REPORT

Results from the Swedish National Screening Programme 2005

Subreport 1: Antibiotics, Anti-inflammatory substances, and Hormones

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Report Summary

Project title

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Title and subtitle of the report

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Sub report 1 Antibiotics, Anti-inflammatory substances and Hormones

Summary

Measurements of pharmaceuticals were performed in 181 samples of water, sludge, manure, sediment and biota at background sites, municipal STPs, landfills, hospital effluents and recipient water from STPs. Bioassays of hormone activity were performed for a selected number of water samples. The NSAIDs were the most frequently detected pharmaceuticals and occurred in the highest concentrations. There were large differences in concentrations both between substances and between sampling sites. A regional trend in the STP effluent water could be observed for the NSAIDs and for some antibiotics with increased concentrations in samples originating from the north. No pattern could be seen for the hormones. Estrogenic effects were detected in STP outlets to the aquatic environment while values obtained for androgenicity were in most samples close to or below the detection limit. Based on the risk asessment (MEC/PNEC) risk quotients >1 was obtained for estradiol, estriol, ethinylestradiol and ibuprofen.

Keyword

Screening, **antibiotics**: doxycycline, lymecycline, oxytetracycline, tetracycline; **anti- inflammatory substances, NSAIDs**; ibuprofen, ketoprofen, naproxen, diclofenac; **hormones**: ethinylestradiol, norethindrone, estradiol, estriol, and progesterone, Sweden

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Sammanfattning

IVL Svenska Miljöinstitutet har på uppdrag av Naturvårdsverket genomfört en sk screening av läkemedel. Följande ämnen ingick i uppdraget: **antibiotika**: doxycyklin, lymecyklin, oxytetracyklin och tetracyklin; **anti-inflammatoriska substanser**; ibuprofen, ketoprofen, naproxen och diklofenak; **hormoner:** etinylestradiol, noretisteron, östradiol, östriol och progesteron.

Huvudsyftet med studien var att bestämma koncentrationer i olika matriser i miljön, att belysa viktiga transportvägar samt att bedöma sannolikheten för pågående emissioner i Sverige. Studiens resultat skall kunna bidra med underlag för beslut om vidare miljöövervakning av dessa ämnen.

Läkemedel är vitt använda ämnen. Det finns cirka 1200 aktiva substanser i 7600 olika produkter på den svenska marknaden (Läkemedelsverket, 2004). Under det senaste decenniet har läkemedel blivit uppmärksammade som problematiska föroreningar i miljön.

En provtagningsstrategi utarbetades utifrån ämnenas möjliga källor. Potentiella punktkällor, diffusa källor (reningsverk) samt bakgrundsstationer valdes ut och provtagning utfördes i vatten, slam, gödsel, sediment och fisk. 15 länsstyrelser bidrog med ytterligare 152 prover fördelat på matriser enligt tabellen nedan.

Program	Gödsel	Vatten	Sediment	Slam	Biota	Totalt
Nationellt	5	14	6	1	3	**29**
Regionalt		91	1	60		**152**
Totalt	***5***	***105***	***7***	***61***	***3***	***181***

Som komplement till de kemiska analyserna utfördes ”bioassays” för hormonell aktivitet i miljöprover för 19 av proverna ovan.

Variationen mellan koncentrationer av läkemedel var stor både mellan olika substanser och mellan olika provtagningsplatser. Den mest frekvent detekterade läkemedelsgruppen var de anti-inflammatoriska ämnena. Band dem var det ibuprofen och naproxen som förekom i högst halter. Tetracykliner och doxyxyklin var de mest frekvent förekommande ämnena antibiotikagruppen emedan progesteron och norethindrone var de hormoner som förekom i högst halter.

Koncentrationen av läkemedel i bakgrundsproverna var mestadels under detektionsgränsen (vatten och sediment). De antinflammatoriska ämnena förekom i en av bakgrundssjöarna som kan ha varit påverkad av enskilda avlopp. Chlorocyklin, progesteron och norethindron uppmättes i enstaka fall i de andra sjöarna

De olika läkemedelsgrupperna förekom frekvent i proverna som samlades in från de olika reningsverken vilket indikerar betydelsen av dessa som källa till spridning av läkemedelsrester. Skillnaderna mellan de olika reningsverken var stor både för inkommande och utgående vatten liksom för slam. Jämförelse mellan inkommande och utgående vatten visar på skillnad i förmågan att eliminera läkemedelsrester mellan de olika reningsverken. I några fall förekom vissa substanser i

högre halter i det utgående vattnet än i det inkommande. En regional trend kunde observeras för de antiinflammatoriska ämnena med högre halter av ibuprofen och naproxen från reningsverken i norra Sverige.

Koncentrationen av läkemedel i recipientvatten taget i närheten av reningsverk i två olika sjöar var lägre eller i samma nivå som bakgrundsproverna. I lakvatten detekterades halter av läkemedel i samma nivåer som i effluenterna från reningsverken. Läkemedelsrester återfanns också i avloppsvatten från sjukhus

Bio-assay undersökningarna påvisade estrogena effekter i utgående vatten från reningsverken. De högsta nivåerna av estrogena effekter var detekterade i avloppsvatten från sjukhus och i ett av reningsverksproverna. Lakvattenproverna hade jämförelsevis lägre estrogena effekter emedan inga estrogena effekter kunde påvisas i proverna som insamlats kring djurstallar.

Värdena för androgena effekter var i de flesta proverna under eller nära detektionsgränsen. De högsta nivåerna för androgena effekter fanns i ett sjukhusavlopp som också hade de högsta koncentrationerna av noretisteron. Obehandlat lakvatten hade också en högre androgen nivå vilket korrelerade med högre noretisteronhalter. I övrigt kunde inga samband påvisas mellan andrgoena effekter och noretisteron.

Riskkvoter för diklofenak, naproxen, oxytetracyklin, tetracyklin och doxyxyklin var <1 i samtliga prover. De ämnen som visade högst risk kvoter var etinylestradiol och östradiol. Ibuprofen hade också riskkvoter >1 i flera av proverna av utgående vatten från reningsverk. I vissa ytvatten i närheten av djurhållning erhölls risk kvoter >1.

Summary

As an assignment from the Swedish Environmental Protection Agency, IVL has performed a screening study of pharmaceuticals. The following substances were included: **antibiotics**: doxycycline, lymecycline, oxytetracycline and tetracycline; **anti-inflammatory substances**; ibuprofen, ketoprofen, naproxen and diclofenac; **hormones**: ethinylestradiol, norethindrone, estradiol, estriol and progesterone

The overall objectives of the screening were to determine the concentrations of the selected substances in a variety of media in the Swedish environment, to highlight important transport pathways, and to assess the possibility of current emissions in Sweden. The outcome of the study is aimed to serve as a basis for decision-making regarding monitoring activities of these chemicals.

Pharmaceuticals are widely used substances. On the Swedish market there are approximately 1200 active compounds in about 7600 different products (Läkemedelsverket, 2004). During the last decade pharmaceuticals have become recognised as relevant environmental contaminants.

A sampling strategy was set up based on the possible sources of these compounds. Potential point sources, diffuse sources (sewage treatment plants) as well as background sites were selected, and sampling was performed in water, sludge, sediment and fish. 15 county administrative boards participated in the study and provided additional samples for analysis. The total number of samples analysed in the study are shown in the table below.

Programme	Manure	Water	Sediment	Sludge	Biota	**Total**
National	5	14	6	1	3	**29**
Regional		91	1	60		**152**
Total	***5***	***105***	***7***	***61***	***3***	***181***

In addition to this 19 samples were selected for bioassays procedures to estimate hormone activity of pharmaceuticals in environmental samples.

There was a great variation in the concentrations of the pharmaceuticals among different sampling sites. The anti-inflammatory substances (NSAIDs) were the most frequently detected pharmaceutical group. Among the NSAIDs, ibuprofen and naproxen occurred in the highest concentrations while diclofenac was in most cases the least abundant. The most frequently found antibiotic substances were tetracycline and doxycycline. Progesterone and norethindrone were the substances that occurred in the highest concentrations among the hormones.

The concentrations of the pharmaceuticals in the background site samples (water and sediment) were, mostly below the detection limits. However the NSAIDs were present in one of the background lakes which might have been affected by private drains. Chlorocycline, norethindrone and progesterone occurred occasionally in low levels in the other lakes.

The pharmaceuticals were frequently found in the samples collected at the STPs indicating the importance of STPs as a source for these substances. There was however a great variation in the concentrations, both in the infuent and effluent waters as well as in the sludge among the sampled STPs located all over Sweden. Comparisons of the concentrations in the influent and effluent water showed that the reduction efficiency in the STP varied among the different substances and occasionally some of the substances occurred in higher concentrations in the effluent than in the effluent.

A regional trend was found for the NSAIDs, with the highest effluent water concentration of ibuprofen and naxproxen from the STPs situated in the northern part of Sweden

The concentrations of the pharmaceuticals in the recipient water samples collected close to STPs were lower or in the same level as in the background water samples. The pharmaceuticals occurred in landfill leachate water samples in concentrations in the same order of magnitude as for the effluents from the STPs. Pharmaceutical residues were also found in the effluents from hospitals where the concentrations of the antibiotics and diclofenac in some of the samples exceeded the concentrations in the STPs effluents.

The bioassay investigations showed that estrogenic effects were detected in STP outlets to the aquatic environment. The highest levels were found in wastewaters from a hospital and one of the municipal STPs, while others had lower levels. Landfill leachate wastewaters had comparatively lower estrogenic effects. No estrogenic effects were detected in outlets from animal farm areas.

Values obtained for androgenicity were in most samples close to or below the detection limit. The highest concentration was found in a hospital wastewater that also had the highest androgenic effects. Untreated landfill leachate water that also had a higher norethindrone concentration correlated with a higher androgenic effect. Otherwise there was no apparent correlation between analysed concentrations of norethindrone and the androgenic effects.

The risk quotas for diclofenac, naproxen, oxytetracycline, tetracycline and doxycycline were all <1. The substances that showed the highest risk quotients were as expected ethinylestradiol and estradiol. Ibuprofene also had risk quotients >1 in several effluent samples for STPs.
Some surface water samples around animal breeding locations also had risk quotients >1 implying that it could be interesting to continue with an investigation around these type of facilities.

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1 Introduction

As an assignment from the Swedish Environmental Protection Agency, IVL has during 2005/2006 performed a "Screening Study" of selected chemicals within the groups pharmaceuticals, biocides and perfluorinated alkylated substances (PFAS). The selected chemicals (Table 1) are emitted and distributed in the environment via a variety of sources, e.g. point sources and via use in consumer products. Pharmaceuticals in particular are frequently spread by domestic use.

The overall objectives of the screening were to determine the concentrations of the selected substances in a variety of media in the Swedish environment, to highlight important transport pathways, and to assess the possibility of current emissions in Sweden. A further aim was to investigate the likelihood of atmospheric transport (biocides and PFAS) and uptake in biota.

This sub-report considers the screening of the selected pharmaceuticals. Results for the other chemical groups included in the screening are given in sub-report 2 & 3.

Table 1. Chemicals selected for the screening 2005

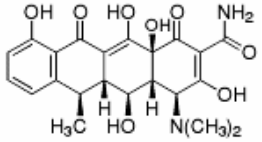
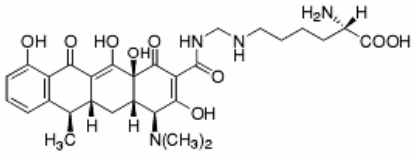
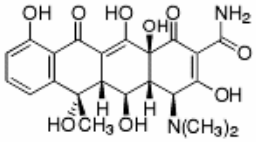
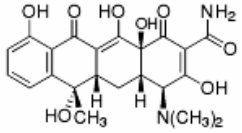
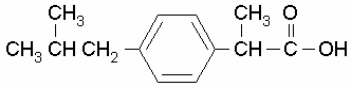
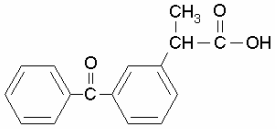
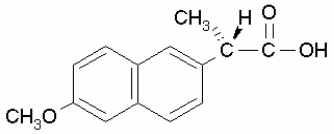
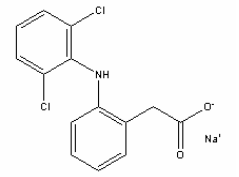
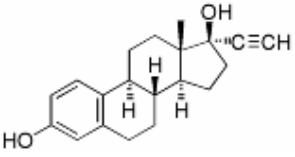
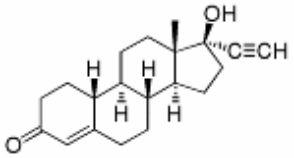
		Report nr.
Pharmaceuticals	**Antibiotics**: Doxycycline, Lymecycline, Oxytetracycline, Tetracycline **Anti- inflammatory substances**: Ibuprofen, Ketoprofen, Naproxen, Diclofenac **Hormones**: Ethinylestradiol, Norethindrone, Estradiol, Estriol, Progesterone	1
Biocides	Bronopol,4-Chloro-3-cresol, Mercaptobenzothiazole, N-didecyl-dimethylammoniumchloride, Propiconazole, Resorcinol, 2-(Tiocyanomethylthio)benzothiazole, Methylparabene, Ethylparabene, Propylparabene, Butylparabene, Benzylparabene	2
PFAS	Perfluorobutane sulfonate (PFBS), Perfluorohexane sulfonate (PFHxS), Perfluorooctane sulfonate (PFOS), Perfluorodecane sulfonate (PFDS), Perfluorohexanoic acid (PFHxA), Perfluoroheptanoic acid (PFHpA), Perfluorooctanoic acid (PFOA), Perfluorononanoic acid (PFNA), Perfluorooctane sulfonamide (PFOSA)	3

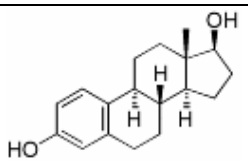
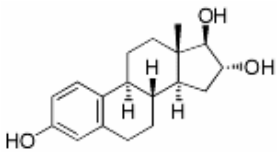
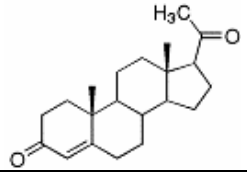
Pharmaceuticals are widely used substances. On the Swedish market there are approximately 1200 active compounds in about 7600 different products (Läkemedelsverket, 2004). During the last decade pharmaceuticals have become recognised as relevant environmental contaminants (Halling-Sörensen et al., 1998, Kümmerer (ed), 2004).

The inherent bioactivity of pharmaceuticals has thus far manifested itself in the environment in a number of cases such as the adverse effects on reproduction and hormonal disturbances of aquatic organisms due to the presence of a synthetic hormone, ethinylestradiol (MacLatchey et al., 1997, Routledge et al. 1998, Larsson et al., 1999). In South East Asia, the use of the anti-inflammatory drug diclofenac in veterinary medicine has resulted in an almost complete extinction of some species of vultures, feeding on cattle carcasses. These birds have shown to experience acute kidney failure syndrome upon exposure to the drug (Oaks et al., 2004). Concerns have also been raised on the topic of bacterial resistance to antibiotics in sludge from wastewater treatment plants (Alexy et al, 2004).

Three major subgroups of pharmaceuticals were included in this screening study; antibiotics, anti-inflammatory substances and hormones. The selected substances are presented Table 2.

Table 2. Pharmaceutical substances included in the screening

Therapeutic use	Substance	Molecular formula	CASnr
Antibiotic	Doxycycline		564-25-0
	Lymecycline		992-21-2
	Oxitetracycline		2058-46-0
	Tetracycline		60-54-8
Anti-inflammatory	Ibuprofen		15687-27-1
	Ketoprofen		22071-15-4
	Naproxen		22204-53-1
	Diclofenac		15307-86-5
Hormone	Ethinylestradiol		57-63-6
	Norethindrone		68-22-4

Estradiol		50-28-2
Estriol		50-27-1
Progesterone		57-83-0

In the investigation of the pharmacokinetics of lymecycline it was evident that the chemical, upon administration to patients, would be immediately and completely metabolised to tetracycline (CAS 64-75-5), the active compound (Fass.se). It was therefore highly unlikely that lymecycline could be found in any samples. The sec-amine group bridging the tetracycline to the methyl lysine-arm of the molecule is very easily cleaved resulting in the transformation of lymecycline to tetracycline. Instead another tetracycline structural analogue, chlorotetracycline (CAS 57-62-5, Mw 478.88, see Figure 1) and in addition to this demecyclocycline (see Figure 2) have instead been included in this study.

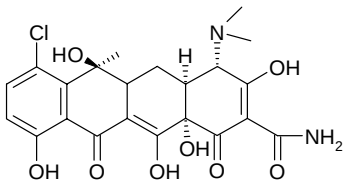


Figure 1. Chlorotetracycline

Demecycline is a tetracyline drug used in the treatment of bacterial infections including pneumonia and other respiratory tract infections; acne; infections of skin, genital, urinary systems as well as hyponatraemia (low blood sodium concentration).

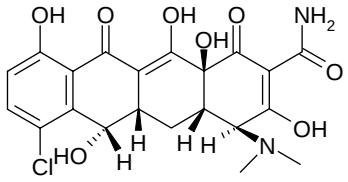


Figure 2. Demeclocycline, CAS 127-33-3

The retrieved results in this screening effort regarding detected tetracycline in the environmental samples thus, rather reflect the summed contribution of both tetracycline and lymecycline.

2 Chemical properties and toxicity

Pharmaceutically active substances are developed and used because of their biological activity. Normally pharmaceuticals are classified according to their therapeutical purpose and within the subgroups of the active ingredient, by chemical structure.

Pharmaceutical chemicals are often complex molecules with physico-chemical properties depending on pH (e.g. dependence of log K_{ow}). Under environmental conditions they can be neutral, cationic, anionic or zwitterionic and often have basic or acidic functionalities.

In the report from the Swedish Medical Product Agency on environmental effects of pharmaceuticals (Läkemedelsverket, 2004), 30 pharmaceutically active compounds were classified with regards to aquatic toxicity, according to the regulation of the Swedish Chemical Inspectorate for classification and labelling for chemical products. The classification of the pharmaceuticals included in this study is presented in Table 3.

Table 3. Environmental classification of pharmaceuticals (Läkemedelsverket, 2004)

Substance	Toxic values	Bioaccumulation	Persistence	Classification
*Doxycycline**				
*Lymecycline**				
Oxitetracycline	3 toxicity values (R50)	Not bioaccumulative	No data	Dangerous for the environment (R50)
Tetracycline	2 toxicity values (R51)	Not bioaccumulative	No data	Not enough information
Ibuprofen	3 toxicity values (R51)	Potential bioaccumulation	Not easily biodegradable	Dangerous for the environment (R51/53)
Ketoprofen	No data	Potential bioaccumulation	No data	Not enough information
Naproxen	3 toxicity values (R52)	Not bioaccumulative	Ambiguous data	Not enough information
Diclofenac	3 toxicity values (R 52)	Potential bioaccumulation	Not easily biodegradable	Dangerous for the environment (R52/53)
Ethinylestradiol	2 toxicity values (R51)	Potential bioaccumulation	Not easily biodegradable	Dangerous for the environment (R51/53)
Norethindrone	1 toxicity value(R50)	Not bioaccumulative	Not easily biodegradable	Dangerous for the environment (R50/53)
Estradiol	1 ambiguous toxicity value	Potential bioaccumulation	Ambiguous data	Not enough information
Estriol	No data	Not bioaccumulative	No data	Not enough information
*Progesterone**				

R50: Very toxic to aquatic organisms; R51: Toxic to aquatic animals; R52: Harmful to aquatic animals; R53: May cause long-term adverse effects in the aquatic environment.

* Not included in the report

In the same report on environmental effects of pharmaceuticals (Läkemedelsverket, 2004) and in Carlsson et al., a predicted no effect concentration (PNEC) was estimated for the pharmaceuticals. The PNECs according to Läkemedelsverket, 2004 and Carlsson et al., 2005 are given in Table 4.

The estimated PNECs of ethinylestradiol and estriol were based on sexual development in Japanese risefish applying a safety factor of 50 for ethinylestradiol and 100 for estriol. Ecotoxicological data used for PNEC-calculation for estradiol was based on induction of vitellogenin in fish. Presently, no significant correlation has been found between vitellogenin induction and long term effects on populations and there are no available test data on effects of reproduction from fish exposed during a full lifecycle, thus the authors consider the risk assessment for estradiol as very uncertain. However, an alternative PNEC derived from a test on induced intersex on Japanese risefish applying a safety factor of 50, gave a PNEC of 0.008 ng/l which further confirms the undesirable environmental effect implied by the vitellogenin test (Läkemedelsverket, 2005).

Ecotoxicological data for diclofenac were based on either reproduction tests or toxicity tests for early life stages using at least three trophic levels, which gave a safety assessment factor of 10 (Läkemedelsverket, 2004). PNECs for oxytetracycline and tetracycline in Läkemedelsverket and in Carlson et al., were derived from test on blue green algae applying a safety factor of 1000. However, according to the guidelines from European Medicine Agency on environmental risk assessment for medicinal products on human use (EMEA, 2006), an assessment factor of 10 is applicable for antibiotics tested for microbial effects. This assessment approach would give a higher PNEC (oxytetracyclin 20 µg/l and tetracycline 9 µg/l) than suggested in the report (EMEA, 2006). The PNECs for ibuprofen and doxycycline were derived from acute toxicity tests applying a safety factor of 1000. The results from ecotoxicological effect studies used for PNEC calculations are presented in Appendix 1 (Läkemedelsverket, 2004).

Table 4. Predicted no effect concentrations.
(Läkemedelsverket, 2004; Carlsson et al, 2005)

Substance	PNEC (µg/l)	Assessment factor
Doxycycline	0.316*	1000
Lymecycline	*No data*	*No data*
Oxytetracycline	20*	10
Tetracycline	9*	10
Ibuprofen	7.1	1000
Ketoprofen	*No data*	*No data*
Naproxen	35	1000
Diclofenac	100	10
Ethinylestradiol	0.00002	50
Norethindrone	*No data*	*No data*
Estradiol	0.00002	50
Estriol	0.00075	100
Progesterone	*No data*	*No data*

* derived from ecotoxicological data in Appendix 1.

Many pharmaceuticals are bio-transformed in the body, which may lead to a change in the chemical structure of the active component and a change in pharmaceutical as well as in physico-chemical properties. This may lead to lower activity or enhanced water solubility. However, metabolism of a substance is in most cases not complete and excretion rates range from 0-100% (Kümmerer, 2004).

Metabolism in humans may occur through two major important pathways. Phase I metabolism occurs through modification of the active compound by hydrolysis, oxidation, reduction, alkylation and dealkylation. Phase II metabolites are phase I metabolites which have been modified by glycoside conjugation i.e. glucoronidation (conjugation with glucuronic acid) or sulphate conjugation (formation of sulphate esters), (Kümmerer, 2004). During the phase II metabolic glucuronidation pathway the liver enzymes involved are aimed to render the metabolites more polar than the mother compound, thus increasing excretion rates. Chemically, glucuronidation involves

the attachment of a glucuronic acid unit to any of the hetero atoms of the pharmaceutical mother compound (e.g., O- glucuronidation or N- glucuronidation). Whether glucuronidation takes place at the oxygen rather than nitrogen hetero atom has consequences for the STP-process. O-glucuronidated compounds are very often transformed in the STP environment back to the mother compound whereas N-glucuronidated drugs seem stable enough to pass the STP process without reformation of the mother compound (Möhle et al., 2001 Ternes et al., 1999, Kozak et al., 2001).

The mammalian metabolic glucuronidation of diclofenac is rather complex and briefly sketched out in Figure 3 (Ebner et al., 1999 Kretz-Rommel et al., 1993, Grillo et al., 2003). The most important metabolic substance in this pathway (as indicated) is diclofenac 1-O-acyl glucuronide (D-1-O-G), being glucuronidated at the hydroxyl group of diclofenac (i.e. O-glucuronidated). During passage through the STP process the mother compound can be reformed.

Other NSAIDs such as ibuprofen and ketoprofen induce the glucuronidation enzyme *uridine diphosphate glucuronosyltransferase* (UDPGA) when metabolised although the stability of their metabolites in the STP seems largely unknown. In laboratory experiments their glucuronidation reactions have been found to be guided by a slower kinetics than for diclofenac (Bolze et al., 2002).

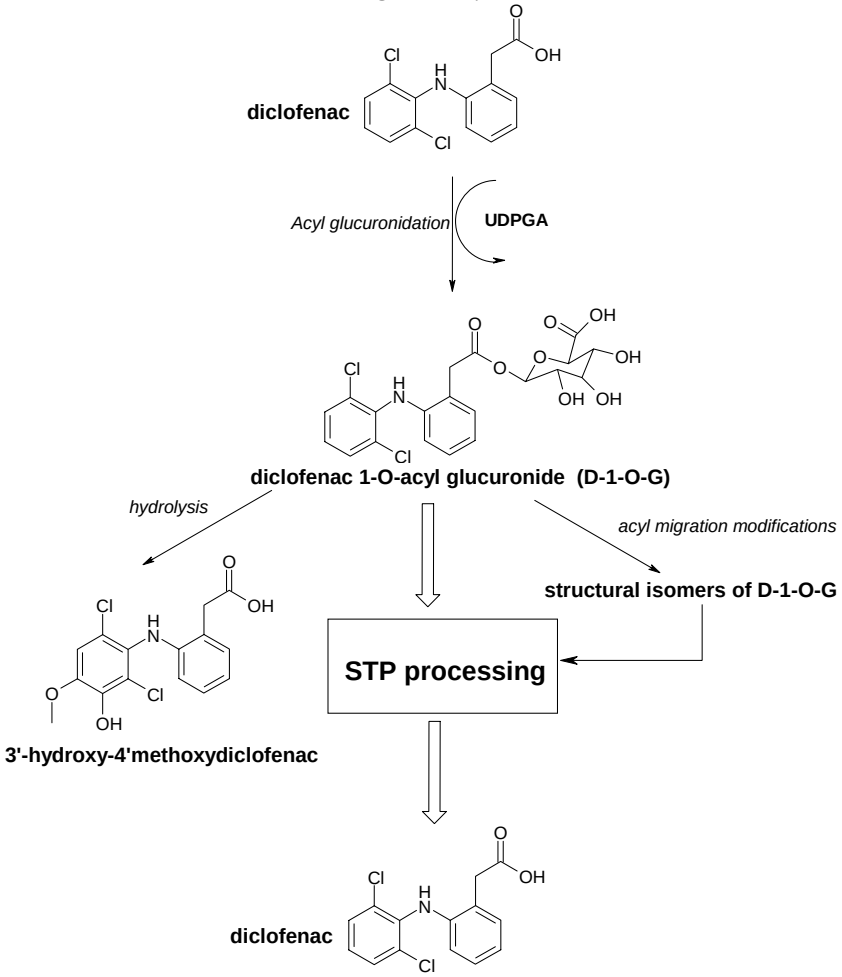


Figure 3. Metabolic pathway of diclofenac

3 Fate

In order to highlight the likely fate and partitioning behaviour of the pharmaceuticals a modelling exercise was performed using the Equilibrium Criterion (EQC) model (Mackay et al., 1996). Ibuprofen, diclofenac, ethinylestradiol and norethindrone were selected as model substances for the fate assessment. The antibiotic substances were purposely omitted from fate modelling due to the huge uncertainties associated with their adsorption to solid matter; chelation, ionic interaction and chemisorption.

Table 5. Chemical and physical data[a].

Substance	MW	W_{sol} (mg/l)	V_p (mm Hg, (25°))	H (Atm m^3/mol)	Log K_{ow}	BCF[c]	K_{oc}(L/kg)
Doxycycline	444.44	630	1.42E-23[b]	4.66E-24	-0.02	3.2	-
Lymecycline	602.64	137.2[b]	1.01E-031[b]	5.780E-033[b]	-3.22[c]	3.2	-
Oxytetracycline	496.89	137.2[b]	3.06E-028[b]	2.138E-033[b]	-3.60[b]	3.2	-
Tetracycline	480.90	24890[b]	7.81E-029[b]	1.994E-034[b]	-3.70[b], (-1.62[d])	3.2	-
Ibuprofen	206.28	21	1.86E-04[c]	1.50E-07[c]	3.97	3.2	-
Ketoprofen	254.28	51	3.72E-07[c]	2.12E-11[c]	3.12	3.2	-
Naproxen	230.26	15.9	1.89E-06[c]	3.39E-10[c]	3.18	3.2	-
Diclofenac	296.15	2.37	6.14E-08[c]	4.73E-12[c]	4.51	3.2	-
Ethinylestradiol	296.41	11.3	2.67E-09[c]	7.94E-12[c]	3.67	130	-
Norethindrone	298.42	7.04	7.31E-09[c]	5.80E-10[c]	2.97	39	-
Estradiol	272.39	3.6	1.26E-08[c]	3.64E-11[c]	4.01	240	-
Estriol	288.39	441[c]	1.97E-10[c]	1.33E-12[c]	2.45	15	-
Progesterone	314.47	8.81	1.30E-06[c]	6.49E-08[c]	3.87	190	-

Physical-chemical properties were taken from Table 5. The degradation half-lives (in hours) used is given in Table 6.

Table 6. Estimated half-lives (hours)[1]

Substance	$T_{½}$ (air)	$T_{½}$ (water)	$T_{½}$ (soil)	$T_{½}$ (sediment)
Ibuprofen	33	360	720	3360
Diclofenac	2	912	1800	8160
Ethinylestradiol	3	1440	2880	12960
Norethindrone	18	1440	2880	12960

[a] Experimental values retrieved from ChemID Advanced Plus, unless otherwise stated.
[b] Estimated value retrieved from the EPIWin software (Meylan, 1999)
[c] Estimated value retrieved from ChemID Advanced Plus
[d] Estimated value retrieved from clogp-software

[1] Values retrieved from the PBT-profiler

Emission rates were set to 1000 kg/h, only for illustrative purposes. The outcome of the modelling exercise is shown in Table 7 and Table 8. The numbers given in the tables should be regarded as indicative, as they are dependent on the model structure as well as on the chemical property data. The calculation gives the steady-state, rather than the equilibrium distribution of a chemical. The chemical is continuously discharged at a constant rate into the chosen environmental media, and achieves a steady-state condition at which input and output rates are equal. The calculation involves the rates of degradation and advection (from half-lives/rate constants and advective flow rates) and it considers the emission. Intermedia transport processes (e.g. wet deposition, evaporation, or sedimentation) are included. The media receiving the primary emission is very important and have a controlling influence on the overall fate of the chemical. Direct emission to air has been purposely omitted since it is very unlikely to occur for the pharmaceuticals.

The overall residence time in the system of both ibuprofen and diclofenac seems to depend on which compartment the chemical is emitted to and it is generally lower for ibuprofen than for diclofenac (~30 days when emitted to all media compared to ~75 days in the case of diclofenac). Due to the higher vapour pressure of ibuprofen compared to diclofenac, a minor fraction of the ibuprofen load will be advected out of the system boundaries from the air compartment. The primary receiving media are likely to be soil and water, based on the neglible volatility and the areas of use of these substances. Both these two anti-inflammatory drugs possess ionisable groups (e.g. carboxylic acid moieties). Thus, at environmentally relevant pH-values, a considerable fraction of the drugs will exist as an-ions and the predicted environmental distribution is likely to be shifted to the water compartment with persistence largely governed by the aqueous degradation kinetics.

