Biocides in wastewater and in sewage sludge

Report of the Nordic Biocide Group 2005

Financed by the Nordic Council of Ministers

Foreword

This report presents the results of a pilot project with the aim to gather information about the fate of some biocidal active substances in water treatment plants using different levels of treatment techniques. The aim was also to investigate whether the water temperature had any effect on the results of the treatment process.

Four substances expected to be found in treatment plants (active substances notified in PT 1, 2, 3, 4, 6 or 9 of the Biocidal Products Directive) were chosen for the project. Samples for analysis were taken from four different treatment plants located in Sweden and representing the most common treatment techniques in the Nordic countries.

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The Nordic Council of Ministers has financed the project.

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1. Summary

The purpose of this study was to conduct a pilot scale survey regarding biocide concentrations in sewage treatment plants (STP) in the Nordic countries. Selected biocidal active substances were analysed from incoming and outgoing water and sewage sludge at plants using different kinds of water treatment techniques. The aim was also to investigate whether the water temperature had any effect on the results of the treatment process, and to estimate if the outgoing water concentrations could pose unacceptable risk to aquatic organisms.

Five biocidal active substances were selected for the study. The choice of substances was made primarily taking into account that they should be able to be found in sewage treatment plants. An indication of this is that they had to be listed for use in product types PT 01, 02, 03, 04, 06 or 09 according to Annex V of the Biocidal Products Directive (98/8/EC). When the choice was made it was also considered as an advantage if the substances were recorded in the SPIN database on the Internet as ones sold or manufactured in a high volume. SPIN (Substances in Preparations in Nordic Countries) provides data on the use of chemical substances in Norway, Sweden, Denmark and Finland. The SPIN project is financed by the Nordic Council of Ministers under the Chemical group and the Product Registries of the contributing countries supply the data (http://www.spin2000.net).

Furthermore, suitable analytical methods had to be available or possible to be set up. It was also regarded as positive if the substances were documented concerning toxicity, lipofilicity, vapour pressure, water solubility, pKa-values etc. The five substances chosen fulfilling these criteria were benzothiazole-2-thiol, 4-chloro-m-cresole, propiconazole and didecyldimethylammonium chloride and triclosan.

Triclosan has been found in STP recipient water in earlier monitoring studies carried out by the Swedish water treatment company Stockholm Vatten and was included in this study as a benchmark substance.

Four sewage treatment plants situated in Sweden were selected for the study. The selection was made to represent the most common kinds of treatment techniques used in the Nordic countries. The number of people connected to and the size of the plants were also taken into account before the choice was made. Furthermore it was regarded as positive if the plant had a hospital connected to it because of the products used as disinfectants in PT 02. Two different seasonal periods were chosen to investigate if the temperature of the water affects the treatment results. The samples were collected in late April or early May after the snow-melting period when the incoming water has a low

temperature and in late August or early September when the incoming water has a higher temperature.

Residual concentrations in water and sludge were determined, as the environmentally important parameters. Degradation as a function of organic load was also determined to evaluate the importance of biological degradation.

The results are briefly as follows:

- Of the substances studied benzothiazole-2-thiol, triclosan and propiconazole were found in treated outgoing water and sludge samples (especially benzothiazole-2-thiol and triclosan). The other substances were not found in any of the analysed samples.
- Benzothiazole-2-thiol (CAS 149-30-4) mainly disappears from the water phase during biological treatment, possibly dependent on the organic load in the STP. Benzothiazole-2-thiol was not found in the sludge after anaerobic sludge treatment. Some other results were difficult to explain. Benzothiazole-2-thiol can possibly be formed from other compounds in the sewage.
- Triclosan (CAS 3380-34-5) is according to the results mainly transferred from water to sludge phase. The concentration that was found in the treated water is probably a function of the amount of sludge leaving the treatment system and the amount of fine suspended matter in the water. Due to the long sludge ages it is very difficult to determine if there is an actual degradation, based upon a few water and sludge samples.
- 4-chloro-m-cresole (CAS 59-50-7) was not found in any of the water or sludge samples.
- Propiconazole (CAS 60 207-90-1) was found in very low concentrations in some water and sludge samples. It was detected just once in biologically treated water.
- Didecyldimethylammonium chloride (CAS 7173-51-5) was not found in any of the four analysed sludge samples. As an extremely lipophilic compound it was not analysed in the water samples.

The temperature differences between the two sampling periods showed no or little effect on the treatment results. This could be due to many physical rather than chemical reactions, or low concentrations rather than degradation capacity as the limiting factor.

The concentrations of the five different substances in the outlet water from the STP were in almost every case lower than the PNEC (Predicted No Effect Concentration) values for each measured chemical. The corresponding calculated PEC (Predicted Environmental Concentration) value was also lower than the PNEC value. This means that the risk of adverse effects to the aquatic environment is low.

2. Background

With the Biocidal Products Directive, 98/8/EC, which reached legal status in the EU Member States in May 2000, a procedure for evaluation and authorisation of active substances and biocidal products in 23 different product types was implemented. A large number of biocidal products and articles treated with biocides are marketed for consumers and different professional users in order to destroy, prevent the action of, or otherwise exert a controlling effect on harmful organisms. A great part of the active substances finally reach the environment often after passing through municipal sewage treatment plants especially when used in biocidal product types PT 01, 02, 03, 04, 06 or 09 (Appendix 4). The substances could thus have an adverse impact on the actual plant or the recipient, due to their toxicity against organisms.

The climatic conditions in the Nordic countries with low average temperature may lead to a decrease in degradation rate for these kinds of substances and it could be expected that they to a greater extent could accumulate in the environment.

On these grounds the Nordic Biocide Group (NBG), a project group of the Nordic Chemicals Group, launched a project in order to collect and gather information about the fate of biocides in sewage treatment plants situated in the Nordic countries. Information about concentrations in wastewater and sludge will be of help within the progressing work of risk assessments of existing biocidal active substances, which started in 2004 in the EU Member States.

The project was financed and carried out within the frames of the Nordic cooperation on the environment under the Nordic Council of Ministers.

It is of great interest to establish to which extent the sewage treatment plants in the Nordic countries representing different treatment techniques actually are able to degrade the compounds. It is also possible that the substances pass the waste water treatment to the recipient or that they are transferred from the water phase to the sludge and then possibly to soil.

Previous studies have showed that active substances used in disinfectant agents have been found in sewage treatment plants and also in the recipient (Norberg et al. 2000). Yet information concerning this topic is scarce, and more knowledge of possible adverse effects is needed.

3. Substances

Five biocidal active substances with different physical-chemical and toxicological properties were selected for this study. It was taken into account if the biocides of

concern were sold or manufactured in a high volume. Furthermore the substances had to be listed for use in product types PT 01, 02, 03, 04, 06 or 09 according to Annex V of the Biocidal Products Directive (98/8/EC) (see Appendix 4). It was regarded as favourable if there was documentation available about toxicity, lipophilicity, vapour pressure, water solubility, pKa-values etc. Furthermore, suitable analytical methods had to be available or possible to set up.

Information about the five selected substances is presented in Table 1. The amount of substance used in the Nordic countries was found in the SPIN database. The structural formulas of the substances are given in Appendix 2.

Table 1. Selected substances, their uses, sales volumes and physical-chemical and ecotoxicological properties. The figures represent total registered volumes. Values in brackets represent the amount used in consumer products.

Substance	Benzothiazole-2-thiol	Triclosan	4-chloro-m-cresole	Propiconazole	Didecyldimethyl ammonium chloride
CAS	149-30-4	3380-34-5	59-50-7	60 207-90-1	7173-51-5
Tonnes (registration year)	2002	2002	2002	2002	2002
S	61,1 (26)	3,1 (2)	9,1 (4)	93,3 (6)	780 (22)
N	7.4	0.3	0.2	22	7.9
FI	26	0.4	13	53	18
DK	1.7	0.4	0.3	12	731
PT01	-	+	+	+	+
PT02	+	+	+	+	+
PT03	-	+	+	-	+
PT04	-	-	+	+	+
PT06	-	-	+	-	+
PT09	+	+	+	+	+
log Kow	2.4	4.8; 5.4	3.1	3.7	3.7
Toxic to aquatic species	Yes	Yes	Yes	Yes	Yes
Vapour pressure (20°C) mPa	62	0,09	6700	0,13	n.f.
Water sol. mg/l	120	10	3800	110	n.f.
pKa	6.9	8.1	9.6	1.1	1.1
Persistence	R	I	R	I	I
Analyt. Method	GC	GC	GC	GC	GC
Det. limit µg/kg TS	50	10	10	1	1
Det. limit µg/l	0.05	0.01	0.05	0.01	n.f.

+ Listed, - Not listed.
R readily biodegradable, I inherently biodegradable.
n.f. no value found.

Benzothiazole-2-thiol is primarily used in accelerators and in cleaning/washing agents. 26 tonnes of the registered 61.1 tonnes in the year of 2002 were used in consumer products in Sweden (SPIN).

In 2002 the registered use of triclosan was totally 3.1 tonnes of which 2 were used in consumer products in Sweden. Most commonly it was used in hygien products such as tooth paste, soap, shower gel and hand cleansing cream (SPIN).

The most common types of products in which 4-chloro-m-cresole have been used are in dyestuff, pigments, adhesives, glues and paints. Of the 9.1 tonnes registered in 2002, 4 tonnes were used in consumer products in Sweden. The total registered volumes used shows that the amounts have gone down by approximately 50% in 2002 compared with 2001 (SPIN).

Propiconazole is a substance mainly used in anti- fungi preservatives. In 2002 the total use was 93.3 tonnes of which 6 was registered in Swedish consumer products (SPIN).

Didecyldimethylammonium chloride is primarily used in different kinds of disinfecting agents. In 2002 nearly 780 tonnes were used according to SPIN. 22 of those were used in Sweden in different kinds of consumer products and 7.9 tonnes in Norwegian consumer products (SPIN).

4 Sampling sites and procedure

4.1 Selection of sewage treatment plants

Mechanical/chemical/biological treatment, with or without denitrification (nitrogen removal) is by far the dominating treatment techniques used in Sweden, Finland and Denmark. In Denmark biological phosphorus reduction is also a common technique and the interest for this technique is growing in Sweden. Particularly in Norway there are many sewage treatment plants (STP's) using only mechanical and chemical treatment. In Iceland the sewage water is treated mechanically. The typical capacity sizes of STP's in the Nordic countries are from 10 000 to 100 000 person equivalents (pe).

Below the different treatment techniques typically used in the Nordic countries, and their abbreviations, are listed:

M	Mechanical
MC	Mechanical and Chemical
MCB	Mechanical, Chemical and Biological
MCBden	Mechanical, Chemical and Biological with denitrification
MBdenBioP	Mechanical and Biological with denitrification and biological phosphorus reduction

In order to cover several different treatment techniques within the frames of the project budget one sewage treatment plant was used to study more than one of the treatment techniques. To get representative samples it was also preferable if the sewage treatment plants studied were of a certain size. Other aspects that were considered were the amount of industrial wastewater led into one plant, which should normally not be greater than a few percent. Moreover it was considered as an advantage if sewage water from hospitals

was connected to the plants, given the frequent use of disinfectants in the health care sector.

The process at the selected sewage treatment plants is presented briefly in sections 4.2-4.6, and in more detail in Appendix 1. All STP's studied in this project except for Ekebyhov have sewage water from hospitals connected. The Ekebyhov plant on the other hand was the only STP, which had a great part of industrial wastewater connected from a soap and detergent manufacturer.

In all four cases some storm water enter the sewer systems. General sampling procedure is given in chapter 4.6.

