

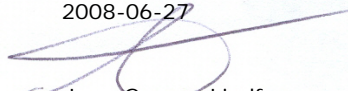
Measurements of Sucralose in the Swedish Screening program 2007

PART II; Sucralose in Biota samples and regional STP samples

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<p>Title and subtitle of the report</p> <p>Measurements of Sucralose in the Swedish Screening program 2007. PART II; Sucralose in Biota samples and regional STP samples</p>	
<p>Summary</p> <p>IVL has performed a "screening study" of sucralose on commission from the Swedish EPA, reported in two consecutive parts. Sucralose is a chlorine containing derivative of sucrose, manufactured by selectively substituting three hydroxyls with chlorine. The substance is used as a sweetener in food products; on a weight basis it tastes ca. 600 times sweeter than the parent compound. The objectives of the study were to determine the concentrations of sucralose in media in the Swedish environment such as biota, wastewater effluents and to highlight important transport pathways. In total 84 samples were analysed. This report constitutes part 2 of the study.</p>	
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Summary

The Swedish Environmental Research Institute, IVL and the Norwegian Institute for Air Research, NILU have performed a "screening study" of sucralose during 2007 as an assignment from the Swedish Environmental Protection Agency. Sucralose is a chlorine containing derivative of sucrose, manufactured by selectively substituting three hydroxyls with chlorine. This substance is used as a sweetener in food products; on a weight basis it tastes ca. 600 times sweeter than the parent compound. The overall objective of the screening was to determine the concentrations of the substance in some compartments of the Swedish environment, focusing on the release into the aquatic environment. The complete screening programme included measurements in background areas and close to potential point sources. Sample types included biota (fish liver tissue, fish muscle tissue and mussels soft tissue), untreated and treated wastewaters, sewage sludge, and surface water samples. This is the second part of the report, complementing part 1 with chemical analysis data on 76 additional samples (not reported in part 1).

The table shows concentration ranges of sucralose in some environmental matrices.

	STP Influent waters (ng/l)	STP Effluent waters (ng/l)	Surface waters (ng/l)	Sludge (ng/g ww)	Biota Fish (ng/g fw)	Biota Mussels (ng/g fw)
# of samples	9	36	13	8	4	2
Sucralose	1700 - 4100	710 - 4900	<2.2 - 470	<0.3 - 19	<0.3 - <1	<0.4 - <0.7
DF (%)	100	100	23	36	0	0

DF = Detection frequency
 STP = Sewage Treatment Plant

In addition, also one leachate water sample was included in the study, which did not contain any sucralose (< 8 ng/l), along with a hospital effluent sample containing 330 ng/l of sucralose. Furthermore, also data from two additional surface water samples have been added.

From this study, part 2, it can be concluded that;

- Sucralose is detected in Swedish surface waters receiving wastewater effluents.
- Untreated municipal wastewater seems to always contain sucralose in µg/l concentrations.
- Wastewater treatment processes has little or no effect on sucralose, removal rates ranges between -47% and 17 % (average -17%) in all paired samples (influent/effluent).
- Sucralose was detected in all 365 effluent samples reported in this part of the study (from 34 STPs throughout Sweden); 710 to 4900 ng/l, with a median of 3500 ng/l.
- Sucralose was not significantly accumulated in sewage sludge. In 63 % of the sludge samples reported in part 2 of the study, sucralose is not detected at all, and the highest concentration of sucralose reported herein is 19 ng/g ww.
- Surface water from reference lakes and water courses upstream of STP effluents contained no measurable sucralose, < 2 ng/l - < 7 ng/l.
- Sucralose uptake in biota seems unlikely, mussels (*Anodonta cygnea*, in cages) exposed to sucralose in effluent discharged from a STP in Stockholm for 8 weeks did not show any traces of sucralose (<0.4-<0,7 ng/g fw). Neither fish liver nor muscle tissue of perch (*Perca fluviatilis*) contained sucralose when analysing fish sampled from Linköping and Stockholm.

This report on sucralose in aqueous samples constitutes the second part of a thorough screening study of sucralose in the Swedish environment.

Sammanfattning

IVL har tillsammans med NILU på uppdrag av Naturvårdsverket genomfört en screening av sötningsmedlet sukralos. Sukralos är en disackarid, som modifierats i tre positioner med klor. Ämnet är ca 600 gånger sötare än sackaros och används efter tillstånd i USA och inom Europeiska unionen, m.fl. länder som tillsats i livsmedel. Ämnet är lösligt i vatten och vid intag utsöndras minst 95 % i oförändrad form. Ingen ackumulering i organismen är känd och nedbrytning eller omvandling har endast påvisats i vattenmiljö under inverkan av mikroorganismer. Tre primära klorinnehållande omvandlingsprodukter har påvisats. De studier i djurförsök som legat till grund för tillståndsgivningen har visat mycket små effekter.

Det huvudsakliga syftet med denna översiktliga kartläggning var att bestämma koncentrationer av sukralos i några olika matriser i miljön, framförallt för att belysa viktiga transportvägar i vattenmiljön i Sverige. Rapporten avser del 2 i en landsomfattande screeningstudie. Totalt bestämdes sukralos i 76 prover varav 68 insamlats av länsstyrelserna i Sverige. I rapport del 1 har sedan tidigare 57 prover redovisats.

Nedan visas en tabell med uppmätta halter i olika provtyper.

	ARV Inkommande vatten (ng/l)	ARV Utgående vatten (ng/l)	Ytvatten (ng/l)	Slam (ng/g ww)	Biota Fisk (ng/g fw)	Biota Mussla (ng/g fw)
# prov	910	36	13	8	4	2
Sukralos	1700 - 4100	710 - 4900	<2.2 - 470	<0.3 - 19	<0.3 - <1	<0.4 - <0.7
DF (%)	100	100	23	36	0	0

DF=Detektionsfrekvens

Dessutom ingick även ett lakvattenprov ifrån en deponi i studien, samt ett utgående vattenprov ifrån ett sjukhus. Vidare har ytterligare två ytvattenprover ifrån bakgrundsområden inkluderats.

Studien visar att;

- Sukralose detekteras i vattenrecipienter i Sverige som tar emot utgående vatten ifrån reningsverk.
- Inkommande vatten till svenska avloppsreningsverk (9 ARV i denna del av studien) innehåller 1700-4100 ng sukralos/l.
- Reningsgraden m a p. sukralos är låg i reningsverk, maximalt uppmättes 17% reningsgrad i de parade prover som analyserats (inkommande/utgående) emedan median- och medelreningsgraden var negativ (-19% respektive -17 %).
- Sukralos detekterades i alla de 36 utgående reningsverksvattenproverna redovisade i denna del av studien (ifrån 34 olika reningsverk i landet); 710 - 4900 ng/l, median 3500 ng/l.
- Det sker ingen ackumulation av sukralos i slam. Av de 8 slamprover som redovisas i denna del av studien uppvisar 63 % ej påvisbara halter (under metodens detektionsgräns), och den högsta sukraloskoncentrationen i slam var 19 ng/g våtvikt.
- I ytvatten ifrån bakgrundsjöar och vattendrag som ligger uppströms reningsverk, har inte sukralos detekterats (< 2 ng/l - < 7 ng/l).

