT	2, 7, & 3/7	PS	X	**Yes** (TBT)
	TPhT, MPhT	1 & 2/7		X	**Yes** (TPhT)
	DOT	1/7			No
Brominated flame retardants	BDEs (47, 99, 100)	6, 5 & 3/7	PS	X	**Yes** (∑BDE)
	HBCDD	7/7		X	No
Phenolic compounds	4-Nonylphenol	4/7	PS	X	**Yes**
	4-t-Octylphenol	2/7	PS	X	**Yes**
	Triclosan	7/7			**Yes**
	Bisphenol A	5/7	Annex III		**Yes**
Metals	Hg	7/7	PS	X	No
	Cd	7/7	PS	X	No
	Pb	7/7	PS		No
	Ag	7/7			**Yes**
	As	7/7			No
	Cu	7/7			**Yes**
NSAIDs	Ibuprofen	7/7			**Yes**
	Naproxen	7/7			**Yes**
	Ketoprofen	7/7			No
	Diclofenac	7/7			**Yes**
Perfluorinated substances	PFOS	7/7	Annex III	X	No
	PFOA	7/7		X	No
	PFOSA	5/7			-
	PFHxA	7/7			-
	PFDcA	7/7			-
Phthalates	DEP	7/7			-
	DIBP	7/7			-
	DBP	7/7			No
	BBzP	4/7			-
	DEHP	6/7	PS		No
Organo-phosphorus esters	TIBP	7/7			**Yes**
	TBP	7/7			No
	TCEP	7/7			No
	TDCP	6/7			No
	TBEP	7/7			**Yes**
	TPhP	7/7			**Yes**
	EHDPP	7/7			-
Volatile organic compounds	n-Hexane	6/7			-
	Benzene	6/7			No
	Toluene	7/7			No
	Ethyl-benzene	5/7			No
	m+p-Xylene	7/7			-
	Styrene	3/7			No
	o-Xylene	6/7			-
	n-Nonan	1/7			-
Volatile halogenated substances	1,1,1-Trichloroethane	3/7			-
	1,2-Dichloroethane	7/7	PS		No
	Chloroform	7/7	PS		No
	Carbon tetrachloride	7/7	PS		No
	Tetrachloroethene	7/7	PS		No
	Trichloroethene	5/7	PS		No
Siloxanes	D6	2/7			-
	MM	1/7			-
	MDM	1/7			-
	MD2M	2/7			-
	MD3M	4/7			-

No clear seasonal variation in concentrations of measured substances was found. The general low variability between samples taken during different months is in agreement with the expected diffuse spreading of the included substances. It should however be kept in mind that the samples taken were single daily samples.

The measurements of chemicals in effluents from Gässlösa STP and in surface waters from Viskan indicated that other sources could be of importance for the chemical load to this water body. The measurements indicated a significant load from the STP of phenolic substances, whereas the concentrations of other compounds measured in recipient water, especially the metals, were in the same range or higher in these samples compared to the effluent samples.

The identification work on ”unknown” compounds in fractions used for the identification of specific substances, turned out to be successful. A large number of substances were identified; several of them at concentrations in μg/l levels. Further, several metabolites or degradation products were identified which could be used as tracers indicating diffuse spreading of their precursors. Thus, the methodology used is a potential option for the identification of new emerging substances.

The identification of several substances at concentrations close to or above toxicity and limit values, and the identification of a large number of “unknown” substances at relatively high concentrations, stress the importance to address the issue of mixed toxicity. This could be done by incorporating effects measurements in a monitoring program. Suitable assays for such measurements should target effects relevant for chronic effects, have a high through put, be cost efficient, and preferably avoid usage of animals. Within this study, some estrogenic effects and results indicating anti-androgenic effects were found. The literature survey on potential additional bioassays suitable for monitoring identified several other effects to be of relevance for a monitoring program.

Based on the results from the chemical and biological characterization of effluent water, the recipient study, as well as the literature survey on potential additional bioassays, a recommendation for a monitoring program for municipal STP effluent is given.

Table of contents

1 Introduction

Chemicals are emitted to the environment via a variety of different sources, both via point sources and via diffusive spreading. Previous screening studies have confirmed that there is a diffuse spreading of organic contaminants and metals to the environment and enriched levels in urban areas suggest that the use in e.g. household products may be important sources for emissions. Effluent water from municipal sewage treatment plants (STPs) may thus constitute an important source to the recipient load of chemicals with a diffusive spread pattern.

However there is s a "cosmos" of different chemicals in effluent water which have different chemical/ physical and biological properties, which may affect both transport processes as well as the biological effects. Except the origin substances there are also transformation products.

EU Water Framework Directive (WFD) has identified 33 priority substances (PS), for which environmental quality standard (EQS) and emission control measures have been established. With the recently adopted HELCOM Baltic Sea Action Plan (BSAP) the Baltic Sea countries have committed themselves to achieve a "Baltic Sea with life undisturbed by hazardous substances", eleven hazardous substances /or groups of substances have been identified to be of specific concern to the Baltic Sea.

Emerging substances are substances that have been detected in the environment, but are currently not included in routine monitoring programs, for which fate, behavior, and (eco) toxicological effects are not well understood. They may be candidates for future legislation due to their adverse effects and / or persistency.

To get an overall picture of the diffusive spreading of chemicals to the environment, the importance of the emissions from sewage treatment plants has to be investigated. A program for chemical and biological monitoring of effluent water from municipal sewage treatment plants is important for the understanding of the spreading of chemicals in the environment, for implementation of directives to identify changes in the use or substitution of chemicals, and to identify possible emissions of "new" emerging substances to the environment.

2 Aim

The overall objectives of this study were to:

- Present basic data for decision-making concerning a program for chemical and biological monitoring in effluent water from municipal sewage treatment plants (STPs).

- Identify "unknown" substances in the effluent water.

To fulfill these objectives the following activities have been carried out:

- Measurements of selected organic substances and metals in effluent water samples.

A characterization of potential biological effects, consisting of bioassay measurements of androgenic and estrogenic activities.

An evaluation of the seasonal variability in effluent chemical composition.

Measurements of selected substances in the adjacent receiving water from a STP.

A broader chemical characterization of effluent waters including directed analyses of a large number of substances and also identification of "unknown" substances.

A literature surveys on potential other bioassays suitable for monitoring purposes has also been undertaken.

3 Monitoring strategy

3.1 Sampling sites, STPs

For the chemical and biological characterization of the effluents, the same seven STPs which are included in Swedish monitoring program on chemicals in sewage sludge were chosen (Haglund and Olofsson, 2007). These STPs differ e.g. in size, load composition, treatment processes, and geographical location. Some of the characteristics of the included STPs are presented in Table 1, and their locations are presented in Figure 1.

Table 1. Information regarding the included STPs (from Haglund and Olofsson, 2007).

STP	Size (kpe)	Influent volume (Mm^3/year)	Load
Henriksdal, Stockholm	835	88	Municipal, hospital, industrial
Ryaverket, Göteborg	772	117	Municipal, storm water, hospital, industrial
Öhn, Umeå	100	12	Municipal and hospital
Ellinge, Eslöv	100	4.6	Municipal, large contribution from food industry
Gässlösa, Borås	98	18	Municipal, textile industry
Nolhaga, Alingsås	37	4.6	Municipal, industrial, laundry, landfill, hospital
Bollebygd	1.7	0.29	Municipal

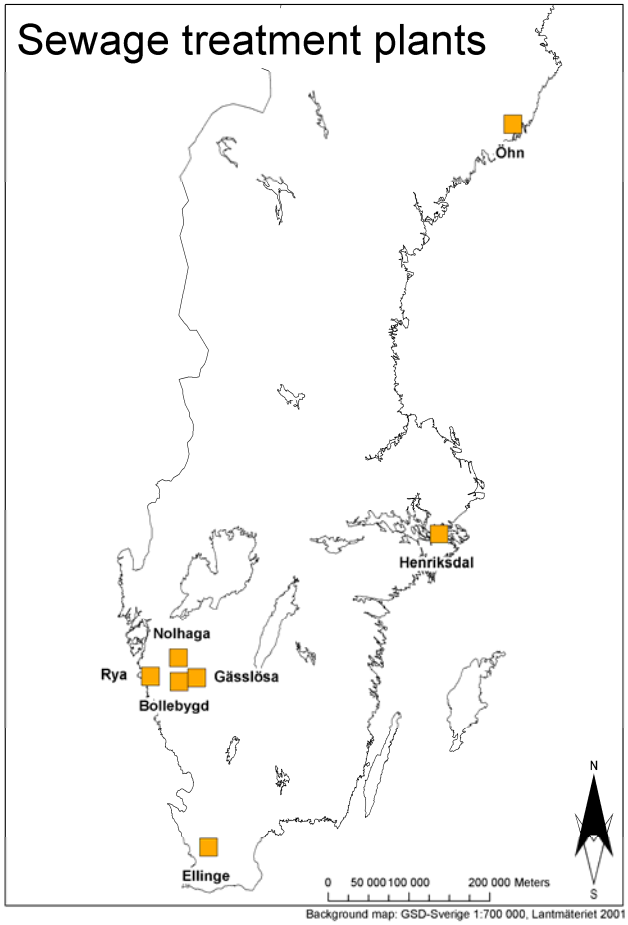


Figure 1. Locations of STPs included for chemical and biological characterization of effluent water.

The samples were collected at one occasion in August-September. In order to evaluate seasonal variation of the chemicals in the effluent water, samples from one of the STPs, Gässlösa, were collected at four occasions during 2008, in the beginning of March, April, and August, and in the end of October.

3.2 Receiving water

Recipient water samples were collected in river Viskan at Gässlösa STP at two occasions, in April and August. The water samples (surface water 0-1 m depth) were taken upstream and 50 m and 2 km downstream, the effluent point in the river Viskan.

3.3 Chemical characterization

3.3.1 Target analytes

Substances subject to specific analysis included in the study are listed in Table 2. For the chemical characterization of effluents, all these substances were analyzed. For the study on seasonal variability in chemical composition, substance groups A to E in Table 2 were measured. These substance groups were also the ones measured in the surface water samples from the recipient Viskan.

Table 2. Compounds measured by directed analysis.

Substance group	Included substances
A. Organic tin compounds	Mono-, di-, and tri-butyl tin (MBT, DBT, TBT) Mono-, di-, and tri-phenyl tin (MPhT, DPhT, TPhT) Mono- and di-octyl tin (MOT, DOT)
B. Brominated flame retardants (BFRs)	Brominated diphenyl ethers; congeners # 47, 85, 99, 100, 153, 154, 209 Hexabromocyclododecane (HBCDD)
C. Phenolic compounds	4-Nonylphenol (branched) 4-t-Octylphenol Triclosan Bisphenol A
D. Herbicides	Glyfosate 4-Chloro-2-methylphenoxy acetic acid (MCPA)
E. Metals	Mercury, total (Hg-tot) Cadmium (Cd) Lead (Pb) Silver (Ag) Arsenic (As) Copper (Cu)
F. Non-steroidal antinflammatory drugs (NSAIDs)	Ibuprofen Naproxen Ketoprofen Diclofenac
G. Perfluorinated substances (PFAS)	Perfluorooctane sulfonate (PFOS) Perfluorooctanoic acid (PFOA) Perfluorooctane sulfonamide (PFOSA) Perfluorohexanoic acid (PFHxA) Perfluorodecanoic acid (PFDcA)
H. Phthalates	Diethyl phthalate (DEP) Diisobutyl phthalate (DIBP) Dibutyl phthalate (DBP) Benzyl butyl phthalate (BBzP) Di-ethylhexyl phthalate (DEHP) Dioctyl phthalate (DOP) Diisononyl phthalate (DINP) Diisodecyl phthalate (DIDP)
I. Organophosphorus esters	Triisobutyl phosphate (TIBP) Tributyl phosphate (TBP) Tris(2-chloroethyl) phosphate (TCEP) Tris(1,3-dichloro-2-propyl) phosphate (TDCP) Tributoxy ethyl phosphate (TBEP) Triphenyl phosphate (TPhP) 2-Ethylhexyl diphenyl phosphate (EHDPP)
J. Volatile organic compounds	3-Methyl-pentane n-Hexane Benzene Toluene n-Octan

Substance group	Included substances
	Ethyl-benzene
	m+p-Xylene
	Styrene
	o-Xylene
	n-Nonan
	1,3,5-Trimethylbenzene (1,3,5-TMB)
K. Volatile halogenated substances	1,1,1-Trichloroethane
	1,2-Dichloroethane
	Dichloromethane
	Chloroform
	Carbon tetrachloride
	Tetrachloroethene
	Trichloroethene
L. Chlorobenzenes	1,3,5-Trichlorobenzene
	1,2,4-Trichlorobenzene
	1,2,3-Trichlorobenzene
	Hexachlorobutadiene
	1,2,3,4-Tetrachlorobenzene
	1,2,3,5- + 1,2,4,5-Tetrachlorobenzene
	Pentachlorobenzene
	Hexachlorobenzene
	Octachlorostyrene
M. Siloxanes	Octamethylcyclotetrasiloxane (D4)
	Decamethylcyclopentasiloxane (D5)
	Dodecamethylcyclohexasiloxane (D6)
	Hexamethyldisiloxane (MM)
	Octamethyltrisiloxane (MDM)
	Decamethyltetrasiloxane (MD2M)
	Dodecamethylpentasiloxane (MD3M)

3.3.2 Identification of non target substances

The extracts used for target analysis of different groups of organic compounds were also used for identification of non target ("unknown") compounds. For this purpose GC-MS chromatograms were recorded in "full scan" mode. Identification of substances was made by comparison of acquired mass spectra with mass spectra in the GC-MS library data base and published mass spectra in the scientific literature, see chapter 4.2.2.

3.4 Biological characterization

The biological characterization of effluent waters consisted of measurements regarding androgenic and estrogenic activities. These measurements were done on effluent waters from six STPs, the STPs included in the chemical characterization with the exception of Bollebygd.

4 Methods

4.1 Sampling

Flow proportional daily samples of effluent waters were taken for analysis of all substances except phthalates. In order to avoid the risk of contamination from sampling equipment used in the STPs

spot samples were used in this case. In the recipient, Viskan, spot samples of surface water (0-1 m) were taken.

Samples were collected in muffled (400 ºC, 3 h) dark glass bottles, or acid rinsed plastic bottles, depending on substance to be analyzed. Samples in plastic bottles were stored frozen, whereas samples in glass bottles were acidified and stored at 4 ºC. All samples were preserved with phosphoric acid (pH 2). Samples for metal analysis were preserved with 1(w/w) % nitric acid.

4.2 Chemical analysis

The chemical analysis of effluents from seven STPs was divided into two separate parts. The initial target analysis was focused on specific compound or groups of compounds. For this purpose sample extracts were fractionated and in some cases derivatized to enrich certain compound groups sharing common properties, see Figure 2. Specially developed analytical methods for the different target analytes were used in conjunction with the GC-MS instrument in high sensitivity and selective mode. To obtain high sensitivity the MS was used in Selected Ion Monitoring (SIR) mode. In SIR mode only selected ions are monitored, typically 2-3 ions. The limitation of the SIR mode is that other compounds present in the sample extracts are not detected.

In the second part the same extracts were analyzed with the MS-instrument in "full scan" mode implying that all compounds that are chromatographed on the GC-column are detected with their characteristic mass spectrum. An attempt was then made to identify as many compounds as possible in the different extracts. The types of compounds expected in the extracts are presented in Figure 2.

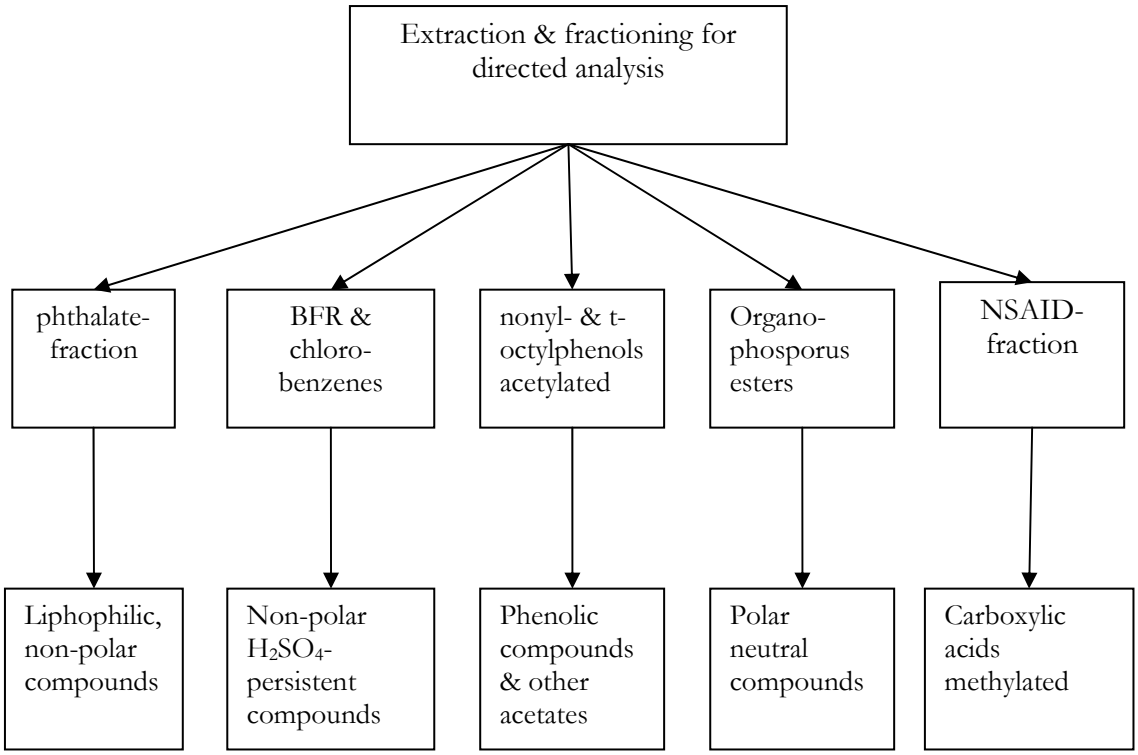


Figure 2: Schematic presentation of the analytical strategy used in this report. Explanation: Brominated flame retardants (BFR).

4.2.1 Determination of specific compounds

Phthalates

Un-filtrated water samples (500 ml) were liquid-liquid extracted with MTBE after addition of sodium chloride (2,5 % w/w). The extracts were dried over sodium sulphate and the solvent was changed to hexane. Clean-up was performed on a solid phase extraction column (ethylendiamine-N-propyl; PSA). Analysis was done using GC-MSD (6890N, 5973N, Agilent) selective detector (MSD) in selected ion monitoring mode (SIM) with an electron ionisation energy of 70 eV.

Brominated flame retardants (BDEs/HBCDD) and chlorobenzenes

Filtrated water (glass fiber filter) was extracted using a SPE-column (C18; 500 mg). The filter was extracted separately using acetone and hexane:MTBE (3:1). The SPE-column eluate and the filter extract were combined and the solvent changed to hexane. The extract was purified using concentrated sulfuric acid. Finally, the extract was chromatographed on a short silica gel column. The BDEs in the extract were analysed with a GC (5890, Agilent) equipped with an electron capture detector (ECD). The chlorobenzenes were analysed on a GC-MSD (6890N, 5973N, Agilent) in SIR mode using negative chemical ionization with methane as reagent gas.

Phenolic compounds

Filtrated water (glass fiber filter) was extracted using a SPE-column (Isolute ENV+, 200 mg) while the filter was extracted separately using acetonitrile and hexane:MTBE (1:1). The SPE-column eluate and the filter extract were combined and the solvent changed to hexane. The extract was acetylated (Allard *et al.*, 1985) and purified on a silica column prior to GC-MS analysis (6890N, 5973N, Agilent). The detector was used in SIM mode with an electron ionisation energy of 70 eV.

Organophosphorus esters

Un-filtrated water samples (500 ml) were liquid-liquid extracted with MTBE after addition of sodium chloride (2,5 % w/w). The extracts were dried over sodium sulphate and the solvent was changed to hexane. Clean-up was performed on a solid phase extraction column (PSA). Analysis was performed on GC-MSD (6890N, 5973N, Agilent) in SIR mode with an electron ionisation energy of 70 eV.

Non-steroidal anti-inflammatory drugs (NSAIDs)

Water samples (500-1000 ml) were filtered (pre-heated GF/C filter) at pH > 8. The filters were discarded. The filtrates were acidified and concentrated on SPE-columns (Oasis HLB 200 mg, Waters). The flow rate during extraction was ~15 ml/min. The NSAIDs were eluated using ethyl acetate. The extracts were subjected to a developed liquid-liquid extraction routine prior to derivatization. The sample was derivatised (methyl esterification) using methyl chloroformate (MCF) according to Weigel *et al.* (2002) and Butz and Stan (1993). Prior to GC-MS analysis, the extracts were subjected to a silica gel chromatographic purification routine where a deactivated silica gel column was prepared in a Pasteur pipette. Analysis was performed on GC-MSD (6890N, 5973N, Agilent) in SIR mode with an electron ionisation energy of 70 eV.

Herbicides

(4-chloro-2-methylphenoxy) acetic acid (MCPA) was extracted and derivatised using the same methodology as for NSAIDs. Samples (10 ml; un-filtrated) for analysis of glyphosate were first reacted with *iso*-butylchloroformate according to Kataoka *et al.*, (1991). The produced derivative of the amine was solvent extracted, dried and concentrated. The phosphorus group in glyphosate was finally reacted with diazomethane. The analysis was performed on a GC-MSD (6890N, 5973N, Agilent) in SIR mode with an electron ionisation energy of 70 eV.

Organic tin compounds

The samples (un-filtrated) were ethylated using sodium tetraethylborate and simultaneously extracted with hexane. The extracts were analyzed using GC-MSD (6890N, 5973N, Agilent) in SIR mode with an electron ionisation energy of 70 eV. The methodology is described in more detail in Lilja *et al.* (2009).

Perfluorinated substances

Water samples (300 ml) were filtered (pre-heated GC/C filter) and extracted using SPE (Oasis HLB 200 mg, Waters). The column was eluted with methanol (8 ml) which were evaporated to 1 ml. The sample extract was analyzed applying high performance liquid chromatography connected to a triple quadrupole mass spectrometer (HPLC-MS/MS) (Prominence UFLC, Shimadzu, API 4000, Applied Biosystem).

Volatile organic compounds and volatile halogenated substances

Water samples (100 ml) were purged with helium and the analytes were trapped on Tenax TA. The analysis of the Tenax TA adsorbent tubes was carried out on an automated thermal desorption instrument (ATD-400, Perkin-Elmer) attached to a gas chromatograph (3400, Varian). The VOCs were detected using a flame ionisation detector (GC-FID) and the halogenated hydrocarbons on an electron capture detector (GC-ECD).

Siloxanes

Siloxanes were analysed according to Kaj *et al.* (2005). The samples were purged with nitrogen and the analytes trapped in adsorbent tubes containing Tenax TA. The tubes were transferred to, and the analytes thermally desorbed in, a dedicated instrument (Unity, Markes) connected to a gas chromatograph with mass specific detector (6890N, 5973N, Agilent).

Metals

^{185}Re was added to the sample and standards as an internal standard. The concentration of the metals Cu, Pb, Hg, Cd, Ag and As in the samples were determined using a high-resolution plasma mass spectrometer (ELEMENT2, Thermo Inc., Germany) at NILU. The data processing and instrument control were performed by the ELEMENT software SWv 3.06.

4.2.2 Identification of unknown compounds in extracts used for specific analysis

A schematic description of the identification strategy is presented in Figure 2 showing what type of compounds the different extracts may contain. Attempts were made to identify all organic compounds in all samples by screening all resolved peaks in the GC-MS chromatogram. Once a positive identification of an organic compound was made the newly identified compound was then screened as a target anlayte in other samples in the same type of extract. Identification of the compounds in the GC-MS chromatograms was made by comparison of the acquired mass spectrum with Wiley and NIST mass spectrum library GC-MS data base, published mass spectra in the scientific literature and by comparison of our oven mass spectrum from compounds analysed in our laboratory. For unambiguous identification requires good mass spectrum agreement and GC-retention match between the unknown compound and the reference compound. However, in this project the demand for a positive identification was first a good MS-library match ($\geq$90 % agreement). Furthermore, was it estimated if the proposed compounds retention time was reasonable in relation to its molecule weight and chemical structure (physical and chemical properties; NLM ChemID Plus) and if it was likely that the compound could be recover in the extract examined considering the analytical protocol.

4.3 Estrogenic and androgenic activity

Estrogenic and androgenic activities of effluent water samples were determined employing the Yeast Estrogen Screen (YES) and Yeast Androgen Screen (YAS) assays. YES is a recombinant yeast strain with the human estrogen receptor α gene incorporated in the main chromosome (Routledge and Sumpter, 1996), whereas YAS is a recombinant yeast strain containing the gene for the human androgen receptor (Sohoni and Sumpter 1998). Binding and activation of these receptors results in transcription of the reporter gene *lacZ* coding for β-galactosidase, which activity can be measured spectrophotometrically. Sample treatment, assays performance, and data treatment were done according to standardized protocols (for details, see e.g. Andersson *et al.*, 2006).

5 Results and discussion

Detailed information regarding concentrations of specific chemicals and hormonal activities for the samples can be found in Table A2-Table A16 in Appendix A.

5.1 Chemical characterization of STP effluents – specific analysis

This section presents the results from the specific chemical characterization of STP effluents. For the evaluation of measured concentrations, comparisons have been made to toxicity and limit values set in legislation or derived by international authorities, These include e.g. the Annual Average Environmental Quality Standards (AA-EQS) found in the European Union Water Framework Daughter Directive (2008/105/EC) and PNEC values derived in European Union Risk Assessment Reports (EU-RARs). For substances not covered by these, comparisons have been made with values derived by national authorities, *e.g.* the risk assessments on siloxanes derived by

the United Kingdom Environment Agency (Brooke *et al.*, 2008a, 2008b, 2008c) and the proposal EQSs for specific pollutants derived by the Swedish Chemical Agency (Swedish EPA, 2008), and/or from the open scientific literature.

