

Measurements of Sucralose in the Swedish Screening Program 2007

-PART I; Sucralose in surface waters and 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 I; Sucralose in surface waters and STP samples.</p>	
<p>Summary</p> <p>IVL has performed a "screening study" of sucralose on commission from the Swedish EPA. 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 related to wastewater effluents and to highlight important transport pathways. In total 57 samples were analysed representing wastewater and sludge from sewage treatment plants as well as surface waters.</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 screening programme included measurements in background areas and close to potential point sources. Sample types included untreated and treated wastewaters, sewage sludge, and surface water samples. In total 57 samples were analysed.

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

	STP Influent waters (ng/l)	STP Effluent waters (ng/l)	Receiving waters (ng/l)	Sludge (ng/g ww)
# of samples	6	29	15	6
Sucralose	3 530-7 920	1 790-10 800	<4-3 560	<1-15
DF (%)	100	100	73	83

DF = Detection frequency

STP = Sewage Treatment Plant

From this study it can be concluded that;

- Sucralose was detected in Swedish surface waters receiving wastewater effluents.
- Untreated municipal wastewater (2 STPs) contained 3 500-7 900 ng sucralose/l.
- Wastewater treatment processes had little effect on sucralose, removal rates < 10 % in all paired samples (influent/effluent).
- Sucralose was detected in all 29 effluent samples, from 25 STPs throughout Sweden; 1 800 to 10 800 ng/l, with a median of 4 900 ng/l.
- Sucralose was not significantly accumulated in sewage sludge.
- Surface water from reference lakes and water courses upstream of STP effluents contained no measurable sucralose, < 4 ng/l.

This report on sucralose in aqueous samples constitutes the first part of a thorough screening study of sucralose in the Swedish environment. The study will be concluded in a forthcoming, second report where the uptake of sucralose in aqueous biota will be examined.

Sammanfattning

IVL har 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 Europaunionen, 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. Totalt bestämdes sukralos i 57 prover.

Nedan visas en tabell med uppmätta halter i olika provtyper.

	Inkommande avloppsvatten (ng/l)	Utgående vatten (ng/l)	Ytvatten (ng/l)	Slam (ng/g våtvikt)
# prov	6	29	15	6
Sukralos	3 530-7 920	1 790-10 800	<4-3 560	<1-15
DF (%)	100	100	73	83

DF=Detektionsfrekvens

Studien visar att;

- Sukralose detekteras i vattenrecipienter i Sverige som tar emot utgående vatten ifrån reningsverk.
- Inkommande vatten till svenska avloppsreningsverk (2 ARV) innehåller 3 500-7 900 ng sukralos/l.
- Reningsgraden m a p. sukralos är låg i reningsverk, maximalt uppmättes 10 % reningsgrad i de parade prover som analyserats (inkommande/utgående).
- Sukralos detekterades i alla de 29 utgående reningsverksvattenproverna ifrån 25 olika reningsverk i landet; 1 800-10 800 ng/l, median 4 900 ng/l.
- Det sker ingen ackumulering av sukralos i slam.

Denna rapport utgör den första delen av en fördjupad screening av sukralos i den svenska miljön. I en fortsättande, kommande rapport kommer resultat ifrån undersökningar av sukralosupptag i akvatiska biotaprover att redovisas.

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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. This substance is used as a sweetener in food products. Sucralose 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 this screening study was to determine concentrations of sucralose 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 uptake and distribution of sucralose in biota.

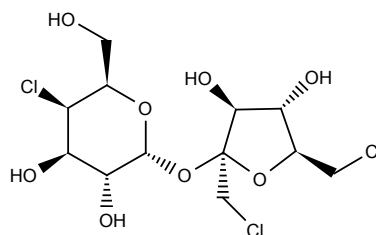
2 Chemical properties, fate and toxicity

2.1 Properties and fate

Pure sucralose is an odourless, white crystalline powder. Figure 1 shows the full name, CAS number, and molecular structure of the substance. The physical and chemical properties of sucralose are summarised in Table 1.

Sucralose: 1,6-Dichloro-
1,6-dideoxy- β -D-
fructofuranosyl-4-chloro-
4-deoxy- β -D-
galactopyranoside

56038-13-2



Source for structure: ChemID plus

Figure 1. Name, CAS Number, and Molecular Structure of Sucralose

Table 1. Physico-chemical properties of Sucralose

Property	Value	Source	
MW (g/mol)	397,64	a	
Melting Point (°C)	130	a	
Vapour Pressure (mm Hg)	3.25×10^{-14}	b	
Water Solubility (g/l)	110	a	
Henry's Law Constant (atm·m ³ /mole)	3.99×10^{-19}	c	
Log Kow (estimated)	-1.0	d	
Degradation	Time (d)	Mineralized (%)	
Lake water	42	1.2	e
	77	1.7-3.6	e
Soil	20	33-37	e
	69	56-60	e
Sewage	123	23	e

a Meylan et al. 1996

d Meylan, Howard 1995

b Neely, Blau 1985

e Labare, Alexander 1993

c Meylan, Howard 1991

The physical and chemical properties indicate that the substance is readily soluble in water and that it is not likely to vaporize. High aqueous solubility and low K_{OW} are properties that prevent accumulation in fat tissues or other hydrophobic compartments in the environment.

The substance has a slow transformation and mineralisation in the environment (Table 1). The degradation of sucralose in aqueous solution (hydrolysis rate) was investigated at 62, 50, 40 and 30 °C at pH 1, 1.5, 2 and 3 to determine the stability of sucralose in beverages. The rate of hydrolysis increased with temperature and decreasing pH, e.g., at pH 1 and 62 °C, 98.8% of sucralose was hydrolysed after 120 hours, at pH 2 and 62 °C only 30.4% was hydrolysed after 120 hours. This study dealt with low pHs, whilst in the environment pH ranges from 4 to 9, therefore significant hydrolysis is unlikely to occur (Tate and Lyle Group Research and Development, 1983 from NA/944, 2001).

In an OECD TG 301E test (biodegradation test in an aerobic aqueous media) the results indicated that 5% of the sucralose had degraded in 28 days. The results indicated that sucralose was not readily biodegradable. Furthermore the biodegradability of sucralose in a sediment/water system and water system was investigated by Imperial Chemicals Industries PLC (1987, referred to in NA/944, 2001). The biodegradation in this investigation was determined by the measurement of the ¹⁴CO₂ generated from the medium after it was inoculated with either soil or micro-organisms population (activated sludge), and shaken at 20°C for 130 days. For two of the soil inocula, the results indicated that approximately 56 days was required for microbial adaptation before degradation began, thus resulting in 63 and 45.2% degradation by day 130. With the third soil inoculum the adaptation period appeared to be 100 days, with 14.2% degradation by day 130. No degradation was observed in the activated sludge inoculum. These results indicate that sucralose is inherently biodegradable but not readily degradable (NA/944, 2001).