4.2 The Enköping plant in Enköping

The plant has an average sewage load of 22 000 pe. There is no nitrogen removal via denitrification. About a 50 % nitrogen removal before outlet to the recipient is achieved by pumping digestion reject and some of the treated water to plantations of willow (*Salix*). The de-watered sludge is presently stored or landfilled. This STP represents **MCB** treatment as defined in 4.1. More details about the plant and sampling points are found in Appendix 1.

At this plant samples were also taken in different steps of the process to simulate Norwegian (**MC**) treatment and Icelandic (**M**) treatment conditions.

The following water and sludge samples were collected at the STP:

W in	Untreated incoming sewage
W (MC)	Water after mechanical and chemical treatment
W (MCB)	Water after total treatment
S (MC)	Undigested primary sludge including excess biological sludge
S (B)	Undigested excess biological sludge
S (MCB)	Digested excess sludge

To simulate Icelandic conditions a volume of 50 litres was collected in a barrel from which first a mixed water sample was taken as the untreated water (**W in)**. To accomplish sedimentation of suspended solids the barrel was then left without disturbance. After one hour a water sample simulating treated water (**W (M)**) was taken from the surface. The barrel was left for sedimentation over night in order to get enough solid material, and not just the biggest particles. This was the sludge sample (**S (M)**).

Water sample corresponding to **MC** (mainly Norway) was taken as indicated, **W (MC)**. The corresponding sludge sample, **S (MC)**, was taken as indicated, but the content in a representative amount of biosludge **S (B)** was subtracted after analyses.

4.3 The Henriksdal plant in Stockholm

The Henriksdal sewage treatment plant has biological nitrogen removal and treats sewage from about 700 000 pe. This corresponds to about 2.8 m^3 water/s in average, but the flow rate is changing a lot, both over the day and due to rainfall. De-watered digested sludge is today used to cover mining waste. This plant represents **MCBden** treatment according to 4.1. More details about the plant and sampling points are found in Appendix 1.

There are two different inlets, with separate pre-precipitation. The analysed inlet sample (**W in)** is a proportional mixture of water from the two inlets after screen and grit chamber. The following samples were taken;

W in (1)	Sewage after screen and sand trap in to the Sickla inlet
W in (2)	Sewage after screen and sand trap in to the Henriksdal inlet
W (MCBden)	Water after total treatment
S (MC)	Primary sludge before digestion (sampled only in the autumn)
S (Bden)	Excess biological sludge before digestion (only in the autumn)
S (MVBden)	Digested excess sludge

4.4 The Koholmen plant in Karlskrona

The Koholmen STP has both nitrogen and phosphorous removal with biological methods **(MBdenBioP)**. It treats sewage corresponding to about 39 000 pe. More details about the plant and sampling points are found in Appendix 1.

Koholmen's STP has a polishing step where iron is added and the sludge is recirculated. This means that the site is not a true BioP but the important thing is that the treatment plant has a step with anaerobic conditions. None of the 20 Swedish BioP plants can cope without chemicals due to the tough demands set by the authorities. De-watered sludge is presently landfilled.

The elevated phosphorous content in the biosludge makes the sand filter necessary. The excess sludge cannot be anaerobically digested, since the stored poly-phosphate will be released under anaerobic conditions. The long sludge retention time is the only sludge stabilisation.

The following samples were collected:

W in	Sewage after screen and sand trap
W (MBdenBioP)	Water after total treatment
S (MBdenBioP)	Excess sludge

4.5 The Ekebyhov plant in Ekerö

The plant treats water corresponding to 17 000 pe. It is a **MCBden**-plant like Henriksdal, but much smaller and without anaerobic sludge digestion. Some industrial wastewater from a manufacturer of hygiene products using biocides and some external sludge is included. Most of the produced excess sludge is used for soil production. More details about the plant and sampling points are found in Appendix 1.

The following samples were taken:

W in	Sewage after screen and sand trap
W (MBden)	Water after total treatment
S (MCBden)	Excess sludge

4.6 Sampling procedure

All samples of water were taken as 24-hours collective samples at a constant rate (not flow-proportional). At least 2 litres were taken from each sampling position. Sludge was collected as single grab samples. 0.2-1 litre was taken, dependent on dry matter content. The samples were cooled down at the plants and sent to IVL where they were frozen until analyses.

To avoid dilution of the wastewater, the samples were taken during periods without rainfall or snow melting. All STPs were run at normal conditions.

Data on the temperature over the year from Henriksdal shows that samples were taken close to the maximum and minimum mean temperatures. The temperature of incoming water normally varies between 12 and 19°C. It is only during heavy autumn rains and some days during snow melting that the temperature goes down to 7-10°C, and these periods were excluded since they are not representative. Exact sampling times are given in the Table of Appendix 1.

5. Materials and methods

5.1 Handling and storage of samples

All samples were collected in glass bottles, which previously had been washed in a laboratory dish washing machine, dried and than heated in an oven at 400°C for at least two hours to avoid contamination of the samples and to oxidise possible organic matter. All samples were stored frozen prior to analysis and the spring and autumn samples were analysed at the same time. The STP personnel, in most cases with their normal collective sampling units took the samples.

5.2 Analytical methods

Analysis of triclosan, 4-chloro-m-cresol, benzothiazole-2-thiol and propiconazole.

The samples were acidified and extracted twice with organic solvents. The combined extracts were derivatized with diazomethane and chromatographed on a silica column. The extracts were analysed by gas chromatograph connected to a mass spectrometric detection as described in more detail in Appendix 2. The laboratory at IVL has accreditation for several analyses, but not for these that were developed during the project.

The quantification result was corrected to the recovery yield of the corresponding recovery standard (Table 1, Appendix 2, assuming that recovery and target compound behave similar in the different analytical steps e.g. during extraction, clean-up procedures and derivatization).

Analysis of didecyldimethylammonium chloride (DDDMA).

DDDMA is a salt and can not be analysed by gas chromatography. But after de-alkylation in a hot GC-injector to the corresponding *tert*-amino compound (decyl dimethylamine) the compound may be analysed on a gas chromatograph (Hind et al. 1997).

The full method is described in Appendix 2. Briefly, DDDMA is extracted from sludge with acidic methanol. The compound counter ion is changed from chloride to iodide. The potassium salt of DDDMA is extracted and injected into a hot GC-injector. Under these conditions, DDDMA is dealkylated to di-methyl-decylamine. The derivative is chromatographed and detected by means of GC-FID.

6. Results and discussion

All analytical data are given here. Due to the restricted budget and the complicated analyses just one determination was made in each case.

6.1 Benzothiazole-2-thiol

Table 2 shows the results from the chemical analyses. As described earlier, water and sludge out from M, (only mechanical treatment as in Iceland), is after only sedimentation of Enköping sewage. Water and sludge out from MC, (mechanical and chemical treatment that is common in Norway) is after the chemical precipitation and pre-sedimentation in Enköping.

The concentrations in incoming water seem to have been generally somewhat higher in the autumn samples (2 after the STP name in Table 2). One reason for this can be the slightly higher solubility at higher temperature. The total organic contents in the waters were somewhat lower in the autumn samples, so the higher concentrations of benzothiazole-2-thiol were not a result of less diluted sewage (Appendix 1).

Table 2. Levels of concentration of benzothiazole-2-thiol in waste water and sludge.

STP	Treatment	Water in µg/L	Water out µg/L	Sludge out from STP µg/kg dry weight
Henriksdal 1	MCBden	1.18	n.d.	n.d.
Henriksdal 2	MCBden	1.3	0.6	n.d.
Ekebyhov 1	MCBden	2.0	0.12	300
Ekebyhov 2	MCBden	2.2	n.d.	5700
Koholmen 1	MBdenBioP	1.4	n.d.	400
Koholmen 2	MBdenBioP	0.9	n.d.	500
Enköping 1	MCB	0.6	n.d.	n.d.
Enköping 2	MCB	0.9	0.9	n.d.
“Norway” 1	MC	0.6	1.3	900
“Norway” 2	MC	0.9	1.5	1200
“Iceland” 1	M	0.6	0.6	600
“Iceland” 2	M	0.9	-	250

The figure1 after the STP name stands for the spring sample, 2 for the autumn sample.
n.d. = not detected.
The detection limit was 0.05 µg/L and 50 µg/kg for water and sludge samples respectively.
– Missing value because the sample was lost (broken glass bottle).

There seems to be a biological removal of benzothiazole-2-thiol from the water phase, but not a mechanical/chemical. Enköping at the second sampling is an exception, and to some extent also Henriksdal 2. A part of the explanation can be found in Figure 1.

The organic loads as g BOD per kg of total suspended solids (TSS) and day are calculated from data given by the STP at each sampling (Appendix 1). Since Ekebyhov does not determine the sludge concentration in the aeration, we have used 2 000 mg/L as a reasonable figure. The plot suggests that there is a negative correlation between removal of benzothiazole-2-thiol and organic load, as expected with a biological degradation. High organic load could then explain the relatively high residual concentrations in Enköping 2 and Henriksdal 2. Note that symbols with arrows in figure

1 probably underestimate the removal capacity. The residual concentration was below detection limit in these cases, so the actual capacity could have been higher.

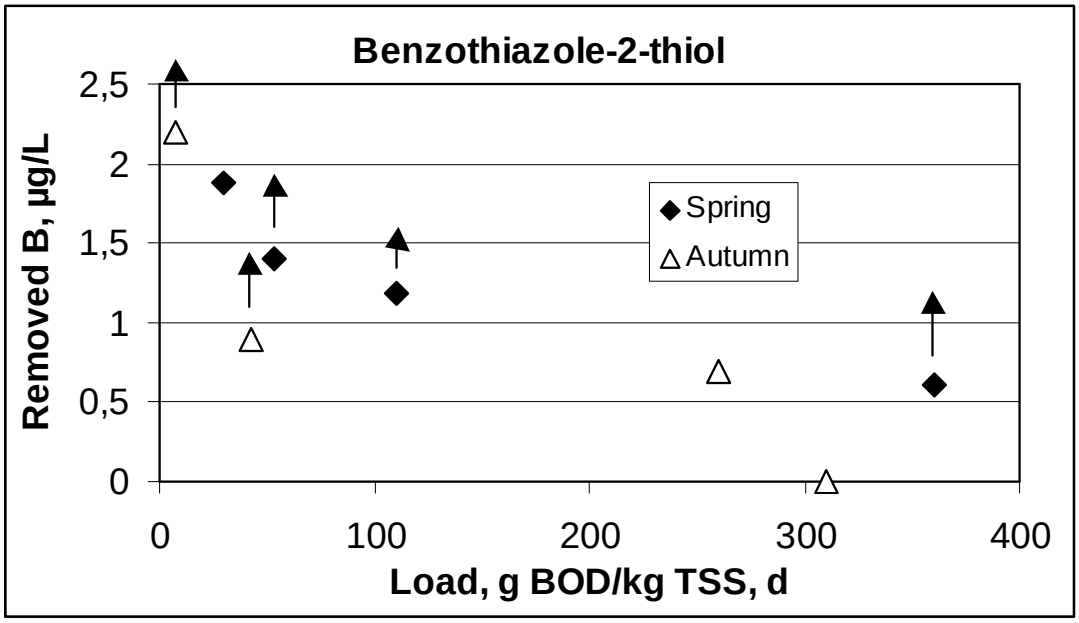

Figure 1. Removal of benzothiazole-2-thiol in biological treatment as a function of organic load in the STP. For 5 of the 8 determinations the residual concentration was below detection limit. The capacity could have been higher, indicated by ↑.

