CLEARANCE RATE AND MASS LOADINGS OF TRICLOSAN AND TRICLOCARBON IN FOUR WASTEWATER TREATMENT PLANTS IN SAVANNAH, GEORGIA, USA

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Abstract

Concentrations of Triclosan (TCS) and Triclocarbon (TCC) were measured in influent and effluent of President Street, Wilshire, Travis Field and Georgetown wastewater treatment plants in Savannah, Georgia, USA. Among treatment plants, Wilshire plant showed elevated concentrations of TCS (influent; 86,161, effluent; 5370 ng/L), while TCC were greater in Georgetown plant influent; 36,221 and Wilshire plant effluent; 4760. Clearance of TCS and TCC were 95% and 92% in President Street plant, 94% and 85% in Wilshire, 99% and 80% in Travis Field plant, and 99% and 99% in Georgetown plant. Loading estimate results showed that President Street plant WWTP discharge 28 g/day TCS and 62 g/day TCC in to the Savannah River.

Introduction

Triclosan "TCS" (5-chloro-2-(2,4-dichlorophenoxy)phenol) $C_{12}H_7Cl_3O_2$ is a widely used antimicrobial, antibacterial agents and preservatives. TCS is listed as "could be" and "suspected to be" contaminated with dioxins in EPA's Draft Dioxin Reassessment[1]. Because of its chemical structure similar to polychloro phenoxy phenol, it is possible that dioxin can be found in TCS as synthesis impurities. TCS are hydrophobic and lipophilic in nature, hydrolytically stable, relatively non-volatile (4×10^{-6} mmHg), low water solubility (10 mg/L), half-life (21-540 h), and octanol-partition co-efficient (K_{oc}) in sludge solids is 47, 500 log K_{oc} and in water is 4.8-5.4 log K_{ow}. In 1970's TCS like hexachlorophene used in soaps which penetrate skin and produce nervous system to kids[2]. An assessment of liquid soaps available on the market in the USA showed that 76% of 395 soaps of different brands contained TCS. In Europe ~350 tons of TCS are produced annually for commercial applications. In Sweden, toothpaste is considered to be the main singular human source of exposure to TCS. An estimated 20-25% of all toothpaste sold in Sweden contains TCS, accounting for ~ 2 tons of use per year. The European Commission stated that more than one third of the TCS used within the EU in 2002 appeared to reach consumers in oral care products, and a similar amount in skin care products. Annual, worldwide usage of TCS and related biocides was projected to grow at a rate of 5.4% per year to a total of $6.9 billion in 2009, according to a recent market assessment conducted by Freedonia group[3].

Since 1957, Triclocarbon "TCC" (N-(4-chlorophenyl)-N'-(3,4-dichlorophenyl) urea) $C_{13}H_9Cl_3N_2O$ has been used for industrial and domestic purpose. Like TCS, TCC also used as an antimicrobial and antibacterial agents. TCC was reportedly released into wastewater in the U.S. at rates of 500,000-1,000,000 pounds/year[4-8]. TCC has a partition coefficient of log K_{ow} (4.2 at 22.6 °C), low water solubility (11 mg/L at 20 °C), vapor pressure (4.6×10^{-11} Pa) and half-life in soil and sediment of 120 and 540 days, respectively. Despite its extensive use over several decades, environmental occurrence data on TCC is scarce. TCC is toxic to humans and other animals[4] it triggers methemoglobinemia, reduces mammal birth/survival rate, and leads to low body weight.

The majority of TCS and TCC is used and disposed of through municipal or on-site wastewater treatment plants (WWTPs). Due to their affinity for organic matter, removal of TCS and TCC from wastewater is primarily due to sorption of sludge. A portion of the TCS entering a WWTP undergoes methylation to form its primary metabolite methyl triclosan. A fraction of TCC in wastewater is also removed through biologically-mediated transformation. Even with a large fraction of TCS and TCC removed in WWTP, significant quantities of these

compounds enter U.S. surface waters. According to the US Geological Survey study of 95 organic wastewater contaminants in US streams, TCS and TCC were one of the most frequently detected compounds. Savannah wastewater treatment plants contain greater concentrations of perfluorinated compounds[9]. Similarly, it is anticipated that TCS and TCC compounds may be even greater than PFCs and therefore, in this study we proposed to determine these compounds in all four WWTPs located in Savannah, Georgia, USA.

Materials and Methods

Influent, effluent, sludge, pond water (in Wilshire plant) samples were collected from four WWTP in Savannah, Georgia, USA during October 2007. Sediment and sludge samples were stored in I-CHEM bottles, while influent and effluent were collected in clean bottles. Aqueous samples were analyzed within one day after collection, sludge samples were analyzed a few days after collection and samples were stored in refrigerator in dark condition.

Known amount of internal standards of ^{13}C-TCS and TCC were spiked into 500 mL to 1-L of aqueous samples which was filtered through pre-cleaned glass fiber filters (GFF; 0.7 μm; Whatman; Florham Park, NJ). Liquid-liquid extraction (3 cycles) with toluene was employed for dissolved aqueous phase samples. Sonification method (3 cycles) was followed for particulate phase (from ½ liter) sample with 50% acetone in 50% methanol (MeOH). Approximately 1-g sludge samples were loaded with Na_2SO_4 in a pressurized fluid extractor (ASE 200; Dionex, Sunnyvale, CA) and extracted after adding a known amount of ^{13}C-TCS and TCC. Extraction (3 cycles) was done with 95% acetone in 5% MeOH with pressure rate of 1500 psi at 100^{o}C for 5 min. All extracted samples were evaporated with TurboVap II (Caliper Life Science, Hopkinton, MA). Fractionation was conducted with 1-g 5% activated silicagel cartridges by fraction collector (Foxy 200; ISCO, Lincoln, NE). In fraction-1 Methyl-TCS (Me-TCS) was eluted with 6-mL 20% dichloromethane but data was not reported. The fraction-2, 1 mL of 100% dichloromethane was eluted and discarded. In fraction-3, 12-mL 50% dichloromethane in MeOH was eluted to collect TCS and TCC. Eluted samples were reduced to dryness and re-constituted with acetonitrile for LC-MS/MS analysis.