It should be kept in mind that toxicity and limit values may vary significantly depending on protection goals covered and the amount of data available for their derivation. The WFD EQSs are intended to protect all compartments, not only the pelagic, but also the benthic community, against secondary poisoning of predators, and to protect human health. This means that an EQS derived for e.g. a persistent bioaccumulative substance may be lower compared to a PNEC for the same substance derived for the protection of the pelagic environment. A pelagic PNEC may thus not always be protective for the most relevant compartment. This might be true e.g. for the perfluorinated substances and HBCDD.

For the comparisons, measured concentrations have not been corrected for dilution effects. Comparisons have also been made with previous measurements in STP effluents.

5.1.1 Organic tin compounds

All of the organic tin compounds included in the study, except DPhT and MOT, were found in the STP effluents, see Figure 3 below and Table A2 in Appendix A. DBT was the butyl tin compound most frequently found; it was detected in effluents from all STPs, whereas MBT and TBT were found in effluents from three and two STPs, respectively. The concentrations were in the ranges 1.3-4.4 ng/l, 1.4-3.1 ng/l and 0.62-1.4 ng/l for MBT, DBT and TBT, respectively. Of the phenyl tin compounds, MPhT was found in effluent from Henriksdal and Bollebygd (5.9 and 1.7 ng/l respectively), whereas TPhT was found in effluent from Henriksdal STP (1.8 ng/l). DOT was only found in effluent from Henriksdal STP (1.3 ng/l).

The effluent from Henriksdal contained the highest number of detected organic tin compounds, and also at the highest concentrations, see Figure 3. As can be seen in Table 3 below and in Table A2 in Appendix A, the concentrations found for most of the organic tin compounds were close to their respective reporting limits.

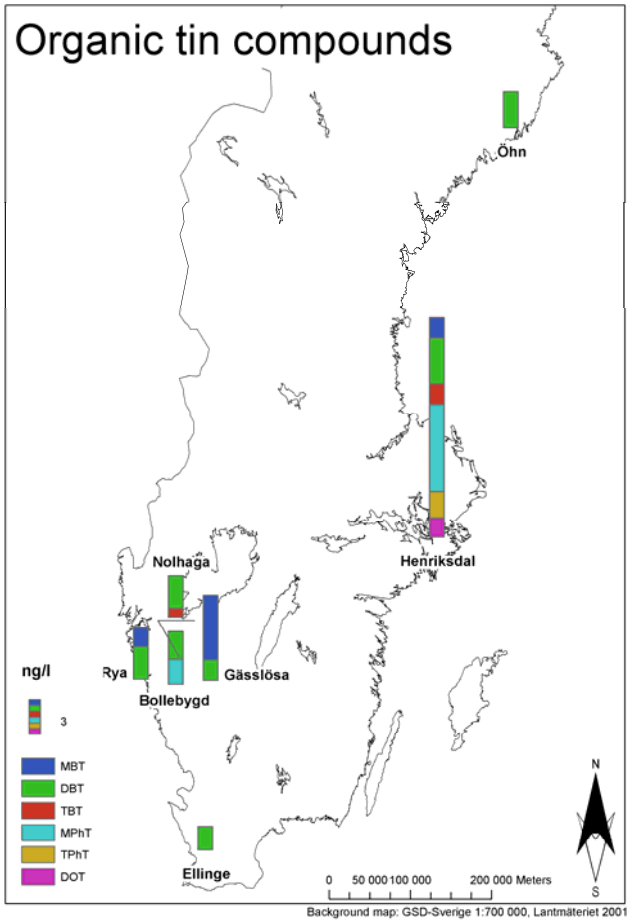


Figure 3. Concentrations of organic tin compounds found in STP effluents.

The concentrations found in this study were lower or in the same range as previously been found. Within the compilation of data done by the HAZARDOUS project (HELCOM, 2009), concentrations of TBT and TPhT in effluent water from STPs were below the reporting limits in all studies but one, for which one sample contained 2.7 and 2.3 ng/l of TBT and TPhT, respectively.

Concentrations of TBT and TPhT in STP effluent waters determined within the Swedish regional screening programme 2008 were below the reporting limits (0.3 and 0.4 ng/l) in all 23 samples analysed (www.ivl.se). The other studied organic tin compounds were however found. Some previous measurements of organic tin compounds, and identified toxicity/limit values for comparisons are presented in Table 3 below.

Table 3. Concentrations of organic tin compounds measured in the present and previous studies, and toxicity/limit values for comparison. Detection frequencies are given within brackets for the present study and some of the previous measurements.

	Compound	Concentration (ng/l)	Reference
Measured concentrations	TBT, DBT, MBT	<0.5-1.4, 1.4-3.1, <1.1-4.4 (2, 7 & 3/7)	This study
	TPhT, DPhT, MPhT	<0.8 & 1.8, <3, <0.6-5.9 (1, 0 & 2/7)	
	MOT, DOT	< 0.8, 1.3 (0 & 1/7)	
Previous measurements	DBT, MBT	1.7-13, 4-110	Sternbeck *et al.*, 2006
	TBT, DBT, MBT	< 0.2, 1-2700, 1.1-710 (0, 12 & 16/23)	Regional Screening 2008 (www.ivl.se)
	TPhT, DPhT, MPhT	< 0.4, 3.1-7.1, 0.69 (0, 10 & 1/23)	
	DOT, MOT	1.2-7.2, 0.84-29 (3 & 8/23)	
Toxicity/limit values	TBT	0.2 (AA-EQS)	2008/105/EC
	DBT	1500	WHO, 2006
	MBT	25000	WHO, 2006
	TPhT	1 (estimated PNEC)	HELCOM, 2009
	DPhT	?	
	MPhT	?	
	DOT	400	WHO, 2006
	MOT	60	WHO, 2006

TBT is listed as a priority substance under the Water Framework Directive, and both TBT and TPhT are among the substances identified to be of specific concern under the Baltic Sea Action Plan (BSAP). The TBT concentrations found in the present study were 2-7 times higher compared to the AA-EQS set under the WFD, and the concentration of TPhT found in the effluent from Henriksdal STP was above the estimated NOEC reported by HELCOM (2009). Concentrations of the other organic tin compounds found in the present study were below the identified toxicity values. However, concentrations found during the Regional Screening 2008 (www.ivl.se) were for some substances (MOT and DBT) close to or above the identified toxicity values.

5.1.2 Brominated flame retardants (BDEs/HBCDD)

Of the measured brominated flame retardants, BDE-47, BDE-99, BDE-100 and HBCDD were found, see Figure 4 and Table 4 below and Table A3 in Appendix A. BDE-47 was found in effluent samples from six of the STPs (concentration range 0.026-0.081 ng/l), BDE-99 in five samples (0.027-0.082 ng/l), and BDE-100 in three samples (0.021-0.030 ng/l). HBCDD was detected in effluent samples from all STPs (0.05-0.27 ng/l). BDE-85, BDE-153, BDE-154 and BDE-209 were not detected in any of the analysed effluent samples.

Previous data on brominated flame retardants concentrations in STP effluents are scarce. In the data compilation done by HELCOM (2009), three BDEs studies are listed, two of which also include HBCDD. Only one of these studies concerns Swedish data, and it only consists of measurements on a single sample. In Table 4 below, results from the present study are compared with this previous measurement and some toxicity and limit values.

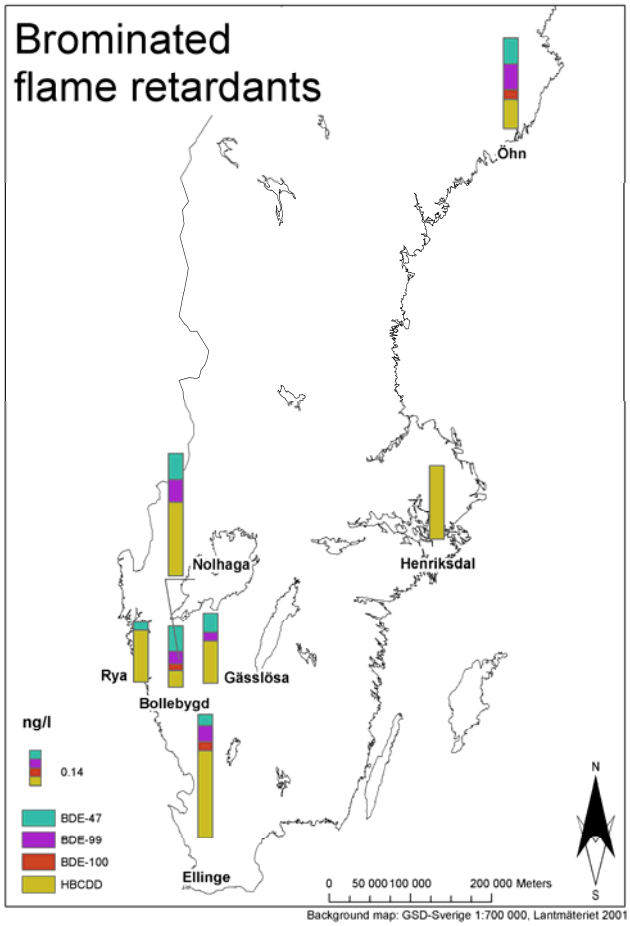


Figure 4. Concentrations of brominated flame retardants in STP effluents.

Table 4. Concentrations of brominated flame retardants, measured in the present study and in a previous study, and toxicity/limit values for comparison. Detection frequencies are given within brackets for the present study and some of the previous measurements.

	Compound	Concentration (ng/l)	Reference
Measured concentrations	BDE-47	<0.02-0.081 (6/7)	This study
	BDE-85	<0.03 (0/7)	
	BDE-99	<0.02-0.082 (5/7)	
	BDE-100	<0.02-0.030 (3/7)	
	BDE-153	<0.03 (0/7)	
	BDE-154	<0.03 (0/7)	
	BDE-209	<0.05 (0/7)	
	HBCDD	0.05-0.27 (7/7)	
Previous measurements	BDE-47	7.0 (single sample)	HELCOM, 2009
	BDE-99	30	
	BDE-100	7.0	
	HBCDD	<1	
Toxicity/limit value	∑BDE*	0.5 (AA-EQS, limnic)	2008/105/EC
		0.2 (AA-EQS, marine)	2008/105/EC
	HBCDD	30 (PNEC marine)	EU-RAR, 2008a

* Sum of congeners #28, 47, 99, 100, 153 & 154

5.1.3 Phenolic substances

All of the phenolic compounds included were found in the STP effluents, see Figure 5 and Table 5 below and Table A4 in Appendix A.

4-nonylphenol was found in four samples (220-270 ng/l), 4-t-octylphenol in two samples (16 and 67 ng/l), triclosan in all samples (16-110 ng/l), and bisphenol A in five samples (270-1300 ng/l). In effluents from Ryaverket and Öhn, all phenolic substances were found, whereas only triclosan was found in effluents from Henriksdal and Ellinge.

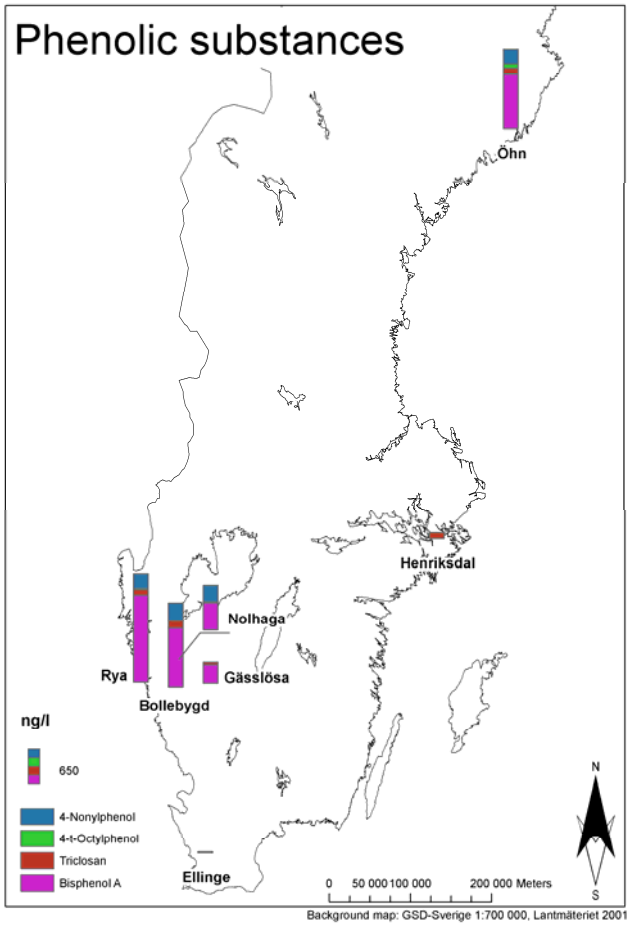


Figure 5. Concentrations of phenolic substances in STP effluents.

In Table 5 below, previously measured concentrations of, and some toxicity and limit values for, the included phenolic compounds are presented.

4-nonylphenol and 4-t-octylphenol are listed as priority substances under the WFD, and are also among the substances identified to be of specific concern for the Baltic Sea according to the BSAP. Concentrations of 4-nonylphenol found in the present study were close to the WFD AA-EQS, concentrations of 4-t-octylphenol above the AA-EQS for marine waters but below the AA-EQS for limnic waters.

Table 5. Concentrations of phenolic compounds in effluent waters measured in the present and previous studies, and toxicity/limit values for comparison. Detection frequencies are given within brackets for the present study and some of the previous measurements.

	Compound	Concentration (ng/l)	Reference
Measured concentrations	4-nonylphenol	<87-270 (4/7)	This study
	4-t-octylphenol	<19-67 (2/7)	
	Triclosan	16-110 (7/7)	
	Bisphenol A	<120-1300 (5/7)	
Previous measurements	4-nonylphenol	median: 110 (<10-1600, 38/42)	Regional Screening 2008 (www.ivl.se)
	4-t-octylphenol	median: 14.5 (<2-290, 36/42)	
	Triclosan	median: 42 (<1-250, 41/42)	
	Bisphenol A	median: 240 (<5-3000, 38/42)	
	4-nonylphenol	30-5500	Remberger *et al.*, 2004
	4-t-octylphenol	5-220	
Toxicity/limit values	4-nonylphenol	300 (AA-EQS)	2008/105/EC
	4-t-octylphenol	100 (AA-EQS, limnic)	2008/105/EC
		10 (AA-EQS, marine)	2008/105/EC
	Triclosan	1550 (PNEC, SSD, HC5)	Capdevielle *et al.*, 2008
		1900 (EC50, reprod. chlorophyte)	Franz *et al.*, 2008
		150 (effect on thyroid system in tadpoles)	Veldhoen *et al.*, 2006
		50 (proposed EQS, limnic)	Swedish EPA, 2008
		5 (proposed EQS, marine)	
	Bisphenol A	1500 (PNEC, limnic)	EU-RAR, 2008b
		150 (PNEC, marine)	EU-RAR, 2008b

Furthermore, in previous studies concentrations almost 20 and 30 times higher compared to AA-EQS have been found for 4-nonylphenol and 4-t-octylphenol, respectively. In addition, high concentrations of their respective ethoxylates have been found in STP effluents.

Concentrations of bisphenol A found in the present study were above the PNEC for marine waters but below the PNEC of limnic waters given in the EU-RAR (2008b). Within the Regional Screening 2008, concentrations also above the PNEC for limnic waters were found. It should however be mentioned that the limit values given in the EU-RAR (2008b) have been criticized, since several studies indicate that exposures to these concentrations may cause effects on fish but particularly on molluscs (Oehlmann *et al.*, 2008).

For triclosan a PNEC of 1550 ng/l, based on a species sensitivity distribution (SSD), has been derived by Capdevielle *et al.* (2008). However, for this PNEC no assessment factor reflecting further uncertainties has been used, and two data points in the SSD, both representing green microalgae, are below the HC5. Green microalgae have been identified to be particularly sensitive to triclosan (Franz *et al.*, 2008) and the PNEC derived by Capdevielle *et al.* (2008) might not be protective for this group. Furthermore, recent data indicate that triclosan may affect bacterial communities at environmentally relevant concentrations (Johnson *et al.*, 2009). In addition triclosan has been identified as an endocrine disrupting chemical, potentially affecting metamorphosis of tadpoles already at 150 ng/l (Veldhoen *et al.*, 2006). The Swedish Chemical Agency has derived proposal EQSs of 5 and 50 ng/l for triclosan. Concentrations found in effluent water in this study and within the Regional Screening 2008 are below the PNEC derived by Capdevielle *et al.* (2008), but in the same range or above the EQSs proposed by the Swedish EPA.

5.1.4 Herbicides

Concentrations of the herbicides MCPA and glyphosate were below the reporting limits, 2 ng/l and 0.5 µg/l respectively, in all analysed STP effluent samples. Proposals on EQS derived by the

Swedish Chemical Agency are presented in Table 6 below. The detection limits in the present study were approximately 200-500 times lower compared to these proposed EQS.

Table 6. Toxicity values for herbicides.

	Compound	Concentration (µg/l)	Reference
Toxicity/limit values	MCPA	1.1 (proposed EQS)	Swedish EPA, 2008
	Glyphosate	100 (proposed EQS)	

5.1.5 Metals

All of the metals included in the study were found in the effluent water samples from all of the STPs, see Figure 6, Figure 7 and Table A6 in Appendix A. The results for Hg, Cd and Ag are presented in Figure 6, the results for Pb, As and Cu in Figure 7.

Copper occurred in the highest concentrations (0.70-6.4 µg/l) followed by As (0.58-0.99 µg/l), Pb (0.038-0.58 µg/l), and Ag and Cd (0.006-0.034 µg/l and 0.003-0.022 µg/l respectively). The concentrations of Hg were in the range 0.39-4.9 ng/l.

The highest concentration of Hg was found in the sample from Bollebygd. For the other metals, concentrations in this sample were in the lower range. The opposite could be seen for the sample from Ellinge. For this sample, the lowest concentration of Hg was found, but for the other metals concentrations were in the higher range.

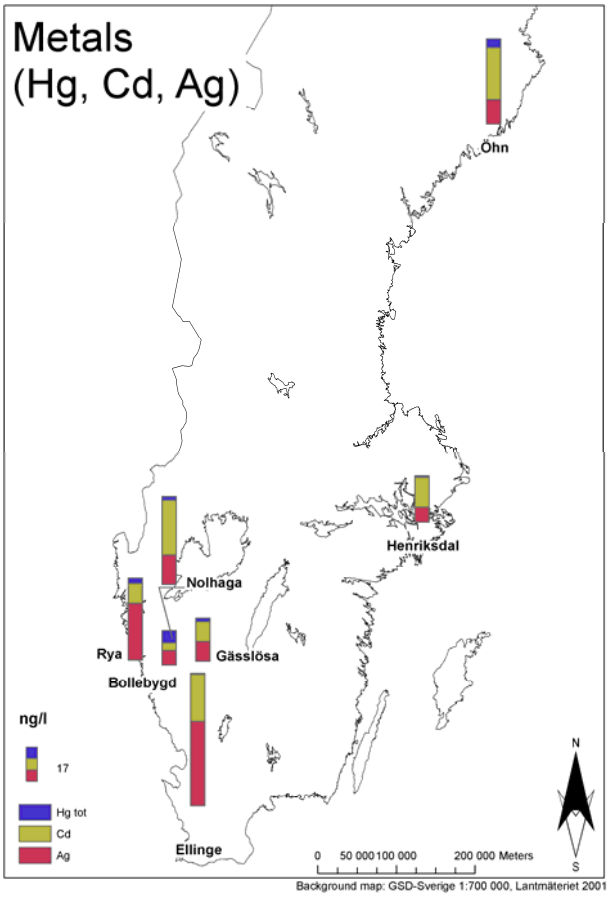


Figure 6. Concentrations of Hg, Cd and Ag in STP effluents.

In Table 7 below, measured concentrations are compared to some toxicity and limit values. The concentrations of Hg, Cd and Pb can be compared to the EQSs given in the daughter directive of the WFD (2008/105/EC), and the concentrations of As can be compared to the drinking water standard (98/83/EC). A review of aquatic toxicity values for Ag has been done by Blaser *et al.* (2008). Ag is expected to be present as silver sulphide clusters (organic and inorganic) in freshwater systems, but the toxicity of free Ag^+ ions should also be considered when silver concentrations exceed concentrations of sulphides (Blaser *et al.*, 2008). In Table 7 below, toxicity values for silver zinc sulphide (Ag-ZnS) and free silver ion (Ag^+) are presented. These are based on highest observed no-effect concentrations (HONEC) and no observed effect concentrations (NOEC) for crustaceans, and the use of assessment factors (Blaser *et al.*, 2008). For Cu the Swedish Chemical Agency has proposed an EQS (Swedish EPA, 2008).

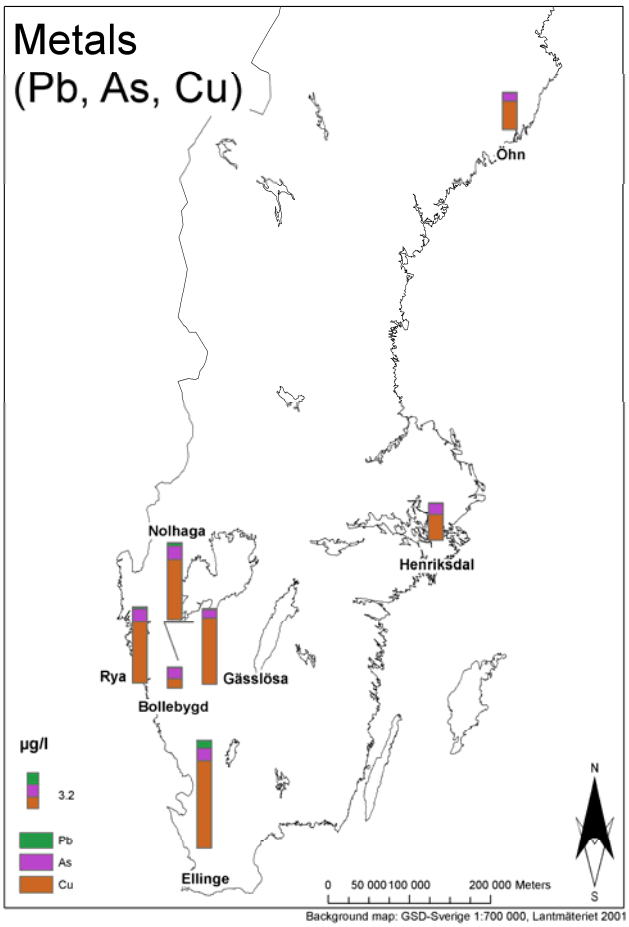


Figure 7. Concentrations of Pb, As and Cu in STP effluents.

Concentrations of Cd, Hg and Pb were below their respective EQS and concentrations of As were below the drinking water standard, in all effluent samples. Concentrations of Cu were just above the proposed EQS in four samples. For Ag however, concentrations were above the PNEC values given by Blaser *et al.* (2008) (3-17 times the PNEC derived for Ag-ZnS, 60-340 times the PNEC derived for Ag^+).

Table 7. Concentrations of metals measured in effluent waters in the present study, and toxicity/limit values for comparison. Detection frequencies are given within brackets for the present study.

	Compound	Concentration (ng/l)	Reference
Measured concentrations	Hg	0.39-4.9 (7/7)	This study
	Cd	3-22 (7/7)	
	Ag	6-34 (7/7)	
	Pb	38-580 (7/7)	
	As	580-990 (7/7)	
	Cu	700-6400 (7/7)	
Toxicity/limit values	Hg	50 (AA-EQS)	2008/105/EC
	Cd	80-250 (AA-EQS)	2008/105/EC
	Ag	2 (PNEC, Ag-ZnS) 0.1 (PNEC, Ag^+)	Blaser *et al.*, 2008
	Pb	7200 (AA-EQS)	2008/105/EC
	As	10000 (drinking water standard)	98/83/EC
	Cu	4000 (proposed EQS)	Swedish EPA, 2008

5.1.6 NSAIDs

The concentrations of NSAIDs, including also salicylic acid and the metabolites of ibuprofen, the hydroxylated ibuprofen (OH-ibuprofen) and carboxylated ibuprofen (ibuprofen-COOH), are presented in Figure 8 below. The concentrations for the individual samples are presented in Tables A7 (ibuprofen, naproxen, ketoprofen and diclofenac) and A15 (ibuprofen-OH, ibuprofen-COOH and salicylic acid) in Appendix A. All the NSAIDs were found in effluents from all STPs. The concentrations were in the range 8-5000 ng/l for ibuprofen, 81-3000 ng/l for naproxen, 68-1400 ng/l for ketoprofen, and 71-620 ng/l for diclofenac.

The highest concentrations were found in the effluent sample from Bollebygd. This sample also contained the highest concentrations of the metabolites ibuprofen-OH and ibuprofen-COOH. The effluent sample from Ellinge contained the lowest concentrations of all analysed NSAIDs except diclofenac. Effluents from STPs located in western Sweden appeared to contain concentrations in the higher range.

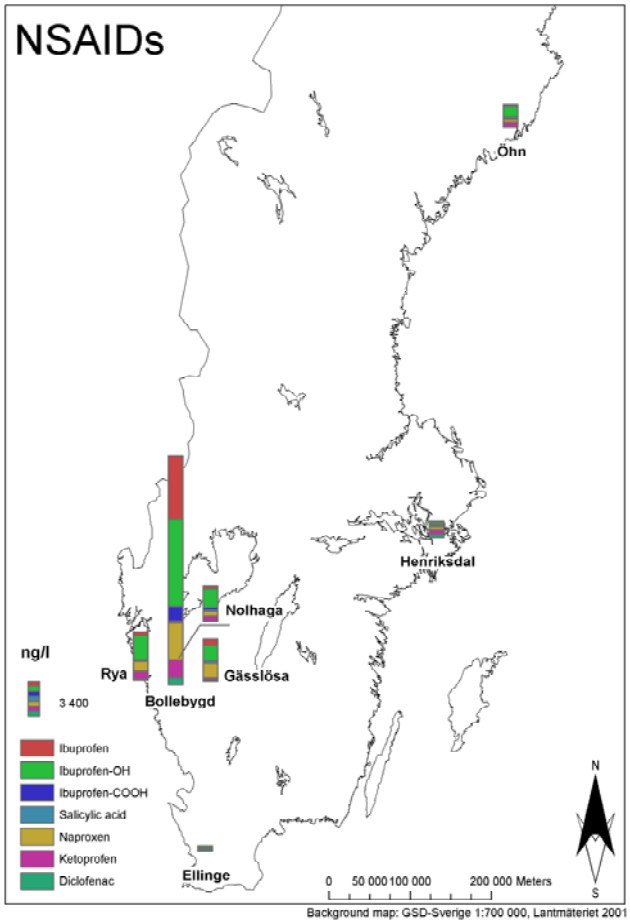


Figure 8. Concentrations of NSAIDs found in STP effluents.