In biological treatment at different loads it is more relevant to give degradation in amounts than in percent. A certain amount of sludge has a certain capacity to degrade a certain amount of a compound under given conditions. If the capacity is 5 µg/L with a certain sludge and load, the percent degradation will be very different if you start with 50 or 5 µg/L. With a low enough load and a pure, biodegradable compound the degradation is of course 100%. In practice this is not always noticeable because the K_M is relatively high and the degradation process is not given enough time. Another reason with substances like this can be too little other organic material when there is a co-metabolism. A figure based upon benzothiazole-2-thiol load gives a similar picture.

Figure 2 gives the removal in percent.

The removal is calculated with 0 µg/L when no residual concentration was detected. If the detection limit value 0.05 µg/L is used, the "total" removal figures would be between 92 and 98%.

Results from only mechanical and chemical treatment are surprising. No biological degradation is expected, but benzothiazole-2-thiol seems to increase in the chemical

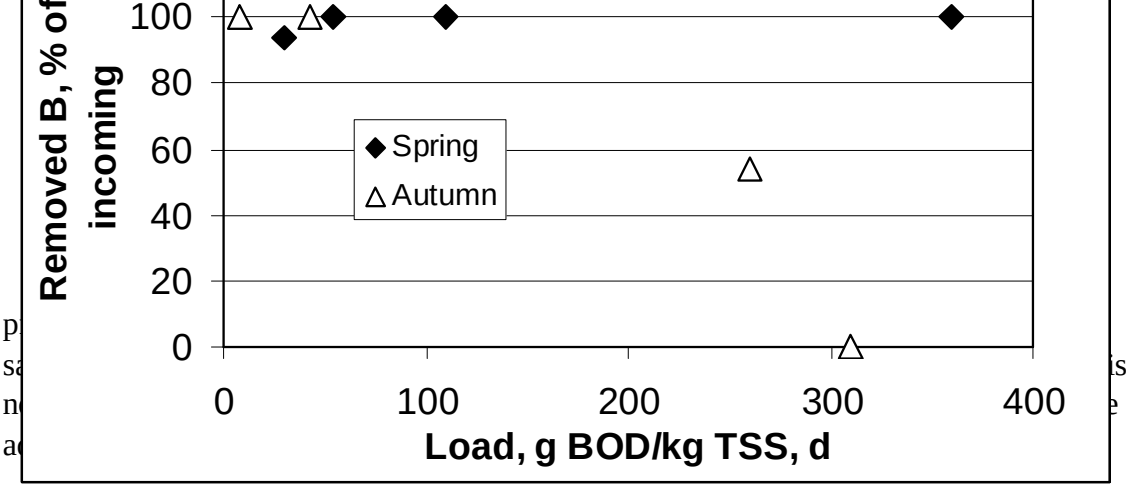

Figure 2. Percent removal of benzothiazole-2-thiol in biological treatment as a function of organic load in the STP.

Only sedimentation, without chemical precipitation did not change the concentration of benzothiazole-2-thiol in the water.

The concentration of benzothiazole-2-thiol in sludge also differs between the STP's. No benzothiazole-2-thiol was detected in sludge from Henriksdal and Enköping, which both have anaerobic treatment of the sludge. In all other sludge samples benzothiazole-2-thiol was found. This suggests that anaerobic conditions are needed to degrade/metabolise benzothiazole-2-thiol in the sludge. Comparing the concentrations in MC-sludge with that from MCB further supports this. As can be seen in Appendix 1 the only difference is that the MCB-sludge is anaerobically treated. Separate analyses of primary sludge and biosludge before anaerobic digestion at Henriksdal also showed that benzothiazole-2-thiol corresponding to 43 kg/year (32 kg/year in primary sludge, S (MC), and 11 kg/year in biological sludge, S (Bden) was completely gone after anaerobic digestion, in S (MCBden).

Data for Ekebyhov differ very much between the samplings. There is no intentional anaerobic treatment of sludge; it is just stored until it is taken for processing to soil. The two samples might be taken from anaerobic and aerobic parts of the storage respectively.

Table 3 shows the calculated amounts of benzothiazole-2-thiol in sewage and sludge handled by the plants per year in different media and STP. Figures are calculated from analysis data and average yearly amounts of treated sewage and produced sludge according to Appendix 1. Given intervals are for concentrations between 0 and the detection limit.

Table 3. Calculated amounts of benzothiazole-2-thiol in sewage and sludge handled by the plants per year, based upon concentrations in the sampling periods.

STP	Treatment	Water in kg/year	Water out kg/year	Sludge out kg/year	Lost %
Henriksdal 1	MCBden	90	0-4	0-0.4	95-100
Henriksdal 2	MCBden	104	48	0-0.4	53-54
Ekebyhov 1	MCBden	3.0	0.18	0.17	89
Ekebyhov 2	MCBden	3.3	0-0.09	3.1	3-5
Koholmen 1	MBdenBioP	7.4	0-0.3	0.52	89-93
Koholmen 2	MBdenBioP	6.2	0-0.3	0.65	85-90
Enköping 1	MCB	1.7	0-0.16	0-0.03	89-100
Enköping 2	MCB	2.0	2.0	0-0.03	-2-0
“Norway” 1	MC	1.7	3.6	0.83	-160
“Norway” 2	MC	2.0	3.3	1.1	-120
“Iceland” 1	M	1.7	1.7	0.16	-9
“Iceland” 2	M	2.0	-	0.008	-

– Missing value because the sample was lost (broken glass bottle).
Negative values in the lost column mean increased amount compared to the inlet water.

In the Henriksdal and Enköping plants, benzothiazole-2-thiol is completely degraded/metabolised when the organic or benzothiazole-2-thiol load is low enough. In the Koholmen plant the removal is also almost total, possibly because the retention time of the sludge in the anaerobic part of the BioP system is quite long. In Ekebyhov the treatment result may be dependent on the actual oxygen level of the sludge during storage.

The “Iceland “ samples shows that only sedimentation doesn’t affect the concentration of benzothiazole-2-thiol. The “Norway” samples show an increased concentration of benzothiazole-2-thiol in the water. One theory that can explain this “production” and also fits with the other results is that benzothiazole-2-thiol is in equilibrium with a reduced metabolite. The “production” of benzothiazole-2-thiol in Enköping is really an oxidation in the aerated sand trap, from the relatively low redox potential in the sewage. Removal in sludge during anaerobic treatment can be just the opposite - a reduction to the metabolite. This means that what looks like aerobic degradation in water phase can to a great extent be adsorption to sludge and in some cases anaerobic reduction there, and very little can be said without analysing the reduced metabolite. The problem is that a reduced metabolite is not known.

Benzotiazol-2-thiol may be produced by degradation of (2-thiocyanomethylthio) benzothiazol (CAS 21564-17-0), but whether this compound can be found in Swedish sewage is not known.

There is no sign of a faster degradation/removal of benzothiazole-2-thiol in the second sampling, with higher water temperature.

If , in spite of the uncertain values, it is supposed that the mean concentration of benzothiazole-2-thiol in untreated sewage in Sweden is 1.3 µg/L, it is possible to calculate the amount that is found in the total domestic wastewater in Sweden. In 2002 the amount of sewage treated in STP receiving sewage from more than 2 000 pe was 1 230 Mm^3 (Statistiska meddelanden 2004). About 120 Mm^3 can be estimated to be treated in STP between 25 and 2 000 pe. At least 1 million persons are not connected to STP at all. This gives an estimated total amount of wastewater of about 1500 Mm^3/year. With 1.3 µg/L this should contain about 2 tonnes of benzothiazole-2-thiol. In 2002 the total registered volume was 26 tonnes divided over 15 preparations. This indicates that either less than 10% of the used benzothiazole-2-thiol goes to the wastewater, or that a great part is chemically changed already in the sewer system.

6.2 Triclosan

Table 4 shows the results from chemical analyses of triclosan. Water and sludge out from M, “Iceland”, is after only sedimentation of Enköping sewage. Water and sludge out from MC, “Norway” is after the chemical precipitation and pre-sedimentation in Enköping.

The concentrations in incoming water seem to have been about at the same level in the spring and autumn samples.

Table 4. Found concentrations of Triclosan in water and sludge.

STP	Treatment	Water in µg/L	Water out µg/L	Sludge out from STP µg/kg dry weight
Henriksdal 1	MCBden	0.57	0.07	3600
Henriksdal 2	MCBden	0.7	0.2	2000
Ekebyhov 1	MCBden	1.6	0.04	2600
Ekebyhov 2	MCBden	1.5	0.09	820
Koholmen 1	MBdenBioP	0.44	0.01	1500
Koholmen 2	MBdenBioP	0.4	0.1	1100
Enköping 1	MCB	0.4	0.1	3900
Enköping 2	MCB	0.7	0.1	2500
“Norway” 1	MC	0.4	0.09	2600
“Norway” 2	MC	0.7	0.8	1700
“Iceland” 1	M	0.4	0.3	1500
“Iceland” 2	M	0.7	-	1300

The detection limit was 0.01 µg/L and 10 µg/kg for water and sludge samples respectively.
- Missing value because the sample was lost (broken glass bottle).

Triclosan was detected in all sewage and sludge samples. Mechanical or mechanical-chemical treatment alone does not seem to remove the substance. The concentration in the biologically treated water was much lower compared to incoming water. Figure 3 shows the relation between removed triclosan and the organic sludge load at the different STP and sampling times.

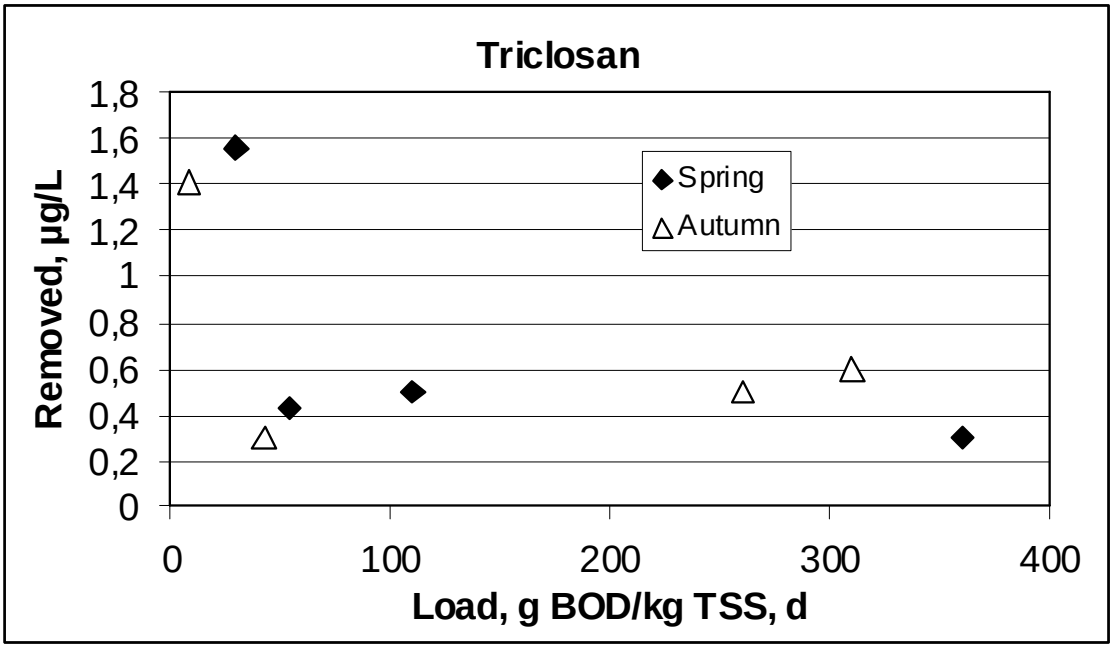

Figure 3. Removal of triclosan as a function of organic sludge load.