- Sukralos förefaller att ej upptagas i biota. I musslor (*Anodonta cygnea*, i burar) exponerade för sukraloshaltigt utspätt utgående avloppsvatten från Henriksdals reningsverk i Stockholms under 8 veckor, kunde sukralos ej detekteras (<0.4 - < 0.7 ng/g fw) i musselvävnad. Varken fiskmuskel eller fisklever ifrån abborre (*Perca fluviatilis*), infångad i Linköping och Stockholm, innehöll heller detekterbara halter av sukralos.

Denna rapport utgör den andra delen av en screening av sukralos i den svenska miljön.

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1 Introduction

The Swedish Environmental Research Institute, IVL and the Norwegian Institute for Air Research, NILU has performed a "screening study" of sucralose as an assignment from the Swedish Environmental Protection Agency. The first results from this screening study have previously been given in the report "Measurements of Sucralose in the Swedish Screening Program 2007:-PART I; Sucralose in surface waters and STP samples" (Brorström-Lundén et al., 2008).

The Swedish county administrative boards had the possibility to add samples to the national sampling programme and this report, Part 2, includes the results from the regional screening. The results from sucralose analyses in biota samples from the national screening study are also given in this report.

Sucralose is used as a sweetener in food products. It is chemically a disaccharide, which has been modified to contain three atoms of chlorine. The substance tastes sweet, more than 600 times sweeter than sucrose, the saccharide of cane sugar.

Like other synthetic sweeteners it replaces sugar in low calorie food products. In comparison to aspartame, another sweetener, it is more stable to elevated temperatures and acid and alkaline conditions. Sucralose does not interfere with levels of glucose and insulin in blood and may therefore be consumed by persons with diabetes.

The overall objective of the sucralose screening study was to determine concentrations of this substance in a variety of compartments in the Swedish environment and to highlight important transport pathways to the environment. A further aim was to investigate the occurrence of sucralose in biota.

For a complete background description on the physico-chemical properties, fate, , as well as the regulatory aspects of sucralose, the reader is referred to part 1 of this report (Brorström-Lundén et al., 2008).

2 Toxicity

Although report part I did disseminate the eco toxicity of sucralose, some data previously unknown to the authors of this report have been identified and are included for completeness. The data are taken from the the "pending petition" (FAP 8A4624), submitted to the US Foods and Drugs Administration by Tate & Lyle to support its use of Sucralose as a general purpose sweetener (FAP 8A4624, Dec., 1998).

The general impression from report part I of sucralose as a virtually non-toxic substance is not altered, however additional chronic eco toxicity data with regard to sucralose have been intensively sought after.

Table 1. Additional eco toxicity data for sucralose

Species	Duration/Endpoint	Toxicity [mg/l]	Reference
Daphnia Magna (<i>crustacean</i>)	21 days, NOEC	1800	FAP 8A4624, Dec., 1998 (BL/B/2915)
Rainbow trout (<i>fish</i>)	96 h, LC ₅₀	>2400	FAP 8A4624, Dec., 1998 (AFT/83/051)
Bluegill sunfish (<i>fish</i>)	96 h, LC ₅₀	>3200	FAP 8A4624, Dec., 1998 (BL/B/2686)
Selenastrum capricornutum (<i>unicellular green algae</i>)	96h, E _r C ₅₀ , 96h, E _b C ₅₀	1800 1800	FAP 8A4624, Dec., 1998 (BL/B/2897)

3 Sampling strategy and study sites

A sampling strategy was developed in order to determine the concentrations of sucralose in different environmental matrices in Sweden and to identify major emission sources as well as important transport pathways. The overall programme included both measurements in background areas and close to potential sources (Brorström-Lundén et al., 2008). However, in this part of the study additional samples from Swedish sludge treatment plants (STPs) have been included along with some surface water samples and the biota samples. All samples but the biota samples were part of a regional sampling conducted by the county administrative boards in Sweden, see above. All sample locations throughout Sweden (both reported herein and the ones previously reported) can be seen in Figure 1.

As previously concluded in part 1 of the study, due to the high aqueous solubility of sucralose, the sampling strategy has been focussed on effluents into the aquatic environment. A primary source related to consumption of food products containing sucralose is the municipal sewage wastewater and hence STP water samples are dominating also in the second part of the study. A summary of the samples included in this report is given in Table 1.