In Table 8 below, some toxicity values for the included NSAIDs are presented. It appears that chronic effects might occur at concentrations predicted to not cause an affect based on acute toxicity data. For ibuprofen a PNEC of 17.5 µg/l has been derived based on acute toxicity to the algae *P. subcapitata* (www.fass.se). However, behavioral effects on the crustacean *G. pulex* has been seen after exposure to 10 ng/l (Santos *et al.*, 2010). Chronic toxicity studies with the cnidarian *H. attenuata* and the mollusc *P. carinatus*, also indicate that chronic effects may occur at concentrations lower compared to the PNEC (Santos *et al.*, 2010). Similarly, diclofenac has been shown to cause chronic effects to fish at concentrations as low as 0.5-1 µg/l, resulting in PNECs in the range 0.005-0.1 µg/l (100-2000 times lower compared to the PNEC based on acute toxicity) depending on choice of assessment factors (Grung *et al.*, 2008; Hoeger *et al.*, 2005). In addition, for both diclofenac and naproxen, there are indications on higher toxicity of their photoderived degradation products (reviewed in Santos *et al.*, 2010).

Table 8. Concentrations of NSAIDs in effluent waters measured in the present study, some previous studies, and toxicity/limit values for comparisons. Detection frequencies are given within brackets for the present study and some of the previous measurements.

	Compound	Concentration (µg/l)	Reference
Measured concentrations	ibuprofen	0.008-5.0 (7/7)	This study
	naproxen	0.081-3.0 (7/7)	
	ketoprofen	0.068-1.4 (7/7)	
	diclofenac	0.071-0.620 (7/7)	
Previous measurements	ibuprofen	0.0032-7.8 (51/51)	Andersson *et al.*, 2006
	naproxen	0.030-15 (51/51)	
	ketoprofen	0.0049-2.9 (51/51)	
	diclofenac	0.014-0.71 (51/51)	
	ibuprofen	3.2 & 3.5 (2 samples from same STP)	Remberger *et al.*, 2008
	naproxen	3.4 & 3.2	
	ketoprofen	0.53 & 0.57	
	diclofenac	0.23 & 0.22	
Toxicity/limit values	ibuprofen	17.5 (PNEC, acute toxicity, algae)	www.fass.se
		1000 ($LOEC_{morphology}$, cnidarian)	Santos *et al.*, 2010
		1000 ($NOEC_{growth}$, chronic, mollusc)	
		0.01 ($LOEC_{behaviour}$, crustacean)	
	naproxen	0.64 (PNEC, chronic, crustacean)	www.fass.se
	ketoprofen	100 (PNEC, acute, crustacean)	www.fass.se
	diclofenac	10 (PNEC, acute, crustacean)	www.fass.se
		0.1 (PNEC, chronic, fish)	Grung *et al.*, 2008
		0.005 (PNEC, chronic, fish)	Hoeger *et al.*, 2005

Concentrations of NSAIDs found in the present study are in a range potentially causing negative impacts to the aquatic environment. Concentrations of ibuprofen were below the PNEC based on acute toxicity. However the effluent from Bollebygd contained a concentration 5 times higher compared to the chronic toxicity values found for a cnidarian and a mollusc, and concentrations in six effluents were above the $LOEC_{behaviour}$ (11-500 times) for the crustacean *G. pulex*. For naproxen concentrations were above the PNEC (1-5 times) in three of the effluent samples. Concentrations of diclofenac were below the PNEC based on acute toxicity, but concentrations in three samples were above the PNEC (1-6 times) reported by Grung *et al.* (2008) and concentrations found in all samples were above the PNEC (14-124 times) given by Hoeger *et al.* (2005).

5.1.7 Perfluorinated substances

The determined concentrations of perfluorinated substances are presented in Figure 9 and Table 9 below and in Table A8 in Appendix A. PFOS, PFOA, PFHxA and PFDcA were found in effluents from all seven STPs, whereas the concentrations of PFOSA were above the reporting limits (0.02 ng/l) in the effluent samples from all STPs but Rya and Bollebygd. Concentrations found were in the range 3.1-19 ng/l for PFOS, 3.6-41 ng/l for PFOA, 0.038-0.18 ng/l for PFOSA, 8.4-21 ng/l for PFHxA, and 6.0-13 ng/l for PFDcA.

No clear differences between the included STPs could be seen. Concentrations of and distribution between the included perfluorinated substances were similar.

In Table 9 below, determined concentrations are compared to some previously measured concentrations and toxicity/limit values. Concentrations found were in the same range or lower compared to previous results, and several orders of magnitude below the limit values presented by OSPAR (2005) and the Swedish EPA (2008).

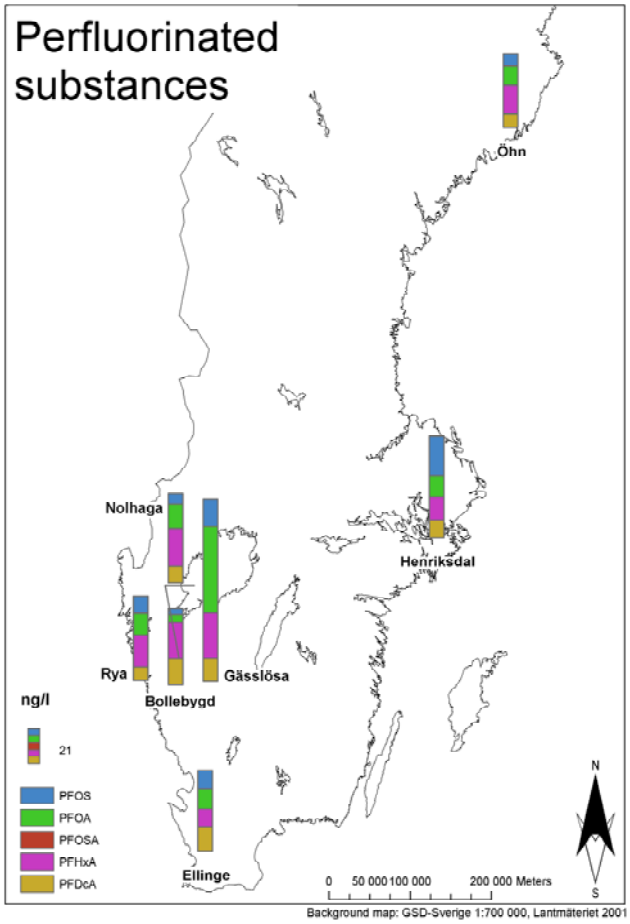


Figure 9. Concentrations of perfluorinated substances in STP effluents.

Table 9. Concentrations of perfluorinated substances in effluent waters measured in the present study, and toxicity/limit values for comparisons. Detection frequencies are given within brackets for the present study and some of the previous measurements.

	Compound	Concentration (ng/l)	Reference
Measured concentrations	PFOS	3.1-19 (7/7)	This study
	PFOA	3.6-41 (7/7)	
	PFOSA	<0.02-0.18 (5/7)	
	PFHxA	8.4-21 (7/7)	
	PFHDcA	6.0-13 (7/7)	
Previous measurements	PFOS	6.7-49 (4/4)	Woldegiorgis *et al.*, 2006
	PFOA	7.4-77 (4/4)	
	PFOSA	<0.5-0.98 (3/4)	
	PFHxA	<2-22 (2/4)	
	PFHDcA	<6.7-48 (3/4)	
Toxicity/limit values	PFOS	25000 (PNEC, limnic) 2500 (PNEC, marine)	OSPAR, 2005
	PFOA	30000 (proposed EQS, limnic) 3000 (proposed EQS, marine)	Swedish EPA, 2008
	PFOSA	?	
	PFHxA	?	
	PFDcA	?	

5.1.8 Phthalates

The determined concentrations of phthalates are presented in Figure 10 and Table 10 below, and in Table A9 in Appendix A. DEP, DIBP and DBP were found in effluent samples from all STPs, whereas DEHP and BBzP were found in effluents from six and four STPs, respectively. DOP, DINP and DIDP could not be detected in any of the effluent samples (reporting limits were 0.01, 1 and 1 µg/l, respectively). Concentrations found were in the range 0.030-1.47 µg/l for DEP, 0.046-0.21 µg/l for DIBP, 0.081-0.28 µg/l for DBP, 0.015-0.050 µg/l for BBzP, and 0.19-0.71 µg/l for DEHP.

The lowest concentrations were generally found in the samples from Henriksdal and Ellinge. The sample from Bollebygd differed from the other samples containing a comparatively elevated concentration of DEP.

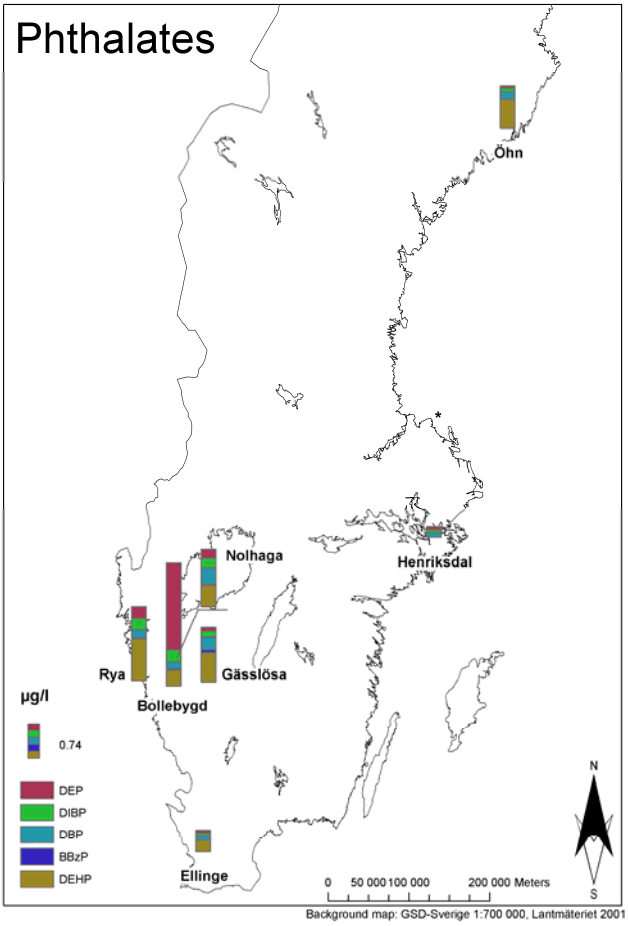


Figure 10. Concentrations of phthalates in STP effluents.

In Table 10, determined concentrations are compared to previous results and some toxicity and limit values. Measured concentrations were in the same range and detection frequencies similar to results from previous studies.

DEHP is listed as a priority substance under the WFD and is also among the substances indentified to be of specific concern under the BSAP. Concentrations of DEHP found were lower than the AA-EQS given in the daughter directive (2008/105/EC). Concentrations of DBP were several orders of magnitude below the PNEC given in the European Union Risk Assessment Report (EU-RAR, 2004). For the other included phthalates there is a lack of toxicity or limit values for the evaluation. According to the risk assessments for DINP and DIDP, aquatic PNECs could not be established due to the lack of toxicity data at concentrations below the solubility for these phthalates (EU-RAR, 2003a and 2003b).

Table 10. Measured concentrations of phthalates in effluent waters in this study, some previous measured concentrations, and toxicity/limit values for comparisons. Detection frequencies are given within brackets for the present study and some of the previous measurements.

	Compound	Concentration (µg/l)	Reference
Measured concentrations	DEHP	<0.1-0.49 (6/7)	This study
	DEP	0.030-1.47 (7/7)	
	DIBP	0.046-0.21 (7/7)	
	DBP	0.081-0.28 (7/7)	
	BBzP	<0.01-0.050 (4/7)	
	DOP	<0.01 (0/7)	
	DINP	<1 (0/7)	
	DIDP	<1 (0/7)	
Previous measurements	DEHP	0.54	(Furtmann, 1993)
	DEP	0.06	(Furtmann, 1993)
	DBP	0.22	(Furtmann, 1993)
	DEHP	median: 0.52 (0.064-8.3, 30/30)	Regional Screening
	DEP	median: 0.025 (0.0015-0.29, 25/30)	2008 (www.ivl.se)
	DIBP	median: 0.016 (0.0072-0.17, 29/30)	
	DBP	median: 0.079 (0.024-0.20, 9/30)	
	BBzP	median: 0.15 (<0.1-0.15, 1/30)	
	DOP	<0.03 (0/30)	
	DINP	median: 2.3 (<0.3-3.9, 2/30)	
	DIDP	median: 0.54 (<0.3-0.76, 2/30)	
Toxicity/limit values	DEHP	1.3 (AA-EQS)	2008/105/EC
	DEP	?	
	DIBP	?	
	DBP	10 ($PNEC_{aquatic}$)	EU-RAR, 2004
	BBzP	?	
	DOP	?	
	DINP	$PNEC_{aquatic}$ could not be established	EU-RAR, 2003a
	DIDP	$PNEC_{aquatic}$ could not be established	EU-RAR, 2003b

5.1.9 Organophosphorus esters

The determined concentrations of organophosphorus esters are presented in Figure 11 and Table 11 below, and in Table A10 in Appendix A. All organophosphorus esters measured were found, and all but TDCP in all analysed effluent samples. Concentrations varied between 0.029-2.8 µg/l for TIBP, 0.019-0.39 µg/l for TBP, 0.19-1.8 µg/l for TCEP, 0.28-0.82 µg/l for TDCP, 0.24-16 µg/l for TBEP, 0.015-0.12 µg/l for TPhP, and 0.0092-0.069 µg/l for EHDPP. Of the organophosphorus esters analysed, except for the sample from Ellinge STP, TBEP was found at the highest concentrations. For the sample from Henriksdal the lowest concentrations or concentrations in the lower range were found, but no clear general differences between the STPs could be seen.

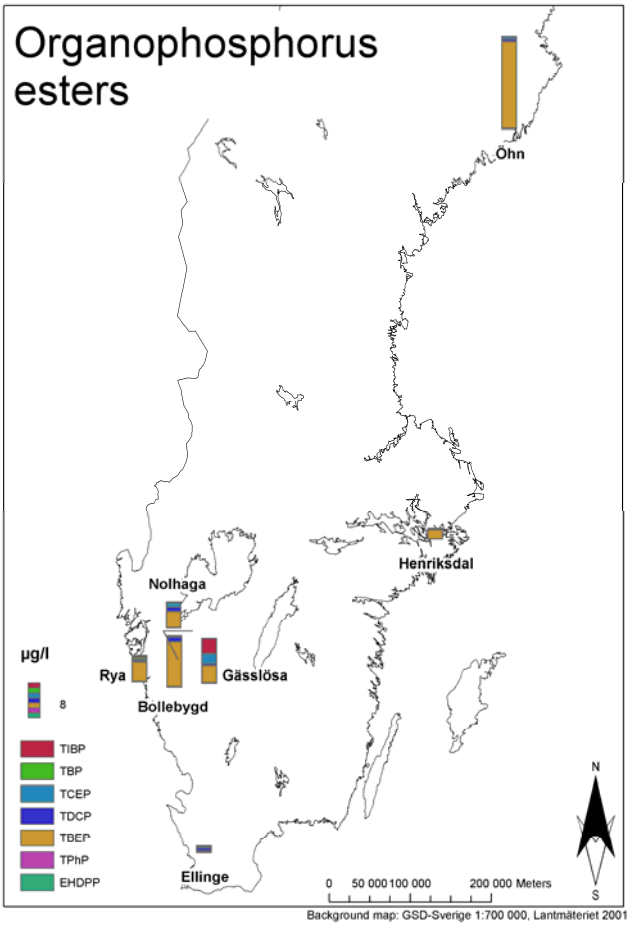


Figure 11. Concentrations of organophosphorus esters in STP effluents.

In Table 11 below, some previous measurements and toxicity/limit values are presented. Concentrations found in this study were in the same range as reported by Bester (2005) and Marklund *et al.* (2005). Concentrations of TCEP and TDCP can be compared to the aquatic PNECs given in the European Union Risk Assessment Reports (EU-RAR, 2008c and 2009). For the remaining organophosphorus esters, except EHDPP, RIVM has derived PNECs for the aquatic compartment (RIVM, 2005). Concentrations of TIBP, TBEP and TPhP exceeded the PNEC derived by RIVM, up to 10 times if compared to the PNECs derived for marine waters.

Table 11. Measured concentrations of organophosphorus esters in effluent waters in this study, some previous measured concentrations, and toxicity/limit values for comparisons. Detection frequencies are given within brackets for the present study.

	Compound	Concentration (µg/l)	Reference
Measured concentrations	TIBP	0.029-2.8 (7/7)	This study
	TBP	0.019-0.39 (7/7)	
	TCEP	0.19-1.8 (7/7)	
	TDCP	<0.008-0.82 (6/7)	
	TBEP	0.24-16 (7/7)	
	TPhP	0.015-0.12 (7/7)	
	EHDPP	0.0092-0.069 (7/7)	
Previous measurements	TIBP	-	
	TBP	0.36-6.1	Marklund *et al.*, 2005□
	TCEP	0.24-0.61; 0.39-0.47	Bester, 2005; Marklund *et al.*, 2005□
	TDCP	0.13-0.34	Marklund *et al.*, 2005□
	TBEP	3.1-30	Marklund *et al.*, 2005□
	TPhP	0.041-0.13	Marklund *et al.*, 2005□
	EHDPP	-	
Toxicity/limit values	TIBP	11 (PNEC, limnic) 1.1 (PNEC, marine)	RIVM, 2005
	TBP	66 (PNEC, limnic) 6.6 (PNEC, marine)	RIVM, 2005
	TCEP	65 ($PNEC_{aquatic}$)	EU-RAR, 2009
	TDCP	10 ($PNEC_{aquatic}$)	EU-RAR, 2008c
	TBEP	13 (PNEC, limnic) 1.3 (PNEC, marine)	RIVM, 2005
	TPhP	0.16 (PNEC, limnic) 0.016 (PNEC, marine)	RIVM, 2005
	EHDPP	?	

5.1.10 Volatile organic compounds and volatile halogenated substances

The determined concentrations of volatile organic compounds and volatile halogenated substances are presented in Figure 12 , Figure 13 and Table 12 below, and in Tables A11 and A12 in Appendix A.

Toluene and m+p-xylene were found in all samples, measured concentrations were in the ranges 8.4-200 and 7.7-35 ng/l, respectively. Benzene, o-xylene and n-hexane were found in six samples, at 1.1-5.5, 13-95 and 2.5-14 ng/l. Ethyl-benzene was found in five samples (1.3-18 ng/l), styrene in three samples (3.1-14 ng/l) and n-nonan in one sample (1.4 ng/l). 3-methyl-pentane, n-octane, and TMB could not be found, reporting limits were 10 ng/l, 1.0 ng/l and 5 ng/l, respectively.

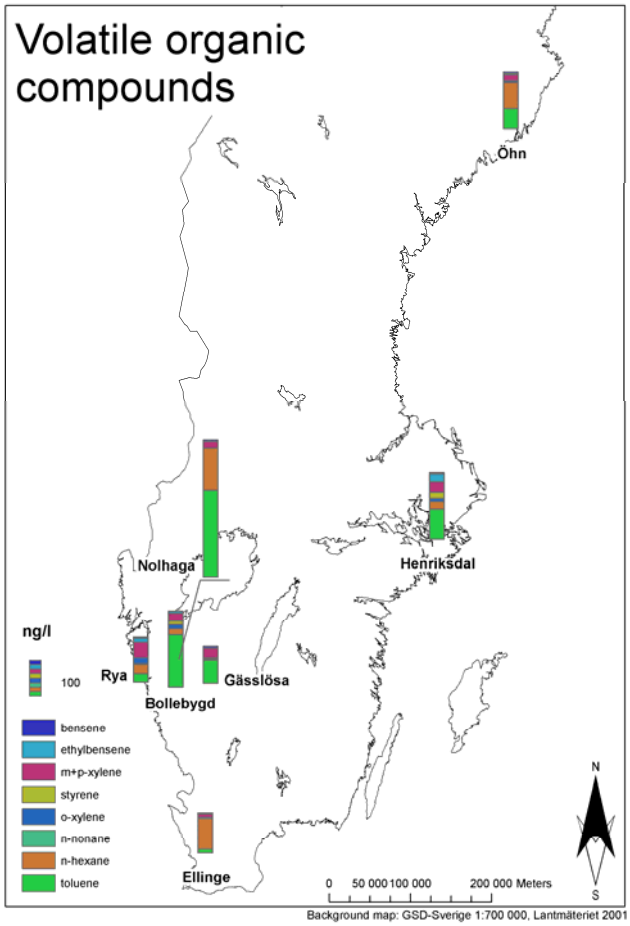


Figure 12. Concentrations of volatile organic compounds in STP effluents.

All of the volatile halogenated substances, except dichloromethane, were found in the effluent samples. 1,1,1-trichloroethane occurred in three out of seven samples, the concentrations were in the range 0.42-0.50 ng/l. 1,2-dichloroethane, chloroform, carbon tetrachloride, and tetrachloroethene were found in all samples. Concentrations found were in the ranges 34-270 ng/l, 14-140 ng/l, 1.6-2.4 ng/l, and 1.1->300 ng/l, respectively. Trichloroethene was found in five samples, concentration range 0.82-5.9 ng/l.

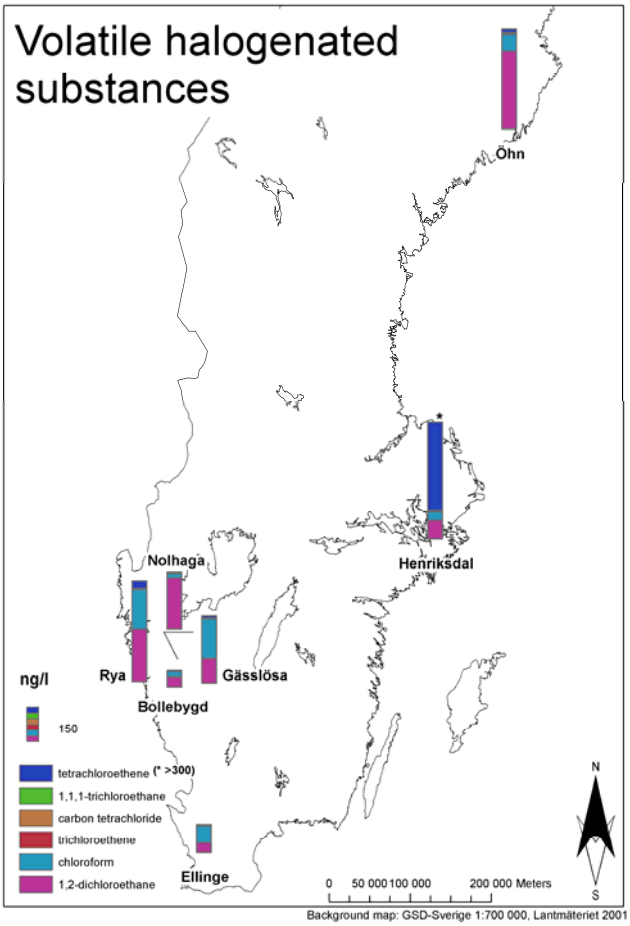


Figure 13. Concentrations of volatile halogenated substances in STP effluents.

In Table 12 below measured concentrations are compared to some toxicity and limit values. For styrene, toluene, benzene and ethyl-benzene, PNECs for the aquatic compartment can be found in their respective European Union Risk Assessment Report (EU-RAR, 2002, 2003c, 2007 and 2008d). For all of the volatile halogenated substances included in the present study but 1,1,1-trichloroethane, limit values are given in the WFD daughter directive (2008/105/EC). Measured concentrations of volatile organic compounds and halogenated substances were below, in general several orders of magnitude, these toxicity and limit values.

Table 12. Concentrations of volatile organic compounds and halogenated substances in effluent waters measured in the present study, and toxicity/limit values for comparisons. Detection frequencies are given within brackets for the present study.

	Compound	Concentration (µg/l)	Reference
Measured concentrations	styrene	<0.0020-0.014 (3/7)	This study
	toluene	0.0084-0.20 (7/7)	
	benzene	<0.0010-0.0055 (6/7)	
	ethyl-benzene	<0.0010-0.018 (5/7)	
	3-methyl-pentane	<0.010 (0/7)	
	n-hexane	<0.0010-0.095 (6/7)	
	n-octan	<0.0010 (0/7)	
	m+p-xylene	0.0077-0.035 (7/7)	
	o-xylene	<0.0020-0.0014 (6/7)	
	n-nonan	<0.0010-0.0014 (1/7)	
	1,3,5-TMB	<0.0050 (0/7)	
	1,1,1-trichloroethane	<0.4-0.50 (3/7)	
	carbon tetrachloride	0.0017-0.0024 (7/7)	
	1,2-dichloroethane	0.034-0.27 (7/7)	
	dichloromethane	<0.0060 (0/7)	
	tetrachloroethene	0.0011->0.30 (7/7)	
	trichloroethene	<0.0003-0.0059 (5/7)	
	chloroform	0.014-0.14 (7/7)	
Toxicity/limit values	styrene	40 ($PNEC_{aquatic}$)	EU-RAR, 2002
	toluene	74 ($PNEC_{aquatic}$)	EU-RAR, 2003c
	benzene	80 ($PNEC_{aquatic}$)	EU-RAR, 2008d
	ethyl-benzene	100 ($PNEC_{aquatic}$)	EU-RAR, 2007
	3-methyl-pentane	?	
	n-hexane	?	
	n-octan	?	
	m+p-xylene	?	
	o-xylene	?	
	n-nonan	?	
	1,3,5-TMB	?	
	1,1,1-trichloroethane	?	
	carbon tetrachloride	12 (AA-EQS)	2008/105/EC
	1,2-dichloroethane	10 (AA-EQS)	2008/105/EC
	dichloromethane	20 (AA-EQS)	2008/105/EC
	tetrachloroethene	10 (AA-EQS)	2008/105/EC
	trichloroethene	10 (AA-EQS)	2008/105/EC
	chloroform	2.5 (AA-EQS)	2008/105/EC

5.1.11 Chlorobenzenes

The concentrations of the chlorobenzenes were below their respective reporting limits. Limit values for chlorobenzenes are given in Table 13 below. The reporting limits for trichlorobenzenes, pentachlorobenzene, hexachlorobenzene, and hexachlorobutadiene were 0.1-2, 0.2, 0.1, 0.2 ng/l, respectively. The reporting limits were below the respective AA-EQSs.