There is no obvious correlation between load and removal of triclosan. The two higher removal figures are from the same STP, Ekebyhov. However, they are probably not different from the others because of the treatment method at Ekebyhov, but because the inlet concentration was higher. This can be seen from Figure 4.

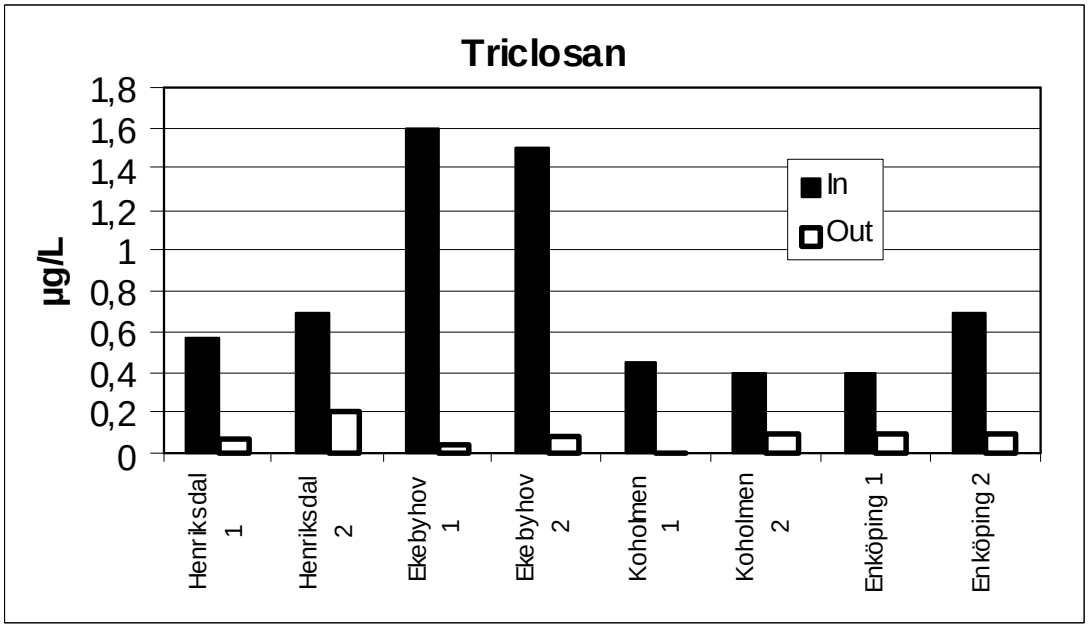

Figure 4. Concentrations of triclosan found in the water before and after treatment.

The residual concentration is low and about the same, irrespective of the starting value. This means that it is probably not a biological process, but rather an adsorption to solid material with very little remaining in solution. This is in line with the high log K_{OW}, see Table 1.

Figure 5 also shows that percent removal is not very informative.

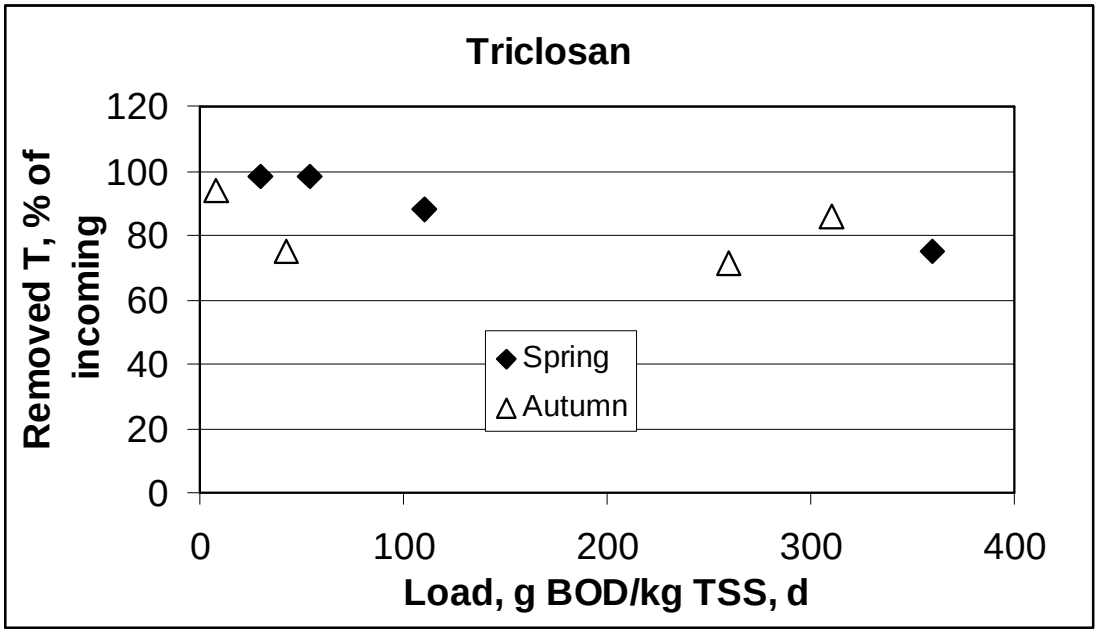

Figure 5. Percent removal of triclosan as a function of organic sludge load.

Percent removal varies between 75 and 94% for about the same residual concentration, the environmentally important parameter.

Table 5 shows the calculated amount per year of triclosan in the different media. Figures are calculated from analysis data and average yearly amounts of treated sewage and produced sludge according to Appendix 1.

Table 5. Calculated amounts of triclosan in wastewater and sludge handled by the plants per year, based upon the sampling periods and yearly average flow and sludge amount.

STP	Treatment	Water in kg/year	Water out kg/year	Sludge out kg/year	Lost %
Henriksdal 1	MCBden	43	5.3	50	-29
Henriksdal 2	MCBden	56	16	28	21
Ekebyhov 1	MCBden	2.4	0.06	1.4	38
Ekebyhov 2	MCBden	2.3	0.14	0.45	74
Koholmen 1	MBdenBioP	2.3	0.05	2.0	11
Koholmen 2	MBdenBioP	2.8	0.69	1.4	25
Enköping 1	MCB	1.1	0.3	1.8	-90
Enköping 2	MCB	1.5	0.2	1.1	13
“Norway” 1	MC	1.1	0.3	1.9	-100
“Norway” 2	MC	1.5	1.8	1.2	-100
“Iceland” 1	M	1.1	0.8	0.4	-9
“Iceland” 2	M	1.5	-	0.04	-

– Missing value because the sample was lost (broken glass bottle).

Negative values in the lost column mean increased amount compared to the inlet water.

The data is difficult to explain. Taking into account the relatively high uncertainty in the analyses, data from Henriksdal and Koholmen does not indicate any degradation/metabolism of triclosan. Ekebyhov data seem to show some degradation, while there seems to be a formation of triclosan in Enköping. One problem is the great influence of relatively little suspended material in the water samples. Because of the high log K_{OW}, most of the triclosan in a water sample is probably attached to solids in the water. A water sample with more than average suspended solids will thus give a too high value, and the other way around. Another reason can be the long sludge age compared to the water-sampling period. Higher or lower concentrations in the influent water during the period before sampling will be seen in the sludge samples, and the concentration in the sludge is really not representative of the concentration in the water samples.

As can be seen from both tables 4 and 5 there is a tendency for higher concentrations of triclosan in water samples from the autumn sampling, with warmer water. At the same time the concentration in the sludge is lower in the autumn samples. It indicates a higher solubility or lower log K_{OW} at higher temperature. There is also an indication that more triclosan is lost (degraded) in the system at the higher temperature. If this is so, the

difference should be in the water treatment and not in the sludge handling, since the sludge digestion temperature is relatively independent of the water temperature.

The extra sludge samples from Henriksdal in the second sampling period showed that the chemical sludge (S MC) had 1.4 mg triclosan/kg sludge, corresponding to about 28 kg/year. The biological sludge (S Bden) contained 1.8 mg/kg, corresponding to about 13 kg/year. This 41 kg had according to the analyses of the digested sludge (S MCBden) decreased to 28 kg/year, or with 32%.

The concentrations of triclosan determined in the present investigation are in agreement with previous reports (Svenson 2002, Remberger et al. 2002, Adolfsson-Erici et al. 2002).

In these previous studies triclosan were found in sewage sludge in the range between 3 and 4 mg/kg. Results from the present investigation combined with earlier results points in the direction that the concentration of triclosan in sludge from STP decreases, but there is too little data to conclude that this actually is the case.

Calculating the amount of triclosan in total domestic wastewater in the same way as for benzothiazole-2-thiol (c.f. Chapter 6.1) gives about 1.2 tonnes/year. If the total volume reported in 2002 (2 tonnes in consumer preparations) is expected to be the same in 2004 it seems to be a high proportion. However, there is a manufacturer for hygienic products connected to the relatively small STP Ekebyhov. This might give exceptionally high concentrations in the sewage. If the mean value of the other 6 sewage samples is used, the annual amount will be about 0.8 tonnes.

6.3 4-chloro-m-cresole

4-chloro-m-cresole was not found in any sample. The limits of detection were 0.05 µg/L and 0.01 mg/kg for water and sludge samples, respectively. Based upon the annual use of 4 tonnes in Sweden and everything ending up in the wastewater expected concentration is about 2.7 µg/L. This would mean that less than 2% of the amount used reaches the STP. It can be strongly adsorbed to other material, or degraded before it reaches the STP. In this case with high water solubility and relatively low persistence (Table 1), it is most probably degraded.

6.4 Propiconazole

Propiconazole was found in low concentrations in a few samples, see Table 6. The concentrations in incoming water seem to have been generally higher in the spring samples.

Table 6. Concentrations of propiconazole (isomer 1+2) in water and sludge samples.

STP	Treatment	Water in µg/L	Water out µg/L	Sludge out from STP µg/kg dry weight
Henriksdal 1	MCBden	0.02	n.d.	10
Henriksdal 2	MCBden	n.d.	n.d.	15
Ekebyhov 1	MCBden	0.02	n.d.	n.d.
Ekebyhov 2	MCBden	n.d.	n.d.	n.d.
Koholmen 1	MBdenBioP	0.01	0.04	n.d.
Koholmen 2	MBdenBioP	n.d.	n.d.	n.d.
Enköping 1	MCB	n.d.	n.d.	30
Enköping 2	MCB	0.02	n.d.	30
“Norway” 1	MC	n.d.	n.d.	n.d.
“Norway” 2	MC	0.02	0.18	n.d.
“Iceland” 1	M	n.d.	n.d.	n.d.
“Iceland” 2	M	0.02	-	n.d.

n.d. = not detected.
The detection limit was 0.01 µg/L and 1 µg/kg for water and sludge samples respectively.
– Missing value because the sample was lost (broken glass bottle).

It is impossible to draw any reliable conclusions from the very few and low concentration values. Generally the concentrations in biologically treated water was lower compared to incoming water but one sample from Koholmen showed the opposite. No conclusions can be made whether mechanical or mechanical-chemical treatment alone is sufficient to remove the substance from the sewage.

Propiconazole was found in sludge samples only in those plants with an anaerobic sludge treatment (Henriksdal and Enköping). This suggests that aerobic conditions are needed to degrade/metabolise propiconazole in the sludge. No propiconazole was detected in the separate Henriksdal sludges (S MC and S Bden) before digestion.

Based upon used amounts in Sweden (4 tonnes/year), the concentration in sewage would be about 9 µg/L if everything ended up in the sewage. It is probable that only a very small portion enters the STP.