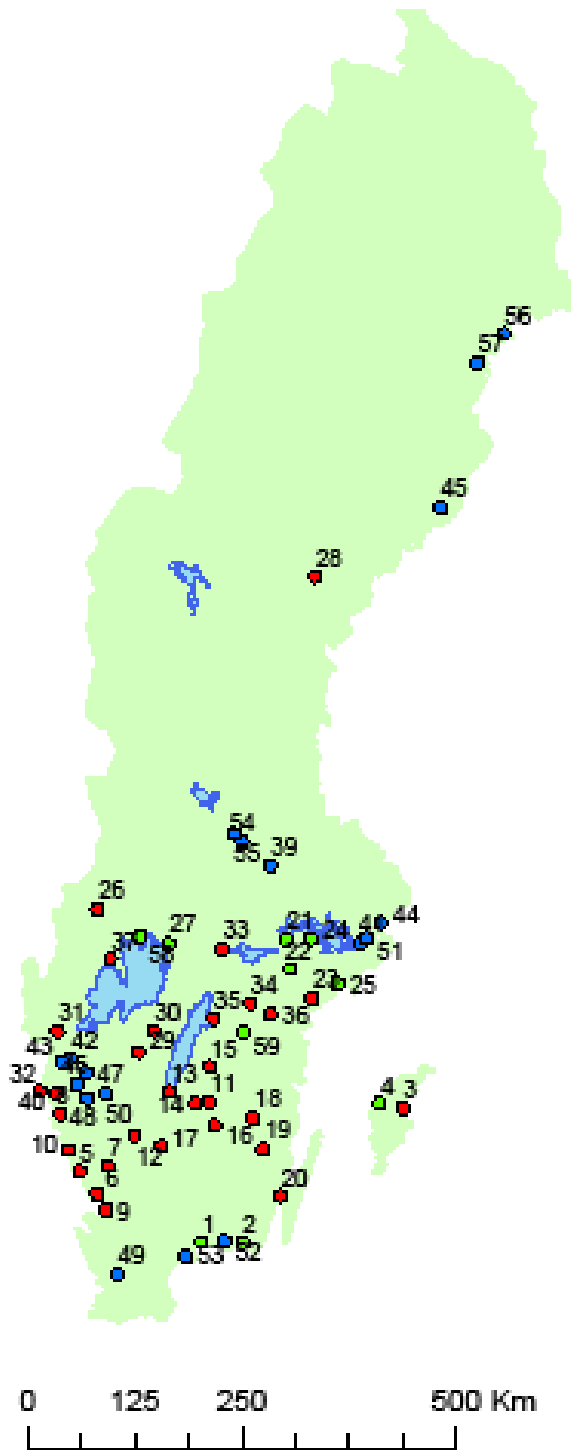


Figure 1. STP water samples and surface waters have been sampled in the locations given by the map (see appendix 1 for locations). The red dots represents sampling locations unique to this part of the study, the blue dots represents sampling locations unique to the first part of the study (Brorström-Lundén et al., 2008), and the green dots represents sampling locations providing samples and data in both parts of the study.

Table 2. Overview of samples collected and reported in this study.

Site	Sewage wastewater, untreated	Sewage wastewater, treated	Surface water	Sludge	Biota	Total
Background locals	-	-	2	-	-	2
Diffuse sources (regional)	9	36	13	8	-	66
STP of densely populated region (Sthlm)	-	-	-	-	4	4
STP, affiliated to dairy industry using sucralose	-	-	-	-	2	2
Total	910	36	15	8	6	74

Not included in the table is a hospital effluent sample as well as depot leachate water sample, making a grand total of 76 samples reported herein.

4 Methods

4.1 Sampling

As the sampling methodology regarding the aqueous samples and the sludge sampling have been extensively described in part 1 (Brorström-Lundén et al., 2008), only the biota sampling and handling are described herein.

Fish

Fish samples were collected by means of fishing net downstream of the outlets from two sewage treatment plants; Henriksdal STP in Stockholm and Linköping (Nykvarnsverket). The netfishing was approved by the fishery authorities in Stockholm (Stockholm ströms FVO) and in Linköping (Hushållnings sällskapet i Östergötland). The collection of the fish was approved by the ethical board for animal testing in Stockholm (Diarie nr 572/07). From the total catch approx. ten individuals of Perch (*Perca fluviatilis*) were selected, representing the second-fifth year classes. Perch was chosen because it is one of the most stationary fish species in both investigated areas. The fish were individually wrapped in cleaned aluminium foil and stored in freezer at -18 °C until analysed.

Fish muscle was dissected from the dorsal muscle for analysis using solvent washed scalpels. Tissue samples from the ten individuals were mixed to a composite sample, frozen and stored at -18 °C until homogenization. The fish abdomen was opened and livers prepared. Ten livers were mixed to a composite sample.

Mussels

Mussels were exposed to treated municipal wastewater for periods up to eight weeks followed by analysis of tissue concentrations of sucralose. Ten specimens of the Swan Mussel (*Anodonta cygnea*) were collected in a forest lake, situated 30 km south of Stockholm, with no known discharges of municipal or industrial effluents. The mussel collection was approved by the community ecologist in Huddinge community. The mussels had a mean length of 105 mm. Age determination conducted

after dissection, showed that all the mussels were older than 20 years of age. The mussels were transported to Henriksdals STP for acclimatization. This was achieved by keeping the animals in an aquarium flown through by Stockholm City tap water at 20°C for 24 h. Exposure for treated wastewater was conducted at 20 ±1.0°C in a 120 l glass aquarium equipped with a flow-through system for undiluted treated wastewater after sand filtration. The flow was adjusted to 400 ml/min corresponding to an average hydraulic residence time of 5 h. A modest aeration was added to the aquaria to maintain at least 70 % of saturated oxygen concentration in the solutions. Mussels were collected after 8 weeks exposure and stored at -18 °C for further investigations. Soft tissues of individual mussels were prepared by dissection and collected in glass jars. The samples were frozen and stored at -18 °C until further preparation. Homogenization and extraction of the mussel tissues samples was performed as described below for fish tissues.

4.2 Analytical procedures

Analysis of water and sludge was done according to the procedures described in part 1 (Brorström-Lundén et al., 2008).

Samples of fish muscle and liver (10 g) were homogenized in acetonitrile (10 ml) using an Ultraturrax homogenizer. The sample was extracted on a reciprocating shaker in 5 min and centrifuged at 10000 RPM (10 min). The supernatant was safeguarded and was subjected to clean up on a solid phase column (300 mg, Isolute-MM, IST, Mid Glamorgan, UK). The acetonitrile content of the eluate was evaporated by means of a Zymark TurboVac II Concentration Workstation (Caliper Life Sciences, Hopkinton, MA, USA).

After the concentration the extract was again centrifuged at 10000 RPM and filtrated through a PTFE-filter in order to remove any precipitate in the solution. Finally, the extract was cleaned up on a SPE-column (Oasis HLB) using the same protocol as for water samples. Briefly, the sample was applied on the column. The column was washed with diluted HCl (10 mM) and dried by N₂ (5 min). Sucralose was eluted from 7 ml of acetone: methanol (5:1). The solvent was exchanged to HPLC-mobile phase prior to analysis.

4.2.1 HPLC/HRMS analysis

Liquid chromatography was performed with an Agilent 1100 liquid chromatography system (Agilent Technologies, Waldbronn, Germany), equipped with an autosampler, a quaternary pump, an on-line degassing system and a diode array detector (UV). The compound separation was performed with a reversed phase C₁₈ column (Atlantis dC18, 2.1 mm ID x 150 mm length, 3 µm, Waters, Milford USA). A stainless steel inlet filter (Supelco, 0.8 µm) was used in front of a pre-column with the same stationary phase as the separation column. Water was used as solvent A and acetonitrile as solvent B. The binary gradient had a flow rate of 0.2 ml min⁻¹ and started with 95 % A. From 0.1 minute solvent B was introduced at a linear rate up to 90% B at 10 minutes and kept isocratic until 16 minutes. At 16.5 minutes solvent B was ramped up to 100% and kept isocratic up to 19.5 minutes. At 19.6 minutes B was set to 5% and the column was equilibrated up to a total runtime of 30 minutes. The analytical detector was a Micromass LCT orthogonal-acceleration time-of-flight (TOF) mass spectrometer (MS) equipped with a Z-spray electrospray ion source and a 4 GHz time to digital converter (TDC) (Micromass Ltd., Wythenshawe, Manchester, UK). The electrospray source parameters were optimised to the following values: Negative mode: sample cone 20 V, capillary voltage 2.7 kV, extraction cone 3 V, source temperature 120 °C, desolvation temperature 350 °C, cone gas flow 4 l h⁻¹ and desolvation gas flow 632 l h⁻¹. The pusher frequency

was operated in automatic mode. The data processing and instrument (HPLC/HRMS) control were performed by the MassLynx software, and quantification was performed with signal extraction of a peak width of 90 amu (typical).

