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Results from the Swedish National Screening Programme

Antibacterial substances

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Summary
As an assignment from the Swedish Environmental Protection Agency, a screening study of antibacterial substances has been performed during 2013. Substances selected for analysis were triclosan, triclocarban, silver and the degradation products methyl triclosan and 3,4-dicloroaniline (3,4-DCA). Samples were taken at six different WWTPs and covered influent and effluent water, sludge and sediments and fish collected in the vicinity of the WWTP outlet. A total of 30 samples were analysed.

Triclosan was found in all matrices sampled, and consistently occurred in highest concentrations of the organic substances, with the exception of sediment, where triclocarban concentrations were in the same order of magnitude. The triclosan concentrations in sludge (890-1800 ng/g dw), incoming water (81-130 ng/l) and outgoing water (27-52 ng/l) from WWTPs were similar to previously reported concentrations and indicate that the decreasing trend in sludge appears to be levelling off. Methyl-triclosan was mainly present in sludge, and the levels were about a factor of 200 lower than triclosan concentrations. Triclocarban was commonly found in WWTP influents, sludge and sediments but was not found in biota and only in one effluent sample. The sediment concentrations were comparable to the triclosan levels whereas concentrations in sludge and WWTP influents were a factor of 7.5 and 4 lower, respectively. 3,4-DCA (and also 4-chloroaniline, another degradation product of triclocarban), were not detected in any sample. Silver was found in sediment and sludge, but not in biota or effluent water and only in one sample of WWTP influent water. The concentrations in sludge were similar to previously reported values.

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Sammanfattning

IVL Svenska Miljöinstitutet har under 2013 på uppdrag av Naturvårdsverket utfört en screeningstudie av antibakteriella substanser i miljön. De ämnen som ingick i studien var triklosan, triklokarban, silver och nedbrytningsprodukterna metyl-triklosan, och 3,4-dikloranilin (3,4-DCA).

Det övergripande syftet med screeningen var att bestämma koncentrationer av de utvalda ämnena i kommunala avloppsreningsverk och bedöma förekomst och nivåer i recipienter i närheten av reningsverken.

Prover togs vid sex olika kommunala reningsverk och omfattade inkommande och utgående avloppsvatten samt slam. Dessutom ingick sediment och fisk som samlats in i närheten av reningsverkens utlopp. Totalt analyserades 30 prover.

Triklosan hittades i alla provtagningsmatriser, och förekom konsekvent i högst koncentrationer av de organiska ämnena, undantaget sediment, där koncentrationerna av triklokarban var i samma storleksordning. Koncentrationerna av triklosan i slam (890-1800 ng/g dw) och inkommande (81-130 ng/l) och utgående (27-52 ng/l) vatten från reningsverk låg i samma storleksordning som tidigare rapporterade halter och indikerar att den tidigare observerade nedåtgående trenden i reningsverksslam tycks börja plana ut.

Metyl-triklosan, återfanns främst i slam, med halter motsvarande ungefär en faktor 200 lägre än koncentrationerna av triklosan.

Triklokarban var allmänt förekommande i inkommande vatten, slam och sediment, men kunde inte detekteras i biota och endast i ett prov av utgående vatten. Halterna i sediment var i samma nivå som triklosan medan halterna i slam och inkommande vatten var en faktor 7,5 respektive 4 lägre än triklosan.

3,4-DCA (och även 4-kloranilin, en ytteliggare nedbrytningsprodukt av triklokarban), kunde inte detekteras i något prov, men detektionsgränserna var högre för dessa föreningar jämfört med moderföreningen. Detektionsgränserna för dessa föreningar var dock betydligt lägre än de koncentrationer av 3,4-DCA som uppmätts i regioner utanför Sverige.

Silver hittades i sediment och slam, men inte i biota eller utgående vatten och endast i ett prov av inkommande vatten till reningsverk. Halterna i slam var i samma storleksordning som tidigare rapporterade koncentrationer.

Summary

As an assignment from the Swedish Environmental Protection Agency, a screening study of antibacterial substances has been performed during 2013. Substances selected for analysis were triclosan, triclocarban, silver and the degradation products methyl triclosan and 3,4-dicloroaniline (3,4-DCA).

The overall objective of the screening was to determine the concentrations of the selected substances in municipal wastewater treatment plants and to assess the occurrence and levels in recipients and local background stations in the vicinity of municipal waste water treatment plants (WWTPs).

Samples were taken at six different WWTPs and covered influent and effluent water, sludge and sediments and fish collected in the vicinity of the WWTP outlet. A total of 30 samples were analysed.

Triclosan was found in all matrices sampled, and consistently occurred in highest concentrations of the organic substances, with the exception of sediment, where triclocarban concentrations were in the same order of magnitude. The triclosan concentrations in sludge (890-1800 ng/g dw) incoming water (81-130 ng/l) and outgoing water (27-52 ng/l) from WWTPs were similar to previously reported concentrations and indicate that the decreasing trend in sludge appears to be levelling off.

Methyl-triclosan was mainly present in sludge, and the levels were about a factor of 200 lower than triclosan concentrations.

Triclocarban was commonly found in WWTP influents, sludge and sediments but was not found in biota and only in one effluent sample. The sediment concentrations were comparable to the triclosan levels whereas concentrations in sludge and WWTP influents were a factor of 7.5 and 4 lower, respectively.

3,4-DCA (and also 4-chloroaniline, another degradation product of triclocarban), were not detected in any sample, but the quantification limits were higher for these compounds than for the mother compound. The quantification limits were, however, substantially lower than measured concentrations of 3,4-DCA in regions outside of Sweden.

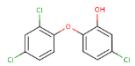
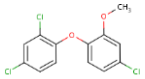
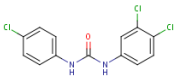
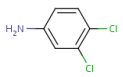
Silver was found in sediment and sludge, but not in biota or effluent water and only in one sample of WWTP influent water. The concentrations in sludge were similar to previously reported values.

1 Introduction

As an assignment from the Swedish Environmental Protection Agency, a screening study of antibacterial substances has been performed during 2013. Substances selected for analysis were triclosan, triclocarban and silver. In addition, methyl triclosan and 3,4-dicloroaniline were also included, which are degradation products of triclosan, and triclocarban respectively.

The overall objective of the screening was to determine the concentrations of the selected substances in municipal wastewater treatment plants (WWTPs) and to assess the occurrence and levels in the receiving waters. The substances included in the current study are given in Table 1 together with their molecular formulas and structures.

Table 1. Substances covered in this study

Name (abbreviation)	Systematic name	CAS	Molecular formula	Structure
Triclosan (TCS)	2,4,4'-Trichloro-2'-hydroxy-diphenylether	3380-34-5	$C_{12}H_7Cl_3O_2$	
Methyl triclosan (M-TCS)	2,4,4'-Trichlor-2'-methoxy-diphenylether	4640-01-1	$C_{13}H_9Cl_3O_2$	
Triclocarban (TCC)	3,4,4'-Trichlorocarbanilide	101-20-2	$C_{13}H_9Cl_3N_2O$	
3,4-Dichloro-aniline (3,4-DCA)	-	95-76-1	$C_6H_5Cl_2N$	
Silver (Ag)	-	7440-22-4	Ag	-

Antibacterial substances are used in many different types of products to prevent bacterial growth and bad smell, and may reach the environment mainly via diffuse spreading and municipal wastewater plants. An increasing use of antibacterial substances and subsequent release to the environment may in the long term lead to

development of resistance among bacteria to substances which may be essential to hygiene and health in other contexts. In addition, some of the antibacterial substances have toxic effects and increasing environmental concentrations may thus lead to negative damage on humans and/or wildlife.

2 Physical-chemical properties and fate

2.1 Organic antibacterial substances

To predict how the chemicals behave in the environment, an evaluative environmental fate model was applied, namely the equilibrium criterion (EQC) model (Mackay et al., 1996). This model represents a standardized environment of about the size of Ontario State in Canada (where the model was originally developed) and comprises four environmental compartments: air, water, soil and sediment. The model can be applied using different settings (levels):

- Level I assumes that the chemical is released into the world, without specifying the receiving compartment. It also assumes that the world is closed and that the chemical cannot degrade or leave the compartment where it first ends up. Level I shows the preferred partitioning behaviour of a chemical based on its physical-chemical properties only.

- Level II, assumes that the unit world is an open system, i.e. the chemical can leave the system through passive transport via air or water or sediment burial and degradation rates are also considered. However, equilibrium between compartments is assumed, thus there can be no transport from air to soil etc. Level II thus gives additional information about important removal processes.

- Level III, assumes a non-equilibrium situation, and therefore considers also the emission mode, i.e. to which compartment the chemical is released. Transport between compartments is considered, as is degradation rates. Level III generates information about overall residence times of the chemical in system for different emission scenarios, and can be used to assess the importance of different chemical loss processes.

Both Level II and III assume steady state, i.e. no changes in concentrations or emissions over time. The EQC model is suitable for comparing partitioning and fate properties for different chemicals based on their physical-chemical properties and degradation half-lives, and should not be used for assessing fate or predicting concentrations in a specific local environment.

A summary of the physical-chemical properties of the organic substances included in this study is given in Table 2. As shown in the table they are fairly soluble in water and have generally low vapour pressures with the exception of 3,4-DCA, which is fairly volatile. Combined with their relatively high hydrophobicities (Log K_{OW} 2.7-5.2) this generates partitioning properties according to the emission mode, but with a preference of aquatic and solid matrices. Figure 1 illustrates the environmental fate of the substances as predicted by the EQC model if steady state and equilibrium are assumed (Level II) as well as the partitioning tendency in a non-equilibrium, steady state system (Level III) with a scenario of emissions only to water, since most of the substances included are mainly released via the sewage system. Figure 2 gives an example of the EQC results for triclosan at level III and Figure 3 Figure 3shows the predicted overall residence time for the evaluative EQC environment in the emission to water scenario as well as the residence times attributed to only degradation or advection.

Table 2. Physical-chemical properties of antibacterial substances at 25°C, unless otherwise noted.

Name		**Triclosan**	**Methyltriclosan**	**Triclocarban**	**3,4-Dichloroaniline**
MW (g/mol)		289.5	303.6	315.6	162
VP (Pa)		1.15×10^{-3} [1]	8.9×10^{-3} [1]	4.8×10^{-7} [1]	0.84[2]
W_{SOL} (mg/L)		10^2 [2] (20°C)	0.4[1]	0.65[1]	92[2] (20°C)
Log K_{ow}		4.76[2]	5.22[1]	4.9[1]	2.69[2]
BCF[1]	**(L/kg ww)**	640	1300	797	28
Half-lives (h)[1]	Air	16	22.5	12	12
	Water	1440	1440	1440	900
	Soil	2880	2880	2880	1800
	Sediment	13000	13000	13000	8100

[1] Estimated values retrieved from the EPIWeb software USEPA. Estimation Programs Interface Suite™ for Microsoft® Windows. United States Environmental Protection Agency, Washington, DC, USA., 2011.
[2] Experimental values retrieved from the EPIWeb software ibid.

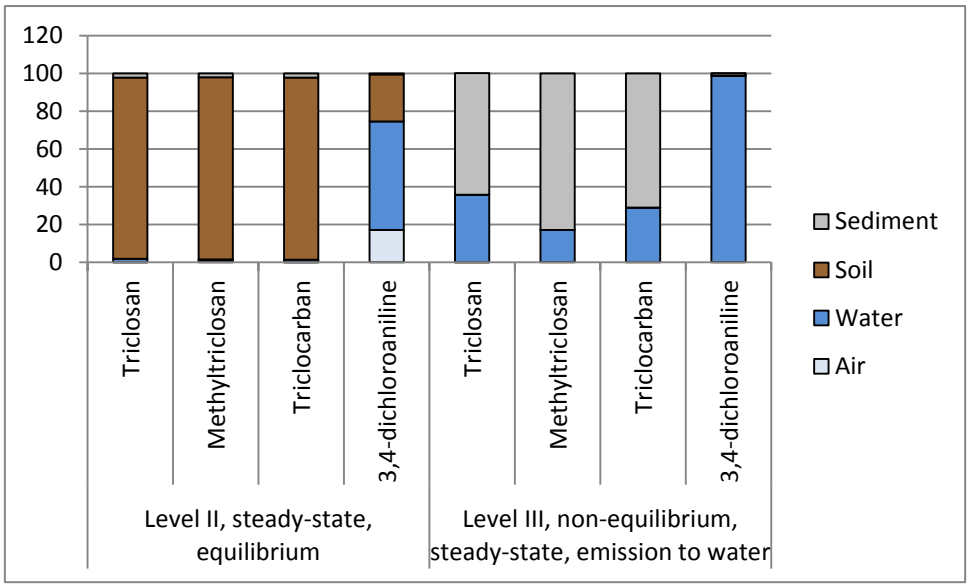


Figure 1. Evaluative fate assessment of target chemicals, as predicted by the EQC model

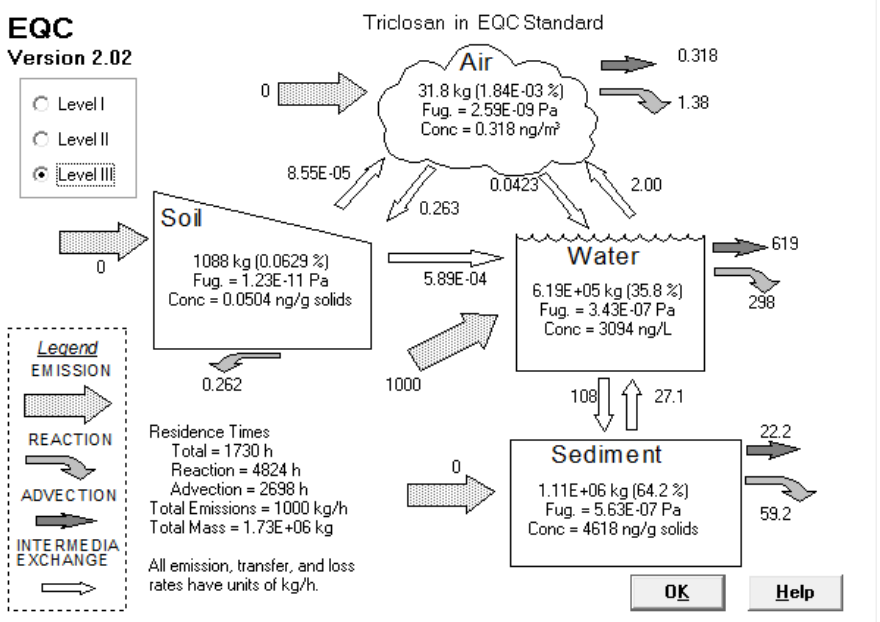


Figure 2. Example figure of EQC output, Level III results for triclosan when emitted to water.