The primary hydrolysis products of sucralose, 4-chloro-4-deoxygalactose (4-CG) and 1,6-dichloro-1,6-dideoxyfructose (1,6-DCF) are also themselves resistant to hydrolysis (Figure 2). Besides these two metabolites two other chlorinated conversion products have been found: an unsaturated aldehyde and an uronic acid (Anon. 2000). The chlorine modification also makes the sucralose molecules resistant to glucoside formation in the mammalian body.

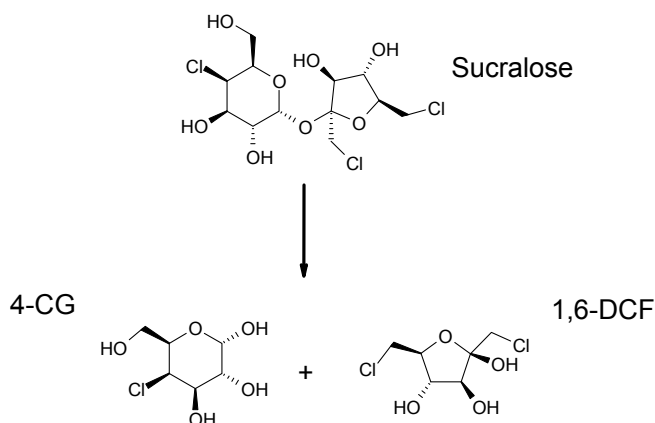


Figure 2. Degradation pattern of sucralose upon hydrolysis

2.2 Toxicity, human exposure and metabolism

An Acceptable Daily Intake or ADI is a measure of the amount of a specific substance (usually a food additive, or a residue of a veterinary drug or pesticide) in food or drinking water that can be ingested (orally) over a lifetime without an appreciable health risk. ADIs are expressed by body mass, usually in milligrams (of the substance) per kilograms of body mass per day. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) allocated an ADI for sucralose of 15 mg/kg body weight daily, based on a NOEL of 1500 mg/kg body weight in a long term rat study and a safety factor of 100 (JECFA 1991).

In a study on rats it was concluded that the animals avoided food with high concentrations of sucralose. No other adverse effects were observed in this study (Anon, 2000).

Teratology studies with rats and rabbits showed no adverse developmental effects even with top doses of sucralose, 2000 and 700 mg/kg body weight/day, respectively (Anon. 2000). Although earlier studies had indicated some effect on the immune system, a later study showed no immunotoxicity effects even at the highest dose, 3000 mg/kg body weight/day (ibid.). Neither sucralose nor its chlorine containing primary degradation products, 4-CG and 1,6-DCF, were found to be mutagenic (ibid.).

Sucralose administered orally is poorly absorbed in mammals like mice, rats, rabbits, dogs or in man (Anon. 2000). Absorption in man ranged from 8 to 22 % of the administered dose, and this fraction was rapidly excreted in urine. The half-life for the elimination of a single dose in man was 25 h. No significant conversion was observed.

Regarding the eco toxicity of sucralose the rather few test results reported indicate a low toxicity towards the species of the three standard trophic levels (Table 2).

Table 2. Ecotoxic parameters of sucralose

Species	Endpoint	Toxicity [mg/l]	Ref
Bluegill sunfish	96 h Acute toxicity	LC50 >3200	NA/944; Imperial Chemicals Industries PLC (1985a).
Daphnia magna	48 h Acute toxicity	EC50 >1800	NA/944; Aquatox Ltd (1984c).
Green Algae	96 h Acute toxicity	E _p C50 >1800	NA/944; Imperial Chemicals Industries PLC (1986b).

3 Production, consumption, emissions and regulation

Sucralose was first synthesized and the sweet taste discovered in 1976 (Nandini on-line Journal, 2007). It has been used commercially in Canada since 1991 and in 1998 it was approved by the US Food and Drug Administration. Today it is used as a sweetening food additive in more than 4000 different food products. Many are liquid food products such as soft drinks and dairy products but sucralose is also used as a sweetener in products like ketchup and cereals (Arla Pressinformation, 050215).

3.1 National and EU legislation

Sucralose has been approved as a food additive by authorities in more than 40 countries (to 2006), Table 3.

Table 3. Compilation of nations that have approved the use of sucralose as a food additive (to 2006)

Argentina	Greece	New Zealand	Switzerland
Australia	Guatemala	Nicaragua	Sweden
Brazil	Honduras	Pakistan	Tadsjikistan
Canada	Hong Kong	Panama	Taiwan
Chile	Indonesia	Paraguay	Trinidad and Tobago
China	Jamaica	Peru	United Kingdom
Colombia	Japan	Republic of South Africa	Uruguay
Costa Rica	Jordan	Romania	USA
Dominica	Lebanon	Russia	Venezuela
Dominican Republic	Malaysia	Singapore	Vietnam
Georgia	Mexico	South Korea	

In Sweden sucralose has been approved by the Swedish Food Administration. In declarations of contents of food products sucralose has been given the code E-955. Due to the lack of scientific evidence regarding harmful effects, a daily intake of not more than 15 mg/kg bodyweight is recommended (according the recommendations by the European Union Scientific Committee).

4 Previous measurements in the environment

Sucralose has not been measured in Swedish environmental samples before. Data from Norwegian studies showed levels from some ng/l to several µg/l (Table 4).

Table 4. Measured concentrations of sucralose in Norwegian environmental samples (Dye C et al., 2007)

Matrix	Sucralose
Sewage sludge [ng/g ww]	1-20
Sewage effluents [µg/l]	0.4-7.3
Biota [µg/kg dw]	-

5 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 programme included both measurements in background areas and close to potential sources.

However, due to the high aqueous solubility of sucralose, the sampling strategy was focussed on effluents into the aquatic environment. A primary source related to consumption of food products containing sucralose would be municipal sewage wastewater. Therefore samples from water flows to and from sewage treatment plants (STPs) as well as surface waters receiving such effluents were included.

Two STPs were selected for investigations of possible impacts in receiving waters, Henriksdal STP in Stockholm and Nykvarnsverket STP in Linköping, where influent and effluent wastewater and sewage sludge were collected on three occasions.

Recipient water close to Henriksdal STP was examined by analysis of surface water samples at six locations (Figure 3).