Table 3. Sucralose ions used for HPLC/HRMS (ES-) analysis

Compound	Mw	Monoisotopic mass	Quantifier	Qualifier {M-H} -
Sucralose	397.6	396	397	395

As a quality control, two fish samples (negative controls, caught in background lakes), were spiked with sucralose (60 and 285 ng) and the sample work-up recoveries were determined as 68 and 39 % respectively.

5 Results and discussion

The concentrations of sucralose in individual samples are given in Appendix together with sample characteristics.

5.1 Sewage wastewaters

Sucralose was detected in all STP influent water samples (10 samples, see Figure 2). The concentration of sucralose in the STP influent samples ranged between 1700 and 4100 ng/l. When these data are compared with the corresponding results from the attributed “point sources” from the report part 1; Nykvarnsverket STP in Linköping and Henriksdal STP in Stockholm, the median concentration of sucralose in STP influent water (2350 ng/l) seems to be a factor of three lower for the “average” Swedish STP (reported herein) compared to Stockholm and Linköping (median 6800 ng/l, Brorström-Lundén et al., 2008). Stockholm is the most densely populated city in Sweden and the load reaching Henriksdal STP is from about 700 000 person equivalents. Also, differences in consumption patterns regarding provisions could be the reason why Stockholm stands out as high in sucralose influent concentration. Nykvarnsverket STP in Linköping receives process water from a nearby dairy production plant using sucralose in its products, and therefore it is not surprising to find elevated concentrations in the influent water to that STP compared to the “averaged” Swedish STP.

Regarding the STP effluent water samples in this report, part 2, sucralose was detected in the samples from all 34 STPs (see Figure 3). The median sucralose concentration in the effluent samples was 3500 ng/l. This is lower than the median effluent concentration reported in part 1 (4900 ng/l). However, in the report part 1, the effluent concentrations measured in the Henriksdal STP stand out as significantly higher than the rest (8500-10800 ng/l, see figure 6 in Brorström-Lundén et al., 2008).

From this data set it is not possible to draw any conclusions on regional trends (north-south or east-west) but it is interesting to note that the effluent water from Ryaverket STP in Göteborg (a big STP receiving waste water from a very densely populated area in Sweden, 600 000 pe) is low (2800 ng/l), compared to the sucralose effluent concentrations in Stockholm and Linköping.

Since the sampling has been performed in a random manner (one sample from each location), it is important not to over interpret the data. For instance sampling a specific STP during a warmer

period will not only impose a decreased water flow through the STP, thus increasing the concentrations of all domestic pollutants, but also specifically increase the concentration of sucralose due to an increased consumption of beverages in the local community.

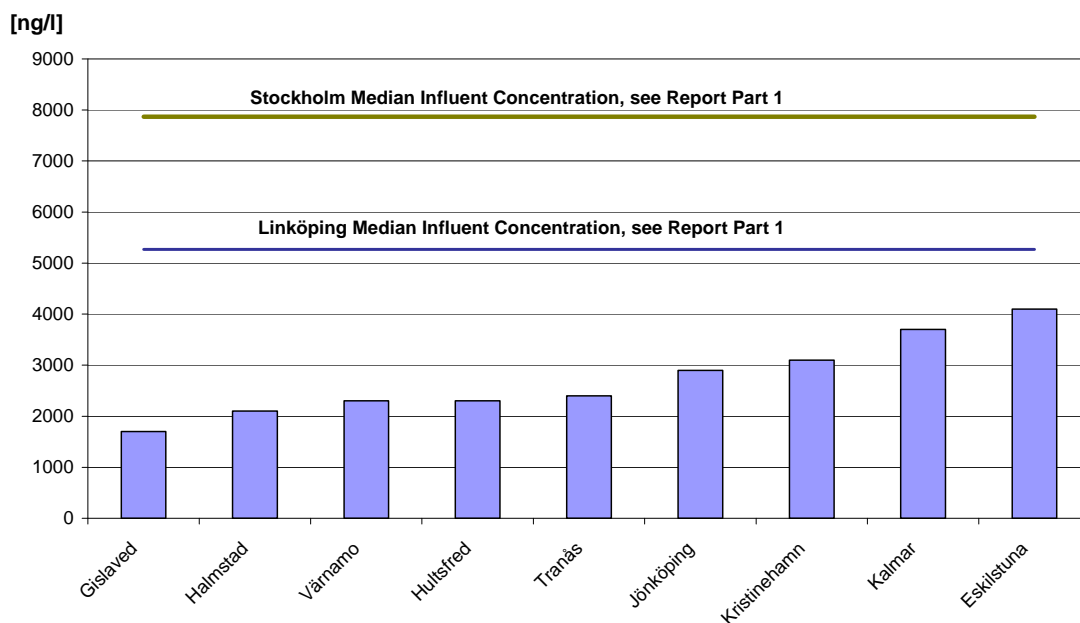


Figure 2. STP influent water concentrations reported in this part. For comparison, influent water concentrations of the Henriksdal STP (Stockholm) and Nykvarn STP (Linköping), from report part 1, have been inserted as solid lines.

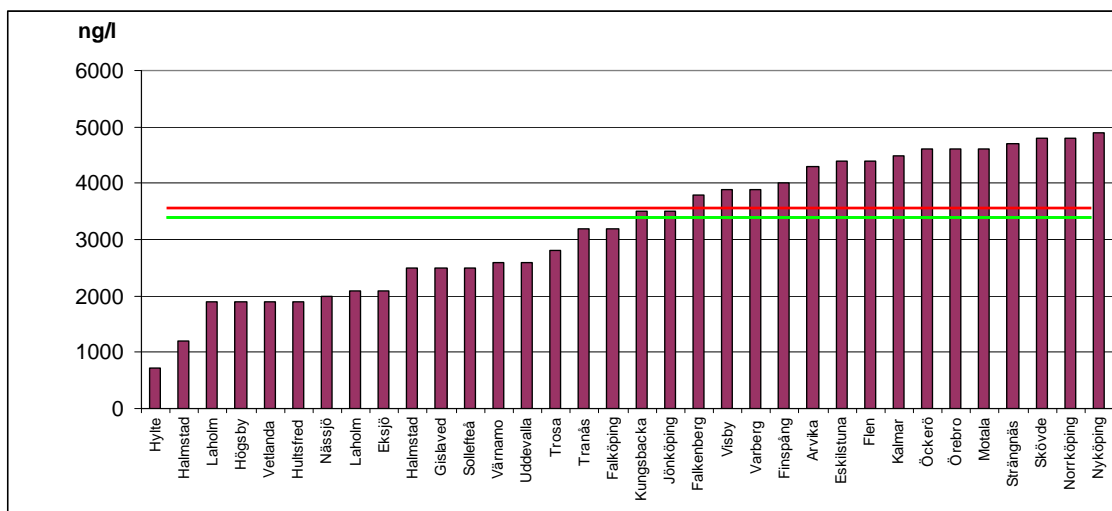


Figure 3. Sucralose effluent concentrations reported in report part 2. For comparison, the median (red) and the mean (green) effluent concentration have been added as solid lines.