6.5 Didecyldimethylammonium chloride

Didecyldimethylammonium chloride was not detected in any of the four analysed sludge samples. The limit of detection was 1 µg/kg. Of the main reason that this lipofilic substance was not found in the sludge samples, no water samples were analysed.

7. Effect of water temperature on removal

In this study the spring samples were collected after the snow melting period to as far as possible avoid the risk of dilution. Thus the largest difference between spring and autumn temperature was 8°C as the water temperature increased at maximum from 12 to 20°C. Comparing the analytical results from the spring and autumn samples no general differences were noticed. However, there are indications that both benzothiazole-2-thiol and triclosan concentrations in the sewage were higher at higher temperatures, and also that more triclosan was lost from/degraded in the system at higher temperatures.

More analyses are needed to allow any final conclusions about the temperature effect on degradation.

8. Environmental risk assessment

To evaluate the risk for the environment the predicted environmental concentration (PEC) and the predicted no effect concentration (PNEC) are calculated for the substance. Comparing the PEC with the PNEC performs a risk characterisation. If the quotient (RCR, Risk Characterisation Ratio) is greater than one, the risk for the environment is considered to be unacceptable. For wastewater emissions the risk assessment should be made for microorganisms from a biological wastewater treatment plant and for the aquatic organisms of the recipient.

The environmental risk assessment for biocidal active substances is done according to the Technical Guidance Document (TGD) on Risk Assessment (ECB, 2003). The computerised way to conduct environmental risk assessment is to use the EUSES 2.0 software.

The EUSES programme is based on the guidelines in TGD on Risk Assessment. From the amount produced and via the distribution of the chemical, the concentration in the effluent of a STP can be calculated and the potential risks assessed. However, in this project it was not possible to make EUSES calculations because not enough data on the total EU tonnages for biocidal uses was available.

To evaluate the environmental effects the Predicted No Effect Concentrations, PNEC, was calculated for each chemical. The calculation procedure was adopted from the TGD on Risk Assessment (ECB, 2003). The PNEC values for the aquatic organisms ($PNEC_{water}$) are presented in table 7. The PEC values for each chemical when entering the recipient (i.e. without dilution) have been calculated on the basis of the measured effluent concentrations according to the TGD on Risk Assessment (ECB, 2003), see Appendix 3. The Risk Characterisation Ratios, (RCR), is the ratio between PEC and PNEC. The results are presented in table 8.

Table 7. $PNEC_{water}$ values for the analysed chemicals. Please note that if the effluent will go to the Baltic Sea or to the Atlantic Sea in Iceland and Norway the assessment factor for the marine PNEC will usually be 10 times higher and therefore the PNEC 10 times lower.

	Benzotiazole-2-tiol	Triclosan	4-chloro-m-cresole	Propiconazole	Didecyldimethyl ammonium chloride
CAS	149-30-4	3380-34-5	59-50-7	60 207-90-1	7173-51-5
Assessment factor	100	1000	10	100	1000
Ecotoxicity data Concentration Organism Species	NOEC 220 µg/l Daphnids *Daphnia magna*	EC 50 250 µg/L Fish *Pimephales promelasm*	NOEC 1 mg/l Fish *Brachydanio rerio*	NOEC 500 µg/l Fish *Salmo trutta*	EC 50 36 µg/l Fish *Menidia beryllina*
Ecotoxicity reference	ESIS, European chemical Substances Information System	ECOTOX Database	ESIS, European chemical Substances Information System	ECOTOX Database	The ECETOC Aquatic Toxicity (EAT) database
PNEC, µg/L	2,2	0,25	100	5	0,036

Table 8. PEC and RCR values

	STP	Henriksdal	Ekebyhov	Koholmen	Enköping	"Norway"	"Iceland"	PNEC
PEC, µg/L without dilution	Benzothiazole-2-thiol	0.3125	0.0725	0.025	0.4625	1.4	0.6	2.2
	Triclosan	0.135	0.065	0.055	0.1	0.445	0.3	0.25
	4-chloro-m-cresole	0.025	0.025	0.025	0.025	0.025	0.025	100
	Propiconazole	0.005	0.005	0.0225	0.005	0.0925	0.005	5
RCR	Benzothiazole-2-thiol	0.14	0.03	0.01	0.21	0.64	0.27	
	Triclosan	0.54	0.26	0.22	0.40	1.78	1.20	
	4-Chloro-m-cresole	0.0003	0.0003	0.0003	0.0003	0.0003	0.0003	
	Propiconazole	0.0010	0.0010	0.0045	0.0010	0.0185	0.0010	

The only concentrations out from the STP that are higher than the PNEC-values are the triclosan concentration from “Norway ” sample 2 and “Iceland” sample 1.

No conclusions can be made on whether the didecyldimethyl ammonium emissions from the STPs would cause adverse effects in the recipient because no water was analysed.

However the discharge from the STP will be diluted in the recipient and the Predicted Environmental Concentration, PEC, will be lower than the measured concentration out. The PEC and RCR values for Triclosan in the recipient have been calculated on the basis of the measured effluent concentrations according to the TGD. The results are presented in table 9.

Table 9. PEC values and PEC/PNEC ratio for triclosan

STP	Water out, µg/L	PEC_{water}, µg/L	PNEC, µg/L	RCR
“Norway” 2	0,8	0,0137	0,25	0,05
“Iceland” 1	0,3	0,0051	0,25	0,02

An RCR exceeding 1 indicates reason for concern. The RCR is less than 1 and therefore the risk of an effect on the environment, in this case the surfaces water, should be low. The driving variable, besides the concentration, is the dilution factor. For this calculation the actual flow values for the STP and recipient is used, giving a dilution factor of approximately 60. A sensitivity analysis with different dilutions factor is shown in table 10.

Table 10. Risk characterisation ratio (RCR) calculations with different dilution factors in Norway 2 and Iceland 1.

	Dilution	PEC, µg/L	PNEC, µg/L	RCR
Norway 2	1	0.3	0.25	1.2
Iceland 1	1	0.8	0.25	3.2
Norway 2	10	0.03	0.25	0.12
Iceland 1	10	0.08	0.25	0.32
Norway 2	58.6	0.0137	0.25	0.055
Iceland 1	58.6	0.0051	0.25	0.020
Norway 2	100	0.003	0.25	0.012
Iceland 1	100	0.008	0.25	0.032

Note that for the Norwegian and Icelandic conditions a dilution factor of 100 or higher is more likely and although the marine $PNEC_{water}$ is then also 10 times lower the RCR may consequently be somewhat lower than reported in table 9.

Regarding PNEC for microorganisms of a biological STP only ecotoxicity data for one compound was found, triclosan. The ecotoxicity data found was an EC 50 value (60

mg/l) for activated sludge. In the TGD the suggested assessment factor for EC 50 values are 100. The calculated PNEC for triclosan is 0.6 mg/l. The values in to the STP:s are all much lower than this value meaning that the RCR will be lower than 1.

9. Conclusions

- The environmentally important parameters are residual concentration in the treated water and the sludge leaving the STP. These concentrations and the resulting amounts give the load on the recipients.
  Biological degradation is normally dependent on the load i.e. the amount of organic matter per unit time compared to the amount of biosludge in the system. Physical adsorption often gives a relatively constant residual concentration in the water phase.
  Percent removal is only of interest combined with the original concentration.

- Benzothiazole-2-thiol mainly disappears from the water phase if there is a biological treatment, possibly dependent on the organic load. With anaerobic sludge treatment it is not found in the sludge. Data indicate that there might be a metabolite in equilibrium with benzothiazole-2-thiol. To be able to say more about the amounts in sludge and also in water the metabolic pathways should be known.

- Triclosan is probably just transferred from water to sludge phase. The concentration that was found in the treated water is probably a function of the amount of sludge leaving the treatment system and the amount of fine suspended matter in the water. Due to the long sludge ages it is impossible to determine if there is an actual degradation, based upon a few water samples. However, there were indications of some biological degradation at the higher temperature in the autumn.

In previous studies (e.g. Svenson 2002, Remberger et al. 2002, Adolfsson-Erici et al. 2002) triclosan were found in sewage sludge in the range between 3 and 4 mg/kg. Residues of triclosan has also been found in the water recipient of the investigated STP and in fish. Results from the present investigation combined with earlier results imply that the concentration of triclosan in sludge from STP is decreasing. It goes beyond the scope of the project to confirm that this actually is the case, and if so what would be the reason for it.

- 4-chloro-m-cresole was not found in any of the water or sludge samples probably due to degradation already in the sewer systems.

- Propiconazole was found in very low concentrations in some water and some sludge samples. It was found in the sludge where anaerobic treatment was used.

- Didecyldimethylammonium chloride was not found in any of the four sludge samples.

- The concentration in the outlet water from the STP are in almost every case lower then the PNEC values for each measured chemical. Only for “Norway” 2 and “Iceland” 1 are the concentrations of triclosan out higher then the PNEC value. The calculated PEC value however is lower then the PNEC value meaning that the risk of affecting the aquatic environment is low.

- The temperature differences between the two sampling periods showed no or little effect on the treatment results, just an indicated loss or degradation of triclosan at higher temperatures. Concentrations of benzothiazole-2-thiol and triclosan in the sewage also seemed to be higher at higher water temperature, but there is not enough data to enable any reliable conclusions.

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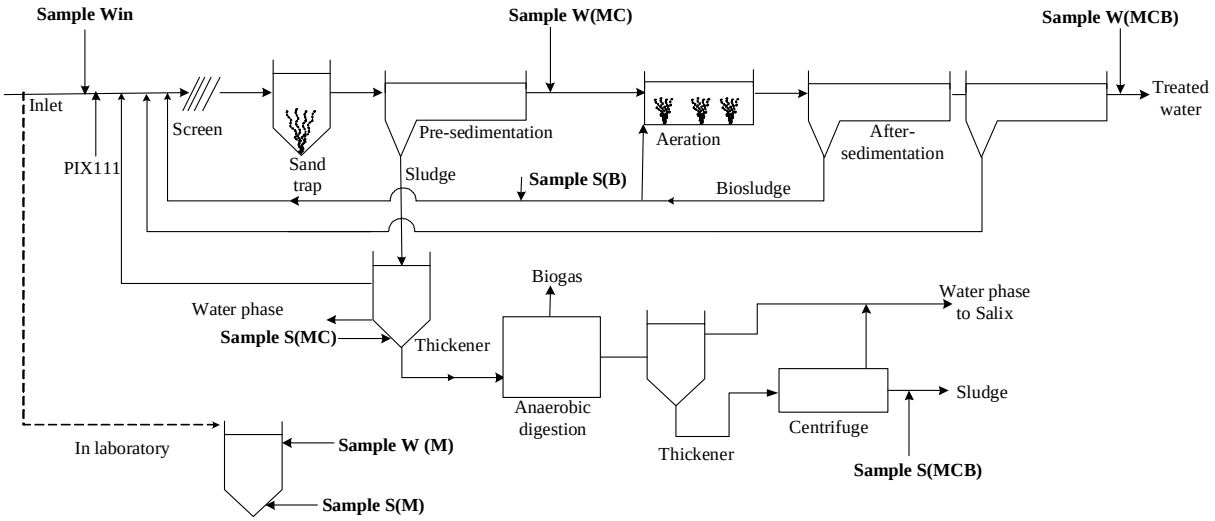
The SPIN data base (www.spin2000.net/spin.html)

Appendix 1
Description of the sewage treatment plants

Enköping STP

The plant has an average load of 22 000 pe, and MCB treatment . About 50% nitrogen recipient is achieved by pumping digestion reject and some of the treated water to *Salix* plantations.
PIX 111 ($FeCl_3$) is added directly at the end of the sewer, before two automatically rinsed screens in parallel. Excess biological sludge is also added to the inlet. About 100 tonnes/year of coarse material is separated in the screens and landfilled. Two aerated sand traps in parallel collect about 10 tonnes/year of sand.
There are four pre-sedimentation basins in parallel, where the settling sludge is separated and pumped for further treatment.
The chemically treated water is pumped to the activated sludge unit with a long completely aerated basin and four sedimentation basins. Most of the separated sludge is recirculated to the aeration, while excess biosludge is recirculated to the inlet.
There is another set of sedimentation basins for the earlier post-precipitation. Now it is just an extra settling, and this small amount of sludge is also recirculated to the inlet water.
Treated water is always released into the Enköping stream, while some of it is used to water a *Salix* plantation during vegetation season.
Excess sludge (primary sludge and biosludge) is thickened with addition of polymer before it is pumped to anaerobic digestion. The biogas is used to produce heat. The digested sludge is thickened and then de-watered in a centrifuge. Water from thickener and centrifuge is stored in ponds and used as nutrient for the *Salix*.
The de-watered sludge is presently stored or landfilled because farmers are afraid of possible contamination.