Table 7. Results from EQC modelling of Ibuprofen (IBU) and Diclofenac (DICLO), using emission rates of 1000 kg/h

Emission medium	% in air		% in water		% in soil		% in sediment		Persistence (h)	
	IBU	DICLO	IBU	DICLO	IBU	DICLO	IBU	DICLO	IBU	DICLO
Water	<0.1	<0.001	89	56	<0.1	<0.01	11	44	380	1000
Soil	<0.01	<0.001	<0.1	<0.1	99.8	99.9	0.01	<0.1	1000	2500
both	***0.02***	***<0.001***	***24***	***15***	***73***	***73***	***3***	***12***	***700***	***1800***

Table 8. Results from EQC modelling of ethinylestradiol (EE) and norethindrone (Nor), using emission rates of 1000 kg/h

Emission medium	% in air		% in water		% in soil		% in sediment		Persistence (h)	
	EE	Nor	EE	Nor	EE	Nor	EE	Nor	EE	Nor
Water	<0.001	<0.001	88	98	<0.001	<0.01	12	2.	750	700
Soil	<0.001	<0.001	0.4	1.8	98	99.9	<0.1	<0.1	4000	4000
both	<0.001	<0.001	***14***	***16***	***84***	***83***	***2***	***0.4***	***2400***	***2250***

Regarding the hormone substances, as evident from Table 5, the vapour pressures of these substances are very low. Since ethinylestradiol has a higher log Kow-value compared to norethindrone, the substance has a slightly longer persistence since a larger fraction of the compound emission will be distributed into the sediment compartment. That explains why,

compared with the anti-inflammatory drugs above, the persistence of the hormones can be anticipated to be longer.

According to Lee et al., most natural sex hormones seem to have organic carbon sorption coefficients of log Koc in the range between 3 and 3.5 [Koc in units of l/kg], thus indicating that normal leaching of these components from soil is limited. Rather, the runoff of soil- and land applied bio solids seem to be the most likely input to surface waters. Furthermore, is seems likely that a significant fraction of these compounds will be associated with sediments (Lee et al., 2003).

4 Regulation, therapeutical use and emissions

The use of pharmaceuticals is regulated within the European Union. Regulations are concerned with both the importance for and the possible impact of the substance on human health. It is also an area that takes national economic and social interests into consideration. The regulation is based on Directive 2001/83/EC of the European Parliament as well as on the Council of 6 November 2001 on the Community code related to medicinal products for human use. Further, it is amended by Directive 2004/27/EC of the European Parliament and of the Council of 31 March 2004 amending Directive 2001/83/EC on the Community code relating to medicinal products for human use.

Veterinary medicine is regulated by Directive 2001/82/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to veterinary medicinal products and amended by directive 2004/28/EC of the European Parliament and of the Council of 31 March 2004 amending Directive 2001/82/EC on the Community code relating to veterinary medicinal products (Läkemedelsverket, 2004).

According to the legislation pharmaceuticals are classified as "substances that are intended to be supplied to humans or animals in order to prevent, point out, relieve or cure a disease or a symptom of a disease" (Läkemedelsverket, 2004; own translation). The purpose of the chemical is a central part of the definition.

Pharmaceuticals are administered orally, dermally or intravenously depending on the substance and the medical circumstances (Kümmerer, 2004). Pharmaceutical agents may enter the environment via two major pathways, the industrial route or the domestic route. Point discharges from the industries have previously been considered as limited since pharmaceuticals are high cost chemicals and the handling of the chemicals often is performed in closed facilities. The pharmaceuticals will be administered to a patient, either at home or in hospitals and the substances are mainly reaching the community sewage treatment plants (STPs) via excretion. In the STPs they will be degraded totally, partially or be released intact to recipient water systems. The pharmaceuticals may also be deposited as waste. Pharmaceuticals are also administered in veterinary applications and may reach the environment via direct excretion, via leachate from waste deposits or via animal manure that is spread in agriculture areas. The major emission pathways of pharmaceuticals to the environment are illustrated Figure 4.

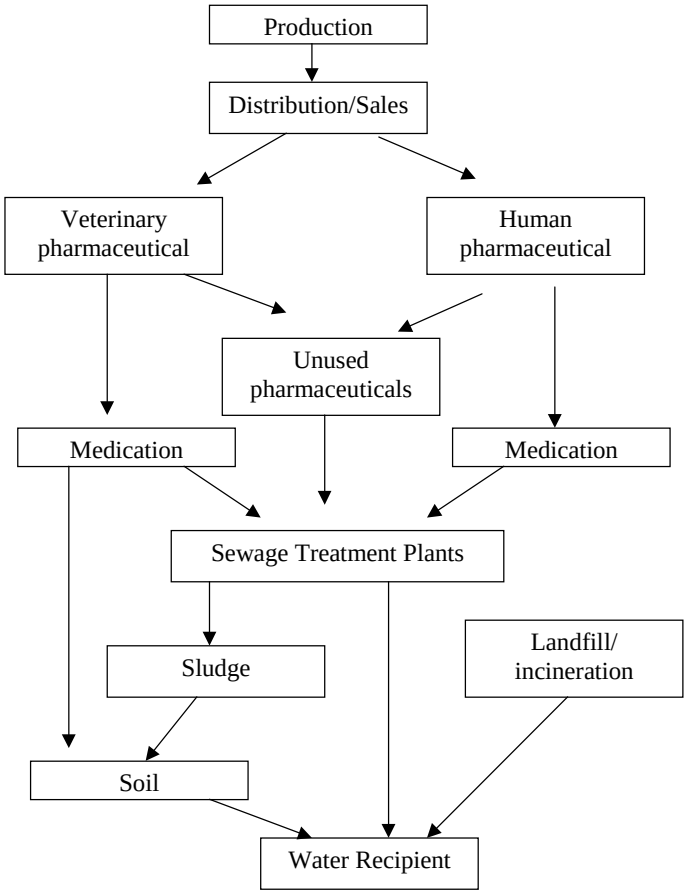


Figure 4. Emission pathways of pharmaceuticals to the environment (translated and redrawn from Bengtsson et al. 2005)

The therapeutical use for the selected pharmaceutical groups are described below.

4.1 Antibiotics

All the antibiotics included in the screening belong to the group of tetracyclines and have bacteriostatic effects. The mode of action of the bacteriostatics is the inhibition of protein synthesis in the bacteria, which means that they inhibit growth and reproduction of the bacteria in contrast to bactericidal antibiotics that instead kill the bacteria.

Doxycycline is amongst others used to treat pneumonia, genital infections or sinus infections when regular antibiotics are not enough. It can also replace regular penicillin in case of allergy reactions. Lymecycline is used to treat pneumonia and genital infections and it is used for treatment of acne. Oxytetracycline is the active ingredient in the pharmaceutical product oxytetral that also is used to treat acne. Tetracycline is used to treat pneumonia and genital infections. It can be used to treat sinus infections when regular penicillin has shown to be ineffective. The medicine can also be used to treat acne or when there is an allergic reaction to regular penicillin (Infomedica, 2006).

4.2 Anti-inflammatory substances (NSAIDs)

All the anti-inflammatory substances in this study are cox-inhibitors i.e., they possess the function to inhibit an enzyme family in the body called cyclooxygenases, abbreviated cox. When this enzyme is inhibited the production of prostaglandins decreases. The prostaglandins are substances that cause pain, inflammation and fever. This type of anti-inflammatory drugs are often referred to as NSAIDs; non-steroid anti-inflammatory drugs.

Ibuprofen is used to cure headache, migraine, toothache and menstrual pain. It can also be used to treat fever in connection with an infection, for example a cold, or when treating chronic or inflammatorily pain such as rheumatic pain. Ketoprofen is used to treat pain and inflammation at rheumatic diseases but also for acute pain and against menstrual pain. Naproxen is used to treat pain and inflammation at rheumatic diseases, acute pain and menstrual pain. It can also be used for migraine. Diclofenac relieves pain, reduce inflammation and fever (Infomedica, 2006).

4.3 Hormones

Ethinylestradiol has a similar function as the natural estrogen estradiol and its metabolites estriol and estrone. Estriol and estradiol are prescribed to women when natural production of estrogens is low for example after menopause. Ethinylestradiol is an ingredient in many contraceptives. Progesterone (also called lutren, lutein, flavolutan, corporin, and luteal hormone) is a C-21 steroid hormone involved in the female menstrual cycle, pregnancy (supports gestation) and embryogenesis of humans and other species. The substance belongs to a class of hormones called progestagens, and is the major naturally occurring human progestagen. Progesterone can also be prescribed to women that are treated for infertility. Norethindrone, essentially an androgen, has the same function as progesterone and is prescribed to women after menopause (Infomedica, 2006).

5 Previous investigations in the environment

5.1 Measurements of pharmaceuticals

A large variety of different medical substances have previously been found in various environmental compartments e.g. in surface water, ground water and drinking water (Kümmerer, 2004). The pharmaceuticals have also frequently been detected in effluents from national care units, in sewage and in the effluent of sewage treatment plants as well as in the effluent from landfill sites. A summary of selected previous investigations found in the literature concerning the occurrence of the pharmaceutical substances in different sample matrixes is given in Table 9.

Table 9. Environmental concentrations of the selected pharmaceutical substances

Substance	Matrix	Concentration	Reference
Doxycycline	Raw sewage	67-333 ng/l	Lindberg et al., 2005
	Final effluent	64-915 ng/l	Lindberg et al., 2005
	Sludge	1.3-1.5 mg/kg dw	Lindberg et al., 2005
	River water	~1μg/l	Läkemedelsverket,2004*
	Surface water	ND	Kolpin et.al., 2002
	Surface water (Canada)	0.038 μg/g	Metcalfe, 2004
Tetracycline	River water	~1μg/l	Halling-Sörensen et al, 1998
	Surface water (Canada)	0.151μg/g	Metcalfe, 2004
Oxytetracycline	Surface water	0.34 μg/l	Kolpin et.al., 2002
	Surface water (Canada)	ND	Metcalfe, 2004
Oxytetracyline (as feed additive in fish farm)	Sediment	0.1-11μg/g	Halling-Sörensen et al, 1998
	Sediment	285μg/g	Halling-Sörensen et al, 1998
Diclofenac	Effluent from sedimentation tank	Up to 2μg/l	Halling-Sörensen et al, 1998
	River Rhine	15-304 ng/l	Halling-Sörensen et al, 1998
	Different Rivers	38-489 ng/l	Halling-Sörensen et al, 1998
	Sewage water (effluent) Canada	0.359 μg/l	Metcalfe, 2004
	Surface water adjacent to wwtp (Canada)	0.026 μg/	Metcalfe, 2004
	Influent sewage water (Switzerland)	310-1920 ng/l	Buser et al., 1999
	Effluent sewage water (Switzerland)	310-930 ng/l	Buser et al., 1999
	Surface water (Switzerland)	12-370 ng/l	Buser et al., 1998
	Surface water (Germany)	26-67 ng/l	Weigel et al., 2004
	Elbe Estuary (Germany)	6.2 ng/l	Weigel et al., 2002
Ibuprofen	Effluent sedimentation from tank	12 μg/l	Halling-Sörensen et al, 1998
	River Rhine	<5-41ng/l	Halling-Sörensen et al, 1998
	Different river water samples	17-139 ng/l	Halling-Sörensen et al, 1998
	Sewage water rheumatic hospital (influent)	77.2-116.3 μg/l	Läkemedelsverket, 2004*
	Sewage water (effluent) Canada	1.885 μg/l	Metcalfe, 2004
	Surface water adjacent to wwtp (Canada)	0.06 μg/l	Metcalfe, 2004

Substance	Matrix	Concentration	Reference
	Untreated leachate water(Norway)	0.62-3.7µg/l	SPFO-rapport: 949/2006
	Treated leachate water (Norway)	0.61-5 µg/l	SPFO-rapport: 949/2006
	Influent sewage water (Norway)	1.5-3.9 µg/l	SPFO-rapport: 949/2006
	Sediment (adjacent to STP, Norway)	2.8 µg/kg	SPFO-rapport: 949/2006
	Surface water (adjacent to STP, Norway)	0.14-0.036 µg/l	SPFO-rapport: 949/2006
	Influent sewage water (Switzerland)	1-3.3 µg/l	Buser et al., 1999
	Surface water (Switzerland)	7.8 ng/l	Buser et al., 1999
	Surface water (Germany)	4.7-32 ng/l	Weigel et al., 2004
	Elbe estuary (Germany)	0.6 ng/L	Weigel et al.,2002
	Sewage water (effluent, Sweden)	7.11 µg/l	Andreozzi et al, 2003
Ketoprofen	Sewage water rheumatic hospital (influent)	Detectable	Läkemedelsverket, 2004*
	Sewage water (effluent) Canada	0.130 µg/l	Metcalfe, 2004
	Surface water adjacent to wwtp (Canada)	0.01µg/2	Metcalfe, 2004
	Elbe estuary (Germany)	n.d	Weigel et al.,2002
	Sewage water (effluent Sweden)	Not detected	Andreozzi et al, 2004
Naproxen	Sewage water (effluent) Canada	0.168 µg/l	Metcalfe, 2004
	Surface water adjacent to wwtp (Canada)	0.09 µg/4	Metcalfe, 2004
	Sewage water rheumatic hospital (influent)	Detectable	Läkemedelsverket, 2004*
	Sewage water (effluent, Sweden)	2.15 µg/l	Andreozzi et al.2003
Estrogens	Raw sewage wastewater	0.2-0.5 nmol/l	Halling-Sörensen et al, 1998
	Treated sewage wastewater for irrigation	Measurable concentrations	Halling-Sörensen et al, 1998
Estrogen/estradiol and estrone Estradiol	Urinary excretion from pregnant women	10 µmol/day	Halling-Sörensen et al, 1998
Ethinylestradiol	Surface water	< 0.2 ng/l	Halling-Sörensen et al, 1998
	Effluent from sedimentation tank	0.3-0.5 ng/l	Halling-Sörensen et al, 1998
	River water	2-15 ng/l	Halling-Sörensen et al, 1998
	Reservoir	1-3 ng/l	Halling-Sörensen et al, 1998
	Drinking water	< 5 ng/l	Halling-Sörensen et al, 1998
	Incoming sewage water (Norway)	0.00046-0.0017 µg/l	SPFO-rapport: 949/2006
	Effluent sewage water (Norway)	0.00034-0.00081 µg/l	SPFO-rapport: 949/2006

* Reference cited in

5.2 Reduction of NSAID´s in STPs

In a study at a rheumatic care hospital in Sweden several medical substances were measured in the separate sewage treatment plant belonging to the hospital in both influent and effluent water.

Ibuprofen, naproxen, ketoprofen were removed to an extent of 80-99%, while diclofenac only was removed by 0-70% (reference cited in Läkemedelsverket, 2004)

The impact of the temperature on the removal of pharmaceuticals in sewage water has been investigated in a STP in Finland. The occurrence of the five pharmaceuticals; ibuprofen, naproxen, ketoprofen and diclofenac, was measured in the influent and effluent water of the STP as well as in recipient- and drinking water. The concentrations of the studied chemicals in the effluent water were 3-5 times higher in wintertime (about 2500 ng/L) than during the other seasons (about 500-900 ng/L). Accordingly, the highest concentrations, up to 129 ng/L, in the recipient water were measured during the wintertime. The substances were also transported longer distances downstream from the STP when the river was covered by ice and snow. During the snow melting there was a drastic increase of water flow and hence a faster transportation of the pharmaceuticals. In wintertime, ibuprofen and ketoprofen were detected in drinking water (~89 ng/L) while they were below detection limit during the summer period (Vieno et al., 2005). A suggested explanation of increased levels of pharmaceuticals during wintertime included a lower biodegradation rate in the STPs when the temperature of the water decreased.

The ability of elimination of pharmaceutical substances in STPs was also investigated in a study including six different STPs in four different countries. Diclofenac was removed to an extent of 10 -80% naproxen 42-93 % and ibuprofen of 52-90% (Andreozzi, 2003).

In a study in Switzerland a removal efficiency for ibuprofen of 96-99.9 % was observed in a STP as the influent concentrations were 25-1000 times higher then effluent concentrations. Degradation kinetics indicated that a residence time of wastewater of 6 h was needed for complete removal of ibuprofene (Buser et al., 1999). In another Swiss study diclofenac was eliminated to an extent of more than 90% in a lake (comparison between influent and effluents, Buser et al., 1998).

6 Sampling programmes and study sites

6.1 National sampling programme

A national sampling strategy was developed in order to determine the environmental concentrations of selected pharmaceuticals in different environmental matrices in Sweden. An additional aim of the sampling programme was to identify major emission sources as well as important transport pathways. The sampling programme was based on identified possible emission sources and the behaviour of the substances in the environment.

Municipal sewage treatment plants (STPs) were identified as the most important source for the occurrence of the pharmaceuticals in the aquatic environment. STP samples e.g. sludge is often used as indicator for diffuse spreading of chemicals to the environment. However samples from STPs were not included in the national program as they were the main focus in the regional screening programme, see 6.2.

One of the depicted routes of pharmaceuticals into the environment is residual medicine being added to the domestic garbage disposal system. Regarding point sources one of the main issues in selecting appropriate sampling schemes resided subsequently in whether pharmaceuticals could be detected in landfill effluents.

In order to identify additional potential point sources the sampling program also included measurements close to livestock facilities such as a pig breeding farm, a horse racing stable as well as a grazing field for cattle. Manure, water and sediment were sampled. As for pig manure, both firm and buoyant manure were included in the sampling. Additionally effluent water samples from hospitals were included.

Environmental background levels in water and sediment were determined in samples from three background lakes where the influence from human activities was considered minor. Finally, to investigate human exposure some foodstuff samples were also analysed.

The sampling program is summarised in Table 10. Site information and sample characteristics of the samples collected within the national program are given in Appendix 2.

Table 10. Sampling scheme for the national sampling programme

Source	Site	Sediment	Water	Sludge/ manure	Biota	Total
Background	Lilla Öresjön	1	1			2
	Stora Envättern	1	1			2
	Tärnan	1	1			2
Point source	Horse stable	1	2	3		6
	Pig Breeding	2	3	2		7
	Cattle breeding		2			2
	Hospital		1			1
	Henriksdal STP			1		1
	Waste disposal		1			1
	Högbytorp waste disposal		2			2
Human exposure	Food stuff				3	3
Total		6	14	6	3	29

In order to investigate the estrogenic and androgenic impact 19 samples were selected for androgenicity and estrogenicity testing on yeast strains and the results were compared with the measured chemical concentrations.

6.2 Regional sampling programmes

Swedish county administrative boards had the possibility to add regional samples to the national sampling programme. The main focus in the regional screening programme was to take samples from municipal sewage treatment plants. A vast majority of samples were taken from either effluent water (54 samples) or STP-originating sludge samples (60 samples). Also influent samples (20 samples) were included. In addition, there were also some leachate samples from landfills (6 samples), effluent water from hospitals (6 samples) and surface water samples (5 samples) as well as one sediment, and one biota sample. In total fourteen regional county administrative boards participated in the regional screening programme.

Detailed information about sampling sites and sample characteristics of the samples included in the regional program are given in Appendix 3 and Appendix 4.

7 Methods

7.1 Sampling

As a guideline for adequate and consequent sampling, a manual for the sampling personnel in the national as well as the regional screening programs was developed. Detailed instructions for sampling, storing and transport were outlined. Sampling protocols for all sample types were included in the manual. The overall aim of the sampling protocols was to:

1. Guide the responsible personnel on how to avoid contamination when sampling.
2. Ensure documentation of the sampling procedure, quality of the sample as well as environmental and physical circumstances during the sampling.

The samples from the regional county administrative boards were sent to IVL Swedish Environmental Research Institute.

7.1.1 Water

Water samples were collected in cleaned plastic bottles and stored at 4°C or -18°C until analysed. A bottle with ultra pure water (Milli-Q), which was exposed to the surrounding environment during the sampling time, was used as a field blank.

7.1.2 Sediment

Sediment samples from lakes were collected by means of a Kajak sampler. The sediment core was sliced and transferred into plastic jars and stored at 4°C or -18°C until analysed. A plastic jar filled with diatomaceous earth (10 % water) that was exposed to the surrounding environment during the sampling time was used as field blank.

7.1.3 Sludge and manure

The staff at the different treatment plants collected the sludge samples from the anaerobic chambers. The sludge was transferred into plastic jars and stored at 4°C or -18°C until analysed. A plastic jar filled with diatomaceous earth (10 % water), which was exposed to the surrounding environment during the sampling, was used as a field blank. Manure samples were collected from live stock facilities (pigs, cattle and horses) and transferred to plastic jars and stored at 4°C or -18°C until analysed.

7.2 Analysis

7.2.1 Chemicals

The solvents (HPLC-quality) acetone, hexane and metyl-*tert*-butylether (MTBE) were delivered from Rathburn (Chemical Ltd., Peeblesshire, Scotland). Solid phase columns containing a macropolymeric sorbent (Oasis SPE-kolonn HLB (200 mg) was delivered from Waters (Waters Corporation, MA, USA). Sodium sulphate and silica gel was delivered from Merck (Darmstadt, Germany) and pre-heated (400°C) prior to use. All solvents and chemicals were checked by GC-MS before use. Ultra pure water was produced by a Milli-Q plus equipment (Millipore Corporation, Bedford, MA, USA). The surrogate standards used for quantification of NSAIDs were; 3-phenylpropionic acid (Fluca Chemie AG, Buchs, Switzerland), 2-(2,4,5-trichlorophenoxy)propanoic acid (2,4,5-TP)(Aldrich Chemie, Steinheim, Germany) and 1-Naphthoic acid. (EGA Chemie Steinheim, Germany). Surrogate standard used for quantification of hormones and antibiotics were D_3,-β-estradiol and meclocycline, respectively. Diphenyl, used as volumetric standard, was delivered from Accustandard, CT, USA.

7.2.2 Extraction

NSAIDs

Water samples (500-1000 ml) analysed with regard to NSAIDs were filtrated (pre-heated GF/C-filter) before solid phase extraction (SPE-extraction). The filters were saved and stored in a freezer. The SPE-columns (Oasis HLB 200 mg) were cleaned and activated prior use with hexane, ethyl acetate, methanol and water. The filtrates were acidified, spiked with surrogate standards and subsequently concentrated on SPE-columns. The flow rate during extraction was ~15 ml/min. The SPE columns were rinsed with acidified water and hexane and the NSAIDs were then eluted using ethyl acetate as mobile phase. The extract was dried over sodium sulphate and evaporated to complete dryness and re-dissolved in hexane. Extracts of sewage waters were back-extracted prior to derivatization (see below).

Solid samples of sediment, sludge and manure were worked-up in a similar manor as water samples. The samples were transferred to test tubes (~5 g of sludge/manure, 10 g of sediment, respectively) where the samples were carefully acidified and extracted with acetone and after that with a mixture of acetone: MTBE. The procedure was performed using an ultra sonic bath and gentle agitation. The extracts were combined and mixed with water and subsequently extracted twice using hexane : MTBE as solvent. The extracts were dried over sodium sulphate for 15 min. After transferring the dried organic phases to new test tubes they were evaporated to complete dryness and re-dissolved in hexane. The hexane phases were then back-extracted, derivatized, cleaned-up on a silica gel column and analysed (GC-MS) as described below.

Fish samples (muscle 10 g) were fortified with recovery standards acidified and homogenized in acetonitrile followed of a 30 minute-period of agitation. The extraction was repeated in the same manor with a second aliquot of acetonitrile. The combined extract was diluted with water and extracted twice with hexane: MTBE. The extracts were dried over sodium sulphate for 15 min.

After transferring the dried organic phases to new test tubes they were evaporated to complete dryness and re-dissolved in hexane. The extracts were then treated (clean-up) in the same manor as the solid samples (see above).

Hormones and antibiotics

The **water** samples (550 ml) were extracted according to Lindsey and Meyer et al., (2001). Briefly, the samples were acidified using a small aliquot of sulphuric acid or hydrochloric acid. A complexing agent, Na_2-EDTA, was added and the samples were slowly agitated for an hour. Most of the water samples were filtrated (pre heated GF/C-filter) before SPE-extraction due to the high content of particulate material. The filters were extracted separately (for details see below). The SPE-columns (Oasis HLB column 200 mg) were cleaned and activated prior use with hexane, ethyl acetate, methanol and water. The filtrates were weighted, spiked with surrogate standards and subsequently concentrated on the SPE-columns. The SPE-columns were rinsed with water, and then eluted using methanol accompanied with ethyl acetate. The eluates were pooled and evaporated to dryness and used for LC-MS analysis (see below).

The filters used were extracted twice with small aliquots of ethyl acetate combined with a 30 minute-period of agitation. Filter-extracts were then pooled with water-SPE extracts (prior to evaporation).

The frozen samples of **sludge, sediment, manure and biota** were thawed and spiked with internal standard (meclocycline in the case of antibiotics and D_3-β- estradiol in the case of hormones). The sample extractions of tetracyclines were performed by means of aqueous ion pair extraction followed by a solid phase extraction clean up step. The hormones were extracted in methanol using whirl mixing and sonication. The sample extracts were further cleaned up (see below).

The sample extractions of hormones in **biota** samples were performed as follows: the samples were thawed, spiked with internal standard (D_3-β- estradiol) and homogenised with potassium sulphate and extracted by use of whirl mixing and sonication. The sample extracts were further cleaned up by liquid-liquid extraction and solid phase extraction (see below).

Sample preparation for tests of androgenic and estrogenic effects

Water samples (0.5 – 1 l) were adjusted to pH 2.5 – 3.1 with concentrated HCl and the salinity was checked by conductivity. If necessary, solid NaCl was added to give a conductance corresponding to that of 5 g NaCl L^{-1}. Extraction of samples was carried out using solid phase extraction (SPE) with prepacked columns (ENV+, Sorbent AB, Västra Frölunda) containing 0.2 g of polystyrene divinylbenzene copolymers according to a published procedure (Körner et al. 1999, Svenson et al. 2003). Before use each SPE column was successively rinsed with two portions of 5 mL acetone and two portions of 5 mL 1 mM HCl. Water samples were then passed through the columns by suction at flow rates of approximately 100 – 500 ml/h. Then columns were washed twice with 5 ml HCl (1 mM) and dried under reduced pressure. Elution was performed with four portions of 2 ml acetone. Dimethylsulfoxide (100 µl, 99.5 %, Sigma-Aldrich Sweden) was added and the eluate mixed and divided into four equal portions. The acetone in each portion was then evaporated with a gentle stream of nitrogen. The final extracts were stored at –18 °C until assay.

7.2.3 Clean-up of sample extracts

NSAIDs

Samples (water, sediment and sludge) containing large amounts of organic debris were also subjected to a developed **back-extraction** (liquid-liquid extraction) routine where the extracts were vortexed with borax buffer and methanol. The buffer phases, containing the NSAIDs, were transferred to a new test tube, diluted with water, acidified and subjected to two rounds of hexane: MTBE extraction. The solvent was removed under a stream of nitrogen before derivatisation.

The sample was derivatised (methyl esterification) using methyl chloroformate (MCF) according to Weigel et al. (2002), Butz and Stan (1993. The dried extracts were resolved in reagent solvent containing acetonitrile/methanol/water/pyridine. A small aliquot of MCF was added and the sample vortexed. Subsequently, another aliquot of MCF was added the samples were vortexed for and left for 10 minutes to complete the reaction. The derivatization was stopped by the addition of water. The samples were then extracted using hexane: MTBE and gently dried and desolved in hexane. Prior to GC-MS analysis, the samples were subjected to a silica gel chromatographic purification routine where a deactivated silica gel column was prepared in a Pasteur pipette. The hexane-dissolved samples were applied on top of the pipette and the neutral non polar compounds were eluted with hexane and then a second fraction, containing the target substances, was eluted using hexane: MTBE. After a gentle drying to reduce the sample volume diphenyl was added as volumetric standard.

Hormones and antibiotics
The hormone extracts prepared from the sludge, sediment, and manure samples were cleaned up by centrifuge steps and solid phase ion exchange in order to reduce the matrix effects. The biota samples used for hormonal analyses were homogenised with potassium sulphate and extracted in MTBE by use of whirl mixing and sonication. The sample extracts were further cleaned up by liquid-liquid extraction and solid phase extraction. The sample treatment of the tetracycline samples included a solid phase extraction clean up step.

7.2.4 GC-MS Analysis

NSAIDs

The NSAID-extracts were analysed on a 6890N gas chromatograph coupled to a 5973N mass selective detector (Agilent). The injection, 1 µl, was done in splitless mode at 275°C. The fused silica capillary column (VF-5MS 30 m x 0.25 mm i.d. x 0.25 µm film thickness, Varian) was held at 45°C for 1 min., ramped 15°C/min to 200°C, 5°C/min until 300°C and held at 300°C for 5 min. Helium was used as carrier gas. The detector was used in selected ion monitoring mode (SIM) with electron ionisation at energy of 70 eV. The analytes were identified by their characteristic retention time and one quantification ion (Q-ion) and one or two supporting ions (S-ion) used to increase specificity was recorded (see Table 11) Quantification was based on comparison of peak abundance to the known response of the internal standard (2.4.5-TP). The reported analyte concentrations were corrected according to the determined surrogate standard losses.

Table 11: Ions utilized in the MS-quantification

Compound	Q-ion	S-ion 1	S-ion 2	Mw
Ibuprofen	161	177	220	220
Diclofenac	214	242	309	309
Ketoprofen	209	268	-	268
Naproxen	185	244	-	244
2,4,5-TP	198	282	223	282

7.2.5 HPLC-MS Analysis

Antibiotics
Liquid chromatography was performed with an Agilent 1100 liquid chromatography system (Agilent Technologies, Waldbronn, Germany), equipped with an autosampler, a quaternary pump, an on-line degassing system and a diode array detector (UV). The separation was performed using a reversed phase C18 column (Atlantis dC18, 2.1 mm ID x 150 mm length, 3 µm, Waters, Milford USA). A stainless steel inlet filter (Supelco, 0.8 µm) was used in front of a pre-column with the same stationary phase as the separation columns. Gradient elution was performed with 0.075% formic acid in water as solvent A and 0.075% formic acid in acetonitrile as solvent B. The binary gradient had a flow rate of 0.2 ml min-1 and started with 100 % A. Solvent B was introduced linear up to 99% at 22 minutes and kept isocratic until 32 minutes. At 32.5 minutes the setting was 100 % A and the column was equilibrated up to a runtime of 40 minutes with increased flow rate (0.5 ml/min). The analytical detector was a Micromass LCT orthogonal-acceleration time-of-flight (TOF) mass spectrometer (MS) equipped with a Z-spray electrospray ion source and a 4 GHz time to digital converter (TDC) (Micromass Ltd., Wythenshawe, Manchester, UK). The instrument was operated in positive ion mode and the electrospray source parameters were optimised to the following values; sample cone cycling 20/30 V, capillary voltage 2.8 kV, extraction cone 3 V, source temperature 130 ^{0}C, desolvation temperature 350 ^{0}C, cone gas flow 24 l h-1 and desolvation gas flow 600 l h-1. The pusher frequency was operated in automatic mode. The data processing and instrument (HPLC/HRMS) control were performed by the MassLynx software, and quantification was performed with signal extraction of a peak width of 90 mDa (typical).

Table 12: Molecular Ion Adduct and Confirming Ions

Compound	MW	$\{M+H\}^+$	Confirming ion
Oxytetracycline	460	461	444
Tetracycline	444	445	428
Chlorotetracycline	478	479	481
Doxycycline	444	445	428
Demeclocycline	463	464	466
Meclocycline-ISTD	476	477	460

Hormones
Liquid chromatography was performed with an Agilent 1100 liquid chromatography system (Agilent Technologies, Waldbronn, Germany), equipped with an autosampler, a quaternary pump, an on-line degassing system and a diode array detector (UV). The compound separation was performed using a reversed phase C18 column (Atlantis dC18, 2.1 mm ID x 150 mm length, 3 µm, Waters, Milford USA). A stainless steel inlet filter (Supelco, 0.8 µm) was used in front of a pre-column with the same stationary phase as the separation columns. Two different gradient elution profiles were used, one for estrogens and one for norethindrone and progesterone. Estrogens;

Water as solvent A and acetonitrile as solvent B and addition of ammonium hydroxide post column in order to improve the analytical sensitivity. The binary gradient had a flow rate of 0.25 ml min-1 and started with 80 % A. Solvent B was introduced linear up to 100% at 10 minutes and kept isocratic until 12 minutes. The flow rate was increased to 0.5 ml/min 12.2 min for column flushing and equilibration. The total runtime was 21 minutes. The same gradient profile was used for norethindrone/progesterone separation without post column addition of ammonium hydroxide. The analytical detector was a Micromass LCT orthogonal-acceleration time-of-flight (TOF) mass spectrometer (MS) equipped with a Z-spray electrospray ion source and a 4 GHz time to digital converter (TDC) (Micromass Ltd., Wythenshawe, Manchester, UK). The instrument was operated in positive mode for norethindrone and progesterone and negative mode for estrogens. The electrospray source parameters were optimised to the following values: Negative mode: sample cone 38 V, capillary voltage 2.85 kV, extraction cone 3 V, source temperature 125 ^{0}C, desolvation temperature 350 ^{0}C, cone gas flow 24 l h-1 and desolvation gas flow 600 l h-1. Positive mode: sample cone 16 V, capillary voltage 3.5 kV, extraction cone 3 V, source temperature 125 ^{0}C, desolvation temperature 350 ^{0}C, cone gas flow 24 l h-1 and desolvation gas flow 600 l h-1The pusher frequency was operated in automatic mode. The data processing and instrument (HPLC/HRMS) control were performed by the MassLynx software, and quantitation was performed with signal extraction of a peak width of 90 amu (typical).