Nykvarnsverket receives wastewater from a dairy production plant using sucralose as an additive in the production. Lake Roxen, the main receiving water from Nykvarnsverket STP was examined by sampling and analysis of surface waters of the lake and the tributaries Svartån, Göta Canal and Stångån (Figure 4). The latter, at which the STP is situated, was sampled both up- and downstream of the outlet of treated wastewater ('effluent discharge point').



Figure 3. Map of the aqueous sample locations in Stockholm. At location 4 (‘Stockholms Ström, Finnboda varv’), the major STP in the region, Henriksdal STP, has its effluent discharge point. At that location another large STP plant, Bromma STP, also has its effluent discharge point. The general flow direction (depending on pertaining wind conditions) is from the lower left part of the map, diagonally, to the upper right part of the map (through Halvkakssundet). Location 6 (‘Stockholm, Hammarby sjö’) is thus upstream of the STP discharge point, but is also connected to Lake Mälaren (large water recipient for other STPs such as Strängnäs, Eskilstuna and Västerås). Locations 5 (‘Stockholms Ström, Kvarnholmen’) and 3 (‘Stockholms Ström, Täckas Udden’) are downstream and in the proximity of the discharge point. Location 2 (‘Stockholms Ström, Mölna strand’) and 1 (‘Stockholms Ström, Käppala’) are further downstream in the direction of the discharge plume of the Henriksdal STP. Location 1 and 2 are simultaneously upstream locations with respect to the Käppala STP (not shown in this map).

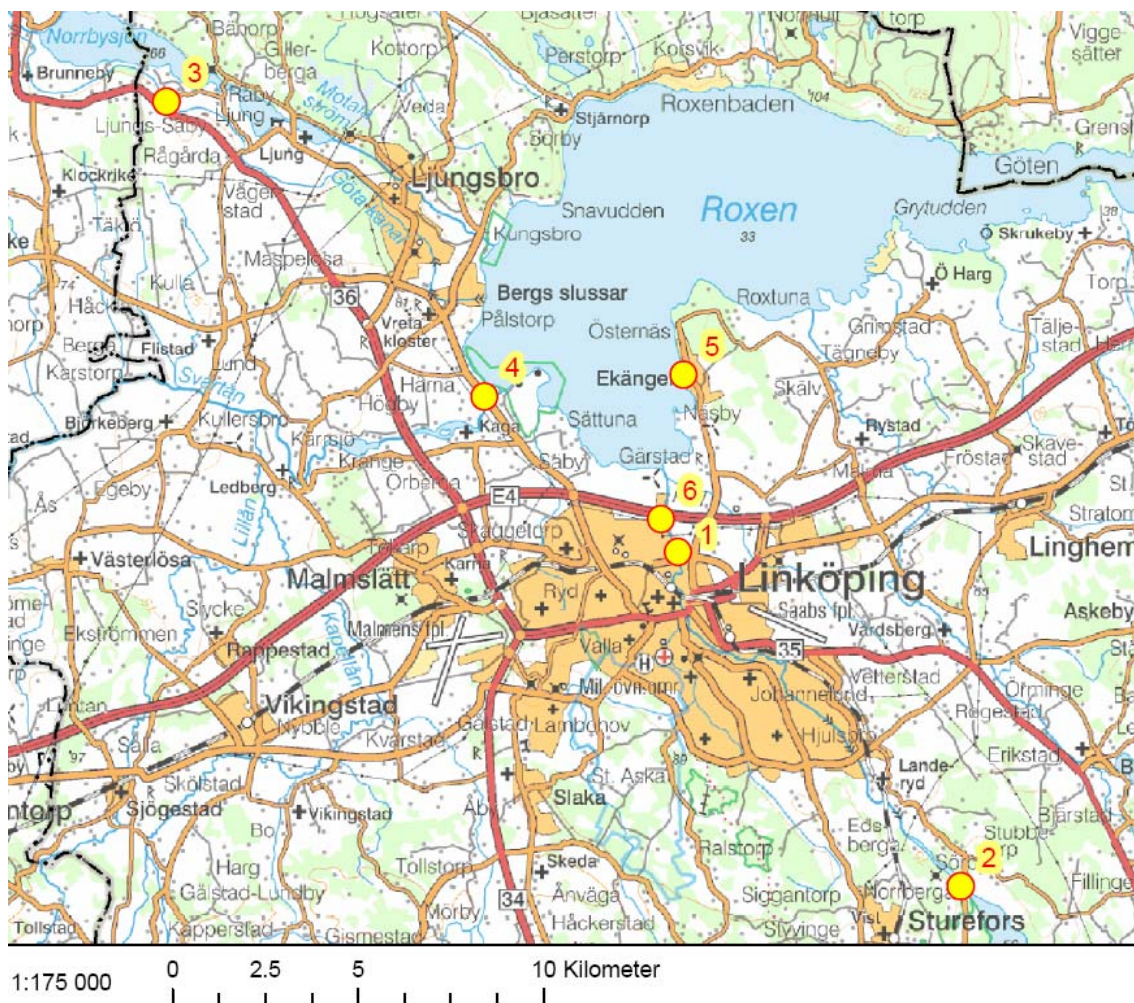


Figure 4. Map of the aqueous sample locations in Linköping. At sampling location 1 (Linköping, Stångån, downstream STP), the Nykvarnsverket STP has its effluent discharge point. Location 2 (Linköping, Stångån, Årlången, upstream STP) is directly upstream of the STP (flow direction is always from 2 to 1). Sampling locations 6 (Linköping, Stångåmyningen, upstream Lake Roxen) and 5 (Linköping, Lake Roxen, Ekängen) are both downstream locations with respect to the STP effluent discharge point. Location 4 (Linköping, Svartåmyningen, upstream Lake Roxen) is a downstream location with respect to smaller STPs along Svartån but not likely to be affected by the Nykvarnsverket STP effluent discharge. Sampling location 3 (Linköping, Göta Canal, Sjöbacka, upstream Lake Roxen) is a downstream sampling point with respect to smaller STPs in the Motala ström region, but not likely to be affected by the Nykvarnsverket STP effluent discharge.

The sampling program also included effluent water samples from another 24 STPs which differ in size and are from different regions in Sweden. Seven of the selected STPs are included in the Swedish Environmental Protection Agency's monitoring programme for environmental pollutants in sludge (Naturvårdsverkets miljöövervakningsprogram av miljögifter i slam).

In order to determine background levels, reference sites were chosen in lakes with no known impacts from wastewater effluents. The sampling programme is summarized in Table 5.