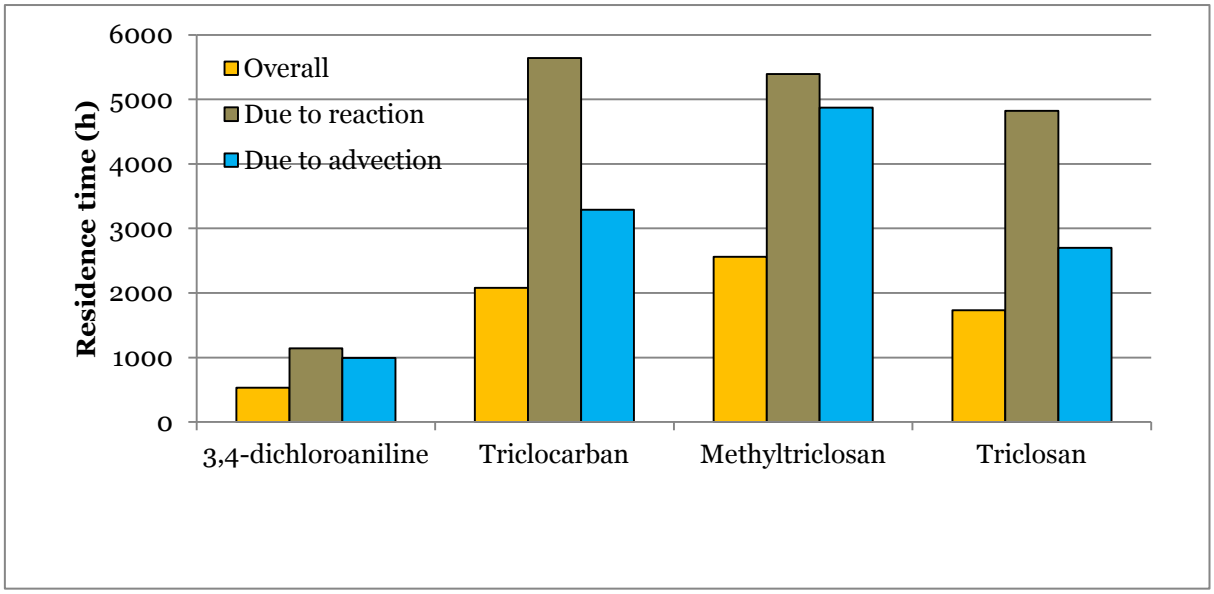


Figure 3. Predicted residence times of target substances in a scenario of emissions only to water

As shown in Figure 1-Figure 3, triclosan, methyltriclosan and triclocarban have high tendencies to partition to soil when equilibrium and steady state is assumed (Level II), as a result of their intermediate log K_{OW} (4.76-5.22), whereas 3,4-DCA, being more volatile and more water soluble tends to partition to air and water to a higher extent. In a non-equilibrium system (Level III), the emission mode plays a large role and when released to water, triclosan, methyltriclosan and triclocarban will be distributed between water and sediment whereas 3,4-DCA preferably partitions to the aquatic phase. Methyltriclosan is predicted to be the most persistent of the four in this emission scenario and environment (Figure 3), with an overall residence time of 107 days, followed by triclocarban, which also may degrade to 3,4-DCA. The major removal route for all substances when released to water is via advection, which is reflected in Figure 3 by the shorter residence time due to advection compared to if only reaction is considered.

2.1.1 Transformation under aerobic conditions

In laboratory experiments with a bench-top continuous activated-sludge system, triclosan showed to be mineralized. Adaptation of the bacterial flora was observed when the reactor was re-dosed with triclosan leading to a more efficient mineralization. In this experiment, using radioactive labelled triclosan, the removal of the parent compound triclosan was nearly quantitative (98.5%) but 15% of the total radioactivity remained indicating production of more stable metabolites. Between 1.5-4.5% of the triclosan in the influent was sorbed to the sludge in the reactor (Federle et al., 2002).

Similar results were reported in a field study (mass balance studies) in a wastewater treatment plant. In these investigations 79% was degraded, 15% adsorbed to sludge and 6% discharged to the surface water (McAvoy et al., 2002; Singer et al., 2002).

In spite of the high removal rate of triclosan in WWTPs significant amounts are discharged to the surface water leading to detectable concentrations of triclosan in the aquatic environment (Singer et al., 2002).

The non-ionized molecule is stable against photochemical reduction while the ionized molecule is not. In surface water with a pH of 7-8 a significant part of the compound is deprotonated and thereby subjected to photochemical reactions. Photolysis was showed to be responsible for about 80% of the triclosan loss in epilimnion in Lake Greifensee in Switzerland (Tixier et al., 2002). It therefore was concluded that during the summer season photo-transformation appears to be the most important degradation pathway of triclosan in surface water (Singer et al., 2002; Tixier et al., 2002).

In laboratory experiments with deprotonated triclosan (high pH) several photoproducts were detected including 4,5-dichloro-(1,1'-bifenyl)-2,2'-diol (10%), 2,4-dichlorophenol (7%), 2,8-dichlorodibenzodioxine (2,8-DCDD; 4%) and 5-chloro-2-(4-chlorophenoxy)phenol (0.5 %) (Kliegman et al., 2013). Analysis of two sediment cores from the depositional zone of waste water impacted sediments provide evidence for the production of 2,8-DCDD from triclosan in the aquatic environment. The level of 2,8-DCDD mirrored the use of triclosan since the introduction in the 1960s. Three other congeners, 2,3,7-TriCDD, 1,2,8-TriCDD and 1,2,3,8-TCDD were also detected and followed similar trend profiles as 2,8-DCDD. The latter tri- and tetrachlorodibenzodioxines are known as photoproducts of chlorinated triclosan, produced during chlorination of water (Buth et al., 2010).

Lindström (2002) and Balmer (2004), detected methyl-triclosan in WWTP-effluent. The authors concluded that the *O*-methylation of triclosan was mediated by bacteria. This hypothesis is supported by the work of Neilson et al. (1983); (1984). Under aerobic conditions (activated sludge) about 1 % of the triclosan was converted to methyl-triclosan (Chen et al., 2011). A retrospective monitoring using archived fish muscle tissue showed frequent occurrence of methyl-triclosan in samples collected in German rivers (2005-2007) receiving effluents from WWTPs. In general, the concentration of the metabolite methyl-triclosan was higher compared to the precursor triclosan (Rüdel et al., 2013).

Both triclosan and methyl-triclosan are lipophilic compounds (logKow >4, Table 2) and have high affinity to particulate matter leading to removal of the compounds from the water column to the sediments (Chen et al., 2011). This has been confirmed in a number of investigations detecting triclosan and methyl-triclosan in sludge (Singer et

al., 2002), suspended particulate matter (Rüdel et al., 2013) and sediment in the vicinity of WWTPs (Pintado-Herrera et al., 2014).

Algae may act as adsorbent for lipophilic compounds in receiving waters. In laboratory experiments it was showed that within one hour more than 50 % of the dissolved triclosan was associated with algae. Algae also proved to have metabolic capacity to de-chlorinate the compound to the di-chloro analogue (Wang et al., 2013).

2.1.2 Transformation under anaerobic conditions

In field studies of dated sediment cores collected in lakes influenced by WWTP effluents triclosan and triclocarban were found in sediment layer as old as over 30-40 years, suggesting that the two substances are persistent under anaerobic conditions (McAvoy et al., 2002; Miller et al., 2008; Singer et al., 2002). This was supported by (Chen et al., 2011), who reported 75 % removal of triclosan in an activated sludge reactor while no reduction was observed under anoxic or anaerobic conditions.

Under anoxic or anaerobic conditions triclocarban was sequentially de-chlorinated to the final product carbanilide. De-chlorination of triclosan was not detected (Miller et al., 2008; Pycke et al., 2014).

Methyl triclosan was significantly removed under the anaerobic digestion process (37-74%) in WWTPs (Pycke et al., 2014). Most likely this reversion process is mediated by anaerobic bacteria (Neilson et al., 1990; Remberger et al., 1986).

2.2 Silver

Silver (Ag), occurs in natural aquatic environments as a free ion (Ag^+) and forms strong complexes with e.g. Cl^- and HS^- but is mainly associated with particles. Dissolved concentrations of Ag are usually very low in natural waters. The average concentration of silver in the earth crust is around 0.07 mg/kg and background concentrations in water are ca 1 ng/L (Sternbeck and Östlund, 1999). As a consequence of its high affinity to particles, most silver entering municipal sewage wastewater will end up in sludge (Naturvårdsverket 1996). Silver has a high bioaccumulation potential and may be taken up by plants and living organisms.

3 Bioaccumulation and toxicity

A recent review on personal care products in the environment, including triclosan, Me-triclosan and triclocarban (Brausch and Rand, 2011) concluded that there are still large

data gaps concerning biomagnification potential and potential toxic effects for aquatic organisms, in particular related to benthic invertebrates, algae and vascular plants. The review also stated that although most of the substances assessed appear to have limited potential to cause acute or chronic effects on a substance per substance basis, a so-called "whole effluent testing (WET)" approach would be more realistic, considering that many of these substances often come together in wastewater effluents.

Triclosan is classified as "irritating to skin and eyes", "very toxic to aquatic organisms" and "may cause long term adverse effects in the environment" (R 36/38;R50/53). It is a suspected endocrine disruptor, but otherwise relatively harmless to human health, albeit the potency towards aquatic organisms is much higher, especially towards algaee, where a no-effect concentration (NOEC; 96-h) of 0,69 µg/l and an effect concentration (EC) of 1.4 µg/has been determined using *Scenedesmus* algae as test organism (Orvos et al., 2002), but a NOEC of 0.5 µg/L has also been observed (Naturvårdsverket, 2008). There is also a concern that triclosan may generate antibiotic cross-resistance (Bergman et al., 2013; Dann and Hontela, 2011). According to the PBT profiler (USEPA, 2012), triclosan exceeds the criteria for persistence in water, soil and sediment and may be a potential persistent and toxic substance. The criterion for bioaccumulation is, however, not exceeded according to the PBT profiler. A PNEC water derived from the most sensitive species for triclosan with a safety factor of 10 is 0.05 µg/l (Reiss et al., 2002). Triclosan belongs to a group of substances that have been classified as "Specific Pollutants" within the UK River Basin Management and a long-term standard of 0.1 µg/L and a short-term standard of 0.28 µg/L has been derived (WFD-UKTAG, 2013). Applying a safety factor of 10 (based on data from three trophical levels) the Swedish Chemical's Agency proposed limit values for triclosan of 0.05 µg/l for inland waters. For "other surface waters" an extra safety factor of 10 was applied due to absence of data for marine species and a limit value of 0.005 µg/l was proposed (Naturvårdsverket, 2008). The corresponding limit value for sediment (based on equilibrium partitioning is 0.2 µg/g dw.

There is not much information available on the toxicity of **methyl triclosan,** a common degradation product of triclosan, but software estimates predict a double bioaccumulation potential compared to triclosan (USEPA, 2011).

Triclocarban is classified as R50/53: "very toxic to aquatic organisms" and may cause long term adverse effects in the environment". The PBT profiler (USEPA, 2012), defined triclocarban as a potentially persistent and toxic substance and it was predicted to exceed the criteria for persistence in water, soil and sediment. The criterion for bioaccumulation is, however, not exceeded according to the PBT profiler.The bioconcentration and metabolism of triclocarban in plants have been investigated by Wu et al. (2010) who reported that triclocarban was taken up by soybean from biosolid

amended soil and was transported up in the aerial part of the plant and was even detected in the beans. Macherius et al. (2012) reported similar results using carrots as test organism: no metabolism (phase 1 or 2) but triclocarban was taken up into the cells.

