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Fate and Effects of Triclosan

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Preface

The extended use of bactericidal substances in ordinary consumers products like cleaning products, products for personal hygiene and textiles has caused concern regarding the risk of developing resistance resulting in reduced efficiency of disinfectants.

In ordinary households, the amendment of e.g. cleaning products with bactericidal substances like Triclosan is not necessary. Investigations have shown that there is no reason to believe that bactericidal cleaning products or cosmetics are more efficient than the usual products. However, the use of disinfectants is necessary in places where bacteria must not be present, e.g. swimming pools. For such purposes, it is important that the efficiency of the products is not reduced due to resistance in the target bacteria.

Bactericidal substances may also be slowly degradable in wastewater treatment plants and in the environment where, in addition, they may cause adverse effects as these substances were developed to be toxic to living organisms (bacteria). Furthermore, potential discharge of sewage containing bactericides with long retention times in the environment may enhance the risk of developing resistance.

On this background – and further supported by a public debate in Denmark – the Danish EPA requested a review of the fate and environmental effects of Triclosan.

For the project management, a small group was established, consisting of:

Annette Orloff (Danish EPA)
Nanna Seidelin (Danish EPA) (2001)
Lærke Ambo Nielsen (Danish EPA) (2002-2003)
Lise Samsøe-Petersen (DHI Water & Environment)

The project was initiated in 2001 by a first phase for the description of the fate of Triclosan in wastewater treatment plants. Subsequent phases – initiated in 2002 and 2003 – are finalised by this report, in which the results of all phases are presented.

Hørsholm, 3 September 2003
Lise Samsøe-Petersen, DHI Water & Environment

Summary

Studies regarding the fate and effects of Triclosan were reviewed in order to describe the fate of Triclosan in wastewater treatment plants (WWTPs) and to make environmental risk assessments of Triclosan.

The reviewed studies (made available by the Danish EPA) showed that Triclosan is degradable under aerobic conditions in WWTPs and is extensively degraded and removed in activated-sludge systems. Furthermore, Triclosan does not adversely impact the treatment processes at levels up to 2 mg/L in the influent. However, monitoring studies indicate that only little or no removal of Triclosan occurs during anaerobic sludge digestion. Monitoring of Triclosan concentrations at WWTPs in the U.S.A., Sweden, Switzerland and Denmark showed the following ranges of Triclosan concentrations:

- Influent: 0.1-16.6 µg/L
- Effluent: 0.10-2.70 µg/L
- Sludge: 0.028-15.6 µg/g (dry weight)

Studies regarding photolysis of Triclosan in surface water have demonstrated that this may be a significant pathway in the upper layers of lakes. E.g. at pH 8, 4.6% of the parent compound was transformed to the dioxin 2,8-DCDD. Such a transformation can, however, only be expected in the upper layers of lakes due to sorption of light in the water column. Therefore, dioxin concentrations in the entire water column are expected to be lower. 2,8-DCDD does not have "the dioxin effect" and the WHO has not assigned any dioxin toxicity equivalent to it.

The estimates of Predicted Environmental Concentrations (PEC) were based on information regarding U.S., Swedish and Swiss conditions. Conservative estimates of PEC values of Triclosan in surface water were calculated by application of the principles used in the EU and those of the Danish EPA. A potential background concentration of Triclosan was not included in the $PEC_{\text{surface water}}$. The resulting ranges of PEC values were:

$PEC_{\text{surface water}}$: 0.009-0.303 µg/L for high technology plants
 $PEC_{\text{surface water}}$: 0.149-1.26 µg/L for low technology plants

The lowest NOEC value for aquatic organisms was a NOEC for algae of 0.5 µg/L. Based on the EU principles for derivation of Predicted No Effect Concentrations (PNEC), the resulting PNEC value was

$PNEC_{\text{surface water}} = 0.05 \mu\text{g/L}$

Based on conservative assumptions regarding dilution of the waste water (water courses with low flow and a dilution factor of 2-10), PEC values were derived from Triclosan concentrations in U.S., Swedish and Swiss waste water. The resulting PEC/PNEC ratios (risk quotients, RQ) for surface water were ≥ 1 for low technology WWTPs ($RQ = 3-25$) and for most of the high technology plants ($RQ = 0.2-6$).

In Denmark, only small local Danish WWTPs may, however, produce effluents and sewage sludge attaining the level of the foreign lower technology plants and, for most discharges from Danish WWTPs, the dilution will be considerably higher than 10. Therefore, Triclosan is not expected to cause effects in Danish surface water unless discharges are from low technology plants to waters with low dilution.

From Danish WWTPs, only one measurement of the Triclosan concentration in the effluent from a WWTP is available and it was below the limit of detection (1 µg/L).

For the soil compartment, the PEC estimate was based on information on concentrations of Triclosan in U.S. and Swedish sewage sludge. For realistic worst-case application rates, the Danish application scheme for sewage sludge was used. The resulting PEC values for the soil compartment were:

Activated sludge: $PEC_{soil} = 0.00004-0.0056$ mg/kg (soil dry weight)
“Bio-filter” sludge: $PEC_{soil} = 0.0005-0.021$ mg/kg (soil dry weight)

The only soil-living organisms, which have been tested with Triclosan, are plants and worms, of which plants are the most sensitive species. Based on the lowest available NOEC value and the principles used in the EU, a preliminary PNEC was estimated to be:

$PNEC_{soil} = 0.096$ µg/kg (soil dry weight)

The terrestrial risk assessment indicated risks of adverse effects on soil-living organisms following application of most of the sewage sludge to agricultural soil. Risk quotients ranged between 0.4 and 219. The lowest value was based on the lowest concentration found in activated sludge from Sweden while the highest was derived from a U.S. trickling filter plant.

For a more realistic terrestrial risk assessment, information regarding concentrations of Triclosan in Danish sewage sludge would be needed. Furthermore, toxicity data of high quality for terrestrial organisms would improve the reliability of the PNEC value.

Due to lack of data on Danish conditions, all information regarding concentrations of Triclosan in waste water, effluents and sewage sludge was from the U.S.A., Sweden or Switzerland. Therefore, the outcome of the risk assessments for surface water and soil cannot be considered conclusive for Danish conditions. Based on information on Danish WWTPs, it is, however, considered likely that most Danish effluent waste water and most Danish sewage sludge will contain concentrations of Triclosan at the level of the lowest values measured in Sweden, Switzerland and The U.S.A. Only at small local WWTPs, effluents and sewage sludge are expected to attain the level of the foreign lower technology plants.

The very sparse information on Danish conditions indicates that, for aquatic environments, risk quotients below 1 may be obtained for discharges for most WWTPs. This is expected for effluents from high technology WWTPs with a high dilution in the recipient. However, discharge of waste water to watercourses with very low flow may lead to risks of effects. For verification, results of analysis could be used, only if measurements are made with low

detection limits. For terrestrial environments, there is no information regarding concentrations of Triclosan in Danish sewage sludge.

Sammenfatning

Projektet har omfattet en gennemgang af undersøgelser vedrørende Triclosans skæbne og effekter med det formål at beskrive Triclosans skæbne i renselanlæg og udarbejde miljømæssige risikovurderinger af Triclosan.

De gennemgæede undersøgelser har vist, at Triclosan er nedbrydeligt under aerobe forhold i renselanlæg, og at det i vidt omfang nedbrydes og fjernes i anlæg med aktivt slam. De har desuden vist, at Triclosan ikke har uønskede effekter på rensprocesserne i koncentrationer på op til 2 mg/L i indløbsvandet. Monitoringsundersøgelser viser dog, at der kun forekommer meget lidt eller slet ingen fjernelse af Triclosan ved anaerob udrådning. Monitoring af Triclosankoncentrationer i amerikanske, schweiziske, svenske og (et enkelt) dansk renselanlæg (aktivt slam og biologiske filtersystemer) viste følgende niveauer af Triclosankoncentrationer:

- Indløb: 0,1-16,6 µg/L
- Udløb: 0,10-2,70 µg/L
- Slam: 0,028-15,6 µg/g (tørvægt)

Undersøgelser af Triclosan i overfladevand har vist, at fotolyse kan være en betydelig omsætningsmekanisme i de øverste lag af søer. F.eks. blev 4,6% af stoffet omsat til dioxinet 2,8-DCDD ved pH 8. En sådan omdannelse kan dog kun forventes at finde sted i de øvre vandlag på grund af sorption af lyset ved nedtrængningen gennem vandsøjlen. Det må derfor forventes, at dioxinkoncentrationerne i vandsøjlen som helhed er lavere. 2,8-DCDD har ikke "dioxineffekt", da den ikke bindes til den såkaldte dioxinreceptor (Ah-receptor) i kroppen. Stoffet er da heller ikke tildelt nogen dioxintoksicitetsækvivalent af WHO og er heller ikke inkluderet i EUs seneste risikovurdering af dioxiner.

Koncentrationerne i miljøet (Predicted Environmental Concentrations, PEC) er beregnet ud fra oplysninger om amerikanske, svenske og schweiziske forhold. En forsigtig beregning af Triclosans PEC-værdier i overfladevand blev foretaget ved at anvende de principper, som bruges i EU, og Miljøstyrelsens principper. Der blev ikke medtaget en potentiel baggrundskoncentration af Triclosan i PEC_{overfladevand}. Resultatet var PEC-værdier i området:

PEC_{overfladevand}: 0,009-0,303 µg/L for højteknologiske anlæg og
PEC_{overfladevand}: 0,149-1,26 µg/L for lavteknologiske anlæg

Den laveste NOEC-værdi for vandlevende organismer var en NOEC for alger på 0,5 µg/L. Anvendelse af EUs principper for beregning af den estimerede nul-effekt koncentration (Predicted No Effect Concentration, PNEC) gav PNEC-værdien:

PNEC_{overfladevand}: 0,05 µg/L

PEC-værdierne blev beregnet ud fra oplysninger om koncentrationer af Triclosan i udløb fra amerikanske, svenske og schweiziske anlæg samt baseret

på meget forsigtige antagelser om fortynding af spildevandet (vandløb med lav vandføring og en fortyndingsfaktor på 2-10). Risikokvotienterne (PEC/PNEC) var 0,2-6 og 3-25 for spildevand fra henholdsvis høj- og lavteknologiske anlæg.

I Danmark er det dog kun på små, lokale renselanlæg, at koncentrationerne i afløb og slam er på niveau med de udenlandske lavteknologiske anlæg. Desuden er fortyndingen af spildevandet højere end en faktor 10 for de fleste danske anlæg. Derfor forventes det ikke, at Triclosan vil give anledning til miljøeffekter i overfladevand, med mindre der er tale om udløb fra lavteknologiske anlæg til vandløb med lav fortynding.

Der findes kun en enkelt måling af Triclosan i udløbsvand fra danske renselanlæg, og den var under detektionsgrænsen på 1 µg/L.