Table 5. Sampling programme

Site	Sewage wastewater, untreated	Sewage wastewater, treated	Surface water	Sludge	Total
Background					
Reference lakes			3		3
Point sources					
STP of densely populated region (Sthlm)	3	3	6	3	15
STP, affiliated to dairy industry using sucralose	3	3	6	3	15
Municipal STPs		24			24
Total	6	30	15	6	57

Individual samples are listed in the Appendix

6 Methods

6.1 Sampling

6.1.1 Surface water and sewage wastewater

Surface water samples were taken at 0-1 m depth. The samples were collected in 1 l polyethylene bottles containing 2 ml 6 M phosphoric acid.

Flow proportional 24 hour composite samples of untreated and treated municipal sewage wastewaters were collected in the same type of bottles. The effluent water was sampled after the influent samples, compensating for the hydraulic retention time (HRT, 20 h at both sites) in the sewage treatment plant. The samples were frozen and stored at -18 °C until extracted.

6.1.2 Sewage sludge

At Nykvarnsverket STP sludge was sampled directly from an anaerobic reactor (one sample) and also after dehydration by centrifugation (two samples). At Henriksdal STP, composite monthly samples of dehydrated sludge from anaerobic reactors (DW approximately 30%) further dried by evaporation were collected. The sludge was transferred into acid rinsed, polypropylene jars and stored in a freezer at -18 °C until extracted.

6.2 Analytical procedures

The methods for sample pre-treatment and analysis were adopted from published procedures developed for analysis of food products (Hatano and Nako 2002, Koyama et al. 2005).

6.2.1 Surface water and sewage wastewater

Samples of surface water (1000 ml) or municipal sewage wastewater (200 ml) were acidified to pH 3 with HCl and filtered through glass fibre filters (GF/C). Samples were then solid phase extracted on to columns (Oasis HLB, 200 mg from Waters Corp., Milford, USA, preconditioned with acetonitrile and diluted HCl). After washing with diluted HCl (10 mM) the columns were dried by suction (5 min) and frozen at -18 °C. Some samples were treated in duplicate, with and without spiking for evaluation of the method efficacy and losses in the work-up procedure. An aliquot of 100 µl of a 1 µg/ml sucralose solution was added to the replicate prior to the solid phase extraction. The columns were then sent to the analytical laboratory.

Before analysis sucralose was eluted from the columns with 7 ml of acetone: methanol (5:1, v/v). To remove matrix compounds the extract was cleaned by passing through a mixed-mode ion exchange SPE-cartridge (Isolute-MM, IST, Mid Glamorgan, UK). Any sucralose remaining in the SPE-resin was washed out with additional acetone: methanol, which was pooled with the extract. The extract volume was then reduced to 1 ml with a Zymark TurboVap II Concentration Workstation (Caliper Life Sciences, Hopkinton, MA, USA). Further extract clean up was performed by passing the extract through another mixed mode ion exchange SPE cartridge (Oasis MAX, Waters Corp., Milford, MA, USA) with subsequent extract volume reduction.

6.2.2 Sewage sludge

Sewage sludge samples (5 g) were shaken in 50 ml diluted HCl for 30 min (if suspension after this procedure was not acidic, the pH was adjusted and the shaking procedure repeated for 10 min). The suspension was then centrifuged at 450 x g for 5 min, and the liquid phase collected. This aquatic sample was then treated as described for water samples (6.2.1).

6.3 Instrumentation

6.3.1 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 6. Sucralose ions used for HPLC/HRMS (ES-) analysis

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

6.3.2 Analytical Quality Control

At the time the project sampling commenced, no isotope labeled sucralose was commercially available. Therefore, selections of the sample matrices were spiked with a known amount of sucralose and the recovery was calculated in order to evaluate the method performance. In Table 7 the results is given for the spiking experiments.

Table 7. Results from recovery experiments

Matrix	Spiked amount (ng)	Average Recovery	Standar dev.	# of exp.
Sludge	200	70 %	± 30	3
Sewage water	200	93.5 %	± 23	2

7 Results and Discussion

The concentrations of sucralose in individual samples are given in Appendix 1 together with sample characteristics.

7.1 Sewage wastewaters

Sucralose was detected in all the untreated wastewater samples from the two STPs, Nykvarn and Henriksdal, where the concentrations varied between 3500 and 7900 ng/l (Figure 5). The sucralose concentrations in samples taken at one month intervals at Henriksdal STP varied between 7600 and 7900 ng/l, whereas those in three samples taken at Nykvarnsverket in Linköping varied within 3500 – 6000 ng/l. The levels were lower in the latter STP despite the fact that this plant received effluents from a dairy production plant using sucralose in its production. However diffuse dispersion from STPs in urban areas seems to be a more important contributor to the sucralose concentrations found in STP waters. Henriksdal is located in the most densely populated area in Sweden, receiving wastewater from approximately 800 000 personal equivalents.

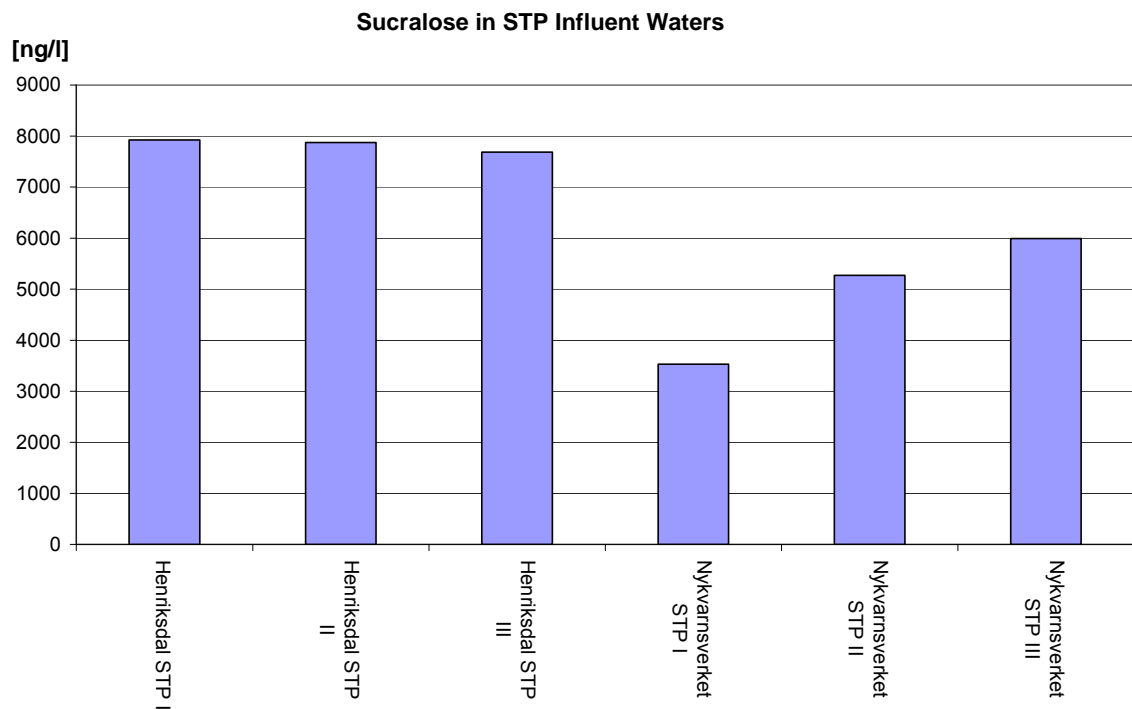


Figure 5. Sucralose concentrations in municipal sewage wastewater before treatment in two Swedish STPs