3,4-Dichloroaniline is a potential degradation product of triclocarban, but also of other pesticides such as diuron (Giacomazzi and Cochet, 2004). A review on 3,4-DCA conducted by Crossland (1990) showed effects on aquatic species such as depression of growth at levels of about 200 µg/L. The acute-chronic ratio for different species varied between 20 and 1000, where the latter was pointed out as unusually high. A PNEC of 1.0 µg/L was determined by Girling et al. (2000). 3,4-DCA belongs to a group of substances that have been classified as "Specific Pollutants" within the UK River Basin Management and a long-term standard of 0.2 µg/L and a short-term standard of 5.4 µg/L has been derived (WFD-UKTAG, 2013). The PBT profiler (USEPA, 2012), defined 3,4-DCA as a potentially persistent and toxic substance and it was predicted to exceed the criteria for persistence in soil and sediment. The criterion for bioaccumulation is, however, not exceeded according to the PBT profiler.

Silver has no known biological function in living organisms and its toxic properties depend on the form of occurrence. The free ion Ag^+ has been shown to be substantially more toxic to fish than different forms of complexes, thus the toxicity of Ag to fish is higher in freshwaters than in marine waters (Sternbeck and Östlund, 1999). The effects of silver on human health are limited. High occupational environmental exposure may lead to symptoms such as included breathing complaints, irritated throat and lungs, and stomach pain (Butterman and Hilliard, 2005), but the main concern for humans is the development of antimicrobial resistance caused by excessive use of silver.

4 Production and use

Antibacterial substances are currently used in many different types of products to resist bacterial growth and bad smell. Examples of application areas are hygienic care products, clothes and cutting boards. Detailed information about the production and use of different substances is more limited.

Triclosan has been used as a biocide in several consumer products for many years. It has been used in e.g. toothpaste to prevent bacterial growth but also in sportswear and various wooden surfaces e.g. cutting boards. Due to recent concerns for environmental effects of TCS many producers have ceased using the compound. Triclosan is an LPV chemical in the European Union with production registered in Switzerland and Italy. (ESIS, 2006). The EU use in 2006 was estimated to 450 tonnes (EC, 2008). The

consumption of triclosan in chemical products in Sweden has been around 2 tonnes per year but seems to have ceased in recent years (Table 3).

Methyltriclosan appears as a degradation product of triclosan and has been found in waste water effluents and environmental samples but is not used in itself. It is produced by aerobic bacteria and is expected to be removed from the water phase by adsorption to sludge due to its high affinity to particles (log Kow of 5.22).

Since 2006**, triclocarban** is not allowed for use as a biocide within the EU, but it may be used as a preservative in cosmetics and hygienic products (maximum concentration of 0.2 %). Triclocarban may enter the Swedish market via import of treated fabrics, whereby it may be released upon washing (KEMI, 2012). It can be degraded to the even more persistent and bioaccumulative metabolite 3,4-DCA. In 2006, the total use of triclocarban in the EU was reported to be 30 tonnes/year.

3,4-Dichloroaniline is exclusively used as an intermediate in chemical industry in the synthesis of e.g. triclocarban, but may also form as a result of degradation of the same as well as other substances, e.g. diuron (Giacomazzi and Cochet, 2004). There is thus no direct use of 3,4-DCA. According to the EU risk assessment report, there were two production sites in the EU in the mid 00's, one in Germany and one in France. Together, they were reported to produce 13500-15500 tonnes/year of which 3700-4600 tonnes were exported. No use was reported in Sweden.

Silver was mined in mid-Sweden (Sala silver mine in Västmanland) until the beginning of the 20th century, but is now extracted from other metal ore (copper, lead) mines in the northern parts of Sweden. In 2013, the Swedish production of silver was 306 metric tonnes, representing 1.3 % of the global production (www.silverinstitute.org). The registered use as a chemical product in the Swedish Products Register is significantly smaller, only 1 tonne in 2012, and the reported import and export as raw material and as chemical product is small in comparison, less than 5 tonnes in 2003 (KEMI, 2013). The discrepancy is mainly due to the fact that enterprises are not required to report the use of pure silver to the Products Register. Silver is mainly used in jewellery and ornaments and as a catalyst in chemical industry. In recent years, silver has found a new market in consumer products like washing machines, refrigerators, socks and shoes, and body lotions, mainly as an antiseptic and anti-odour agent. Table 3 shows the estimated annual silver production in Sweden, assuming that the share of the global production has been constant since 2000. The Swedish use of silver in different forms is also shown in the Table.

Table 3. Estimated production and use of antibacterial substances in Sweden (metric tonnes)

Year	Estimated silver production*	Consumption			
		Silver	Silver nitrate	Silver cyanide	Triclosan
2000	230	15.2	1.5	4.2	4.9
2001	243	3.7	1.4	3.2	4.1
2002	260	1.2	0.9	3	3.3
2003	244	6.8	1.5	3.2	2.9
2004	256	1	1.6	5.2	3.1
2005	251	0.8	1.9	3	2.7
2006	263	0.8	1	4.5	2.2
2007	270	15.1	0.8	4.5	2.8
2008	277	8.3	1	7	2.2
2009	278	7.5	0.7	2.9	2.2
2010	300	1.2	0.6	2.5	0
2011	309	1.2	0.7	2.6	0
2012	331	1.1	0.3	2.3	
2013	306				
Reference	(USGS, 2014)	(KEMI, 2013)	(KEMI, 2013)	(KEMI, 2013)	(KEMI, 2013)

*Estimated as 1.3% of the global production

5 Previous measurements in the environment

5.1 Sweden

The environmental occurrence of the substances included in the current study has been studied to varying extent in previous studies. For example, prior to the recent screening study, conducted in 2012-2013 (Törneman et al., 2014) triclocarban had not been measured in the outdoor Nordic environment (Frankki, 2012). Two previous reviews have summarized Swedish data on triclosan (Frankki, 2012; Sternbeck et al., 2007). Extensive monitoring activities have been carried out within the national screening programme, funded by the Swedish Environmental Protection Agency, the results of which are stored in a joint national database hosted by IVL[3]. Table 4 summarizes the data found in this national database (10.06.14). The variability in reported levels can to some extent be explained by the fact that samples were collected in different years and that data are generated in different projects. In addition, Table 4 does not reveal whether samples represent background sites or locally polluted sites. Most often, screening projects performed on contract by the EPA include samples from background sites as well as suspected source regions. Thus the data presented below can be viewed as an overall picture of typical levels found in the Swedish environment. As evident from the Table, data on triclocarban and methyltriclosan are scarce. No data on 3,4-DCA have been reported to the national database.

[3] The national screening database can be found at http://www.ivl.se – Tjänster – Datavärdskap – Miljögifter i biologiskt material och screening – Databas: Screening, Access date: 23.06.14

Table 4. Average concentrations from previous measurements in the Swedish environment (Source National Screening database, www.ivl.se).

	BIOTA		SEDIMENT	SLUDGE*	WWTP INFLUENT	WWTP EFFLUENT	SURFACE WATER
Substance	µg/kg ww	µg/kg lw	ng/g dw	ng/g dw	ng/L	ng/L	ng/L
Triclosan	3.2 (0.3-8.4) n=37	5.2 (1.1-12) n=21	12 (1-56), n=66	3400 (nd-43000) n=135	275 (0.91-1100) n=24	62 (0.2-820) n=97	129 (0.01-2000) n=122
Methyl triclosan							n.d n=15
Triclo-carban							n.d n=77
Silver	0.63 (0.3-1) n=24		4700 (42-44000) n=101	9800 (120-46000) n=89	66 (9-280) n=47	36 (2-1400) n=104	17 (1-63) n=18

*Sludge samples include a mixture of primary, secondary and digested sludge. No significant concentration differences were observed for the different sludge types.

Triclosan and silver are included in the national monitoring programme for sludge. Figure 4 and Figure 5 show the measured concentrations of triclosan and silver in WWTP sludge over time. The samples include a mixture of primary, secondary and digested sludge. No significant concentration differences were observed between the different sludge types, reflecting the limited anaerobic degradation of triclosan.

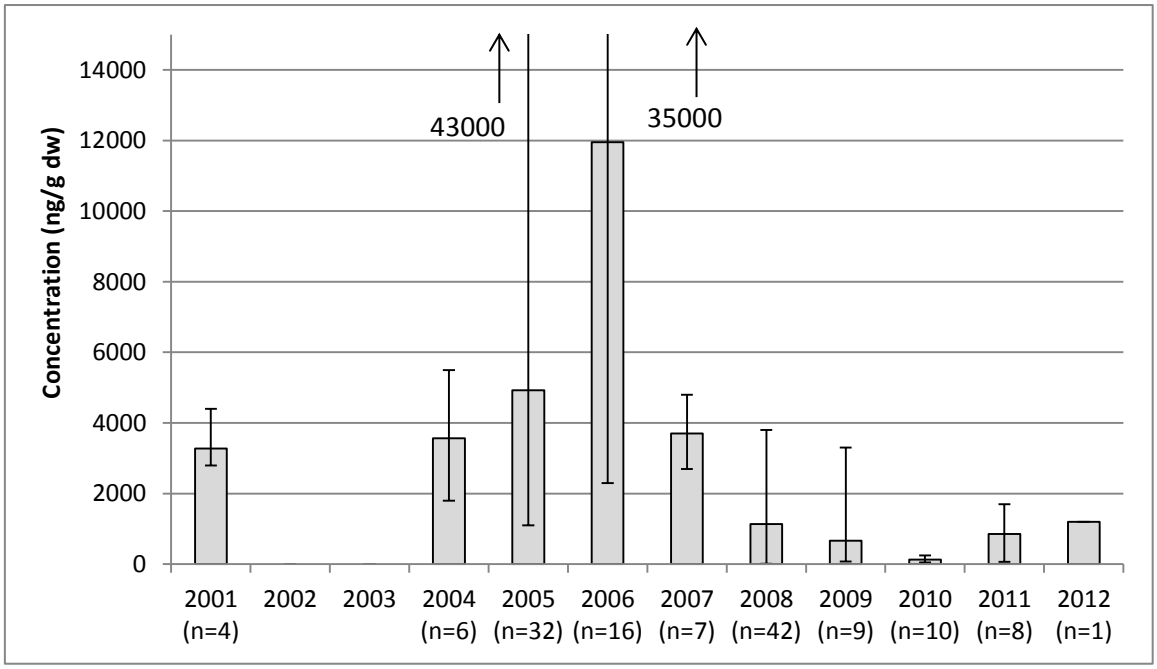


Figure 4. Average concentrations of triclosan in WWTP sludge as reported in the national screening database (See footnote 3). The error bars represent the min and max concentrations reported.

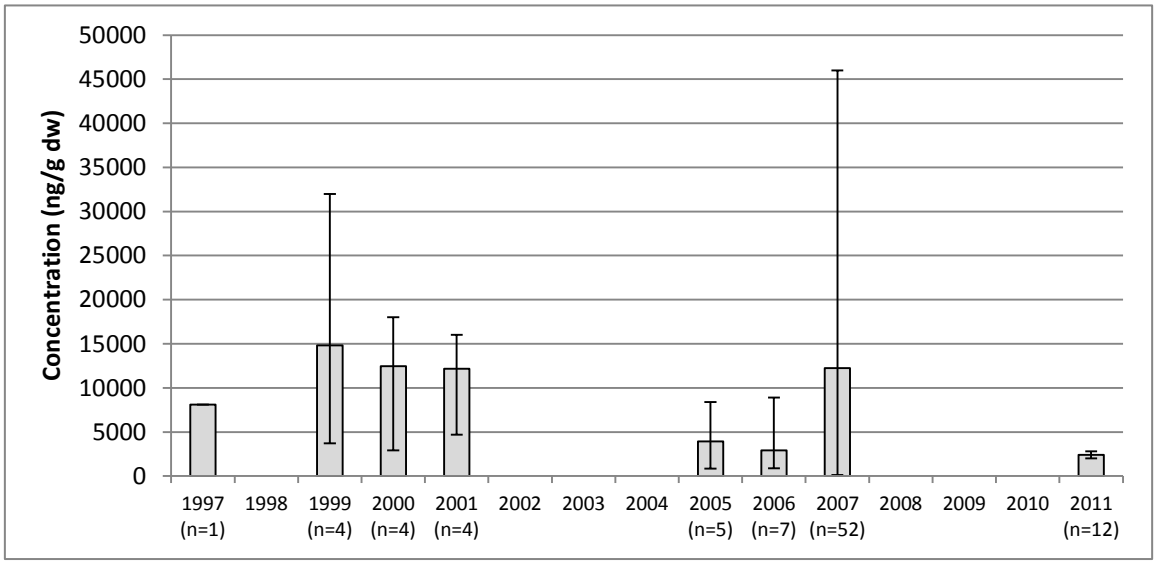


Figure 5. Average concentrations of silver in WWTP sludge as reported in the national screening database (See footnote 3). The error bars represent the min and max concentrations reported.

5.2 Other countries

Numerous studies have reported concentrations of triclosan in European waters, recently compiled by Bedoux et al. (2012) and Brausch and Rand (2011). Some studies report concentrations of methyltriclosan, but the few studies available on triclocarban have mainly been conducted in North America (see Table 5). Despite the frequent occurrence of toxicity studies in the scientific literature, environmental data on 3,4-DCA and 4-chloroaniline are very scarce, and we have only found one study, from Germany in 1988 .