For jordmiljøet er PEC-estimatet baseret på oplysninger om Triclosankoncentrationer i amerikansk og svensk spildevandsslam. For at opnå realistiske "worst-case" doseringer er de danske værdier for anvendelse af spildevandsslam brugt i beregningerne. De fremkomne PEC-værdier for jordmiljøet var:

Aktiveret slam: $PEC_{jord} = 0,00004-0,0056 \text{ mg/kg (jord tørvægt)}$
"Bio-filter" slam: $PEC_{jord} = 0,0005-0,021 \text{ mg/kg (jord tørvægt)}$

De eneste jordlevende organismer, som er blevet testet med Triclosan, er planter og orme, hvor planterne var de mest følsomme. Ud fra den laveste, tilgængelige NOEC-værdi og EUs principper blev PNEC beregnet til:

$PNEC_{jord} = 0,096 \text{ µg/kg (jord tørvægt)}$

Den terrestriske risikovurdering tydede på risiko for uønskede effekter på jordlevende organismer efter udbringning af det meste af slammet på landbrugsjord. De estimerede risikokvotienter (PEC/PNEC) lå i intervallet 0,4-219, hvor den laveste værdi var baseret på den laveste koncentration fundet i svensk aktivt slam, mens den højeste værdi er afledt fra slam fra et amerikansk anlæg med biologisk filter.

En mere realistisk risikovurdering bør baseres på oplysninger om koncentrationer af Triclosan i dansk spildevandsslam. Desuden ville det øge pålideligheden af PNEC-værdien, hvis den kunne baseres på toksicitetsdata af høj kvalitet for jordlevende organismer.

Da det ikke har været muligt at finde data for danske forhold, stammer alle informationer om Triclosankoncentrationer i spildevand, udløbsvand og spildevandsslam fra USA, Sverige eller Schweiz. Resultatet af risikovurderingerne for overfladevand og jord kan derfor ikke direkte overføres til danske forhold. Ud fra den tilgængelige viden om danske renselanlæg anses det dog for sandsynligt at det meste danske slam vil indeholde Triclosan i koncentrationer, der er på niveau med de lavest målte værdier i Sverige, Schweiz og USA. Kun på små, lokale renselanlæg forventes det at Triclosankoncentrationerne i slam og udløbsvand vil være ligeså høje som de udenlandske lavteknologianlæg.

De meget sparsomme oplysninger om danske forhold tyder på, at risikokvotienterne for vandmiljø kan blive lavere end 1 kan opnås for de fleste

renseanlæg. Dette gælder for højteknologiske anlæg, hvor der er høj fortynding i recipienten. Derimod kan udledning til vandløb med lav vandføring give anledning til risiko for miljøeffekter. Disse antagelser kan verificeres ved analyse af prøver af udløbsvand fra danske anlæg, under forudsætning af, at der kan analyseres for Triclosan med lave detektionsgrænser. Der foreligger ingen oplysninger om koncentrationer af Triclosan i dansk spildevandsslam, der kunne bruges som grundlag for en risikovurdering for jordmiljøet under danske forhold.

1 Introduction

Triclosan (TCS; 2,4,4'-trichloro-2'-hydroxydiphenylether) is an antimicrobial agent, which is widely used in personal care products such as shampoos, soaps, cosmetics, lotions and toothpaste.

The aims of the project are to evaluate the environmental properties of Triclosan and assess the risks of effects in aquatic and terrestrial environments.

The project was limited to a review of the literature, which was made available by the Danish EPA and did not leave resources for inclusion of supplementary literature.

At present, almost no information is available regarding the occurrence of Triclosan in sewage, effluents or sewage sludge in Denmark. Therefore, the assessments were mainly based on data from other countries as available, i.e. the U.S.A., Sweden and Switzerland.

The present report reviews several studies describing the fate of Triclosan in wastewater treatment plants (WWTPs) as well as fate and effects of the substance in surface water and soil. Laboratory and field data are integrated for preliminary aquatic and terrestrial risk assessments for Triclosan.

2 Fate of Triclosan in wastewater treatment plants

2.1 Studies of Triclosan degradation in laboratory tests

Triclosan is not readily or inherently degradable in standardised screening tests like OECD 301C (MITI I) or OECD 302C (MITI II). The negative results in these tests may be a consequence of the bacterial toxicity of Triclosan at the high substrate concentration required for these biodegradability screening tests. Therefore, a batch activated sludge mineralisation test and a continuous activated sludge (CAS) test were conducted by use of ^{14}C -labelled Triclosan as radiotracer measurements allow the application of a relatively low, non-toxic concentration in mineralisation experiments (Federle *et al.* 2002).

2.1.1 Batch activated sludge mineralisation test

Triclosan was uniformly ^{14}C -labelled in the 2,4-dichlorophenoxy ring, which represents the most recalcitrant part of the molecule. The activated sludge mineralisation test was performed by use of activated sludge from the Avondale Sewage Treatment Plant in Avondale, Pennsylvania, which primarily receives domestic waste water. Triplicate samples of the sludge (1 L; 2,500 mg/L) were dosed with the test chemical and incubated in 2-L flasks, which were continuously mixed on a shaker at $22^\circ \pm 1.5^\circ\text{C}$. The head-space of each flask was continuously purged with CO_2 -free air, and evolved $^{14}\text{CO}_2$ was trapped in 1.5 N KOH and quantified by liquid scintillation counting.

Mineralisation of Triclosan began immediately in the batch activated sludge test, and the final recovery of $^{14}\text{CO}_2$ after 71 days attained 30.9%, 44.7% and 52.3%, for initial test substance concentrations of 20 $\mu\text{g/L}$, 100 $\mu\text{g/L}$ and 200 $\mu\text{g/L}$, respectively. Two of the 200- $\mu\text{g/L}$ test systems were re-dosed with 1 mg/L and, following a lag period of 3 or 10 days, degradation proceeded, resulting in 78.9% and 80.9% of the second dose being recovered as $^{14}\text{CO}_2$ after 52 days.

2.1.2 CAS test

The CAS systems were initially inoculated with activated sludge from either Avondale or Downingtown Regional Water Pollution Control Center, Pennsylvania, which also predominantly receives domestic waste water. Parallel systems, each consisting of a 6-L aeration chamber connected to a 2-L cylindrical clarifier with recycle discharge in the bottom and an effluent discharge tube at the side were used. The systems were incubated at $22^\circ \pm 2^\circ\text{C}$. Experiments were performed with groups of either two or five CAS systems. In each experiment, one of the systems served as a control unit receiving either waste water only or waste water and a constant influent concentration of Triclosan. The remaining systems received a mixture of waste water and variable influent concentrations of Triclosan. The radioactivity of Triclosan and its intermediates was quantified by liquid

scintillation counting of samples of influent, effluent and sludge solids. The incorporation of ^{14}C into constituents of biomass was determined by fractionation of extracted solids and quantification by liquid scintillation counting. Various experiments were conducted with CAS systems to examine the degradation of Triclosan and to study the effect of a shock loading with pulses of a high concentration of Triclosan.

When the influent concentration of Triclosan was increased gradually from 40 to 2,000 $\mu\text{g/L}$, no intact ^{14}C Triclosan was detected in the effluents of the CAS system, and removal of the parent compound exceeded 98.5%. The ^{14}C present in the effluent consisted of polar intermediates, which were extractable into ethyl acetate (0.4-7.2%), and very polar intermediates, which could not be extracted with ethyl acetate (2.3-10.5%). At steady state, 76-90% of the added Triclosan was mineralised to $^{14}\text{CO}_2$ whereas 2.2-6.8% of the labelled ^{14}C was incorporated into the biomass. The amount of Triclosan sorbed to the activated sludge equalled 1.5-4.5% of the total ^{14}C dosed to the influent. Primary degradation of Triclosan exceeded 94% whereas complete degradation (i.e. mineralisation and incorporation into biomass) exceeded 80% of the dosage in the influent. Approximately the same pattern was observed at lower concentrations of Triclosan, which were more representative of the actually expected concentrations in waste water (i.e. 7.5 to 50 $\mu\text{g/L}$). The fate and distribution of Triclosan added at 10 $\mu\text{g/L}$ are shown in Table 2.1. Neither the removal of parent Triclosan or the mineralisation and formation of intermediates showed any correlation with the Triclosan concentration in the low concentration range. No adverse effects on the treatment process were observed at any influent concentration.

Shock loading with Triclosan (i.e. 750 $\mu\text{g/L}$ for 4 hours), which is representative of a situation in which a sewage treatment plant receives a consistent low level of Triclosan but with periodical pulses of higher levels, did not change the removal pattern significantly. In addition, the impact of shock loading of Triclosan on the operation of the CAS system was minimal and short term, as evidenced by changes in removal of solids, biological and chemical oxygen demand as well as nitrification.

Table 2.1
Steady-state distribution of radioactivity in CAS system dosed with ¹⁴C Triclosan at 10 µg/L (Federle *et al.* 2002)

	Per cent
<i>Present in effluent as</i>	
Parent Triclosan	5.3
Polar intermediates	3.2
Non-extractables	2.2
<i>Sorbed to sludge solids as</i>	
Parent Triclosan	1.0
Polar intermediates	2.6
Non-polar intermediates	0.6
<i>Incorporated into biomass</i>	
	6.0
<i>Mineralised to ¹⁴CO₂</i>	
	79.1
<i>Removal</i>	
Parent Triclosan	94.7
Total radioactivity	89.3
<i>Triclosan undergoing</i>	
Primary degradation	94.7
Complete degradation (mineralised or incorporated into biomass)	85.1

2.2 Monitoring of Triclosan at wastewater treatment plants in the U.S.A.

A monitoring study was conducted with the purpose of obtaining field data that could be used for assessing the environmental exposure concentrations of Triclosan (McAvoy *et al.* 2002). In this study, samples of influent, primary effluent (when available) and final effluent were collected and analysed for Triclosan, a methylated byproduct of Triclosan and higher chlorinated closans, which may potentially be formed during wastewater treatment. The WWTPs selected for the monitoring of Triclosan included two activated sludge treatment plants (located in Columbus, Ohio and Loveland, Ohio) and two trickling filter treatment plants (located in Glendale, Ohio and West Union, Ohio).

The wastewater samples were extracted and, following derivatisation, the samples were analysed by gas chromatography-mass spectrometry (GC-MS) (McAvoy *et al.* 2002). The substances included in the analyses were Triclosan, Tetra II (2,3',4,4'-tetrachloro-2'-hydroxydiphenylether), Tetra III (2,4,4',5'- tetrachloro-2'-hydroxydiphenylether), Penta (2,3',4,4',5'-pentachloro-2'-hydroxydiphenylether) and Triclosan-OMe (2,4,4'-trichloro-2'-methoxydiphenylether). An evaluation of the analytical method showed that the average recovery (n = 4) of laboratory spiked sludge was ≥79% of Triclosan, ≥65% of Tetra II, ≥91% of Tetra III, ≥75% of Penta and ≥70% of Triclosan-OMe.