Treated municipal sewage wastewater also contained sucralose. In 29 effluent water samples from 25 different STPs the concentrations varied from 1800 to 10800 ng/l, with a median of 4 900 ng/l. (Figure 6).

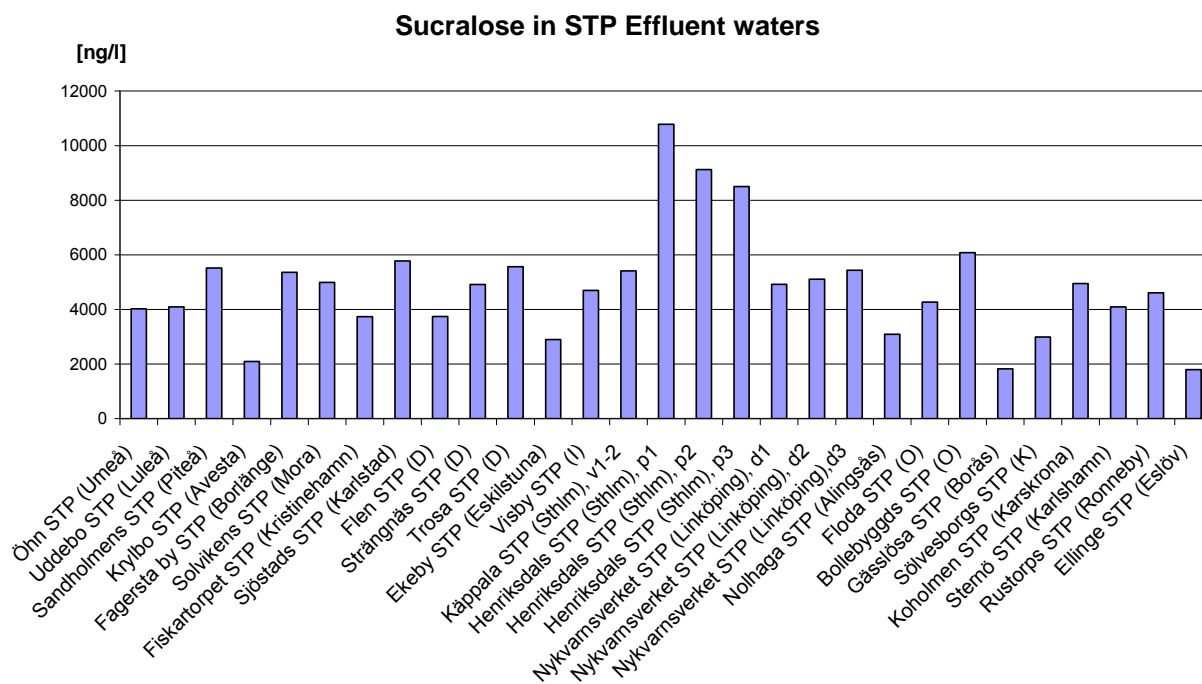


Figure 6. Sucralose concentrations in treated municipal sewage wastewaters.

The STPs in Figure 6 are arranged according to the location, from north to south (left to right). The three highest concentrations of sucralose were obtained in the effluent water samples from Henriksdal STP in Stockholm, the most densely populated area in Sweden, receiving wastewater from approximately 800 000 personal equivalents.

The annual water discharge from all STPs in Sweden is 1 362 917 000 m³ (value for the year 2000, SCB 2004). If the median sucralose concentration found in this study is representative for all STPs in Sweden the annual total discharge would correspond to 6.6 tonnes.

The removal efficiency with respect to sucralose in the two investigated STPs, Henriksdal and Nykvarnsverket, was poor. In fact most effluent concentrations were higher than in the corresponding influent wastewaters. Sucralose removal efficiency was at most 10 %, but in four cases negative values from -10 to -40 % were obtained. Negative removal of substances in the STP may indicate that a fraction of the incoming load is conjugated or complexed, and that the conjugates (or complexes) are re-transformed back to the mother compound somewhere along the STP process.

As previously stated, the sampling of STP water samples were conducted in such a manner that the hydraulic retention time (HRT) of each sampled STP was taken into consideration. Thus, both measured influent and effluent concentrations should originate from the same “water package”.

7.2 Reference surface waters and effluent receiving waters

Sucralose was, as expected, not detected in surface water from the background reference lakes (Figure 7). Stockholm Ström, receiving effluents from Henriksdal, Bromma and sometimes also the Käppala STP, contained 400 – 900 ng/l sucralose in its surface water. The concentrations were 10-15-fold lower than the STP effluent water.

Sucralose was not detected in Stångån upstream Linköping (Linköping loc 2, Figure 4). At the discharge point of Linköping STP in Stångån (Linköping loc 1) the sucralose concentration was 3600 ng/l. This corresponds to a dilution of the effluent water of only 1:1.4. Further downstream in Stångån (Linköping loc 6) the concentration was 410 ng/l, thus consequently a dilution of 1:12. Stångån then falls into Lake Roxen and at two sampling points in Roxen (Linköping loc 4 and 5) the sucralose concentration was 62 and 52 ng/l respectively. An additional flow into Roxen is Motala ström. No sucralose was found in the sampling site upstream of Lake Roxen (Sjöbacka Linköping loc 3).