Table 13. Limit values for chlorobenzenes.

	Compound	Concentration (ng/l)	Reference
Limit values	Trichlorobenzenes	400 (AA-EQS)	2008/105/EC
	Pentachlorobenzene	7 (AA-EQS, limnic)	2008/105/EC
		0.7 (AA-EQS, marine)	2008/105/EC
	Hexachlorobenzene	10 (AA-EQS)	2008/105/EC
	Hexachlorobutadiene	100 (AA-EQS)	2008/105/EC

5.1.12 Siloxanes

Results on siloxanes are presented in Figure 14 below and in Table A14 in Appendix A. Of the siloxanes analysed, D6, MM, MDM, MD2M and MD3M could be found. D4 and D5 could not be found (reporting limits were 0.09 and 0.04 µg/l, respectively). D6 was found in the samples from Henriksdal and Ellinge, at 0.065 and 0.040 µg/l, respectively. MM was found in the effluent sample from Henriksdal, at 0.0021 µg/l. MDM was found in the sample from Bollebygd at a concentration of 0.0003 µg/l, whereas MD2M was found in the sample from Bollebygd at a concentration of 0.00067 µg/l and in the sample from Henriksdal at 0.00059 µg/l. MD3M could be detected in the samples from Nolhaga, Ellinge, Henriksdal and Bollebygd, concentrations varied between 0.00059-0.0017 µg/l.

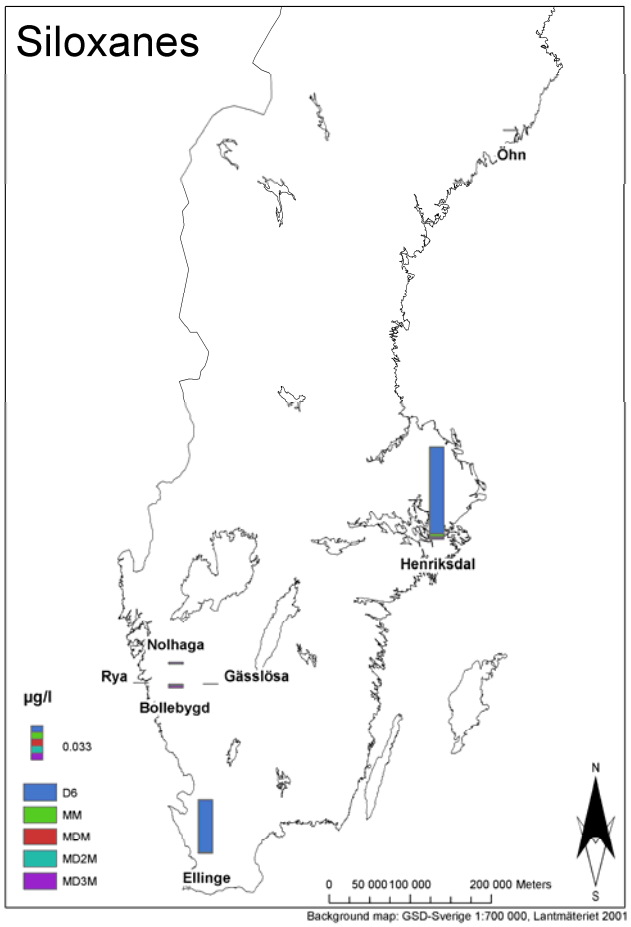


Figure 14. Concentrations of siloxanes in STP effluents.

In Table 14 below concentrations found in this study are compared to some previous measurements and toxicity/limit values. Measured concentrations were in the same range or lower compared to the results by Kaj *et al.* (2005 and 2007). The reporting limit for D4 was below the PNEC for the aquatic compartment presented by Brooke *et al.* (2009a). According to the risk assessments for D5 and D6, aquatic PNECs could not be established due to the lack of toxicity data at concentrations below the solubility for these siloxanes.

Table 14. Measured concentrations of siloxanes in effluent waters in this study, some previous measured concentrations, and toxicity/limit values for comparisons. Detection frequencies are given within brackets for the present study and some of the previous measurements.

	Compound	Concentration (ng/l)	Reference
Measured concentrations	D4	< 90 (0/7)	This study
	D5	< 40 (0/7)	
	D6	< 30-65 (2/7)	
	MM	< 1-21 (1/7)	
	MDM	< 0.2-0.3 (1/7)	
	MD2M	< 0.3-7.6 (2/7)	
	MD3M	< 0.4-1.7 (4/7)	
Previous measurements	D4, D5, D6	< 60, < 40-51, < 40-230 (0, 1 & 5/12)	Kaj *et al.*, 2005
	MM, MDM, MD2M, MD3M	< 0.5 (0/12)	
	D4, D5, D6	60-260, 440-2300, 11-59	Kaj *et al.*, 2007
	MM, MDM, MD2M, MD3M	< 0.5, < 0.5-80, < 0.5-8.9, < 5 (3 samples, same STP)	
Toxicity/limit values	D4	440 ($PNEC_{aquatic}$)	Brooke *et al.*, 2009a
	D5	$PNEC_{aquatic}$ could not be established	Brooke *et al.*, 2009b
	D6	$PNEC_{aquatic}$ could not be established	Brooke *et al.*, 2009c
	MM	?	
	MDM	?	
	MD2M	?	
	MD3M	?	

5.2 Estrogenic and androgenic activity of STP effluents

Estrogenic activity was found for all effluent waters determined. Estrogenic activity was not determined for Bollebygd STP. The activities were in the range 2.0-4.2 ng estradiol units/l, see Figure 15. These values are within what has previously been found for STP effluent waters (< 0.1 – 15 ng estradiol units/l) Svenson *et al.* (2002).

No androgenic activity could be detected in the YAS assay. The detection limit was 1 ng DHT units/l. However, the results indicated dose-dependent anti-androgenic activities.

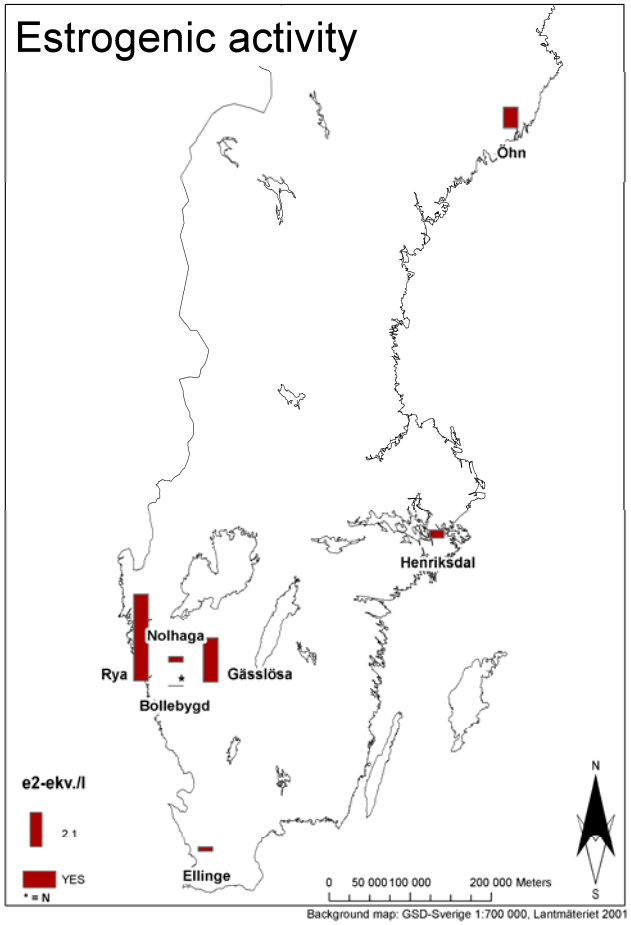


Figure 15. Estrogenic activity of STP effluents. N: not determined.

5.3 Seasonal variability in chemical composition of STP effluents

Of the organic tin compounds found during the chemical characterization of the seven STP effluents, MBT, DBT and DPhT could be found in the sample from Gässlösa, see Figure 16. MBT and DBT were found at approximately the same concentrations in samples taken in March, April, and August, but could not be found in the sample from October. DPhT could only be found above the reporting limit in October.

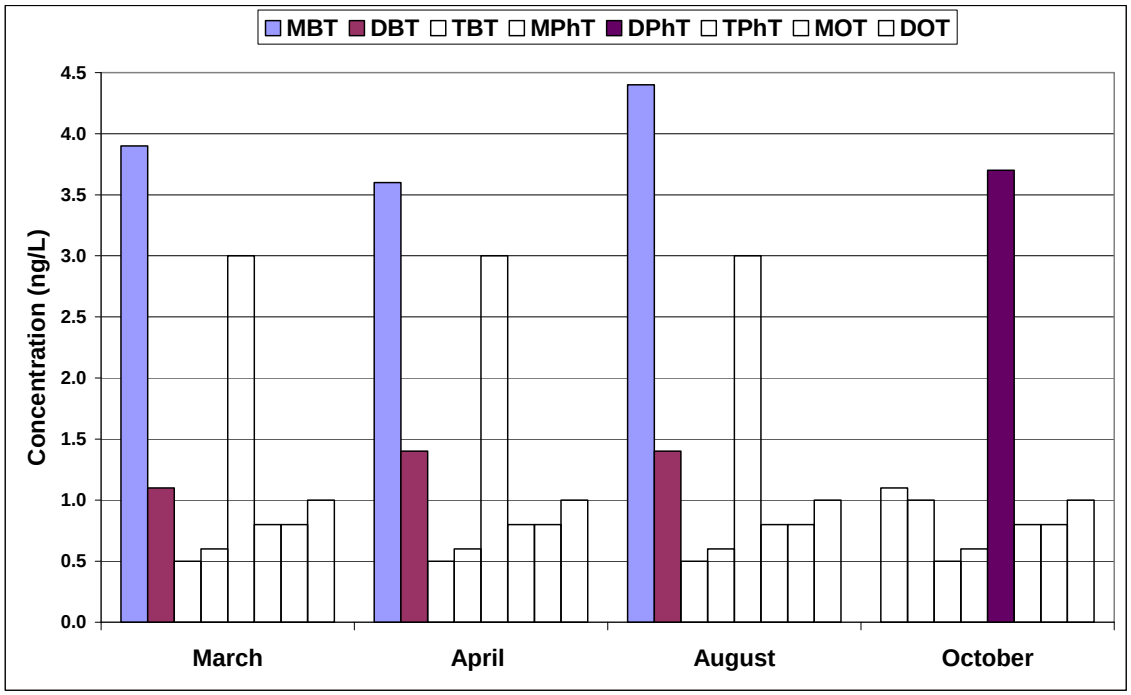


Figure 16. Organic tin compounds in effluent water from Gässlösa STP sampled in March, April, August and October 2008. An unfilled bar indicates that the compound could not be found, the height shows the reporting limit.

Results on seasonal variation in concentrations of brominated flame retardants in effluents are presented in Figure 17. BDE-47, BDE-99 and HBCDD were found in effluent water from Gässlösa STP. For the samples taken in March and April, only BDE-209 and HBCDD were analysed. For HBCDD, concentrations were higher in March and April compared to August and October. BDE-47 and BDE-99 were found at approximately the same concentrations in samples taken in August and October.

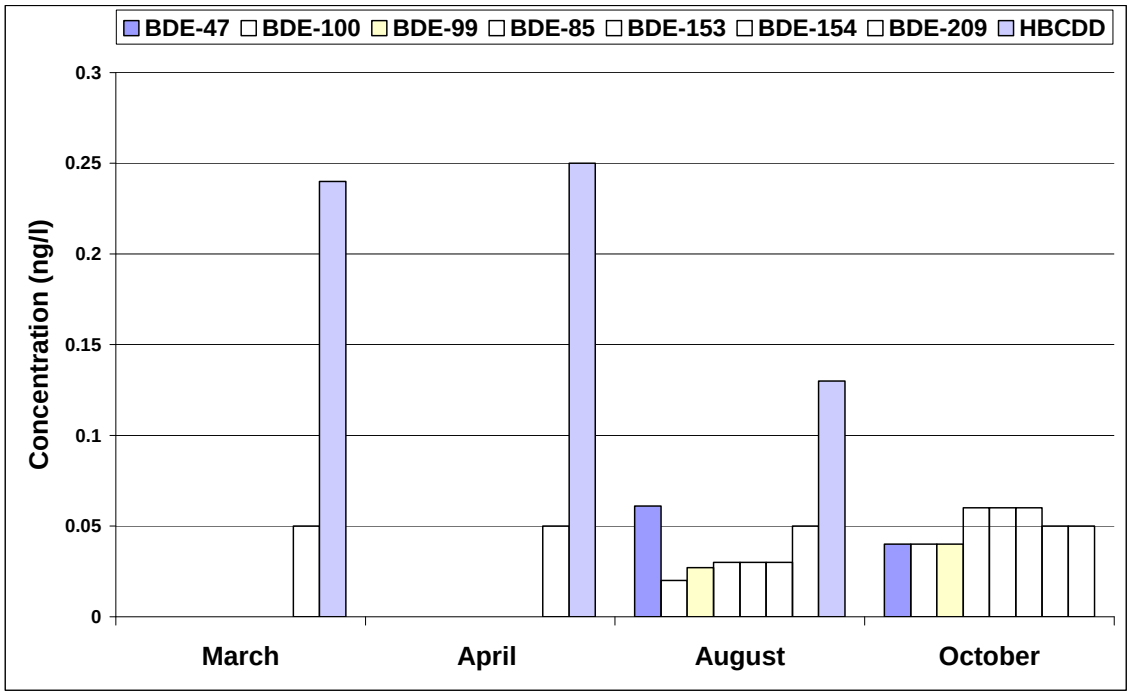


Figure 17. Concentrations of brominated flame retardants in effluents from Gässlösa STP sampled in March, April, August and October 2008. An unfilled bar indicates that the compound could not be found, the height shows the reporting limit. For the samples taken in March and April, only BDE-209 and HBCDD were analysed.

Concentrations of 4-nonylphenol, 4-t-octylphenol, triclosan and bisphenol A are presented in Figure 18. The concentrations of 4-nonylphenol and 4-t-octylphenol were close to the reporting limits, and for 4-nonylphenol it was below the reporting limit in August, whereas the concentrations of 4-t-octylphenol were below the reporting limit in March and August. Triclosan and bisphenol A were found in all four samples. No clear differences in concentrations could be seen for 4-nonylphenol, 4-t-octylphenol and triclosan, whereas for bisphenol A the concentration in the April sample was approximately ten times higher compared to the other months.

All the metals included in the study were found in effluents from Gässlösa STP, see Figure 19. Cadmium and silver were found in three out of four samples in the ranges < 0.005-0.013 µg/l and < 0.005-0.022 µg/l respectively. Lead (0.036-0.147 µg/l), arsenic (0.04-0.846 µg/l), copper (2.6-4.9 µg/l) and mercury (1.5-3.2 ng/l) were found in all four samples. No clear seasonal pattern in concentrations could be seen.

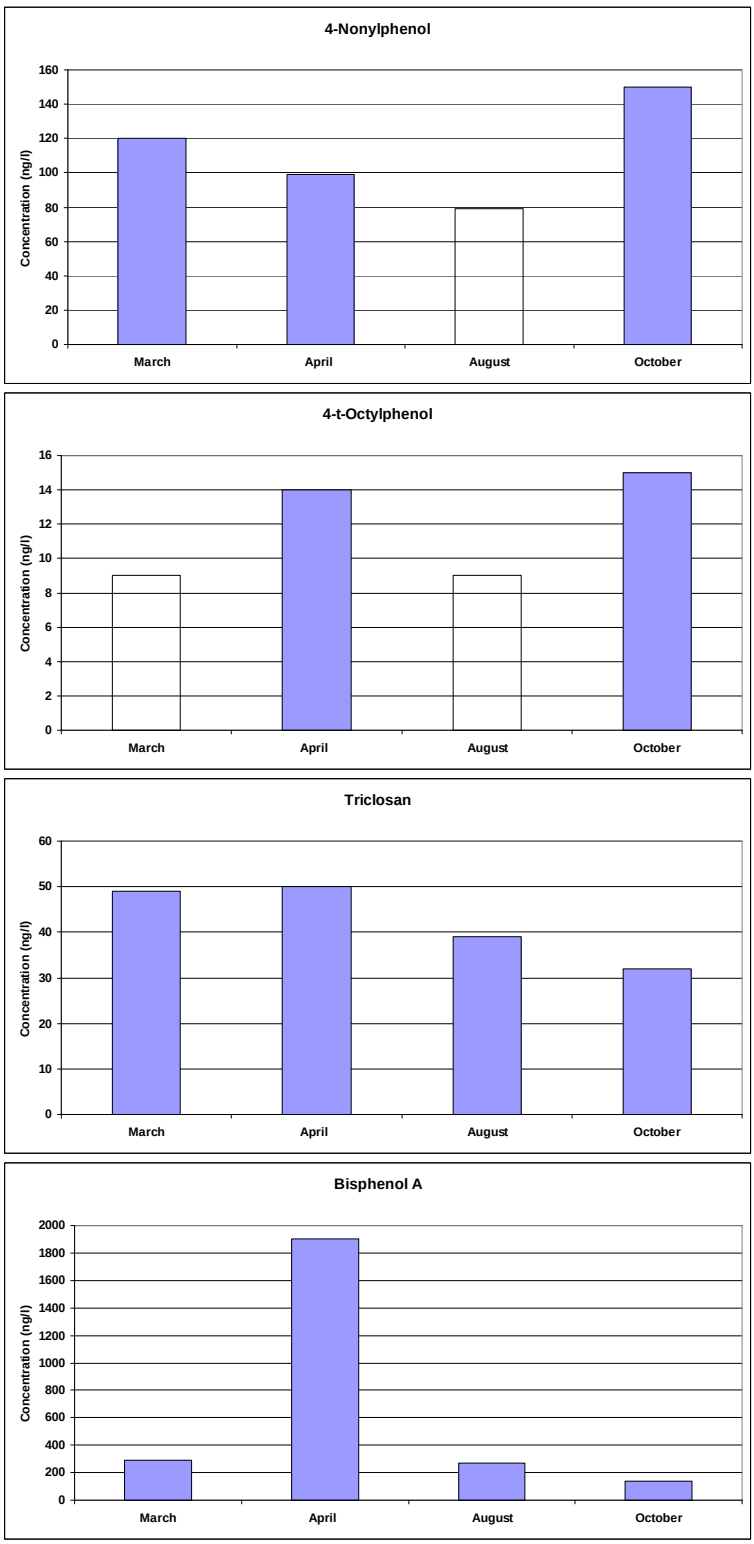


Figure 18. Concentrations of phenolic compounds in effluents from Gässlösa STP sampled in March, April, August and October 2008. An unfilled bar indicates that the compound could not be found, the height shows the reporting limit.

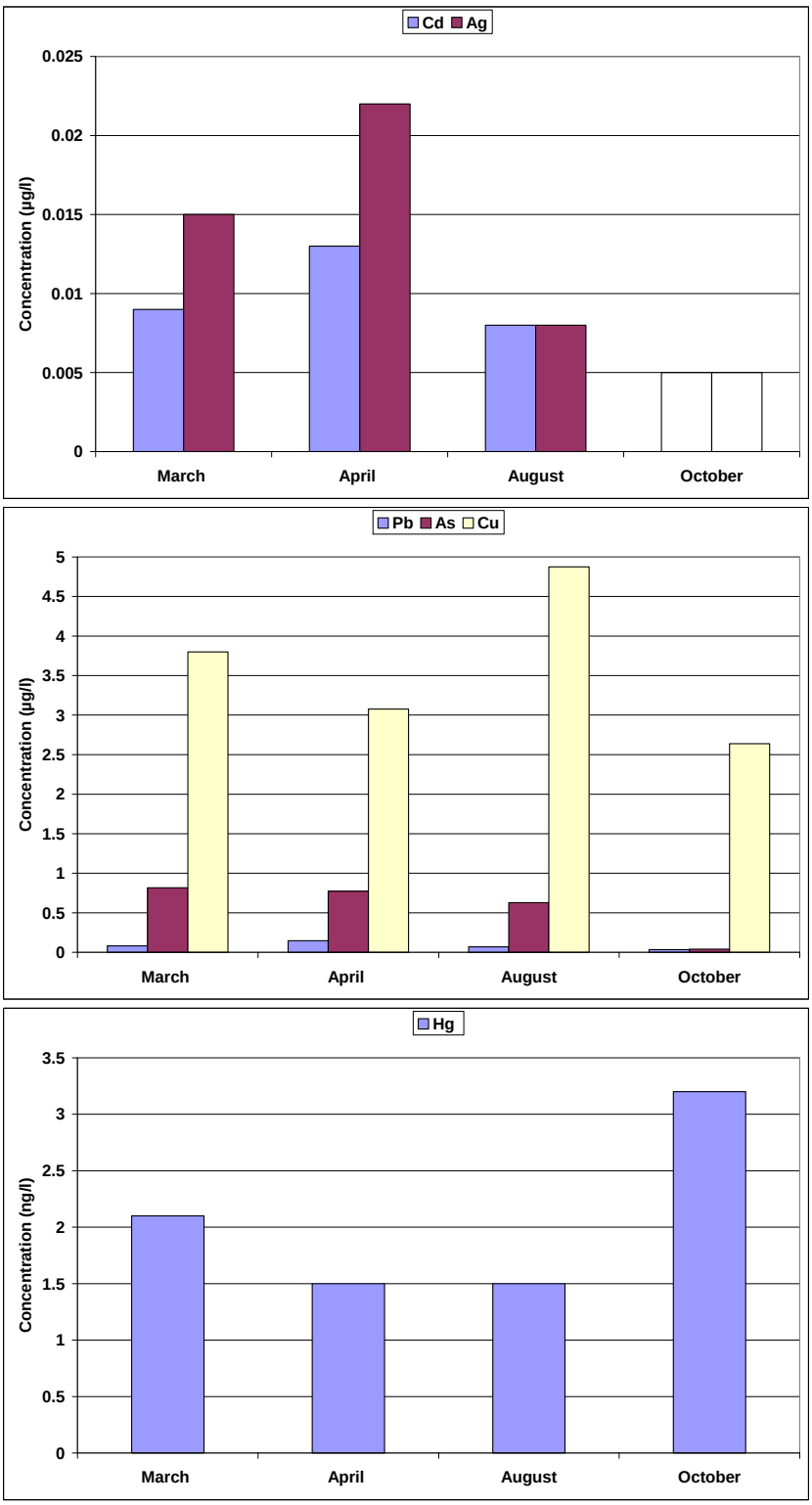


Figure 19. Metal concentrations in effluents from Gässlösa STP sampled in March, April, August and October 2008. An unfilled bar indicates that the compound could not be found, the height shows the reporting limit.

Concentrations of the herbicides MCPA and glyphosate were below the limits of detection, 2 ng/l and 0.5 µg/l respectively, in all water samples.

No general seasonal differences in concentrations could be seen. For several of the analysed compounds (MBT and DBT, BDE-47 and HBCDD, triclosan, bisphenol A, and all metals but mercury) concentrations were slightly lower or below the reporting limits in October. Further, concentrations of bisphenol A were more than six times higher in April compared to the other months. On the other hand, the highest concentrations of 4-nonylphenol, 4-t-octylphenol and mercury were found in the October sample. It should also be kept in mind that the samples taken were single daily samples and might thus not represent the different sampling periods.

5.4 Recipient water

MBT and DBT were detected in surface water samples from Viskan, see Figure 20. In April, MBT was found in the samples taken downstream of the effluent point, whereas in August MBT and DBT were found both upstream and in one of the downstream samples.

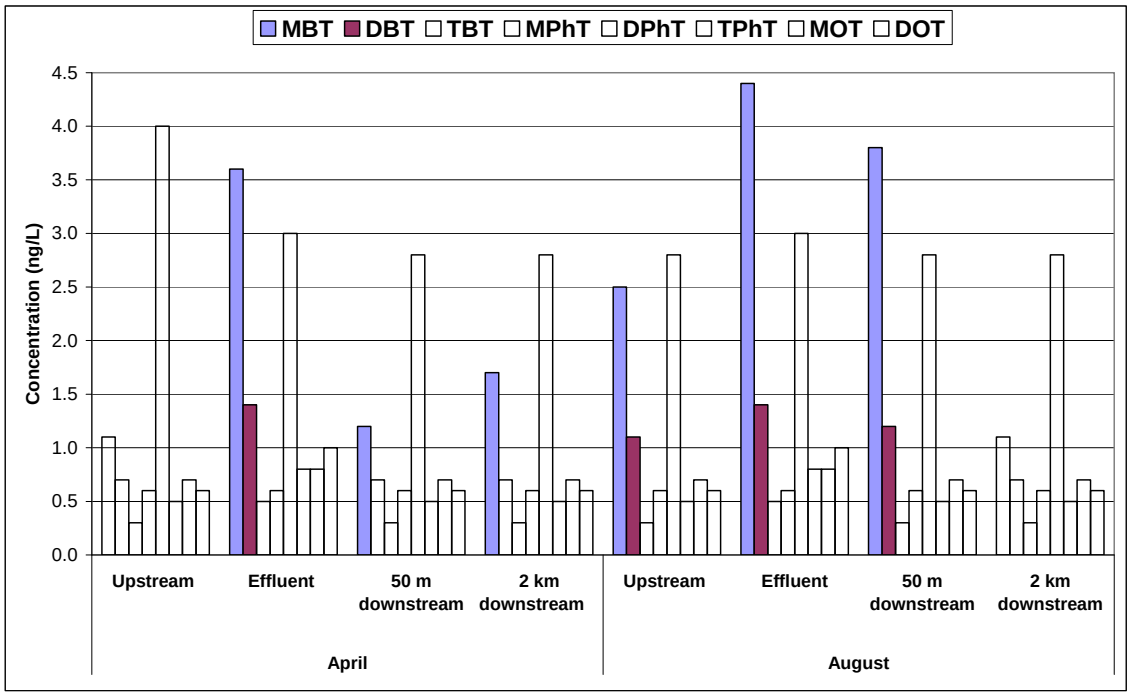


Figure 20. Organic tin compounds in surface water from the river Viskan and in effluents from the Gässlösa STP, sampled in April and August 2008. An unfilled bar indicates that the compound could not be found, the height shows the reporting limit.