The concentrations of Triclosan in the influent waste water ranged between 3.8 and 16.6 µg/L (McAvoy *et al.* 2002). The authors explain this variation by the per capita water usage among the four communities, in which the

individual WWTPs are located. The concentrations of Triclosan in primary effluent were reported to range between 3.4 and 8.0 µg/L while final effluent concentrations were 0.24-0.41 µg/L for activated sludge treatment and ranged from 1.6 to 2.7 µg/L for trickling filter treatment. A high overall removal of 95.4-96.2% of the influent Triclosan was seen at the activated sludge treatment plants, and this is in good agreement with the reported removals in laboratory CAS systems (Section 2.1; Table 2.1). The overall removal of Triclosan by trickling filter treatment was lower as the removals ranged from 58.0 to 86.1%.

The higher chlorinated closans (Tetra II, Tetra III and Penta) were either not detected in influent and effluent waste water or were present at very low concentrations, generally <0.3 µg/L. The methylated product Triclosan-OMe was qualitatively detectable but the concentration was below the limit of quantification (0.1 µg/L) in all of the samples.

The concentrations of Triclosan in sludge samples from the selected WWTPs ranged from 0.53 to 15.6 µg/g (dry weight), the sum of Tetra II, Tetra III and Penta ranged from the detection limit to less than 1 µg/g and Triclosan-OMe ranged from below quantification to 1.03 µg/g. The concentrations of Triclosan measured in sludge samples indicate that Triclosan is rapidly removed during the aerobic sludge digestion process (reduction from 12.2 µg/g in primary sludge to 1.5 µg/g in aerobically digested sludge at West Union; Table 2.2). However, at the trickling filter treatment plant in Glendale, little or no removal of Triclosan occurred during anaerobic sludge digestion as indicated by the presence of 7.5 µg/g in primary sludge and 15.6 µg/g in anaerobically digested sludge. This lack of anaerobic degradation of Triclosan has also been confirmed in laboratory studies (McAvoy *et al.* 2002).

Table 2.2 presents the occurrence of Triclosan, Triclosan-OMe and higher chlorinated closans in waste water and sludges from the activated sludge treatment plant in Loveland and the trickling filter treatment plant in West Union.

Table 2.2

Concentrations of Triclosan (TCS), TCS-OMe, Tetra II, Tetra III and Penta chloro-hydroxydiphenylethers in wastewater and sludge samples collected at the activated sludge treatment plant (AS) in Loveland and the trickling filter treatment plant (TF) in West Union (McAvoy *et al.* 2002)

Sludge type	TCS	TCS-OMe	Tetra II	Tetra III	Penta
	Influents, effluents: µg/L; sludge: µg/g (dry weight)				
<i>Loveland (AS)</i>					
Raw influent	10.70	n.q. ^a	(0.03) ^b	0.08	0.05
Primary effluent	7.00	n.q.	0.04	0.08	0.05
Final effluent	0.41	n.q.	n.d. ^c	(0.03)	n.d.
Primary sludge	14.70	0.05	0.04	0.10	0.07
Secondary sludge	4.20	1.03	0.06	0.04	0.09
<i>West Union (TF)</i>					
Raw influent	15.4	n.q.	0.62	0.25	0.19
Primary effluent	8.00	n.q.	0.34	0.16	0.16
Secondary effluent	2.70	n.q.	0.29	0.07	0.07
Primary sludge	12.20	0.14	0.07	0.12	0.12
Secondary sludge	7.30	0.45	0.09	0.05	0.08
Aerobic digested sludge	1.50	0.13	n.d.	n.d.	n.d.

- a: n.q., below limit of quantification. The limit of quantification was 0.1 µg/L and 0.07 µg/g for wastewater and sludge samples, respectively.
- b: Values in parentheses were below the limit of quantification, hence these are approximate concentrations.
- c: n.d., below limit of detection. The detection limit was 0.01 µg/L and 0.04 µg/g for wastewater and sludge samples, respectively.

2.3 Occurrence of Triclosan at wastewater treatment plants in Switzerland

Twenty-four-hour flow proportional samples of influent to the biological stage (effluent from the primary sedimentation) and of treated effluent emitted to rivers and lakes were collected from Swiss WWTPs in 1997 and 2001 (Lindström *et al.* 2002). The WWTPs are all modern 3- or 4-stage mechanical/biological/chemical plants serving populations from 4,500 to 36,000 persons.

It was suspected that Triclosan might undergo biological methylation in WWTPs. Therefore, an analytical method was developed to distinguish between parent and methylated compounds. The samples were acidified for complete recovery of Triclosan by solid-phase extraction, ethylated, cleaned up and analysed for Ethyl Triclosan and Methyl Triclosan, the former compound representing parent Triclosan. Some of the WWTP samples were analysed directly for Triclosan and Methyl Triclosan without pH adjustment and derivatization. Furthermore, the samples collected in 1997 had been acidified, solid-phase extracted, and then methylated. The data from these samples included the combined amount of Triclosan and Methyl Triclosan. Aliquots of the sample extracts were analysed by GC-MS. Evaluation of the methods showed a recovery of Triclosan and Methyl Triclosan of 50-90% under acidic (pH ≈ 2) and neutral conditions (pH ≈ 7-8).

Triclosan in the influent to the biological stage was found at concentrations of 0.6-1.3 µg/L and Methyl Triclosan was detected in much lower concentrations, from <0.001 to 0.004 µg/L. The corresponding effluent concentrations were between 0.11 and 0.65 µg/L (Triclosan) and <0.002 and

0.011 µg/L (Methyl Triclosan) (Table 2.3). The higher concentrations of Methyl Triclosan in the effluent than in the corresponding influent indicate a formation of this compound during the biological treatment.

Table 2.3
Concentrations of Triclosan in wastewater samples collected from WWTPs in Switzerland (Lindström *et al.* 2002)

WWTP	Persons (PE)	Sampling time	Influent to the biological stage		Final effluent	
			Triclosan (µg/L)	Methyl Triclosan (µg/L)	Triclosan (µg/L)	Methyl Triclosan (µg/L)
Maur	4,500	Mar 2001	0.98	<0.001	0.650	<0.002
Pfäffikon	9,200	Feb 2001	1.044	0.004	0.250	0.004
Uster	36,000	Feb 2001	1.300	0.0025	0.110	0.011
Wetzikon	19,000	Mar 2001	0.584	<0.001	0.183	<0.002
Gossau	11,000	Jan 2001	0.970	<0.001	0.136	0.035
Gossau	10,500	Oct 1997	1.000	a	0.100	a
Gossau	10,500	Dec 1997	0.500	a	0.070	a

a: All samples were methylated, precluding differentiation between Triclosan and Methyl Triclosan.

There is no information about the type of biological treatment process or other process conditions during the sampling except the flow rate. Therefore, it is not possible to draw any conclusions regarding the influence of the wastewater treatment process on the removal rate of Triclosan. The lowest removal is, however, found in the small size WWTP Maur.

2.4 Triclosan in samples of sludge and waste water from Swedish WWTPs

2.4.1 Sludge samples

Sludge from 19 WWTPs was sampled during 2001-2002 and analysed for a series of organic pollutants including Triclosan. The samples were collected from WWTPs of different size and geographic location. Furthermore, the WWTPs were selected to obtain loadings with variable types of industrial waste water. Only one sludge sample was collected from each WWTP. The samples were generally taken from freshly produced and stabilised sludge (Svensson 2002).

The concentration of Triclosan in the sludge ranged from 0.028 to 6.4 µg/g d.w. (Table 2.4). The lowest concentration was found in a WWTP consisting of a mechanical process followed by an activated sludge treatment and a chemical precipitation (the Åmål plant). The highest concentration was found in a plant, in which the biological treatment process consisted of a bio-filter. The Triclosan concentration was generally between 0.2 and 0.5 µg/g d.w. in WWTPs with the activated sludge treatment. A concentration of 3.7 µg/g d.w. was, however, seen in Rävlanda WWTP receiving waste water from households only. A Triclosan concentration of 0.04 µg/g d.w. was found in the sludge sample taken from the largest of the investigated WWTPs (Ryaverket), which is treating waste water from approx. 600,000 persons.

Table 2.4
Wastewater treatment plants investigated in Sweden and
concentrations of Triclosan in sludge samples (Svensson 2002)

WWTP	Process	PE	Leachate		Anaerobic digesting of sludge	Important connection	Sludge production year 2001 (ton dw)	Triclosan in sludge (mg/kg dw)
			Yes	No				
<1,000 PE								
Bohus- Malmö	MC	400		X		House hold	23	2.1
1,000-5,500 PE								
Donsö (Göteborg)	M B ¹ C	2,000		X		House hold	40	0.47
Brålanda (Vänersborg)	M B ² C	2,250		X		Food industry	217	0.34
Rävlanda	M B ² PC	2,600		X		House hold	105	3.7
Herrljunga	M B ² C	3,880		X		Photo laboratory	57	0.35
Munkedal	M B ¹ C	4,000		X		House hols	240	0.53
Tranemo	MC B ²	5,000		X		Glas & color industry	497	0.34
Karlsborg	M B ² C	5,200		X		Military & mechanical industry	181	0.18
5,500-20,000 PE								
Vara	MC B ² C	6,360		X	X	Laundry	160	0.45
Lysekil	M B ¹ C	8,000	X		X	Fish industry	643	1.2
Ulricehamn	M B ¹ C	8,725	X		X	Mixed industry	293	5.1
Åmål	M B ² C	10,000		X	X	Tool industry	264	0.028
Skara	M B ^{1,3,4} C	14,000	X		X	Dairywork slaughterhouse	655	0.38
Stenungsund	M B ⁴ PC	14,500	X			Mixed industry	380	0.20
Skene	M B ² C	17,220	X			Textile industry	992	0.26
>20,000 PE								
Alingsås	M B ¹ C	23,931	X		X	Laundry & food industry	801	6.4
Lidköping	M B ⁴ C	28,160	X			Mixed industry	1,246	1.3
Uddevalla	MC B ² C	38,000		X	X	Mixed industry	1,038	0.18
Ryaverket	M B ² C	605,526	X		X	Large city	1,3512	0.035

M: Mechanical; C: Chemical; B: Biological; P: Biological phosphate removal

1: Bio-filter

2: Activated sludge

3: Sequency Batch Reactor (SBR)

4: Activated sludge – aerobic and anoxic treatment

The data do not allow further assessment of the influence of the treatment process on the concentration of Triclosan in sludge. There are no parallel analyses of influent and effluent waste water and it is not possible to evaluate whether e.g. a high sludge concentration is due to a high loading rate of Triclosan or the type of treatment process.

Another investigation of sludge samples from four Swedish WWTPs in 2001 showed Triclosan concentrations between 2.8 and 4.4 µg/g d.w. in anaerobically digested sludge (Remberger *et al.* 2002). This is within the range found in the investigation of the nineteen WWTPs presented above. For one of the WWTPs (Bromma), both a primary and an anaerobically digested sludge sample were analysed. The results of these analyses supported the findings in the study performed by McAvoy *et al.* (2002) that little or no removal of Triclosan occurs during anaerobic digestion.