Table 13: Molecular Ion Adduct

Compound	MW	$\{M-H\}^-$	$\{M+H\}^+$
Estriol	288	287	
Estradiol	272	271	
D_3-Estradiol-ISTD	275	274	
Ethinyl estradiol	296	295	
Norethindrone	298		299
Progesterone	314		315

7.2.6 Androgenicity assay procedure

A recombinant yeast strain of *Saccharomyces cerevisiae* was used for assay of androgenicity as previously described (Sohoni and Sumpter 1998). The genome of this strain contains the constitutively expressed gene for the human androgen receptor protein. This protein controls the expression of the reporter gene *lac-Z* that produces β-galactosidase. Enzyme activity is measured using the chromogenic substrate, chlorophenol red-β-D-galactopyranoside (CPRG), which forms a red product. Assays were performed on SPE extracts of wastewaters in microtitre plates with serial dilutions of the samples and standard solutions. Each plate contained a negative control containing only growth medium, a dilution series (23 - 15000 ng/l) of dihydrotestosterone (DHT) as positive control, and a single series of 12 dilutions of 1 to 6 effluent extracts. The dilution factor was 1.8 in the positive control and 2.0 otherwise. For each run, three plates were incubated in parallel for 3-4 days until the colour of the positive control was fully developed. The absorbance was measured spectrophotometrically at 540 nm using an automatic plate reader (Spectracount, Packard Instrument Co., Meriden, USA).

Absorbance measurements for samples, and positive and negative controls were used to calculate EC_{50} values by non-linear regression of the dose-response curves. The calculated optical density (OD_{calc}) was obtained from the following equation:

$OD_{calc} = OD_{min} + (OD_{max}-OD_{min}) \times (C/EC_{50})^s/(1+(C/EC_{50})^s)$

where OD_{min} is the baseline (blank) optical density, OD_{max} is the optical density of the fully induced cells, C is the concentration of the compound in the positive control, EC_{50} is obtained in concentration units, and s is the slope of the dose curve. The Microsoft Solver Program was used to calculate the EC_{50} and slopes for the minimum of the sum of the squares of the deviations between calculated and observed optical densities by variation of the values of EC_{50} and slopes. Assays of wastewater extracts were evaluated similarly using dilution factors as concentrations. The EC_{50} values were expressed as dilutions and recalculated to ng DHT units L^{-1}.

7.2.7 Estrogenicity assay procedure

The test of estrogenicity in SPE extracts of wastewater samples was performed with a recombinant yeast strain, *Saccharomyces cerevisiae,* with the human estrogen receptor α gene incorporated in the main chromosome (Routledge, Sumpter, 1996). The yeast cell also contained plasmids carrying the estrogen response element and the reporter gene *lacZ* coding for β-galactosidase. This enzyme released into the cell medium will catalyse the conversion of the chromogenic substrate, chlorophenol red-β-D-galactopyranoside, CPRG, into a red product, which is measured by spectrophotometry.

The composition of media and microtitre plate procedure followed published descriptions (Routledge, Sumpter, 1996; Beresford et al., 2000). Each test was run in triplicate. A positive control, 17β-estradiol in ethanol, was run on each plate. This control sample was serially diluted with ethanol in twelve concentrations with a dilution factor of 1.8 and wastewater extracts with a dilution factor of 2.0. The plates were incubated for three days in darkness at 30 °C. After incubation each plate was shaken for 30 seconds and left to settle an hour before the absorbance was read at 540 nm in a plate reader (Spectracount, Packard).

Dose-response curves measured as absorbance at 540 nm at different concentrations of controls and sample extracts were evaluated by a non-linear, exponential fit to the experimental data using the Solver programme in Microsoft Excel. EC_{50} values and slopes (s) of dose curves were derived from a minimisation of the sum of deviations of the non-linear fit and the experimental data calculated according to the equation:

$OD_{calc.} = OD_{min} + (OD_{max}-OD_{min})*(C_i/EC_i)^s/(1+(C_i/EC_i)^s)$

where OD_{min} is the baseline (blank) optical density, OD_{max} is the optical density of the fully induced cells, C is the concentration of the compound in the positive control, EC_{50} is obtained in concentration units, and s is the slope of the dose curve. EC_{50} values were expressed in number of dilutions and those of the positive controls in ng/l. By combination of these data, the estrogenicity in wastewater was calculated in estradiol units and expressed in ng E2 units/l.

7.2.8 Immunoassay procedure

Ethinylestradiol (EE2) in extracts of water samples was analysed using an immunoassay (ELISA) analysis kit (Japan Envirochemicals Ltd, Tokyo, Japan).

8 Results and discussion

The concentrations of the antibiotics, the anti-inflammatory substances and the hormones found in the different samples from the national and regional screening are given in Appendix 5 and Appendix 6. The results for the different pharmaceutical groups are summarised and discussed below.

8.1 Antibiotics

8.1.1 Background sites

The analysed antibiotics were not detected in the samples collected at the background sites with the exception of one water sample from Lake Tärnan where chlorocycline was found in a concentration of 0.001 µg/l. The detection limits are given in Table 14.

Table 14. Detection limits for antibiotics in background samples

Site	matrix	Unit	Oxytetra-cycline	Tetra-cycline	Demeclo-cycline	Chloro-cycline	Doxy-cycline
Tärnan	water	µg/l	<0.0001	<0.0003	<0.0005	*0.0010*	<0.0007
Stora Envättern	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004
Lilla Öresjön	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004
Tärnan	sediment	µg/kg dw	<4	<2	<4	<8	<4
Stensjön	sediment	µg/kg dw	<7	<3	<7	<13	<7
Lilla Öresjön	sediment	µg/kg dw	<8	<4	<8	<17	<8

The variation in the detection limit for the same analyte depends on factors such as differences in sample dry weight and recovery during the analytical procedures.

8.1.2 Diffusive sources - municipial sewage treatment plants

Influent and effluent water
The concentrations of the antibiotic substances found in influent (20 samples) and effluent sewage waters (54 samples) are summarised in Figure 5. The concentrations and distribution among the selected substances in the influent and effluent water at the different STPs are shown in Figure 6 and Figure 7.

A great variation in the concentrations among the different STPs as well as in the distribution of the analysed substances was observed. The most frequently found antibiotic substance in the influent water was tetracycline, which was detected in 17 out of 20 influent samples. Oxytetracycline and doxycycline were detected in 12 and 10 samples respectively, chlorocycline in

two and demeclocycline in one influent sample (the Sternö STP in Karlshamn). The concentration ranges were: oxytetracycline <0.0003 - 0.79 µg/l, tetracycline<0.0002 - 1.8 µg/l, demeclocycline <0.0003 - 0.049 µg/l chlorocycline <0.0005 - 0.34 µg/l and doxycycline <0.0004 - 2.3 µg/l.

17 out of 54 effluent water samples contained antibiotic residues in concentrations above LOD. The most frequently found antibiotic drug; tetracycline was detected in 10 influent samples whereas chlorocycline and doxycycline were only found occasionally and demeclocycline was not detected at all (LOD>0.0003).

The antibiotics found in the effluent water mostly occurred in lower concentrations than in influent water. Chlorocycline however, occurred occasionally in higher concentration in effluent water compared to the influent water samples. The concentration ranges was: oxytetracycline <0.0003-0.26 µg/l, tetracycline <0.0002 - 0.20 µg/l, chlorocycline <0.0005 - 0.68 and doxycycline <0.0004-0.22 µg/l.

Lindberg et al have previously reported levels of 0.064-0.9 µg/l of doxycycline in Swedish effluent waters from STPs (Lindberg et al., 2005).

The STPs that provided samples are sorted in approximate geographical order from north to south in Figure 7. Concentrations of antibiotics in effluent water seemed to be higher in samples originating from STPs located in the north part of Sweden even though the pattern is less clear than in the case of the NSAIDs (see chapter 8.2.2). Chlorocycline stands out as the main contributor to this pattern. This substances was detected in increased concentrations in the samples from the Kavaheden STP in Gällivare (0.56 µg/l) and the Åredalen STP in Åre (0.68 µg/l). The STP-processes may attribute to the observed differences in concentrations e.g. via biodegradation of chlorocycline which may be temperature dependent and these observations may thus reflect the differences in climate rather than consumption as no regional differences were identified for the nfluent water.

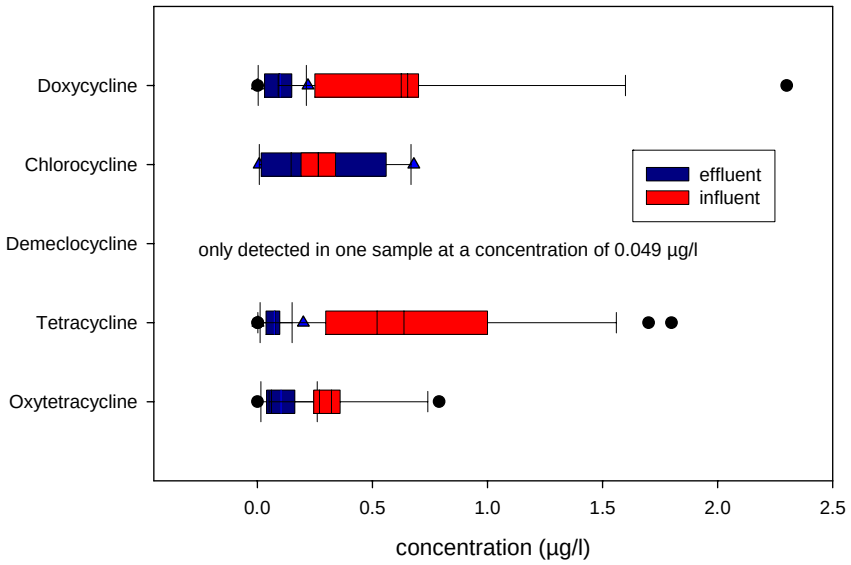


Figure 5. Concentrations of antibiotics in influent (20 samples) and effluent water (54 samples) from municipal STPs. The lower and upper boundaries of the box represent the 25- and 75-percentiles, the line within the box is the median concentration. The whiskers represent the 10- and 90-percentiles, and the dots (influent) and triangles (effluent) are individual results outside this range.

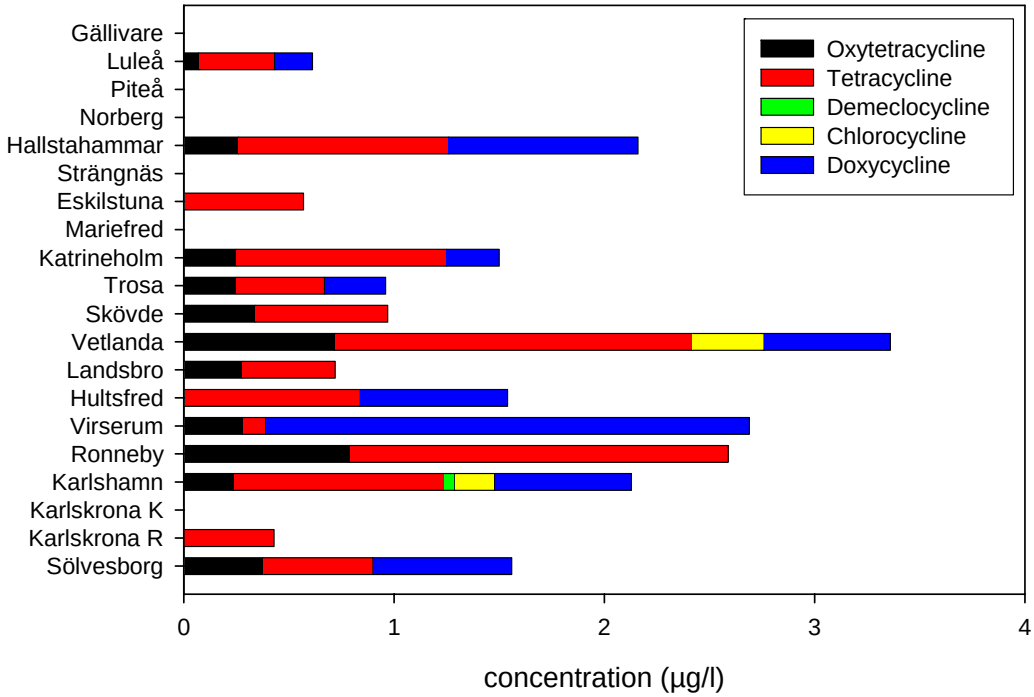


Figure 6. Concentrations of antibiotics in influent water to municipal STPs

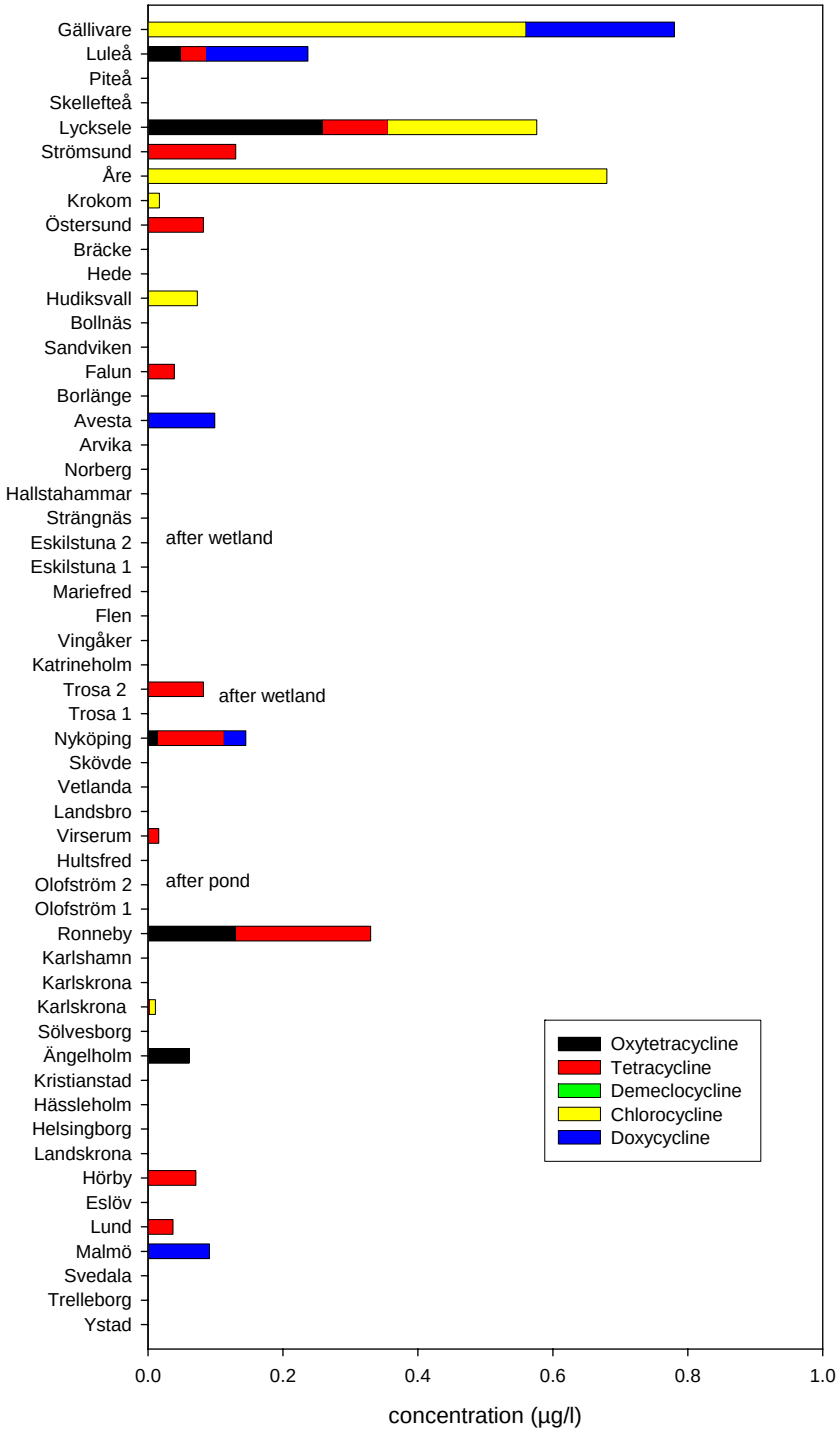


Figure 7. Concentrations of antibiotics in effluent samples from municipal STPs approximately sorted from north to south. In Eskilstuna and Trosa samples were taken both before and after a wetland (marked with the figure 2 in the picture) and in Olofström samples were taken before and after an aeration pond (marked with the figure 2 in the picture).

Sludge

The concentrations of the antibiotic substances found in sludge are summarised in Figure 8. The concentrations and distribution in sludge from individual STPs are shown in Figure 9. Most of the sludge samples included in the screening were processed i.e., digested and dehydrated. Anaerobic digestion of sludge results in loss of roughly half of the solids, and digested sludge is therefore denser than the raw sludge. In the dehydrated sludge the water content has been reduced by physical means, for instance by centrifugation or mechanical pressing.

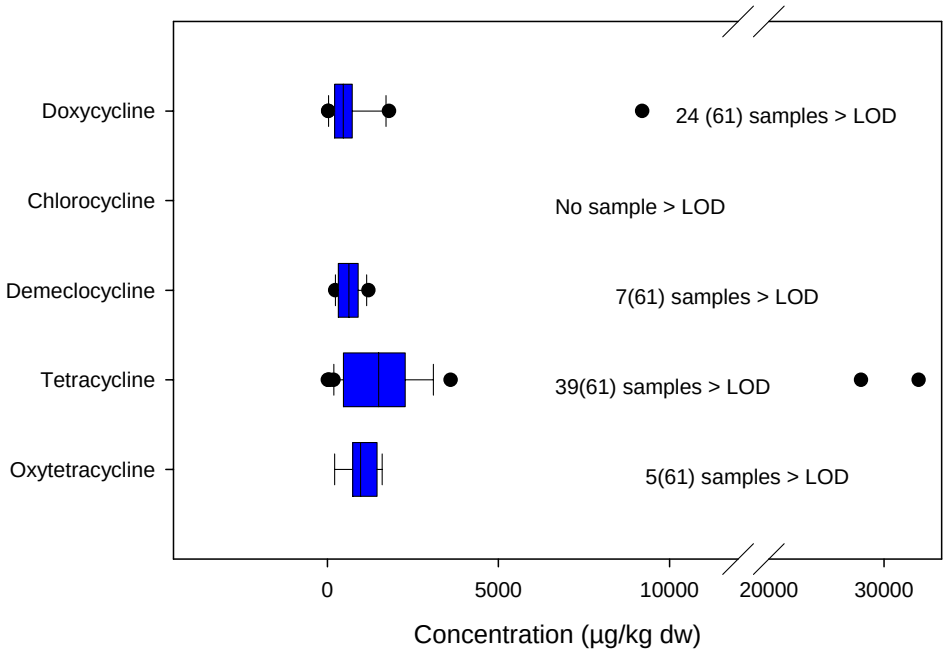


Figure 8. Concentrations of antibiotics in sludge from municipal. The lower and upper boundaries of the box represent the 25- and 75-percentiles, the line within the box is the median concentration. The whiskers represent the 10- and 90-percentiles, and the dots are individual results outside this range

Tetracycline and doxycycline were the most frequently found substances in sludge. They were detected in 39 and 24 samples respectively out of 61 analysed samples. Demeclocycline and oxytetracycline were only found in a few of the samples while chlorocycline was not detected in any of the sludge samples. No obvious pattern in the distribution among the analysed substances between the different STPs could be observed.

There was a great variation in concentration of the different substances. Tetracycline varied between <3 - 33 000 µg/kg dw. The concentrations of the other substances varied from not detected to a maximum of: oxytetracycline 1400 µg/kg dw, demeclocycline 1200 µg/kg dw and doxycycline 9200 µg/kg dw. The highest concentrations of antibiotics were found in semi-processed sludge, subjected to neither digestion nor dehydration. However, in the non-dehydrated sludge, with the highest levels of tetracycline and doxycycline (Figure 9, Vetlanda STP, Västerås STP and Landsbro STP), the other antibiotic substances where not detected at all. Lindberg et al., have previously reported concentrations of 1300-1500 µg/kg dw of doxycycline in Swedish STP sludge (Lindberg et al., 2005).

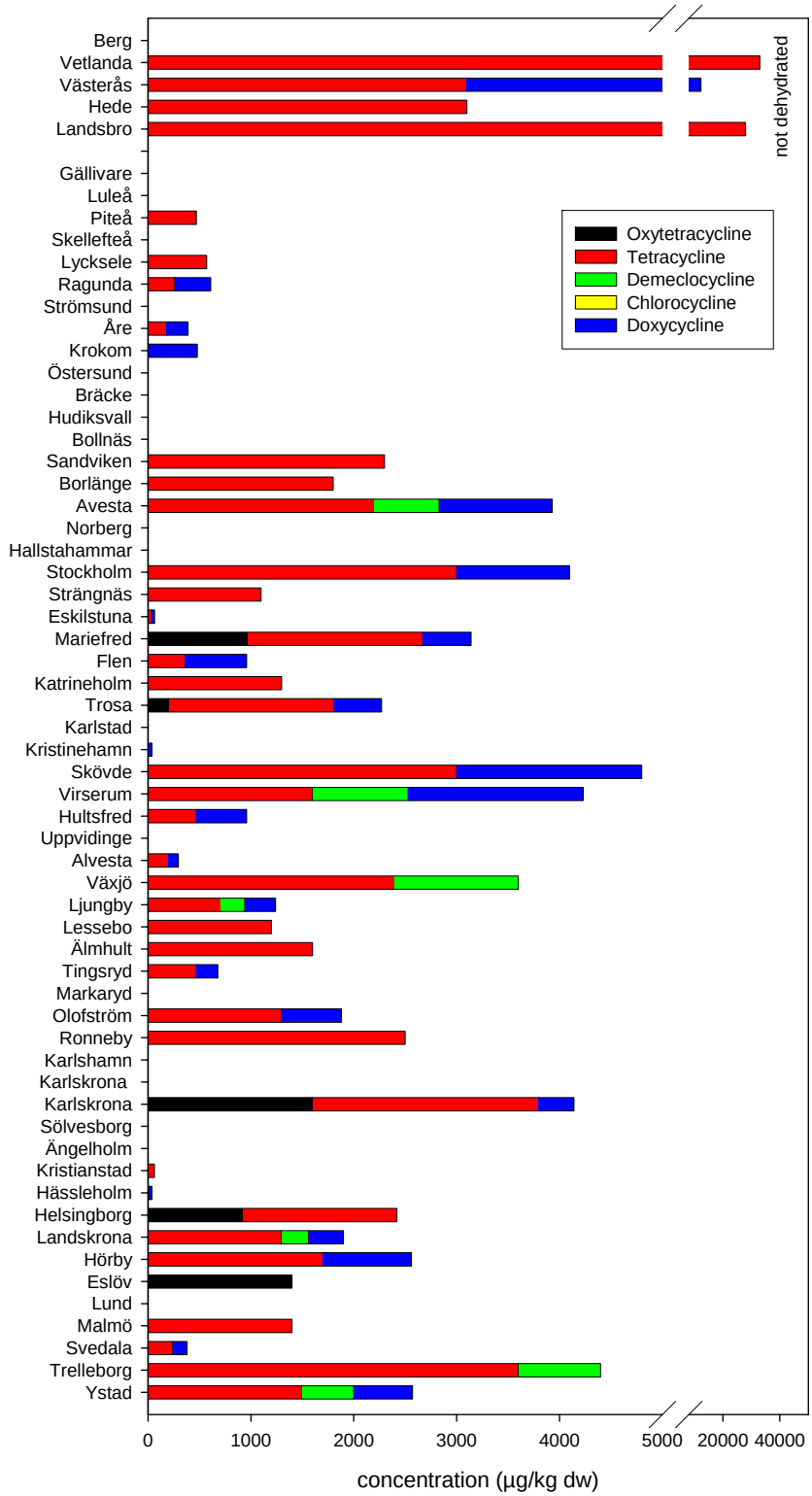


Figure 9. Concentrations and distribution of antibiotics in sludge from municipal STPs, approximately sorted from north to south.

8.1.3 Recipient water

The concentrations of the antibiotics in water samples collected in 2 different recipient lakes for STPs were below the detection limits. LOD were as follows: oxytetracycline <0.0003 µg/l; tetracycline <0.0002 µg/l; demeclocycline <0.0003 µg/l; chlorocycline <0.0005 mg/l and doxycycline <0.0004 µg/l.

8.1.4 Point sources - landfill

The concentrations of the analysed antibiotics were below the detection limits in the leachate water samples collected at landfills with the exception of the leachate sample from the Spillepeng landfill in Malmö. Concentrations where in following order; tetracycline, chlorocycline and doxycycline: 0.003, 0.005 and 0.091 µg/l. These concentrations are however lower than what was found for effluent water for STPs. LODs are given in appendix 5 och appendix 6.

8.1.5 Point source - hospital

In effluent water samples from hospitals, antibiotic residues were detected in 5 out of 7 samples. Tetracycline and doxycycline occurred in the highest concentrations (Figure 10) and these concentrations exceeded those found in influent water to STPs. Chlorocycline was only found in the sample from Huddinge (0.79 µg/l) and demeclocycline and oxytetracycline was not found in any hospital sample.

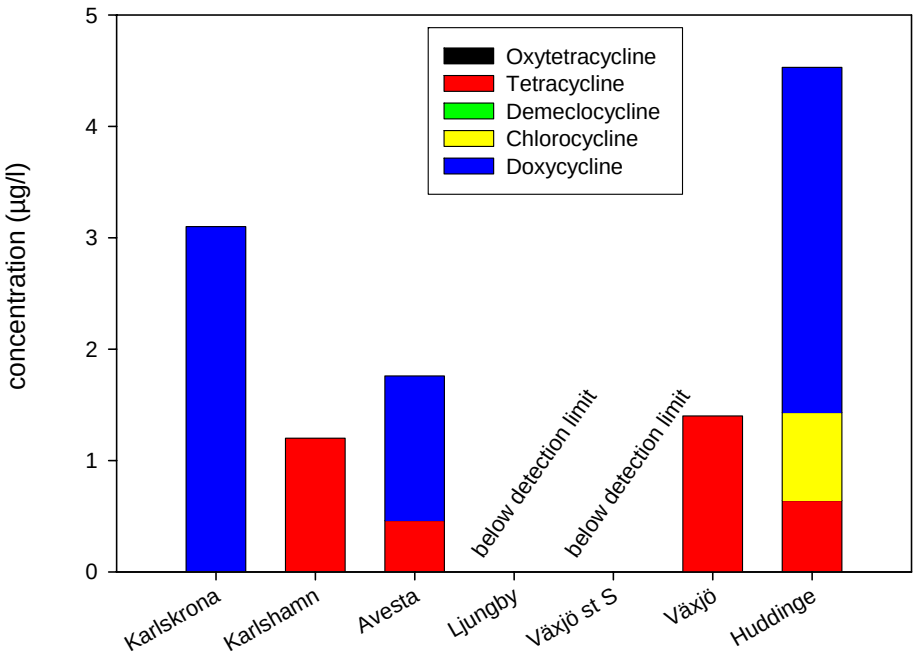


Figure 10. Concentrations of antibiotics in hospital effluents

8.1.6 Point source- animal breeding

The only antibiotic substance that was found in manure sample (horse) was tetracycline (400 µg/kg dw).

Antibiotics were not found in the water samples with the exception of the surface water collected in the proximity of a grazing field for cattle in concentrations as follows; oxytetracycline 0.010 µg/l, tetracycline 0.002 µg/l and chlorocycline 0.001 µg/l.

The only antibiotic substance that was found in detectable amounts in sediment samples was instead demeclocycline that occurred in the sediment sample from the proximity of the horse track area at a concentration of 29 µg/kg dw.

Table 15. Results and LOD for antibiotics in samples from animal keeping

Sample ID	Site	Matrix	unit	Oxytetra cycline	Tetra cycline	Demeclo cycline	Chloro-cycline	Doxy cyline
4425	Cattle farm	Water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004
4424	Cattle farm	Water	µg/l	*0.01*	*0.002*	<0.0003	*0.001*	<0.0004
4438	Horse track area	Manure	µg/kg dw	<5	*400*	<5	<10	<5
4439	Horse track area	Manure	µg/kg dw	<6	<3	<6	<12	<6
4440	Horse track area	Manure	µg/kg dw	<5	<3	<5	<11	<5
4456	Horse track area	Sediment	µg/kg dw	<2	<1	*29*	<3	<2
4435	Horse track area	Water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004
4436	Horse track area	Water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004
4431	Pig farm	Manure	µg/l	<1	<0.5	<1	<2	<1
4430	Pig farm	Manure	µg/kg dw	<6	<3	<6	<12	<6
4432	Pig farm	Sediment	µg/kg dw	<2	<1	<2	<3	<2
4433	Pig farm	Sediment	µg/kg dw	<2	<1	<2	<3	<2
4427	Pig farm	Water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004
4428	Pig farm	Water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004
4426	Pig farm	Water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004

8.1.7 Human exposure

The antibiotics were not found in any of the analysed food stuff samples. The limits of detection were: oxytetracycline <1 µg/kg ww; tetracycline <0.5 µg/kg ww; demeclocycline <1 µg/kg ww; chlorocycline <2 µg/kg ww; doxycycline <1 µg/kg ww.

8.2 Anti-inflammatory substances (NSAIDs)

8.2.1 Background sites

The anti-inflammatory substances were detected in one of the background lake water samples, Lilla Öresjön. The concentrations were: ibuprofen 0.041 µg/l, naproxen: 0.021 µg/l; ketoprofen: 0.008 µg/l and diclofenac: 0.002 µg/l. Several cottages were located around this lake and private drains not affiliated with any STPs may have affected it. A concentration of 0.0071 µg/l of ketoprofen was also detected in the lake Tärnan. No detectable amounts of the other anti-inflammatory substances were found neither in Tärnan nor in the third background lake water samples or in any of the sediment samples from the three background lakes. The detection limits are given in Table 16.

The NSAIDs were also detected in a source for drinking water in Kristinehamn (naproxen; ketoprofen, diclofenac 0.0023 µg/l;0.0017 µg/l and 0.0025 µg/l respectively). However, the concentrations were close to the limit of detection and in the same level or lower than concentrations found in the background lakes.

Table 16. Detection limits for the anti-inflammatory substances in samples from background areas

Detection limit	Matrix	Unit	Ibuprofen	Naproxen	Ketoprofen	Diclofenac
Background lakes	Water	µg/l	<0.003	<0.002	<0.002	<0.001
Tärnan	Sediment	µg/kg dw	<1	<1	<2.5	<1
Stensjön	Sediment	µg/kg dw	<1.8	<1.8	<4.6	<1.8
Lilla Öresjön	Sediment	µg/kg dw	<3.4	<3.4	<8.4	<3.4

8.2.2 Diffuse sources - Municipal sewage treatment plants

Influent and effluent waters

The concentrations of the anti-inflammatory substances found in the influent (21 samples) and the effluent sewage waters (54 samples) are summarised in "box plots" in Figure 11. The concentrations and distribution of the analysed substances found in the different STPs are shown in Figure 12 and Figure 13. The anti-inflammatory substances were detected in all samples with ibuprofen and naproxen found in the highest concentrations in both influent and effluent water samples.