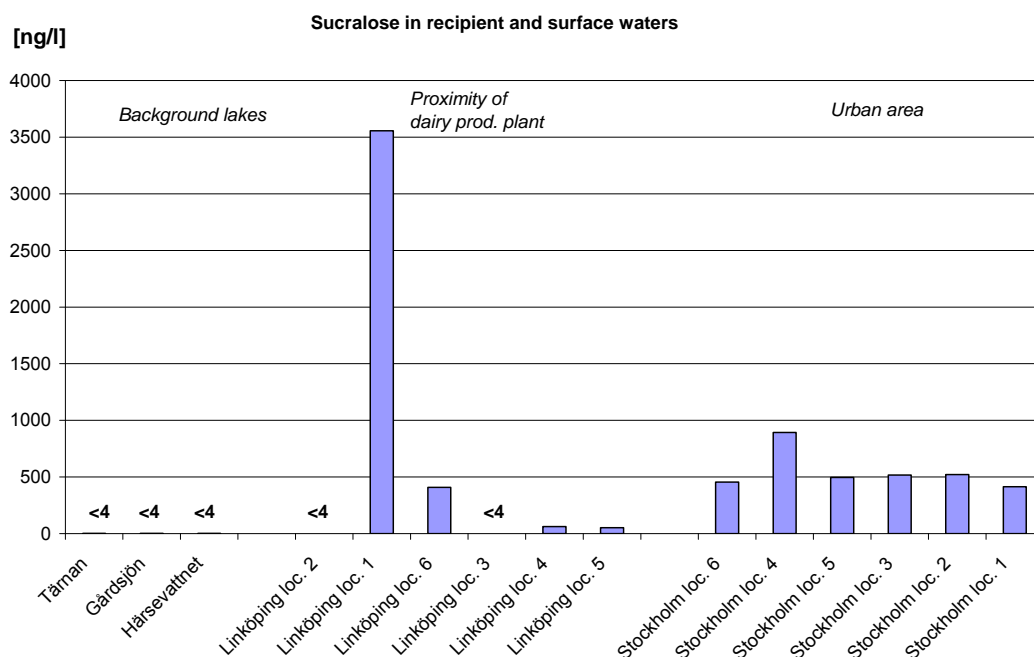


Figure 7. Sucralose concentrations in reference surface waters and effluent receiving waters. The sampling locations in Linköping and Stockholm (see Figure 3 and Figure 4 respectively) have been plotted in order of appearance with respect to the prominent water flow direction.

To put this detected concentrations in some perspective the following comparison can be made.

The average sucralose concentration in soft drinks and beverages sold in Norway is around 100 mg/l and the average person in Norway is estimated to drink some 5 liters of such soft drinks per year (Dye C et al., 2007). Thus, some 1.4 mg of sucralose is excreted per person and day (corresponds to 14 ml of Coca Cola light per person and day). Using this number for Sweden, a total annual consumption of 4.5 tonnes can be expected in Sweden (9 million inhabitants). This can

be converted to a standard Predicted Environmental Concentration (PEC, equation from EMEA ERA 2002 draft guidance) of 700 ng/l in Swedish surface waters. The predicted concentration is thus comparable with the concentrations presented in Figure 7. The concentrations detected in this study (Measured Environmental Concentrations, MECs) in surface waters in Stockholm, thus corroborates this calculation.

Furthermore, when a MEC-value is derived from STP-effluent concentrations (median effluent concentration diluted 10 times), the calculation yields a national MEC-value of 490 ng/l of sucralose (based on 25 STP effluents).

The surface water concentrations of sucralose in this study ($\sim 0.5 \mu\text{g/l}$) is more than 3 orders of magnitude lower than the lowest PNEC value of aquatic species hitherto reported for sucralose (*Daphnia magna*, $\sim 2 \text{ mg/l}$).

When comparing the measured concentrations of sucralose in surface waters with other common pollutants with low log Kow-values (polar compounds), it is interesting to note that sucralose is being detected in concentrations similar to several high-volume use substances (Figure 8).

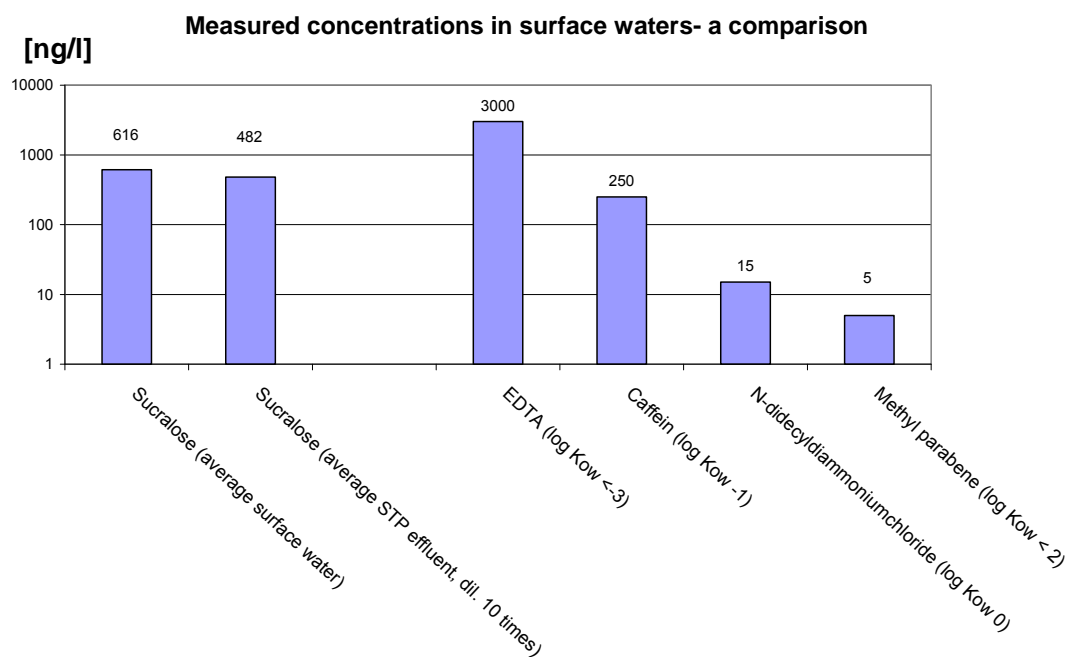


Figure 8. Comparison between the average surface water concentration of sucralose (12 samples), the nominal surface water concentration based on STP effluent concentrations diluted by a factor of 10 (29 samples), and the reported surface water concentrations of 4 other polar high-volume chemicals; EDTA (CAS 60-00-4, reported in Remberger 2001) is annually used in quantities of 14-58 tonnes (SPIN database 1999-2005), caffeine (CAS 58-08-2, reported in Woldegiorgis et al., 2007) is annually used in quantities of 15-27 tonnes (SPIN database 1999-2005, caffeine from coffee and beverages not included), N-didecyldiammoniumchloride (CAS 7173-51-5, reported in Remberger et al., 2006) is annually used in quantities of 13-31 tonnes (SPIN database 1999-2005), and methyl parabene (CAS 99-76-3, reported in Remberger et al., 2006) is annually used in quantities of 30-59 tonnes (SPIN database 1999-2005). Note that the ordinate axis is logarithmic.