2.4.2 Wastewater samples

In 1995, one-month composite samples of influent and effluent from six Swedish WWTPs of different size were collected and analysed for Triclosan and other organic compounds (Paxéus unpublished). The composite samples were prepared by combining daily random samples or daily flow proportional samples of the waste water. Three groups of WWTPs were represented:

- Small WWTPs receiving solely domestic waste water (Ölmanäs and Donsö WWTPs)
- Medium-sized WWTPs receiving domestic waste water and effluents from small industries, workshops, hospitals, gasolines etc. (Lerum and Skansverket WWTPs)
- Large WWTPs receiving domestic waste water and discharges dominated by industries and car-washing facilities (GRYAAB and Henriksdal WWTPs)

The Triclosan concentrations of the influent (0.1-1.5 µg/L) were higher than in effluent samples (up to 0.2 µg/L) as shown in Table 2.5. The lowest influent concentration was found in the small-size WWTPs while the highest concentration was found in the large WWTPs in Stockholm loaded with waste water from larger industries including fabricated metal production, pharmaceutical and circuit board production. Paxéus concluded that the removal of organic compounds in the treatment process appears to be independent of the WWTP size. Considering the removal of Triclosan from the water phase of the six Swedish WWTPs, it is, however, obvious that the highest removals are obtained in the medium-sized WWTP Skansverket and the large WWTP Henriksdal.

Table 2.5
Triclosan in influent and effluent of Swedish WWTPs (Paxéus unpublished)

WWTP	Information about the biological process	Influent (µg/L)	Effluent (µg/L)
Ölmanäs	Activated sludge	0.1	0.1
Donsö	Activated sludge	0.2	0.1
Lerum	Activated sludge	0.3	0.2
Skansverket (Uddevalla)	Activated sludge	0.4	-
GRYAAB (Göteborg)	Sequency batch reactor	0.2	0.1
Henriksdal (Stockholm)	Activated sludge	1.5	-

"-": Not found (the detection limit is not stated)

2.5 Measurements of Triclosan at two Danish WWTPs

Several monitoring programmes have been running at the Danish WWTPs Renseanlæg Lynetten (RL) and Renseanlæg Damhusåen (RD) in Copenhagen. The plants serve the population and the industries in the area of Copenhagen and the capacities of RL and RD are approx. 750,000 and 350,000 PE, respectively. Both plants are modern WWTPs with biological wastewater treatment based on the activated sludge process and fully upgraded to nitrogen and phosphate removal. Analysis of Triclosan was included in the monitoring programme performed in June - July 2002.

Samples of waste water were collected from the influent, the effluent of the primary sedimentation and the final effluent at RL and from the influent at RD. All samples were 24-h flow proportional samples collected and analysed after mixing either as whole week samples or as Saturday-Sunday and Monday-Friday samples. The wastewater samples were acidified and extracted with dichloromethane. Then the extracts were dried over sodium sulphate and analysed by GC-MS. The limit of detection was 1 µg/L (Pedersen & Nielsen 2003).

The average Triclosan concentration in the influent samples of RL was 2.5 µg/L in the workday samples and 1.7 µg/L in the weekend samples. The concentration seems to be reduced significantly during the primary sedimentation process indicating that Triclosan is removed by sorption. In the effluent of RL, no concentration of Triclosan was found above the detection limit.

Table 2.6
Triclosan in wastewater samples from two Danish WWTPs (Pedersen & Nielsen 2003)

WWTP	Date of sampling	Influent (µg/L)	Effluent after primary sedimentation (µg/L)	Final effluent (µg/L)
RL	Week 26 2002, workdays	2.6	1.8 ^a	<1 ^a
	Week 27 2002, workdays	2.4	1.4 ^a	<1 ^a
	Week 26 2002 Weekend	1.6		
	Week 27 2002, Weekend	1.8		
RD	Week 26 2002	2.7 ^a		
	Week 27 2002	3 ^a		

a: Analysis of week sample

2.6 The relative quality of Danish WWTPs

The monitored U.S. WWTPs represent two different types of biological treatment, i.e. activated sludge and trickling filter. The data from Switzerland originate from 3- or 4-stage modern WWTPs of different size and all with biological treatment. However, the type of biological treatment is not described. In Sweden, the reported monitoring studies were performed mainly at WWTPs with activated sludge or bio-filter. The monitoring of sludge included WWTPs with and without primary sedimentation and WWTPs with capacities between 400 and 600,000 PE. Nutrient removal was included at some but not all of the Swedish WWTPs. Danish WWTPs were represented by a single monitoring study of a modern treatment plant based on the activated sludge process and upgraded to nutrient removal.

Activated sludge treatment represents the major part of treated wastewater flows in Denmark. It is obvious that the best removal of Triclosan in the U.S. plants is obtained in the activated sludge treatment. The examined plant - located in Loveland - has a capacity of approx. 27,000 PE. The plant receives mainly municipal waste water (industrial flow contribution <1%), has a total hydraulic retention time of approx. 6 h, with approx. 3 h in the aeration basin

and its effluent BOD₅ and total suspended solids (TSS) concentrations are generally <5 mg/L. During the study, the BOD₅ and TSS removal were very high (98.3% and 96.5%, respectively) and the plant thus represents a very well-functioning activated sludge plant with high removal efficiency.

The Loveland plant was probably not upgraded for nutrient removal, which is the case for the WWTPs treating the major part of the municipal waste water in Denmark (approx. 90% (Danish EPA 2001)). Generally, both the hydraulic retention time (HRT) and the sludge retention time (SRT) are increased when a plant is upgraded for nutrient removal. This would eventually increase the time of biodegradation and of establishing a microbial population capable of degrading Triclosan. The lower effluent concentrations found in the monitoring studies in Switzerland and Sweden could be the results of a longer treatment period in the biological step but also a result of a lower loading with Triclosan.

The results obtained in Sweden indicate that the highest removal is obtained in the larger WWTPs. However, the limited information and data material from the presented monitoring studies in Switzerland, Sweden and Denmark do not allow a more detailed evaluation concerning the role of the treatment process. It is expected that the efficiency of Triclosan removal in the majority of the Danish WWTPs (upgraded for nutrient removal and with high effluent quality) would be at the same level as in the Loveland plant or even better.

The influent concentrations of Triclosan at the Danish WWTPs are higher than the concentrations measured in Switzerland and Sweden. This could lead to the conclusion that the Triclosan concentration in the effluents of Danish WWTPs might be higher than in Switzerland and Sweden. Based on the limited Danish analytical data, it is, however, not possible to draw any conclusions regarding the general level of Triclosan in WWTP effluents in Denmark.

It is expected that the discharge of Triclosan from the WWTPs is dependent on the loading of Triclosan, the type of treatment plant and the operational conditions. Therefore, the concentrations of Triclosan in effluents from plants with e.g. only mechanical treatment or mechanical-chemical treatment will probably be higher than the concentrations in effluents from plants with biological treatment. In Denmark, waste water discharged from WWTPs without any biological treatment constituted, however, less than 2% of the total amount in 1998 (Danish EPA 2000). As mentioned above, the HRT and SRT may influence the overall efficiency of Triclosan removal together with parameters like temperature, loading rate and sludge treatment.

Different sludge treatments were used at the WWTPs reported above. The highest removal of Triclosan was achieved when the sludge was aerobically digested while only negligible removal was seen during anaerobic digestion. The highest Triclosan concentration (16 µg/g d.w.) was found in a U.S. WWTP with trickling filter and anaerobic sludge treatment. The influent concentration of Triclosan at this WWTP was 3.8 µg/L and thus slightly above the influent concentration found at a Danish WWTP (RD) (approx. 3 µg/L). Therefore, it is not possible to exclude that Triclosan concentrations in sludge at this level (16 µg/g d.w.) might be found at local small size WWTPs in Denmark. However, the main part of the Danish sewage sludge would probably contain Triclosan in concentrations close to the level in the activated sludge plants in Sweden and the U.S.A. (0.028-4.2 µg/g d.w.).

2.7 Summary of the fate of Triclosan in WWTPs

The reviewed studies showed that Triclosan is degradable under aerobic conditions in WWTPs. Furthermore, the monitoring studies indicated that only little or no removal of Triclosan occurred during anaerobic sludge digestion.

Degradation experiments in CAS laboratory systems showed that Triclosan was extensively degraded and removed in activated-sludge systems. Furthermore, Triclosan did not adversely impact the treatment processes at levels of up to 2 mg/L in the influent.

The ranges of Triclosan concentrations in influent, effluent and sludge obtained by the presented monitoring studies at WWTPs in the U.S.A., Switzerland, Sweden and Denmark are summarised in Table 2.7.

Table 2.7
Ranges of Triclosan concentrations in wastewater and sludge samples from WWTPs

Country (number of plants)	Influent (µg/L)	Effluent (µg/L)	Sludge (µg/g dw)
U.S.A. (2)	5.2-10.7 ^a	0.24-0.41 ^a	0.9 - 4.2 ^a
U.S.A. (2)	3.8-16.6 ^b	1.61-2.70 ^b	0.53-15.6 ^b
Switzerland (5)	0.5-1.3	0.10-0.65	-
Sweden (6 for water, 19 for sludge)	0.1-1.5	up to 0.2	0.028-3.7 ^a 0.38-6.4 ^b
Denmark (2 for influent, 1 for effluent)	1.6-3.0	<1	-

a: Activated sludge

b: Trickling filter or other bio-filters

Based on the limited number of observations available, it seems that influent concentrations of Triclosan are higher in the U.S.A. than in Europe. The Triclosan removal was better in the best U.S. WWTP than in the Swiss plants while the removal in the Danish plant cannot be evaluated due to a high limit of detection.

Based on information on Danish WWTPs, it is considered likely that most Danish effluent waste water and most Danish sewage sludge will contain concentrations of Triclosan at the level of the lowest values presented in Table 2.7. Only at small local WWTPs, effluents and sewage sludge will attain the level of the lower technology foreign plants.

3 Aquatic risk assessment of Triclosan

3.1 Fate of Triclosan in aquatic environments

3.1.1 Triclosan concentrations in U.S. rivers

The results presented in Chapter 2 of the monitoring of Triclosan in wastewater treatment plants (WWTPs) in the U.S.A. have shown Triclosan concentrations in wastewater effluent from 0.24 to 2.7 µg/L (McAvoy *et al.* 2002). Therefore, there is a potential risk of adverse effects in aquatic environments caused by Triclosan discharged from WWTPs.

A probabilistic assessment has been performed with the purpose of determining the frequency at which Triclosan river concentrations might exceed levels of ecological concern (Reiss *et al.* 2001). The majority of the U.S. WWTPs discharges their effluent to rivers. Therefore, only discharges to rivers were included in the analysis. Discharges to e.g. oceans and lakes as well as septic system discharges were assumed to have a smaller ecological impact than the river discharges.