Table 5. Environmental concentrations of methyltriclosan, triclocarban and 3,4-DCA measured outside of Sweden.

Substance	Site	Water ng/l	Sediment ng/g dw	Sludge ng/g dw	Biota (fish) ng/g lw	Reference
Triclosan	Different European sites (data publ. 2002-2009)	Influent:52-21900 Effluent:10-2210 Surface:<LOQ-285	<0.4-131			(Bedoux et al., 2012)
	"All published studies"	Surface: <0.1-2300 (median 48) n=710				(Brausch and Rand, 2011)
	Germany, 20 WWTPs			1000 -8000		(Bester 2003)
Methyl-triclosan	Guadalete river, Spain	27-191, n=11	n.d.-1.8, n=13			Pintado-Herrera et al. (2014)
	German rivers (2005-2007)				68-1010 N =12 (pooled samples)	Rüdel et al. (2013)
	Northwest Spain, rivers and estuaries and WWTPs from several cities		n.d, n=4	83 (15-191), n=11		González-Mariño et al. (2010)
	"All published studies"	Surface: 0.5-74, n=4				(Brausch and Rand, 2011)
Triclocarban	"Typical US WWTP"	Influent: 6100, n=6 Effluent: 170, n=22		51000±1500, n=3		Heidler et al. (2006)
	Activated sludge and treated biosolids, Ontario, Canada			4200 (2200-6000), n=6		Chu and Metcalfe (2007)
	"All published studies"	Surface: 19-1425 (median: 95) n=29				(Brausch and Rand, 2011)
3,4-DCA	German waste water	1800-3300 (3,4-DCA) 1100-67000 (4-CA)				Scholz and Palauschek (1988)

6 Sampling programme

The sampling programme was designed with a strong focus on diffuse emissions from urban areas reflected in samples from waste water treatment plants (WWTPs) and their receiving waters. Samples collected include influent, effluent and sludge from five WWTPs. Sediment and fish were collected near the effluent point at three of the WWTPs. From one of them sediment and fish were also sampled upstream the WWTP and at one additional site downstream. Sediment and fish from one background lake (included among the reference lakes sampled by the Swedish Museum of Natural History) was also included. In total, 30 samples were collected. An overview of the sampling program is presented in Table 6. Table 7 lists the technical specifications of the WWTPs included in the sampling programme. All individual samples are listed in Appendix 1.

Table 6. Sampling programme, number of samples

Type	Municipality	Site	Infl	Effl	Sludge	Sed	Fish
Background	Vallentuna	Tärnan				1	1
Diffuse	Stockholm	Henriksdal WWTP	1	1	1	1	1
	Göteborg	Ryaverken WWTP	1	1	1	1	1
	Borås	Gässlösa WWTP	2	2	2	3	3
	Umeå	Ön WWTP	1	1	1		
	Bollebygd	Bollebygd WWTP	1	1	1		

Table 7. Technical specification of the sampled WWTPs. Data from 2011.

	Henriksdal, Stockholm	Rya, Göteborg	Gässlösa, Borås	Ön, Umeå	Bolle-bygd
Influent water (1000 m^3/y)	90 900	141 000	13 800	12 500	260
p.e	680 000	730 000	76 500	131 000	4 100
Share of industrial water	~0 %	29 %	?	22 %	~0 %
COD removal	93 %	85 %	93 %	92 %	?
Nitrogen removal process	Yes	Yes	Yes	No	No
Nitrogen removal	80 %	63 %	61 %	21 %	?
Anaerobic sludge treatment	Yes	Yes	Yes	Yes	No

7 Methods

7.1 Sampling

Samples of sediment and fish from Tärnan were provided by the Swedish Museum of Natural History.

Influent and effluent 24 h composite samples and de-watered sludge from the anaerobic chambers were collected by local staff at the individual WWTPs. The samples were collected in heat treated (400 ºC) glass bottles (water) and PE jars (sludge). The sludge from Bollebygd WWTP was treated in a reed bed from which a combined sample was taken. The samples were stored frozen (-18°C) until analysis. Surface sediments (0-2 cm) were collected by means of a Kajak sampler. The sediment was transferred into heat treated (400 ºC) glass jars. Fish were caught using fishing nets and stored frozen until dissection and analysis.

7.2 Sample preparation and analysis

7.2.1 Triclosan and methyl triclosan

Water: Acidified water was spiked with internal standard ($^{13}C_{12}$-triclosan, $^{13}C_{12}$-methyl triclosan) and concentrated on a polymeric SPE column. The column was eluted successively with MeOH and MTBE. Water was added and the sample was extracted with hexane:MTBE (9+1). Finally the extract was acetylated and subjected to GC-MS-MS (MRM mode) determination.

Sludge and sediment: The sample was acidified and extracted with acetone followed by aceton:hexane. The extract was concentrated and the acetone washed away with acidified water (0.05 M HCl). Co-extracted ”matrix compounds” was separated from the extract by the aid of SPE-columns. The extract was acetylated and subjected to GC-MS-MS (MRM mode) determination.

Fish: Fish liver was homogenised and extracted twice with acetonitrile. The solvent was diluted with water and the analytes extracted with hexane:MTBE (3+1). Co-extracted ”matrix compounds” was separated from the extract by the aid of SiO_2-columns. The extract was acetylated and subjected to GC-MS-MS (MRM mode) determination.

7.2.2 Triclocarban

Water: Acidified water was spiked with internal standard ($^{13}C_6$-triclocarban) and concentrated on a polymeric SPE column. The column was eluted successively with MeOH and MTBE. Water was added and the sample was extracted with hexane:MTBE (9+1). The organic solvent was removed and changed to MeOH prior to analysis with HPLC-MS-MS (MRM mode).

Sludge and sediment: The sample was extracted with acetone followed by aceton:hexane. The extract was concentrated and the acetone washed away with water. Co-extracted ”matrix compounds” were separated from the extract by the aid of SiO_2-columns. The extract was subjected to HPLC-MS-MS (MRM mode) determination.

Fish: Fish liver was homogenised and extracted twice with acetonitrile. The solvent was diluted with water and the analytes extracted with hexane:MTBE (3+1). Co-extracted ”matrix compounds” was separated from the extract by the aid of SiO_2-columns. The extract was subjected to HPLC-MS-MS (MRM mode) determination.

7.2.3 4-Chloroaniline and 3,4-dichloroaniline

Published works dealing with determination of anilines in solid matrices are scarce. Initially, one quite new published method (Akyüz and Ata, 2006), was used in the analytical work. However, we experienced serious difficulties. For example we obtained low extraction and derivatisation recovery. Furthermore, the back-extraction protocol used, crucial for the clean-up of sludge and sediment extracts, could in our hands not be reproduced. Thus, we decided to combine the method outlined by of Schultz et al., (1988) and Kasprzyk-Horden et al., (2008), Lavén et al., (2009) and our acquired experiences obtained in this project.

Unfortunately, the achieved LOQ with the adopted method was too high to meet the needed sensitivity for the samples in the present screening. Thus, further method development is needed to enhance the sensitivity.

***Water**:* Water was concentrated on an SPE column. The column was rinsed with water (pH 9) before eluting the analytes with acetonitrile. The eluate was concentrated under nitrogen gas, diluted with water (pH 9) and extracted twice with MTBE. The combined MTBE extract was dried over sodium sulfate and evaporated after addition of a keeper (bis(2-ethylhexyl)phosphate,BEHPA). The dried extract was dissolved in a “reaction solvent” containing acetonitrile, pyridine and *iso*-butyl alcohol and reacted with *iso*-butyl chloroformate (IBCF) before GC-MS-MS determination.

Sludge and sediment: Freeze dried sludge or sediment was, after addition of KOH, extracted twice with DCM:methanol. The pooled extract was concentrated with the aid of nitrogen gas, diluted with water and extracted twice with MTBE. The MTBE was exchanged to hexane and subjected to an HCl back-extraction protocol. The final MTBE extract was dried over sodium sulfate and evaporated after addition of a keeper (BEHPA). The extract residue was derivatised with IBCF prior to GC-MS-MS determination.

Fish: Fish liver was mixed well with sodium sulfate and extracted twice with acetonitrile. The solvent was exchanged to hexane. The extract was chromatographed on a silica gel column. The analytes were eluted with hexane:MTBE. The solvent was evaporated after addition of a keeper (BEHPA). The extract residue was derivatised with IBCF prior to GC-MS-MS determination.

7.2.4 Silver

Water: HNO_3 was added and the sample digested in a microwave oven.

Sludge and sediment: The sample was dried and digested using aqua regia.

Fish*:* Freeze dried fish muscle was digested in a microwave oven in closed teflon vessels using HNO_3 / H_2O_2.

Instrumental analysis was done using ICP-SFMS (Inductively Coupled Plasma Sector Field Mass Spectrometry). Sample preparation and analysis was carried out by ALS Scandinavia AB, Luleå.

7.3 Quality control

One important question is whether a sample is representative for a given time period or a given region. In this screening only few samples were collected of each matrix, the samples were collected within a narrow time frame and at different geographical locations. The results obtained here therefore only represent a snapshot of the real situation.

In order to avoid contamination of the samples during extraction and clean-up plastic materials were avoided for triclosan, methyl-triclosan and triclocarban. Glass equipment and silica gel were heated (400°C) prior to use. Using these precautions, we experienced no problems concerning background levels in the field- and analytical blank samples.

When determining anilines, exclusively plastic materials were used to avoid adsorption of the analytes to silanol groups in glass equipment.

The following quality criteria were used to ensure correct identification and quantification of the target compound: (a) the retention times should match those of the standard compounds within ± 0.05 min, (b) the intensity ratios of the selected ions should be within ± 15% of the expected / theoretical value and (c) the signal-noise ratios should be greater than 3:1.

^{13}C-labelled internal standard (recovery standard added to the samples) approach was used for quantification of triclosan, methyl triclosan and triclocarban. For the chloro-anilines d_5-anilin and 4-*tert*-butylanilin were used.

All reported values were recovery-corrected according to the recovery standard. Method blanks were included for each sample batch and analysed to assess background interferences and possible contamination of the samples.

The uncertainty of the chemical analysis is governed by losses during extraction and clean-up, interference from other compounds, trueness of analytical standards, instrumental parameters, and contamination. The uncertainty is expected to be larger for compounds which are analysed the first time, in this case chloroanilines, than for compounds which previously have been analysed or where similar compounds have been analysed earlier. For the determined chloroanilines the analytical uncertainty is estimated to be in the range 20-40%.

The limit of quantification (LOQ) was defined as 10 times the blank samples noise but in consideration of the actual sample noise in the target analytes "retention window" in the chromatograms. Possible background levels of analytes were subtracted from measured sample values. Limits of quantification (LOQ) for the analytes in different sample types are summarized in Table 8.

Table 8. LOQ for the analytes in different sample types

Type	unit	Triclo-san	Methyl triclosan	Triclo-carban	3,4-Dichloro aniline	4-Chloro aniline	Ag
Influent, effluent	ng/L	5-10	5-10	1	100	100	500
Sludge	ng/g dw	10	10	1	20	20	50
Sediment	ng/g dw	1	1	0.3	10	20	50
Fish, liver	ng/g ww	1	1	0.5	100	100	4

8 Results and discussion

The results from the measurements are presented in detail in Appendix 2.

An overview of the concentrations found in the current study is shown in Table 9. Triclosan consistently occurred in highest concentrations of the detected organic substances, with the exception of sediment, where triclocarban concentrations were in the same order of magnitude. Methyl-triclosan was mainly present in sludge, and the levels were about a factor of 200 lower than triclosan concentrations. Triclocarban was commonly found in WWTP influents, sludge and sediments but was not found in biota and only in one effluent sample. 3,4-DCA and 4-chloroaniline was not detected in any sample, but quantification limits were higher than for their mother compound triclocarban. Silver was found in sediment and sludge, but not in biota, effluent water and only in one sample of WWTP influent water.

The triclosan levels found in effluent water were well below established risk concentrations, but the limit value for sediment was exceeded on one occasion. The LOQ for 3,4-DCA was below the limit value proposed by the UK. In the following, the results are presented in more detail.

Table 9. Median and range of measured concentrations of substances included in the screening.

	BIOTA (ng/g ww)	SEDIMENT (ng/g dw)	SLUDGE (ng/g dw)	WWTP INFLUENT (ng/l)	WWTP EFFLUENT (ng/l)
Triclosan	4.1 (3-52)	7 (<1-290)	1550 (890-1800)	87 (81-130)	38 (27-52)
Me-triclosan	<1 (<1-3)	<1 (<1 – 2.3)	7.6 (3.5-23)	<10 (<10 – 19)	<5
Triclocarban	<0.5	7.7 (<0.3-180)	205 (59-770)	20 (7.5-53)	<1 (<1 –1.8)
3,4-DCA	<100	<10	<20	<100	<100
4-Chloroaniline	<100	<20	<20	<100	<100
Silver	<4	4900 (260-73000)	1850 (860-3100)	<500 (<500-660)	<500

8.1 Triclosan, methyl triclosan, triclocarban

8.1.1 WWTPs

8.1.1.1 Influent and effluent water

Triclosan was detected in all WWTP influents and effluents (six pairs, Figure 6). The concentration range was 81-130 ng/l in influents and 27-60 ng/l in effluents. The

calculated median reduction was 55% (range 42-78 %), which is somewhat lower the reported elsewhere (see 2.1.1).