The concentrations of NSAIDs (sum of the analysed substances) varied between 5.1 and 33 µg/l in the influent water and the average concentrations of ibuprofen, ketoprofen, naproxen and diclofenac were 7.5, 3.0, 7.3 and 0.37 µg/l respectively. Bendz et al. have reported influent concentrations of these compounds from the Källby STP outside the city of Lund as 3.59, 0.94, 3.65 and 0.16 µg/l respectively (Bendz et al., 2005). The average concentrations found in this study were in the same order of magnitude and the average relative proportions among the different NSAIDs (normalised to diclofenac concentrations) seemed to argue very well; 20: 8: 20: 1 in this study compared to 22: 6: 22: 1 from the Källby STP.

Most of the NSAIDs were detected in the effluent water from the STPs throughout Sweden, with concentrations of the NSAIDs up to 24 μg/l (sum of the analysed substances). Also in effluent water there was a great variation in concentrations between the different STPs. There may be a number of possible explanations for this finding such as the type of STP process and the time of sampling.

The highest effluent water concentrations of naproxen and ibuprofen found in this study were from the STPs located in the northern part of Sweden e.g. Piteå, Luleå, Lycksele, Sandviken and Hede. These results indicate that there is a geographical trend in the concentration of naproxen and ibuprofen in effluent water from the STPs, which is illustrated in Figure 13. A similar geographic trend was not found for ketoprofen and diclofenac. A possible explanation for this finding may be that lower temperatures in the north may affect the degradation of NSAIDs in the STPs as no north-south geographical trends could be seen for NSAIDs in STP influent waters.

An influence of the water temperature was also suggested as an explanation for seasonal variation in STP effluent concentrations, with elevated concentration during wintertime observed in Finland (Vieno et al., 2005).

The average concentrations in effluent water from the STPs found in this study were 1.3, 0.98, 1.7 and 0.23 μg/l respectively (substances in the order ibuprofen, ketoprofen, naproxen and diclofenac). Bendz et al., reports lower effluent concentrations from the Källby STP in Lund 0.15, 0.33, 0.25 and 0.12 μg/l respectively. However, these concentrations agree with several of the concentrations detected in this study, especially from the STPs located in the south part of Sweden and in particular with the sample from the same STP (Källby STP, Lund). In another study conducted in Sweden, ibuprofen was detected in STP effluent water at concentrations around 7 μg/l and naproxen at 2 μg/l (Andreozzi et al., 2003).

Paxéus has performed an extensive study of the STP effluent concentrations of diclofenac, ibuprofen and naproxen, including Swedish as well as European samples; France, Italy, Greece and Denmark (Paxéus, 2004). The Swedish sampling campaign was performed at Ryaverken STP in Göteborg and the Källby STP in Lund. The concentrations of diclofenac detected in the Swedish samples were 0.19 and 0.16 μg/l for Ryaverken STP and Källby STP respectively, while the overall European median value reported for diclofenac was 0.29 μg/l. The corresponding values for ibuprofen (in the same order of appearance) were 0.49 (Rya), 0.15 (Källby) and 0.11 μg/l as the European median value. Naproxen concentrations were 0.88 (Rya), 0.25 (Källby) and 0.41 μg/l as the European median value. Thus the concentrations reported in this screening study are slightly higher than the ones found in the article by Paxéus.

In a review article by Dlugolecka et al., a summary of the scientific literature on this topic concludes that the reported effluent concentrations of NSAIDs (diclofenac, ibuprofen and naproxen) in Swedish STP samples (the Paxéus study) were slightly lower than the corresponding values reported for European STP effluent samples (Dlugolecka et al., 2006). It can thus be concluded that the concentrations reported herein are in agreement with previously reported European STP effluent values while previously published data from Swedish investigations are generally reporting lower NSAID-concentrations in the effluent samples.

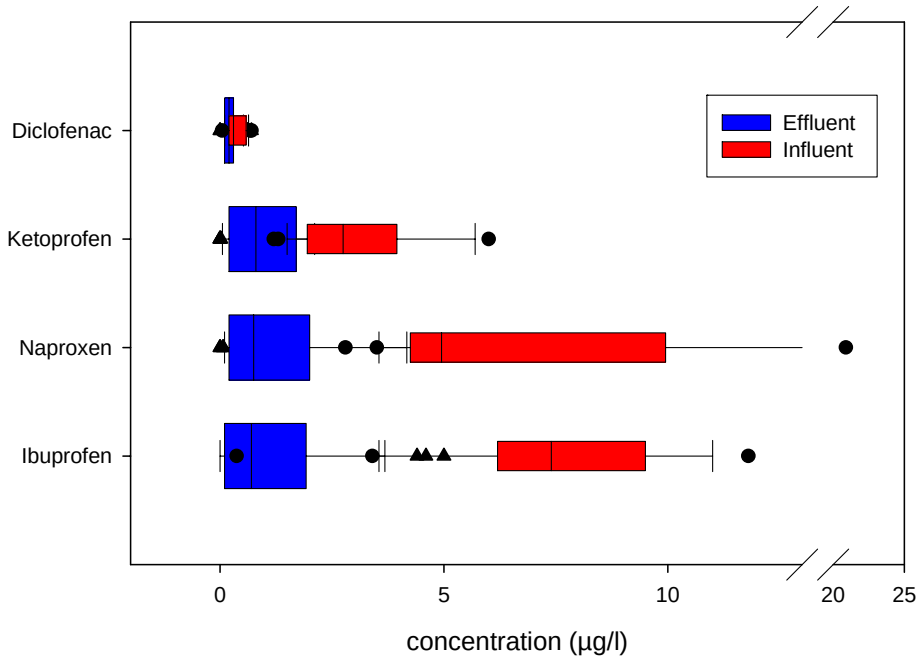


Figure 11. Anti-inflammatory substances in influent and effluent sewage water (20 influent samples, 54 effluent samples). The lower and upper boundaries of the box represent the 25- and 75-percentiles, the line within the box is the median concentration. The whiskers represent the 10- and 90-percentiles, and the dots (influent) and triangles (effluent) are individual results outside this range.

Comparison between the concentrations in influent and effluent water from the individual STPs showed that ibuprofen and naproxen to a great extent were removed in the STP processes. The reduction of ibuprofen varied between 4-100 % with an average of 77% and of naproxen between 12-99 % with an average of 68%. However, there was a great variation in reduction in the different STPs. Despite the high removal efficiency of ibuprofen in the STPs as well as faster degradation kinetics in water (i.e. estimated half-lives in water, Table 5), this substance was still found in highest concentrations in the effluent waters compared to the other analysed substances.

Diclofenac and ketoprofen, which occurred in lower concentrations in the influent water than ibuprofen and naproxen, showed somewhat lower reduction efficiencies in the STPs: ketoprofen 5-91 % with an average of 55%, diclofenac 14-75 % with an average of 50%. The removal of diclofenac in the STP process has previously been reported to be rather low (Heberer et al, 2002 & 2005). At several STPs diclofenac was detected in higher concentrations in effluent than influent water. This could possibly be attributed to the degradation of the metabolite diclofenac 1-O-acyl glucuronide (D-1-O-G) back to diclofenac during the STP passage (figure 3, chapter 2).

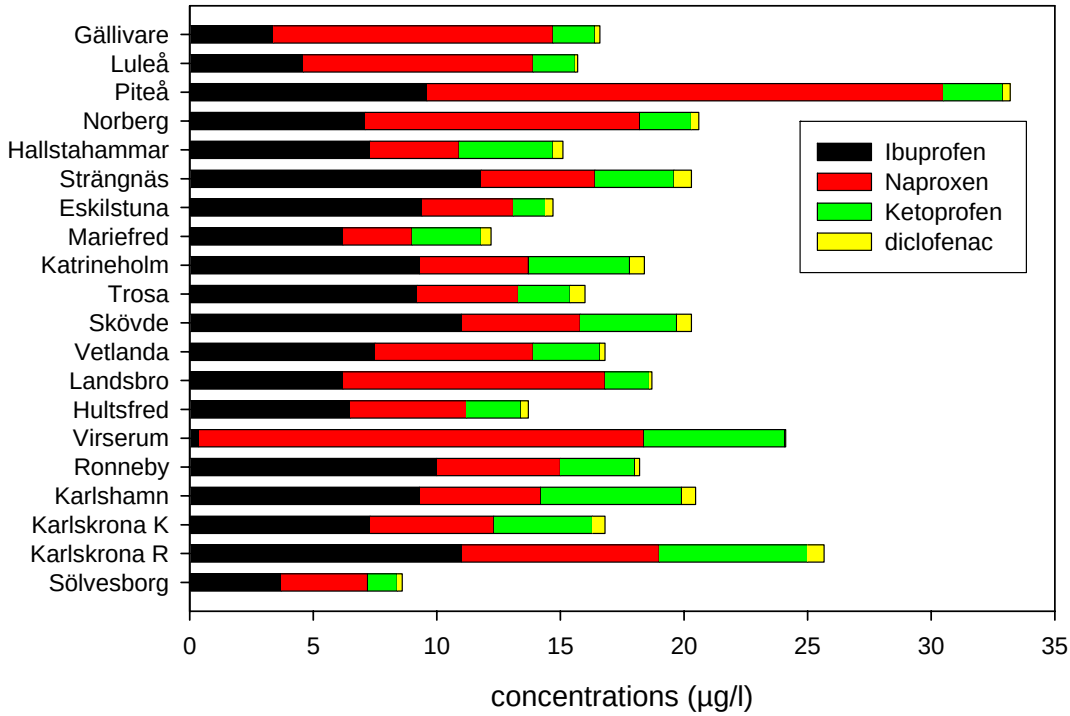


Figure 12. Concentrations of anti-inflammatory substances in influent water to STPs (approximately sorted from north to south).

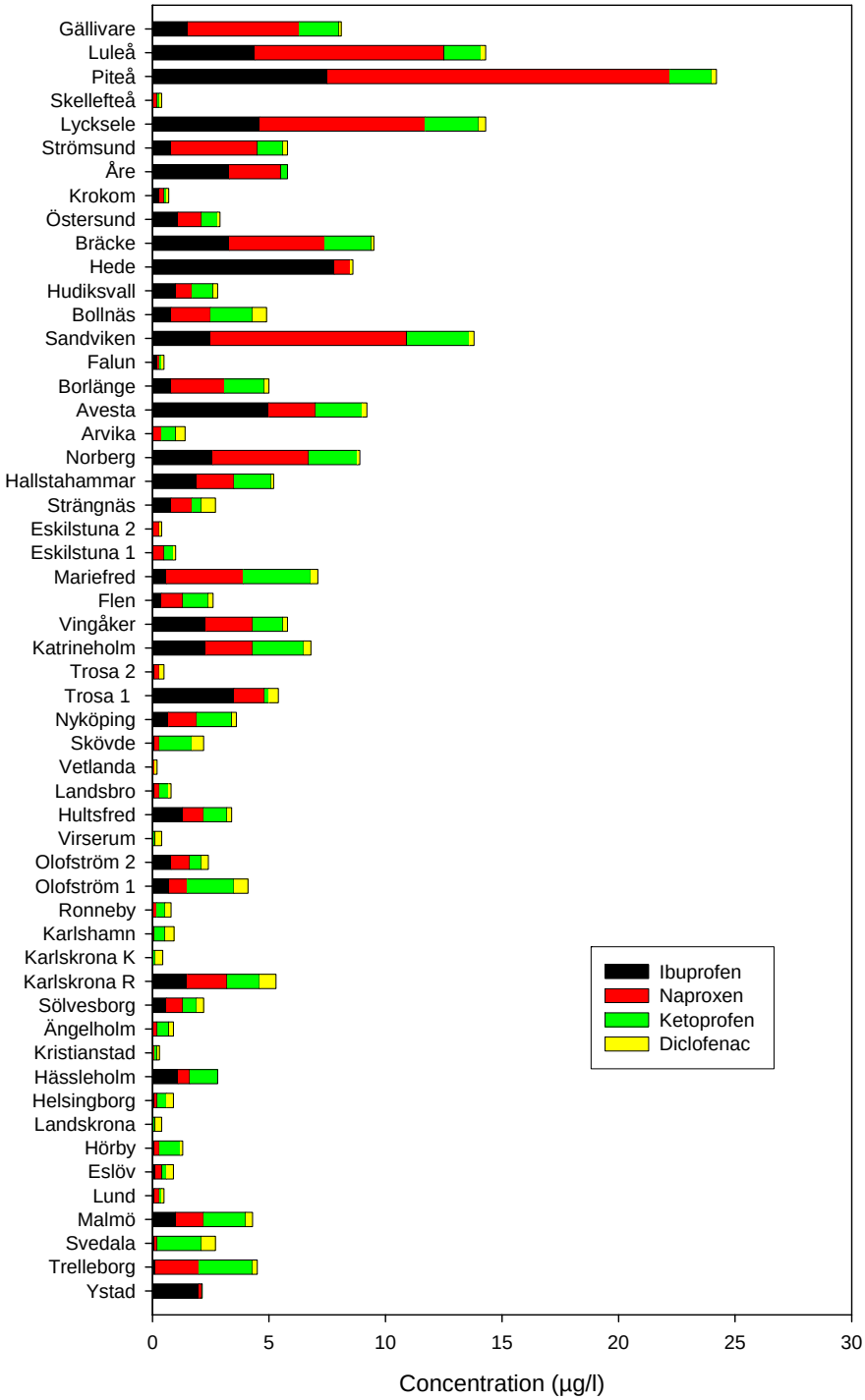


Figure 13. Concentrations of anti-inflammatory substances in STP effluents (approximately sorted from north to south). In Eskilstuna and Trosa samples were taken both before and after a wetland (marked with the figure 2 in the picture) and in Olofström samples were taken before and after an aeration pond (marked with the figure 2 in the picture).

Sludge
The NSAIDs were detected in almost all the sludge samples. The concentrations are summarised in box plots in
Figure 14 and the concentrations found at the individual STPs are shown in Figure 15.

The variation in the concentrations among the different STPs was large and most pronounced regarding ibuprofen which occurred in the highest concentrations among the NSAIDs. The concentration varied between 4 and 560 µg/kg dw with median of 66 µg/kg dw. Naproxene, also frequently found, varied between 3 and 350 µg/kg dw with a median of 18 µg/kg dw. Ketoprofen varied between 5 and 580 µg/kg dw, median 17 µg/kg dw, and diclofenac between 4 and 77 µg/kg dw, median 23 µg/kg dw.

A small number of sludge samples were only semi-processed, i.e., either non-digested or non-dehydrated. No significant difference between digested or non digested sludge was observed but the highest concentrations of NSAIDs could be seen in sludge subjected to neither dehydration nor digestion. The concentrations and the distribution of the NSAIDs of semi-processed sludge can be seen in the samples from Hede, Vetlanda and Berg (Hackås) STP (Figure 15).

The semi-processed sludge sample from the STP in Vetlanda contained proportionally, very high content of ketoprofen, 580 µg/g dw, compared to the approximately 30 µg/g dw for the other NSAIDs (diclofenac was not detected). The sludge that contained the highest concentrations of all NSAIDs on a dry weight basis originated from the Tomta STP in Västerås. The sample was taken directly from storage tanks recieving sewage from private sewage tanks. The sludge was neither digested nor dehydrated and had a very low dry weight (0.31%). The concentrations was 22000, 14000, 1500 and 460 µg/kg dw for ibuprofen, naproxen, ketoprofen and diclofenac respectively. (This figures are excluded from the concentration ranges given above and have been purposely omitted from

Figure 14 and Figure 15. The results can also be given on wet weight basis as 68, 43, 4.7 and 1.7 µg/l with the substances in the same order. The concentrations of ibuprofen, naproxen and diclofenac are then 5-15 times higher than the average concentrations for STP influent water.

Data on NSAIDs analysed in Swedish STP sludge have not been found in the literature. Khan and Ongerth have reported concentrations of ibuprofen in 'primary sludge' (non-processed) as low as 4 µg/kg dw and 0.006 µg/kg dw in digested sludge. The corresponding values for naproxen were 1 µg/kg dw and 0.001 µg/kg dw, respectively (Khan and Ongerth, 2002). However, the results reported reflect current conditions in Australia.

The Swedish sale of ibuprofen in 2002 was 46 000 000 DDD (Defined Daily Dose) (Läkemedelsverket 2004). As one DDD is 1.2 g (WHO 2006) the sale equaled 56 000 kg. This figure does not include the amount sold as a component in anti-inflamatoric gels. The corresponding figures for naproxen, diklofenac and ketoprofen was 14 000, 2 800 and 1 600 kg respectively.

Each year a total of 240 000 kg dw of sludge is produced in the around 400 Swedish municipal STPs (SCB 2004). If the median concentration of ibuprofen (66 µg/kg dw) found in sludge analysed in this study is used as an estimate for the concentration in all Swedish sludge, a total of 16 kg ibuprofen ends up in sludge annually. This is only 0.03% of the annual sale (56 000 kg). A similar calculation for naproxen, ketoprofen and diclofenac gives 4.3, 4.1 and 5.5 kg respectively in sludge annually which equals 0.03, 0.15 and 0.35% of the annual sales. Thus, a quite small part of the used quantities of the NSAIDs ends up in sludge.

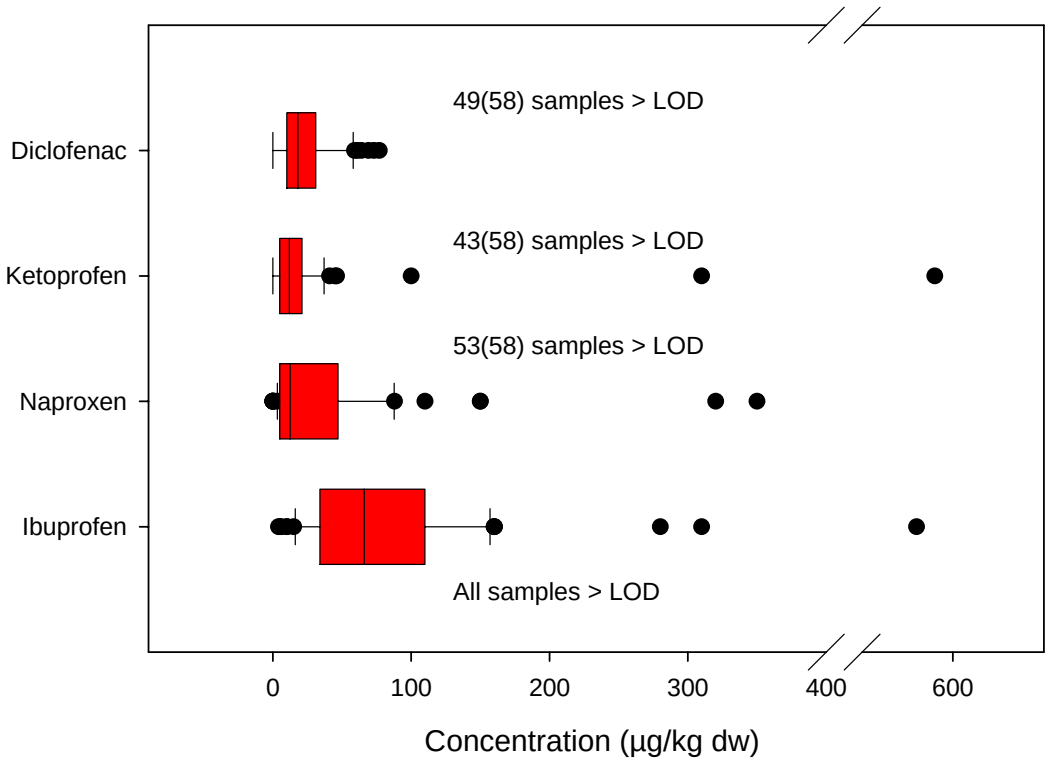


Figure 14. Anti-inflammatory substances in sludge from municipal sewage treatment plants. The lower and upper boundaries of the box represent the 25- and 75-percentiles; the line within the box is the median concentration. The whiskers represent the 10- and 90-percentiles, and the dots are individual results outside this range.

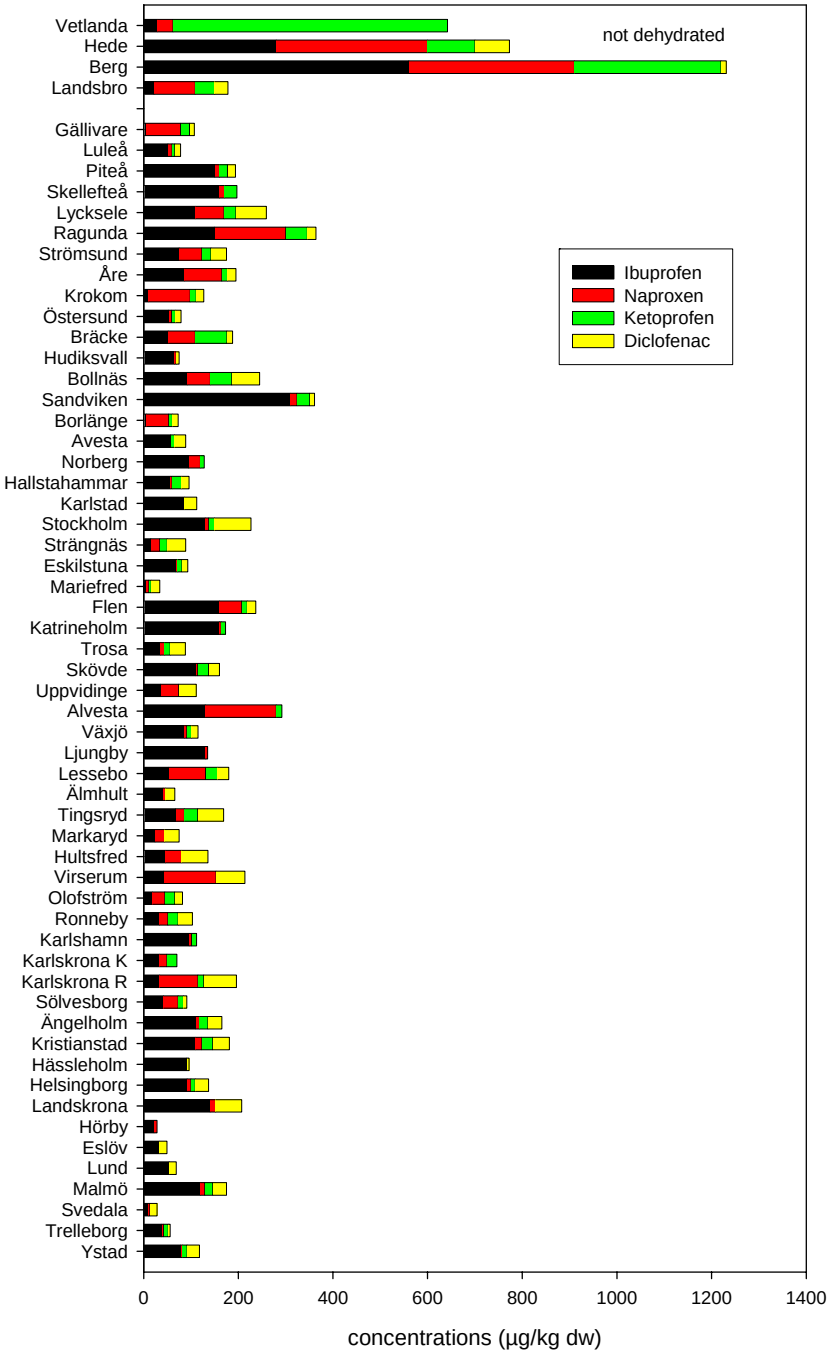


Figure 15. Concentrations of anti-inflammatory substances in sludge from municipal STPs

8.2.3 Recipient water samples

The NSAIDs were found in varying concentrations in surface waters collected in the proximity of STPs in Vänern (Figure 16; ibuprofen 0.0056 -0.017 µg/l; naproxen 0.002-0.009 µg/l; ketoprofen 0.002-0.008 µg/l; diclofenac 0.002 µg/l). However these concentrations are close to the detection limit and they were lower than the concentrations found in the "background" lakes, Lake Öresjön and Lake Tärnan.

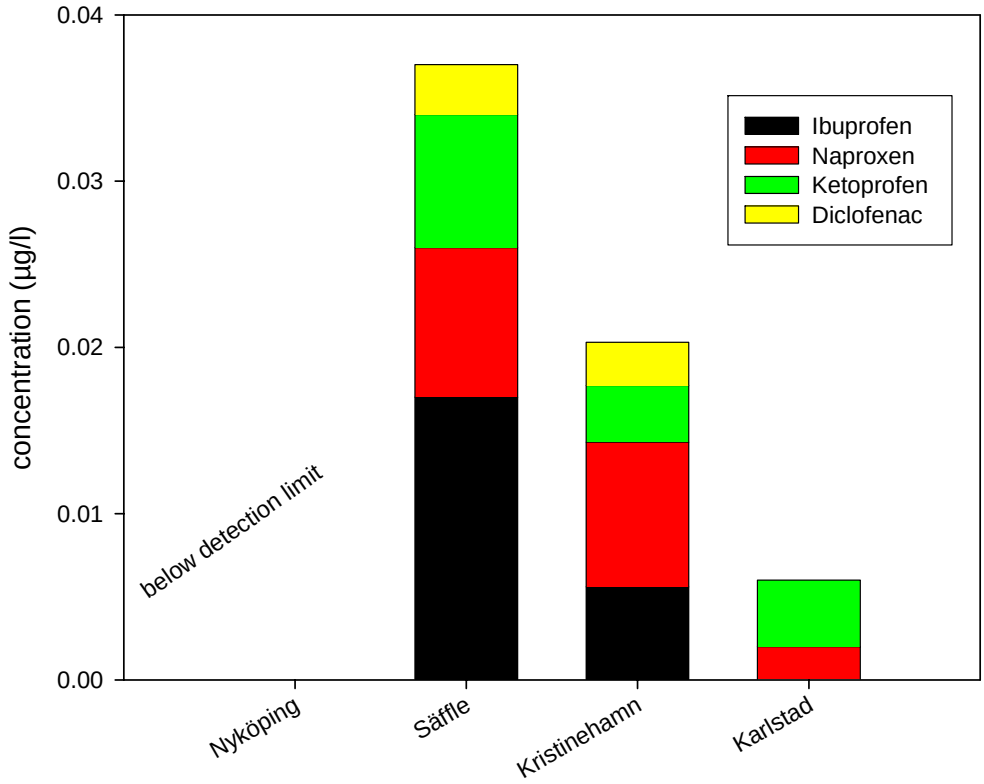


Figure 16. Concentrations of anti-inflammatory substances in recipient waters for STPs

8.2.4 Point sources - landfills

When leachate water samples from landfills were analysed with regard to NSAIDs, ibuprofen, and to some extent ketoprofen, were found in highest concentrations (0.30-2 µg/l; 0.040-0.50 µg/l respectively) while the concentrations of diclofenac and naproxen were generally lower (0.0098-0.028; 0.009-0.040 µg/l respectively). The concentrations and distribution of the NSAIDs among different landfills is shown in Figure 17.

The measured concentrations of NSAIDs in one untreated leachate sample from Högbytorp landfill in Bro were increased compared to the other landfill samples (ibuprofen 200 µg/l; naproxen 0.033 µg/l; ketoprofen 0.58 µg/l and diclofenac 0.10 µg/l) and have purposely been omitted from

the figure. However the concentrations were significantly reduced after the leachate water treatment in the landfill (see Bro sample Figure 17).

The concentrations of ibuprofen occurred in the same level or somewhat lower compared to the concentrations found in the effluent water from the STPs while the three other substances generally were lower than in the STP effluents. In a Norwegian screening, ibuprofen in leachate waters varied between 0.61 and 5 µg/l (SPFO-report 949/2006), which is in the same order of magnitude as in this study.

Whether the measured concentrations found at the landfills can be interpreted, as solemnly a result of domestic disposal of pharmaceuticals through the garbage disposal system is still elusive since STP-originating sludge may occasionally contribute to landfill build-up.

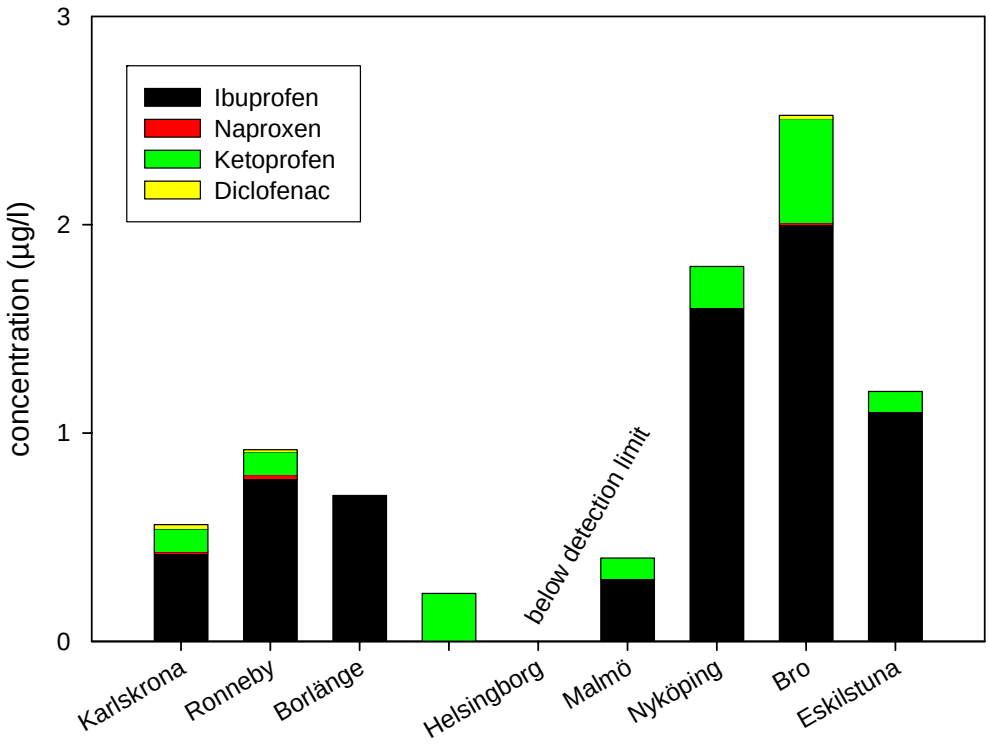


Figure 17. Concentrations of anti-inflammatory substances in leachate water from landfills

8.2.5 Point sources - hospitals

The concentrations of the NSAIDs found in hospital effluents are shown in Figure 17. Ibuprofen was the most abundant anti-inflammatory pharmaceutical also in the effluent from the hospitals. The concentrations of ibuprofen, naproxen and ketoprofen were 0.0034-26 µg/l; 0.1-11 µg/l; 1.3-6.1; respectively, which were in the same range as in the influent water samples to municipal STPs. The concentrations of diclofenac varied between 0.1 and 5.0 µg/l and the concentrations in some of the samples from the hospital exceeded the concentrations found in STP influent water. The hospital effluents are led to different STPs where concentrations are reduced before reaching the recipient.

One of the samples, Huddinge, deviated from the others with substantially lower concentrations (ibuprofen: 0.0034, naproxen: <0.002; ketoprofen 0.0025 and diclofenac <0.001 µg/l) and was purposely omitted from the figure.

The concentrations of ibuprofen found in the effluent from the hospitals included in this study were lower than what previous has been reported for a rheumatic hospital (ibuprofen: 77.2-116.3 µg/l; Läkemedelsverket, 2004).