7.3 Sewage sludge

Sucralose occurred at low concentrations in the sludge collected at Henriksdal STP in Stockholm and Nykvarnsverket STP in Linköping. The measured concentrations are shown in Figure 9.

The sucralose concentration in effluent water (5 000 - 10 000 ng/l) corresponds to 5 - 10 ng/g. This is also the concentration range (<1 - 15 ng/g w w) measured in the sludge. The values are close to the method detection limit and the analytical uncertainty is thus large. However, these results indicate that there was no significant accumulation to the solid material in the sludge.

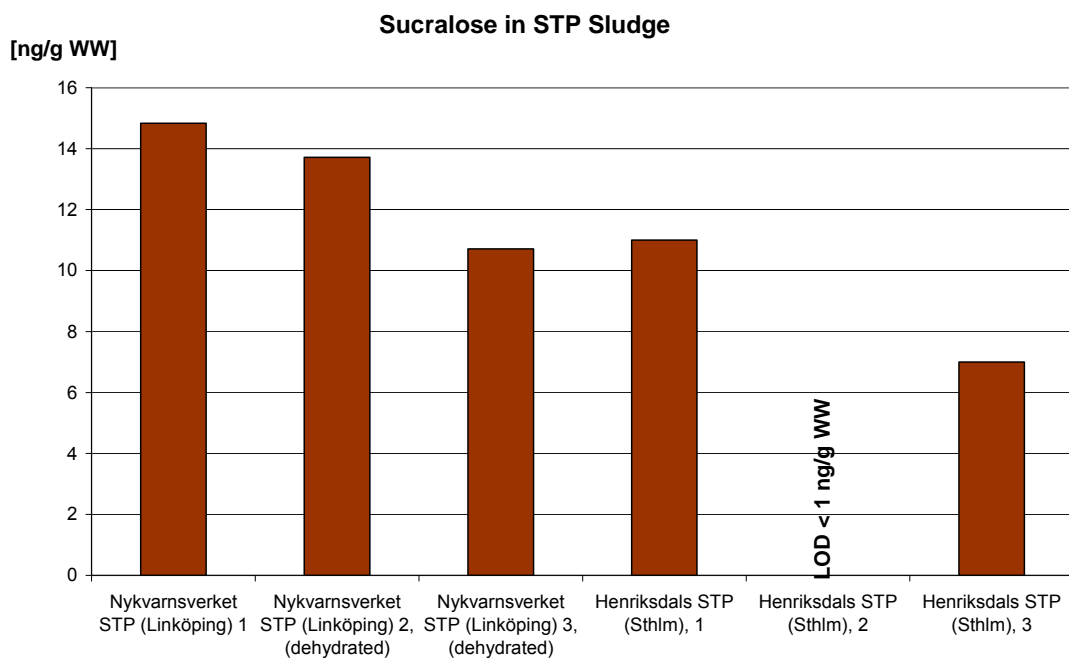


Figure 9. Concentration of sucralose in sludge from two municipal STPs.

8 Conclusions

- Sucralose was detected in Swedish surface waters receiving wastewater effluents.
- Untreated municipal wastewater (2 STPs) contained 3500-7900 ng sucralose/l.
- Wastewater treatment processes had little effect on sucralose removal rates.
- Sucralose was detected in all 29 effluent samples, from 25 STPs throughout Sweden; 1800 to 10800 ng/l, with a median of 4 900 ng/l.
- Sucralose was not significantly accumulated in sewage sludge.
- Surface water from reference lakes and water courses upstream of STP effluents contained no measurable sucralose, < 4 ng/l.

Sucralose was detected in all STP influent and effluent samples. The concentrations of sucralose in effluent water samples were up to several µg per litre. In some instances the effluent concentration exceeds the corresponding influent concentration from the same STP. When possible, the STP removal rate have been calculated and sucralose is poorly retained in the STP (% removed; -40 – 10 %). Negative removal of substances in the STP may indicate that a fraction of the incoming load is conjugated or complexed, and that the substance-conjugates (or complexes) are re-transformed back to the mother compound somewhere along the STP process.

Sucralose was also occasionally detected in STP sludge. However the concentrations were rather low and it is important to note that the sludge samples analysed within this study are primary sludge ('non-processed') or dehydrated sludge with a large residual water contents. Re-calculating the sludge data with respect to solid contents indicates that the water fraction of the sludge samples contain sucralose concentrations similar to the effluent concentrations measured within this study.

The annual water discharge from all STPs in Sweden is 1 362 917 000 m³ (value for the year 2000, SCB 2004). If the median sucralose concentration found in this study is representative for all STPs in Sweden the annual total discharge of sucralose to recipients in Sweden would correspond to 6.6 tonnes, given the uncertainties accompanying such a calculation.

When compared with other high-volume use substances of a similar polarity used in Sweden, the reported surface water concentrations of sucralose herein are comparable with surface water concentrations previously reported for caffeine, a chemical being used in quantities of at least 15-27 tonnes annually in Sweden. Caffeine is, contrary to sucralose, proven to be almost completely removed from STP influent waters (removal rate > 95 %).

At this stage it is also a bit premature to conduct the MEC/PNEC-based risk assessments; the limited number of eco-toxicological data (3 endpoints, all acute) suggests that the measured environmental concentrations of sucralose in surface water (~ 0.5 µg/l) is at least 3 orders of magnitude lower than the lowest PNEC value (*Daphnia magna*, ~ 2 mg/l).

In a continuation of this study, biota samples (fish) have been collected from the vicinity of the Henriksdal effluent discharge point as well as from Lake Roxen discharge point. These biota samples, along with caged mussels exposed to the water environment of Stockholm Ström and Lake Roxen, will be analysed with respect to sucralose. The biota monitoring programme aims to answer the question of whether sucralose can be detected in biota tissue and furthermore, whether there is a sucralose uptake mechanism in these species.

In the continuation of the study, an inter laboratory comparison (ISO/IEC Guide 43-1:1997) from several participating laboratories, with respect to the determination of sucralose, will be undertaken. Since sucralose is best described as an emerging pollutant, there are no generally accepted methods of sample work-up and analysis, hence the results of the inter laboratory comparison will be most welcomed.

9 Acknowledgements

We thank staff members at the local municipalities that took part in the sampling of wastewater effluents and sludge. The study was funded by the Environmental Monitoring programme at the Swedish Environmental Protection Agency.

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Appendix Sample Characteristics and Results of Sucralose Analysis.