Methyl triclosan was detected in one out of six influents (19 ng/l at Henriksdal) but was not found in any of the effluent samples (Figure 6).

Triclocarban was detected in all influents but only in one effluent sample at a low concentration of 1.8 ng/l (Figure 6).

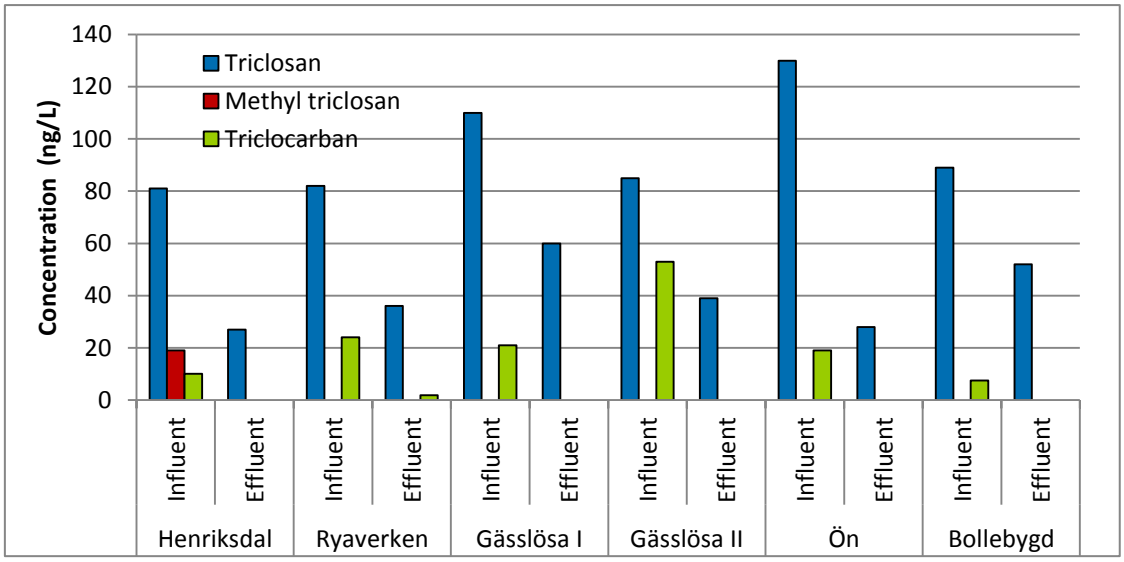


Figure 6. Measured concentrations of triclosan, methyl triclosan and triclocarban in influents and effluents from WWTPs.

A comparison between the triclosan concentrations found in the current study, and concentrations reported to the Swedish national screening database is shown in Figure 7, indicating that the current measurements are in line with, or slightly lower than previous measurements in Sweden in the time period from 2005 to 2012. Levels are in the lower end of reported concentrations from European countries as summarized in a recent review, which have been reported to be in the range 10-2210 ng/L (Table 5).

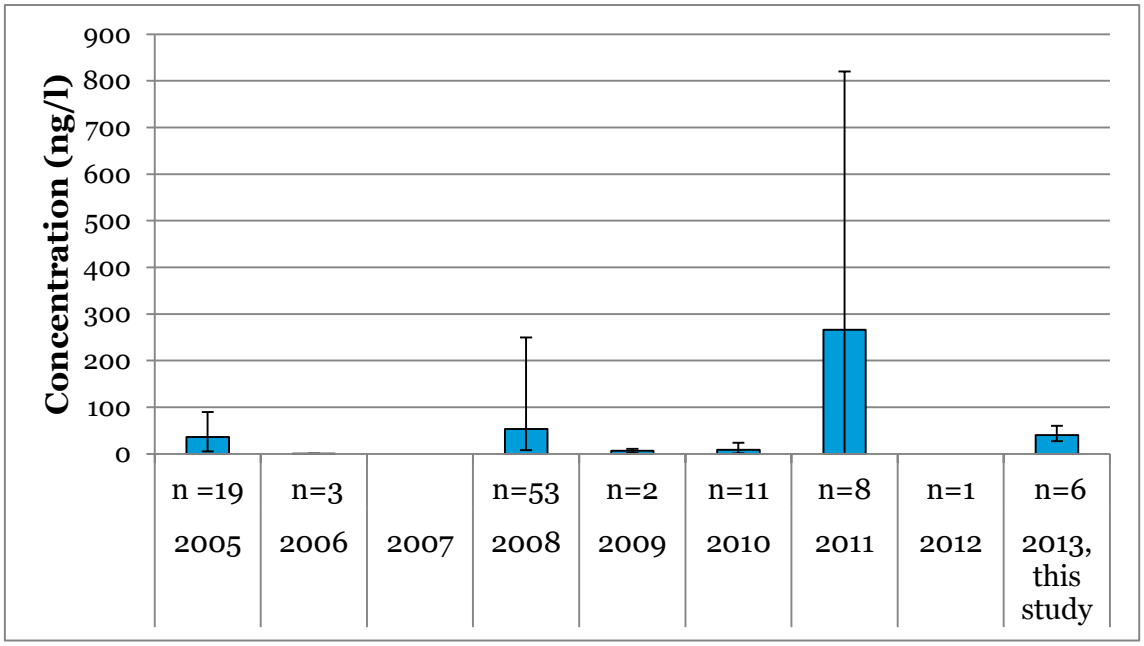


Figure 7. Comparison between previously reported triclosan concentrations in WWTP effluent water and concentrations observed in this study. The bars represent average values and the error bars represent the min and max concentrations reported. The data for comparison has been derived in different studies conducted within the Swedish national screening programme and were extracted from the Swedish national screening database (see section 5.1). Data below the LOQ were treated as LOQ/2.

The concentration of methyl-triclosan in incoming water to Henriksdal is slightly higher than the levels reported by Lindström et al. (2002) for Swiss WWTPs in the early 00's (influent: <1-4 ng/l, effluent <2-11 ng/l). It was lower than concentrations found in surface water in southern Europe (i.e. the Spanish river Guadalete; (Pintado-Herrera et al., 2014), see Table 5).

Triclocarban is efficiently removed from effluent water in the WWTPs (Figure 6) which has been observed also in North American WWTPs, where removal efficiencies have been reported to be 88-97 % (LANXESS, 2010). This is likely a consequence of sorption to sludge but degradation may also be important under aerobic conditions (Halden and Paull, 2005). Incoming triclocarban concentrations to Swedish WWTPs were at the lower end compared to concentrations measured in river surface water in Baltimore and Texas in the US (see Table 5), and much lower than observed concentrations in influent and effluent samples from a typical US WWTP, where incoming concentrations were 6100 ng/l and effluent concentrations 170 ng/l (Heidler et al., 2006).

8.1.1.2 Sludge

Triclosan and triclocarban were detected in all six sludge samples (Figure 8). This is probably a consequence of their high log K_{OW} (Table 1). The concentration range for triclosan was 890-1800 ng/g dw and for triclocarban 59-770 ng/g dw. The concentrations of the two compounds appear to correlate, the triclosan levels being on average 8 times higher than the triclocarban levels. This type of correlation has been reported previously and was explained by similar properties, usage, disposal and environmental life-time of the compounds (Halden and Paull, 2005). Methyl-triclosan was detected in all six sludge samples, albeit at much lower concentrations (3.5-23 ng/g dw). The appearance in sludge is expected according to its high log K_{OW} (5.22; Table 1). The adsorption of the compound probably explains the low detection rate in effluent waters (see above). No clear relationship between triclosan and methyl triclosan concentrations was observed.

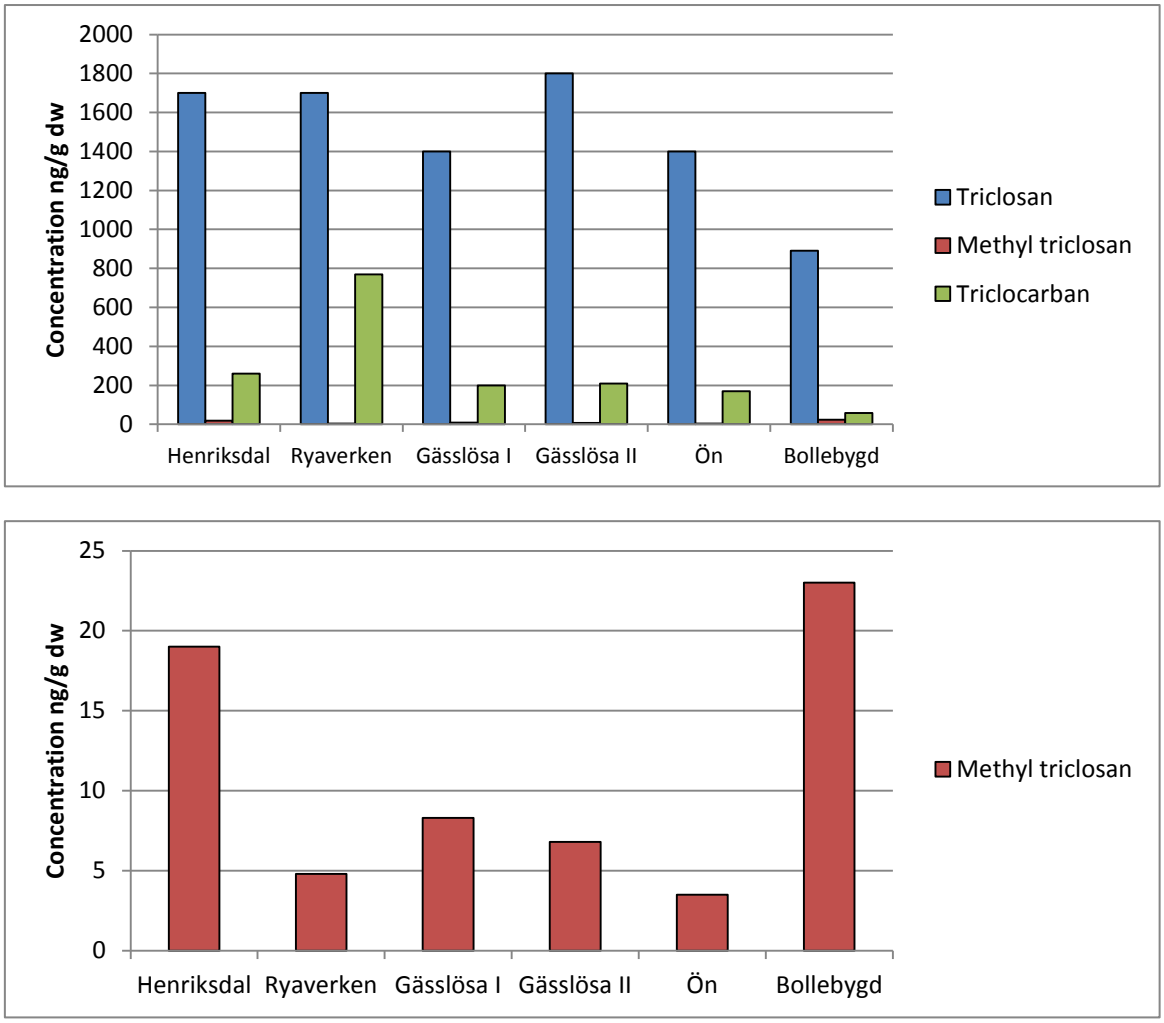


Figure 8. Measured concentrations of triclosan, methyl triclosan and triclocarban in sludge from WWTPs. The bottom figure shows methyl triclosan levels using a different scale on the y-axis.

A comparison between the triclosan concentrations found in the current study to previously detected levels in Swedish WWTP sludge is shown in Figure 9. The current levels are in line with measured concentrations in sludge the last few years. The levels are a factor of 20-370 lower than levels reported from other European sludge samples as compiled in a recent review (Table 5).

It appears as if the decreasing trend from 2006 has levelled off. The concentrations observed in the current study (2013) are slightly higher than the concentrations in 2008-2012. A more detailed trend assessment can be conducted by studying the concentration over time at an individual WWTP, as shown in Figure 10 for Henriksdal WWTP.