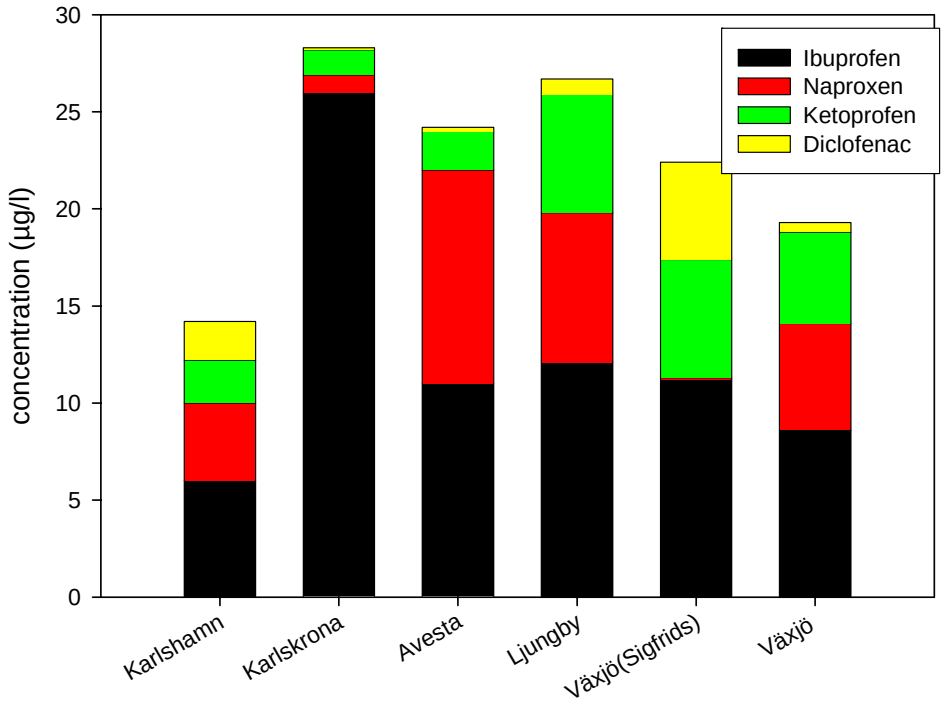


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Figure 18. Concentrations of anti-inflammatory substances in hospital effluents

8.2.6 Point sources - animal breeding

The results of the measurements of the NSAIDs in water samples collected close to animal breeding facilities are summarised in

Figure 19. There was a great variation in concentrations as well as in the distribution of the NSAIDs.

Pig-farm

Ketoprofen was detected in both firm and buoyant manure from the pig breeding facility (14 µg/kg dw and 0.56 µg/l respectively). A veterinary drug, Romifen, having ketoprofen as the active component, that had been administered through intramuscular injection (personal communication with the pig farmer) could be attributed to this finding.

In a nearby ditch, approximately 200 meters from the farm, ketoprofen and diclofenac were detected in concentrations of 0.031 and 0.021 µg/l respectively (stack 3,

Figure 19). However, the emission of NSAIDs to that water stream may not necessary originate from the pig farm since a couple of settlements nearby have their domestic sewage directed untreated to the water stream. Higher concentrations was found in a water sample taken from another water-filled ditch, approx. 20 meters from the farm, where all the NSAIDs were detected; ibuprofen 4.2 µg/l, naproxen 0.023 µg/l, ketoprofen 1.0 µg/l and diclofenac 0.039 µg/l .This ditch also receives contributions from local domestic settlements but run-off water from the manure stacks and the manure-contaminated soil may also contribute to the NSAID-content of this water. NSAIDs could not be found in the sediment samples from either of the two ditches.

Ketoprofene was also detected in the well that was used for water supply (stack 1,

Figure 19). The concentration was 0.0018 µg/l, which is lower than what was found in some of the background lake waters.

Horse stable

None of the NSAIDs were detected in the manure samples from a horse track area a (LOD 2-6 µg/kg dw) while they were found in the water samples taken from the Bällsta water stream, running through the stables and racing track.

In the water sample collected at the entrance point of the stables area, all four NSAIDs were detected at concentrations of ibuprofen 0.052 µg/l, naproxen 0.014 µg/l, ketoprofen 0.0039 µg/l and diclofenac 0.0025µg/l. The other water sample was collected in the middle of the racing track and ketoprofen was detected at a concentration of 0.003 µg/l. Along the whole of the Bällsta water stream domestic sewage is reaching the stream untreated, which could give a contribution to the contents of pharmaceutical residues.

The sediment sample collected at the entrance of the horse stable area did not contain any detectable amounts of the NSAIDs. Unfortunately, it was too difficult to collect sediment samples from the pond in the centre of the racetrack due to limited access to the area with the necessary equipment.

Cattle farm

NSAIDs were analysed in a recipient for runoff water from a grazing field for cattle. Two water samples were collected where as one of them contained increased concentrations of NSAIDs; ibuprofen 28 µg/l, naproxen 12 µg/l, ketoprofen 0.66 µg/l and diclofenac 0.25 µg/l and the other sample were below detection limit. These results have therefore purposely been omitted from Figure 19.

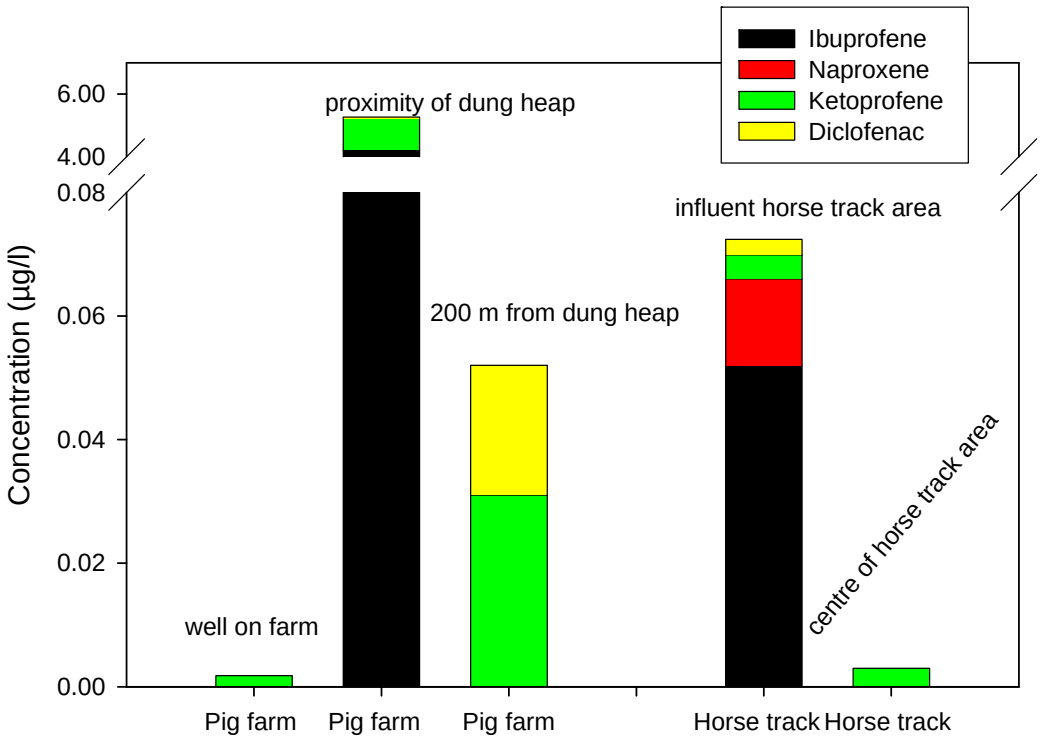

Figure 19. Concentrations of NSAIDs in water samples from a pig farm facility and from the Solvalla horse stables (Bällsta water stream).

8.2.7 Human exposure

Concentrations of NSAIDs in food stuff were all below detection limit (ibuprofen: <0.6 µg/kg ww, naproxen: <0.2 µg/kg ww, ketoprofen: <0.5 µg/kg ww, diclofenac :<0.2 µg/kg ww).

8.3 Hormones

8.3.1 Background sites

Estradiol, norethindrone and progesterone were detected in one of the background water samples while the other hormones were below detection limits in all the samples. The concentrations and detection limits are given in Table 15. Results and LOD for antibiotics in samples from animal keeping are given in Table 17.

Estriol and progesterone were also found in a sample from a drinking water source in Kristinehamn at concentrations of 0.0033 and 0.003 µg/l respectively.

Table 17. Hormones in background samples (<LOD)

Site		Unit	Estriol	Estradiol	Ethinylestra-diol	Norethind-rone	Progeste-rone
Lilla Öresjön	water	µg/l	<0.0001	<0.0003	<0.0005	<0.002	intf
Stensjön	water	µg/l	<0.0001	0.0020	<0.0005	0.0050	0.007
Tärnan	water	µg/l	<0.0001	<0.0003	<0.0005	<0.002	<0.0007
Lilla Öresjön	sediment	µg/kg dw	<4	<5	<7	<10	intf
Stora Envättern	sediment	µg/kg dw	<3	<4	<5	intf	intf
Tärnan	sediment	µg/kg dw	<2	<2	<3	<6	<8

8.3.2 Diffuse sources - Municipal sewage treatment plants

Influent and effluent waters

The concentrations of the hormonal substances found in the influent (20 samples) and the effluent sewage waters (54 samples) are summarised in "box plots" in Figure 20. The concentrations and distribution of the analysed substances found in the different STPs are shown in Figure 21 and Figure 22.

In several of the STPs the concentrations of the hormones were higher in the effluent than in the influent water, which is illustrated in Figure 20. This was found in the water samples from the following STPs; Kavaheden STP (progesterone), Sternö STP (ethinylestradiol, progesterone), Koholmen STP (norethinedrone), Uddebo STP (progesterone), Perbo STP (estradiol, norethinedrone), Sandholmen STP (estriol), Rustorp STP (progesterone), Stadskvarn STP (progesterone), Strängnäs STP (estriol, norethindrone and progesterone), Sölvesborg STP (progesterone) and Trosa STP (progesterone).

In the influent water samples progesterone and norethindrone were detected in 18 and 15 out of 20 samples while the other hormones were only found occasionally. The norethindrone concentrations, in samples with concentrations above the LOD (<0.007-0.002 µg/l) were 0.001-0.02 µg/l while the corresponding values for progesterone were 0.004-0.03 µg/l (LOD <0.002-0.003 µg/l).

The highest detected concentration of ethinylestradiol 0.05 µg/l was found in a sample from the Koholmen STP in Karlskrona. Matrix interference problems with respect to norethindrone discarded one influent sample from the Hultsfred STP.

In previous studies Johnsen et al. reported STP influent concentration of estradiol in the range <0.0005 to 0.044 µg/l and estriol <0.0005 to 0.010 µg/l, while Lagana et al. reported median estradiol and estriol concentrations of 0.025 µg/l and 0.031 µg/l respectively in the STP influent. No comparable STP influent data is found in literature on androgens. However, in a survey of the organic wastewater contaminants affecting 139 U.S. streams (Kolpin et al.) progesterone was reported with maximum concentration of 0.199 µg/l and median 0.110 µg/l /l. The maximum concentration of norethindrone was 0.872 µg/l with a median of 0.048 µg/l, maximum concentration of ethinylestradiol of 0.831 µg/l and median of 0.073 µg/l.

In our study the concentrations of the hormones found in effluent water samples from STPs are presented in Figure 22. Progesterone was the most frequently detected substance of the hormones with concentrations above LOD (<0.007) in 45 out of 54 samples, norethindrone in 22 samples out

of 54 samples (LOD< 0.003 - 0.0001) while ethinylestradiol, the least frequently encountered hormone, was only detected in one sample at a concentration of 0.04 μg/l (Sternö STP in Karlshamn; LOD<0.0005 - 0.002). The concentrations of progesterone varied between 0.001-0.11 μg/l while the concentration range for the other detected hormones was 0.001-0.06 μg/l. It should also be noted that in two effluent water samples, matrix interference problems[2] prevented the quantification of progesterone (Hässleholms STP and the Sandholmen STP in Piteå, where matrix interference also prevented the quantification of norethindrone). These samples are designated as non-detects.

In other studies Labadine et al. reported an average estradiol concentration of 0.0044 μg/l and average estriol concentration of 0.0029 μg/l from the effluent of the Eysines STP (France) and below detection limit in two other campaigns (Labadine et al., 2006). Norethindrone was below detection limit in al three campaigns while the progesterone measurements were flagged with analytical interferences in two of the campaigns. In the effluent from the wastewater treatment plant and the Seine river estuary Labadine et al. reported estriol concentrations of 0.0035 μg/l at one occasion, while estradiol, ethinylestradiol, progesterone and norethindrone were reported below detection limits in most of the samples. Lagana et al. reported median estradiol and estriol concentrations of 0.006 μg/l and 0.001 μg/l respectively, in the STP effluent. Ethinylestradiol was below detection limit. A one year STP study reported by Shore et al. revealed estradiol effluent concentrations ranging between 0.0064 μg/l and 0.050 μg/l depending on the STP operating conditions.

The concentration level of the estrogens (estradiol, ethinylestradiol, estriol) found in this work complies with the levels reported in literature (below detection limit to several tens of ng/l). Based on our work no obvious and consistent distribution pattern is found for the estrogens. However, this is also the case for the data reported in literature. The concentration levels of the norethindrone and progesterone in this work are somewhat higher than the very few data reported for STPs in literature. However, the survey by Kolpin et al. shows that norethindrone and progesterone is present in the environment at significant amounts. As for the estrogens, a reliable distribution pattern of androgens is not obvious to find in the data set.

[2] Non-desired components of complex samples which cannot be chromatographically distuinguished from the analytes of interest, despite rigorous sample preparation.

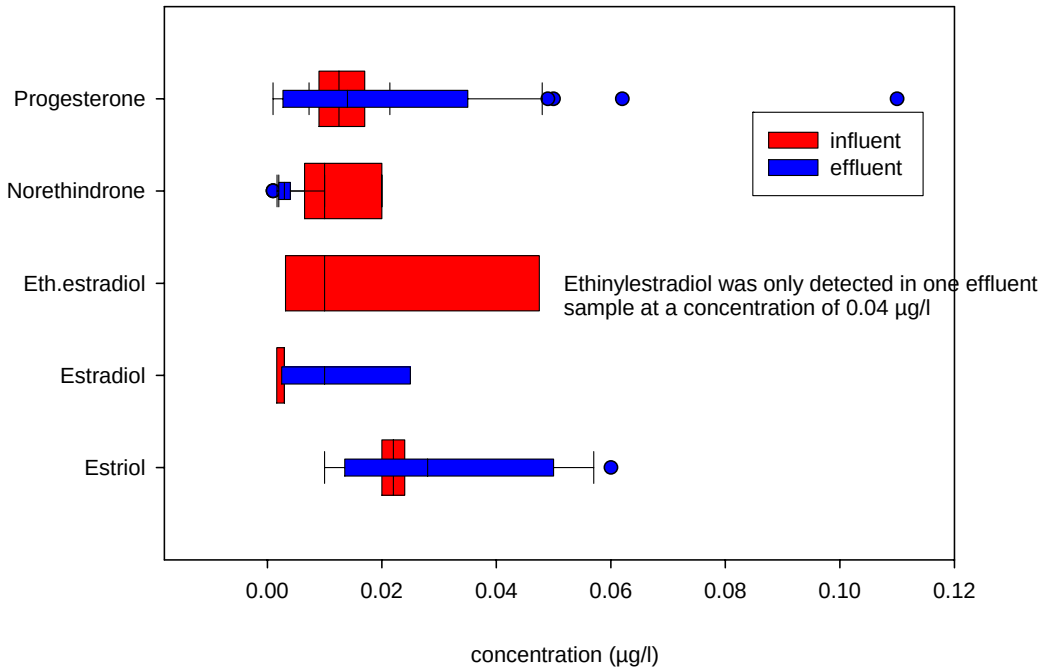

Figure 20. Concentrations of hormones in influent and effluent water in STPs

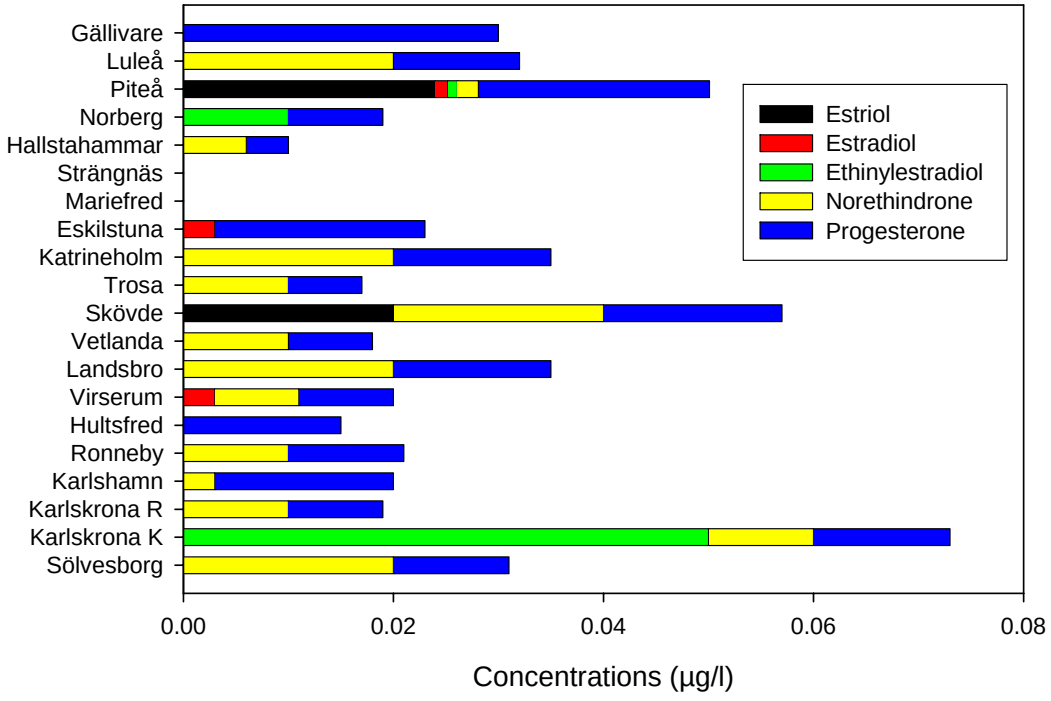

Figure 21. Concentrations of hormones in influent water to municipal STPs

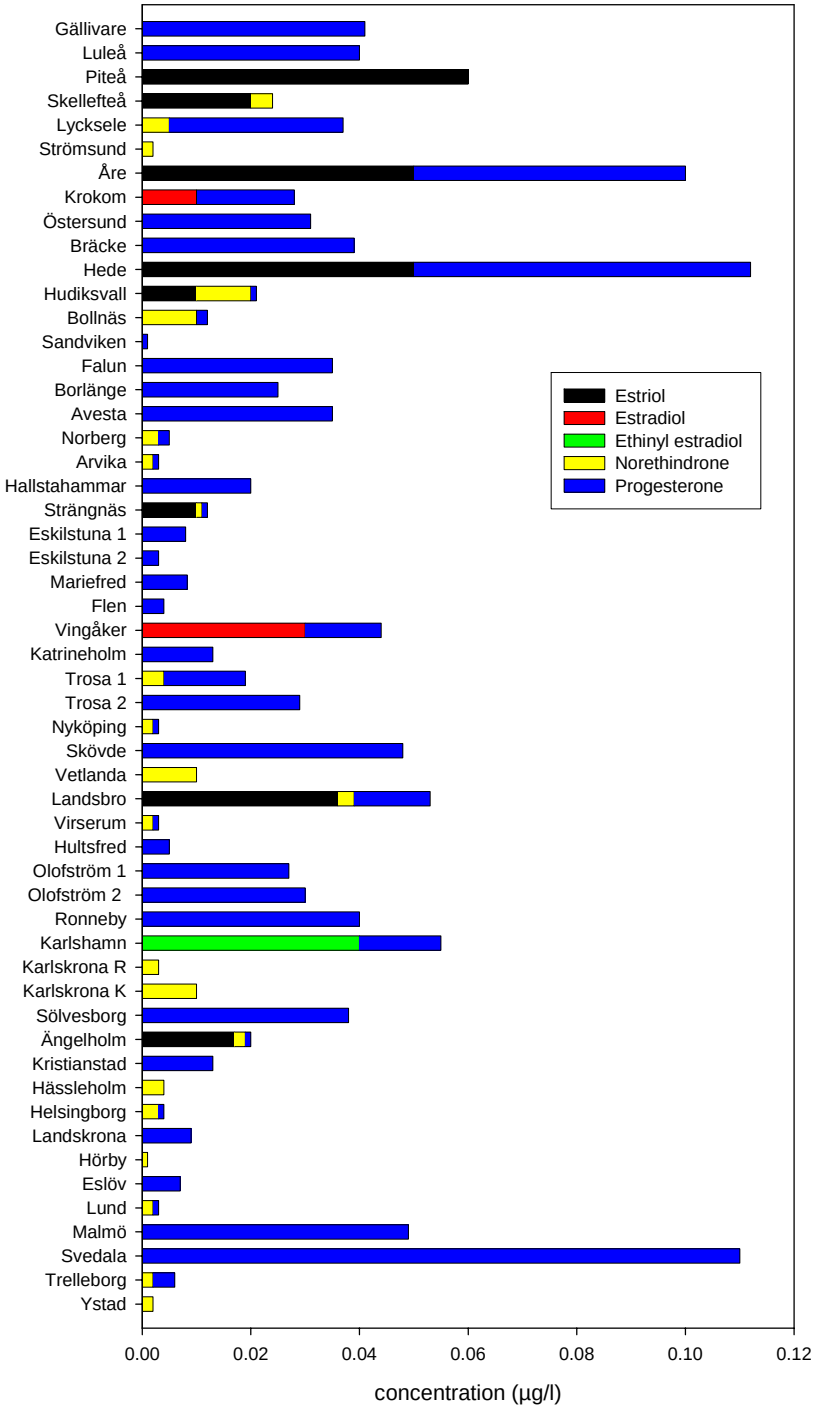

Figure 22. Concentrations of hormones in effluent water samples from municipal STPs. In Eskilstuna and Trosa samples were taken both before and after a wetland (marked with the figure 2 in the picture) and in Olofström samples were taken before and after an aeration pond (marked with the figure 2 in the picture).

Sludge

The concentrations of the hormonal substances found in sludge are summarised in Figure 23 and the concentrations and distribution found at the different STPs are shown in Figure 24.

Progesterone was the most frequently detected hormone with concentrations above LOD in 45 out of 61 sludge samples (LOD<10 – 17 µg/kg dw) while estradiol, the least detected hormone, was detected in 4 out of 61 samples (LOD<2 - 80 µg/kg dw). Norethindrone was detected in 19 out of 61 sludge samples (LOD <2 - 80 µg/kg dw), Ethinylestradiol in 7 out of the 61 samples analysed (LOD <3 - 260 µg/kg dw) and estriol in 6 out of the 61 samples analysed (LOD< 1- 4 µg/kg dw). A great variation in the concentrations was found for all the analysed hormones with the non dehydrated sample in particular deviating from the others. The ranges of detected concentrations of hormones were : estriol 14 - 3900 µg/kg dw, estradiol 3.3-310 µg/kg dw, ethinylestradiol 3.3 - 6800 µg/kg dw, norethindrone 8.7- 6100 µg/kg dw and progesterone 14 -1900 µg/kg dw.

In other studies of activated sludge Wenzel et al found estradiol in the concentration range 4.2- 111 µg/kg dw and estriol in the concentration range 18.1- 31.4 µg/kg dw. In activated sludge Ternes et al. found estrogens in the range <4 - 37 µg/kg dw while digested sludge contained estrogens in the range <2 - 49 µg/kg dw. Data of the concentrations of norethindrone and progesterone in sludge are limited in literature. However, Barcelo et al. determined the concentration of norethindrone and progesterone in sediments of the Cardener River (Catalonia, NE Spain) and found progesterone in the range 0.2 - 6.8 µg/kg and norethindrone in the range 0.2-1 µg/kg.

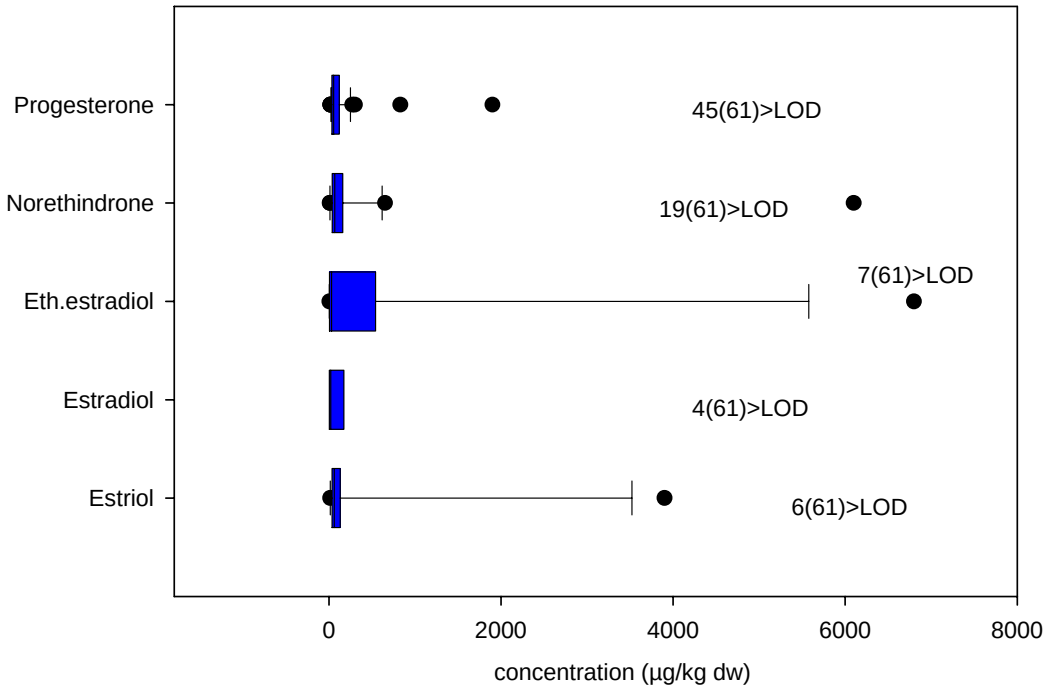

Figure 23. Concentrations of hormones in sludge from municipal STPs

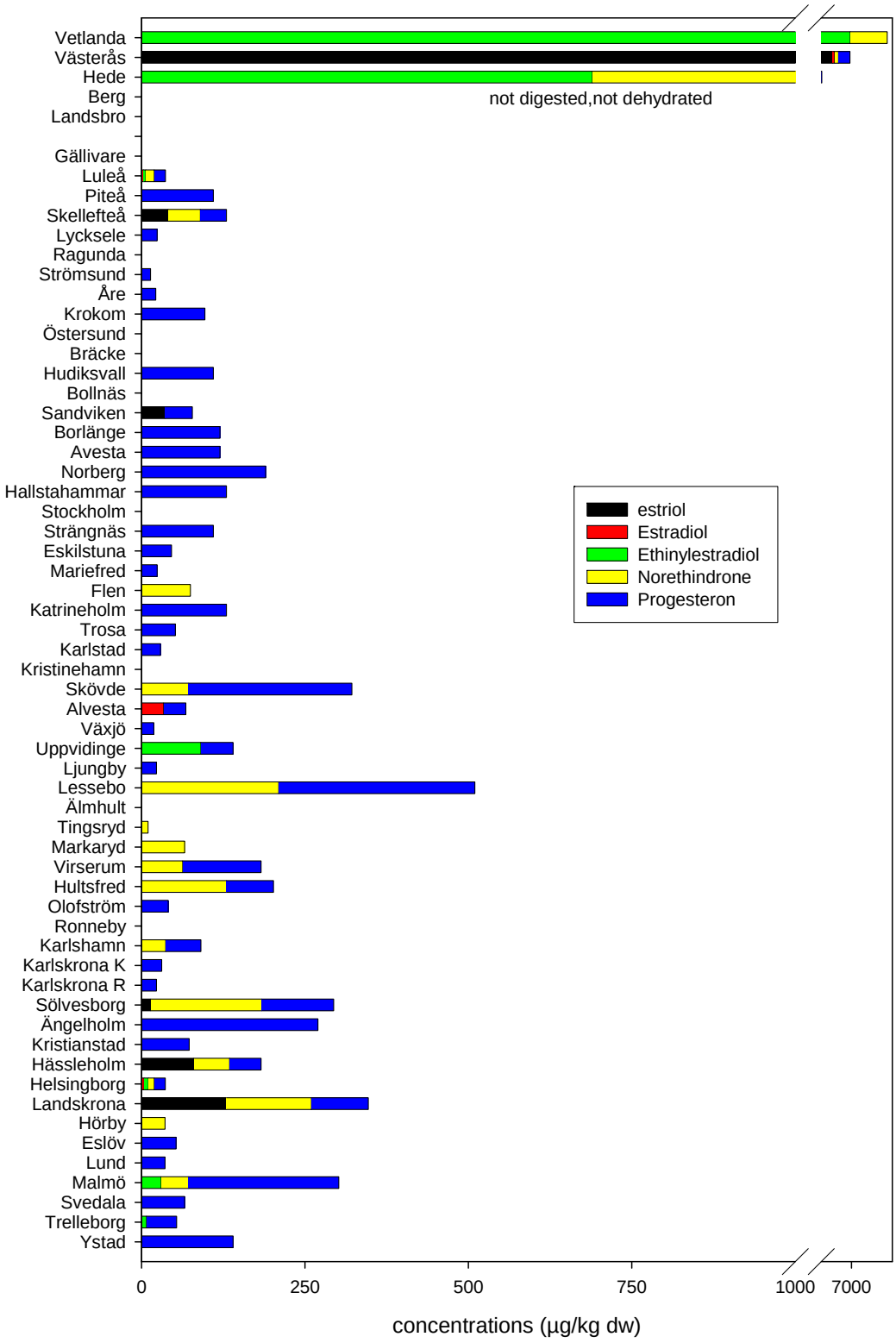

Figure 24. Concentrations of hormones in sludge from municipal STPs.

Recipient water

Hormones were only detected in one of the recipient water samples, from Nyköping, which contained norethindrone in a concentration of 0.001 µg/l. All the other substances were below the limit of detection.

8.3.3 Point source - landfill

The concentrations of the hormones found in the leachate water samples are summarised in figure 25. Progesterone occurred in all leachate water samples in concentrations ranging from 0.001 to 0.02 µg/l. Norethindrone was found in all samples except in the samples from Karlskrona and Nyköping in concentrations of 0.004-0.04 µg/l. Ethinylestradiol, estradiol and estriols were however more sporadically found and then in concentrations of 0.004-0.035 µg/l; 0.001-0.8 µg/l; 0.005-0.02 µg/l respectively. Concentrations of hormones in leachate water are in the same order of magnitude as in the municipal effluent samples while the distribution of the different hormones was slightly different.

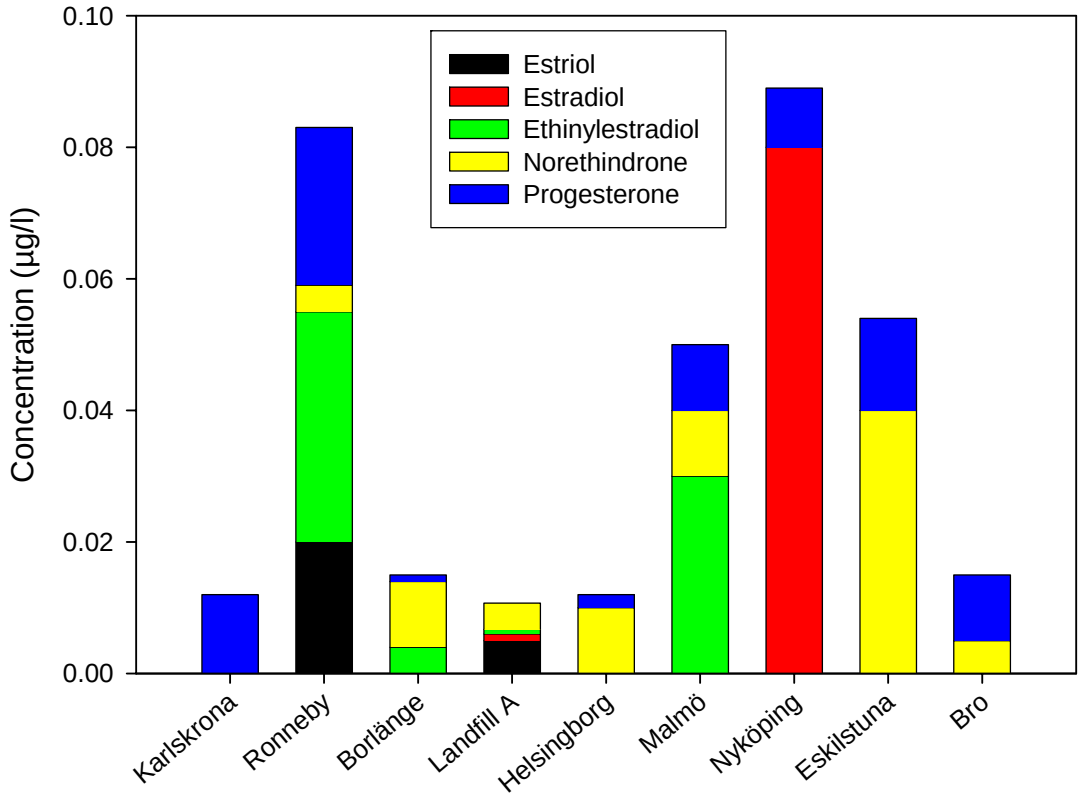

Figure 25. Concentrations of hormonal substances in leachate water from landfill

8.3.4 Point source - hospital

The concentrations of hormones found in hospital effluents are summarised in Figure 26. Progesterone and norethindrone were detected in five out of seven samples in concentrations of 0.006-0.016 µg/l and 0.01-0.034 /l respectively. Ethinylestradiol was found in 2 out of 7 samples in concentrations of 0.01-0.02 µg/l. Estriol was found in one sample at a concentration 0.05 µg/l while estradiol was below LOD in all samples. Concentrations of hormones in effluents from hospitals are in the same range as municipal effluents.