The probabilistic assessment was based on a compilation of all U.S. WWTP data (U.S. EPA in 1996) and a series of databases of national hydrological data, which uniquely identify and interconnect the stream segments or reaches that comprise the U.S. surface drainage system. The hydrological databases include data on the volume of flow, stream velocity, width, depth and pH. Data on the stream reaches, into which the WWTPs discharge, were obtained. Risk estimates were calculated for mean flows and low flows of 11,010 and 9,860 WWTPs, respectively. For the mean flow dilution of WWTP effluent, the lower 5th, 10th and 50th percentiles were 15, 33 and 583, respectively, while the lower 5th, 10th and 50th percentiles were 1.0, 1.4 and 27 for the low dilutions. These dilutions were calculated for a situation in which the waste water is completely mixed with the receiving water. However, for toxic substances, a different approach should be used since critical environmental conditions may occur near the discharge before complete mixing with the receiving water (U.S. EPA, 1991; Tørsløv *et al.* 2002).

Triclosan concentrations measured in two activated sludge treatment plants and two trickling filter treatment plants located in Ohio (McAvoy *et al.* 2002) were used for the assessment. It was assumed that the influent concentration was independent of the treatment type or percentage removal and it is stated that one of the five influent concentrations found by McAvoy *et al.* (2002) was chosen for each treatment plant. However, there is no description of how this selection of the influent concentration was made. The removal percentages of Triclosan from the 11,010 WWTPs were selected among the data obtained in Ohio depending on the treatment system used in the specific WWTPs and used to calculate an effluent concentration.

The physico-chemical properties of Triclosan were evaluated and data were selected for estimation of environmental exposures at the discharge point of a WWTP (Reiss *et al.* 2001). The removal of Triclosan by sorption to organic matter, i.e. through contact with the suspended solids from the wastewater effluent, with the suspended sediment and with the dissolved organic carbon from the river, was considered for the estimation of the exposure concentration at the discharge point. The pKa of Triclosan is 8.14 at 20°C and a significant amount of the substance may be found in ionised form in the water bodies. The ionisation of Triclosan was considered and it was assumed that only the unionised Triclosan sorbs to the organic matter.

The Triclosan dissipation downstream was estimated using standard first-order kinetics. A first-order rate constant, k , of 0.054 hour⁻¹ was used. The constant was estimated based on a river die away study by Morral *et al.* (not dated) and corrected for dilution. This study by Morral *et al.* is, however, still unpublished and has not been available for the present evaluation. The physico-chemical properties collected by Reiss *et al.* (2001) are summarised in Table 3.1.

Table 3.1
Physico-chemical properties of Triclosan (Reiss *et al.* 2001)

Property	Value
Molecular weight	289.6
Water solubility	12 mg/L
Dissociation constant (pKa)	8.14 at 20°C
Vapor pressure	$7 \cdot 10^{-4}$ Pa at 25°C
Partition coefficient (log K_{ow})	4.8
Aerobic biodegradation in soil	17.4 – 35.2 day half-life
Aqueous photolysis	41 min. half-life at pH 7 and 25°C
Adsorption to suspended solids (K_{oc})	47,454 mL/g

The following aspects were either not considered significant for the estimation of the exposure concentrations or data were not available (Reiss *et al.* 2001):

- Aquatic biodegradation or anaerobic degradation – no available studies
- Sorption to biota - no available data
- Biodegradation in benthic sediments – considered negligible
- Aquatic photolysis - considered negligible in the water bodies

The estimate used for the suspended solid concentration of effluent at 5 mg/L is conservative. The recommended concentration of a WWTP effluent in Europe is 30 mg/L for a generic risk assessment (European Commission 2003). Suspended sediment and the dissolved organic carbon data for stream reaches were obtained from national water-quality monitoring networks in the U.S.A. Finally, the organic carbon content of the suspended sediment was estimated on the basis of a log-linear relationship obtained by using 131 sets of measurements in U.S. rivers.

The available data on the dissipation of Triclosan during the river die-away study by Morral *et al.* and the water velocity in each stream reach were used to estimate the dissipation of Triclosan in the stream reaches at one and five miles downstream from the discharge point of the WWTPs. The results of the modelling are summarised in Table 3.2.

Table 3.2
 Modelled distribution of bioavailable Triclosan concentrations ($\mu\text{g/L}$) in rivers following discharge (Reiss *et al.* 2001)

Dilution	Location	Percentiles		
		5 th	50 th	95 th
Mean flow	Discharge point	$6.9 \cdot 10^{-6}$	$7.0 \cdot 10^{-4}$	0.043
	One mile ¹ downstream	$6.6 \cdot 10^{-6}$	$6.4 \cdot 10^{-4}$	0.038
	Five miles ¹ downstream	$5.0 \cdot 10^{-6}$	$3.2 \cdot 10^{-4}$	0.015
Low flow	Discharge point	$5.8 \cdot 10^{-6}$	0.016	0.53
	One mile ¹ downstream	$5.6 \cdot 10^{-6}$	0.015	0.47
	Five miles ¹ downstream	$4.3 \cdot 10^{-6}$	$7.9 \cdot 10^{-3}$	0.25

1: 1 mile = approx. 1.6 km

One significant uncertainty is the possibility of Triclosan discharges from several point sources into the same water body, which was not considered. This problem is discussed by Reiss *et al.* (2001) leading to the conclusion that accumulation may occur in some rivers, which is, however, not likely to be a major problem even in the most vulnerable rivers. Another critical point is that it is not possible to assess whether the combination of influent concentration and WWTP is reasonable. Furthermore, the removal rate of a substance in a WWTP may not only depend on the type of the biodegradation process (e.g. activated sludge or trickling filter) but also on the overall design of the plant, operational conditions, characteristics of the biomass etc.

3.1.2 Triclosan concentration in surface water and sediment in Sweden

Water samples from three cost stations located at Stenungssund in Sweden were analysed for Triclosan as part of an investigation of the environmental fate of Triclosan and tetrabromobisphenol A in Sweden. Stenungssund represents an area with an extensive chemical production and heavy industry. Triclosan was only detected at one of the stations in a concentration at 160 ng/L in a bottom sample taken very close to the industrial area. Apparently, the concentrations at the other stations were very low ($< 2 \text{ ng/L}$) (Remberger *et al.* 2002). It should be mentioned that the report by Remberger *et al.* (2002) does not include details regarding sampling procedures or analytical methods so it has not been possible to evaluate the quality of the investigations.

Samples of sediment were taken from the same three stations at Stenungssund and near to a closed wood preservation plant at Boro in the municipality of Vetlanda. Triclosan was found in sediment samples from all three locations at Stenungssund. The concentrations ranged between 8 and 17 $\mu\text{g/kg d.w.}$, which indicates that Triclosan may be accumulated in the sea sediment. The Triclosan concentration in sediment samples down stream from Boro was 2-25 $\mu\text{g/kg d.w.}$ while $< 2 \mu\text{g/L}$ was found at a reference station at Boro (Remberger *et al.* 2002).

3.1.3 Occurrence of Triclosan in surface water in Switzerland

Water samples from the lakes Greifensee, Zürichsee and Jörisee were analyzed for Triclosan and Methyl Triclosan (Lindström *et al.* 2002). Both Greifensee and Zürichsee receive considerable inputs from WWTPs. Additionally, samples taken from the river Glatt at its source (Greifensee) and its fall into

the river Rhine were analysed. The principle of the analytical procedure used in the investigation is presented in the Section 2.3 above.

Triclosan was detected in the lakes Greifensee and Zürichsee in concentrations up to 0.014 and 0.0031 µg/L, respectively, but remains undetected (<0.0004 µg/L) in the mountain lake Jörisee. The analyses of the water samples from the river Glatt showed Triclosan concentrations of 0.011 µg/L (source) and 0.074 µg/L (outfall). The river Glatt receives waste water from a population of 260,000. An estimation of the emission of Triclosan and Methyl Triclosan into the river Glatt showed a total input of Triclosan to the river between its source and outfall corresponding to 2.8 g/10,000 PE/day. This was found to be in reasonable agreement with the data obtained from the examination of individual WWTPs (Section 2.3).

Estimates of the expected load of Triclosan entering Greifensee and Zürichsee and the resulting concentrations in the lakes showed that the actual concentrations were much lower than the expected concentrations of 0.050 and 0.008 µg/L, respectively. This together with observation of seasonal variations of the concentrations in different layers of the lakes indicated removal by photochemical transformation (Table 3.3). As a result of these observations, experiments with photolysis were performed (see Section 3.1.4).

Table 3.3
Triclosan and Methyl Triclosan in Lake Greifensee (Lindström *et al.* 2002)

Date	Depth in the lake (m)	Concentration (µg/L)	
		Triclosan	Methyl Triclosan
Feb 2, 1998	5	0.013	a
Feb 2, 1998	20	0.014	a
July 21, 1998	5	0.0014	a
July 21, 1998	20	0.010	a
Dec 7, 1998	5	0.012	0.0005
Dec 7, 1998	20	0.010	a
Aug 16, 1999	2	0.0034	0.0008

a: Methylated samples analysed, precluding differentiation between Triclosan and Methyl Triclosan

The removal of Triclosan and Methyl Triclosan in Greifensee was simulated using photolysis rates of Triclosan obtained in laboratory experiments and including removal by sorption/sedimentation. A dynamic lake model was set up based on a previous model developed to describe the behaviour of other organic pollutants in Greifensee. The results of the model calculation were consistent with the measured data indicating a pronounced seasonal variation in Triclosan concentration near the lake surface as a consequence of seasonally varying sunlight intensity (Lindström *et al.* 2002).

3.1.4 Photochemical transformation of Triclosan in surface water

A rapid removal of Triclosan was observed in the lake Greifensee in the investigation 1998-1999 by Lindström *et al.* (2002). The removal rate in the lake could not be explained by removal by sorption/sedimentation only. Indications of photochemical transformation being a further removal process came from Triclosan measurements at different depths in Greifensee (Table 3.3). An experiment was therefore carried out in which Triclosan and Methyl

Triclosan were exposed to natural sunlight in fortified lake water at different pH values. Neither Triclosan nor Methyl Triclosan were transformed at pH 5.6. Triclosan was, however, transformed at pH 8.0 whereas Methyl Triclosan was still not transformable. A parallel experiment performed at pH 8.0 in distilled water showed that the transformation of Triclosan was independent of the presence of natural organic matter and other water constituents. The results indicate that Triclosan is transformed via direct photolysis and that the dissociation of Triclosan governs its susceptibility to photooxidation. The transformation of Triclosan followed first order kinetics. At pH 8, the rate constant (2.7 h^{-1}) was similar to that of dichlofenac, which was used as a reference compound.

The photolysis experiment of Lindström *et al.* (2002) was carried out under clear sky conditions on 23 August 2001. The experimental conditions and the data evaluation are described in a previous investigation and are, therefore, not included in the present report.

Triclosan has previously been detected in rivers along with structurally related compounds, including 2,8-dichlorodibenzo-p-dioxin (2,8-DCDD), which was hypothesized to be derived from the synthesis of Triclosan (e.g. Lopez-Avila & Hites 1980 quoted by Latch *et al.* 2003). Based on these considerations, the finding of the loss of Triclosan in Lake Greifensee and the general concern about dioxins in the environment, a study of the photochemical behaviour of Triclosan was performed (Latch *et al.* 2003).