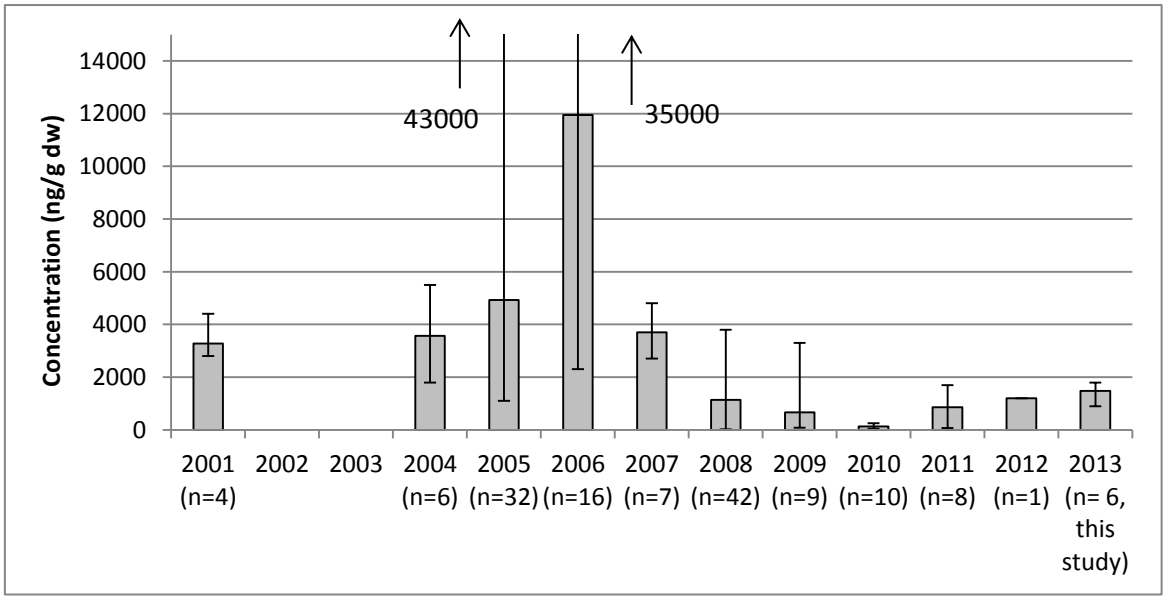


Figure 9. Comparison of previously reported triclosan concentrations in sludge and concentrations observed in this study. The bars represent average values and the error bars represent the min and max concentrations reported. The data for comparison has been derived in different studies conducted within the Swedish national screening programme and were extracted from the Swedish national screening database (see section 5.1).

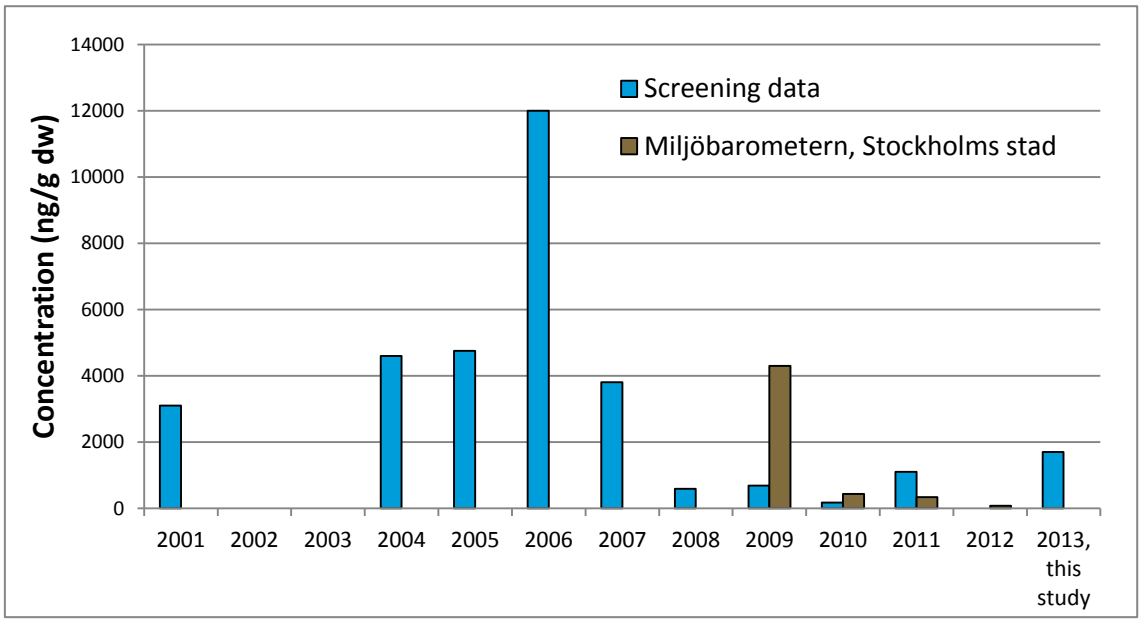


Figure 10. Temporal trend of triclosan concentrations in sludge from Henriksdal WWTP, based on data generated within the Swedish national screening programme (extracted from the Swedish national screening database (see section 5.1)), data from the City of Stockholm (Miljöförvaltningen, 2014) and the current study.

According to Figure 10 it appears as if the triclosan concentrations may even be increasing, if considering only the national screening data (n=1 per year), whereas the data obtained from the environmental monitoring of the city of Stockholm show the opposite trend.

The methyl-triclosan concentrations (3.5-23 ng/g dw) are lower than or in the lower end of measured concentrations in WWTP sludge from north western Spanish cities, where methyl-triclosan was found at an average concentration of 83 (15-191) ng/g dw (González-Mariño et al., 2010). The triclosan concentrations in the Spanish samples were similar to those detected in the current study (1400 (270-2640) ng/g dw compared to 1480 (890-1800) ng/g dw in this study). McAvoy et al. (2002) reported a concentration range of 130-1030 ng/g dw for methyltriclosan (secondary and digested sludge in the US), which is much higher than the concentrations found in the current study, reflecting the more extensive use of triclosan in North America.

Triclocarban levels (59-770 ng/g dw) were substantially lower than measured levels in Ontario Canada (2200-6000 ng/g dw, (Chu and Metcalfe, 2007)) and US sludge (51000 ±1500 ng/g dw, (Heidler et al., 2006)), reflecting the lower use of the substance in Sweden compared to North America.

8.1.2 Sediment

Triclosan and triclocarban were detected in all sediment samples except for the background sample from Lake Tärnan (Figure 11). The concentration range for triclosan was 2.1-290 ng/g dw and for triclocarban 1-180 ng/g dw. As in the case of sludge, the concentrations of triclosan and triclocarban appear to correlate in sediments.

The sediment sampled in the vicinity of the discharge point of Henriksdal WWTP contained the highest concentrations of triclosan (290 ng/g dw) and triclocarban (180 ng/g dw). This was also the only site where methyl-triclosan was detected (2.3 ng/g dw), see (Figure 11). Considering its absence in WWTP effluents it is not surprising that methyl-triclosan was not found in the majority of sediments, even though it would be expected to be found there according to its physical-chemical properties (Figure 1).

The sediment samples collected in a gradient from Gässlösa WWTP, showed low concentrations (just above the LOQ) of triclosan and triclocarban in the upstream sample and in the sample collected at the discharge point (downstream I). In the sample from about 5 km downstream the discharge point (downstream II) the concentrations of triclosan and triclocarban were 61 and 130 ng/g dw respectively. This is somewhat surprising as one might expect the highest concentrations near the

discharge point. A possible explanation is that the sampling point at the outlet has the characteristics of an erosion area whereas the second sampling point 5 km further downstream (downstream II) is a typical sedimentation area.

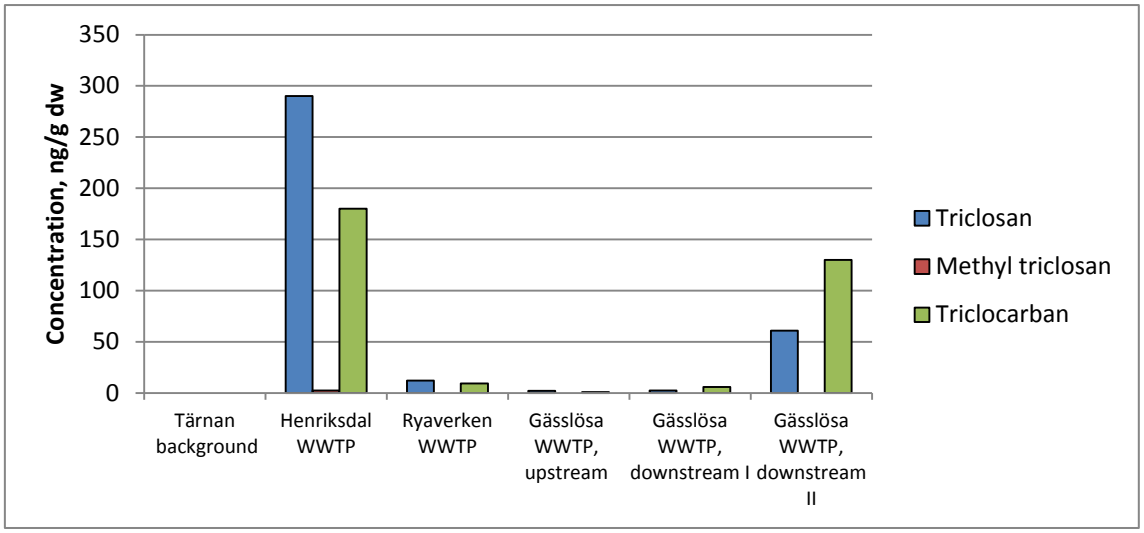


Figure 11. Measured concentrations of triclosan, methyl triclosan and triclocarban in sediments.

Previously reported sediment concentrations of triclosan, extracted from the national screening database[4] are in the range 1-56 ng/g dw (average 12 ng/g dw) and at other European sites <0.4-131 ng/g dw (see Table 4 and Table 5), which is in the same order of magnitude as the concentrations in this study, except for the sediment sample outside Henriksdal, where concentrations were substantially higher (about a factor of 24 compared to previous Swedish measurements). Despite the fact that influent and effluent concentrations are similar at Henriksdal as in the other WWTPs, the sediment concentrations outside the plant are significantly higher. This may be an effect of accumulation zones, where hydrodynamic conditions are favourable for sedimentation. Elevated concentrations of PAHs have been found in the same sediment sample (Hansson et al., 2014).

8.1.3 Biota

Triclosan was neither detected in liver from fish collected in the background Lake Tärnan nor in fish collected upstream Gässlösa WWTP. It was, however, found in liver from fish caught in the vicinity of WWTP effluent points (Figure 12).

[4] The national screening database can be found at http://www.ivl.se – Tjänster – Datavärdskap – Miljögifter i biologiskt material och screening – Databas: Screening, Access date: 23.06.14

At Gässlösa, an increasing concentration of triclosan in fish liver was observed along the gradient from upstream the WWTP plant to 5 km downstream (Figure 12). Methyl triclosan was not detected in these samples.

Fish liver collected outside the two large WWTPs Henriksdal and Ryaverken showed lower concentrations (3 and 4 ng/g ww), probably an effect of the high dilution of sewage effluents that occurs outside these plants. Methyl triclosan was also detected in these two samples at concentrations just above LOQ (2.7 and 2.8 ng/g ww) i.e. in the same concentration level as the mother compound.

One difference between present data and literature data is that we determined the analytes in fish liver whereas literature data refer to fish muscle. Quick metabolism of the methyl-triclosan (de-methylation) in the liver may thus be one explanation to the differences (Neilson et al., 1991).

Triclocarban could not be detected in any of the fish liver samples (LOQ 0,5 ng/g ww). As mentioned in section 3, triclocarban has been shown to be taken up by plants, but according to the PBT profiler (USEPA, 2012) it does not exceed the criterion for bioaccumulation. Our results do not contradict this conclusion.

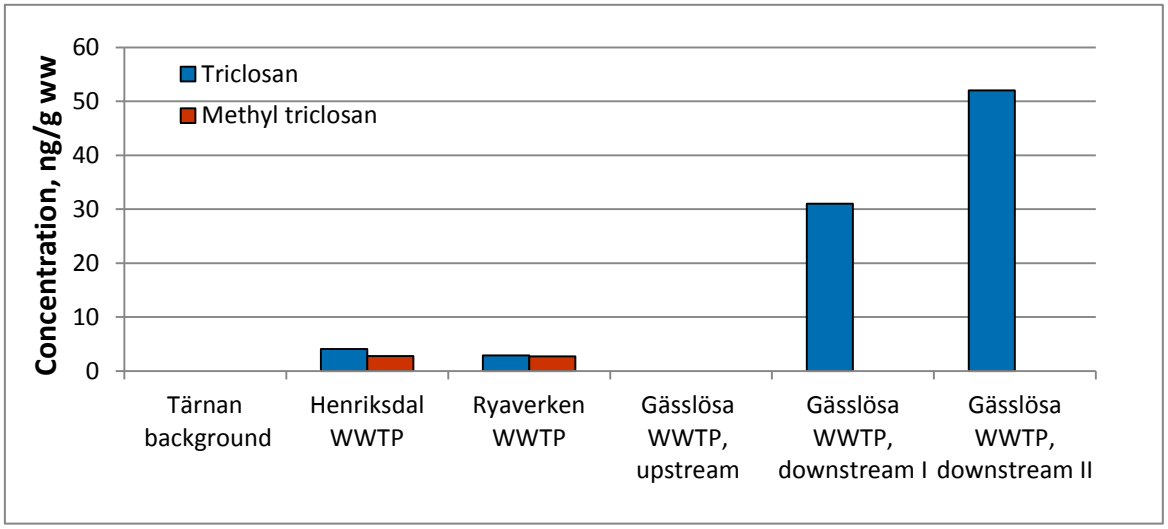


Figure 12. Measured concentrations of triclosan and methyl triclosan in fish liver. Triclocarban was not detected.