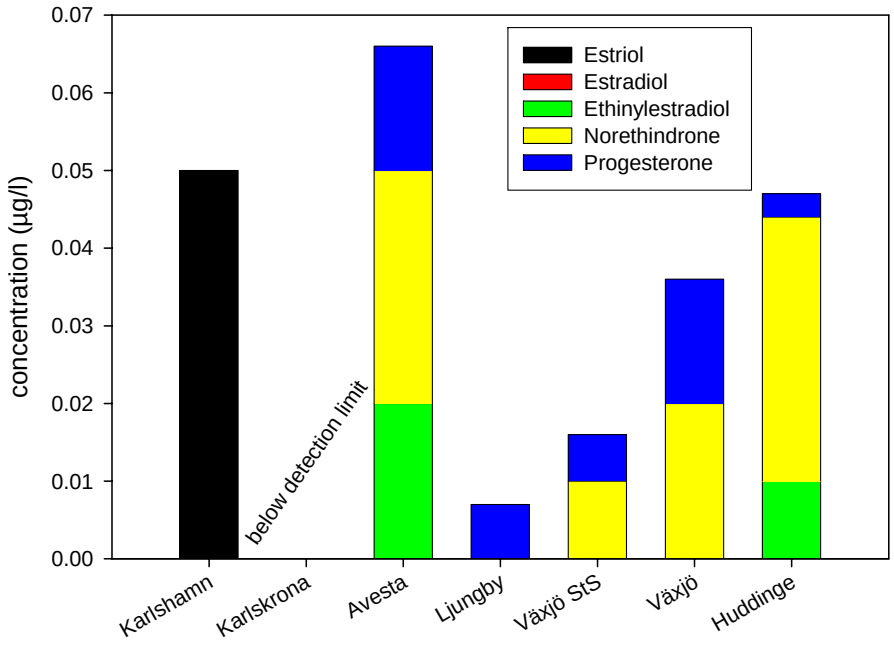

Figure 26. Hormonal substances in hospital effluents

8.3.5 Point sources-animal breeding

Concentrations of the hormones around animal breeding are illustrated in Figure 27.Progesterone was found in two out of three manure samples collected at the horse track area (78-200 µg/kg dw) and one out of two manure samples from the pig farm (60 µg/kg dw). Norethindrone occurred in one manure sample from the horse track area and one from the pig farm (42 µg/kg dw and 62 µg/kg dw respectively). Ethinylestradiol was found in one manure sample from horsetrack area in concentration of 82 µg/kg dw.

Norethindrone was found in all surface water samples adjacent to animal keeping (0.001 - 0.01 µg/l) except in the sample taken from the proximity of a dung heap at the pig farm. Ethinylestradiol occurred in surface water adjacent to grazing fields for cattle in concentrations of

0.001-0.003 µg/l. The source of this compound is elusive since ethinylestradiol is not used in veterinary medecines in Sweden (fass.se). Progesterone was found in surface water samples from both the pig farm (0.005-0.032 µg/l) and in storm water from the grazing field (0.001-0.003 µg/l). Norethindrone and progesterone were also detected in the sediment sample from the ditch in proximity of the dung heap in concentrations of 12 µg/kg dw and 32 µg/kg dw respectively.

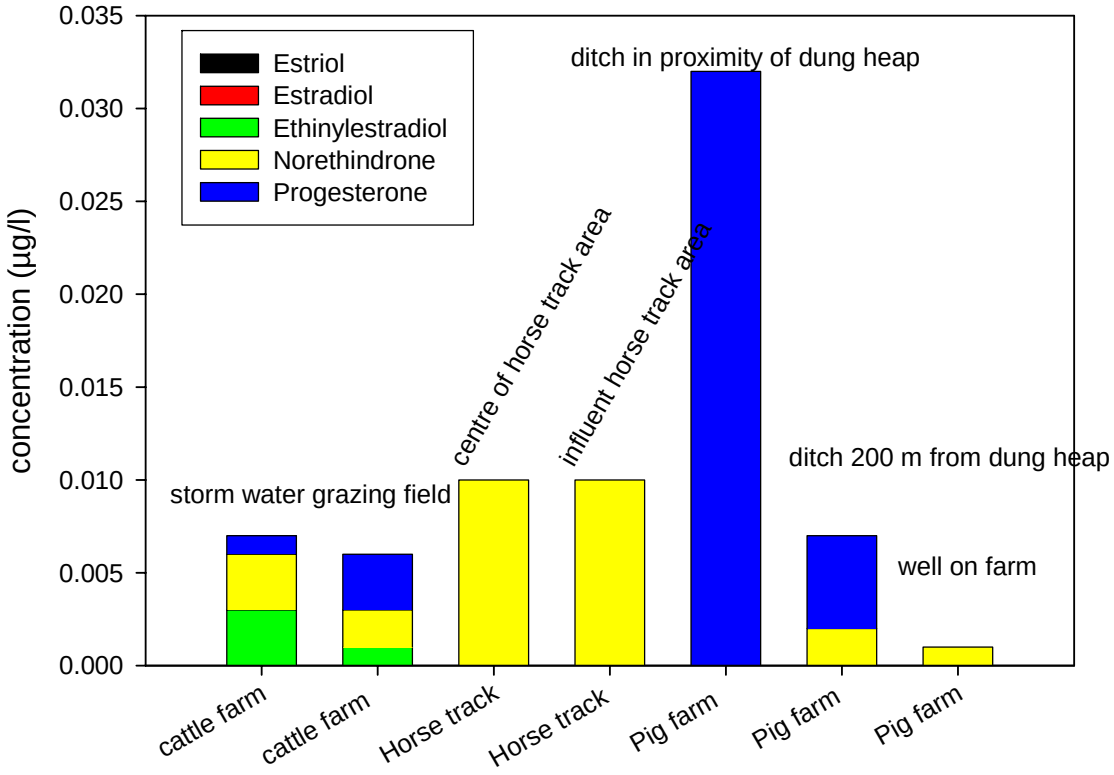

Figure 27. Concentrations of hormones in surface water adjacent to point sources

8.3.6 Human exposure

In food stuff samples the only hormone above LOD was ethinylestradiol which was found in both cultivated and wild fish, both in concentrations of 0.90 µg/kg ww.

8.3.7 Reflections about the chemical analytical hormonal results

Most research on the occurrence of hormones in the environment has been conducted on water samples, and solid samples have to some degree been overlooked. In addition, the chemical analysis has been focused on estrogens. As a consequence, comparable data on androgens (norethindrone

and progesterone in water and solid samples) are limited in the literature as well as data on estrogens in solid samples.

In all efforts to draw conclusions and compare data is important to keep in mind the nature of the STP samples and all factors that possibly will influence the results. The number of factors increases significantly when the data are compared with literature data. Following list indicates some of the complexity:

- The sample matrix within each STP is very complex and the composition may continuously change depending on operating conditions and seasonal variations. Braga et al. have reported how different STP processing influences steroid concentrations.
- The variability in sample matrix composition from one STP to another is a challenge for the chemical analyses.
- The reported data in literature have all been produced with different sampling strategies to serve different project aims.
- The analytical target compounds and the chemical analytical methods are not standardised.
- The data reporting is not standardised, i.e. for solid samples the dry weight vs. wet weight can constitute significant differences.
- Metadata, which enables adequate data comparisons, are very often limited available in literature.

Again, the transformation of gucuronidated metabolic conjugates may also be reflected in these results since only O-glucuronidation is possible for these drugs.

8.4 Bioassay procedures to estimate hormone activity of pharmaceuticals in environmental samples

Steroid hormones may also be screened in bioassay procedures. Both androgens and estrogens are highly active specific compounds that exert their hormonal function at low concentrations. The hormones are excreted, usually after a reversible metabolic conversion, and appear in sewage wastewaters. Despite dilution, the hormones in treated wastewaters may affect biota in the receiving waters. Conventional chemical analytical procedures may not always quantify these low concentrations. Instead their occurrence can be detected indirectly by their biological function in specifically designed bioassays. These assays measure an effect of all present androgens or estrogens. If the compositon of the hormones is not entirely known these assays will detect the integrated effect of all specific components present in an environmental sample that can cause a hormonal response. Furthermore, as these samples in many cases contain mixtures of contaminants in very low concentrations, it may be difficult to assess the risk based on chemical analysis of a limited amount of known hormonally active compounds. It is also apparent that interactions between different endocrine systems occur. Therefore bioassays are used since they reflect the actual *in vivo* situation that might occur upon exposure to complex mixtures in the aquatic environment.

8.4.1 Androgenic effects

Androgens are steroids, released by testis and the adrenal cortex, that control male reproductivity and anabolic processes. The hormone function involves binding of the steroids to specific androgen receptors. This property has been adopted in a bioassay format and is used to characterise environmental samples regarding androgenic effects. Testosterone and dihydrotestosterone (DHT) are considered the most important male hormones, but other derivatives are also used therapeutically and in veterinary medicine.

The androgen receptor test was used to detect hormonal effects in a selection of environmental samples in the screening program. The results of tests of androgenic effects in samples of outlets to receiving waters are given in Table 18.

Table 18. Androgenicity and norethindrone concentrations in extracts of samples of outlets to receiving waters in Sweden

Localities	Date	Androgenicity (ng DHT units /l, ± s.d., n=3)	Norethindrone (ng/l)
Background lakes, surface water			
Tärnan	2005-11-14	< 2	1.0
Stora Envättern	2005-11-13	< 2	5.0
Lilla Öresjön	2006-01-12	< 2	<2
Animal farms			
Horserace camp, pond	2005-12-06	< 5	6.9
Horserace camp, inlet	2005-12-06	< 5	13
Pig farm, 20 m from urine container	2005-12-07	< 5	< 4
Pig farm, ditch 200 m downstream	2005-12-07	< 5	2
Pig farm, farm well	2005-12-07	< 5	1.0
Cattle farm, outlet	2005-12-10	< 5	2.0
Cattle farm, outlet	2005-12-11	< 5	3.0
Hospital sewage wastewater			
Hospital A	2005-11-25	420 (380-460)	34
Landfill leachate water			
Landfill A, leachate water pond	2004-06-28	12 (8.8-15)	4.0
Högbytorp, untreated	2005-11-22	75 (70-80)	n.a
Högbytorp, biological treatment	2005-11-22	< 10	5.0
Municipal sewage wastewater			
Uddebo STP, Luleå	2005-10-25	37 (33-41)	< 4
Strömsund STP	2005-10-04	< 5	2.0
Reffelmansverket STP, Hudiksvall	2005-10-26	< 5	5.0
Kristianstad STP	2005-10-25	< 5	< 4
Främby STP, Falun	2005-10-04	< 5	< 4

n.a. = not available

No androgenic effects were detected in any of the background lake samples. The detection limit was calculated as 2 ng DHT units/l. The results were congruent with those of previous investigations (Svenson, Allard 2002a, 2004a). In extremely humic lakes, however low but significant androgenic effects has previously been detected.

Androgenic effects were tested in samples of treated wastewater from five municipal sewage treatment plants. The results showed that no effects were detected in four of the STPs. The

detection limit was calculated as 5 ng DHT units/l. The results were similar to those previously reported, where it was found that substances responsible for the androgenic effects were easily transformed into products with less androgenic effects (Svenson, Allard 2002b, 2004b). Biological treatment in STPs usually reduces the levels of androgenic effects in municipal sewage wastewater. Treated wastewater from Uddebo STP in Luleå contained 37 ng DHT units/l. This STP uses a biological reactor treatment preceded and followed by aluminium precipitation, which in previous investigations have given somewhat lower reduction of estrogenic effects in the same purification plant (Svenson et al. 2003).

Leachate water from two landfills, in one case without and with biological treatment, was assayed for androgenicity. The results show that substances with androgenic effects were detected in these outlets. Effects corresponding to 12 and 75 ng DHT units /l were measured. After treatment of leachate water at the landfill at Högbytorp the level decreased to below the detection limit (< 10 ng DHT units/l). Androgenic effects in leachate water have previously been reported (Svenson, Allard 2002b, 2004c, Svenson et al. 2004, 2005). Levels from < 1 to 72 ng DHT units/l were found. The reduction in androgenicity upon biological treatment of leachate water at Högbytorp was also in accordance with previous findings.

The results from storm water and sewage water from animal breeding locations showed no androgenic effects. The detection limit was 5 ng DHT units/l.

One hospital sewage wastewater was tested for androgenic effects *in vitro*. The hospital released substances with androgenic effects in its sewage wastewater corresponding to 420 ng DHT units/l. The effect level was close to the highest levels that have been measured in wastewaters in Sweden (Svenson et al. 2004).

Norethindrone was analysed in samples of water tested for androgenicity. The detection limit of the analytical procedure was 2-4 ng/l. Values obtained in most samples were close to or below the detection limit. The highest concentration was found in a hospital wastewater that also had the highest androgenic effects. Untreated landfill leachate water also had a higher norethindrone concentration correlated with a higher androgenic effect. Otherwise there was no apparent correlation between analysed concentrations of norethindrone and the androgenic effects.

Previously known androgenic properties in outlets from landfills and in some cases municipal sewage treatment works were confirmed in this investigation. Hospitals contributed to the release of substances with androgenic effects. The specific effect of norethindrone in the androgenicity test is not known. Probably it is lower than that of dihydrotestosterone that was used as a positive control in the androgenicity test.

8.4.2 Estrogenic effects

Estrogens control the female reproductive system development and function in vertebrates. The main estrogens are estradiol and its metobolic conversion products estrone and estriol. Some of them are also used as pharmaceuticals. The derivative ethinylestradiol is used as an active component in contraceptive pills. The hormones are targeted to specific receptors. As in the case of the androgens, specific bioassays for measurements of estrogenic effects have been developed based on the principle of receptor interaction.

The estrogen receptor test was used to detect hormonal effects in a selection of environmental samples in the screening program. The results of tests of estrogenic effects in samples of outlets to receiving waters are given in Table 19.

Table 19. Estrogenicity and estrogenic compounds in extracts of samples of outlets to receiving waters in Sweden

Localities	Date	Estrogenicity (ng E2 units/l, ± s.d., n = 3)	EE2 ng/l ELISA	EE2 ng/l	E2 ng/l	E3 ng/l
Background lakes, surface water						
Tärnan	2005-11-14	< 0.1		< 0.5	< 0.3	< 0.1
Stora Envättern	2005-11-13	< 0.1		< 0.5	2.0	< 0.1
Lilla Öresjön	2006-01-12	< 0.1		< 0.5	< 0.3	< 0.1
Animal farms						
Horserace camp, pond	2005-12-06	< 0.1		< 0.5	< 0.3	< 0.1
Horserace camp, inlet	2005-12-06	< 0.1		< 0.5	< 0.3	< 0.1
Pig farm, 20 m from urine container	2005-12-07	< 0.1		< 2	< 1	< 0.5
Pig farm, ditch 200 m downstream	2005-12-07	< 0.1		< 0.5	< 0.3	< 0.1
Pig farm, farm well	2005-12-07	< 0.1		< 0.5	< 0.3	< 0.1
Cattle farm, outlet	2005-12-10	< 0.1		1.0	< 0.3	< 0.1
Cattle farm, outlet	2005-12-11	< 0.1		3.0	< 0.3	< 0.1
Hospital sewage wastewater						
Hospital A	2005-11-25	17 (16-18)	0.69	12	< 0.3	< 0.1
Landfill leachate water						
Landfill A, leachate water pond	2004-06-28	0.62 (0.52-0.72)		0.7	1	5
Högbytorp, untreated	2005-11-22	< 1.0		n.a	n.a	n.a
Högbytorp, biological treatment	2005-11-22	< 1.0		< 0.5	< 0.3	< 0.1
Municipal sewage wastewater						
Uddebo STP, Luleå	2005-10-25	50 (41-61)	0.81	< 2	< 1	< 0.5
Strömsund STP	2005-10-04	1.9 (1.7-2.1)	0.52	< 0.5	< 0.3	< 0.1
Reffelmansverket STP, Hudiksvall	2005-10-26	4.2 (4.0-4.4)	0.24	< 0.5	< 0.3	6.0
Kristianstad STP	2005-10-25	2.5 (2.3-2.8)	0.72	< 2	< 1	< 0.5
Främby STP, Falun	2005-10-04	0.3 (0.2–0.4)	0.63	< 2	< 1	< 0.5

n.a. = not available

No estrogenic effects were detected in any of the background lakes in accordance to previous investigations of uncontaminated lake water (Svenson et al. 2003). The detection limit was 0.1 ng E2 units/l.

Samples of treated municipal wastewaters were tested for estrogenic effects. In one of the treatment plants (Uddebo STP, Luleå) an estrogenic effect corresponding to 50 ng E2 units/l was found, which is the highest level measured we have found in a treatment plant in Sweden. The level was higher than < 0.1 – 15 ng/l that previously have been recorded in treated municipal sewage wastewaters (Svenson, Allard 2002a-b, Svenson et al. 2000, 2002, 2003). The treatment plant using a bioreactor and aluminium precipitation both prior to and after the biological step has had a high estrogenicity in its treated water before. The extreme value may to some extent also depend on

different sampling procedure and sampling period of the year. In the four remaining STPs, levels between 0.3 and 5 ng E2 units/l were found, in accordance with previous investigations.

Wastewater from two landfills were sampled and assayed for estrogenicity. As shown in the table low or not quantifiable levels were detected in these outlets. In the extraction of samples of these wastewaters, also substances with toxic, cell growth inhibiting properties on the yeast cells used in the bioassay were extracted. The detection limit was therefore higher than that usually obtained in these assays. The estrogenicity in leachate water from the landfill at Högbytorp was 2.6 ng E2 units/l in an earlier study, and from Landfill A somewhat lower (Svenson et al. 2004, 2005). The differences were not greater than expected considering the differences in sampling period and procedure. As no measurable effect was detected in wastewater from the landfill at Högbytorp before purification treatment, the effect of the wastewater treatment may not be evaluated.

No estrogenic effects were detected for storm water or wastewater from the animal farm areas. The detection limit was 0.1 ng E2 units/l. Low levels may depend on that samples were collected at some distance from the possible source, but otherwise estrogenic effects should be expected in waters contaminated with urine and faeces from domestic animals.

A hospital sewage wastewater was assayed in the estrogenicity test. The estrogenic effects in this wastewater corresponded to 17 ng E2 units/l, a level that also may be found in untreated municipal wastewaters. The level is high enough to produce effects in aquatic organisms especially fish, if exposed in receiving waters.

The estrogenic hormones estradiol (E2), estriol (E3) and ethinylestradiol (EE2) were analysed in samples of wastewater, the latter also using a specific immunoassay analysis. Estriol was only found in one of the samples, treated municipal wastewater from the STP in Hudiksvall. Levels in other samples were below the detection limit. Estriol at the detected concentration or below may not contribute to the overall estrogenicity in the wastewater samples, because the specific activity in the receptor test is much lower than for e.g. estradiol, estrone and ethinylestradiol (Svenson et al. 2003). Estradiol was detected in a sample of surface water from a reference lake, Stora Envättern. There are no known outlets into the lake that could explain the occurrence of the hormone. Analyses of estradiol in wastewaters were below the detection limits. Ethinylestradiol was detected downstream an area where cattle was kept, in a hospital sewage wastewater and in a leachate water from a landfill. The occurrence in water flows on the cattle farm remains to be explained. There was no support in the results of the receptor test, where ethinylestradiol has a higher specific activity than estradiol (Svenson et al. 2003). The occurrence in hospital wastewater may be expected, but the level 12 ng/l was high in comparison to other untreated wastewater outlets. The level may account for the effect in the receptor test although contributions from estrone should be expected. The leachate wastewater from the landfill at Högbytorp contained 8 ng ethinylestradiol/l. This compound, but at lower concentrations, has been detected in leachate wastewater before. The origin of the compound in this type of wastewater is however obscure.

Ethinylestradiol was also analysed in sewage wastewater from a hospital and municipal treatment works using an immunoassay. The levels varied within 0.2 and 0.8 ng/l. In municipal wastewaters the concentrations made up parts of the estrogenicity tested in the bioassay (expressed in ng E2 units/l). No apparent correlation between this estrogenicity and the concentrations of ethinylestradiol was found. The hospital wastewater contained 0.69 ng ethinylestradiol/l as compared to 12 ng/l in the chemical analysis.

The investigation showed that estrogenic effects were detected in STP outlets to the aquatic environment. The highest levels were found in wastewaters from a hospital and from one of the

municipal STPs, while others had lower levels. The lower levels were in accordance to those found in previous investigations. Landfill leachate wastewaters had comparatively lower estrogenic effects. No estrogenic effects were detected in outlets from animal farm areas.

8.5 Risk assessment (MEC/PNEC)

An alternative way to relate the measured concentrations to known environmental effects is to perform a risk assessment and derive risk characterisation quotient based on the measured concentrations (MEC) and the predicted no effect concentrations (PNEC). When the MEC/PNEC ≤ 1 no negative effects of the substance is expected but when MEC/PNEC ≥1 the substance is considered to be problematic in the environment and further investigations are needed (TGD, 2003). Risk quotients are presented in Table 20 and PNECs for the risk assessment are according to Table 4. In order to use the effluent data from the STPs where no recipient water samples were provided a dilution factor of 10 was applied to all effluent concentrations (TGD, 2003). Since the PNEC calculation is associated with certain uncertainties depending on availability and quality of data, applied safety factors and that the screening results are not statistically reliable but rather "a snap shot" of the situation the results should be interpreted with care and regarded as indications to where there is a need for further investigations. This is especially important regarding the point source samples that are limited in number.

No risk assessment was performed for ketoprofen, demeclocycline and chlorocycline, norethindrone and progesterone due to lack of PNEC data. The risk quotas for diclofenac, naproxen, oxytetracycline, tetracycline and doxycycline were all <1 The PNECs used for the risk assessment for antibiotic did not though include end points for antibiotic resistance in nature which is one of the undesirable environmental effects associated with antibiotics. The risks associated with these compounds can therefore not be totally discarded based on the results.

The substances that showed the highest risk quotients were as expected ethinylestradiol and estradiol. There were only a few samples that had detectable concentrations of these substances and hence risk quotients >1. However, the PNECSs are so low for these substances that the risk for effects in samples below LOD can not be totally discarded since effects could exist in concentrations lower than what the analytical procedure could detect. None of the STP effluent samples that showed estrogenic effect in the estrogenicity testing had for example risk quotients > 1. At the same time the two surface water samples, adjacent to the cattle farm that showed increased levels of ethinylestradiol and hence had a risk quotients >1, showed no estrogenic effects.

Risk quotients of eight STP effluent samples and 2 surface water samples adjacent to point sources was >1 regarding ibuprofen. Eight other STP effluent samples and one recipient water sample for STPs had risk quotients >1 for Estriol.

Only one available recipient water sample had risk quotients >1 (estriol) however for the effluents that had elevated risk quotients no samples were taken from the corresponding STP recipient. A future recommendation is therefore to analyse samples from STP recipients especially during the cold season when concentrations seem elevated regarding anti-inflammatory substances and antibiotics. This in order to see to what extent the effluents from the STPs are diluted and at what distance from the source effects could be anticipated. Some surface water samples around animal breeding locations also had risk quotients >1 implying that it could be interesting to continue investigating the risks associated with this type of facilities.

Table 20. Samples with risk quotients >1

Sample ID	Site	Sample characteristics	MEC/PNEC Ibuprofen	MEC/PNEC Estriol	MEC/PNEC Estradiol	MEC/PNEC Ethinyl-estradiol
4162	Avesta STP	Effluent	1.4	-	-	-
4185	Bräcke STP	Effluent	1.1	-	-	-
4239	Hede STP	Effluent	2.5	-	-	-
4224	Åre STP	Effluent	1.1	-	-	-
4282	Luleå STP	Effluent	1.4	-	-	-
4256	Karlshamn STP	Effluent	-	-	-	190
4239	Hede STP	Effluent	-	6.4	-	-
4219	Krokom STP	Effluent	-	-	70	-
4224	Åre STP	Effluent	-	6.3	-	-
4332	Landsbro STP	Effluent	-	4.8	-	-
4271	Piteå STP	Effluent	2.4	7.3	-	-
4373	Ängelholm STP	Effluent	-	2.3	-	-
4150	Strängnäs STP	Effluent	-	1.2	-	-
4242	Vingåker STP	Effluent	-	-	145	-
4304	Skellefteå STP	Effluent	-	2.9	-	-
4300	Norberg STP	Effluent	-	-	10	-
4188	Trosa STP	Effluent	1.1	-	-	-
4342	Lycksele STP	Effluent	1.4	-	-	-
4246	Kristinehamn	Recipient STP	-	4.4	-	-
4428	Pig farm	Surface water	1.3	-	-	-
4424	Cattle farm	Surface water	-	-	-	50
4425	Cattle farm	Surface water	88.6	-	-	150
4367	Södertälje	Background surface water	-	-	100	-

9 Conclusions

The overall objective of this study were to determine the concentrations of the selected pharmaceuticals in a variety of media in the Swedish environment, to highlight important transport pathways, and to assess the possibility of current emissions in Sweden. The sampling program utilised in this screening study is designed to yield a "snapshot" picture of concentrations rather than statistically significant comparisons, which has to be taken in consideration for the interpretation of the results.

The anti-inflammatory substances (NSAIDs) were the most frequently detected pharmaceutical group compared to the antibiotics and the hormones both in the environmental samples and in samples from the STPs.

There was a great variation in the concentrations of the pharmaceuticals among different sampling sites. The NSAIDs were generally found in the highest concentrations. However the other pharmaceutical substances exceeded at some sites the NSAIDs concentrations.

Among the NSAIDs, ibuprofen and naproxen, occurred in the highest concentrations while diclofenac in most cases was the least abundant. The most frequently found antibiotic substances were tetracycline and doxycycline.

In most of the samples, progesterone and norethindrone, were the hormones that occurred in the highest concentrations. The concentration levels of these compounds were found in somewhat higher concentrations than the very few data reported for STPs in litterature. The levels of the estrogens complied with the levels reported in literature. However, no obvious and consistent distribution pattern was found for the estrogens.

The concentrations of the pharmaceuticals in the background site samples (water and sediment) were, mostly below the detection limits. However the NSAIDs were present in one of the background lakes which might have been affected by private drains. Chlorocycline, norethindrone and progesterone occurred in low levels occasionally in the other lakes.

The pharmaceuticals were frequently found in the samples collected at the STPs indicating the importance of STPs as a source for these substances. There was however a great variation in the concentrations, both in the infuent and effluent waters as well as in the sludge among the STPs located all over Sweden. The differences may be due to different consumption pattern, number of person equivalents contributing to the STP influent, as well as the treatment process in the STP.

Comparisons of the concentrations in the influent and effluent water showed that the reduction efficiency in the STP varied among the different substances and occasionally some of the substances occurred in higher concentrations in the effluent. The degradation pathway and stability of the major metabolites seems to be important in understanding the fate of a drug in the STP process and further research in this area is needed.

A regional trend was found for the NSAIDs, with the highest effluent water concentration of ibuprofen and naxproxen from the STPs situated in the northern part of Sweden.

The concentrations of the pharmaceuticals in the recipient water samples collected close to STPs were lower or at the same level as in the background water samples. However only few samples were included in the screening and the possible transport from the STPs to the recipient ought to be more thoroughly investigated.

Despite the fact that quite a small part of the used quantites of the NSAIDs ends up in sludge they nevertheless constitute unwanted substances if the sludge is going to be used in agriculture or forestry.

The pharmaceuticals occurred in landfill leachate water samples in concentrations in the same order of magnitude as for the effluents from the STPs.

Pharmaceutical residues were also found in the effluents from hospitals where the concentrations of the antibiotics and diclofenac in some of the samples exceeded the concentrations in the STPs effluents.

Livestock facilities may constitute point sources of pharmaceuticals to the environment as increased concentrations were found in some of the samples. However these results should be seen as an indication and more measurements are needed to evaluate the importance of animal breeding.

The bioassay investigations showed that estrogenic effects were detected in STP outlets to the aquatic environment. The highest levels were found in wastewaters from a hospital and one of the municipal STPs, while others had lower levels. The lower levels were in accordance to those found in previous investigations. Landfill leachate wastewaters had comparatively lower estrogenic effects. No estrogenic effects were detected in outlets from animal farm areas.

Values obtained for androgenicity were in most samples close to or below the detection limit. The highest androgenic effects was found in a hospital wastewater that also had the highest concentration of norethindrone . Untreated landfill leachate water that also had a higher norethindrone concentration correlated with a higher androgenic effect. Otherwise there was no apparent correlation between analysed concentrations of norethindrone and the androgenic effects.

The risk quotients for diclofenac, naproxen, oxytetracycline, tetracycline and doxycycline were all <1 The PNECs used for the risk assessment for antibiotics did not though include end points for antibiotic resistance which is one of the undesirable environmental effects associated with antibiotics. The risks associated with these compounds can therefore not be discarded based on these results. The substances that showed the highest risk quotients were as expected ethinylestradiol and estradiol. Ibuprofene also had risk quotients > 1 in several effluent samples for STPs.

Several effluent samples had risk quotients > 1. On the other hand only one available recipient water sample had risk quotient > 1 (estriol). However for the effluents that had elevated risk quotients no sample from the corresponding STP recipient was anlysed. A future recommendation would therefore be to analyse STP recipients samples especially during the cold season when concentrations seem elevated regarding in particular anti-inflammatory substances and in some extent the antibiotics.

Some surface water samples around animal breeding locations also had risk quotients > 1 implying that it could be interesting to continue with investigation around these type of facilities.