Category	Sample ID	Site	Matrix	Sampling date	N (RT90)	E (RT90)	Concentration (ng/l)
Reference lake water	5642	Gårdsjön	Surface water	2007-04-11			< 4
Reference lake water	5563	Tärnan	Surface water	2007-03-24			< 4
Reference lake water	5643	Härsevattnet	Surface water	2007-04-11			< 4
Sewage wastewater	6027	Henriksdal STP, Stockholm	Influent ww	2007-08-31			7920
Sewage wastewater	6028	Henriksdal STP, Stockholm	Effluent ww	2007-08-31			10800
Sewage wastewater	6033	Henriksdal STP, Stockholm	Influent ww	2007-09-13			7870
Sewage wastewater	6034	Henriksdal STP, Stockholm	Effluent ww	2007-09-13			9120
Sewage wastewater	6377	Henriksdal STP, Stockholm	Influent ww	2007-10-05			7680
Sewage wastewater	6378	Henriksdal STP, Stockholm	Effluent ww	2007-10-05			8500
Sewage wastewater	5714	Nykvarnsverket STP, Linköping	Influent ww	2007-06-19			3530
Sewage wastewater	5715	Nykvarnsverket STP, Linköping	Effluent ww	2007-06-19			4920
Sewage wastewater	5716	Nykvarnsverket STP, Linköping	Influent ww	2007-06-19			5270
Sewage wastewater	5717	Nykvarnsverket STP, Linköping	Effluent ww	2007-06-19			5110
Sewage wastewater	5727	Nykvarnsverket STP, Linköping	Influent ww	2007-06-19			5990
Sewage wastewater	5728	Nykvarnsverket STP, Linköping	Effluent ww	2007-06-19			5440
Sewage wastewater	6066	Öhn STP, Umeå	Effluent ww	2007-09-11			4030
Sewage wastewater	6077	Floda STP	Effluent ww	2007-09-13			4270
Sewage wastewater	6072	Nolhaga STP, Alingsås	Effluent ww	2007-09-13			3090
Sewage wastewater	6146	Bollebygd STP	Effluent ww	2007-09-21			5340
Sewage wastewater	6593	Ellinge STP, Eslöv	Effluent ww	2007-11-02			1790
Sewage wastewater	6384	Gässlösa STP, Borås	Effluent ww	2007-10-05			1820
Sewage wastewater	5510	Käppala STP, Lidingö	Effluent ww	2007-02-28			5410
Sewage wastewater	4982	Rustorp STP, Ronneby	Effluent ww	2006-09-20			4610
Sewage wastewater	5022	Sternö STP, Karlshamn	Effluent ww	2006-10-09			4090
Sewage wastewater	5043	Koholmen STP, Karlskrona	Effluent ww	2006-10-09			4950
Sewage wastewater	5261	Sölvesborg STP, Sölvesborg	Effluent ww	2006-11-29			2990
Sewage wastewater	5010	Solviken STP, Mora	Effluent ww	2006-09-28			4990
Sewage wastewater	5058	Krylbo STP, Avesta	Effluent ww	2006-10-10			≥ 2100

Category	Sample ID	Site	Matrix	Sampling date	N (RT90)	E (RT90)	Concentration (ng/l)
Sewage wastewater	5070	Fagersta By STP, Borlänge	Effluent ww	2006-10-16			5360
Sewage wastewater	5109	Visby STP	Effluent ww	2006-10-23			4690
Sewage wastewater	5080	Uddebo STP, Luleå	Effluent ww	2006-10-19			4090
Sewage wastewater	5084	Sandholmen STP, Piteå	Effluent ww	2006-10-19			5520
Sewage wastewater	4975	Strängnäs STP	Effluent ww	2006-09-14			4910
Sewage wastewater	4997	Flen STP	Effluent ww	2006-09-22			3740
Sewage wastewater	5049	Trosa STP	Effluent ww	2006-10-09			5560
Sewage wastewater	5134	Ekeby STP, Eskilstuna	Effluent ww	2006-11-09			2900
Sewage wastewater	4987	Sjöstad STP, Karlstad	Effluent ww	2006-09-21			5780
Sewage wastewater	4992	Fiskartorpet STP, Kristinehamn	Effluent ww	2006-09-21			3730
Receiving water	6344	Stockholms Ström, Käppala	Surface water	2007-10-03	6583297	1637710	415
Receiving water	6345	Stockholms Ström, Mölna strand	Surface water	2007-10-03	6582321	1635350	522
Receiving water	6346	Stockholms Ström, Täckå Udden	Surface water	2007-10-03	6580189	1633291	518
Receiving water	6347	Stockholms Ström, Finnboda varv	Surface water	2007-10-03	6579642	1631993	895
Receiving water	6348	Stockholms Ström, Kvarnholmen	Surface water	2007-10-03	6579736	1632768	497
Receiving water	6349	Stockholm, Hammarby sjö	Surface water	2007-10-03	6578903	1630836	455
Receiving water	5721	Linköping, Stångån, downstream STP	Surface water	2007-06-19	6477950	1489772	3557
Receiving water	5722	Linköping, Stångån, Årlången, upstream STP	Surface water	2007-06-19	6468901	1497401	< 4
Receiving water	5723	Linköping, Göta kanal, Sjöbacka, upstream Lake Roxen	Surface water	2007-06-19	6490148	1475953	< 4
Receiving water	5724	Linköping, Svartåmynningen, upstream Lake Roxen	Surface water	2007-06-19	6482165	1484539	62
Receiving water	5725	Linköping, Lake Roxen, Ekängen	Surface water	2007-06-19	6482747	1489921	52
Receiving water	5726	Linköping, Stångåmynningen, upstream Lake Roxen	Surface water	2007-06-19	6478844	1489323	408
Sewage sludge	6035	Henriksdal STP, Stockholm	Sludge (96 % DW)	2007-09-13			11 (ng/g ww)
Sewage sludge	6379	Henriksdal STP, Stockholm	Sludge (68.2 % DW)	2007-10-05			< 1 (ng/g ww)
Sewage sludge	6584	Henriksdal STP, Stockholm	Sludge (99.7 % DW)	2007-11-01			7 (ng/g ww)
Sewage sludge	5718	Nykvarnsverket STP, Linköping	Sludge (3.5 % DW)	2007-06-19			15 (ng/g ww)
Sewage sludge	5719	Nykvarnsverket STP, Linköping	Sludge (26.5 % DW)	2007-06-19			14 (ng/g ww)
Sewage sludge	5720	Nykvarnsverket STP, Linköping	Sludge (25.6 % DW)	2007-06-19			11 (ng/g ww)