Henriksdal STP

The Henriksdal sewage treatment plant treats sewage from about 700 000 persons in a MCBden process. This means about 2.8 m^3/s in average, but the flow rate is changing both over the day and due to rain. All the treatment is underground, blasted into a rock.

Sewage comes to the STP from two directions (Henriksdal and Sickla), and there are separate pre-treatment stations. Treatment starts with a course screen (3 mm between bars), followed by a grit chamber or sand trap. A solution of ferrous sulphate is added to precipitate most of the phosphate and some organic material. Addition is made in an aerated channel to oxidise ferrous ions to ferric ions. After the aerated channel the two sewage streams enter a block of 13 primary sedimentation basins. Primary sludge is pumped to anaerobic digestion.

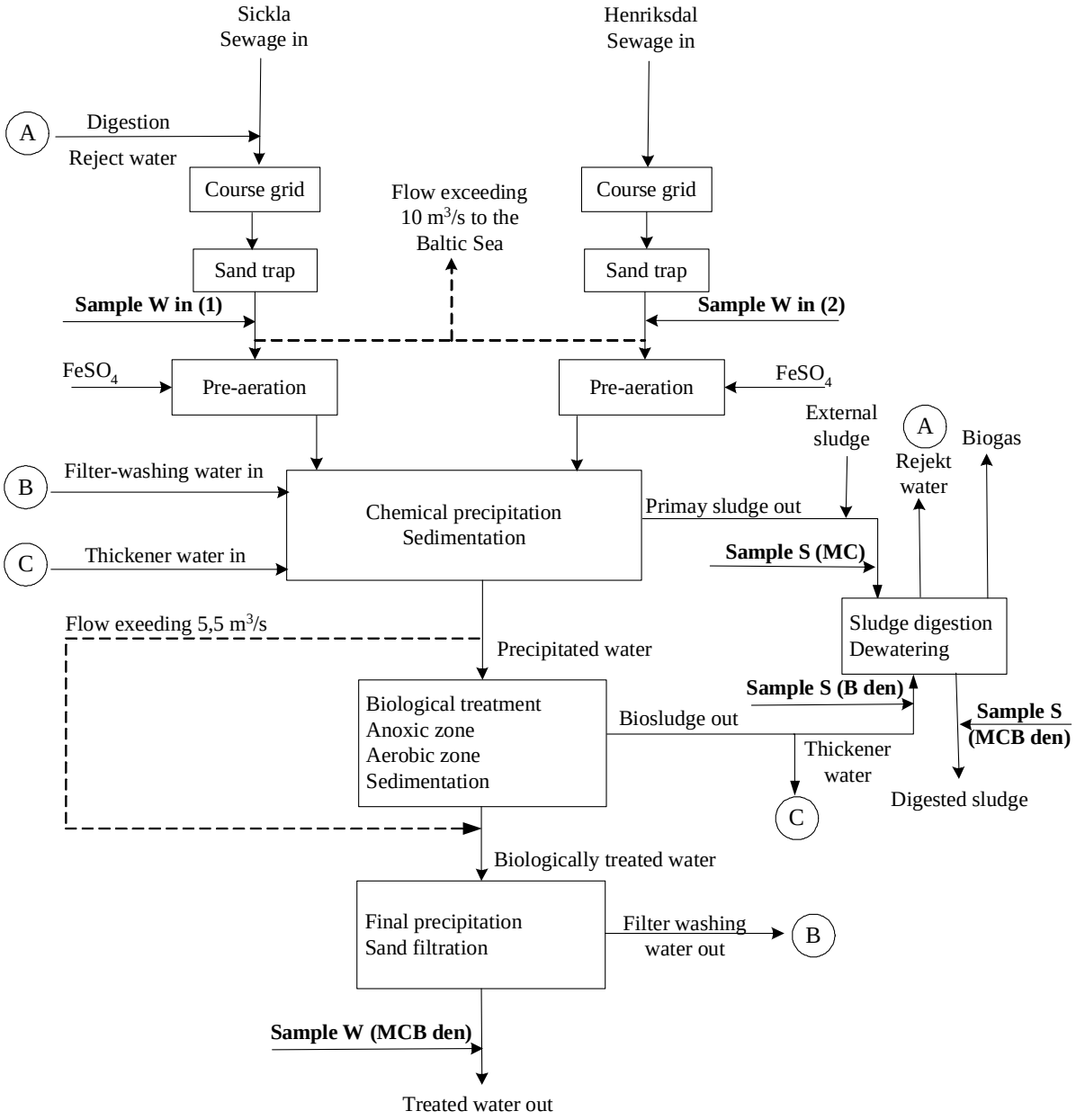
The water phase is pumped into the biological stage. This is 7 parallel long and deep basins with a total volume of about 200 000 m^3. Each basin is divided into one anoxic mixing zone, six aerated zones and one final de-aeration zone. In the mixing zone denitrification occurs, and some organic material is oxidised. Nitrate is reduced to form nitrogen gas. To this zone nitrate-rich water from the de-aeration zone and circulating bio-sludge from the secondary sedimentation is pumped.

In the aerated zones remaining organic compounds and ammonium are oxidised. In the de-aeration zone the remaining oxygen is depleted. Nitrate-rich water is circulated to the mixing zone for denitrification. A water stream corresponding to the total load from the primary sedimentation enters the secondary sedimentation basins. The bio-sludge is settled, and most of the sludge is recirculated to the anoxic mixing zone to keep a high concentration of active sludge in the system. Some bio-sludge has to be taken out, and this is digested anaerobically with the primary sludge.

Water after secondary sedimentation is pumped to sand filters. It still contains some particles, and there can be a too high residual concentration of phosphate. When the concentration of total phosphorous, both in particles and in phosphate, tends to be higher than 0.3 mg P/L ferrous sulphate is added before the sand filters. These, 60 filters in parallel, remove some particles and precipitated phosphate. The sand filters are back-washed with air and water when they tend to have to high flow resistance. The washing water is recirculated to the pre-precipitation step.

Treated water passes heat pumps to recover energy, and is then discharged to the Stockholm archipelago via an outlet tube.

Primary sludge and de-watered excess bio-sludge are anaerobically digested. The produced biogas is presently used for both electricity and heat generation, and an increasing portion is upgraded to vehicle gas quality. Reject water from dewatering of digested sludge is pumped back to the pre-sedimentation. De-watered digested sludge is today used to cover mining waste.


Sickla
Sewage in
Henriksdal
Sewage in
A
Digestion
Reject water
Course grid
Course grid
Flow exceeding
10 m3/s to the
Baltic Sea
Sand trap
Sand trap
Sample W in (1)
Sample W in (2)
FeSO4
Pre-aeration
Pre-aeration
FeSO4
External
sludge
A
Biogas
Rejekt
water
B
Filter-washing water in
Chemical precipitation
Sedimentation
Primay sludge out
C
Thickener water in
Sample S (MC)
Sludge digestion
Dewatering
Flow exeeding 5,5 m3/s
Precipitated water
Biological treatment
Anoxic zone
Aerobic zone
Sedimentation
Sample S (B den)
Biosludge out
Sample S
(MCB den)
Thickener
water
Digested sludge
C
Biologically treated water
Final precipitation
Sand filtration
Filter washing
water out
B
Sample W (MCB den)
Treated water out

Koholmen STP

The Koholmen STP plant has a MBdenBioP process and an average load of 39 000 persons . The sewage first passes two automatically rinsed fine grids, and then two sand- and fat traps. Sand is settling and fat is separated by flotation.
After this mechanical treatment the water enters the biological treatment, designed for biological phosphorous and nitrogen removal in two parallel lines. Each line comprises:

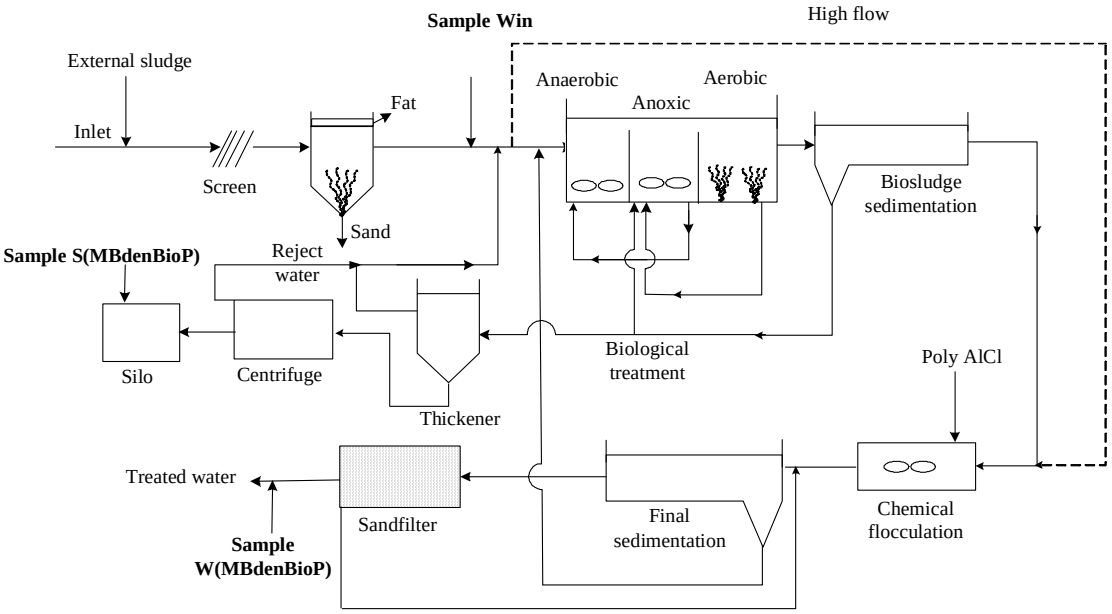
- Anaerobic step (release of orto-phosphate from the sludge)
- Anoxic step (denitrification and dosing of chemicals if simultaneous precipitation is used)
- Oxidation step (nitrification, uptake of orto-phosphate and aerobic degradation of organic material)
- De-oxidation step (to deplete oxygen before the anoxic step)

Water and sludge from the anoxic step is pumped to the anaerobic step for the biological P-removal (1 000 m^3/h). From the de-oxidation step 3 x Q (a flow that is three times the sewage flow) is pumped to the anoxic step for denitrification.