Concentrations of brominated flame retardants in surface water from the recipient and in effluents from Gässlösa, sampled in April and August 2008, are presented in Figure 21. Of the BDE congeners analysed, BDE-47, BDE-100, BDE-99, BDE-153, BDE-154 and BDE-209 were found in the recipient water.

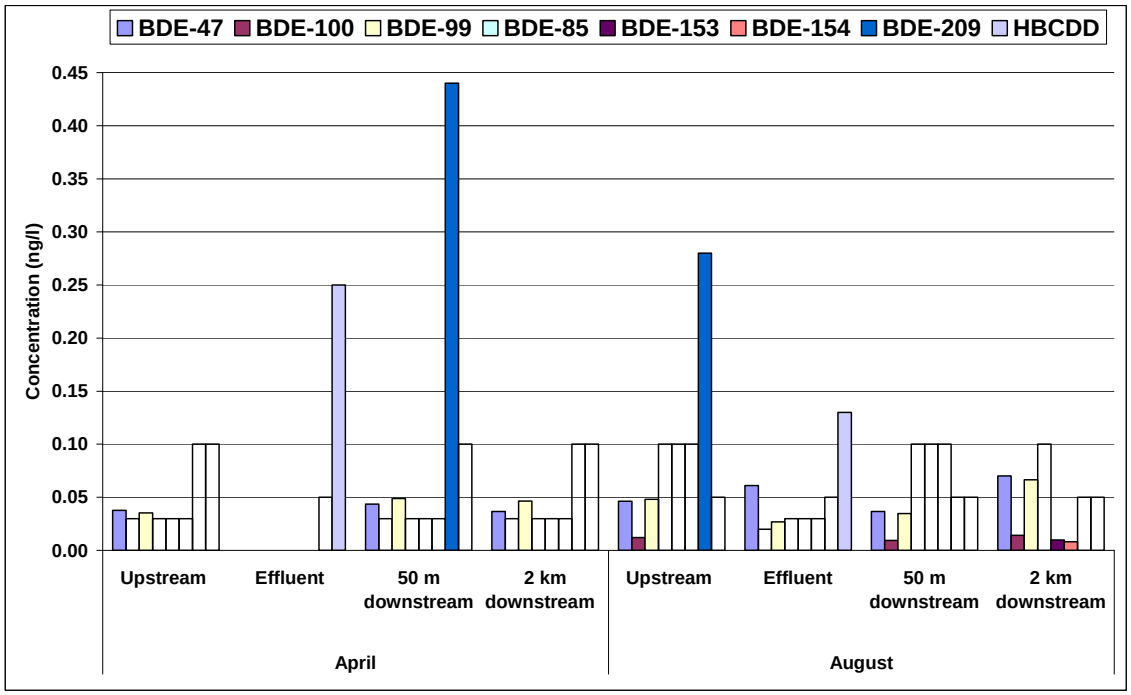


Figure 21. Concentrations of brominated flame retardants in surface waters from the river Viskan, and in effluents from the Gässlösa STP, sampled in April and August 2008. An unfilled bar indicates that the compound could not be found, the height shows the reporting limit. For the effluent sample taken in April, only BDE-209 and HBCDD were analysed.

Concentrations of 4-nonylphenol, 4-t-octylphenol, triclosan and bisphenol A in surface water from the recipient and in effluents from Gässlösa sampled in April and August are presented in Figure 22. All detected concentrations in the effluent samples were higher compared to the upstream surface water samples. Concentrations found were in the same range or slightly higher downstream compared to upstream of the effluent point, but for 4-nonylphenol and 4-t-octylphenol in April, and for triclosan and bisphenol A in both April and August, the highest concentrations were found in the surface water sample taken 2 km downstream. Thus, the STP contributes to the load of the compounds to the recipient, but the patterns indicate that other sources are of importance.

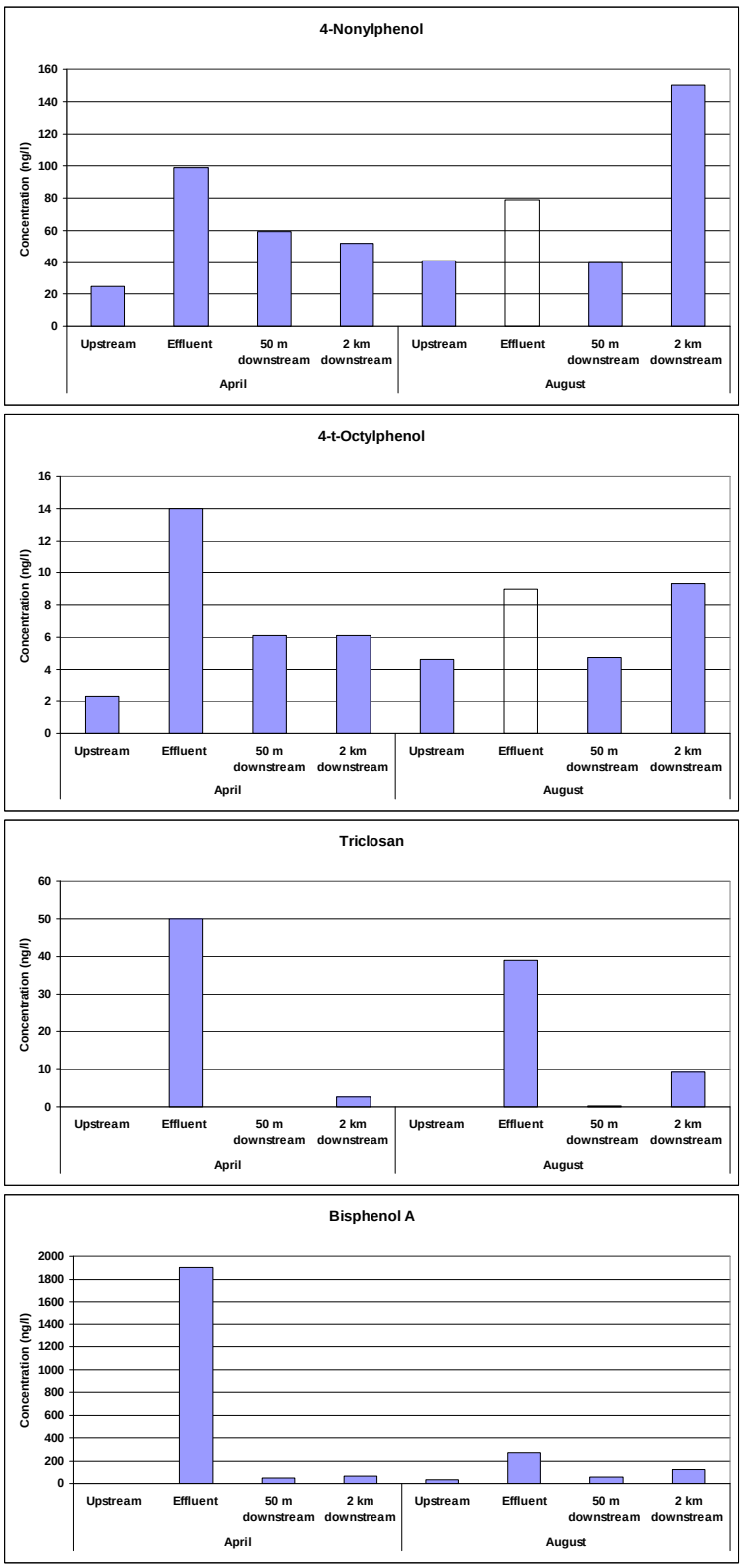


Figure 22. Concentrations of phenolic compounds in surface waters from the river Viskan, and in effluents from the Gässlösa STP, sampled in April and August 2008. An unfilled bar indicates that the compound could not be found, the height shows the reporting limit.

All metals could also be found in the surface waters of Viskan, see Figure 23. Concentrations found were in the same range or higher compared to what was found for the effluent water samples, and no clear differences could be seen between the samples taken upstream and downstream of the effluent point.

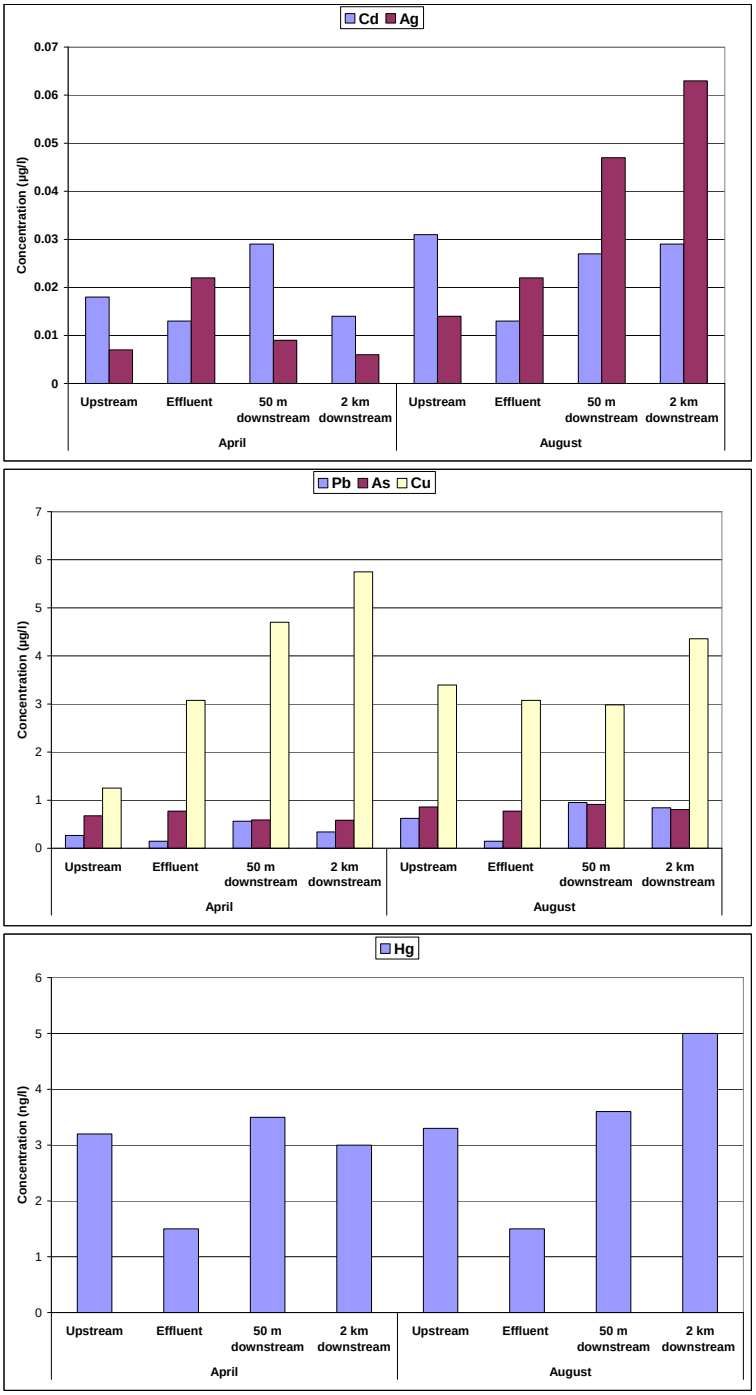


Figure 23. Metals in surface water from the river Viskan and in effluents from the Gässlösa STP, sampled in April and August 2008.

Concentrations of the herbicides MCPA and glyphosate were below the limits of detection, 2 ng/l and 0.5 µg/l respectively, in all analysed surface and STP effluent water samples.

5.5 Chemical characterization of STP effluents - identification of unknown compounds

5.5.1 Determination of lipophilic non-polar compounds

The extraction and clean-up methodology used for phthalates was directed to relatively un-polar neutral compounds (Figure 2). This restricted the possible number of compounds in the final extracts. Consequently, the GC-chromatograms from the phthalate extracts contained only a few peaks. Two main groups of compounds were identified besides the phthalates. The first group was different normal and branched aliphatic hydrocarbons. Cyclotetradecane, squalane, decaline, oktacosane and others were detected. The origin of the hydrocarbons was probably different petroleum products. Squalene is a hydrocarbon and a triterpene, and the biochemical precursor to steroids, cholesterol and vitamin D in the human body. Squalene is also used in cosmetics. The second identified group was cholesterol derivatives (e.g. Cholest-5-en-3-one; Figure 24). The source of these derivatives is human or more specific, human bile and faeces.

The concentrations of the petroleum related compounds were generally lower compared to DEHP. The cholestan derivative was high in one sample, equal to DEHP, but low in the other STPs.

Galaxolide-1 and squalene were present in all samples at concentrations comparable to dibutylphthalate.

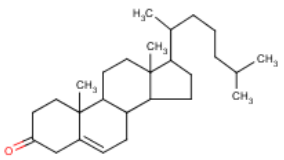


Figure 24. Cholest-5-en-3-one (CAS 601-54-7).

5.5.2 Determination of polar neutral compounds

The chromatograms from the extracts used for quantitative determination of organic phosphorous esters contained, except the target compounds, decaline (CAS 88-29-9) and normal- and branched aliphatic hydrocarbons. The dominant compound in most of the chromatograms was paracetamol (Figure 25). Paracetamol was however not detected in Henriksdal and Bollebygd STPs.

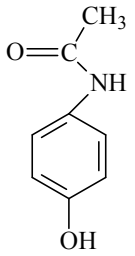


Figure 25. Paracetamol

5.5.3 Determination of phenolic compounds and other acetates

The phenolic compounds in the extracts were converted into their corresponding acetates prior to GC-MS analysis. In these extracts a few compounds were identified besides the target compounds. The acetylated form of 4-hydroxybenzoic acid was detected in all samples. The compound is primarily used for the preparation of parabenes, which are used as preservatives in cosmetics. The compound is also ubiquitous in plants. (1-Phenyletyl)phenol (CAS 4237-44-9) was tentatively identified (Figure 26). Poly-(1-phenylethyl)phenol-polyethoxylate or polyoxypropylenated is used as an industrial surfactant (US Patent 5082591). The compound is also used in rubber manufacturing, as a lubricant oil additive (corrosion inhibitor), stabilizer or plasticizer with phosphoric acid (http://chemicalland21.com/industrialchem/organic/4-CUMYLPHENOL.htm). Finally, tetradecanol was detected. It is an ingredient in cosmetics such as cold creams due to its emollient properties (soften and soothe the skin) and is a key component in the manufacture of lipstick, lotions, and other cosmetic products. The compound is also used as an intermediate in the chemical synthesis of the alcohol sulphate, an emulsifier in cosmetics.

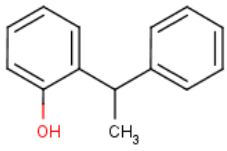


Figure 26. (1-Phenyletyl)phenol CAS 4237-44-9).

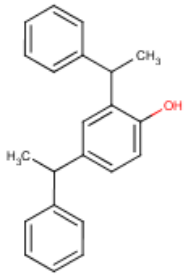


Figure 27. 2,4-Bis(1-phenylethyl)-phenol (CAS 2769-94-0).

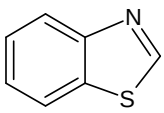
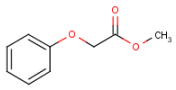
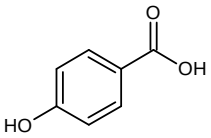
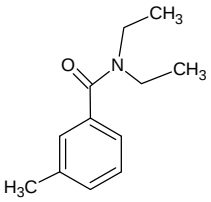
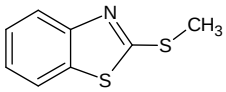
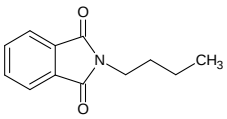
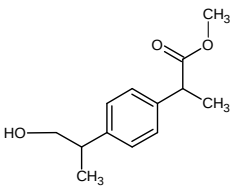
In two samples, Gässlösa and Öhn STP, two isomeric compounds were tentatively identified as 2,4-bis(1-phenylethyl)-phenol (CAS 2769-94-0; Figure 27).

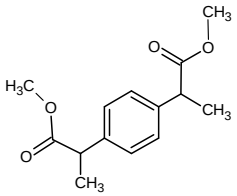
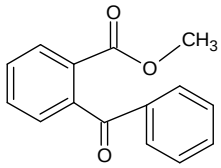
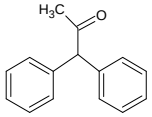
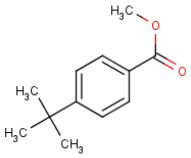
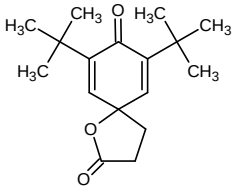
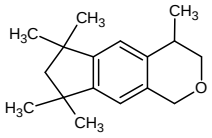
5.5.4 Determination of carboxylic acid methyl esters

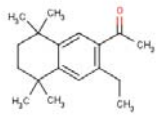
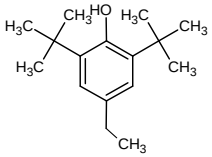
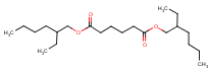
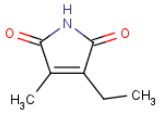
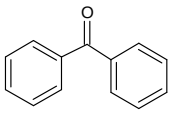
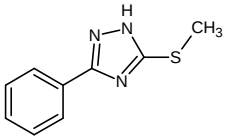
The analytical method used for the determination of non-steroidal anti-inflammatory drugs (NSAIDs) is made for carboxylic acids in general and the extracts contained indeed a great number of carboxylic acids. The other identified compounds and their possible use and origin are summarized in Table 15.

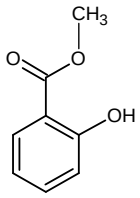
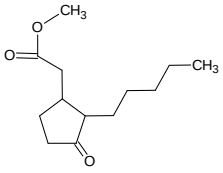
All carboxylic acids were detected as methyl esters but the compounds were probably present as free acids in the native samples. A number of compounds without carboxylic acids groups were also detected in these extracts.

Table 15. Identified compounds in the extracts used to determine NSAIDs

Benzothiazole 	Used as an intermediate in chemical industry. It is also a degradation product. See also Figure 28.
Phenoxyacetic acid methylester CAS 2065-23-8 	Used as an intermediate for manufacturing dyes, pharmaceuticals, pesticides and fungicides. It is also used in flavoring. (methylated prior to GC-MS)
4-Hydroxy benzoic acid CAS 99-76-3. 	Natural product in plants. Possible degradation product of parabenes and ethyl-4-ethoxybenzoate, a additive to plastic (Skjevrak *et al.*, 2005). (methylated prior to GC-MS)
Diethyltoluamide (DEET) CAS 134-62-3 	Insect repellent.
2-Methylthio-benzothiazole CAS 615-22-5 	Accelerator in rubber manufacturing and possible degradation product of 2(thiocyanomethylthio)benzothiazol. (Reemtsma *et al.*, 1995) (Is not methylated by the reagent metylchloroformat)
N-Butylphthalatamide 	Chemical in pigment manufacturing.
Hydroxy-ibuprofen 	Human metabolite of ibuprofen. See Figure 29. (Was methylated prior to GC-MS)

Carboxy-ibuprofen 	Human metabolite of ibuprofen. See Figure 29. (methylated prior to GC-MS)
(2-Benzoyl)benzoic acid methylester CAS 606-28-0 	Derivative of UV-filter? (methylated prior to GC-MS)
1,1-Diphenyl-propanone CAS 781-35-1 	
C14-C18 fatty acid methylesters	Natural products in all living organisms.
4-t-Butylbenzoic acid methyl ester CAS 26537-19-9 	Antimicrobial compound used in sunscreen products. Probably methylated prior to GC-MS)
7,9-Di-t-butyl-1-oxaspiro(4,5)deca -6,9-dien-2,8-dion CAS 82304-66-3 	Oxidized metabolite of 3,5-(di-tert-butyl-4-hydroxyphenyl)propionic acid. The reaction is described in Figure 31.
Galaxolide 1 CAS 1222-05-5 	Fragrance substance

Versalide CAS 88-29-9 	Fragrance substance
2,6-Di-t-butyl-4 ethylphenol CAS 4130-42-1 	Antioxidant. Possible degradation product from Irgafos- and Irganox-type antioxidats (Skjevrak *et al.*, 2005).
Di-(2-ethylhexyl)adipat DEHA CAS 103-23-1 	Plastiziser
3-Ethyl-4-methyl-1H-pyrrole-2,5-dione CAS 20189-42-8 	Probably natural flavour and fragrance substance.
Bensophenon CAS 119-61-9 	Benzophenone can be used as a photo initiator in UV-curing applications. Benzophenone is used as an UV-filter in protecting scents and colors in products such as perfumes and soaps. It can also be added to the plastic packaging as a UV blocker in order to protect the product without using opaque or dark packaging. Important intermediate in the production of medicines and cosmetics
CAS 7747-19-5 	

Salicylic acid CAS 69-72-7 	Natural compound in plants. Metabolite of acetylsalicylic acid. Salicylic acid was identified in STP water. Was methylated prior to GC-MS)Was methylated prior to GC-MS)
3-Oxo-2-pentyl-cyclopentan acetic acid methylester CAS 24851-98-7 	Ethyl dihydrojasmonate is used as a fragrance for cosmetics.

Occurrence and concentrations of identified compounds in NSAID-extracts
The occurrence and concentrations of some of the identified compounds in the seven STP effluent studied are summarized in Table 16. Authentic reference compounds were not available for all new identified compounds. The quantification was therefore calculated as ibuprofen equivalents.

The concentrations (semi quantitative) of the identified compounds are in the same range as bisphenol A, NSAIDs, ibuprofen-OH (metabolite of ibuprofen), some organic phosphorus esters (TCEP, TDCP) and DEHP (Table A4, Table A7, Table A15, Table A10 and Table A9) but lower than TBEP (organic phosphorous esters).

The most frequently found "new" compounds, detected in 5-7 STPs out of 7, were benzothiazole, 2-metylthio-benzothiazole, dietyltolylamide, Galaxolid, the degradation product of (3,5-di-tert-butyl-4-hydroxyphenyl)propionic acid and 1,1-diphenyl-propanone (Table 16). The most important sources for the compound in Table 16 are probably cosmetics, personal care products, plastic and rubber materials.

Table 16: Occurrence and concentrations of identified compounds in NSAID-extracts Concentrations calculated as ibuprofen equivalents.

Compound	Approximate concentration range, µg/l	Detection frequency
Benzothiazole	0.1-1	6/7
Phenoxyacetic acid methylester	0-6	2/7
4-t-Bensoic acid methylester	0-0.5	4/7
Diethyltolylamide	0-0.6	6/7
Benzophenon	traces	4/7
2,6-Di-t-butyl-4-ethylphenol	0-0.5	4/7
2-Methylthio bezothiasole	0-1	5/7
N-n-Butylpthalatamide	0-1	1/7
Methylthiophenyl triazole	0-0.5	3/7
Galaxolid	0-1	7/7
Quinone-lactone	0.2-0.6	6/7
Benzoyl(benzoic acid)	0-0.7	1/7
1,1-Diphenyl-propanone	0-0.6	6/7

Explanation: quinone-lactone = 7,9-di-t-butyl-1-oxaspiro(4,5)deca -6,9-dien-2,8-dion

Transformation products in the NSAID extracts

The origin of 2-methylthio-benzothiazole is probably rubber products (it is used as accelerator in production of rubber). It is also a degradation product of the biocide 2(thiocyanomethylthio)benzothiazol (Figure 28). This compound is not methylated by the reagent used (methylchloroformat) but excited probably as methyl-thio-derivative in the native sample.

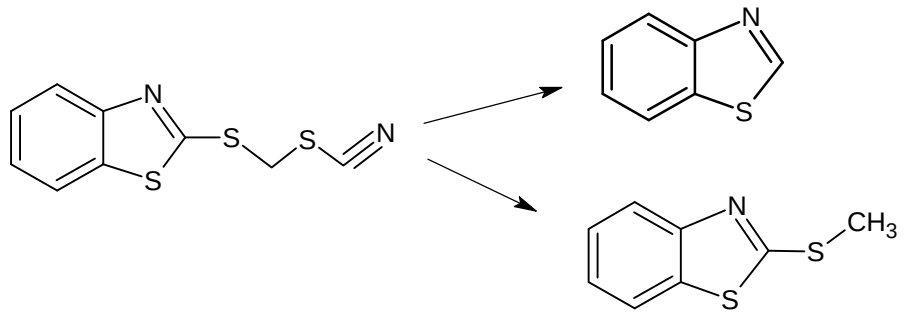


Figure 28: Possible degradation pathway of the fungicide 2(thiocyanomethylthio)benzothiazol.

Ibuprofen is conjugated in the liver and excreted via the kidneys. However, ibuprofen is also metabolised to hydroxyl-ibuprofen (2-(4-(3-hydroxy-2-methylpropyl)phenyl)propanoic acid) and carboxy-ibuprofen (2-[4-(1-hydroxy-1-oxopropan-2-yl)phenyl]propanoic acid; Figure 29; see 5.1.6). These two metabolites have been detected both in STP-effluents and surface water in concentrations higher than the mother compound (Buser *et al.*, 1999; Weigel *et al.*, 2004; Remberger *et al.*, 2008a). Since commercial standards were not available the identification of these compounds must be considered as tentative but the mass spectrum obtained was in good agreement with previously published spectra (Buser *et al.*, 1999; Weigel *et al.*, 2004).

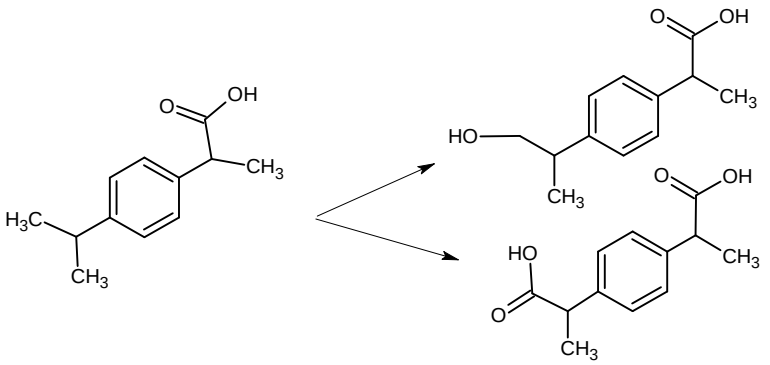


Figure 29: Human transformation products of ibuprofen.