As can be seen from Figure 4, sucralose is poorly retained in the STPs. From seven of the STPs, reported herein, paired influent-effluent concentration data indicate that the removal rate of sucralose is negative, i.e., the concentration of sucralose is systematically higher in the effluent water

stream leaving the STP, compared with the influent concentration entering the STP). The average removal rate was -18 %. These phenomena when passing the STP is not uncommon for pollutants that are being excreted from the human body while possessing hydroxyl groups. The hydroxyl groups of sucralose may very well serve as anchoring points for complex formation, aggregation or conjugation with glucuronic acid. The sucralose-aggregate complex (which is probably not disintegrated during the analytical sample work-up) may then be disrupted somewhere along the STP-passage when the chemical and biochemical conditions change causing re-formation of free sucralose, again susceptible for the analytical method.

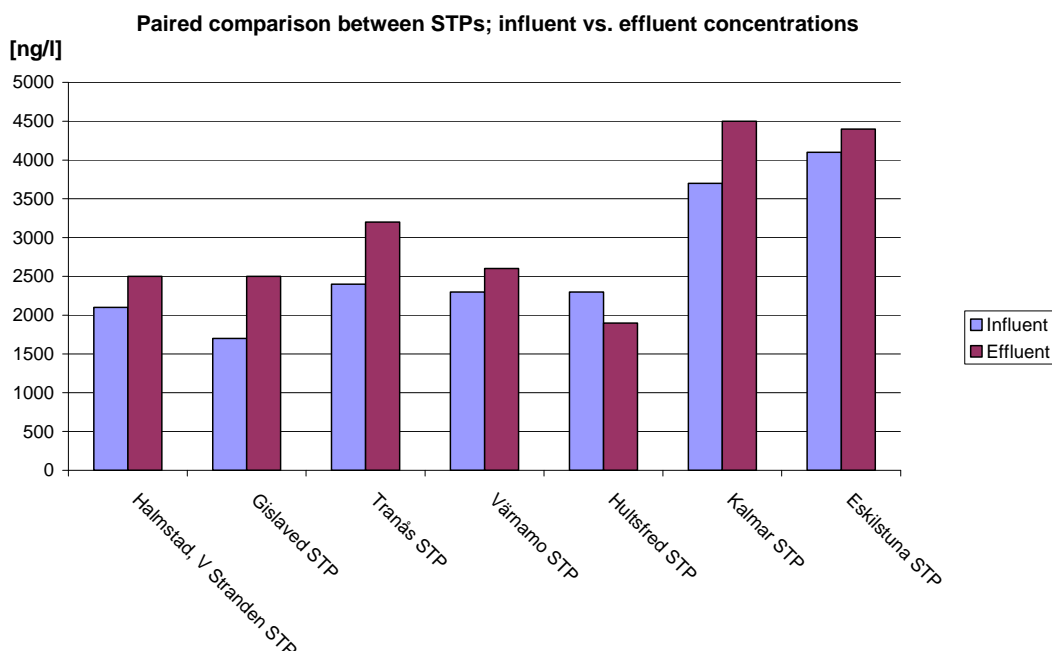


Figure 4. Paired comparison between different STPs; influent- versus effluent sucralose concentrations.

As expected from an inspection of the physico-chemical properties of sucralose (high water solubility and low log Kow-value, see Table 1 in Brorström-Lundén et al., 2008), the concentrations of sucralose found in STP sludge are rather low; <0.3-19 ng/g ww (Figure 5). These concentrations are in good agreement with the results reported in part 1. The measured concentrations herein (4-19 ng/g ww) are comparable with effluent concentrations of 5-20 ng/l, thus the pore water of the sludge may very well be the 'true' reservoir of sucralose in these samples. The sludge samples analysed herein had a dry weight of 14-31 %, and can thus be considered as typical for Swedish STPs.

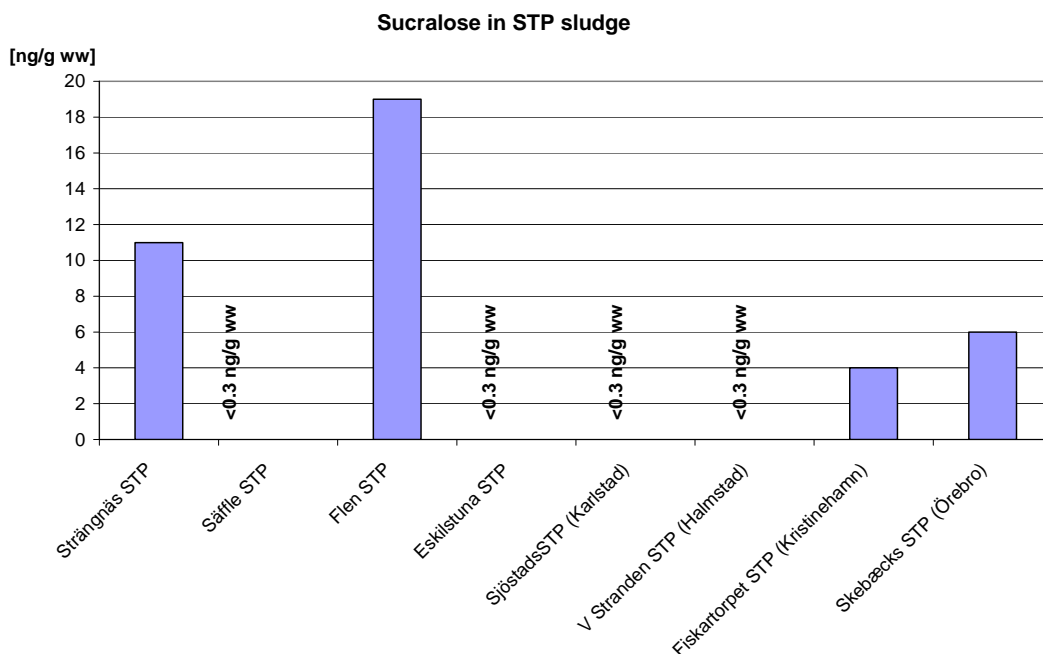


Figure 5. Sucralose concentration in STP sludge.