Triclosan has previously been measured in fish muscle within the Swedish national screening program, and levels have been in the range 0.3-8.4 ng/g ww (Kaj et al., 2010; Remberger et al., 2002 ; Remberger et al., 2006), which is similar to the levels

observed in fish liver outside Henriksdal and Ryaverken in this study, but lower than the levels observed at Gässlösa.

Methyl-triclosan was reported in biota already in 1984 (Miyazaki et al., 1984) at a concentration range of 1-38 ng/g on a lipid basis.

In a retrospective study (1994-2008) of triclosan and methyl-triclosan in archived fish samples collected from German rivers the concentration of methyl-triclosan ranged from 1-33 ng/g ww (re-calculated from lipid normalized concentrations). The concentrations showed an increasing trend from 1994 to the peak years in 2003-2005, after which concentrations decreased. The triclosan concentrations in the samples were considerably lower and ranged <0.2-3.4 ng/g ww. Thus, Rüdel et al., (2013), concluded that methyl-triclosan accumulation in fish muscle tissue is higher compared to triclosan although the difference in log K_{ow} is small (Table 2).

Balmer et al.,(2004) reported that methyl-triclosan was only detected in fish from Swiss lakes receiving effluents from WWTPs. The concentration range was 4.2-365 ng/g lw (corresponding to 0.4-4.9 ng/g ww).

In conclusion, the concentrations in biota observed in the current study are in the same order of magnitude as observed levels in previous studies in Sweden, Germany and Switzerland but with the Swedish results in the lower concentration range.

8.2 3,4-Dichloroaniline and 4-chloroaniline

3,4-Dichloroaniline and 4-chloroaniline, which are known degradation products of triclocarban, were below the limit of quantification in all samples of influent and effluent water, sludge, sediment and fish (LOQ see Table 7). It should be noted that, with the exception of sludge, the LOQs for anilines were higher than the detected concentrations of the other antibacterial substances.

German data from waste and surface water showed concentrations in the range 1.1-67 µg/l of 4-chloraniline and 1.8-3.3 µg/l of 3,4-dichloroaniline (Scholz and Palauschek, 1988). Considering that our detection limits were well below these measured ranges it is obvious that the anilines did not occur in such high concentrations in the influent and effluent waters in the current study.

Anilines are rapidly sorbed to sediment. This adsorption is reversible but is followed by a slower irreversible sorption process. The latter process include oxidative co-polymerisation with phenolic moieties or production of different dimers (Colón et al., 2002). The reaction product is likely to be incorporated into the sludge (Ononye and Graveel, 1994; Simmons et al., 1989). Another possible pathway for the elimination of

the amines is microbial oxidation of the amine to hydroxylamine and nitrosamine (Kaufman et al., 1973).

8.3 Silver

8.3.1 WWTPs

Silver was detected in one out of six samples of WWTP influent water, at a concentration of 660 ng/L. All other samples of influent and effluent water were below the limit of quantification (500 ng/L).

Silver was detected in sludge from WWTPs, in levels between 860 and 3 100 ng/g dw (Figure 13). The concentrations are similar to the concentrations in sludge from 2011 as reported in the national screening database, and are lower than concentrations reported during the 00's (Figure 14).

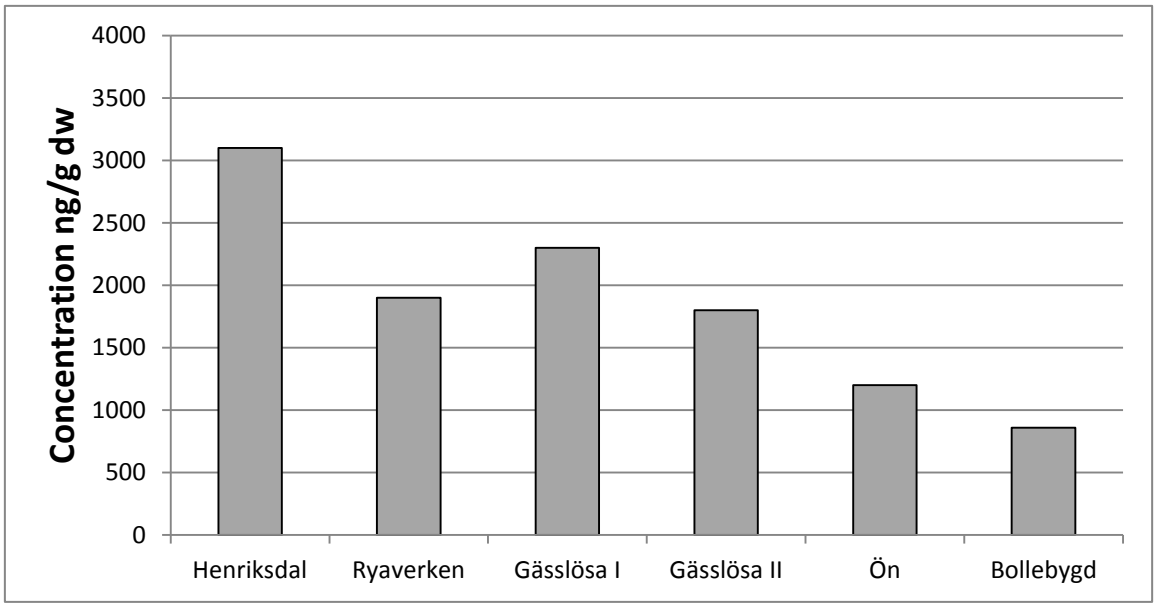


Figure 13. Measured concentrations of silver in WWTP sludge.

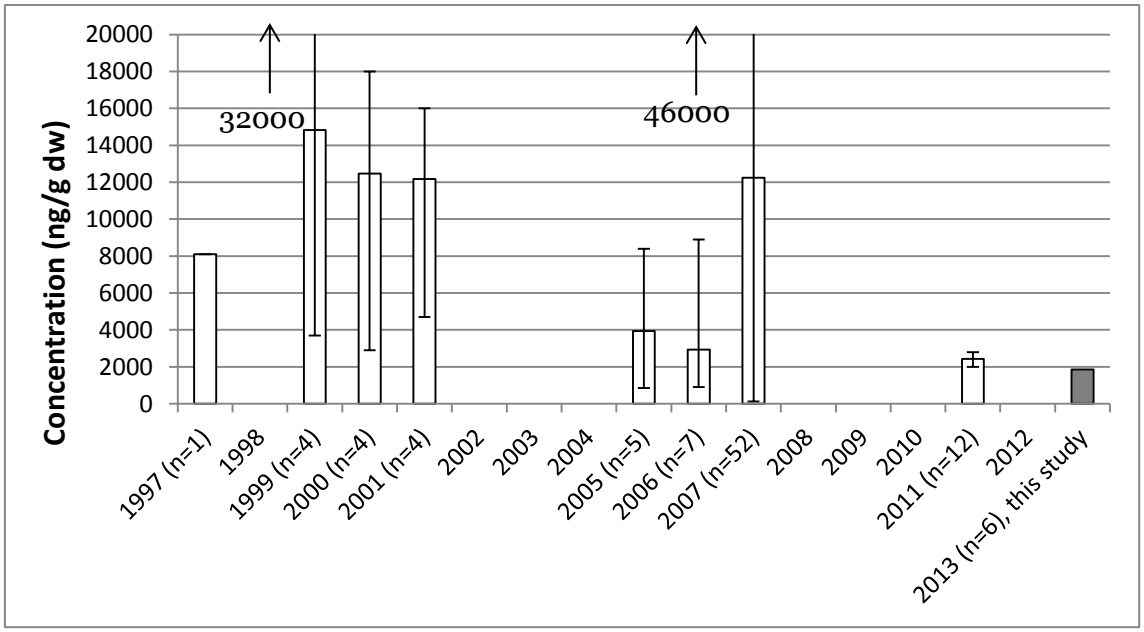


Figure 14. Comparison between previously reported silver concentrations in sludge and concentrations observed in this study. The bars represent average values and the error bars represent the min and max concentrations reported. The data for comparison has been derived in different studies conducted within the Swedish national screening programme and were extracted from the Swedish national screening database[5] (see section 5.1).

8.3.2 Sediment

Figure 15 shows the measured concentrations of silver in sediments near municipal WWTPs. Elevated levels were found in sediments outside Henriksdals WWTP in Stockholm and outside Gässlösa WWTP (downstream I) in Borås. 5 km downstream of Gässlösa (downstream II), levels were a factor of 30 lower compared to the close proximity of the plant. Outside Ryaverken in Göteborg, the levels were similar to background concentrations in Lake Tärnan. The measured concentration in sediments from Tärnan (260 ng/g dw) is similar to the average concentration in sediments from Swedish reference lakes in 2007, which was 267 (42-820) ng/g dw (measured under the national environmental monitoring programme, data extracted from the national database[5]).

[5] The national screening database can be found at http://www.ivl.se – Tjänster – Datavärdskap – Miljögifter i biologiskt material och screening – Databas: Screening, Access date: 23.06.14

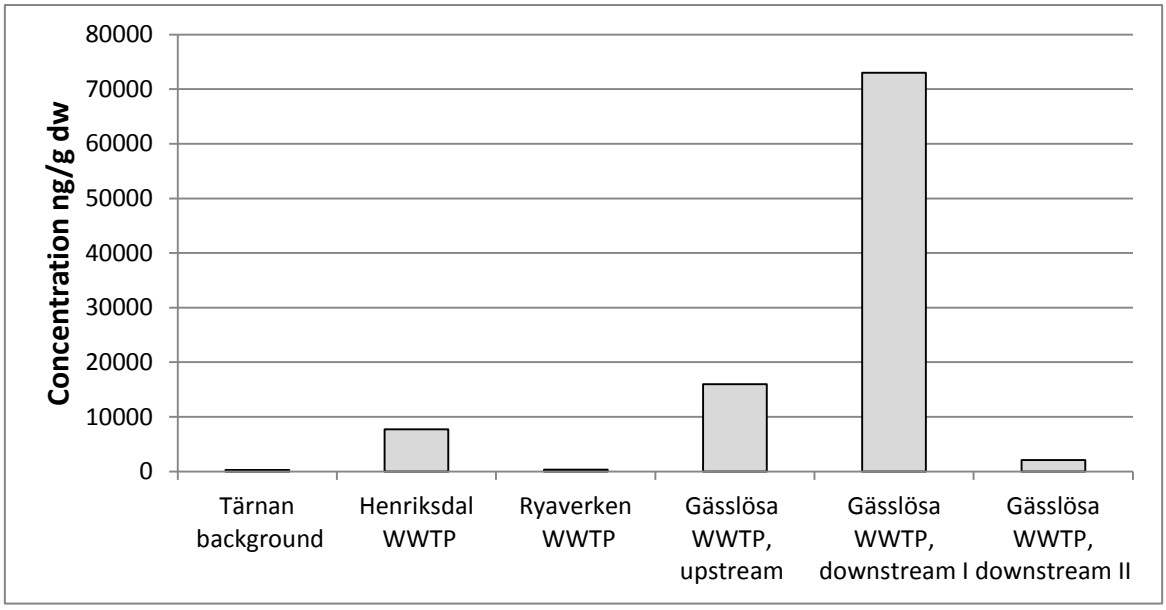


Figure 15. Measured concentrations of silver in sediments outside WWTPs.

8.3.3 Biota

The levels of silver in fish (n = 6) were all below the limit of quantification (<4 ng/g ww).

9 Simplified risk assessment

Typical for a screening study is to cover many sampling points and environmental matrices, therefore limited data are derived at each sampling point, making statistical evaluations difficult. However, it is possible to conduct a simplified risk assessment, which can help to indicate whether observed concentrations can be expected to lead to negative consequences for the environment.

The concentration of triclosan in effluent water exceeded the proposed limit value for inland waters of 0.05 µg/L on two occasions (Gässlösa I and Bollebygd). However, since the proposed limit value applies to surface water rather than concentrated effluents, a direct comparison is not relevant. A standard procedure (ECHA, 2008)is to consider a dilution factor of 10 to the effluent concentrations, which we have done in this assessment. The diluted concentration was divided by the limit value of 0.05 µg/L, generating risk ratios as shown in Figure 16. As evident from the figure the measured levels in effluent water are unlikely to exceed the limit value in the final recipient, thus they should not lead to any negative toxic effects.

This finding is in agreement with a previous modelling study in rivers following WWTP discharge by Reiss et al. (2002). In their study, the discharge of triclosan in WWTP effluent to river water was simulated by the model. Concentrations were calculated based on the characteristics of the watercourse, the estimated concentration of triclosan in the WWTP effluent, and the physicochemical properties of the compound. The model showed that no acute or chronic risks were expected for fish and invertebrates.

For the sediment compartment the situation is somewhat different. The proposed limit value for sediment is exceeded in the sediment outside Henriksdal. Thus, toxic effects at this site cannot be outruled. It should be emphasized, however, that the limit value for sediment is based on the limit value for water, applying equilibrium partitioning methodology. To verify the accuracy of this value, complementary toxicity tests should be performed using sediment dwelling organisms.