Precursor compounds to 7,9-di-t-butyl-1-oxaspiro(4,5)deca-6,9-dien-2,8-dion (Table 15) are probably different esters of 3,5-(di-tert-butyl-4-hydroxyphenyl)propionic acid (CAS 123173-45-5) used in many applications as antioxidants in plastic.

One possible candidate is the compound octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate (CAS 2082-79-3). This compound is used to protect plastic materials against thermo-oxidative degradation (antioxidant). The compound has indeed been detected in the Swedish environment (Remberger *et al.*, 2008b).

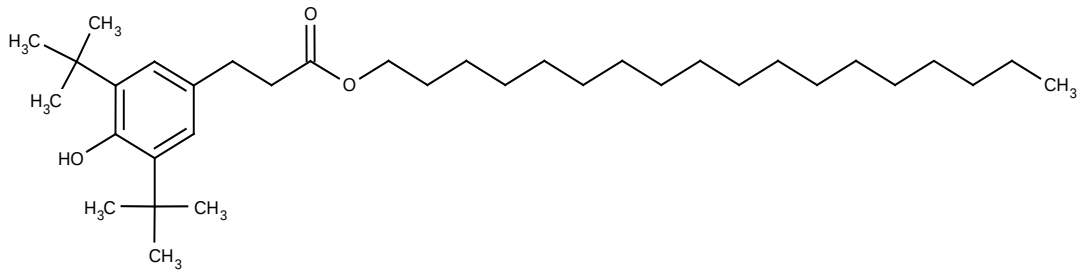


Figure 30: Octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate (CAS 2082-79-3).

The free acid, the 3-(3,5-di-t-butyl-4-hydroxyphenyl)-propionic acid (fenozan; d-t-BPA), (hydrolysis product of the esters) was identified in low concentrations in all studied STP effluent samples (Table A15).

The transformation of the antioxidant is described in Figure 31. The oxidated form of 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate has been described previously (Jenke *et al.*, 2005; Skjevrak *et al.*, 2005).

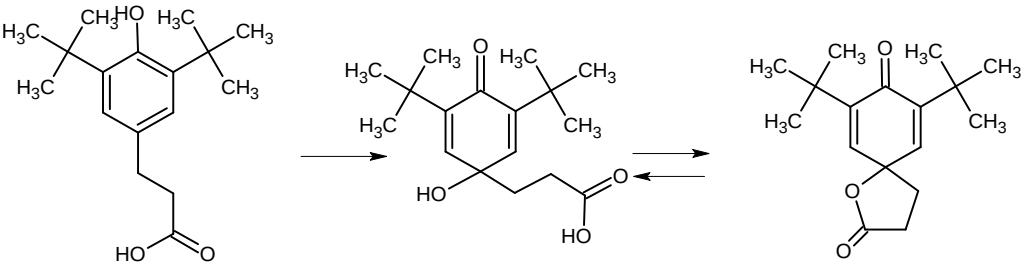


Figure 31: Oxidation of the antioxidant 3,5-(di-tert-butyl-4-hydroxyphenyl)propionic acid (CAS 123173-45-5) to a keton and formation of a lactone.

The compound 1,1´-biphenyl-4-propanone could not be conclusively identified due to co-eluting compounds but the substance in question is probably a biphenyl with a propanone group in the structure.

Summary on the identification work

An attempt was made to identify as may organic compounds as possible in the seven STP effluent samples. The common method used for a broad spectrum analysis and identification of unknown compounds is an extraction of the sample with an organic solvent or a solid phase column (SPE) and then analysed with GC-MS in full scan. The resulting chromatograms are often very complex especially for samples from polluted areas and extracts of STP-effluents are for natural reasons very complex with many co-eluting compounds. In this investigation we used a different approach. Instead for using the raw-extract (no fractionation or derivatisation) we used the extracts for "directed analysis" for full scan identification. The chromatograms from the different extracts were less complex compared to a un-fractionated raw extracts which facilitated the identification. This was true for extracts used for analysis of phthalates, phenols and to some extent organ phosphorous esters. However, the extracts used for NSAIDs were still very complex and difficult to interpret. It was therefore not possible to fully attain the aim to identify all compounds behind every peak in the chromatograms. The main problem was the "mixed" MS-spectrum that frequently

appeared since all compounds were not fully separated making the identification difficult or impossible. Another problem was that not all acquired MS-spectra in the chromatograms gave a match in the mass spectrum library (GC-MS database) due to low concentrations of the compounds, mixed MS-spectrum or the unknown spectrum were not present in the library. A more fine tuned fractionation of the extracts may overcome some of the problem and should be considered. It is also important to keep in mind that the sample preparation, including enrichment, cleanup, derivatisation and fractionation, probably has excluded an unknown portion of the organic compounds in the raw-extracts. Finally, the gas chromatograph restricts what compounds can be analysed.

6 Multivariate data analysis

A multivariate data analysis was performed on the results from the screening. In the initial phase substances found at concentrations above the reporting limits in more than four out of seven samples were included in the Principal Component Analysis, PCA. The explained variation in this initial phase was not acceptable in the PCA, therefore some of the substances were removed that had a low variability between the observations and did not contribute to the explained variation in the model. In Table 17 the substances that were included in the initial and final PCA model are presented.

Table 17. The initial and final PCA was performed on the following substances.

Initial PCA	**Final PCA**	
Name	**Name**	**Class**
DBT		Organic tin compounds
BDE-47	BDE-47	Brominated flame retardants
BDE-99		Brominated flame retardants
HBCDD	HBCDD	Brominated flame retardants
4-Nonylphenol	4-Nonylphenol	Phenolic substances
Triclosan	Triclosan	Phenolic substances
Bisphenol A	Bisphenol A	Phenolic substances
Hg tot	Hg tot	Metals
Cd	Cd	Metals
Pb	Pb	Metals
Ag	Ag	Metals
As		Metals
Cu	Cu	Metals
Ibuprofen	Ibuprofen	NSAIDs
Naproxen	Naproxen	NSAIDs
Ketoprofen	Ketoprofen	NSAIDs
Diclofenac	Diclofenac	NSAIDs
PFOS	PFOS	PFAS
PFOA		PFAS
PFOSA	PFOSA	PFAS
PFHxA		PFAS
PFDcA		PFAS
DEP	DEP	Phthalates
DIBP	DIBP	Phthalates

Initial PCA	**Final PCA**	
Name	**Name**	**Class**
DBP		Phthalates
BBzP		Phthalates
DEHP		Phthalates
TIBP		Organophosphorus compounds
TBP		Organophosphorus compounds
TCEP		Organophosphorus compounds
TDCP		Organophosphorus compounds
TBEP		Organophosphorus compounds
TPhP	TPhP	Organophosphorus compounds
EHDPP		Organophosphorus compounds
n-Hexane	n-Hexane	Volatile halogenated substances
Benzene	Benzene	Volatile halogenated substances
Toluene		Volatile halogenated substances
Ethylbenzene		Volatile halogenated substances
m+p-Xylene		Volatile halogenated substances
o-Xylene	o-xylene	Volatile halogenated substances
1.2-Dichloroethane		Volatile halogenated substances
Chloroform		Volatile halogenated substances
Carbon tetrachloride	Carbon tetrachloride	Volatile halogenated substances
Tetrachloroethene		Volatile halogenated substances
Trichloroethene		Volatile halogenated substances
MD3M		Siloxanes
YES	YES	Hormones
Salicylic acid		Additional
Ibuprofen-OH	Ibuprofen-OH	Additional
Ibuprofen-COOH	Ibuprofen-COOH	Additional
d-t-BPA	d-t-BPA	Additional

The final PCA with three components explains 82% of the variation in data. To visualize the PCA a score plot that describes the relations between the different STPs is shown in Figure 32 and a loadings plot describing the substances in Figure 33. These plots below are for the two first components in the model and they explain 68% of the variation.

From the score plot three groups are identified, marked with dashed lines in Figure 32; Bollebygd, Ellinge and the other STPs. Bollebygd differ from the other STPs in substances that lies in the lower right in Figure 33, i.e. the following substances; all substances in the NSAIDs and Additional class, Carbon tetrachloride and DEP. The sample from Ellinge is more affected by the substances in the lower let of the Figure 33. These substances are mainly: Pb, Ag, Cu, PFOSA and HBCDD.

The third component, not shown, describes mainly the difference in content of PFOS, BDE-47 and n-hexane. No obvious groups are seen but there is a spread between the samples.

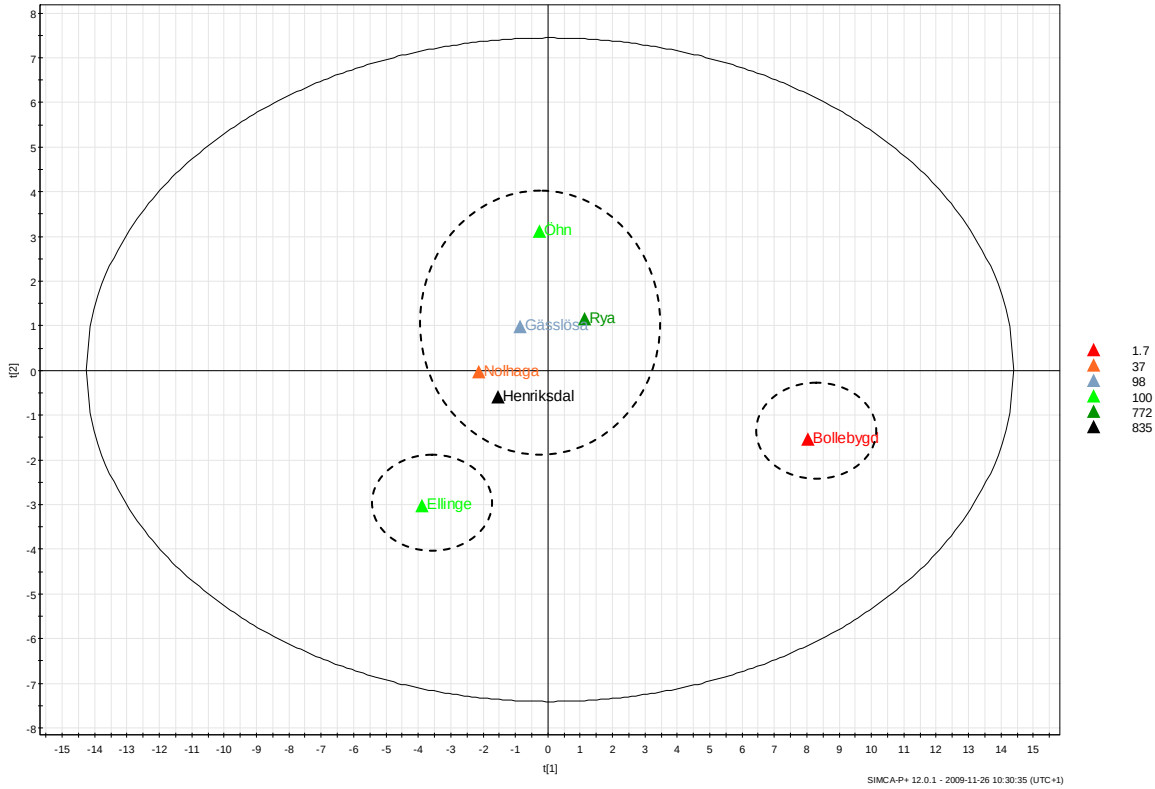


Figure 32. Score plot. The STPs (samples) are color coded after their incoming load in KPE.

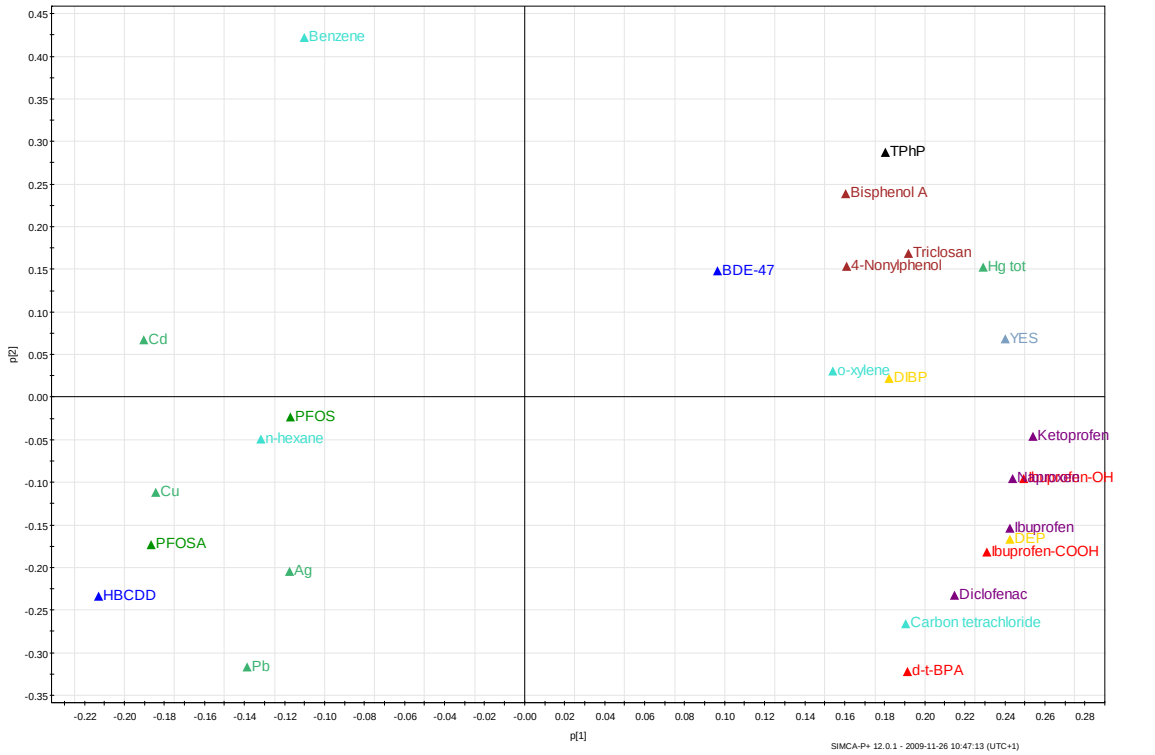


Figure 33. Loadings plot. The substances are color coded after their different classification.

7 Summary and conclusions

Effluent water
The detection frequencies and the comparison of measured concentrations with toxicity and limit values, for those substances found above the reporting limits, are summarized in Table 18. It is also indicated if the substances are among the priority substances (PS) or Annex III substances of the Water Framework Daughter Directive (2008/105/EC), and/or among the substances identified to be of specific concern for the Baltic Sea under the BSAP.

All substance groups but the herbicides and chlorobenzenes were found in the effluent waters sampled in August-September 2008.

The concentration levels of the substances found in this study were in general in the same range or lower compared to other studies.

The concentrations were in the same range or above toxicity or limit values for organic tin compounds (TBT and TPhT), brominated flame retardants (∑BDEs), phenolic compounds (all), metals (Ag and Cu), NSAIDs (ibuprofen, naproxen and diclofenac) and organophosphorus esters (TIBP, TBEP and TPhP).

For some of the substances the concentrations exceeded or were in the same range as toxicity or limit values, but the detection frequencies were low, e.g. for the organic tin compounds, the brominated diphenyl ethers, 4-nonylphenol and 4-t-octylphenol. For these substances there should be a focus on method development in order to lower the reporting limits. For example TBT and TPhT were found in two and one sample, respectively, but when found, concentrations exceeded the toxicity and limit values.

Substances not found were the organic tin compounds DPhT and MOT, the brominated flame retardants BDE congeners 85, 153, 154 and 209, the phthalates DOP, DINP and DIDP, the volatile organic carbons 3-methyl-pentane, n-octan and 1,3,5-TMB, the volatile halogenated substance dichloromethane, and the siloxanes D4 and D5.

The biological characterization, consisted of measurements of estrogenic and androgenic activities, revealed some estrogenic, but no androgenic, activity. The assay on androgenic activity did however indicate anti-androgenic properties of the samples.

The multivariate data analysis showed that, in general, there is relatively low variability in chemical composition in municipal STP effluents. However, differences between Ellinge and Bollebygd compared to the other STPs were found. The difference between Ellinge and the other STPs was explained by the metals Cu, Ag and Pb, PFOSA and HBCDD. For Bollebygd, the difference was explained by the NSAIDs including the metabolites of ibuprofen, d-t-BPA, DEP and carbon tetrachloride.

Table 18. Summary of substances found above the reporting limits, their detection frequencies, results on comparisons with toxicity/limit values, and information regarding WFD and BSAP status.

Substance group	Substance	Detection frequency	WFD	BSAP	Conc. in the same range or above toxicity / limit value
Organic tin compounds	TBT, DBT, MBT	2, 7, & 3/7	PS	X	**Yes** (TBT)
	TPhT, MPhT	1 & 2/7		X	**Yes** (TPhT)
	DOT	1/7			No
Brominated flame retardants	BDEs (47, 99, 100)	6, 5 & 3/7	PS	X	**Yes** (∑BDE)
	HBCDD	7/7		X	No
Phenolic compounds	4-Nonylphenol	4/7	PS	X	**Yes**
	4-t-Octylphenol	2/7	PS	X	**Yes**
	Triclosan	7/7			**Yes**
	Bisphenol A	5/7	Annex III		**Yes**
Metals	Hg	7/7	PS	X	No
	Cd	7/7	PS	X	No
	Pb	7/7	PS		No
	Ag	7/7			**Yes**
	As	7/7			No
	Cu	7/7			**Yes**
NSAIDs	Ibuprofen	7/7			**Yes**
	Naproxen	7/7			**Yes**
	Ketoprofen	7/7			No
	Diclofenac	7/7			**Yes**
Perfluorinated substances	PFOS	7/7	Annex III	X	No
	PFOA	7/7		X	No
	PFOSA	5/7			-
	PFHxA	7/7			-
	PFDcA	7/7			-
Phthalates	DEP	7/7			-
	DIBP	7/7			-
	DBP	7/7			No
	BBzP	4/7			-
	DEHP	6/7	PS		No
Organo-phosphorus esters	TIBP	7/7			**Yes**
	TBP	7/7			No
	TCEP	7/7			No
	TDCP	6/7			No
	TBEP	7/7			**Yes**
	TPhP	7/7			**Yes**
	EHDPP	7/7			-
Volatile organic compounds	n-Hexane	6/7			-
	Benzene	6/7			No
	Toluene	7/7			No
	Ethyl-benzene	5/7			No
	m+p-Xylene	7/7			-
	Styrene	3/7			No
	o-Xylene	6/7			-
	n-Nonan	1/7			-
Volatile halogenated substances	1,1,1-Trichloroethane	3/7			-
	1,2-Dichloroethane	7/7	PS		No
	Chloroform	7/7	PS		No
	Carbon tetrachloride	7/7	PS		No
	Tetrachloroethene	7/7	PS		No
	Trichloroethene	5/7	PS		No
Siloxanes	D6	2/7			-
	MM	1/7			-
	MDM	1/7			-
	MD2M	2/7			-
	MD3M	4/7			-

The STPs included in this study were those also studied in the Swedish monitoring program on chemicals in sewage sludge, chosen to represent STPs differing in size, geographical location, load composition and treatment processes. Based on this, together with the results from the comparisons with previous measurements, it is concluded that this set of STPs may be suitable also for a monitoring program on effluent water.

Some differences, although no clear seasonal patterns, in concentrations of measured substances were found. The general low variability between samples taken during different months is in agreement with the expected diffuse spreading of the included substances. In October the concentrations were lower or below reporting limits for several of the compounds. On the other hand, the highest concentrations of 4-nonylphenol, 4-t-octylphenol and mercury were found in the October sample. For bisphenol A the concentrations were more than six times higher in April compared to the other months. It should however be kept in mind that the samples taken were single daily samples.

For long term monitoring purposes, sampling once a year may be suitable. Sampling should however be done during a defined same time period each year, and samples should preferably represent a longer time span than daily samples to reduce variability.

Recipient water
The measurements of chemicals in surface waters from Viskan and in effluents from Gässlösa STP indicated that other sources could be of importance for the chemical load to this water body.

The results from the measurements indicated a significant load via the STP of phenolic substances, whereas other compounds measured in recipient water, especially the metals, were in the same range or higher in these samples compared to the effluent samples.

Identification of "unknown" compounds
For the identification work on "unknown" compounds, the methodology employed, the identification of "unknowns" in fractions used for the identification of specific substances, turned out to be successful.

A large number of "new" substances were identified; several of them at concentrations in μg/l levels. Further, several metabolites or degradation products were identified, which could be used as tracers indicating diffuse spreading of their precursors.

The methodology used in this study may be a potential option in the identification of new emerging substances. Further the results visualize the lack of knowledge regarding the chemical "cosmos" entering the environment from the society.

Biological characterization of effluent water
The identification of several substances at concentrations close to or above toxicity and limit values, and the identification of a large number of "unknown" substances at relatively high concentrations, stress the importance to address the issue of mixed toxicity. This could be done by incorporating effects measurements in a monitoring program. Suitable assays for such measurements should target effects relevant for chronic effects, have a high through put, be cost efficient, and preferably avoid usage of animals. Within this study, some estrogenic effects and results indicating anti-androgenic effects were found. The literature survey on potential additional bioassays suitable for monitoring identified several other effects to be of relevance for a monitoring program, see Appendix B. Based on this, a battery of assays targeting estrogenic, anti-estrogenic, androgenic and

anti-androgenic effects, an assay on binding to the aryl hydrocarbon receptor ("dioxin like" effects) and an assay of genotoxic effects, is initially suggested.

7.1 Monitoring program outline

Based on the results from the chemical and biological characterization of effluent water, the recipient study, as well as the literature survey on potential additional bioassays, a recommendation for a monitoring program of municipal STP effluent is given.

Substance groups and substances suggested to be included in a monitoring program are presented in Table 19 below. These substances are chosen based on the measured concentrations in relation to toxicity and limit values, detection frequencies, and cost efficiency (analysis costs). Further, they represent different source categories resulting in diffuse emissions such as plastic materials, personal care products, pharmaceuticals and textiles. This set of suggested targeted substances should not be considered a fixed set, but should be perceived as an initial battery, subject to changes as a result of e.g. new legislations, coming additions of new substances under the WFD, new or refined risk assessments and the identification of new emerging substances.

Table 19. Suggested substance groups and substances for the monitoring program.

Substance group	Substances	Reason for inclusion
Organic tin compounds	All measured	Excedances, WFD, BSAP
Brominated flame retardants	BDEs	Excedances, WFD, BSAP
	HBCDD	BSAP
Phenolic substances	4-nonylphenol	Excedances, WFD (PS), BSAP
	4-t-octylphenol	Excedances, WFD (PS), BSAP
	Bisphenol A	Excedances, WFD (Annex III), cost efficient to determine together with the other phenolic compounds
	Triclosan	Excedances, emerging substance, cost efficient to determine together with the other phenolic compounds
Metals	All measured but Hg	Excedances, WFD (PS), BSAP, varied between STPs
NSAIDs	All measured	Excedances, high detection frequencies, emerging substances, varied between STPs
Perfluorinated substances	All measured	WFD (Annex III), BSAP, high detection frequencies
Organophosphorus esters	All measured	Excedances, high detection frequencies, emerging substances

The effect based monitoring is suggested to be based on a battery of assays targeting estrogenic, anti-estrogenic, androgenic and anti-androgenic effects, an assay on binding to the aryl hydrocarbon receptor ("dioxin like" effects) and an assay of genotoxic effects. It is also suggested also to revise the effect based monitoring regularly. This is an expanding field and assays targeting other mechanisms of relevance for chronic toxicity, such as the thyroid system, are continuously being validated.

The following substances will not be recommended to be included in an effluent monitoring program:

Herbicides and chlorobenzenes since these were not found above the reporting limits.

Volatile organic compounds and volatlie halogenated substances based on the fact that measured concentrations were far below toxicity and limit values.

Siloxanes and phthalates due to low detection frequencies and low concentrations respectively. For these two substance groups, knowledge concerning toxicity and also environmental concentrations is limited. They are however covered in the monitoring of sludge (Haglund and Olofsson, 2007).

Mercury is not suggested to be included even though this metal is a priority substance under the WFD and also identified to be of specific concern under the BSAP. The results showed that concentrations of mercury in effluent water were low compared to in recipient water.

Considering the relatively low variability in chemical composition found, and the fact that measured concentrations generally were in the same range as found in previous studies, monitoring is suggested to be performed on effluent water from the STPs included in the present study. Further, this would make it possible to synchronize the program with the monitoring of sludge. This could potentially increase the amount of information possible to extract during the evaluation of obtained data. Among these STPs, there are however only two, Öhn and Henriksdal, with discharges to the Baltic Sea. In order to be able to utilize the obtained data also for compliance with the objectives of the BSAP and the Marine Directive, it is therefore suggested to also include a STP with an effluent point in the southern Baltic Sea, e.g. in Kalmar or Karlskrona, in the monitoring program.

Sampling is suggested to be done once a year preferably coordinated with the program for monitoring of sludge. Further, samples representing a longer time period, e.g. weekly samples, in order to reduce variability, is recommended.

After some years of monitoring it is suggested to revaluate the obtained entire data set by multivariate statistics.

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Appendix A. Sample characteristics and results

Table A1. Sample characteristics.