A hospital effluent sample from; the Blekinge Hospital in Karlshamn, had a sucralose concentration of 330 ng/l.

A leachate water sample from a depot, also in Blekinge (Bubbetorp depot in Karlskrona), did not show any traces of sucralose (LOD < 8 ng/l).

5.2 Surface waters and Receiving waters

In an attempt to assess the risk that sucralose is involuntarily being used in the production of drinking water in local water works, surface water was sampled at four locations in the vicinity of the inlet stream to the local water works; the Långa Lake (used in the Karlshamn water works, Blekinge), the Lyckeby Stream (used in the Lyckeby water works, Blekinge), the Godthem stream (used as a fresh water reservoir in Gotland) and the Bergsjö Lake (used in the Kristinehamn water works, Värmland). Sucralose was not detected (<2.2 - <6.7 ng/l) in any of these samples and hence the risk of involuntary exposure of sucralose through the drinking water seems unlikely. In two cases, the Karlshamn water works and the Lyckeby water works (both in Blekinge), also the produced drinking water was sampled and analysed without detecting sucralose (LOD; <3 ng/l).

In some cases the recipient water system of a specific STP showed minute concentrations of sucralose (29-470 ng/l) in the surface water (Figure 6), indicating that the limnic environment are in fact exposed to sucralose. Even though the number of STPs sampled in this manner (samples collected both from the STP effluent stream and the nearby recipient water) is limited, the finding is in agreement with data from report part 1 (Stockholm Ström and Lake Roxen, Brorström-Lundén et al., 2008).

Two additional surface water samples from background locals were collected and analysed (from Lake Gårdsjön and Lake Hårsevatten, see appendix). As expected no sucralose could be detected in those samples (LOD <2 ng/l).

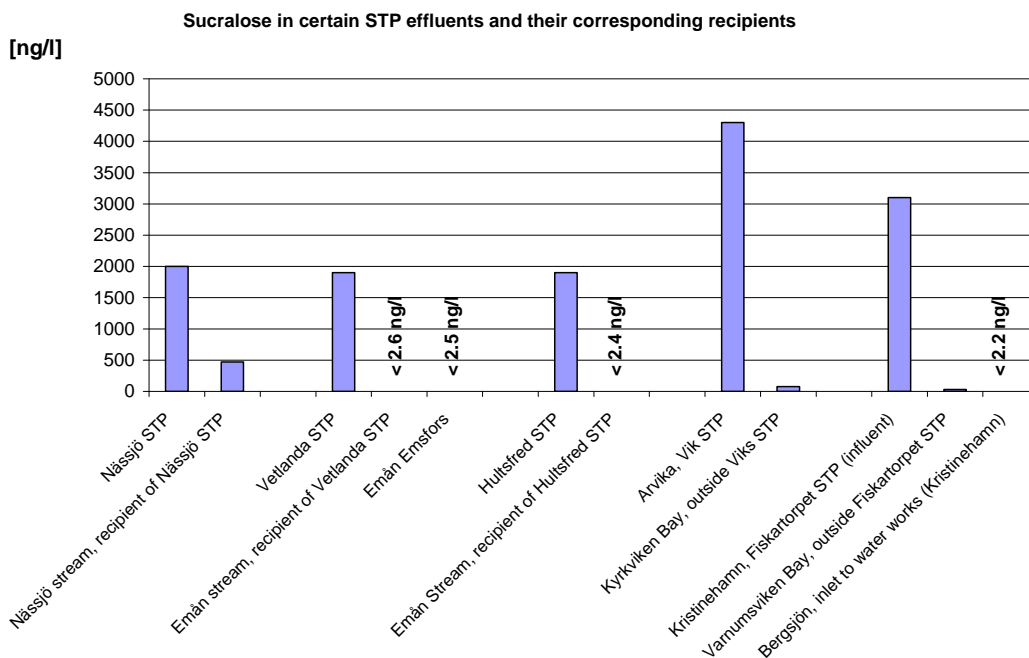


Figure 6. Sucralose concentration in a selected number of STP effluents where the corresponding STP water recipient have also been sampled and analysed. Note that the data from Fiskartorpet STP in Kristinehamn is based on the measurement in the STP influent water stream. However, as illustrated by figure 4; the sucralose STP influent concentration is probably of similar magnitude as the effluent concentration.

5.3 Biota samples

Sucralose was not detected in any of the collected samples from Stockholm and Linköping (fish and mussels). The LODs for these type of matrices (fish muscle, fish liver and mussel tissue) were between <0.3 and <0.7 ng/g fw.

Table 4. LODs in the individual biota samples.

Site	Species	Matrix	LOD
Stockholm Ström (negative control)	Mussels, (<i>Anodonta cygnea</i>)	Muscle tissue	<0.4 ng/g fw
Stockholm Ström (8 week exposure)	Mussels, (<i>Anodonta cygnea</i>)	Muscle tissue	<0.7 ng/g fw
Stockholm Ström	Fish, (<i>Perca fluviatilis</i>)	Muscle tissue	<0.3 ng/g fw
Stockholm Ström	Fish, (<i>Perca fluviatilis</i>)	Liver tissue	<1 ng/g fw
Linköping (outside Nykvarnsverket STP)	Fish, (<i>Perca fluviatilis</i>)	Muscle tissue	<0.6 ng/g fw
Linköping (outside Nykvarnsverket STP)	Fish, (<i>Perca fluviatilis</i>)	Liver tissue	<1 ng/g fw

6 Conclusions

The results of the sucralose screening study given in this report, part 2, further corroborates the impression from the report part 1, that sucralose is ever present in probably all STP water streams in Sweden. Altogether (report part 1 and 2) effluent samples from 54 different STPs in Sweden have been analysed with respect to sucralose and all effluent waters contained sucralose in µg/l concentrations (0.71-11 µg/l).

Out of, altogether, 13 paired influent-effluent measurements; the removal efficiency is negative in 10 cases. This clearly suggests that current cleaning process technology employed by ordinary Swedish STPs is not adequate to retain this substance from entering the environment.