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PBT-profiler	http://www.pbtprofiler.net/
clogp	http://www.daylight-eu.com/daycgi/clogp
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Appendix 1. Ecotox data

(Läkemedelsverket, 2004)

Substance	Species	Test	Data
Oxitetracyclin	Folsomira fimetaria (springtail)	EC50, reproduction, 21 days	>5000mg/kg dw*
	Folsomira fimetaria (springtail)	NOEC, reproduction, 21 days	>5000mg/kg dw*
	Folsomira fimetaria (springtail)	NOEC, survival adult, 21 days	>5000mg/kg dw*
	Enchytraeus crypticus (white pot-worm)	EC50, reproduction, 21 days	2701 mg/kg dw*
	Enchytraeus crypticus (white pot-worm)	NOEC, reproduction, 21 days	2000 mg/kg dw*
	Enchytraeus crypticus (white pot-worm)	LC50, survival, 21 days	>5000mg/kg dw*
	Enchytraeus crypticus (white pot-worm)	NOEC, survival, 21 days	3000mg/kg dw*
	Aporrectodea caliginosa (earth worm)	EC50, reproduction, 21 days	4420 mg/g dw*
	Aporrectodea caliginosa (earth worm)	NOEC, reproduction, 21 days	3000 mg/g dw*
	Aporrectodea caliginosa (earth worm)	NOEC, survival adult, 21 days	>5000 mg/kg dw*
	Aporrectodea caliginosa (earth worm)	EC50, hatching, 21 days exposure	>5000 mg/kg dw*
	Aporrectodea caliginosa (earth worm)	NOEC, hatching, 21 days exposure	>5000 mg/kg dw*
	Aporrectodea caliginosa (earth worm)	EC50, growth, 21 days exposure	>5000 mg/kg dw*
	Aporrectodea caliginosa (earth worm)	NOEC, growth, 21 days exposure	3000 mg/kg dw*
	Daphnia Magna	EC50 reproduction, 21 days	3000 mg/kg dw*
	Microcystis aeruginosa (cyanobacterai)	EC50	0.207 mg/l*
	Selenastrum capricomutum (green algae)	EC50	4.5 mg/ml*
	R. Salina (algae)	EC50	1.6 mg/l*
	Fish	EC50, ECOSAR	1.66e-5 mg/l*
	Daphnid	EC50, ECOSAR	2432mg/l*
	Algae	EC50, ECOSAR	2294 mg/l*
	Penaeus setiferus	LC50, 24 h	>5 mg/l*
	Morone Saxatilis	LC50, 24,48,72,96 h	150,125,100,75 mg/l*
	Lemna Gibba	EC50,growth, ww, 7 days	1010μg/l*
	Lemna Gibba	LOEC,growth, ww, 7 days	1000μg/l*
	Selenastrum capricomotum (green algae)	EC50, 72 h	4.18 mg/L*
Tetracycline	Daphnia magna	EC50, reproduction, 21 days	44.8mg/l*
	Microcystis aeruginosa (limnic cyanobacteria)	EC50,growth, 7 days	0.09 mg/l*
	Selenastrum capricomutum (green algae)	EC50, growth, 3 days	2.2 mg/l*
	Fish	EC50, ECOSAR	16 mg/l*
	Daphnid	EC50, ECOSAR	550 mg/L*
	Algae	EC50, ECOSAR	475 mg/l*
	Nitszscha closterium	EC50, 72 h	16 mg/l*
	Salvenius namaycush (fish–lake trout)	LC50, 24/96h	220 mg/l*
	Morone saxatilis (fish-stripped bass)	LC50,24,48,96 h	>182mg/L*
	Lemna Gibba	EC50, growth, ww 7 days	723 μg/l*

Substance	Species	Test	Data
	Lemna Gibba	LOEC, growth ww, 7 days	1000 µg/l*
Doxycyclin	Lemna gibba	EC50, growth wet weight, 7 days	316 µg/l*
	Lemna gibba	LOEC, growth, 7 days	300 µg/l *
Diclofenac	Vulture	Acute EC50, food,	0.007 mg/kg*
	Vibrio Fischeri (bacteria)	EC50, 30 min	11.45 mg/kg*
	Vibrio Fischeri (bacteria)	EC 50,Tox alert,15min	13.5 mg/l*
	Vibrio Fischeri (bacteria)	EC50, Microtox	13.7 mg/l*
	Pseudokirchnerellia subcapitata= S. capricomutum (green algae)	NOEC, 96 h, growth	10 mg/L*
	Pseudokirchnerellia subcapitata= S. capricomutum (green algae)	LOEC, 96 h, growth	20 mg/l*
	Daphnia Magna	EC50, 48 h	224.3 mg/l*
	Cerodaphnia dubia (kräftdjur)	EC50, 48 h	22.7 mg/l*
	Cerodaphnia dubia (kräftdjur)	NOEC, 7 days, reproduction	1.0 mg/l*
	Cerodaphnia dubia (kräftdjur)	LOEC, 7 days, reproduction	2 mg/l*
	Brachionus calyciflorus (rotifer)	NOEC, 48 h, reproduction	12.5 mg/l*
	Brachionus calyciflorus (rotifer)	LOEC, 48 h reproduction	25 mg/l*
	Danio rerio (zebra fish)	NOEC, 10 days, ELS	4 mg/l*
	Danio rerio(zebra fish)	LOEC, 10 days, ELS	8 mg/l*
	Desmodesmus subspicatus (green algae)	EC50, salted with Sodium, 72 h	72 mg/l*
	Daphnia magna	EC50, salted with sodium, 48 h	68 mg/l*
	Lemna Minor (duck weed)	EC50, growth, 7 days	7.5 mg/l*
Ibuprofen	Lemna gibba	NOEC, growth, 7 days	>1000 µg/l*
	Vibrio fischier (bacteria)	EC50, Tox Alert, 15 min	12.1 mg/l*
	Vibrio Fischirie (bacteria)	EC50, microtox	19.1 mg/l*
	Lepomis macrochirus	LC50, 96 h, static	173 mg/l*
	Lepomis macrochirus	NOEC, 96 h, static	10 mg/l*
	Daphnia Magna	LC50, 48 h, static	9.06 mg/l*
	Daphnia Magna	NOEC, 48 h, static	3.37 mg/l*
	Skeletonema costatum (alg)	EC50,96 h	7.1 mg/l*
Ketoprofen	Fish	EC50, ECOSAR	32 mg/l*
	Daphnid	EC50, ECOSAR	248 mg/l*
	Algae	EC50, ECOSAR	164 mg/l*
	Vibrio fischerie (bacteria)	EC50, Microtox	19.3 mg/l*
	Vibrio Fischeri (bacteria)	EC50,Tox Alert 100, 15 min	15.6 mg/l*
Naproxen	Vibrio Fischerie (bacteria)	EC50,Tox Alert 100, 15 min	21.2 mg/l*
	Vibrio fischeri (bacteria)	EC50, Microtox	35 mg/l*
Estradiol	Oryzias latipes (japansk risefish)	LOEL, induced intersex (testis-ova),exposure from hatching)	0.004 µg/l*
	Oryzias latipes (japansk risefish)	NOEL, induced intersex (testis-ova)	0.0004 µg/l*
Estriol	Oryzias latipes (japansk risefish)	LOEL, induced intersex (testis-ova),exposure from hatching, 90 days	0.75 µg/l*

Substance	Species	Test	Data
	Oryzias latipes (japansk risefish)	NOEL, induced intersex (testis-ova),exposure from hatching-90 days	0.075*
	Oryzias latipes (japansk risefish)	LOEL, biased sexual differentiation(significant more females than males) exp. as above	0.01 µg/l*
Etinylestradiol	Invertibrattoxicitet	NOEC, reproduction	0.01 mg/l*
		EC50 reproduction	0.105 mg/l*
		EC50 acute	5.7 mg/l*
	Algae toxicity	EC50	0.84 mg/l*
	Oryzias latipes (fish-japanese risefish)	LOEL induced intersex (testis-ova) exposure from hatching	0.03 ng/l *

*Reference cited in Läkemedelsverket, 2004

Appendix 2. Sample characteristics national samples

City	Site	Matrix	Sample Information	Site information	Coordinates	Sampling date	DW(%)	Sample ID
	provisions, fish 1	biota	cultivated salmon	Stockholm, 3.3 % LW				4588
Human exposure	provisions, fish 2	biota	wild salmon	Nordingrå, 8.3 % LW				4589
	provisions, fish 3	biota	wild salmon	Baltic sea, 9.1 % LW				4590
	Horse track stable	manure		Outside veterinary hospital		2005-12-06	19.2	4438
	Horse track stable	manure		Stable		2005-12-06	16.8	4439
	Horse track stable	manure		Guest stable		2005-12-06	19.1	4440
Point source	Horse track stable	sediment		Influent horsetrack area		2005-12-06	58.8	4456
	Horse track stable	water	surface water	centre of horse track		2005-12-06		4435
	Horse track stable	water	surface water	Influent horsetrack area		2005-12-06		4436
	Pig farm	manure	firm			2005-12-07	16.7	4430
	Pig farm	manure	buoyant			2005-12-07		4431
	Pig farm	sediment		ditch 200 m from dung heap		2005-12-07	57.1	4432
	Pig farm	sediment		ditch in proximity of dung heap		2005-12-07	63.0	4433
	Pig farm	water	from well	well on farm		2005-12-07		4426
	Pig farm	water	surface water	ditch 200 m from dung heap		2005-12-07		4427
	Pig farm	water	surface water	ditch in proximity of dung heap		2005-12-07		4428
	Cattle farm	water	surface water	storm water from grazing field	6602869; 1661090	2005-12-11		4425
	Cattle farm	water	surface water	storm water from grazing field	6603544; 1661440	2005-12-10		4424
	Landfill	water	leachate					4609
	Högbytorp	water	leachate before treatment	Bro				4381
	Högbytorp	water	leachate after treatment	Bro				4382
	Hospital	water	effluent hospital					4390
	Recieving STP	sludge					26.0	4586
	Lilla Öresjön	sediment		Göteborg		2004-08-31	11.9	4504
	Stensjön	sediment		Södertälje		2004-08-12	15.1	4506
Background	Tärnan	sediment		Stockholm		2004-10-06	24.8	4505
	Lilla Öresjön	water	surface water	Göteborg		2006-01-12		4483
	Stora Envättern	water	surface water	Södertälje		2005-11-13		4367
	Tärnan	water	surface water	Stockholm	6608828; 645102			4368

Appendix 3. Sample information regional sludge samples

County	City	Site	Sample information	Site information	Dimen-sion (STP) pe	Affiliated (STP) pe	STP-treatment	Coor-dinates	Provtagning	DW, %	Sample identity
Blekinge	Karlshamn	Sternö STP		smaller industries,hospital and sludge from private sewage	30000	20 000	UCT	622560; 144004	2005-10-18	24	4258
	Karlskrona	Koholmens STP	not digested, random sample	nursing home and old person home	3300	2000	simultaneous sedimentation	622775; 149885	2005-10-25	16	4298
	Karlskrona	Ramdala STP	random sample	Dairy, leachate water and hospital	57000	40000	Bio-P och N, post sedimentation , sandfilter	622523; 148846	2005-10-25	22	4295
	Olofström	Olofström STP	not digested	Household, nursig home, old person home, industry	19500	12000	pretreatment, biol treatment active sludge/chem sedimentaion, aerob sludge tretment.-slambh aerob stab.+centrifugavv.	6233651; 1420545	2005-10-17	20	4237
	Ronneby	Rustorp STP	not digested, random sample	paper industry	24000	19000	pretreatment, biol treatment active sludge/chem sedimentaion, aerob sludge tretment.-slambh aerob stab.+centrifugavv.	6228468; 1468366	2005-10-11	18	4196
	Sölvesborg	Sölvesborgs STP	not digested, random sample	household, smaller industries, slaughterhouse, sludge froom private sewage and smaller STPs	19500	8600	mech/chem/biol	6213461; 1423579	2005-10-26	18	4311
Dalarna	Avesta	Krylbo STP		Steal,brewery, slaghterhouse, industry landfill, metal, wood, small industries, car wash, waste incineration	30000	17000	pre sedimentation/active sludge		2005-10-05 kl.09.00	23	4163

County	City	Site	Sample information	Site information	Dimen-sion (STP) pe	Affiliated (STP) pe	STP-treatment	Coor-dinates	Provtagning	DW, %	Sample identity
	Borlänge	Fagersta By STP		Houshold, smaller industries, car wash,land fill	60000	44000	pre sedimentation, simultaneous sedimentation and active sludge		2005-10-05	29	4160
	Bollnäs	Arbrå STP							2005-10-	21	4321
Gävleborg	Hudiksvall	Reffelmansverket						6844618; 568169	2005-10-26	28	4303
	Sandviken	Hedåsen							2005-11-09	24	4358
Jämtland	Berg	Hackås STP	not digested, not dehydrated	houshold, aluminium foundry, old age home	1700		mech/chem		2005-10-03	1,4	4145
	Bräcke	Bräcke STP	not digested	Household, car wash, gas stations, metal-, wood industry	3000	1800	mech/chem/biol		2005-10-05	14	4186
	Hede	Hede STP	not digested, not dehydrated biosludge	House hold ,bio sludge and chem sludge mixed and analysed as one sample	2500	2147	mech/chem/biol		2005-10-10	3	4240
	Hede	Hede STP	not digested, not dehydrated chemical sludge	House hold ,bio sludge and chem sludge mixed and analysed as one sample	2500	2147	mech/chem/biol		2005-10-10		4241
	Krokom	Hissmofors STP	not digested	Houshold, saw mill, mechanical repair, car repair, gas stations, car wash	6500	3600	mech/chem/biol		2005-10-12	18	4221
	Ragunda	Hammarstrand STP	not digested	Houshold, health center, nursing home, old age home	1800	1500	mech/chem/biol		2005-10-12	16	4223

County	City	Site	Sample information	Site information	Dimen-sion (STP) pe	Affiliated (STP) pe	STP-treatment	Coor-dinates	Provtagning	DW, %	Sample identity
Jämtland	Strömsund	Strömsund STP	not digested	Houshold, wood impregnation, metal industry, plastic industry, car repair, laundry,	7000	4000	mech/chem/biol		2005-10-04	21	4147
	Åre	Åredalen STP	not digested	Houshold, tourist activities	25000	8200	mech/chem +infiltr.		2005-10-10/11	18	4225
	Östersund	Göviken STP		Houshold, hospital, Dairy, Mejeri,charkuteri, metal industry	110000	49200+ind.belastning 15600	mech/chem/biol		2005-10-06	20	4182
Jönköping	Landsbro	Landsbro STP	not digested, not dehydrated						2005-11-16	0,80	4377
	Vetlanda	Vetlanda STP	not digested						2005-11-16	0,90	4376
Kalmar	Hultsfred	Hultsfred STP	not digested						2005-11-16	21	4378
	Virserum	Virserum STP							2005-11-16	13	4375
Kronoberg	Alvesta	Alvesta STP			12 000	8 000	mech/chem/biol	6307670; 1423430	2005-11-07	29	4354
	Lessebo	Lessebo STP			9 000	6 200	mech/chem/biol	6291100; 1466460	2005-10-04	18	4340
	Ljungby	Ljungby STP			33 000	20 000	mech/chem/biol	6300900; 1385300	2005-10-19	22	4260
	Markaryd	Ribersdals STP			6000	5 000	mech/chem/biol	6262850; 1362850	2005-10-31	18	4318
	Tingsryd	Tingsryds STP			42 000	15 000	mech/chem/biol	6264950; 1449750	2005-11-02	20	4338
	Uppvidinge	Åseda STP	not digested		6000	2 700	mech/chem/biol	6337800; 1473850	2006-01-02	18	4481
	Växjö	Sundet STP			80 000	65 000	mech/chem/biol	6303900; 1436200	2005-10-10	21	4191
	Älmhult	Älmhults STP			22 700	15 000	mech/chem/biol	6268000; 1396200	2005-10-10	21	4193
	Gällivare	Kavaheden STP		Hospital, health centers	20 000	18500	Mech/chem/biol	7287700; 1795760	2005-10-12	20	4234
Norrbotten	Luleå	Uddebo STP			85000	77300	mech/chem/biol	7254250; 1763850	2005-10-25	30	4283

County	City	Site	Sample information	Site information	Dimen-sion (STP) pe	Affiliated (STP) pe	STP-treatment	Coor-dinates	Provtagning	DW, %	Sample identity
Skåne	Piteå	Sandholmen STP		Hospital, health centers	35000	30500	mech/chem/biol	7458000; 1714000	2005-10-19	25	4273
	Eslöv	Ellinge ARV						6190879; 1343012	2005-10-20	17	4285
	Helsingborg	Öresundsverket							2005-10-25	23	4290
	Hässleholm	STP							2005-10-20	29	4276
	Hörby	Lyby STP	not digested; 0,3 % polyakrylamid i T.S.						2005-11-21	20	4380
	Kristianstad	Centrala Reningsverket							2005-10-25	19	4308
	Landskrona	STP							2005-11-30	22	4414
	Lund	STP							2005-10-27	33	4316
	Malmö	STP							2005-11-02	24	4329
	Svedala	STP	not digested, sample taken from reed bed						2005-10-19	14	4265
	Trelleborg	STP	sample collected during october fron centrifuge						2005-10-20	26	4267
	Ystad	STP							2005-11-11	29	4365
	Ängelholm	STP							2005-11-14	27	4374
Södermanland	Eskilstuna	Ekeby STP							2005-10-04	28	4168
	Flen	Flen STP							2005-10-03	12	4133
	Katrineholm	STP Rosenholm							2005-10-18	19	4245
	Mariefred	Mariefred STP							2005-10-03	25	4156
	Strängnäs	Strängnäs STP							2005-10-03	19	4151
	Trosa	Trosa STP							2005-10-05	23	4190
	Nyköping	Mellanfjärden	recipient					1573817; 6513935	2005-11-07		4351*

County	City	Site	Sample information	Site information	Dimen-sion (STP) pe	Affiliated (STP) pe	STP-treatment	Coor-dinates	Provtagning	DW, %	Sample identity
Värmland	Karlstad	Sjöstad STP		Regional hospital, provisional industry, dental clinics, chemical clinicssmaller metal industriies, waste incineration	97000	55800	mech/chem/biol and nitrogen reduction			28	4201
	Kristinehamn	Fiskartorpet STP		hospital, industries	18000	16300	mech/chem/biol and nitrogen reduction	6578124; 1401080	2005-10-18	23	4250
Västerbotten	Lycksele	STP		industrial , hospital		9000			2005-11-02	25	4344
	Skellefteå	Tuvan STP				43000		175009; 719024	2005-10-24	23	4305
Västmanland	Hallstahammar	Mölntorp STP		1300 pe from industri	25000	11241	chem/bio	6602672; 1525476	2005-10-12	27	4208
	Norberg	Persbo STP		300 pe from industry	8000		chem/bio	6659138; 1507145	2005-10-25	19	4301
	Västerås	Tomta	not digested, not dehydrated						2005-11-09	0,31	4356
Västra Götaland	Skövde	Stadskvarn STP							2005-10-12	17	4217

Appendix 4. Sample characteristics regional water samples

County	City	Site	Site information	Sample Information	Dimension (STP)	Affiliated pe (STP)	STP-treatment	Coordinates	Sampling date	Sample Identity
	Ronneby	Angelskog landfill		leachate				6228411; 1469130	2005-10-05	4184
	Karlskrona	Bubbetorp landfill		leachate				6235012; 1487337	2005-10-03	4131
	Karlskrona	Hospital	hospital	Effluent; collected during 24 h hospital				6228230; 1487610	2005-10-18	4252
	Karlshamn	Hospital		effluent hospital; collected during 24 h				6229180; 1440830	2005-10-17/18	4253
Blekinge	Karlskrona	Koholmens STP	nursing home and old person home	Influent; collected during 24 h	3300	2000	simultaneous sedimentation	622775; 149885	2005-10-24/25	4296
	Karlskrona	Koholmens STP	nursing home and old person home	Effluent;collected during 24 h	3300	2000	simultaneous sedimentation	622775; 149885	2005-10-24/25	4297
	Olofström	Olofström STP	Household, nursing home, old person home, industry	effluent	19500	12000	Pre-treatment, biol treatment active sludge/chem sedimentaion, aerob sludge tretment.- slambh aerob stab.+centrifugavv.	6233100; 1420564	2005-10-17	4235
	Olofström	Olofström STP	Household, nursig home, old person home, industry	effluent after pond; random sample	19500	12000	Pre-treatment, biol treatment active sludge/chem sedimentaion, aerob sludge tretment.- slambh aerob stab.+centrifugavv.	6233603; 1420579	2005-10-17	4236
	Karlskrona	Ramdala STP	Dairy, leachate water and hospital	Influent; collected during 24 h	57000	40000	Bio-P och N, post sedimentation , sandfilter	622523; 148846	2005-10-24/25	4293
	Karlskrona	Ramdala STP	Dairy, leachate water and hospital	effluent ; collected during 24 h	57000	40000	Bio-P och N, post sedimentation , sandfilter	622523; 148846	2005-10-24/25	4294
	Ronneby	Rustorp STP	paper industry	Influent; collected during 24 h	24000	19000	Pre-treatment, biol treatment active sludge/chem sedimentaion, aerob sludge tretment.	6228468; 1468364	2005-10-11	4194
	Ronneby	Rustorp STP	paper industry	effluent ; collected during 24 h	24000	19000	Pre-treatment, biol treatment active	6228468; 1468365	2005-10-11	4195

County	City	Site	Site information	Sample Information	Dimension (STP)	Affiliated pe (STP)	STP-treatment	Coordinates	Sampling date	Sample Identity
							sludge/chem sedimentaion, aerob sludge treatment..			
	Sölvesborg	Sölvesborgs STP	household, smaller industries, slaughterhouse, sludge froom private sewage and smaller STPs	Influent; collected during 24 h	19500	8600	mech/chem/bio	6213461; 1423577	2005-10-26	4309
	Sölvesborg	Sölvesborgs STP	household, smaller industries, slaughterhouse, sludge froom private sewage and smaller STPs	effluent ;collected during 24 h	19500	8600	mech/chem/bio	6213461; 1423578	2005-10-26	4310
	Karlshamn	Sternö STP	smaller industries,hospital and sludge from private sewage	influent ; collected during 24 h	30000	20000	UCT	622560; 144000	2005-10-17/18	4254
	Karlshamn	Sternö STP	smaller industries,hospital and sludge from private sewage	Effluent; collected during 24 h	30000	20000	UCT	622560; 144002	2005-10-17/18	4256
Dalarna	Borlänge	Fågelmyra Landfill		leachate					2005-10-05	4157
	Borlänge	Fagersta By STP	Houshold, smaller industries, car wash,land fill	effluent	60000	44000	Pre-sedimentation, simultaneous sedimentation and active sludge		2005-10-05	4158
	Falun	Främbyverket	Houshold, smaller industries, car wash, land fill	effluent	51000	38500	Pre-sedimentation/active sludge		2005-10-04	4148
	Avesta	Hospital		effluent hospital					2005-10-05	4181
	Avesta	Krylbo STP	Steal,brewery, slaghterhouse, industry landfill, metal, wood, small industries, car wash, waste incineration	effluen	30000	17000	Pre-sedimentation/active sludge			4162
Gävleborg	Bollnäs	Arbrå STP		effluent						4320
	Sandviken	Hedåsen		effluent ;collected during 24 h					2005-11-09	4357
	Hudiksvall	Reffelmans-		effluent				6844618;	2005-10-26	4302

County	City	Site	Site information	Sample Information	Dimension (STP)	Affiliated pe (STP)	STP-treatment	Coordinates	Sampling date	Sample Identity
		verket						1568169		
Jämtland	Åre	Åredalen STP	Houshold, tourist activities	effluent	25000	8200	mech/chem +infiltr.		2005-10-10/11	4224
	Bräcke	Bräcke STP	Household, car wash, gas stations, metal-, wood industry	effluent	3000	1800	mech/chem/bio		2005-10-05	4185
	Östersund	Göviken STP	Houshold, hospital, Dairy, Mejeri,charkuteri, metal industry	effluent	110000	49200 +ind. 15600	mech/chem/bio		2005-10-06	4183
	Hede	Hede STP	House hold	effluent	2500	2147	mech/chem/bio		2005-10-10/11	4239
	Krokom	Hissmofors STP	Houshold, saw mill, mechanical repair, car repair, gas stations, car wash	effluent	6500	3600	mech/chem/bio		2005-10-11/12	4219
	Strömsund	Strömsund STP	Houshold, wood impregnation, metal industry, plastic industry, car repair, laundry,	effluent	7000	4000	mech/chem/bio		2005-10-04	4146
Jönköping	Landsbro	Landsbro STP		influent ; collected during 24 h					2005-11-02	4330
	Landsbro	Landsbro STP		Effluent; collected during 24 h					2005-11-02	4332
	Vetlanda	Vetlanda STP		influent ; collected during 24 h					2005-11-02	4334
	Vetlanda	Vetlanda STP		Effluent; collected during 24 h					2005-11-02	4336
Kalmar	Hultsfred	Hultsfred STP		influent ; collected during 24 h					2005-11-02	4348
	Hultsfred	Hultsfred STP		effluent ; collected during 24 h					2005-11-02	4349
	Virserum	Virserum STP		influent ;collected during 24 h					2005-11-02	4346
	Virserum	Virserum STP		Effluent; collected during 24 h					2005-11-02	4347
Kronoberg	Ljungby	Ljungby Hospital		effluent hospital				6302517;1385856	2005-10-18/19	4277
	Växjö	St. Sigfrid's Hospital		effluent hospital				6304930; 1440383	2005-10-11/12	4209
	Växjö	Växjö Hospital		effluent hospital				6305733; 1439141	2005-10-10/11	4211

County	City	Site	Site information	Sample Information	Dimension (STP)	Affiliated pe (STP)	STP-treatment	Coordinates	Sampling date	Sample Identity
Norrbotten	Gällivare	Kavaheden STP	Hospital, health centers	influent	20 000	18500	mech/chem/bio	7287700; 1795760	2005-10-12	4232
	Gällivare	Kavaheden STP	Hospital, health centers	effluent	20 000	18500	mech/chem/bio	7287700; 1795760	2005-10-12	4233
	Piteå	Sandholmen STP	Hospital, health centers	Influent; collected during 24 h	35000	30500	mech/chem/bio	7458000; 1714000	2005-10-19	4269
	Piteå	Sandholmen STP	Hospital, health centers	Effluent; collected during 24 h	35000	30500	mech/chem/bio	7458000; 1714000	2005-10-19	4271
	Luleå	Uddebo STP	Hospital, health centers, minor industries, dairy production	influent	85000	77300	mech/chem/bio	7254250; 1763850	2005-10-25	4281
	Luleå	Uddebo STP	Hospital, health centers, minor industries, dairy production	effluent	85000	77300	mech/chem/bio	7254250; 1763850	2005-10-25	4282
Skåne	Kristianstad	Centrala Reningsverket		effluent					2005-10-25	4307
	Eslöv	Ellinge ARV		effluent				6190879; 1343012	2005-10-19/20	4284
	Helsingborg	Helsingborg landfill		leachate ;NSR, Sample taken at the edge of "industry platform"					2005-10-25	4292
	Hörby	Lyby STP		effluent ;collected during 24 h					2005-11-21	4379
	Helsingborg	Öresundsverket		effluent					2005-10-25	4288
	Malmö	Spillepeng landfill, SYSAV		Leachate; collected at Malmö STP					2005-11-02	4328
	Svedala	STP		effluent					2005-10-18/19	4264
	Trelleborg	STP		effluent					2005-10-19/20	4266
	Hässleholm	STP		Effluent; collected during 24 h					2005-10-20	4275
	Lund	STP		effluent					2005-10-26/27	4314
	Malmö	STP		effluent ; collected during 24 h					2005-11-01	4327
	Ystad	STP		Effluent; sample collected during 24 h					2005-11-11	4363
	Ängelholm	STP		effluent ; collected during 24 h					2005-11-15	4373
	Landskrona	STP		effluent					2005-11-30	4413

County	City	Site	Site information	Sample Information	Dimension (STP)	Affiliated pe (STP)	STP-treatment	Coordinates	Sampling date	Sample Identity
Södermanland	Nyköping	Björshult landfill		leachate					2005-10-11	4205
	Nyköping	Brandholmen STP		effluent					2005-10-11	4204
	Eskilstuna	Ekeby STP		influent					2005-10-04	4164
	Eskilstuna	Ekeby STP		effluent					2005-10-04	4165
	Eskilstuna	Ekeby STP		effluent after wetland					2005-10-04	4166
	Flen	Flen STP		effluent					2005-10-03	4132
	Eskilstuna	Lilla Nyby landfill		leachate before cleaning					2005-10-05	4170
	Mariefred	Mariefred STP		influent					2005-10-03	4154
Södermanland	Mariefred	Mariefred STP		effluent					2005-10-03	4155
	Nyköping	Mellanfjärden		surface water				1573817; 6513935	2005-11-07	4350
	Katrineholm	STP Rosenholm		influent					2005-10-18	4243
	Katrineholm	STP Rosenholm		effluent					2005-10-18	4244
	Strängnäs	Strängnäs STP		influent					2005-10-03	4149
	Strängnäs	Strängnäs STP		effluent					2005-10-03	4150
	Trosa	Trosa STP		influent					2005-10-05	4187
	Trosa	Trosa STP		effluent					2005-10-05	4188
	Trosa	Trosa STP		effluent after wetland					2005-10-05	4189
	Vingåker	Vingåker STP		effluent					2005-10-12	4242
Värmland	Kristinehamn	Bergsjön		surface water				6586800; 1408540	2005-10-17	4246
	Säffle	Byälven		surface water				6557520; 1334089	2005-10-06	4180
	Karlstad	Klarälven	Kaplandsåd, down stream STP	surface water recipient				658699; 137257	2005-10-20	4268
	Kristinehamn	Varnumsviken		surface water					2005-10-12	4238
	Arvika	Vik STP	Hospitals industries	Effluent; collected during 24 h	27000	18000	mech/chem/biol and nitrogen reduction		2005-10-25	4306
Västerbotten	Lycksele	STP	industrial,hospital	effluent		9000		163808	2005-11-01/02	4342
	Skellefteå	Tuvan STP		effluent		43000		175009	2005-10-24	4304

County	City	Site	Site information	Sample Information	Dimension (STP)	Affiliated pe (STP)	STP-treatment	Coordinates	Sampling date	Sample Identity
	Hallsta-hammar	Mölntorp STP	1300 pe from industri	influent	25000	11241	chem/bio	6602672; 1525476	2005-10-12	4206
Västmanland	Hallsta-hammar	Mölntorp STP	1300 pe from industri	effluent	25000	11241	chem/bio	6602672; 1525476	2005-10-12	4207
	Norberg	Persbo STP	302 pe from industry	influent	8000		chem/bio	6659138; 1507145	2005-10-24/25	4299
	Norberg	Persbo STP	301 pe from industry	effluent	8000		chem/bio	6659138; 1507145	2005-10-24/25	4300
Västra Götaland	Skövde	Stadskvarn STP		influent					2005-10-12/13	4213
	Skövde	Stadskvarn STP		effluent					2005-10-12/13	4215