The influence of pH and irradiation wavelength on the reaction was studied in buffered solutions of osmosis-purified laboratory water. Furthermore, an investigation of the importance of the reaction under environmental conditions was performed in natural water taken from the Mississippi River. Aqueous solutions of Triclosan ($3.5\text{-}76 \mu\text{M}$) exposed to air in quartz bottles were irradiated in a merry-go-around reactor with filtered light (>280 , >290 (Pyrex) or $>320 \text{ nm}$). The experimental temperature is not stated. Small aliquots of samples were withdrawn at intervals for analyses of the kinetics, and substrate decay and product growth were determined by High Performance Liquid Chromatography (HPLC) analysis. Rate constants were determined from a least-squares fit of the data to numerically integrated solutions of differential equations. The presence of dioxin in the irradiated samples was confirmed by GC-MS, HPLC (UV absorbance) and Nuclear Magnetic Resonance (NMR) spectroscopy.

The results showed that the ring closure to 2,8-DCDD was obtained in aqueous solution buffered at pH 8 or above (Figure 3.1). The quantum yields for Triclosan transformation (Φ_T) and 2,8-DCDD formation (Φ_D) were found to be sensitive to pH ($\text{p}K_a$ of Triclosan is 7.9) suggesting that the phenolate form is the photoreactive species.

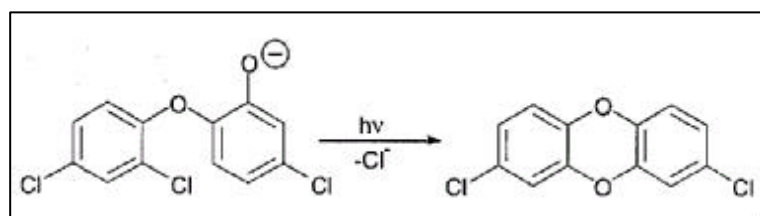


Figure 3.1
Triclosan ring closure by photolysis yielding 2,8-DCDD

The transformation yields (Φ_D/Φ_T) varied with pH and irradiation wavelength but were within the range of 1-4.6% at pH 8 (relevant for most Danish water bodies) and up to 12% at pH 11.5 (not realistic for Danish conditions). This indicates that conversion to 2,8-DCDD is a significant loss process but not the dominant one. Polymers derived from Triclosan are likely to be the main reaction products of a photolysis. The dioxin product 2,8-DCDD is further phototransformed. The decomposition products have, however, not been identified. They may include dechlorinated congeners or rearranged products (Latch *et al.* 2003). The transformation quantum yield of 2,8-DCDD was 2-20 times lower than the apparent quantum yield. The photolysis investigations with Mississippi water confirmed the finding in the buffered solutions indicating that Triclosan is likely to be converted to dioxin in sunlight-irradiated surface waters.

Environmental risk assessments of the transformation products of Triclosan are outside the scope of this report and not included here. However, a simple “very worst case” calculation of a possible environmental concentration of 2,8-DCDD resulting from photolysis of Triclosan can be based on the local PEC values for Triclosan derived in Section 3.1.5 ($PEC_{\text{local surface water}} = 0.009-1.26 \mu\text{g/L}$) and the transformation of Triclosan to 2,8-DCDD at the relevant pH of 8 (4.6%). This gives concentrations of the dioxin in the range of 0.0004-0.06 $\mu\text{g/L}$. Such a transformation can, however, only be expected in the upper water layers due to sorption of light in the water column and, therefore, dioxin concentrations in the entire water column are expected to be lower.

In terms of human toxicology, 2,8-DCDD does not have “the dioxin effect”, because it is not bound to the so-called dioxin receptor (Ah-receptor) in the human body. This is in line with the fact that the WHO has not assigned any dioxin toxicity equivalent to this substance (van den Berg *et al.*, 1998) and with the fact that it was not included in the latest EU human risk assessment of dioxines (SCF 2000, 2001).

3.1.5 Triclosan at the discharge point of WWTPs

The Danish EPA recently published recommendations for the establishment of requirements to and regulation of hazardous substances in waste water (Tørsløv *et al.* 2002). In this publication, it is recommended to define a mixing zone on the basis of a dilution, which is achieved for the discharged waste water within a specific distance from the out-fall. As a starting point, a dilution factor of 2-10 may be chosen for watercourses and chronic criteria must be met at the edge of the mixing zone. Furthermore, protection against acute adverse effects of isolated peak concentrations of the substance should be ensured, e.g. by fixing a maximum value for the discharged concentration of a substance.

A concentration range of Triclosan in surface water (C_{local_water}) was estimated using the mixing zone approach mentioned above, the principles of the EU Technical Guidance Document (European Commission 2003) and the effluent data of WWTPs presented in Chapter 2. It was assumed that the concentration of Triclosan would be between 0.10 and 0.65 $\mu\text{g/L}$ in effluents from modern WWTPs of the activated sludge type while the effluents from low technology WWTPs could reach concentrations of 1.6-2.7 $\mu\text{g/L}$ (Table 2.7).

The local concentration in surface water was calculated as follows (European Commission 2003):

$$C_{local_water} = \frac{C_{local_eff}}{(1 + F_{OC_susp} \cdot K_{OC} \cdot SUSP_{water} \cdot 10^{-6}) \cdot DILUTION} \quad (\mu\text{g/L})$$

where

C_{local_eff} : The concentration of Triclosan in WWTP effluent ($\mu\text{g/L}$)

F_{OC_susp} : Weight fraction of organic carbon in suspended matter (kg/kg)
= 0.1 kg/kg (European Commission 2003)

K_{OC} : Partition coefficient organic carbon-water (L/kg)
= 47,454 L/kg (Reiss *et al.* 2001)

$SUSP_{water}$: Concentration of suspended matter in the river (mg/L)
= 15 mg/L (European Commission 2003)

F: The dilution factor for the effluent in the mixing zone
= 2 to 10 for watercourses (Tørsløv *et al.* 2002)

The calculation resulted in concentrations of Triclosan in surface water between 0.009 and 0.303 $\mu\text{g/L}$ at the edge of the mixing zone for high technology plants and between 0.149 and 1.26 $\mu\text{g/L}$ for low technology plants. For the high technology plants, the estimated concentration range is within the range obtained in the probabilistic assessment made by Reiss *et al.* (2001) ($6.9 \cdot 10^{-6}$ to 0.53 $\mu\text{g/L}$) at the discharge point even if it is at the upper end. For the low technology plants, the estimated concentrations exceed the maximum value of Reiss *et al.* (2001). Furthermore, the lowest concentration for the mixing zone is a factor of 1,348 higher than the 5th percentile found in the probabilistic assessment. In the probabilistic assessment, this very low concentration is caused by the use of dilution factors of up to 48,700 (the highest 95th percentile).

The Triclosan concentrations of the Swiss river Glatt (0.011-0.074 $\mu\text{g/L}$) as well as the maximum concentrations of 0.16 $\mu\text{g/L}$ found in Swedish surface water and of 0.014 $\mu\text{g/L}$ in the Swiss lake Greifensee are within the concentration range obtained by the above EU TGD calculations (0.009-1.26 $\mu\text{g/L}$). The concentrations of Triclosan in the lake Zürichsee (maximum of 0.0031 $\mu\text{g/L}$) are, however, below the estimated values.

Based on this, the concentration estimates are considered to be realistic worst case estimates. Considerable dilution in the water body will, however, take place in most Danish waters, because only very few WWTPs discharge into small water courses with a dilution as low as 2-10, while most others discharge into the sea (Danish EPA 2001). Therefore, for most of the Danish waste water, the dilution is expected to be a factor of 10-50.

3.1.6 PEC for the aquatic compartment

The most conservative estimates of predicted Triclosan concentrations in the aquatic environment resulted from application of the principles of the EU TGD (European Commission 2003) and those of the Danish EPA (Tørsløv *et al.* 2002). Therefore, the estimation of the predicted local environmental concentration for surface water ($PEC_{\text{surface water}}$) was based on these results:

$PEC_{\text{surface water}}$: 0.009-0.303 µg/L for high technology plants
 $PEC_{\text{surface water}}$: 0.149-1.26 µg/L for low technology plants

It shall be noted that a potential background concentration of Triclosan was not included in the $PEC_{\text{surface water}}$.

3.2 Effects of Triclosan in aquatic environments

3.2.1 Toxicity

The available literature regarding the toxicity of Triclosan to aquatic organisms comprises the following studies:

- Environmental assessment of Triclosan, report prepared by NIVA (Hansen & Källqvist 2001)
- Review of a series of laboratory toxicity and bioaccumulation studies, journal article (Orvos *et al.* 2002)
- An ecological risk assessment for Triclosan, including aquatic and terrestrial toxicity data, manuscript (Reiss *et al.* 2001)

Results of toxicity and bioaccumulation tests quoted in this literature are summarised in Table 3.3. Below, the papers are summarised with respect to the possibilities of evaluating the quality of the studies, which they quote.

In the NIVA report, Hansen & Källqvist (2001) quote results of toxicity tests with algae, crustaceans and fish, reported by CIBA and collected from Internet available databases. Apart from a discussion of the results of the test showing the lowest EC_{50} value (algal growth inhibition), the quality of the studies/reports is not evaluated. The algal growth inhibition test under discussion was conducted with one of the species, which are recommended in international test guidelines like the OECD TG No. 201; *Pseudokirchneriella subcapitata* (formerly called *Selenastrum capricornutum*). The test procedure followed international guidelines except for the fact that measurements of algal populations were made only at the start and at test termination, which was after 96 hours as opposed to the recommended 72 hours for algal growth inhibition tests. Hansen & Källqvist (2001) conclude that a calculated $E_t C_{50}$ value of 15.7 µg/L after 96 hours will be rather close to the corresponding value, which could have been obtained after 72 hours. In addition to the data presented in Table 3.3, Hansen & Källqvist (2001) quotes data from safety data sheets and toxicity data, which are not measured after the standardised exposure periods. These data are all higher by up to one order of magnitude than the data presented in Table 3.3.

The review paper by Orvos *et al.* (2002) summarises a series of fate, bioaccumulation and toxicity studies with Triclosan in detail.

It is stated that all the toxicity tests conformed to Good Laboratory Practice procedures as prescribed by either the OECD or the U.S. EPA. However, not all tests were conducted according to international or national (U.S.) guidelines although most tests were based on such guidelines (developed for testing of chemicals or effluents) from the OECD, the U.S. EPA or the ASTM. Most tests were conducted under semi-static or flow-through conditions. The results of most tests were presented as measured concentrations. Therefore, these studies must be considered as being high quality studies.