In the part 1 report, a calculation based on the total annual STP effluent discharge in Sweden (1 362 917 000 m³) yielded an annual total discharge of sucralose to recipients in Sweden corresponding to 6.6 tonnes, based on the assumption that the median effluent concentration is representative for the average Swedish STP. When recalculating, also taking the STP effluent data presented herein, the total annual discharge of sucralose is 5.5 tonnes, given the uncertainties accompanying such a calculation.

STP sludge seems not to be any major sink for sucralose. If sufficiently dehydrated, STP sludge contains only very low residual concentrations of sucralose.

Sucralose was not found in any of the biota samples reported herein. In the case of fish, both liver and muscle tissue homogenisates were analysed without detecting sucralose.

Whether sucralose poses any environmental risks at the current level of use (at the measured environmental concentrations) is difficult to answer. Based on the eco toxicological data currently available, the measured environmental concentrations (MECs) reported in this study (part 1 and 2) do not suggest any environmental risks (MEC/PNEC < 0.00025). However, the eco toxicological dossier openly accessible contains only data on the chronic toxicity of sucralose with respect to *Daphnia magna*. Neither is it possible to address the issue of environmental risks associated with the abiotic and biotic transformation products of sucralose (at least two of them are chlorinated).

Due to the fact that sucralose is considered to be persistent (5 % degradation in an OECD 301E-test and 45 % degradation in 130 days in a soil inoculum, references in Brorström-Lundén et al., 2008) the current level of domestic use (estimated from STP effluent concentrations) may on a long term basis, lead to a build up of sucralose in the aquatic environment.

7 Acknowledgements

We thank staff members at the local municipalities that took part in the sampling of wastewater effluents and sludge. The study was funded Swedish Environmental Protection Agency together with the Swedish county administrative boards.

8 References

Brorström-Lundén E, Svenson A, Viktor T, Woldegiorgis A, Remberger M, Kaj L, Dye C, Bjerke A, Schlabach M. (2008), "Measurements of Sucralose in the Swedish Screening Program 2007 -PART I; Sucralose in surface waters and STP samples." IVL B1769, January 2008.

Sucralose - Environmental Assessment Amendment (FAP 8A4624), test performed by McNeil Specialty Products Company, 501 George Street, New Brunswick, NJ US. December 2, 1998.

Appendix Sample Characteristics and Results of Sucralose Analysis.

Category	# on map	Sample ID	Site	Matrix	Sampling date	County	Concentration (ng/l)
Reference lake water	42	6038	Gårdsjön	Surface water	070913	National	< 2
Reference lake water	43	6039	Härsevattnet	Surface water	070913	National	< 2
Surface water	1	6217	Karlshamn, influent to water works	Surface water	2007-09-25	Blekinge (K)	<6.5
Drinking water	1	6220	Karlshamn, from water works	Drinking water	2007-09-25	Blekinge (K)	<3.3
Sewage wastewater	1	6592	Karlshamn, Blekinge Hospital	Effluent ww	2007-10-31	Blekinge (K)	330
Surface water	2	6158	Karlskrona, influent to water works	Surface water	2007-09-25	Blekinge (K)	<6.7
Drinking water	2	6162	Karlskrona, effluent from water works, before active carbon adsorbent filter	Drinking water	2007-09-25	Blekinge (K)	<3.2
Drinking water	2	6165	Karlskrona, effluent from water works	Drinking water	2007-09-25	Blekinge (K)	<3.1
Leachate	2	6075	Karlskrona, the Bubbetorp Depot	Leachate ww	070905-12	Blekinge (K)	<8
Surface water	3	6310	Godthem stream, Gotland	Surface water	2007-10-01	Gotland (I)	<2.3
Sewage wastewater	4	6307	Visby STP, Gotland	Effluent ww	070925-1002	Gotland (I)	3900
Sewage wastewater	5	6269	Falkenberg STP, Falkenberg	Effluent ww	070919-25	Halland (N)	3800
Sewage wastewater	6	6317	Busör STP, Halmstad	Effluent ww	070924-1002	Halland (N)	1200
Sewage wastewater	6	6325	Västra Stranden STP, Halmstad	Influent ww	070924-1001	Halland (N)	2100
Sewage wastewater	6	6327	Västra Stranden STP, Halmstad	Effluent ww	070924-1001	Halland (N)	2500
Sewage wastewater	7	6452	Hyltebruk STP, Hylte	Effluent ww	2007-09-26	Halland (N)	710
Sewage wastewater	8	6236	Hammargård STP, Kungsbacka	Effluent ww	070919-25	Halland (N)	3500
Sewage wastewater	9	6240	Laholms STP, Laholm	Effluent ww	070918-24	Halland (N)	1900
Sewage wastewater	9	6245	Hedhuset STP, Laholm	Effluent ww	070918-24	Halland (N)	2100
Sewage wastewater	10	6064	Varberg STP, Varberg	Effluent ww	070904-10	Halland (N)	3900
Sewage wastewater	11	6544	Eksjö STP, Eksjö	Effluent ww	071015-22	Jönköping (F)	2100
Sewage wastewater	12	6547	Gislaved STP, Gislaved	Influent ww	2007-10-23	Jönköping (F)	1700
Sewage wastewater	12	6549	Gislaved STP, Gislaved	Effluent ww	2007-10-23	Jönköping (F)	2500
Sewage wastewater	13	6465	Simsholmen STP, Jönköping	Influent ww	071008-15	Jönköping (F)	2900
Sewage wastewater	13	6466	Simsholmen STP, Jönköping	Effluent ww	071008-15	Jönköping (F)	3500
Sewage wastewater	14	6285	Nässjö STP, Nässjö	Effluent ww	070924-30	Jönköping (F)	2000
Receiving water	14	6287	Nässjö stream, recipient of Nässjö STP	Surface water	070924-30	Jönköping (F)	470
Sewage wastewater	15	6580	Tranås STP, Tranås	Influent ww	071022-29	Jönköping (F)	2400