Biologically treated water is settled in 6 parallel sedimentation basins. Separated sludge is pumped to the anoxic step, and excess sludge is de-watered with polymer. Since the Bio-P doesn't give low enough residual P, poly-aluminium chloride (PAX) is added to the biologically treated and settled water. Chemical sludge is separated in 3 parallel basins, and the sludge is pumped either to the inlet to the biology or to chemical sludge storage. Eventual floating sludge is pumped to the inlet of the STP.

Water after chemical precipitation is filtered in 7 parallel sand filters and finally discharged in the Baltic Sea south of the island Verkö via an outlet tube.

Biological excess sludge and sometimes chemical sludge is finally de-watered in centrifuges with addition of polymer. Reject water is pumped to the inlet of the biology. De-watered sludge is presently landfilled.



Ekebyhov STP

The plant treats water corresponding to 17 000 person-equivalents. It is a MCBden-plant like Henriksdal, but much smaller and without anaerobic sludge digestion. Some industrial wastewater and external sludge is included. Sewage and external sludge goes to a sand trap and a mechanically rinsed fine grid.

The biological treatment is an activated sludge system with pre-denitrification and comprises:

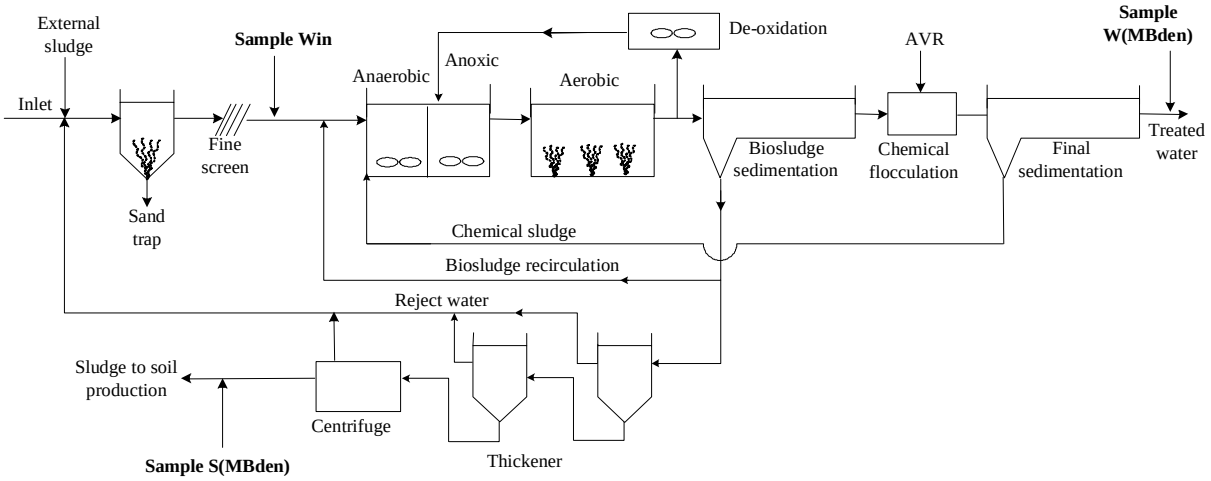
- One anaerobic and one anoxic zone for denitrification, both with mixers
- One aerated basin for oxidation of organic material and ammonium, oxygen level is kept above 2 mg O_2/L with membrane aerators.
- One de-oxidation basin to remove dissolved air before recirculation of nitrate-rich water to the anoxic zone
- Two parallel sedimentation basins for separation of biosludge

Half of the water from the aerated basin is recirculated through the de-oxidation basin, the rest is directed to the sedimentation. Most of the settled sludge is recirculated to a mixing zone before the anaerobic zone, while the rest is pumped to the sludge treatment.

The plant has after-precipitation of phosphorous with AVR (aluminium sulphate) in 6 parallel flocculation basins and 3 sedimentation basins. The chemical sludge is recirculated to the anaerobic zone.

The treated water is released via a 400 meters long tube to Fiskarfjärden in northern Lake Mälaren. Flow rate is measured and flow-proportional samples are taken for analyses.

Mixed sludge (biological and chemical) is thickened in two stages to about 3 % TS and stored before dewatering in centrifuge with polymer addition to about 20 % TS. Most of the sludge is used for soil production.



Wastewater treatment plants with sampling for biocide analyses

Parameter	Unit	Karlskrona	Henriksdal	Enköping	Ekerö
Normal conditions					
N-reduction		Yes	Yes	No	Yes
P-reduction		BioP+PAX	$FeSO_4$	PIX111	AVR
Sludge treatment		Aerobic	Anaerobic	Anaerobic	Stabilising
Load	pe	39 000	700 000	16 500	17 000
Water flow, annual mean	m^3/day	15 600	241 000	8 500	4 800
Sludge production, total	kg TS/day	3 500	38 000	1 250	1 520
Sludge production, primary	kg TS/day	0	55 000	2 000	-
Sludge production, biosludge	kg TS/day		20 000		-
HRT total, mean	h	48	29	23	47
HRT biology, mean	h	31	20	9	34
Wet volume, total	m^3	31 000	290 000	7 400	9 460
Wet volume, biology	m^3	20 000	200 000	3 200	6 770
Sludge age	days	5-20	20	-	-
Spring sampling (cold water)		**26 April**	**4 May**	**4 May**	**12 May**
Water flow	m^3/day	14 400	207 000	7 790	4 030
Water temperature	°C	12	14.5	13.5	14.7
TSS in biology	mg/L	2 900	2 400	1 070	2 000?
COD in	mg/L	850	232	320	2 400
COD load	g COD/kg TSS, d	210	100	730	710?
COD out	mg/L	33	10	43	22
COD reduction	%	96	96	87	99
BOD in	mg/L	190	260	160	100
BOD load	g BOD/kg TSS, d	54	110	360	30?
BOD out	mg/L	<3	3	9	<3
BOD reduction	%	>98	97	94	>97
N reduction	%	86	87	-	78
P reduction	%	95	99	97	>99
Autumn sampling (warmer water)		**31 Aug**	**1 Sept**	**25 Aug**	**25 Aug**
Water flow	m^3/day	18 800	219 000	6 090	4 200
Water temperature	°C	20	19	17	18
TSS in biology	mg/L	3 700	2 300	790	2 000?
COD in	mg/L	450	145	310	1 000
COD load	g COD/kg TSS, d	110	69	750	310?
COD out	mg/L	39	8,2	28	22
COD reduction	%	91	94	91	98
BOD in	mg/L	170	260	130	25
BOD load	g BOD/kg TSS, d	43	120	310	8?
BOD out	mg/L	<3	1	3.6	<3
BOD reduction	%	>98	>99	97	>88
N reduction	%	83	85	-	76
P reduction	%	97	99	97	99
			TOC instead of COD		

HRT = hydraulic retention time TSS = total suspended solids

Appendix 2
Description of analytical methods

Chemicals

The solvents, HPLC-quality, methanol, 2-propanol (IPA), acetone, hexane, pentane, methyl-*tert*-butylether (MTBE) were delivered from Rathburn (Chemical Ltd., Peeblesshire, Scotland). Ethyl acetate was delivered from J. T. Baker (Gross-Gerau, Germany).

Merck (Darmstadt, Germany) delivered diethyl ether, (ethanol-stabilised) phosphoric acid, ammonium acetate, ascorbic acid, sodium sulphate and silica gel.

Potassium iodide and hydrochloric acid were delivered from Fluka (Buchs, Switzerland).

Sodium sulphate and silica gel were preheated (400°C) prior use. The silica gel was deactivated (5%) before used.

A Milli-Q plus (Millipore Corporation, Bedford, MA, USA) was used to produce ultra pure water.

Buffer (pH 7) was prepared by mixing potassium dihydrogen phosphate (50 ml; 0.1 M) and sodium hydroxide (29.1 ml; 0.1 M) (West et al. 1988; table D-145).

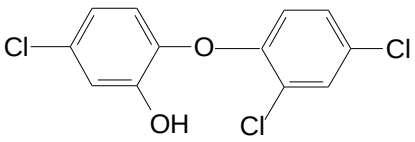
The analytes, injection standard (IS), and recovery standard (RS) used in this study are summarised in Table 1 and the structures of the analytes are presented in Figure 1 and 2. The recovery standards are added in order to improve the identification and quantification (se below).

Propiconazole is not a single compound but two isomers but the analytical results in present report are reported as the sum of these two isomeres.

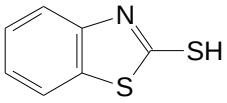
Table 1 CAS-number, purity and suppliers of the target compounds (analytes) and recovery standards (RS).

Target compound (analytes)	**CAS**	**Purity**	**Supplier**
Triclosan	3380-34-5	100	Ciba
4-chloro-m-cresole	59-50-7	99	Aldrich
Benzothiazole-2-thiol	149-30-4	98	Aldrich
Propiconazole	60207-90-1	97	Dr.Ehrendtorfer
Didecyldimethylammonium chloride	7173-51-5	98	Aldrich
Recovery standards (RS)	**CAS**	**Purity**	**Supplier**
6-chloro-3-methylphenol	-	-	TCI
Atrazin	1912-24-9	99	Dr.Ehrendtorfer
Chlorfenvinphos	470-90-6	94	Dr.Ehrendtorfer
3-ethyl-4-chlorphenol	-	97	Aldrich
Tetrachlorbisphenol-A	-	98	Aldrich
N,N-dimethylundecylamine	17373-28-31	98	Aldrich
Biphenyl IS	-	99	-

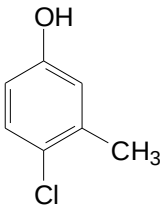
Explanation: Recovery Standard (RS); Injection Standard (IS). The quaternary ammonium compound (Didecyldimethylammonium salt) was pursed from Aldrich as the bromine salt.



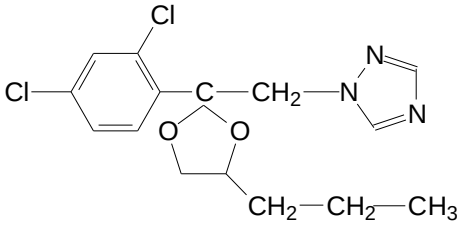
Triclosan (Irgasan DP 300)



Benzothiazole-2-thiol



4-chloro-m-cresole



Propiconazole

Figure 1. The structures of the chemical compounds. These compounds were analysed together using the same method.

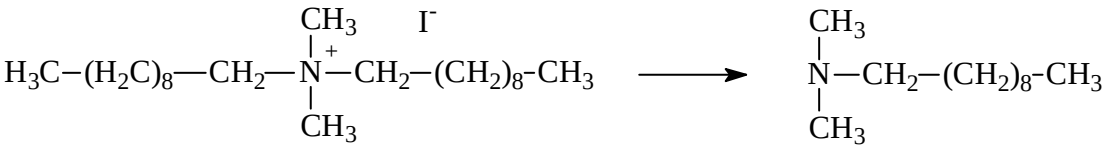


Figure 2: The structure of the cationic surfactant didecyldimethylammonium iodide and the derivative produced (decyldimethylamin) in the GC-injector.

Analytical methods

Recovery standard method

During the sample preparation losses of analytes due occur. It is therefore important to determine this loss and make it in that way possible to correct. This can be accomplished by adding recovery standards to the sample in the beginning of the analytical work.

Obviously, compounds used as surrogate standards must have similar physical and chemical properties as the target substance (analytes). This means that the analytes and the recovery compound must behave similarly in the different analytical steps e.g. extraction, clean-up procedures and derivatization.

In Table 2 the analytes and the corresponding recovery standards are summarised.

The compounds triclosan, 4-chloro-m-cresol, benzothiazole-2-thiol and propiconazole were analysed together using the same analytical procedure. For the quaternary amine didecyldimethylammonium chloride a quite different technique was needed and this substance was therefore analysed separately. Both methods are described in detail below.