Sample ID	City	Sampling site	Matrix	Comment	Sampling date	Coordinates
MR 6900-6901+6906	Borås	Viskan upstream April	Surface water	spot sample	02-04-2008	6401626; 1328642
MR 6902-6903+6907	Borås	Viskan 50 m downstream April	Surface water	spot sample	02-04-2008	6401530; 1328487
MR 6904-6905+6908	Borås	Viskan 2 km downstream April	Surface water	spot sample	02-04-2008	6400019; 1327280
MR 7009	Borås	Viskan upstream August	Surface water	spot sample	05-08-2008	6401626; 1328642
MR 7010	Borås	Viskan 50 m downstream August	Surface water	spot sample	05-08-2008	6401530; 1328487
MR 7011	Borås	Viskan 2 km downstream August	Surface water	spot sample	05-08-2008	6401626; 1328642
MR 6875-6877	Borås	Gässlösa STP March	Effluent	daily sample*	04-03-2008	
MR 6897-6899	Borås	Gässlösa STP April	Effluent	daily sample*	03-04-2008	
MR 7545	Borås	Gässlösa STP October	Effluent	daily sample*	23-10-2008	
MR 7005-7008	Borås	Gässlösa STP August	Effluent	daily sample*	06-08-2008	
MR 7075-7087	Allingsås	Nolhaga STP	Effluent	daily sample*	19-08-2008	
MR 7062-7074	Eslöv	Ellinge STP	Effluent	daily sample*	19-08-2008	
MR 7030-7035	Umeå	Öhns STP	Effluent	daily sample*	12-08-2008	
MR 7098-7110	Gothenburg	Rya STP	Effluent	daily sample*	27-08-2008	
MR 7168-7179	Stockholm	Henriksdal STP	Effluent	daily sample*	07-09-2008	
MR 7224-7236	Bollebygd	Bollebygd STP	Effluent	daily sample*	11-09-2008	

* for phthalates and organophopsphorus esters spot samples were taken.

Table A2. Concentrations of organic tin compounds (ng/l). < indicate reporting limits.

Sample ID	Sampling site	MBT	DBT	TBT	MPhT	DPhT	TPhT	MOT	DOT
MR 6900-6901+6906	Viskan upstream April	<1.1	<0.7	<0.3	<0.6	<4	<0.5	<0.7	<0.6
MR 6902-6903+6907	Viskan 50 m downstream April	1.2	<0.7	<0.3	<0.6	<2.8	<0.5	<0.7	<0.6
MR 6904-6905+6908	Viskan 2 km downstream April	1.7	<0.7	<0.3	<0.6	<2.8	<0.5	<0.7	<0.6
MR 7009	Viskan upstream August	2.5	1.1	<0.3	<0.6	<2.8	<0.5	<0.7	<0.6
MR 7010	Viskan 50 m downstream August	3.8	1.2	<0.3	<0.6	<2.8	<0.5	<0.7	<0.6
MR 7011	Viskan 2 km downstream August	<1.1	<0.7	<0.3	<0.6	<2.8	<0.5	<0.7	<0.6
MR 6875-6877	Gässlösa STP March	3.9	1.1	<0.5	<0.6	<3	<0.8	<0.8	<1
MR 6897-6899	Gässlösa STP April	3.6	1.4	<0.5	<0.6	<3	<0.8	<0.8	<1
MR 7545	Gässlösa STP October	<1.1	<1	<0.5	<0.6	3.7	<0.8	<0.8	<1
MR 7005-7008	Gässlösa STP August	4.4	1.4	<0.5	<0.6	<3	<0.8	<0.8	<1

MR 7075-7087	Nolhaga STP	<1.1	2.2	0.62	<0.6	<3	<0.8	<0.8	<1
MR 7062-7074	Ellinge STP	<1.1	1.6	<0.5	<0.6	<3	<0.8	<0.8	<1
MR 7030-7035	Öhn STP	<1.1	2.5	<0.5	<0.6	<3	<0.8	<0.8	<1
MR 7098-7110	Rya STP	1.3	2.2	<0.5	<0.6	<3	<0.8	<0.8	<1
MR 7168-7179	Henriksdal STP	1.4	3.1	1.4	5.9	<3	1.8	<0.8	1.3
MR 7224-7236	Bollebygd STP	<1.1	1.9	<0.5	1.7	<3	<0.8	<0.8	<1

Table A3. Concentrations of brominated flame retardants (ng/l). < indicate reporting limits.

Sample ID	Sampling site	BDE-47	BDE-100	BDE-99	BDE-85	BDE-153	BDE-154	BDE-209	HBCDD
MR 6900-6901+6906	Viskan upstream April	0.038	<0.030	0.0354	<0.030	<0.030	<0.030	<0.1	<0.1
MR 6902-6903+6907	Viskan 50 m downstream April	0.044	<0.030	0.049	<0.030	<0.030	<0.030	0.44	<0.1
MR 6904-6905+6908	Viskan 2 km downstream April	0.037	<0.030	0.046	<0.030	<0.030	<0.030	<0.1	<0.1
MR 7009	ViskanUpstream August	0.046	0.012	0.048	<0.10	<0.10	<0.10	0.28	<0.05
MR 7010	Viskan 50 m downstream August	0.037	0.010	0.035	<0.10	<0.10	<0.10	<0.05	<0.05
MR 7011	Viskan 2 km downstream August	0.070	0.014	0.066	<0.10	0.010	0.008	<0.05	<0.05
MR 6875-6877	Gässlösa STP March	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	<0.05	0.24
MR 6897-6899	Gässlösa STP April	n.a.	n.a	n.a.	n.a.	n.a.	n.a.	<0.05	0.25
MR 7545	Gässlösa STP October	<0.04	<0.04	<0.04	<0.06	<0.06	<0.06	<0.05	<0.05
MR 7005-7008	Gässlösa STP August	0.061	<0.02	0.027	<0.03	<0.03	<0.03	<0.05	0.13
MR 7075-7087	Nolhaga STP	0.078	<0.02	0.070	<0.03	<0.03	<0.03	<0.05	0.23
MR 7062-7074	Ellinge STP	0.035	0.028	0.053	<0.03	<0.03	<0.03	<0.05	0.27
MR 7030-7035	Öhns STP	0.081	0.030	0.082	<0.03	<0.03	<0.03	<0.05	0.09
MR 7098-7110	Rya STP	0.026	<0.02	<0.02	<0.03	<0.03	<0.03	<0.05	0.16
MR 7168-7179	Henriksdal STP	<0.02	<0.02	<0.02	<0.03	<0.03	<0.03	<0.05	0.23
MR 7224-7236	Bollebygd STP	0.079	0.021	0.039	<0.03	<0.03	<0.03	<0.05	0.05

Table A4. Concentrations of phenolic substances (ng/l). < indicate reporting limits.

Sample ID	Sampling site	4-Nonylphenol	4-t-Octylphenol	Triclosan	Bisphenol A
MR 6900-6901+6906	Viskan upstream April	25	2.3	<0.2	<5
MR 6902-6903+6907	Viskan 50 m downstream April	59	6.1	<0.2	50
MR 6904-6905+6908	Viskan 2 km downstream April	52	6.1	2.6	62
MR 7009	Viskan upstream August	41	4.6	<0.2	30
MR 7010	Viskan 50 m downstream August	40	4.7	0.3	55

MR 7011	Viskan 2 km downstream August	150	9.3	9.4	120
MR 6875-6877	Gässlösa STP March	120	<9	49	290
MR 6897-6899	Gässlösa STP April	99	14	50	1900
MR 7545	Gässlösa STP October	150	15	32	140
MR 7005-7008	Gässlösa STP August	<79	<9	39	270
MR 7075-7087	Nolhaga STP	240	<9	20	410
MR 7062-7074	Ellinge STP	<87	<9	16	<120
MR 7030-7035	Öhns STP	220	67	95	810
MR 7098-7110	Rya STP	220	16	82	1300
MR 7168-7179	Henriksdal STP	<85	<9	87	<120
MR 7224-7236	Bollebygd STP	270	<19	110	880

Table A5. Concentrations of herbicides. < indicate reporting limits.

Sample ID	Sampling site	MCPA (ng/l)	Glyfosate (µg/l)
MR 6900-6901+6906	Viskan upstream April	< 2	< 0.5
MR 6902-6903+6907	Viskan 50 m downstream April	n.a.	< 0.5
MR 6904-6905+6908	Viskan 2 km downstream April	n.a.	< 0.5
MR 7009	Viskan upstream August	< 2	< 0.5
MR 7010	Viskan 50 m downstream August	< 2	< 0.5
MR 7011	Viskan 2 km downstream August	< 2	< 0.5
MR 6875-6877	Gässlösa STP March	< 2	< 0.5
MR 6897-6899	Gässlösa STP April	< 2	< 0.5
MR 7545	Gässlösa STP October	< 2	< 0.5
MR 7005-7008	Gässlösa STP August	< 2	< 0.5
MR 7075-7087	Nolhaga STP	< 2	< 0.5
MR 7062-7074	Ellinge STP	< 2	< 0.5
MR 7030-7035	Öhns STP	< 2	< 0.5
MR 7098-7110	Rya STP	< 2	< 0.5
MR 7168-7179	Henriksdal STP	< 2	< 0.5
MR 7224-7236	Bollebygd STP	< 2	< 0.5

Table A6. Concentrations of metals. < indicate reporting limits.

Sample ID	**Sampling site**	**Hg tot** (ng/l)	**Cd** (µg/l)	**Pb** (µg/l)	**Ag** (µg/l)	**As** (µg/l)	**Cu** (µg/l)
MR 6900-6901+6906	Viskan upstream April	3.2	0.018	0.27	0.007	0.68	1.3
MR 6902-6903+6907	Viskan 50 m downstream April	3.5	0.029	0.56	0.009	0.59	4.7
MR 6904-6905+6908	Viskan 2 km downstream April	3.0	0.014	0.34	0.006	0.58	5.7
MR 7009	ViskanUpstream August	3.3	0.031	0.62	0.014	0.86	3.4
MR 7010	Viskan 50 m downstream August	3.6	0.027	0.95	0.047	0.91	3.0
MR 7011	Viskan 2 km downstream August	5.0	0.029	0.84	0.063	0.81	4.4
MR 6875-6877	Gässlösa STP March	2.1	0.009	0.083	0.015	0.82	3.8
MR 6897-6899	Gässlösa STP April	1.5	0.013	0.15	0.022	0.77	3.1
MR 7545	Gässlösa STP October	3.2	<0.005	0.036	<0.005	0.04	2.6
MR 7005-7008	Gässlösa STP August	1.5	0.008	0.07	0.008	0.63	4.9
MR 7075-7087	Nolhaga STP	1.5	0.022	0.24	0.012	0.99	4.4
MR 7062-7074	Ellinge STP	0.39	0.019	0.58	0.034	0.89	6.4
MR 7030-7035	Öhns STP	3.6	0.021	0.048	0.01	0.58	2.1
MR 7098-7110	Rya STP	2.2	0.008	0.15	0.023	0.99	4.5
MR 7168-7179	Henriksdal STP	0.64	0.012	0.038	0.006	0.86	1.9
MR 7224-7236	Bollebygd STP	4.9	0.003	0.051	0.006	0.76	0.70

Table A7. Concentrations of NSAIDs (ng/l). < indicate reporting limits.

Sample ID	**Sampling site**	**Ibuprofen**	**Naproxen**	**Ketoprofen**	**Diclofenac**
MR 7005-7008	Gässlösa STP August	500	1100	220	79
MR 7075-7087	Nolhaga STP	280	370	380	71
MR 7062-7074	Ellinge STP	8.0	81	68	140
MR 7030-7035	Öhns STP	120	220	400	81
MR 7098-7110	Rya STP	350	790	570	100
MR 7168-7179	Henriksdal STP	110	200	370	270
MR 7224-7236	Bollebygd STP	5000	3000	1400	620

Table A8. Concentrations of perfluorinated substances (PFAS) (ng/l). < indicate reporting limits.

Sample ID	**Sampling site**	**PFOS**	**PFOA**	**PFOSA**	**PFHxA**	**PFDcA**
MR 7005-7008	Gässlösa STP August	13	41	0.038	21	11
MR 7075-7087	Nolhaga STP	4.8	12	0.18	18	7.5
MR 7062-7074	Ellinge STP	8.9	9.5	0.15	8.4	12
MR 7030-7035	Öhns STP	5.9	9.1	0.066	14	6.6

MR 7098-7110	Rya STP	8.4	10	< 0.02	15	6.0
MR 7168-7179	Henriksdal STP	19	10	0.089	11	8.1
MR 7224-7236	Bollebygd STP	3.1	3.6	< 0.02	17	13

Table A9. Concentrations of phthalates (µg/l). < indicate reporting limits.

Sample ID	Sampling site	DEP	DIBP	DBP	BBzP	DEHP	DOP	DINP	DIDP
MR 7005-7008	Gässlösa STP August	0.060	0.10	0.22	0.050	0.49	< 0.01	< 1	< 1
MR 7075-7087	Nolhaga STP	0.14	0.15	0.28	0.020	0.36	< 0.01	< 1	< 1
MR 7062-7074	Ellinge STP	0.047	0.046	0.084	<0.01	0.19	< 0.01	< 1	< 1
MR 7030-7035	Öhns STP	0.030	0.061	0.13	<0.01	0.48	< 0.01	< 1	< 1
MR 7098-7110	Rya STP	0.20	0.21	0.13	0.015	0.71	< 0.01	< 1	< 1
MR 7168-7179	Henriksdal STP	0.052	0.046	0.081	<0.01	<0.1	< 0.01	< 1	< 1
MR 7224-7236	Bollebygd STP	1.47	0.21	0.11	0.025	0.26	< 0.01	< 1	< 1

Table A10. Concentrations of organophosphorus esters (µg/l). < indicate reporting limits.

Sample ID	Sampling site	TIBP	TBP	TCEP	TDCP	TBEP	TPhP	EHDPP
MR 7005-7008	Gässlösa STP August	2.8	0.052	1.8	0.39	3.0	0.072	0.069
MR 7075-7087	Nolhaga STP	0.052	0.11	0.77	0.82	3.1	0.041	0.017
MR 7062-7074	Ellinge STP	0.061	0.038	0.32	0.54	0.24	0.020	0.015
MR 7030-7035	Öhns STP	0.044	0.074	0.43	0.42	16	0.12	0.031
MR 7098-7110	Rya STP	0.33	0.39	0.24	0.28	3.4	0.074	0.045
MR 7168-7179	Henriksdal STP	0.057	0.019	0.20	< 0.008	1.7	0.015	0.0092
MR 7224-7236	Bollebygd STP	0.029	0.088	0.19	0.82	8.2	0.11	0.013

Table A11. Concentrations of volatile organic compounds (ng/l). < indicate reporting limits.

Sample ID	Sampling site	3-metyl-pentane	n-hexane	Bensene	Toluene	n-octan	Etyl-bensene	m+p-xylene	Styrene	o-xylene	n-nonan	1,3,5-TMB
MR 7005-7008	Gässlösa STP August	<10	<1.0	4.1	52	<1.0	<1.0	22	3.1	2.7	<1.0	<5.0
MR 7075-7087	Nolhaga STP	<10	95	3.8	200	<1.0	<1.0	15	<2.0	<2.0	<1.0	<5.0
MR 7062-7074	Ellinge STP	<10	72	1.1	8.4	<1.0	1.3	7.7	<2.0	2.5	<1.0	<5.0
MR 7030-7035	Öhns STP	<10	59	5.5	46	<1.0	2.1	15	<2.0	3.8	<1.0	<5.0
MR 7098-7110	Rya STP	<10	22	2.6	18	<1.0	9.7	35	<2.0	14	1.4	<5.0
MR 7168-7179	Henriksdal STP	<10	17	3.7	71	<1.0	18	24	14	7.4	<1.0	<5.0
MR 7224-7236	Bollebygd STP	<10	13	<1.0	120	<1.0	4.9	19	8.4	9.7	<1.0	<5.0

Table A12. Concentrations of volatile halogenated substances (ng/l). < indicate reporting limits.

Sample ID	Sampling site	1,1,1-trichloro-ethane	1,2-dichloro-ethane	Dichloro-methane	Chloroform	Carbon tetrachloride	Tetrachloro-ethene	Trichloro-ethene
MR 7005-7008	Gässlösa STP August	<0.4	84	<6.0	140	1.7	7.6	1.1
MR 7075-7087	Nolhaga STP	<0.4	180	<6.0	14	1.7	4.4	<0.3
MR 7062-7074	Ellinge STP	<0.4	34	<6.0	59	1.7	2.2	0.82
MR 7030-7035	Öhns STP	0.42	270	<6.0	58	1.6	12	4.4
MR 7098-7110	Rya STP	<0.4	180	<6.0	140	1.6	26	2.2
MR 7168-7179	Henriksdal STP	0.50	65	<6.0	29	2.0	*>300*	5.9
MR 7224-7236	Bollebygd STP	0.46	35	<6.0	17	2.4	1.1	<0.3

Table A13. Concentrations of chlorobenzenes (ng/l). < indicate reporting limits.

Sample ID	Sampling site	1,3,5-Trichloro-benzene	1,2,4-Trichloro-benzene	1,2,3-Trichloro-benzene	Hexachloro-butadiene	1,2,3,4-Tetrachloro-benzene	1,2,3,5 + 1,2,4,5-Tetrachloro-benzene	Pentachloro-benzene	Hexachloro-benzene	Octachloro-styrene
MR 7005-7008	Gässlösa STP August	<0.2	<2	<0.1	<0.2	<0.2	<0.2	<0.2	<0.1	<0.1
MR 7075-7087	Nolhaga STP	<0.2	<2	<0.1	<0.2	<0.2	<0.2	<0.2	<0.1	<0.1
MR 7062-7074	Ellinge STP	<0.2	<2	<0.1	<0.2	<0.2	<0.2	<0.2	<0.1	<0.1
MR 7030-7035	Öhns STP	<0.2	<2	<0.1	<0.2	<0.2	<0.2	<0.2	<0.1	<0.1
MR 7098-7110	Rya STP	<0.2	<2	<0.1	<0.2	<0.2	<0.2	<0.2	<0.1	<0.1
MR 7168-7179	Henriksdal STP	<0.2	<2	<0.1	<0.2	<0.2	<0.2	<0.2	<0.1	<0.1
MR 7224-7236	Bollebygd STP	<0.2	<2	<0.1	<0.2	<0.2	<0.2	<0.2	<0.1	<0.1

Table A14. Concentrations of siloxanes (µg/l). < indicate reporting limits.

Sample ID	Sampling site	D4	D5	D6	MM	MDM	MD2M	MD3M
MR 7005-7008	Gässlösa STP August	<0.09	<0.04	<0.03	<0.001	<0.0002	<0.0003	<0.0004
MR 7075-7087	Nolhaga STP	<0.09	<0.04	<0.03	<0.001	<0.0002	<0.0003	0.0012
MR 7062-7074	Ellinge STP	<0.09	<0.04	0.040	<0.001	<0.0002	<0.0003	0.00059
MR 7030-7035	Öhns STP	<0.09	<0.04	<0.03	<0.001	<0.0002	<0.0003	<0.0004
MR 7098-7110	Rya STP	<0.09	<0.04	<0.03	<0.001	<0.0002	<0.0003	<0.0004
MR 7168-7179	Henriksdal STP	<0.09	<0.04	0.065	0.0021	<0.0002	0.00059	0.0014
MR 7224-7236	Bollebygd STP	<0.09	<0.04	<0.03	<0.001	0.0003	0.00076	0.0017

Table A15. Additional substances (ng/l). < indicated reporting limits.

Sample ID	Sampling site	Acetyl salicylic acid	Salicylic acid	Ibuprofen-OH	Ibuprofen-COOH	d-t-BPA
MR 7005-7008	Gässlösa STP August	< 50	182	1236	<5	10
MR 7075-7087	Nolhaga STP	< 50	160	1428	176	13
MR 7062-7074	Ellinge STP	< 50	24	118	<5	123
MR 7030-7035	Öhns STP	< 50	176	808	10	9
MR 7098-7110	Rya STP	< 50	110	1887	7	43
MR 7168-7179	Henriksdal STP	< 50	85	161	106	73
MR 7224-7236	Bollebygd STP	< 50	128	6877	1197	293

3-(3,3-di-*t*-butyl-4-hydroxyphenol)-propionic acid (d-t-BPA); Carboxy-ibuprofen- metabolite (Ibuprofen-COOH); Hydroxy-buprofen metabolite (Ibuprofen-OH);

Table A16. Estrogenic activity (YES) expressed as estradiol units/l and androgenic activity (YAS) expressed as DHT units/l.

Sample ID	Sampling site	YES	± SD	YAS
MR 7005-7008	Gässlösa STP August	2.1	0.06	< 1
MR 7075-7087	Nolhaga STP	0.26	0.08	< 1
MR 7062-7074	Ellinge STP	0.21	0.06	< 1
MR 7030-7035	Öhns STP	1.0	0.03	< 1
MR 7098-7110	Rya STP	4.2	0.1	< 1
MR 7168-7179	Henriksdal STP	0.39	0.02	< 1
MR 7224-7236	Bollebygd STP	n.a.		n.a.

Appendix B. Literature survey on potential additional bioassays

Choice of bioassays

There are a wide range of aspects to consider when choosing which bioassays to use. These include e.g the relevance of the effect measured, the sensitivity and reproducibility of the assay, factors affecting the throughput of the assay such as scale and time needed, equipment and training/skill requirements, but also cost effectiveness.

Swedish municipal sewage effluents contain mixtures of thousands of chemicals, but of low concentrations that are further diluted in the receiving recipients. Thus acute toxic effects are not expected, but the effluents may have the potential to cause long term chronic effects. Some highly sensitive assays on general toxicity, e.g. the *Vibrio fisheri* test, may detect effects in undiluted or concentrated effluents, but the predicitive value regarding chronic toxicity from such tests has been questioned (discussed in Brack, 2003). Thus for the monitoring of municipal sewage effluents, assays that measure effects of relevance for chronic toxicity should be preferred.

There are a large number of chronic effects that may be of relevance for the monitoring. These include e.g.

- Genetic toxicity
- Carcinogenicity
- Endocrine disrupting effects
- Teratogenic effects
- Neurotoxicity
- Immunotoxicity
- Alterations of detoxification systems

Results from in vivo tests better reflect potential effects in the environment, but, considering all the other aspects mentioned above and also ethics, for an initial monitoring, in vitro assays should be considered.

In the following sections in vitro bioassays detecting genetic toxicity and endocrine disrupting effects are presented. This should not be interpreted as that these effects are comparatively of a higher relevance, but for these effects well established in vitro bioassays often employed in the context of monitoring exist. Further, all of the other effects listed may occur as a result of endocrine disrupting effects.

Hormonal effects

Observations on physiological alterations linked to hormonal systems has been made for several decades, and 15 years ago, the concept of endocrine disruption as a general mechanism of toxicity was introduced (reviewed by Sumpter, 2005 and Hotchkiss, 2008). The first studied effects were feminization and masculinization of aquatic wildlife caused by disruption of the estrogenic and

androgenic hormonal systems. Since then, the field has expanded to also recognize effects on other hormonal systems such as the thyroid system, the glucocorticoid system, and the retinoid system. Also the knowledge on targets affected by EDCs has increased beyond the first recognized reproductive effects. There is increasing evidence for effects on the cardiovascular system, the digestive system, on adipose tissue, effects on the immune system, and also on the central nervous system (Hotchkiss *et al.*, 2008).

The complexity of environmental exposures complicates the identification of clear linkages between observed effects and causative chemicals. Despite this the number of cases where effects of endocrine distrupting chemicals (EDCs) on wildlife has been shown with a strong causal support steadily increases due to intensive research efforts. Among these are e.g. imposex in marine snails caused by organic tin compounds, egg shell thinning due to DDE exposure, feminization and masculinization of fish caused by sewage, pulp and paper mill effluents, reproductive failure in minks, otters and seals, immune dysfunction in seals, and demasculinization of polar bears caused by PCBs, reduced hatchability of fertilized fish eggs due to dioxin-like compounds, etc. (Hotchkiss *et al.*, 2008).

The assays on endocrine disruption covered are mainly based on the binding and activation of cellular receptors. Endocrine disruption may also be caused by competitive binding or alteration of the structure of a receptor preventing its activation (antagonistic effects). Antagonistic effects are not as structurally dependent as agonistic effects, and may thus be caused by a wider range of compounds. Anti-androgenic effects have been well studied, but this mechanism is also of relevance for the other receptors covered. Thus antagonistic effects for these assays should also be considered.

(Anti)-estrogenic and (anti)-androgenic effects

(Anti)-estrogenic and (anti)-androgenic effects caused by industrial and municipal waste water effluents have been well studied and the underlying mechanisms for the effects will not be presented here.

Exposure to sewage effluent has been shown to cause feminization of fish, including induction of vitellogenin, the occurence of intersex gonads, and reproductive failure (reviewed by Sumpter, 2005 and Hotchkiss *et al.*, 2008). Bioassays to detect effects on the estrogen hormone system include assays on vitellogenin induction and assays on binding and activation of the estrogen receptor.

The first indications of androgenic effects came from observations of masculinized female fish in recipients receiving pulp mill effluent (reviewed by Sumpter, 2005 and Hotchkiss *et al.*, 2008). Masculinization of fish has also been shown in recipients in areas where the steroidal growth promoter trenbolone are used in the cattle industry. Androgens have also been shown to be present in municipal sewage effluents. Sewage effluents often also contain chemicals causing anti-androgenic effects. To detect (anti)-androgenic effects, assays that measure binding to and activation/inhibition of the androgen receptor can be employed.

Yeast based assays
Estrogenic effects of Swedish municipal effluents has been found in the range <0.1-15 ng/l estradiol equvivalents using the yeast based assay YES (Svensson *et al.*, 2000). Using the same assay, estrogenic effects in recipient waters at concentrations with potential to affect local fish populations have also been measured (Svensson and Allard, 2002).

Cell line based assays
There are also cell line based assays detecting binding to and activation of the androgen- and estrogen-receptors. These include e.g. the ER-CALUX and AR-CALUX commercially available from Biodetection systems.

Thyroidal effects

The thyroidal hormone system is involved in a wide range of physiological processes especially related to development and reproduction in mammals, fish, amphibians and birds, and disruption of the thyroid axis may thus impair normal development, growth and reproduction (reviewed in Boas *et al.*, 2006; Zoeller *et al.*, 2007; Blanton & Speckler, 2007; McNabb, 2007; Fort *et al.*, 2007).

In mammals, it has also been shown that impairment of the system may affect the developing nervous system, resulting in permanent effects on brain structure and functioning (Boas et al., 2006; Mastorakos *et al.*, 2007; Darras, 2008). In vivo experiments have shown effects on the thyroid hormone system by a wide range of chemicals such as PCBs, dioxins, flame retardants, phenolic compounds, phthalates, pesticides, and metals (reviewed in e.g. Boas *et al.*, 2006; Darras, 2008; Mastorakos *et al.*, 2007).