Category	# on map	Sample ID	Site	Matrix	Sampling date	County	Concentration (ng/l)
Sewage wastewater	15	6581	Tranås STP, Tranås	Effluent ww	071022-29	Jönköping (F)	3200
Sewage wastewater	16	6485	Vetlanda STP, Vetlanda	Effluent ww	071011-16	Jönköping (F)	1900
Receiving water	16	6487	Emån stream, recipient of Vetlanda STP	Surface water	2007-10-15	Jönköping (F)	<2.6
Receiving water	16	6486	Emån stream at Emsfors, Vetlanda	Surface water	2007-10-16	Jönköping (F)	<2.5
Sewage wastewater	16	6488	Emån stream, downstreams of Hultsfred STP (see sample ID 6259)	Surface water	2007-10-15	Jönköping (F)	<2.4
Sewage wastewater	17	6468	Värnamo STP, Värnamo	Influent ww	071008-14	Jönköping (F)	2300
Sewage wastewater	17	6470	Värnamo STP, Värnamo	Effluent ww	071008-14	Jönköping (F)	2600
Sewage wastewater	18	6258	Hultsfred STP, Hultsfred	Influent ww	070920-27	Kalmar (H)	2300
Sewage wastewater	18	6259	Hultsfred STP, Hultsfred	Effluent ww	070920-27	Kalmar (H)	1900
Sewage wastewater	19	6355	Högsby STP, Högsby	Effluent ww	07 okt	Kalmar (H)	1900
Sewage wastewater	20	6206	Kalmar STP, Kalmar	Influent ww	070922-26	Kalmar (H)	3700
Sewage wastewater	20	6207	Kalmar STP, Kalmar	Effluent ww	070922-26	Kalmar (H)	4500
Sewage wastewater	21	6271	Eskilstuna STP, Eskilstuna	Influent ww	070917-21	Södermanland (D)	4100
Sewage wastewater	21	6275	Eskilstuna STP, Eskilstuna	Effluent ww	070917-21	Södermanland (D)	4400
Sewage wastewater	21	6277	Eskilstuna STP, Eskilstuna (after wet land)	Effluent ww	070917-21	Södermanland (D)	3400
Sewage wastewater	22	6262	Flen STP, Flen	Effluent ww	2007-09-19	Södermanland (D)	4400
Sewage wastewater	23	6339	Brandholmen STP, Nyköping	Effluent ww	070925-1001	Södermanland (D)	4900
Sewage wastewater	24	6022	Strängnäs STP, Strängnäs (after wet land)	Effluent ww	070820-27	Södermanland (D)	4700
Sewage wastewater	25	6343	Trosa STP, Trosa (after wet land)	Effluent ww	070925-1001	Södermanland (D)	2800
Sewage wastewater	26	6190	Vik STP, Arvika	Effluent ww	070917-21	Värmland (S)	4300
Receiving water	26	6069	Kyrkviken Bay, Arvika, recipient of Vik STP	Surface water	2007-09-12	Värmland (S)	77
Sewage wastewater	27	6440	Fiskartorpet STP, Kristinehamn	Influent ww	071002-08	Värmland (S)	3100
Receiving water	27	6018	Varnums Bay, Station Kr70, recipient of Fiskartorpet STP, Kristinehamn	Surface water	2007-08-28	Värmland (S)	29
Surface water	27	6435	Bergsjön lake, Kristinehamn, influent to water works	Surface water	2007-10-08	Värmland (S)	<2.2
Sewage wastewater	28	6185	Hågesta STP, Sollefteå	Effluent ww	070917-24	V-Norrland (Y)	2500
Sewage wastewater	29	6032	Falköping STP, Falköping	Effluent ww	2007-08-31	V-Götaland (O)	3200
Sewage wastewater	30	6255	Skövde STP, Skövde	Effluent ww	070917-24	V-Götaland (O)	4800
Sewage wastewater	40	6845	Ryaverket STP, Göteborg	Effluent ww	080110	V-Götaland (O)	2800
Sewage wastewater	31	6472	Uddevalla STP, Uddevalla	Effluent ww	070925-1001	V-Götaland (O)	2600
Sewage wastewater	32	6135	Öckerö STP, Öckerö	Effluent ww	070911-17	V-Götaland (O)	4600
Sewage wastewater	33	6682	Skebæck STP, Örebro	Effluent ww	2007-11-06	Örebro (T)	4600
Sewage wastewater	34	6154	Axsäter STP, Finspång	Effluent ww	2007-09-17	Ö-Götaland (E)	4000

Category	# on map	Sample ID	Site	Matrix	Sampling date	County	Concentration (ng/l)
Sewage wastewater	35	6104	Karshult STP, Motala	Effluent ww	070910-16	Ö-Götland (E)	4600
Sewage wastewater	36	6058	Slottshagen SPT, Norrköping	Effluent ww	2007-09-05	Ö-Götland (E)	4800
Sewage sludge	24	6026	Strängnäs STP, Strängnäs	Sludge (20.1 % DW)	070820-27	Södermanland (D)	11 (ng/g ww)
Sewage sludge	22	6264	Flen STP, Flen	Sludge (14.2 % DW)	2007-09-19	Södermanland (D)	19 (ng/g ww)
Sewage sludge	21	6279	Eskilstuna STP, Eskilstuna	Sludge (27.4 % DW)	070917-21	Södermanland (D)	<0.3 (ng/g ww)
Sewage sludge	6	6326	Västra Stranden STP, Halmstad	Sludge (27.8 % DW)	070924-1001	Halland (N)	<0.3 (ng/g ww)
Sewage sludge	33	6683	Skebäck STP, Örebro	Sludge (31.1 % DW)	2007-11-06	Örebro (T)	6 (ng/g ww)
Sewage sludge	37	6202	Säffle STP, Säffle	Sludge (20.9 % DW)	2007-09-18	Värmland (S)	<0.3 (ng/g ww)
Sewage sludge	58	6284	Sjöstad STP, Karlstad	Sludge (30.2 % DW)	2007-09-28	Värmland (S)	<0.3 (ng/g ww)
Sewage sludge	27	6437	Fiskartorpet STP, Kristinehamn	Sludge (22.3 % DW)	2007-10-08	Värmland (S)	4 (ng/g ww)
Biota	41	6020	Mussels, (<i>Anodonta cygnea</i>), Stockholm Ström (negative control)	Tissue	2007-08-30	National	<0.4 ng/g fw
Biota	41	6577	Mussels, (<i>Anodonta cygnea</i>), Stockholm Ström (8 week exposure)	Tissue	2007-10-31	National	<0.7 ng/g fw
Biota	59	5729a	Fish, (<i>Perca fluviatilis</i>), Linköping (Nykvarnsverket STP)	Muscle tissue	2007-06-19	National	<0.6 ng/g fw
Biota	59	6394a	Fish, (<i>Perca fluviatilis</i>), Stockholm Ström	Muscle tissue	2007-10-05	National	<0.3 ng/g fw
Biota	59	5729b	Fish, (<i>Perca fluviatilis</i>), Linköping (Nykvarnsverket STP)	Liver tissue	2007-06-19	National	<1 ng/g fw
Biota	41	6394b	Fish, (<i>Perca fluviatilis</i>), Stockholm Ström	Liver tissue	2007-10-05	National	<1 ng/g fw
Sewage wastewater	39	5058*	Krylbo STP, Avesta	Effluent ww	2006-10-10	Dalarna	5400

* The effluent water sample from Krylbo STP in Avesta was reported as ">2100 ng/l" due to a Force majeure in report part 1, and has now been re-analysed.