Studies with synthetic secondary effluent from laboratory scale CAS units, to which Triclosan had been added or not, were conducted with fish (*Pimephales promelas*) and crustaceans (*Ceriodaphnia dubia*). The methods applied were U.S. EPA methods for testing of effluents. The results of these tests showed no difference between effects of effluent from CAS systems with and without Triclosan, indicating that Triclosan had been degraded and/or sorbed in the CAS systems.

Experimental details and results of the standard toxicity tests are summarised in Table 3.3.

Reiss *et al.* (2001) focus on the integration of results of fate and effects studies in a probabilistic risk assessment of Triclosan. Therefore, data quality is not evaluated. However, in this paper, the same studies as reviewed by Orvos *et al.* (2002) are used and reference is made to original study reports. From this paper, it is obvious that most studies were carried out during the 1990s by laboratories known to be GLP contract laboratories. Therefore, this paper supports the above conclusions that these studies were high quality studies. In Table 3.3, the studies quoted by Reiss *et al.* (2001) are indicated.

Table 3.3
Summary of aquatic toxicity tests with Triclosan

Taxonomy	End point	Parameter	Exposure time ¹	Result ² µg/L	Reference ³
Algae					
<i>Scenedesmus subspicatus</i>	E _r C ₅₀	Growth ⁴	72 h	2.8 m	Reiss, Orvos
<i>Scenedesmus subspicatus</i>	NOEC	Growth ⁴	72 h	0.5 m	Reiss, Orvos, NIVA
<i>Scenedesmus subspicatus</i>	E _b C ₅₀	Biomass ⁴	72 h	0.7 m	Reiss, Orvos, NIVA
<i>Scenedesmus subspicatus</i>	EC ₅₀	Growth ⁵	96 h	1.4 m	Reiss, Orvos
<i>Scenedesmus subspicatus</i>	NOEC	Growth ⁵	96 h	0.69 m	Reiss, Orvos
<i>Selenastrum capricornutum</i>	EC ₅₀	Growth	96 h	4.46 n	Reiss, Orvos
<i>Selenastrum capricornutum</i>	EC ₂₅	Growth	96 h	2.44 n	Reiss, Orvos
<i>Skeletonema costatum</i>	EC ₅₀	Growth	96 h	>66 n	Reiss, Orvos
<i>Skeletonema costatum</i>	EC ₂₅	Growth	96 h	>66 n	Reiss, Orvos
<i>Navicula peliculosa</i>	EC ₅₀	Growth	96 h	19.1 n	Reiss, Orvos
<i>Navicula peliculosa</i>	EC ₂₅	Growth	96 h	10.7 n	Reiss, Orvos
<i>Anabaena flos-aquae</i>	EC ₅₀	Growth	96 h	0.97 n	Reiss, Orvos
<i>Anabaena flos-aquae</i>	EC ₂₅	Growth	96 h	0.67 n	Reiss, Orvos
Crustaceans					
<i>Daphnia magna</i>	EC ₅₀	Immobility	48 h	390 m	Orvos
<i>Ceriodaphnia dubia</i>	EC ₅₀	Immobility	48 h	130 (?)	NIVA
<i>Daphnia magna</i>	NOEC	Reproduction	21 d	40 m	Orvos
<i>Daphnia magna</i>	LOEC	Reproduction	21 d	200 m	Orvos
<i>Ceriodaphnia dubia</i>	NOEC	Reproduction, pH=7	7 d	6 m	Orvos, NIVA
<i>Ceriodaphnia dubia</i>	NOEC	Reproduction, pH=8.5	7 d	182 m	Orvos
Fish					
<i>Lepomis macrochirus</i>	LC ₅₀	Mortality (1969)	96 h	370 n	Orvos
<i>Pimephales promelas</i>	LC ₅₀	Mortality	96 h	260 n	Orvos, NIVA
<i>Oncorhynchus mykiss</i>	NOEC	Growth and development (ELS)	61 d	34.1 m	Orvos, NIVA
<i>Oncorhynchus mykiss</i>	LOEC	Growth and development (ELS)	61 d	71.3 m	Orvos
Vascular plants					
<i>Lemna gibba</i>	EC ₅₀	Growth	7 d	>62.5 n	Reiss

1: h = hours, d = days

2: n = nominal concentration, m = measured concentration

3: Reiss = Reiss *et al.* 2001, Orvos = Orvos *et al.* 2002, NIVA = Hansen & Källqvist 2001

4: Acetone as carrier, may have resulted in free radicals (Reiss *et al.* 2001), exposure time from Orvos *et al.* (2002)

5: Study designed to avoid free radicals, i.e. NaOH as carrier (Reiss *et al.* 2001), exposure time from Orvos *et al.* (2002)

The toxicity database for Triclosan is comprehensive and most studies referred in Table 3.3 seem to be high quality studies. The database comprises L/EC₅₀ values from acute toxicity tests with the three main groups of aquatic organism (algae, crustaceans and fish) as well as NOEC values from chronic tests with organisms from all three taxonomic groups. According to the EU

TGD (European Commission 2003), this justifies the estimation of a Predicted No Effect Concentration (PNEC) by dividing the lowest NOEC value by an application factor of 10.

However, the choice of the lowest NOEC value is not evident. The discussion of Reiss *et al.* (2001) points to the fact that the lowest value of 0.5 µg/L (from an algal test with acetone as the solvent) may be overestimating the toxicity of Triclosan due to the possibility of acetone inducing formation of free radicals by photolysis. According to these authors, the NOEC value obtained with sodium hydroxide (0.69 µg/L) is more realistic. This test, however, raises another problem (as pointed out by Hansen & Källqvist (2001) who did not have access to the other study), viz. that the exposure time in the test with sodium hydroxide (96 hours) is deviating from the standard exposure time of 72 hours. In their discussion, Hansen & Källqvist (2001) emphasize that it is not possible to estimate whether a 72-hour effect concentration would be higher or lower than one measured at 96 hours. Therefore, the NOEC value estimated after the standard exposure period (72 hours) is preferred for the PNEC derivation.

The lowest NOEC value is thus 0.5 µg/L and the assessment factor is 10, which results in a PNEC = 0.05 µg/L of Triclosan in freshwater aquatic environments.

For marine water, only data from a single test with the marine alga *Skeletonema costatum* are available. The results of the test (nominal concentration) are not feasible for calculation of a PNEC value as both EC₅₀ and EC₂₅ are given as >66 g/L. Therefore, only data for freshwater environments are used.

3.2.2 Bioaccumulation in fish

Orvos *et al.* (2002) refer a bioaccumulation study with zebra fish (*Danio rerio*) as being conducted according to methods modified from OECD TG 305C in a continuous flow system. Accumulation and depuration periods were five and two weeks, respectively, and results were based on measured exposure concentrations (3 and 30 µg/L).

From this study, bioaccumulation factors (BCF) and depuration rates were calculated at the two exposure concentrations. At 3 µg/L, BCF = 4157 and, at 30 µg/L, BCF = 2,532. The depuration rate constants were 0.142 and 0.141/day, respectively.

Hansen & Källqvist (2001) quote results from the Japanese MITI database, showing lower values with *Cyprinus carpio*: BCF: 2.7-44 and 15-90 at 3 and 30 µg/L, respectively. Furthermore, they quote data from a safety data sheet stating BCF values of 3,730-8,400.

With the highest BCF values exceeding 500, Triclosan is considered to be bioaccumulative.

The possibility of finding Triclosan in biota is confirmed by the results obtained in an investigation of twelve different locations in Sweden (Remberger *et al.* 2002). Analyses of samples of different aquatic species showed concentrations ranging from < 0.1 to 13 µg/kg wet weight.

3.3 Risk assessment of Triclosan in surface water

For surface water, the risk quotients based on the above PEC and PNEC values can be calculated as summarised in Table 3.4.

Table 3.4

Risk quotients for surface water, based on U.S., Swedish and Swiss measurements of Triclosan concentrations in effluents from WWTPs

WWTP technology	PEC _{surface water} µg/L	PNEC _{surface water} µg/L	Risk quotient PEC/PNEC
High	0.009-0.303	0.05	0.2-6
Low	0.149-1.26		3-25

Based on the results of the test with the most sensitive species (the alga *Scenedesmus subspicatus*) and on PEC values, which are derived from Triclosan concentrations in U.S., Swedish and Swiss waste water, the risk quotients for surface water are ≥ 1 for low technology WWTPs and for some of the high technology plants.

However, with respect to the PEC values, it was concluded in section 2.7 that only at small local WWTPs, effluents and sewage sludge will attain the level of the lower technology foreign plants and for most waste water discharges from Danish WWTPs, the dilution will be considerably higher than 10. Therefore, Triclosan is not expected to cause effects in surface water unless discharges are from low technology plants to waters with low dilution.

Most Danish WWTPs are high technology plants but the present information on concentrations of Triclosan in Danish waste water is from only one WWTP and is, furthermore, based on a rather high detection limit (1 µg/L). Therefore, no conclusions can be drawn regarding Danish conditions.

Simple calculations – e.g. with an effluent concentration of < 1 µg/L and the use of dilution factors of 2-10, resulting in PEC values in the range < 0.5 - < 0.1 µg/L and risk quotients of < 2 - < 10 – indicate that, in general, the risk from Triclosan in Danish waste water may be moderate. Information on concentrations in Danish effluents as well as a site specific use of the higher dilution factors would be needed to confirm the above assumption regarding the majority of the Danish WWTPs leading to risk quotients below 1.

4 Terrestrial risk assessment of Triclosan

The main sources of data on fate and effects of Triclosan in terrestrial environments are Reiss *et al.* (2001) and a manuscript/report, reviewing a series of studies (Mones not dated).

4.1 Fate of Triclosan in soil

The main route of exposure to soil is expected to be via the application of sewage sludge to agricultural soil. The studies reviewed by Mones include sorption and biodegradation in soil.

4.1.1 Sorption and mobility in soil

In the review made by Mones, the water solubility of Triclosan is given as 10 mg/L while Reiss *et al.* (2001) state that it is 12 mg/L. Both are in agreement as regards the octanol water partition coefficient, which is $\log K_{ow} = 4.8$. Based on this information, Triclosan is considered to have a potential for sorbing to organic matter and particles in sludge and soil (Mones).

In a recent (1997) study based on American standardised sorption/desorption methods, the sorption of Triclosan to sludge was measured. Furthermore, results from an early (1988) study of the mobility in soil, based on German guidelines, are available (Mones).

The sorption study resulted in a K_d value of 21,529 ($\log K_d = 4.3$) and a K_{oc} value of 47,454 ($\log K_{oc} = 4.7$) in sludge (Mones).

In the mobility study, sludge spiked with ^{14}C -labelled Triclosan was placed on top of columns of soil (German standard agricultural (LUFA) soil) and for 48 hours, distilled water was allowed to leach through the columns from the top. Samples of eluate collected at the bottom of the columns as well as soil samples from different levels of the columns were analysed by liquid scintillation counting. The study showed no leaching (i.e. no radioactivity) of Triclosan below a level of 15 cm in the columns during 48 hours (Mones).

Based on this information, it is considered to be a reasonably realistic worst case assumption for the effect assessment that Triclosan, which is entering soil, will not be removed by leaching.