Appendix 5. Results regional samples

Sample identity	County	City	Information	Matrix	Unit	Oxytetra cycline	Tetra cycline	Demeclo cycline	Chloro cycline	Doxy cycline	Ibuprofe n	Napro xen	Ketoprof en	Diclofe nac	Estriol	Estradiol	Ethinyl estradiol	Norethi ndrone	Proges terone
4258	Blekinge	Karlshamn		sludge	µg/kg DW	<4	<2	<4	<8	<4	96	4.8	11	<3	<1	<2	<3	37	54
4256	Blekinge	Karlshamn	Effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	0.008	0.067	0.46	0.40	<0.0001	<0.0003	0.04	<0.0007	0.015
4253	Blekinge	Karlshamn	effluent hospital	water	µg/l	<0.0003	1.200	<0.0003	<0.0005	<0.0004	6.0	4.0	2.2	2.0	0.05	<0.0003	<0.0005	<0.0007	Intf
4254	Blekinge	Karlshamn	Influent	water	µg/l	0.24	1.0	0.049	0.19	0.65	9.3	4.9	5.7	0.57	<0.0001	<0.0003	<0.0005	0.003	0.017
4295	Blekinge	Karlskrona		sludge	µg/kg DW	<5	<2	<5	<9	<5	34	81	12	69	<1	<2	<3	<7	23
4294	Blekinge	Karlskrona	Effluent	water	µg/l	<0.0003	0.003	<0.0003	0.008	<0.0004	1.5	1.7	1.4	0.7	<0.0001	<0.0003	<0.0005	0.00	<0.0007
4297	Blekinge	Karlskrona	Effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	<0.0023	0.07	0.06	0.31	<0.0001	<0.0003	<0.0005	0.01	<0.0007
4252	Blekinge	Karlskrona	effluent hospital	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	3.1	26	0.9	1.3	0.1	<0.0005	<0.001	<0.002	<0.004	Intf
4293	Blekinge	Karlskrona	Influent	water	µg/l	<0.0003	0.430	<0.0003	<0.0005	<0.0004	11	8.0	6	0.67	<0.0001	<0.0003	<0.0005	0.01	0.009
4296	Blekinge	Karlskrona	Influent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	7.3	5.0	4.0	0.5	<0.0001	<0.0003	0.05	0.01	0.013
4131	Blekinge	Karlskrona	leachate	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	0.42	0.01	0.11	0.02	<0.0005	<0.001	<0.002	<0.004	0.012
4298	Blekinge	Karlskrona	not digested	sludge	µg/kg DW	1600	2200	<6	<12	340	34	15	21	<4	<2	<2	<4	<9	31
4235	Blekinge	Olofström	effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	0.7	0.8	2.0	0.6	<0.0005	<0.001	<0.002	<0.004	0.027
4236	Blekinge	Olofström	effluent after pond	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	0.8	0.8	0.5	0.3	<0.0005	<0.001	<0.002	<0.004	0.030
4237	Blekinge	Olofström	not digested	sludge	µg/kg DW	<5	1300	<5	<10	580	19	26	21	16	<2	<2	<4	<8	41
4195	Blekinge	Ronneby	effluent	water	µg/l	0.13	0.200	<0.0003	<0.0005	<0.0004	0.05	0.14	0.35	0.26	<0.0005	<0.001	<0.002	<0.004	0.040
4194	Blekinge	Ronneby	influent	water	µg/l	0.79	1.800	<0.0003	<0.0005	<0.0004	10	5	3	0.2	<0.0001	<0.0003	<0.0005	0.01	0.011
4184	Blekinge	Ronneby	leachate	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	0.78	0.02	0.11	0.01	0.020	<0.001	0.035	0.004	0.024
4196	Blekinge	Ronneby	not digested	sludge	µg/kg DW	<6	2500	<6	<11	<6	33	18	22	30	<2	<2	<4	<9	intf
4310	Blekinge	Sölvesborg	effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	0.6	0.7	0.6	0.3	<0.0005	<0.001	<0.002	<0.004	0.038
4309	Blekinge	Sölvesborg	influent	water	µg/l	0.38	0.520	<0.0003	<0.0005	0.66	3.7	3.5	1.2	0.2	<0.0001	<0.0003	<0.0005	0.02	0.011
4311	Blekinge	Sölvesborg	not digested	sludge	µg/kg DW	<6	<3	<6	<11	<6	42	31	11	7	14	<3	<4	170	110
4163	Dalarna	Avesta		sludge	µg/kg DW	<4	2200	630	<9	1100	57	<2	8	24	<1	<2	<3	<7	120
4162	Dalarna	Avesta	effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	0.099	5	2	2	0.2	<0.0005	<0.001	<0.002	<0.004	0.035

Sample identity	County	City	Information	Matrix	Unit	Oxytetracycline	Tetracycline	Demeclocycline	Chlorocycline	Doxycycline	Ibuprofen	Naproxen	Ketoprofen	Diclofenac	Estriol	Estradiol	Ethinylestradiol	Norethindrone	Progesterone
4181	Dalarna	Avesta	effluent hospital	water	µg/l	<0.0003	0.46	<0.0003	<0.0005	1.3	11	11	2	0.2	<0.0001	<0.0003	0.02	0.03	0.016
4160	Dalarna	Borlänge		sludge	µg/kg DW	<4	1800	<4	<7	<4	6	47	8	12	<1	<1	<2	<5	120
4158	Dalarna	Borlänge	effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	0.8	2.3	1.7	0.2	<0.0005	<0.001	<0.002	<0.004	0.025
4157	Dalarna	Borlänge	leachate	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	0.7	0.0	0.0	0.0	<0.0001	<0.0003	0.00	0.01	0.001
4148	Dalarna	Falun	effluent	water	µg/l	<0.0003	0.039	<0.0003	<0.0005	<0.0004	0.2	0.1	0.1	0.1	<0.0005	<0.001	<0.002	<0.004	0.035
4321	Gävleborg	Bollnäs		sludge	µg/kg DW	<5	<2	<5	<9	<5	92	49	45	59	<2	<3	<4	<7	Intf
4320	Gävleborg	Bollnäs	effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	0.8	1.7	1.8	0.6	<0.0001	<0.0003	<0.0005	0.01	0.002
4303	Gävleborg	Hudiksvall		sludge	µg/kg DW	<5	<2	<4	<7	<4	65	4	<7	6	<1	<1	<3	<5	110
4302	Gävleborg	Hudiksvall	effluent	water	µg/l	<0.0003	<0.0002	<0.0003	0.073	<0.0004	1.0	0.7	0.9	0.2	0.01	<0.0003	<0.0005	0.01	0.001
4358	Gävleborg	Sandviken		sludge	µg/kg DW	<4	2300	<4	<9	<4	310	14	27	10	36	<3	<3	<6	42
4357	Gävleborg	Sandviken	effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	2.5	8.4	2.7	0.2	<0.0001	<0.0003	<0.0005	<0.0001	0.001
4224	Jämtland	Åre	effluent	water	µg/l	<0.0003	<0.0002	<0.0003	0.68	<0.0004	3.3	2.2	0.3	0.0	0.05	<0.0003	<0.0005	<0.0007	0.050
4225	Jämtland	Åre	not digested	sludge	µg/kg DW	<6	180	<6	<11	210	86	79	12	18	<2	<2	<4	<8	22
4145	Jämtland	Berg	not digested, not dehydrated	sludge	µg/kg DW	<70	<40	<7	<150	<70	560	350	310	11	<20	<30	<50	<100	<100
4185	Jämtland	Bräcke	effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	3.3	4.1	2.0	0.1	<0.0005	<0.001	<0.002	<0.004	0.039
4186	Jämtland	Bräcke	not digested	sludge	µg/kg DW	<7	<4	<7	<14	<7	52	57	67	12	<2	<3	<5	<10	intf
4239	Jämtland	Hede	effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	7.8	0.7	0.0	0.1	0.05	<0.0003	<0.0005	<0.0007	0.062
4240	Jämtland	Hede	not digested, not dehydrated biosludge	sludge	µg/kg DW	<40	3100	<40	<80	<40	280	320	100	73	<12	<16	690	570	830
4219	Jämtland	Krokom	effluent	water	µg/l	<0.0003	<0.0002	<0.0003	0.017	<0.0004	0.3	0.2	0.1	0.1	<0.0001	0.01	<0.0005	<0.0007	0.018
4221	Jämtland	Krokom	not digested	sludge	µg/kg DW	<6	<3	<6	<11	480	10	88	13	16	<2	<2	<4	<9	97
4182	Jämtland	Östersund		sludge	µg/kg DW	<5	<3	<5	<10	<5	53	6	8	12	<1	<2	<3	<7	<10
4183	Jämtland	Östersund	effluent	water	µg/l	<0.0003	0.0820	<0.0003	<0.0005	<0.0004	1.1	1.0	0.7	0.1	<0.0005	<0.001	<0.002	<0.004	0.031
4223	Jämtland	Ragunda	not	sludge	µg/kg	<6	260	<6	<13	350	150	150	46	18	<2	<3	<4	<9	<13

Sample identity	County	City	Information	Matrix	Unit	Oxytetracycline	Tetracycline	Demeclocycline	Chlorocycline	Doxycycline	Ibuprofen	Naproxen	Ketoprofen	Diclofenac	Estriol	Estradiol	Ethinylestradiol	Norethindrone	Progesterone
			digested		DW														
4146	Jämtland	Strömsund	effluent	water	µg/l	<0.0003	0.13	<0.0003	<0.0005	<0.0004	0.8	3.7	1.1	0.2	<0.0001	<0.0003	<0.0005	0.00	<0.0007
4147	Jämtland	Strömsund	not digested	sludge	µg/kg DW	<5	<2	<5	<9	<5	75	48	19	33	<1	<2	<3	<7	14
4332	Jönköping	Landsbro	effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	0.1	0.2	0.4	0.1	0.036	<0.0003	<0.0005	0.003	0.014
4330	Jönköping	Landsbro	influent	water	µg/l	0.28	0.440	<0.0003	<0.0005	<0.0004	6.2	10.6	1.8	0.1	<0.0001	<0.0003	<0.0005	0.02	0.015
4377	Jönköping	Landsbro	not digested, not dehydrated	sludge	µg/kg DW	<100	28000	<100	<200	<100	22	87	41	28	<60	<80	<100	intf	intf
4336	Jönköping	Vetlanda	effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	0.0	0.1	0.0	0.1	<0.0001	<0.0003	<0.0005	0.01	<0.0007
4334	Jönköping	Vetlanda	influent	water	µg/l	0.72	1.700	<0.0003	0.34	0.6	7.5	6.4	2.7	0.2	<0.0001	<0.0003	<0.0005	0.01	0.008
4376	Jönköping	Vetlanda	not digested	sludge	µg/kg DW	<100	33000	<100	<200	<100	29	33	580	<15	<60	<70	6800	6100	<200
4349	Kalmar	Hultsfred	effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	1.3	0.9	1.0	0.2	<0.0005	<0.001	<0.002	<0.004	0.005
4348	Kalmar	Hultsfred	influent	water	µg/l	<0.0003	0.840	<0.0003	<0.0005	0.7	6.5	4.7	2.2	0.3	<0.0001	<0.0003	<0.0005	intf	0.015
4378	Kalmar	Hultsfred	not digested	sludge	µg/kg DW	<5	470	<5	<10	490	46	34	<9	56	<2	<3	<4	130	72
4375	Kalmar	Virserum		sludge	µg/kg DW	<8	1600	930	<16	1700	43	110	<12	61	<4	<5	<6	63	120
4347	Kalmar	Virserum	effluent	water	µg/l	<0.0003	0.016	<0.0003	<0.0005	<0.0004	0.0032	0.0036	0.078	0.027	<0.0001	<0.0003	<0.0005	0.002	0.001
4346	Kalmar	Virserum	influent	water	µg/l	0.28	0.110	<0.0003	<0.0005	2.3	0.37	18	5.7	0.046	<0.0001	0.00	<0.0005	0.01	0.009
4193	Kronoberg	Älmhult		sludge	µg/kg DW	<5	1600	<5	<9	<5	41	5	<5	20	<1	<2	<3	<7	intf
4354	Kronoberg	Alvesta		sludge	µg/kg DW	<3	200	<3	<7	96	130	150	12	<5	<2	34	<3	<5	34
4340	Kronoberg	Lessebo		sludge	µg/kg DW	<6	1200	<6	<11	<6	53	78	25	24	<3	<3	<5	210	300
4260	Kronoberg	Ljungby		sludge	µg/kg DW	<5	710	230	<9	300	130	5	<8	<4	<1	<2	<3	<7	23
4277	Kronoberg	Ljungby	effluent hospital	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	12.1	7.7	6.1	0.8	<0.0001	<0.0003	<0.0005	intf	0.007
4318	Kronoberg	Markaryd		sludge	µg/kg DW	<6	<3	<6	<11	<6	24	20	<9	31	<3	<3	<4	66	intf
4338	Kronoberg	Tingsryd		sludge	µg/kg DW	<5	470	<5	<10	210	67	19	28	55	<3	<3	<4	10	<10
4481	Kronoberg	Uppvidinge	Not digested	sludge	µg/kg DW	<5	<3	<5	<11	<5	36	38	<9	37	<3	<3	91	<8	49
4191	Kronoberg	Växjö		sludge	µg/kg DW	<5	2400	1200	<10	<5	86	5	10	14	<1	<2	<3	<7	19

Sample identity	County	City	Information	Matrix	Unit	Oxytetra cycline	Tetra cycline	Demeclo cycline	Chloro cycline	Doxy cycline	Ibuprofe n	Napro xen	Ketoprof en	Diclofe nac	Estriol	Estradiol	Ethinyl estradiol	Norethi ndrone	Proges terone
4209	Kronoberg	Växjö	effluent hospital	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	11.2	0.1	6.1	5.0	<0.0001	<0.0003	<0.0005	0.01	0.006
4211	Kronoberg	Växjö	effluent hospital	water	µg/l	<0.0003	1.400	<0.0003	<0.0005	<0.0004	8.6	5.5	4.7	0.5	<0.0001	<0.0003	<0.0005	0.02	0.016
4234	Norrbotten	Gällivare		sludge	µg/kg DW	<5	<3	<5	<10	<5	4	74	19	10	<1	<2	<3	<7	<10
4233	Norrbotten	Gällivare	effluent	water	µg/l	<0.0003	<0.0002	<0.0003	0.56	0.22	1.5	4.8	1.7	0.1	<0.0005	<0.001	<0.002	<0.004	0.041
4232	Norrbotten	Gällivare	influent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	3.4	11.3	1.7	0.2	<0.0005	<0.001	<0.002	<0.004	0.030
4283	Norrbotten	Luleå		sludge	µg/kg DW	<3	<2	<3	<7	<3	51	9	6	12	<2	3.3	3.3	13	17
4282	Norrbotten	Luleå	Effluent	water	µg/l	0.049	0.038	<0.0003	<0.0005	0.15	4.4	8.1	1.6	0.2	<0.0005	<0.001	<0.002	<0.004	0.040
4281	Norrbotten	Luleå	Influent	water	µg/l	0.072	0.360	<0.0003	<0.0005	0.18	4.6	9.3	1.7	0.1	<0.0001	<0.0003	<0.0005	0.02	0.012
4273	Norrbotten	Piteå		sludge	µg/kg DW	<4	470	<4	<8	<4	150	10	17	17	<1	<2	<3	<6	110
4271	Norrbotten	Piteå	Effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	7.5	14.7	1.8	0.2	0.06	<0.0003	<0.0005	intf	intf
4269	Norrbotten	Piteå	Influent	water	µg/l	0.0006	0.001	<0.0003	<0.0005	<0.0004	9.6	20.9	2.4	0.3	0.024	0.0012	0.0009	0.002	0.022
4374	Skåne	Ängelholm		sludge	µg/kg DW	<4	<2	<4	<7	<4	110	8	18	29	<2	<2	<3	intf	270
4373	Skåne	Ängelholm	effluent	water	µg/l	0.061	<0.0002	<0.0003	<0.0005	<0.0004	0.0	0.2	0.5	0.2	0.02	<0.0003	<0.0005	0.002	0.001
4285	Skåne	Eslöv		sludge	µg/kg DW	1400	<3	<6	<12	<6	33	<4	<9	16	<2	<2	<4	<9	53
4284	Skåne	Eslöv	Effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	0.1	0.3	0.2	0.3	<0.0005	<0.001	<0.002	<0.004	0.007
4276	Skåne	Hässleholm		sludge	µg/kg DW	<3	14	<3	<7	24	91	<3	<6	5	80	<1	<2	55	48
4275	Skåne	Hässleholm	Effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	1.1	0.5	1.2	0.0	<0.0001	<0.0003	<0.0005	0.004	intf
4290	Skåne	Helsingborg		sludge	µg/kg DW	920	1500	<4	<9	<4	92	8	9	28	<2	4.3	6.5	8.7	17
4288	Skåne	Helsingborg	Effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	0.1	0.1	0.4	0.3	<0.0001	<0.0003	<0.0005	0.00	0.001
4292	Skåne	Helsingborg	Leachate	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	<0.005	<0.003	<0.003	<0.002	<0.0001	<0.0003	<0.0005	0.01	0.002
4379	Skåne	Hörby	effluent	water	µg/l	<0.0003	0.071	<0.0003	<0.0005	<0.0004	0.1	0.2	0.9	0.1	<0.0001	<0.0003	<0.0005	0.001	<0.0007
4380	Skåne	Hörby	not digested	sludge	µg/kg DW	<5	1700	<5	<10	860	22	6	<10	<4	<3	<3	<4	36	intf
4308	Skåne	Kristianstad		sludge	µg/kg DW	<3	63	<5	<11	<5	110	13	23	35	<2	<2	<4	<8	73
4307	Skåne	Kristianstad	effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	0.0	0.1	0.1	0.1	<0.0005	<0.001	<0.002	<0.004	0.013
4414	Skåne	Landskrona		sludge	µg/kg DW	<5	1300	260	<9	340	140	12	<8	55	130	<3	<4	130	87
4413	Skåne	Landskrona	effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	0.0	0.0	0.1	0.3	<0.0005	<0.001	<0.002	<0.004	0.009
4316	Skåne	Lund		sludge	µg/kg DW	<3	<2	<3	<6	<3	54	<3	<6	15	<1	<1	<2	<5	36

Sample identity	County	City	Information	Matrix	Unit	Oxytetracycline	Tetracycline	Demeclocycline	Chlorocycline	Doxycycline	Ibuprofen	Naproxen	Ketoprofen	Diclofenac	Estriol	Estradiol	Ethinylestradiol	Norethindrone	Progesterone
4314	Skåne	Lund	effluent	water	µg/l	<0.0003	0.037	<0.0003	<0.0005	<0.0004	0.1	0.2	0.1	0.1	<0.0001	<0.0003	<0.0005	0.00	0.001
4329	Skåne	Malmö		sludge	µg/kg DW	<4	1400	<4	<8	<4	120	9	17	29	<2	<3	30	42	230
4327	Skåne	Malmö	effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	0.091	1.0	1.2	1.8	0.3	<0.0005	<0.001	<0.002	<0.004	0.049
4328	Skåne	Malmö	leachate	water	µg/l	<0.0003	0.003	<0.0003	0.005	0.091	0.3	0.0	0.1	0.0	<0.0001	<0.0003	0.03	0.01	0.010
4264	Skåne	Svedala	Effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	0.1	0.1	1.9	0.6	<0.0005	<0.001	<0.002	<0.004	0.110
4265	Skåne	Svedala	not digested	sludge	µg/kg DW	<7	240	<7	<14	140	10	3	<8	15	<2	<3	<5	<10	66
4267	Skåne	Trelleborg		sludge	µg/kg DW	<4	3600	800	<8	<4	38	4	10	4	<1	<2	7.8	<6	46
4266	Skåne	Trelleborg	Effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	0.1	1.9	2.3	0.2	<0.0001	<0.0003	<0.0005	0.002	0.004
4365	Skåne	Ystad		sludge	µg/kg DW	<4	1500	500	<7	570	78	4	10	26	<2	<2	<3	intf	140
4363	Skåne	Ystad	effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	2	0.12	0.02	<0.001	<0.0001	<0.0003	<0.0005	0.0020	<0.0007
4168	Södermanland	Eskilstuna		sludge	µg/kg DW	<4	36	<4	<7	29	68	4	9	12	<1	<1	<2	<5	46
4165	Södermanland	Eskilstuna	effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	0.0	0.5	0.4	0.1	<0.0001	<0.0003	<0.0005	<0.0007	0.008
4166	Södermanland	Eskilstuna	effluent after wetland	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	0.0	0.3	0.0	0.1	<0.0005	<0.001	<0.002	<0.004	0.003
4164	Södermanland	Eskilstuna	influent	water	µg/l	<0.0003	0.57	<0.0003	<0.0005	<0.0004	9.4	3.7	1.3	0.3	<0.0001	0.00	<0.0005	<0.0007	0.020
4170	Södermanland	Eskilstuna	leachate before cleaning	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	1.1	0.0	0.1	0.0	<0.0001	<0.0003	<0.0005	0.04	0.014
4133	Södermanland	Flen		sludge	µg/kg DW	<8	360	<8	<17	600	160	47	12	18	<3	<3	<6	75	<17
4132	Södermanland	Flen	effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	0.4	0.9	1.1	0.2	<0.0005	<0.001	<0.002	<0.003	0.004
4245	Södermanland	Katrineholm		sludge	µg/kg DW	<5	1300	<5	<11	<5	160	4	9	<4	<2	<2	<4	<8	130
4244	Södermanland	Katrineholm	effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	2.3	2.0	2.2	0.3	<0.0005	<0.001	<0.002	<0.004	0.013
4243	Södermanland	Katrineholm	influent	water	µg/l	0.25	1.000	<0.0003	<0.0005	0.25	9.3	4.4	4.1	0.6	<0.0001	<0.0003	<0.0005	0.02	0.015
4156	Södermanland	Mariefred		sludge	µg/kg DW	970	1700	<4	<8	470	5	6	5	18	<1	<2	<3	<6	24
4155	Södermanland	Mariefred	effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	0.6	3.3	2.9	0.3	<0.0005	<0.001	<0.002	<0.004	0.0083
4154	Södermanland	Mariefred	influent	water	µg/l	<0.0003	0.0030	<0.0003	<0.0005	<0.0004	6.2	2.8	2.8	0.4	<0.0005	<0.001	<0.002	<0.002	<0.002
4204	Södermanland	Nyköping	effluent	water	µg/l	0.015	0.098	<0.0003	<0.0005	0.032	0.7	1.2	1.5	0.2	<0.0001	<0.0003	<0.0005	0.00	0.001
4205	Södermanland	Nyköping	leachate	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	1.6	0.0	0.2	0.0	<0.0001	0.08	<0.0005	<0.0007	0.009
4351	Södermanland	Nyköping	recipient	sediment	µg/kg DW	<2	<1	<2	<4	<2	<1	<1	<2	<1	<1	<1	<2	<3	Intf
4350	Södermanland	Nyköping	surface water	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	0.011	0.010	0.0088	0.0020	<0.0001	<0.0003	<0.0005	0.00	<0.0007

Sample identity	County	City	Information	Matrix	Unit	Oxytetra cycline	Tetra cycline	Demeclo cycline	Chloro cycline	Doxy cycline	Ibuprofe n	Napro xen	Ketoprof en	Diclofe nac	Estriol	Estradiol	Ethinyl estradiol	Norethi ndrone	Proges terone
4151	Södermanland	Strängnäs		sludge	µg/kg DW	<5	1100	<5	<11	<5	15	19	16	39	<2	<2	<4	<8	110
4150	Södermanland	Strängnäs	effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	0.8	0.9	0.4	0.6	0.01	<0.0003	<0.0005	0.00	0.001
4149	Södermanland	Strängnäs	influent	water	µg/l	<0.0003	0.0020	<0.0003	<0.0005	0.001	11.8	4.6	3.2	0.7	<0.0005	<0.001	<0.002	<0.003	<0.003
4190	Södermanland	Trosa		sludge	µg/kg DW	210	1600	<4	<9	460	35	9	12	32	<1	<2	<3	<6	52
4188	Södermanland	Trosa	effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	3.5	1.3	0.2	0.4					
4189	Södermanland	Trosa	effluent after wetland	water	µg/l	<0.0003	0.082	<0.0003	<0.0005	<0.0004	0.1	0.2	0.0	0.2	<0.0005	<0.001	<0.002	<0.004	0.029
4187	Södermanland	Trosa	influent	water	µg/l	0.25	0.42	<0.0003	<0.0005	0.29	9.2	4.1	2.1	0.6	<0.0001	<0.0003	<0.0005	0.01	0.007
4242	Södermanland	Vingåker	effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	2.3	2.0	1.3	0.2	<0.0001	0.03	<0.0005	<0.0007	0.014
4306	Värmland	Arvika	effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	0.0	0.4	0.6	0.4	<0.0001	<0.0003	<0.0005	0.002	0.001
4201	Värmland	Karlstad		sludge	µg/kg DW	<4	<2	<4	<7	<4	86	<2	<4	26	<1	<1	<3	<5	29
4268	Värmland	Karlstad	surface water recipient	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	<0.0024	0.002	0.004	<0.001	<0.0005	<0.001	<0.002	<0.004	intf
4250	Värmland	Kristine-hamn		sludge	µg/kg DW	<4	<2	<4	<9	38	-	-	-	-	-	-	-	-	-
4238	Värmland	Kristine-hamn	surface water	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	0.0056	0.0087	0.0034	0.0026	<0.0005	<0.001	<0.002	<0.004	Intf
4246	Värmland	Kristine-hamn	surface water	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	<0.0024	0.002	0.002	0.002	0.0033	<0.0003	<0.0005	<0.0007	0.003
4180	Värmland	Säffle	surface water	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	0.017	0.0089	0.0080	0.0027	<0.0001	<0.0003	<0.0005	<0.002	<0.0007
4344	Västerbotten	Lycksele		sludge	µg/kg DW	<4	570	<4	<8	<4	110	60	25	64	<2	<2	<3	<6	24
4342	Västerbotten	Lycksele	effluent	water	µg/l	0.26	0.096	<0.0003	0.22	<0.0004	4.6	7.1	2.3	0.3	<0.0001	<0.0003	<0.0005	0.005	0.032
4305	Västerbotten	Skellefteå		sludge	µg/kg DW	<4	<2	<4	<9	<4	160	11	26	<3	41	<3	<4	49	40
4304	Västerbotten	Skellefteå	effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	0.0	0.2	0.1	0.1	0.02	<0.0003	<0.0005	0.004	<0.0007
4208	Västmanland	Hallsta-hammar		sludge	µg/kg DW	<4	<2	<4	<7	<4	56	4	20	16	<1	<2	<3	<6	130
4207	Västmanland	Hallsta-hammar	effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	1.9	1.6	1.6	0.1	<0.0005	<0.001	<0.002	<0.004	0.020
4206	Västmanland	Hallsta-hammar	influent	water	µg/l	0.26	1.000	<0.0003	<0.0005	0.9	7.3	3.6	3.8	0.4	<0.0001	<0.0003	<0.0005	0.006	0.004
4301	Västmanland	Norberg		sludge	µg/kg DW	<5	<3	<5	<11	<5	95	25	8	<4	<2	<2	<4	<8	190
4300	Västmanland	Norberg	effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	2.6	4.1	2.1	0.1	<0.0001	0.00	<0.0005	0.00	0.002

Sample identity	County	City	Information	Matrix	Unit	Oxytetra cycline	Tetra cycline	Demeclo cycline	Chloro cycline	Doxy cycline	Ibuprofen	Naproxen	Ketoprofen	Diclofenac	Estriol	Estradiol	Ethinyl estradiol	Norethindrone	Progesterone
4299	Västmanland	Norberg	Influent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	7.1	11.1	2.1	0.3	<0.0001	<0.0003	0.01	0.00	0.009
4356	Västmanland	Västerås	not digested, not dehydrated	sludge	µg/kg DW	<300	3100	<300	<600	9200	22000	14000	1500	560	3900	310	<260	650	1900
4217	Västra Götaland	Skövde		sludge	µg/kg DW	<6	3000	<6	<12	1800	110	4	23	23	<2	<2	<4	72	250
4215	Västra Götaland	Skövde	effluent	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	0.1	0.2	1.4	0.5	<0.0005	<0.001	<0.002	<0.004	0.048
4213	Västra Götaland	Skövde	influent	water	µg/l	0.34	0.630	<0.0003	<0.0005	<0.0004	11.0	4.8	3.9	0.6	0.02	<0.0003	<0.0005	0.02	0.017

Appendix 6. Results national samples

Sample Identity	County	Site	Matrix	Unit	Oxytetra cycline	Tetra cycline	Demeclo cycline	Chloro cycline	Doxy cycline	Ibupro fen	Napro xen	Ketopro fen	Diclo fenac	Estri ol	Estra diol	17alfa-Etinyl estradiol	Noretis terone	Proges terone
4504	Background	Lilla Öresjön	sediment	µg/kg DW	<8	<4	<8	<17	<8	<3.4	<3.4	<8.4	<3.4	<4	<5	<7	<10	intf
4506	Background	Stensjön	sediment	µg/kg DW	<7	<3	<7	<13	<7	<1.8	<1.8	<4.6	<1.8	<3	<4	<5	intf	intf
4505	Background	Tärnan	sediment	µg/kg DW	<4	<2	<4	<8	<4	<1	<1	<2.5	<1	<2	<2	<3	<6	<8
4483	Background	Lilla Öresjön	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	0.041	0.021	0.008	0.002	<0.0001	<0.0003	<0.0005	<0.002	intf
4367	Background	Stora Envättern	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	<0.003	<0.002	<0.002	<0.001	<0.0001	0.002	<0.0005	0.005	0.007
4368	Background	Tärnan	water	µg/l	<0.0001	<0.0003	<0.0005	0.001	<0.0007	<0.003	<0.002	0.0071	<0.001	<0.001	<0.003	<0.005	<0.002	<0.0007
4586	Diffuse	Henriksdal STP	sludge	µg/kg DW	<4	3000	<4	<8	1100	130	7.6	12	77	<1	<2	<3	<6	intf
4438	point source	Horse track stable	manure	µg/kg DW	<5	400	<5	<10	<5	<2	<2	<5	<2	<3	<3	<4	42	78
4439	point source	Horse track stable	manure	µg/kg DW	<6	<3	<6	<12	<6	<2.5	<2.5	<6.2	<2.5	<2	<2	82	<9	200
4440	point source	Horse track stable	manure	µg/kg DW	<5	<3	<5	<11	<5	<2.2	<2.2	<5.5	<2.2	<2	<2	<4	<8	intf
4430	point source	Pigfarm	manure	µg/kg DW	<6	<3	<6	<12	<6	<2.6	<2.6	14	<2.6	180	<4	<5	66	60
4431	point source	Pigfarm	manure	µg/l	<1	<0.5	<1	<2	<1	<0.04	<0.02	0.56	<0.02	<1	<1	<1	<2	<2
4456	point source	Horse track stable	sediment	µg/kg DW	<2	<1	29	<3	<2	<0.5	<0.5	<1.2	<0.5	<1	<1	<1	<3	<3
4432	point source	Pigfarm	sediment	µg/kg DW	<2	<1	<2	<3	<2	<0.6	<0.6	<1.6	<0.6	<1	<1	<1	<3	intf
4433	point source	Pigfarm	sediment	µg/kg DW	<2	<1	<2	<3	<2	<0.4	<0.4	<1	<0.4	<0.5	<1	<1	12	32
4609	point source	Landfill	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	<0.005	<0.003	0.23	<0.002	0.005	0.001	0.0007	0.004	intf
4381	point source	Högbytorp	water	µg/l	n a	n a	n a	n a	n a	200	0.033	0.58	0.10	n a	n a	n a	n a	n a
4382	point source	Högbytorp	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	2.0	0.0094	0.50	0.015	<0.0001	<0.0003	<0.0005	0.005	0.010
4390	point source	Hospital	water	µg/l	<0.0003	0.640	<0.0003	0.79	3.1	0.0034	<0.002	0.0025	<0.001	<0.0001	<0.0003	0.01	0.034	0.003
4425	point source	Cattle breeding	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	28	12	0.66	0.25	<0.0001	<0.0003	0.003	0.003	0.001
4424	point source	Cattle breeding	water	µg/l	0.010	0.002	<0.0003	0.001	<0.0004	<0.003	<0.002	<0.002	<0.001	<0.0001	<0.0003	0.001	0.002	0.003

Sample Identity	County	Site	Matrix	Unit	Oxytetra cycline	Tetra cycline	Demeclo cycline	Chloro cycline	Doxy cycline	Ibupro fen	Napro xen	Ketopro fen	Diclo fenac	Estri ol	Estra diol	17alfa-Etinyl estradiol	Noretis terone	Proges terone
4435	point source	Horse track stable	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	<0.003	<0.002	0.003	<0.001	<0.0001	<0.0003	<0.0005	0.01	intf
4436	point source	Horse track stable	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	0.052	0.014	0.0039	0.0025	<0.0001	<0.0003	<0.0005	0.01	intf
4426	Point source	Pigfarm	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	<0.003	<0.002	0.002	<0.001	<0.0001	<0.0003	<0.0005	0.0010	<0.0007
4427	point source	Pigfarm	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	<0.003	<0.002	0.0031	0.0021	<0.0001	<0.0003	<0.0005	0.002	0.005
4428	point source	Pigfarm	water	µg/l	<0.0003	<0.0002	<0.0003	<0.0005	<0.0004	4.2	0.023	1.0	0.039	<0.0005	<0.001	<0.002	<0.004	0.032
4588	human exp	Food, fish	biota	µg/kg ww	<1	<0.5	<1	<2	<1	<0.6	<0.2	<0.5	<0.2	<0.3	<0.4	0.90	<1.5	<2
4589	human exp	Food, fish	biota	µg/kg ww	<1	<0.5	<1	<2	<1	<0.6	<0.2	<0.5	<0.2	<0.3	<0.4	<0.7	<1.5	<2
4590	human exp	Food, fish	biota	µg/kg ww	<1	<0.5	<1	<2	<1	<0.6	<0.2	<0.5	<0.2	<0.3	<0.4	0.90	<1.5	<2

[c] Estimated value retrieved from ChemID Advanced Plus
[d] Estimated value retrieved from clogp-software