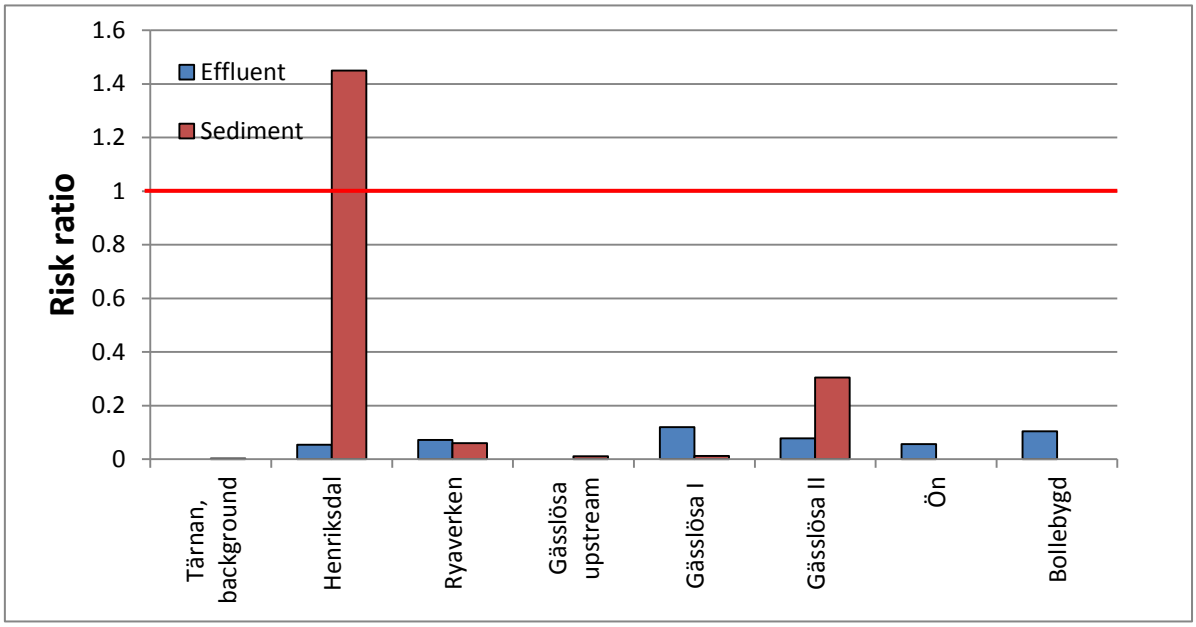


Figure 16. Risk ratio for triclosan when a dilution factor of ten is applied to the concentrations measured in effluents. A ratio < 1 implies no risk.

3,4-DCA was below the LOQ in all effluent waters. The LOQ (100 ng/L) is a factor of 2 lower than the proposed long-term limit value from the UK EPA (WFD-UKTAG, 2013).

10 Conclusions

The following conclusions can be drawn from the current screening study:

- Triclosan concentrations in water were all well below the proposed limit values.
- The triclosan concentration in sediment exceeded the limit value for sediment in one sample from Henriksdal, thus a risk for toxic effects cannot be excluded.
- Triclosan concentrations in sludge were slightly higher than the levels measured in Sweden in recent years (2008-2012), but lower that concentrations observed prior to 2008.
    - Continued monitoring in sludge will help to determine if the concentrations remain on the same level or if an increase is taking place.
- Triclocarban levels in WWTP influents appear to correlate with triclosan and were about a factor of 4 lower than the triclosan levels.
- The median removal efficiency of triclosan in WWTPs was 55%, whereas triclocarban was efficiently removed (~100 %).
- Continued monitoring of triclocarban is recommended due to
    - the frequent occurrence in sediments
    - its persistent properties
    - the general scarcity of data from the Swedish environment
- Methyl-triclosan was mainly found in low concentrations in sludge (200 times lower than triclosan), but also in fish liver samples collected outside two large WWTPs.
- 3,4-DCA was not detected in any of the samples analysed. The LOQs were higher than measured levels for the other compounds, but well below observed levels in other countries, and below the limit value proposed in the UK.
- Silver was detected in sludge and sediment, with sludge levels being similar to previously reported concentrations.

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Appendix 1
Sample list

ID	ID, Fish liver	Type	Municipality	Site	Matrix	Date	WGS84 N	WGS84 E
2859		Backgr	Vallentuna	Tärnan	Sediment	2013-10-04	59° 34.4013′	18° 22.163′
1206	2860	Backgr	Vallentuna	Tärnan	Fish (Perch)	2013-10-04	59° 34.4013′	18° 22.163′
2553		Diffuse	Stockholm	Henriksdal WWTP	Influent	2013-10-08	59° 18.585′	18° 6.458′
2554		Diffuse	Stockholm	Henriksdal WWTP	Effluent	2013-10-08	59° 18.585′	18° 6.458′
2555		Diffuse	Stockholm	Henriksdal WWTP	Sludge	2013-10-08	59° 18.585′	18° 6.458′
2598		Diffuse	Stockholm	Henriksdal WWTP	Sediment	2013-09-25	59° 19.0856′	18° 7.0222′
2778	2779	Diffuse	Stockholm	Henriksdal WWTP	Fish (Perch)	2013-09-25	59° 18.9932′	18° 7.1888′
2541		Diffuse	Göteborg	Ryaverken WWTP	Influent	2013-09-25	57° 41.830′	11° 53.500′
2542		Diffuse	Göteborg	Ryaverken WWTP	Effluent	2013-09-25	57° 41.830′	11° 53.500′
2543		Diffuse	Göteborg	Ryaverken WWTP	Sludge	2013-09-25	57° 41.830′	11° 53.500′
2784		Diffuse	Göteborg	Ryaverken WWTP	Sediment	2013-11-25	57° 41.339′	11° 53.257′
2789	2789	Diffuse	Göteborg	Ryaverken WWTP	Fish, (Saithe + Cod)	2013-11-27	57° 41.339′	11° 53.257′
2494		Diffuse	Borås	Gässlösa WWTP	Influent	2013-08-27	57° 42.2950′	12° 55.5994′
2495		Diffuse	Borås	Gässlösa WWTP	Effluent	2013-08-27	57° 42.2950′	12° 55.5994′
2497		Diffuse	Borås	Gässlösa WWTP	Sludge	2013-08-30	57° 42.2950′	12° 55.5994′
2498		Diffuse	Borås	Gässlösa WWTP	Influent	2013-08-30	57° 42.2950′	12° 55.5994′
2499		Diffuse	Borås	Gässlösa WWTP	Effluent	2013-08-30	57° 42.2950′	12° 55.5994′
2500		Diffuse	Borås	Gässlösa WWTP	Sludge	2013-08-30	57° 42.2950′	12° 55.5994′
2501		Diffuse	Borås	Öresjö, upstream Gässlösa	Sediment	2013-08-27	57° 45.6746′	12° 56.7161′
2504	2505	Diffuse	Borås	Öresjö, upstream Gässlösa	Fish (Perch)	2013-08-28	57° 45.6746′	12° 56.7161′
2502		Diffuse	Borås	Viskan, downstream Gässlösa WWTP	Sediment	2013-08-28	57° 45.6746′	12° 56.7161′
2506	2507	Diffuse	Borås	Viskan, downstream Gässlösa WWTP	Fish (Perch)	2013-08-28	57° 41.5101′	12° 54.5323′
2503		Diffuse	Borås	Rydboholm, approx. 5 km downstream Gässlösa	Sediment	2013-08-29	57° 39.6464′	12° 53.5154′

ID	ID, Fish liver	Type	Municipality	Site	Matrix	Date	WGS84 N	WGS84 E
2508	2509	Diffuse	Borås	Rydboholm, approx. 5 km downstream Gässlösa	Fish (Perch)	2013-08-29	57° 39.6464′	12° 53.5154′
2538		Diffuse	Umeå	Ön WWTP	Influent	2013-09-18	63° 48.2516′	20° 17.4809′
2539		Diffuse	Umeå	Ön WWTP	Effluent	2013-09-18	63° 48.2516′	20° 17.4809′
2540		Diffuse	Umeå	Ön WWTP	Sludge	2013-09-18	63° 48.2516′	20° 17.4809′
2487		Diffuse	Bollebygd	Bollebygd WWTP	Influent	2013-08-27	57° 39.6897′	12° 31.3355′
2488		Diffuse	Bollebygd	Bollebygd WWTP	Effluent	2013-08-27	57° 39.6897′	12° 31.3355′
2490		Diffuse	Bollebygd	Bollebygd WWTP	Sludge	2013-08-29	57° 39.7759′	12° 32.1868′

Appendix 2 Results table

Ag was measured in fish muscle and reported on a wet weight basis (ww). Organic substances were measured in fish liver.

ID	ID, Fish liver	Type	Municipality	Site	Matrix	DW %	Unit	Ag	Triclosan	Methyl triclosan	Triclo-carban	4-Chloro aniline	3,4-Dichloro aniline
2859		Backgr	Vallentuna	Tärnan	Sediment	8.3	ng/g dw	260	<1	<1	<0.3	<20	<10
1206	2860	Backgr	Vallentuna	Tärnan	Fish (Perch)	-	ng/g ww	<4	<1	<1	<0.5	<100	<100
2553		Diffuse	Stockholm	Henriksdal WWTP	Influent	-	ng/l	660	81	19	10	<100	<100
2554		Diffuse	Stockholm	Henriksdal WWTP	Effluent	-	ng/l	<500	27	<5	<1	<100	<100
2555		Diffuse	Stockholm	Henriksdal WWTP	Sludge	26	ng/g dw	3100	1700	19	260	<20	<20
2598		Diffuse	Stockholm	Henriksdal WWTP	Sediment	20	ng/g dw	7700	290	2.3	180	<20	<10
2778	2779	Diffuse	Stockholm	Henriksdal WWTP	Fish (Perch)	-	ng/g ww	<4	4.1	2.8	<0.5	<100	<100
2541		Diffuse	Göteborg	Ryaverken WWTP	Influent	-	ng/l	<500	82	<10	24	<100	<100
2542		Diffuse	Göteborg	Ryaverken WWTP	Effluent	-	ng/l	<500	36	<5	1.8	<100	<100
2543		Diffuse	Göteborg	Ryaverken WWTP	Sludge	25	ng/g dw	1900	1700	4.8	770	<20	<20
2784		Diffuse	Göteborg	Ryaverken WWTP	Sediment	34	ng/g dw	340	12	<1	9.30	<20	<10
2789	2789	Diffuse	Göteborg	Ryaverken WWTP	Fish, (Saithe + Cod)	-	ng/g ww	<4	2.9	2.7	<0.5	<100	<100
2494		Diffuse	Borås	Gässlösa WWTP	Influent	-	ng/l	<500	110	<10	21	<100	<100
2495		Diffuse	Borås	Gässlösa WWTP	Effluent	-	ng/l	<500	60	<5	<1	<100	<100
2497		Diffuse	Borås	Gässlösa WWTP	Sludge	23	ng/g dw	2300	1400	8.3	200	<20	<20
2498		Diffuse	Borås	Gässlösa WWTP	Influent	-	ng/l	<500	85	<10	53	<100	<100
2499		Diffuse	Borås	Gässlösa WWTP	Effluent	-	ng/l	<500	39	<5	<1	<100	<100
2500		Diffuse	Borås	Gässlösa WWTP	Sludge	20	ng/g dw	1800	1800	6.8	210	<20	<20
2501		Diffuse	Borås	Öresjö, upstream Gässlösa	Sediment	17	ng/g dw	16000	2.1	<1	1.0	<20	<10
2504	2505	Diffuse	Borås	Öresjö, upstream Gässlösa	Fish (Perch)	-	ng/g ww	<4	<1	<1	<0.5	<100	<100
2502		Diffuse	Borås	Viskan, downstream Gässlösa WWTP	Sediment	69	ng/g dw	73000	2.3	<1	6.0	<20	<10
2506	2507	Diffuse	Borås	Viskan, downstream Gässlösa WWTP	Fish (Perch)	-	ng/g ww	<4	31	<1	<0.5	<100	<100
2503		Diffuse	Borås	Rydboholm, ca 5 km downstream Gässlösa	Sediment	18	ng/g dw	2100	61	<1	130	<20	<10

ID	ID, Fish liver	Type	Municipality	Site	Matrix	DW %	Unit	Ag	Triclosan	Methyl triclosan	Triclo-carban	4-Chloro aniline	3,4-Dichloro aniline
2508	2509	Diffuse	Borås	Rydboholm, ca 5 km downstream Gässlösa	Fish (Perch)	-	ng/g ww	<4	52	<1	<0.5	<100	<100
2538		Diffuse	Umeå	Ön WWTP	Influent	-	ng/l	<500	130	<10	19	<100	<100
2539		Diffuse	Umeå	Ön WWTP	Effluent	-	ng/l	<500	28	<5	<1	<100	<100
2540		Diffuse	Umeå	Ön WWTP	Sludge	32	ng/g dw	1200	1400	3.5	170	<20	<20
2487		Diffuse	Bollebygd	Bollebygd WWTP	Influent	-	ng/l	<500	89	<10	7.5	<100	<100
2488		Diffuse	Bollebygd	Bollebygd WWTP	Effluent	-	ng/l	<500	52	<5	<1	<100	<100
2490		Diffuse	Bollebygd	Bollebygd WWTP	Sludge	13	ng/g dw	860	890	23	59	<20	<20

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