4.1.2 Biodegradation in soil

In a contract laboratory study from 1994, the biological degradation of ^{14}C -labelled Triclosan was investigated in agricultural soil spiked with the test substance at concentrations of 40 and 600 $\mu\text{g}/\text{kg}$ (soil dry weight) for 577 days. The agricultural soil originated from a sludge-amended field. A series of controls with sterilised soil was included for control of laboratory conditions and photolysis. The latter was being kept outside in daylight. Degradation was measured as CO_2 production.

The results of this study indicated that approximately half of the parent substance was mineralised during the 577 days while the identity of the labelled substance remaining in the soil could not be unequivocally demonstrated to be different from the parent substance (Mones).

A 64-day study (conducted by another contract laboratory in 1994) included measurement of CO₂ evolution from three different types of agricultural soil, to which sludge was added in the laboratory, and the matrix was spiked with 200 µg/kg of labelled Triclosan. The sludge used for the experiments was adapted as its origin was an industrial sewage treatment facility receiving Triclosan in the waste stream. Therefore, the results of this study cannot be taken as a general indication of the biodegradability of Triclosan in sludge-amended soil.

After 64 days, between 12 and 20% of the Triclosan was mineralised as measured by CO₂ evolution. However, characterisation of the substances remaining in the soil demonstrated that most of the Triclosan had been transformed to extractable metabolites in the soil. A major metabolite was identified as 2'-methoxy 2,4,4'-trichloro-diphenyl ether. Only between 4.4 and 23% of the parent compound was intact after 64 days. The half-life of Triclosan was calculated to be in the range of 17.4-35.2 days for the three experimental soils (Mones).

4.1.3 Concentrations in Swedish soil samples

In an investigation of soil in Sweden, samples were collected from two contaminated areas (an area with a former wood preservation plant in Boro and the area of a plastic production plant in Ystad) and from an unaffected forest area at Gårdsjön (Remberger *et al.* 2002). The results showed Triclosan concentrations between <3 and 15 µg/kg d.w. in the contaminated sites while concentrations in the forest soil were below the detection limit of 3 µg/kg d.w. soil.

4.1.4 PEC for the soil compartment

As sewage sludge is the major source of Triclosan contamination of soil, the concentrations in sewage sludge (Table 2.7) were used for the estimation of a PEC value for the soil compartment. Concentrations in U.S. and Swedish sewage sludge from plants with activated sludge are reported to range from 0.028-4.2 µg/g (=mg/kg), based on sludge dry weight. The Triclosan concentrations in sludge from trickling filter or other bio-filter plants from the same countries were in the range of 0.38-15.6 mg/kg, all based on sludge dry weight.

In Denmark, the “realistic worst-case” conditions for sewage sludge application to agricultural soil, which are used for risk assessment, are maximum application rate = 3 tonnes dry weight per hectare once every three years, mixing depth = 15 cm, soil density = 1.5 kg/L. This results in a dilution factor of 750.

Based on the above concentrations and assumptions, the following PEC values for Triclosan can be estimated:

Activated sludge:	PEC _{soil} = 0.00004-0.0056 mg/kg soil
“Bio-filter” sludge:	PEC _{soil} = 0.0005-0.021 mg/kg soil

4.2 Effects of Triclosan on terrestrial organisms

4.2.1 Toxicity to soil-living organisms

A 2-week acute toxicity test with compost worms (*Eisenia fetida*) in soil was carried out according to the OECD TG No. 207 (Mones, Reiss *et al.* 2001). In this study, the artificial soil was deviating from that described in the TG as 21% of the sand was exchanged for natural soil, giving a higher sorptive capacity. Furthermore, the test substance was mixed with dry soil, which is known to give maximum sorption. Therefore, the test may have underestimated the toxicity of Triclosan to the worms. No significant effects on worm survival or weight were measured at the highest concentration of Triclosan tested (1,026 mg/kg, soil dry weight).

Mones and Reiss *et al.* (2001) quote a seedling growth test with six plant species (corn, ryegrass, wheat, cucumber, soybean and tomato). Reiss *et al.* (2001) report that this study showed cucumber to be the most sensitive species with NOEC = 96 µg/kg. Furthermore, both mention an additional test with cucumber (the most sensitive species), which is described by Mones. The result of this study is quoted by Mones as NOEL for all parameters (shoot length, shoot and root weight) of “>424 1000 µg/kg” (presumably: >424-1,000 µg/kg) while Reiss *et al.* (2001) state that there were no effects up to 1,000 µg/kg. Chemical analysis of the test substrate indicated that only 34% of the test substance was left intact at the end of the study.

In the terrestrial toxicity section, Mones quotes a study, which was carried out with “activated sludge-mixed liquor”, as being relevant to the top layers of soil. Exposure time was 15 minutes and inhibition of bacterial heterotrophic activity was measured, resulting in an EC₅₀ = 239 mg/L.

As the bacterial test mentioned cannot be considered relevant to soil-living organisms, only results of tests with two groups of soil-living organisms are available. Both tests must be considered to be acute tests and no L/EC₅₀ value is reported. Therefore, the calculation of a PNEC for the soil compartment must be based on the only effect concentration available (NOEC = 96 µg/kg) and an assessment factor of 1,000. Thereby, a PNEC_{soil} can be estimated, which must be considered as preliminary due to lack of data. It is most likely that availability of results from standardised quality studies could lead to the use of an L/EC₅₀, which was lower than the 96 µg/kg. Furthermore, if a large dataset could be used, the assessment factor could be lowered. However, such data are not available at present and the preliminary PNEC value must be used for the terrestrial risk assessment:

$$\text{PNEC}_{\text{soil}} = 0.096 \text{ } \mu\text{g/kg}.$$

4.2.2 Toxicity to birds

The available toxicity studies with birds include two 14-day acute oral toxicity studies with mallard duck and bobwhite quail (Mones, Reiss *et al.* 2001) and an 8-day acute dietary study with bobwhite quail (Mones). All studies seem to have been carried out according to standardised test guidelines in contract laboratories during the 1990s.

The mallard duck study showed no significant effects on body weight, feed consumption or gross pathology at doses up to 2,150 mg/kg body weight. Therefore, the NOEC = 2,150 mg/kg body weight.

The acute oral study with bobwhite quail resulted in a LD₅₀ of 862 mg/kg body weight and diarrhoea was noted in the lowest concentration test group (147 mg/kg body weight) and therefore no NOEL could be established.

The 8-day acute dietary study with bobwhite quail indicated no mortality up to 1,250 mg/kg food. However, at 2,500 mg/kg food, one death and, at 5,000 mg/kg food, 4 deaths (10 birds/group) were recorded. The LC₅₀ was > 5,000 mg/kg food but no conclusion is drawn by Mones regarding the significance and interpretation of the mortality recorded at 2,500 and 5,000 mg/kg food.

It is not possible to apply this information to a risk assessment as there is no effect concentration for dietary intake of Triclosan.

4.3 Risk assessment for the soil compartment

For the soil compartment, the risk quotients based on the above PEC and PNEC values can be calculated as summarised in Table 4.1.

Table 4.1
Risk quotients for the soil compartment, based on U.S. and Swedish measurements of Triclosan concentrations in sewage sludge

Sludge type	PEC _{soil} mg/kg soil	PNEC _{soil} µg/kg	Risk quotient PEC/PNEC
Activated sludge	0.00004-0.0056	0.096	0.4-58
"Bio-filter" sludge	0.0005-0.021		5-219

Based on the preliminary PNEC value as discussed above and measurements in US and Swedish sludge, the majority of the risk quotients for the soil compartment are > 1. Therefore, all the sludge concentrations measured in the U.S.A. and Swedish samples, except for the one with the lowest concentration of Triclosan, would be expected to cause effects in the soil immediately after application of the maximum amount used in Denmark.

For a more realistic terrestrial risk assessment, information regarding concentrations of Triclosan in Danish sewage sludge would be needed. Furthermore, if toxicity data of high quality for terrestrial organisms were available, more confidence could be laid on the PNEC value.

5 Discussion and conclusions

As there is almost no information available on concentrations of Triclosan in Danish waste water, WWTPs or sewage sludge, the assessments were based on information from other countries.

The available studies regarding the fate of Triclosan in WWTPs showed that Triclosan is degradable under aerobic conditions in WWTPs and that the substance is extensively degraded and removed in activated sludge systems. Furthermore, Triclosan does not seem to affect the treatment processes at levels up to 2 mg/L in the influent. However, the available information indicates that only little or no removal of Triclosan occurs during anaerobic sludge digestion.

Apart from microorganisms (which Triclosan is designed to inhibit), the group of organisms most sensitive to Triclosan is plants (algae and cucumber). It must be stressed that the available data on effects of Triclosan on aquatic and soil-living organisms are very limited – especially for the soil compartment.

Therefore, the risk assessments had to be based on very cautious assumptions and, consequently, they are very conservative.

Based on conservative assumptions regarding dilution of the waste water (water courses with low flow and a dilution factor of 2-10), PEC values were derived from Triclosan concentrations in U.S., Swedish and Swiss waste water. The resulting PEC/PNEC ratios (risk quotients, RQ) for surface water were ≥ 1 for low technology WWTPs ($RQ = 3-25$) and for part of the modern plants, because the RQ range was between 0.2 and 6.

In Denmark, only small local Danish WWTPs may however produce effluents and sewage sludge attaining the level of the foreign lower technology plants and, for most waste water discharges from Danish WWTPs, the dilution will be considerably higher than 10. Therefore, Triclosan is not expected to cause effects in Danish surface water unless discharges are from low technology plants to waters with low dilution.

Based on a preliminary PNEC value and measurements in U.S. and Swedish sludge, the majority of the risk quotients for the soil compartment are > 1 . Except for the sample with the lowest concentration of Triclosan, all the concentrations of Triclosan in sludge measured in U.S. and Swedish samples would thus be expected to cause effects in the soil immediately after application of the maximum amount used in Denmark.

For a more realistic terrestrial risk assessment, information regarding concentrations of Triclosan in Danish sewage sludge would be needed. Furthermore, toxicity data of high quality for terrestrial organisms would improve the reliability of the PNEC value.

The outcome of the risk assessments for surface water and soil cannot be considered conclusive for Danish conditions. Based on information on Danish

WWTPs, it is, however, considered likely that most Danish effluent waste water and most Danish sewage sludge will contain concentrations of Triclosan at the level of the low values measured in Sweden, Switzerland and the U.S.A.. Only at small local WWTPs, effluents and sewage sludge are expected to attain the level of the foreign lower technology plants.

The very sparse information on Danish conditions indicates that, for aquatic environments, risk quotients below 1 may be obtained for discharges for most WWTPs. This is expected for effluents from high technology WWTPs with a high dilution in the recipient. However, discharge of waste water to watercourses with very low flow may lead to risks of effects. For verification, results of analysis could be used, only if measurements are made with low detection limits. For terrestrial environments, there is no information regarding concentrations of Triclosan in Danish sewage sludge.

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