Thyroid hormone homeostasis is regulated through the hypothalamic-pituitary-thyroid (HPT) axis, illustrated in Figure B1. The physiology of the HPT-axis is reviewed in Mastorakos *et al.* (2007) and the above mentioned references, and is briefly described here. Thyrothropin-releasing hormone (TRH) from the hypothalamus stimulates the pituitary gland to secrete thyroid-stimulating hormone (TSH). TSH regulates thyroid gland activity. The thyroid gland secretes the thyroid hormones (THs), thyroxine (T_4) and triiodothyronine (T_3) to the blood. In the blood T_4 and T_3 are transported bound to different transport proteins (thyronine-binding protein, transthyretin and albumin). The active hormone T_3 is formed by enzymatic deiodination of the prehormone T_4 in different organs such as the liver, kidney, thyroid and pituitary gland. A small fraction of the THs in blood are unbound and responsible for the hormonal activity. T_3 has affinity for the thyroid receptor, whereas the prehormone T_4 is involved in negative feedback regulation of the HPT-axis.

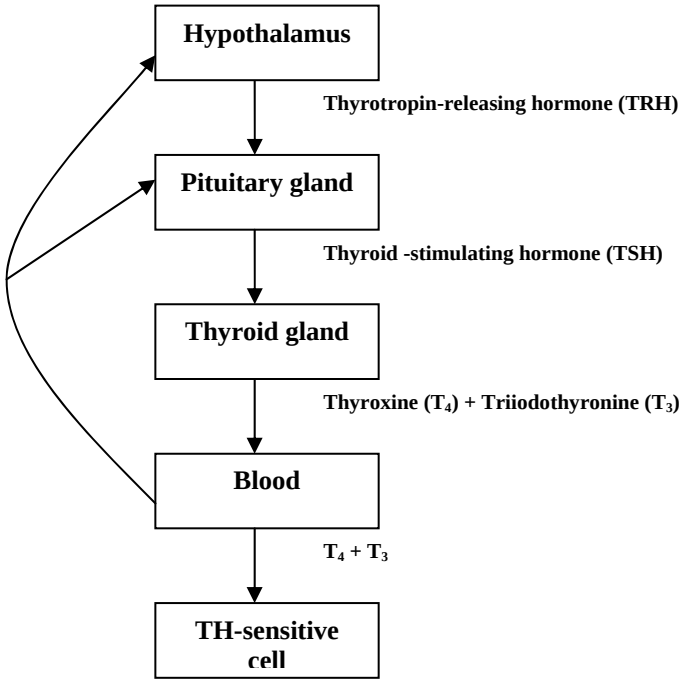

Figure B1. A simplified illustration of the hypothalamic-pituitary-thyroid axis (based on Boas *et al.,* 2006).

Chemicals may affect thyroidal homeostasis at several sites along the HPT-axis (Boas *et al.*, 2006). They may i.e. affect the synthesis of THs in the thyroid gland, the binding to transport proteins in the blood, modify the metabolism of THs, affect cellular uptake and interfere at the thyroid receptor level.

The in vitro bioassays available to study effects on the thyroidal system include assays that measure binding to and/or activation or inhibition of the thyroid receptor and assays measuring the binding to the transport protein transthyretin.

Thyroid receptor (TR-) assays

Thyroid receptor assays may be based on the competitive binding to purified receptors from recombinant bacteria or the binding and activation of the receptor in yeast or mammalian cell lines. Using a method with purified thyroid receptor, Murata and Yamauchi (2008) found competitive binding of extracts from domestic sewage effluent.

A yeast based assay on thyroid receptor activation and inhibition has been used by e.g. Li *et al.* (2008) who found agonistic effects of two phenolic compounds and antagonistic effects of several polyhalogenated aromatic hydrocarbons, and by Inoue *et al.* (2009) who found agonistic effects of surface waters. Effects in cell line based assays has e.g. been detected for halogenated bisphenol A derivatives (Kitamura *et al.*, 2005), halogenated phenolic and phenol compunds (Jugan *et al.*, 2007), sewage effluent water (Murata and Yamauchi, 2008; Ishihara *et al.*, 2009), and surface water and waste waters from paper manufacturing plants (Ishihara *et al.*, 2009).

A cell line based assay, the TR-CALUX, has also been developed by Biodetection Systems, but is yet not commercially available.

Transthyretin-binding (TTR) assay

Effects in TTR-assays have been shown for e.g. the herbicide ioxinyl and several polybrominated diphenyl ethers of which some showed higher affinity than the natural ligand (Morgado *et al.*, 2007), chlorinated derivatives of bisphenol A and nonylphenol (Yamauchi *et al.*, 2003), 2,4,6-tribromophenol and 2,3,4,5,6-pentachlorophenol which were found to contribute to 40-70% effect in indoor dust (Suzuki *et al.*, 2008). Further, Yamauchi *et al.* (2003) also found effects of effluent water from, and of recipient waters downstream of, a paper manufacturing plant. In addition to effects on the thyroid receptor, Murata and Yamauchi (2008) also investigated competitive binding in a TTR-assay. Domestic sewage effluent had an inhibitory effect also in this assay. Further, fractioning of the eluates with RP-HPLC showed that different fractions of the eluates were responsible for the effects seen in the TR- and TTR-assays and that the effects seen in the TR-assay were likely to be caused by unknown chemicals with thyroid hormone activity.

The aryl hydrocarbon receptor (AhR)

The aryl hydrocarbon receptor (AhR) is a cytosolic ligand-activated transcription factor. It is known to mediate most of the "dioxinlike" toxicity and carcinogenicity of a wide range of environmental pollutants in vertebrates. When a ligand such as TCDD enters the cell it binds to AhR causing translocation to the cell nucleus, see Figure B2 (Okey, 2007). Within the nucleus AhR dimerizes with the aryl hydrocarbon nuclear translocator (ARNT), and the complex binds to regulatory gene sequences. This causes both induction and down regulation of target genes. Among the gene products regulated are several phase I and II enzymes causing both activation and detoxication of xenobiotics. Other genes regulated are involved in growth, differentiation and metabolism, and dysregulation of these genes are thought to cause "classic" dioxin toxicity, including effects such as wasting, hepatotoxicity, immunotoxicity, carcinogenicity, and reproductive and developmental toxicity. Binding to AhR may also cause activation of the estrogen receptor (ER) (Ohtake *et al.*, 2003). Thus substances without affinity for the ER may cause estrogenic effects indirect via the AhR

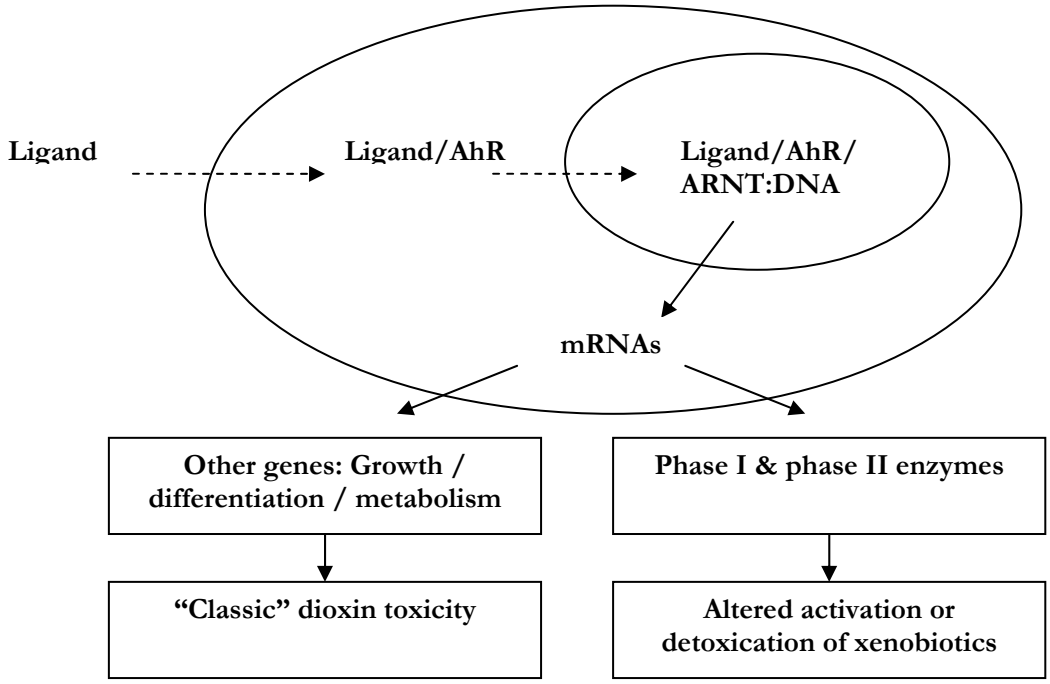

Figure B2. Regulation of gene expression by AhR (based on Okey, 2007). A ligand such as TCDD enters the cell and binds to AhR in the cytosol, causing translocation into the nucleus. Whithin the nucleus, the receptor dimerizes with ARNT and binds to DNA-sequences regulating synthesis of mRNAs. This causes induction of phase I and II enzymes, but also dysregulation of genes involved in growth, differentiation and metabolism, which is percieved to cause "classic" dioxin toxicity.

Cell line based assays

A widely used assay to detect AhR ligands is the DR-CALUX assay. This assay is based on a rat hepatoma cell line with a luciferase reporter gene. It has e.g. been used to detect effects of single substances and additive effects of mixtures of phenolic compounds and plasticizers (Krüger *et al.*, 2008). It has also been used in several studies to detect effects of environmental samples.

Keiter *et al.* (2008) used the assay to characterize sediments from the Danube River. The sediment samples were chemically characterized regarding PAHs, PCBs, PCDDs and PCDFs, and the assay was applied to crude extracts and extracts treated to remove non-persistent compounds. By comparison of the results from the chemical analyses and the bioassays, it could be concluded that the persistent organic pollutants only contributed to a minor part of the response. A somewhat higher proportion of the response could be explained by the analysed PAHs, but in most samples most of the activity was due to unknown nonpriority substances.

Effects of sediments were also analysed by Brack *et al.* (2008a). Extracts from river Elbe sediments were fractionated, to be able to determine activities of fractions corresponding to PCBs, to PCDDs/PCDFs, and to PCNs. Extracts containing PCDDs/PCDFs elicited the highest response, but PCNs were also shown to contribute to the total AhR-activity.

Among other environmental applications, the assay has also been used to detect AhR-activity of substances bound to suspended particulate matter during flood events (Wöltz *et al.*, 2008), and to evaluate the effectiveness in reducing toxicity of constructed wetlands receiving sludge (Gustavsson *et al.*, 2007).

The DR-CALUX assay is available from Biodetections Systems (www.biodetectionsystems.com).

Yeast based assays

A yeast based assay to detect AhR ligands has been developed by Miller III (1999). In this assay receptor affinity causes transcription of the reporter β-galactosidase and induction can be measured spectrophotometrically. This assay has been used by several authors to detect effects of both individual compounds and of complex mixtures (see e.g. Noguerol *et al.*, 2006; Boronat *et al.*, 2007; Sugihara *et al.*, 2008; Alnafasi *et al.*, 2007; Chou *et al.*, 2007; Leskinen *et al.*, 2008).

Alnafasi *et al.* (2007) determined AhR activity of 16 individual PAHs, different PAH mixtures, and extracts from soil and sediment samples. All but two tested PAHs activated the receptor, and several were considered strong activators. Mixtures resulted in both additive and synergistic effects and significant correlations between receptor activation and PAH content were found for the soil and sediment samples.

Chou *et al.* (2007) used the assay to identify AhR ligands in wastewater effluents containing dyes. They found induction already at an extract dilution corresponding to a 250-fold dilution of the wastewater. By an effect directed analysis approach, they identified HPLC fractions causing strong induction and found two dyes, Disperse Yellow 64 and 3'-hydroxybenzo[b]quinophthalone, which attributed to approximately 25% of the response.

Leskinen *et al.* (2008) modified the assay by adding the expression of a receptor cofactor resulting in a 50% increase in maximum induction, and changed the reporter to luciferase allowing the quantification from living cells. They applied the assay on previously chemically characterized sediments, and found the assay to be consistent with contamination levels and also in a comparison with results from the H4IIE-luc/DR-CALUX assay. Further, using sulfuric acid to degrade the less persistent compounds in the extracts, they found these to significantly contribute to the AhR-response.

The strain developed by Miller III (1999) is available form the American Type Culture Collection.

Genotoxic effects

Several studies have shown different kinds of DNA damage such as DNA-adducts and DNA-strand breaks in aquatic organisms (see e.g. Ohe *et. al.*, 2004; Hansson *et al.*, 2006). In the review by Ohe *et al.* (2004) covering the literature published since 1990 on mutagenicity of surface waters, 7% and 15% of the water samples analyzed were considered mutagenic in the Ames test using the *Salmonella* TA98 and TA100 test stains respectively. Further, 3-5% of the samples were classified as highly mutagenic or extremely mutagenic. Several studies have also shown genotoxicity of effluent waters from wastewater treatment plants (e.g. Isidori *et al.*, 2007; Dizer *et al.*, 2002; Jolibois *et al.*, 2003). Thus waste water is a potential source of genotoxic compounds to receiving water bodies. This may pose a risk to aquatic organisms, but also to humans through accumulation in the food chain or if the water is used for drinking water production.

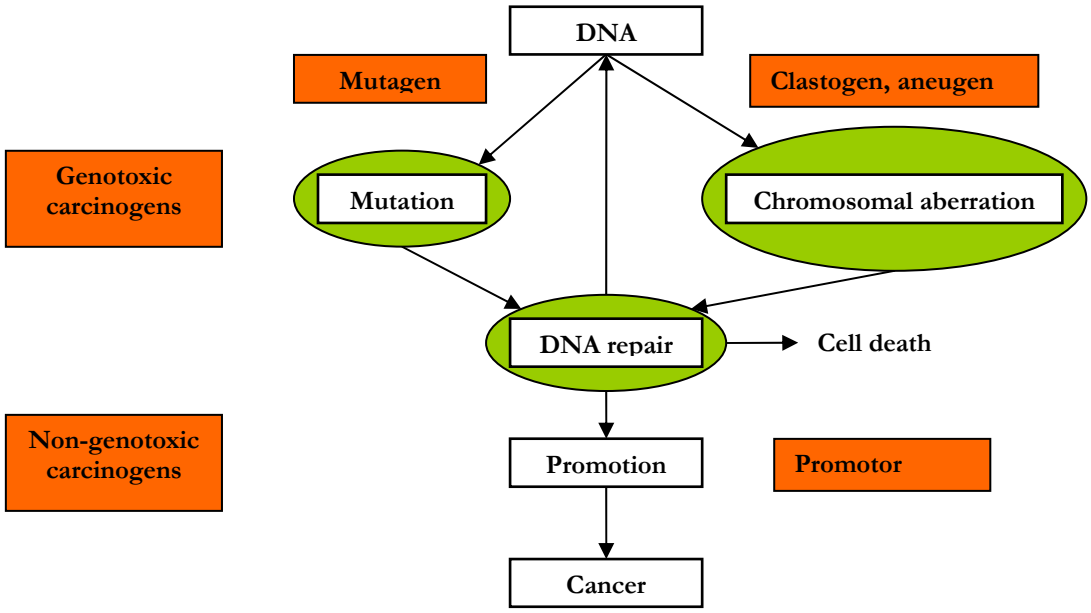

Figure B3. A simplified schematic illustration of how contaminants may cause cancer.

Today, there exist several bioassays with the ability to detect genotoxic carcinogens. There are bioassays that detect mutations, chromosomal aberrations, and activation of cellular DNA repair systems (green in Figure B3). With regards to non-genotoxic carcinogens, there are so far no bioassays available suitable for environmental monitoring.

Bioassays that detect mutations include the bacterial Ames- and Ames II-tests, and different tests employing mammalian cell lines. Assays to detect chromosomal aberrations include tests on sister chromatid exchange, chromosomal aberrations, and micro nucleus and comet assays. Assays on DNA-repair make use of the activation of the cellular DNA-repair system and include the Umu- and GreenScreen-assays. For monitoring purposes, mutation tests based on mammalian cell lines have several drawbacks compared to procaryotic and yeast assays, causing lower throughput and higher costs. This is also true for assays detecting chromosomal aberrations. These tests are thus less suitable in a first screening of large numbers of samples, but should be under consideration if a more thoroughly characterization of the genotoxic potential would become necessary. The following of this section presents some of the procaryotic and yeast assays available detecting mutations and DNA-repair.

Mutation tests (Ames)

The most commonly used assay to detect mutagens in water is the Ames test (Ohe *et al.*, 2004). The Ames test is based on different strains of *Salmonella typhimurium* modified to detect base-pair substitutions and frame shifts resulting in the ability to grow in histidine free medium. In the traditional Ames test each strain is exposed to the test compound or sample at different concentrations, grown on agar plates with medium lacking histidine, and revertants able to form colonies counted. The traditional Ames test has several drawbacks limiting its use in environmental monitoring. Each strain has to be plated on a separate plate at several concentrations. Thus to detect all possible base-pair substitutions and frame shifts, relatively large amounts of both test

sample/compound and assay material is needed. This also makes the test relatively time consuming. Further, handling of large amounts of mutagenic chemicals used as positive controls may pose a health problem.

To improve the throughput of the assay, the Ames II test has been developed (www.aniara.com). The assay has been modified to grow the bacteria in liquid microtiter format, instead of using agar plates. Further, a mixture of bacterial strains detecting all base-pair substitutions is used instead of testing each strain separately. In addition, the Ames II test can also be used to detect cytotoxicity. The performance of the test has been validated in a collaboration study, showing high concordance with results from the standard Ames test and low inter-laboratory variability (Flückiger-Isler *et al.*, 2004). It is now used for screening of genotoxicity in drinking water in the Netherlands (Heringa *et al.*, 2007).

Procaryotic DNA-repair assays

Mutation assays like the Amest test measure damage to a particular genetic locus. In comparison, assays based on cellular DNA-repair monitor damage occurring in the entire genome. Theoretically, DNA-repair assays should therefore have a higher sensitivity. There are several procaryotic DNA-repair assays available.

The Umu genotoxicity assay using a modified *Salmonella typhimurium* strain is based on the SOS-repair system. Upon DNA damage the SOS-repair system is activated, causing production of β-galactosidase and the enzyme activity can be measured spectrophotometrically. The Umu assay can be performed in microtiter format and run according to an ISO-standard. The Umu-assay has been employed to detect genotoxicity in effluent and surface waters in several studies (see e. g. Ohe *et al.*, 2004; Escher *et al.*, 2008; Muller *et al.*, 2007).

Among other procaryotic DNA-repair assays available are the Lux-Fluoro and the Vitotox assays.

Yeast based DNA-repair assays

In order to be able to detect genotoxic compounds that are positive in eukaryotic cells only, different yeast based assays have been developed. One of these tests is the GreenScreen assay (Gentronix Ltd.). In this assay expression of green fluorescent protein has been coupled to the the *RAD54* promoter. This promoter is induced in response to DNA damage, but compared to the SOS-response in *Salmonella*, do not respond to metabolic starvation, heat or osmotic chock, or non-genotoxic oxidative or reductive stresses (Knight *et al.*, 2007). The assay shows high specificity (low numbers of false results), and detects a different but overlapping range of genotoxicants compared to procaryotic tests (Cahill *et al.*, 2004; Knight *et al.*, 2007). It may thus be be used to be able to detect genotoxic compounds not positive in procaryotic assays. It is available in formats suitable for environmental monitoring in the field, but also in a microtiterformat for high throughput in the lab. In environmental monitoring the assay has been used e.g. by Daniel *et al.* (2004) for the evaluation of genotoxicity of industrial effluent waters.

Cytotoxicity / general toxicity

Since effluent waters from waste water treatment plants are expected to contain complex mixtures of pollutants but of low concentrations, the major concern is possible long term effects and thus this report focuses on bioassays targeting mechanisms that may cause chronic effects. It is not unlikely that undiluted or concentrated effluent water also would cause effects in assays on more

acute toxicity, such as the *Vibrio fischeri* bioluminescence inhibition test, but for evaluation of the possible chronic effects the predictive value from those tests are limited. No specific tests targeting general acute toxicity are thus covered in this report, but several of the assays covered also generate information on general toxicity. Cytotoxicity, measured as growth inhibition, is evaluated in the yeast based assays on receptor binding and also in some of the procaryotic genotoxicity assays.

Bioassays

Initial monitoring

Since individual assays sometimes yield false negative or positive results, it is often suggested to use a battery of assays targeting the same effect in order to increase the accuracy (see e.g. Nelson *et al.*, 2007). This could be done if the focus of the monitoring is a specific effect, but for a more general monitoring there has to be a trade off between number of effects targeted and the risk of false responses. The different assays covered in this literature survey are summarized in Table B1 below.

Table B1. Summary of identified assays.

Bioassay	Procaryotic/Yeast/Cell line	Microtiter-format	Special requirements	Standardisation	Commercially available?
Hormonal effects					
Estrogenic:					
YES	Y	Yes		Well established protocols	No
ER-Calux	C	Yes	Cell culture facilities	Yes	Yes
Androgenic:					
YAS	Y	Yes		Well established protocols	No
AR-Calux	C	Yes	Cell culture facilities	Yes	Yes
Thyroidal:					
TTR	Purified receptor	?		No?	No
TR Yeast	Y	Yes		No	No
TR-CALUX	C	Yes	Cell culture facilities	Yes	Under development
"Dioxin like":					
AhR Yeast	Y	Yes		No?	Yeast strain available from ATCC
DR-CALUX	C	Yes	Cell culture facilities	Yes	Yes
Genotoxic effects					
Ames II	P	Yes		Yes, (ISO-standard for the traditional Ames exists)	Yes
Umu	P	Yes		ISO/DIS 13829, 2000	Not as an assay but the bacterial strains are available
GreenScreen	Y	Yes		Yes	Yes

Proposed battery of assays:

- Estrogenic and androgenic effects also including antagonistic effects (YES and YAS, or ER- and AR-CALUX)
- Aryl hydrocarbon receptor for "dioxin like" effects (yeast based or DR-CALUX)
- A procaryotic genotoxicity test (e.g. Ames II or a DNA-repair assay)

Further testing/verifying observed effects

Results from in vitro tests may be indicative, but for a proper risk assessment, effects found needs to be further evaluated. In vitro assays show that an effect might, but not will, occur. They do not, or only partly, reflect uptake, distribution and metabolism (resulting in both detoxification and activation) relevant for potential in vivo effects. Further they do not integrate different toxic mechanisms that may alter the toxic response. Thus if the initial monitoring reveals that decided threshold values are reached, further testing should be considered.

Effects on the hormonal systems covered by the proposed assays may affect both reproduction and cause teratogenic effects. If threshold values are reached in (one or several of) these tests, potential effects could be further evaluated by fish reproduction and early life stage development tests.

The receptor based assays proposed are all vertebrate specific. Thus the assays do not predict specific effects on evertebrates, but if they show effects, it is not unlikely that also other groups of organisms could be affected. If effects above decided threshold values are found, an evaluation of chronic toxicity in an evertebrate test, e.g a reproduction test with *Nitocra spinipes* could be considered.

If genotoxic effects are found additional in vitro tests, preferably also targeting chromosomal aberrations, could be employed.

Indentification of causative compounds/Effect Directed Analysis

Effect directed analysis (EDA) is a method to non-selectively identify effect-causing compounds in the environment. The concept of this method is that the complex mixture of compounds in any matrix, e.g. tissue, water or sediment, is extracted and tested for biological effects. The effect-causing compounds are identified with sequential fractionation and chemical analysis. (See e.g. Brack 2003; Brack *et al.* 2008b; Hewitt and Marwin 2005; Scheurell *et al.* 2007 for a more detailed review of EDA).

The complex extract is tested for effects using one or several bioassays depending on the purpose of the study; the content of the original mixture that will be exposed to the bio-testing is determined by the choice of extraction method (Brack 2003). If the purpose of the EDA is to identify what compound is causing an observed effect a test covering the mechanism of action causing such an effect should be applied, e.g. vitellogenin induction in fish hepatocytes or YES to search for the cause of feminization in male fish (Brack 2003; Grung *et al.* 2006). If, on the other hand, the purpose of the study is to identify any toxic compound of the mixture an often applied method is the *Vibrio fischeri* acute bioluminescence inhibition test (Brack 2003; Scheurell *et al.* 2007).

The choice of bioassay naturally determines what effects and compounds that will be identified. The test(s) should be reproducible and be able to provide quantitative results that can differentiate between toxic and non-toxic fractions using statistic methods. At last confirmation of the toxicity of the identified compounds is a necessary step to establish cause-effect relationships (Brack 2008b).

Some examples of the use of the EDA approach have already been given in the previous chapters presenting the bioassays, e.g. the studies by Keiter *et al.* (2008), Brack *et al.* (2008a), Chou *et al.* (2007) and Leskinen *et al.* (2008). EDA was also used by Grung *et al.* (2007), who identified steroid estrogens, alkylphenols, benzophenone and methylparaben as compounds contributing to the estrogenic activity of untreated waste water. The same study identified i.a. PAHs, nitro-PACs and carbazoles as CYP1A1 inducers using the EROD assay. Burgess *et al.* (1995) concluded that the cause of toxicity of effluent water from a plant receiving household and industrial waste water varied over time, they also concluded that the chlorination process in the plant was responsible for some toxicity. Scheurell *et al.* (2007) could with their study of water polluted by diffuse sources, industrial and municipal sewage effluents show that the compounds causing toxicity in the acute bioluminescence inhibition test were different in the WWTP-sample compared with the other two. Amato *et al.* (1992) identified the insecticide diazinon as a waste water toxicant using the TIE methodology. This study made the importance of the choice of bioassay clear as the sample showed no toxicity in a test with fish (*Pimephales promelas*) but exhibited acute toxicity in a water flea bioassay (*Ceriodaphnia dubia).*

EDA has proven to be a successful tool to identify various effect-causing compounds in waste water. In many cases it may however not be possible to explain the whole effect seen since the mechanism of action is not fully elucidated for several effects and some effects can be caused by a plentitude of compounds. Further research concerning ecotoxicological and chemical analytical method will probably increase the efficiency of EDA in the future.

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