Table 2. Target compounds and the corresponding recovery standards.

Target compound	Recovery compound (RS)
4-chloro-m-cresole	6-chloro-3-methylphenol; 3-ethyl-4-chlorophenol
Benzothiazole-2-thiol	Tetrachlorobisphenol-A
Triclosan	Tetrachlorobisphenol-A
Propiconazole	Atrazin; Chlorfenvinphos
Didecyldimethylammonium chloride	N,N-dimethylundecylamine

Analysis of triclosan, 4-chloro-m-cresole, benzothiazole-2-thiol and propiconazole

Extraction

Water samples

The sample (200 ml) was spiked with RS (Table 2), ammonium acetate (10 ml; 2 M) and ascorbic acid (1 ml; 1 M) were added and the sample was carefully mixed. The sample was acidified (pH 2-6) by adding phosphoric acid (3-500 µl; 6 M). The sample was extracted twice with pentane: diethyl ether (1:1; 50 ml).

The extracts were combined and concentrated first on a RotoVap (40°C) and then with nitrogen gas. Polar and acidic compounds were washed out by shaking the extract with buffer (5 ml; pH 7). The extract was dried over sodium sulphate and derivatized as described below.

Sludge samples

The sample (10 g) was spiked with recovery standards (Table 2). Ammonium acetate (2 ml; 2 M) and ascorbic acid (0.25 ml; 1 M) were added and the sample was carefully mixed. Phosphoric acid (3-500 µl; 6 M) was added to a final pH of 2-6. The sample was extracted twice with methanol (10 + 5 ml). The extracts were combined and diluted with water (35 ml) and extracted in two cycles with pentane: diethyl ether (1:1; 10 ml). The extract was concentrated, extracted with buffer, dried in the same manner as for the water samples and finally derivatized as described below.

Derivatization

The phenols and benzothiazole-2-thiol were derivatized for two reasons. Derivatization improved (a) the clean up of the extract and (b) the chromatographic properties and separation on the GC-column.

The extract, solvent-exchanged into ethyl acetate and / or diethyl ether containing 20-25% methanol, was reacted with ethereal diazomethane (200 µl) at room temperature. After 4-6 hours more diazometan was added (100 µl) and the sample was left over night in darkness.

The reagent excess was evaporated with the aid of a nitrogen jet. The methanol was washed away by shaking the extract with water (5 ml) after addition of hexane (1 ml). The extract was dried over sodium sulfate and concentrated (0.5 ml) before clean up on a silica gel column as described below.

Clean up by silica chromatography

The deactivated silica gel column (5%; 0.5 g) was prepared in a Pasteur pipette. Two fractions were collected: (a) fraction F-2, containing the *O*-methylated phenols and *S*-methylated benzothiazole-2-thiol, was obtained by eluting the column with pentane: MTBE (9:1) and (b) fraction F-6, containing propiconazole, was obtained by eluting with MTBE with IPA (10%).

Fraction F-6 was extracted with water and dried over sodium sulfate. Both fractions F-2 and F-6 were concentrated with nitrogen and finally spiked with IS (Table 1) before GC-MS-analysis.

The extraction and work-up procedure is summarized schematically in Figure 2.

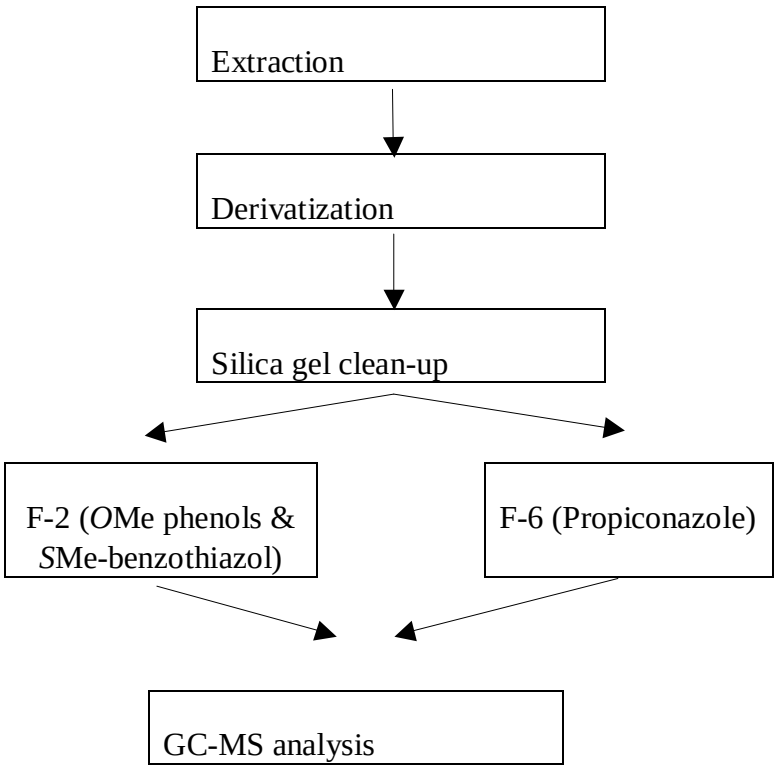


Figure 2: The analytical scheme for phenol compounds, benzothiazole-2-thiol and propiconazole.

Analysis of didecyldimethylammonium chloride (DDDMA)

DDDMA was analysed according to (Fernándes, Alder et al. 1996; Hind, Bhargave et al. 1997; Ding, Liao et al. 2003).

Extraction of sludge samples

The sample (1-2 g) was spiked with recovery standard (N, N´-dimethylundecylamine), acidified with HCl and extracted three times with methanol (3 x 2 ml). The combined methanol extract was evaporated to dryness and re-dissolved in water (2-3 ml).

Derivatization

The pH was adjusted to <2. Potassium iodide (KI) was added and the sample was mixed for 10 min. The pH was adjusted to 10-11 with NaOH. After 10 min mixing the sample was extracted three times with dichloromethane (3 x 2 ml). The extracts were combined, dried and concentrated. Before injection on GC a volumetric standard was added (biphenyl). The GC-MS data were the same as for the analytes in table 2 but the injector temperature was increased to 300°C. At these conditions DDDMA is dealkylated to dimethyldecylamine (Figure 2). The derivative is chromatographed and detected by means of GC-MS. The ions monitored are summarised in Table 4.

GC-MS analysis

The extracts were analyzed on a 6890N gas chromatograph with a 5973N mass selective detector (Agilent). The injection was done at 250°C in pulsed splitless mode. 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 and 5°C/min to 300°C held at 300°C for 2 min. Helium was used as carrier gas. The mass spectrometer transfer line temperature was 280°C. The detector was used in selected ion monitoring mode with electron ionisation at an energy of 70 eV. Their characteristic retention time, one target ion (T-ion) used for quantification of the analytes and one qualifier ion used (Q-ion) to increase specificity was recorded (Table 3 and 4). Quantification was based on comparison of peak abundance to the known response of the internal standard (biphenyl). The reported analyte concentrations were corrected for the losses according to the determined recovery standard as described above.

Table 3: Retention time, molecule weight (Mw), target ion and qualifier ion used for the GC-MS quantification.

Compound	Rt	Mw	T-ion	Q-ion
4-Chloro-m-cresol-*O*Me	**8,23**	**156**	**156**	**141**
6-Chloro-3-methylphenol-*O*Me RS	8,38	156	156	158
3-Ethyl-4-chlorophenol-*O*Me RS	8,98	170	170	155
Biphenyl IS	9,94	154	154	153
benzothiazole-2-thiol *S*-Me	**11,94**	**181**	**181**	**148**
Atrazin RS	13,07	216	215	200
Chlorfenvinfos RS	16,51	351	323	267
Triclosan-*O*Me	**17,41**	**304**	**302**	**252**
Propiconazole	**20,31**	**342**	**259**	**173**
Tetrachlorobisphenol-A-(*O*Me)2 US	23,27	394	379	377

Retention time (Rt; GC); molecule weight (Mw); Target ion (T-ion); Qualifier ion (Q-ion). Injection standard (IS); Recovery standard (RS); *O*-methylether (*O*Me); S-methylated (*S*Me). Compounds marked with bold text are the studied compounds in the present investigation.

Table 4: Retention time, molecule weight (Mw), target ion and qualifier ion used for the GC-MS quantification.

Compound	Rt	Mw	T-ion	Q-ion
Decyldimethylamin	**13,82**	**185,35**	**184**	**58**
N,N-dimethylundecylamin RS	7,34	199,38	58	-

Decyldimethylamin is the derivative of didecyldimethylammonium iodide produced in the GC-injector. Compounds marked with bold text are the studied compounds in the present investigation.

Appendix 3
PEC calculations

Below are the equations used for calculation of PEC. All equations are taken from the TGD.

$$Clocal_{water} = \frac{Clocal_{eff}}{(1 + Kp_{susp} \cdot SUSP_{water} \cdot 10^{-6}) \cdot DILUTION}$$

Explanation of symbols

$Clocal_{eff}$	concentration of the substance in the STP effluent	[mg · l^{-1}]
Kp_{susp}	solids-water partitioning coefficient of suspended matter	[l · kg^{-1}]
$SUSP_{water}$	concentration of suspended matter in the river	[mg · l^{-1}]
DILUTION	dilution factor	[-]
$Clocal_{water}$	local concentration in surface water during emission episode	[mg · l^{-1}]

$$Kp_{comp} = Foc_{comp} \bullet Koc \quad with\ comp \in \{soil, sed, susp\}$$

Explanation of symbols

Koc	partition coefficient organic carbon-water	[l · kg^{-1}]	data set/Ch. 4
Foc_{comp}	weight fraction of organic carbon in compartment *comp*	[kg · kg^{-1}]	Table 5
Kp_{susp}	partition coefficient solid-water in suspended matter	[l · kg^{-1}]	
Kp_{sed}	partition coefficient solid-water in sediment	[l · kg^{-1}]	
Kp_{soil}	partition coefficient solid-water in soil	[l · kg^{-1}]	

Values used:

Flow	345600	m^3/day
Effluent STP	6000	m^3/day
Dilution	58.6	
Suspwater	15	mg/l
Kpsusp	0.000315	l/kg

Appendix 4
Product types according to Annex V of the Biocidal Products Directive (98/8/EC).

MAIN GROUP 1: Disinfectants and general biocidal products

These product types exclude cleaning products that are not intended to have a biocidal effect, including washing liquids, powders and similar products.

Product-type 1: Human hygiene biocidal products

Products in this group are biocidal products used for human hygiene purposes.

Product-type 2: Private area and public health area disinfectants and other biocidal products

Products used for the disinfection of air, surfaces, materials, equipment and furniture which are not used for direct food or feed contact in private, public and industrial areas, including hospitals, as well as products used as algaecides.

Product-type 3: Veterinary hygiene biocidal products

Products in this group are biocidal products used for veterinary hygiene purposes including products used in areas in which animals are housed, kept or transported.

Product-type 4: Food and feed area disinfectants

Products used for the disinfection of equipment, containers, consumption utensils, surfaces or pipework associated with the production, transport, storage or consumption of food, feed or drink (including drinking water) for humans and animals.

MAIN GROUP 2: Preservatives

Product-type 6: In-can preservatives

Products used for the preservation of manufactured products, other than foodstuffs or feedingstuffs, in containers by the control of microbial deterioration to ensure their shelf life.

Product-type 9: Fibre, leather, rubber and polymerised materials preservatives

Products used for the preservation of fibrous or polymerised materials, such as leather, rubber or paper or textile products and rubber by the control of microbiological deterioration.