Instrumental analysis was conducted using high-performance liquid chromatography (Agilent HPLC, USA)-interfaced with tandem mass spectrometer (Applied Bio Systems 3200 LC-MS/MS, USA). Acetonitrile and nano purewater were delivered at 0.2-μL/min with the Agilent HPLC. An aliquot (20 μL) of sample was injected onto Ultra IBD HPLC column with Trident integral inlet fitting; 2.1 mm x 150 mm; 5 μm (Restek, USA). The gradient method was adapted for acetonitrile and nano purewater mobile phase. The detector was an Applied Biosystems API-3200 tandem mass spectrometer operated in an electrospray interface in the negative ionization mode. The electron multiplier was set at 1.5 kV while the nebulizer gas was nitrogen. The recoveries of ^{13}TCS and ^{13}TCC spiked into water samples was >90% in all samples. A minimum five calibration points (0.1, 1, 5, 10, 50 and 100 ng/mL) of all TCS and TCC were freshly prepared for each batch and used to calculate the sample concentrations which gives the r^2 = 0.999. ^{13}C-TCS and native TCS was detected with the *m/z* of 301.00 (daughter ion 35.00) and 289.00 (daughter ion 35.00), respectively. ^{13}C-TCC and native TCC was detected with the *m/z* of 315.00 (daughter ion 162.00) and 313.00 (daughter ion 160.00), respectively with the negative ionization method. Blank sample were analyzed for each batch and trace concentration was detected in blank samples. Concentrations of TCS and TCC would have originated from soap and other antibacterial agents used to clean glasswares. Concentrations of analytes in water and sludge expressed on ng/L and ng/g dry wt, respectively.

Results and Discussion

Concentrations of TCS in aqueous samples (influent and effluent) have been illustrated in Table 1. Particulate phase from President Street and Wilshire WWTPs had significantly higher concentrations of TCS than the dissolved phase. In contrast, the dissolved phase from Travis Field and Georgetown WWTP contained significantly higher concentrations. Influent contained greater TCS concentrations than effluent. This trend is

similar to several other available studies that treatment plant shown to remove TCS efficiently. The results showed clearance efficiency in the following order: Travis Field and Georgetown plants (99% clearance), President Street plant (95%) and Wilshire plant (94%). Total TCS concentrations were recorded in Wilshire plant (92 μg/L) followed by Georgetown plant (33 μg/L), President Street plant (20 μg/L) and Travis Field plant (14 μg/L). Blank samples analyzed showed 140 ng/L and 1.4 ng/L in dissolved and particulate phase, respectively. These results show cleaning of glass wares with antibacterial soap and other agents would have been the possible explanation. Observed TCS concentrations were greatest among other reported studies[3, 10-12]. When compared to the concentrations of aqueous phase, sludge sample contained significantly higher concentration, 423 ng/gm basis (Table 1) against ng/L.

Table 1. Concentrations of triclosan (ng/L) in four WWTP samples from Savannah, Georgia.

Plant Names	Dissolved Phase		Particulate Phase		Total TCS		Total	Sludge (ng/g)	Clearance Rate (%)
	Influent	Effluent	Influent	Effluent	Influent	Effluent			
President St.,	6178	836	12672	200	18850	1036	19886	423	95
Travis Field	11914	152	1789	28	13703	180	13883	NA	99
Wilshire	38255	4760	47906	610	86161	5370	91531	NA	94
Wilshire Pond	2368		606				0	NA	
George Town	22254	261	10385	13	32639	274	32913	NA	99
Blank	140		1.4		141	0	141	ND	

Concentrations of TCC in aqueous samples (influent and effluent) have been illustrated in Table 2. Similar to TCS, President Street and Wilshire WWTPs particulate phase had significantly higher concentrations than dissolved phase. The dissolved phase from Travis Field and Georgetown WWTP contained significantly higher concentrations. Influent contained greater TCC concentrations than effluent. The results showed that clearance efficiency was in the following order; Georgetown plant (99% clearance), President Street plant (92%), Wilshire plant (85%) and Travis Field plant (80%). Total TCS concentration was recorded in Georgetown plant (37 μg/L) followed by President Street plant (28 μg/L), Wilshire plant (20 μg/L) and Travis Field plant (12 μg/L). Blank samples analyzed showed 32 ng/L and 8.7 ng/L in dissolved and particulate phase, respectively. Studies from Baltimore[5] and New Jersey[6] reported ND-6000 and 6650-6750 ng/L of TCC from WWTP samples. Another study[7] from the east coast U.S. showed 6.1 and 0.17 μg/L TCC from influent and effluent, respectively. The average effluent concentration of TCC at 10 secondary sewage treatment facilities located throughout the United States was 5.4 μg/L (range = 2.1 -12.0 μg/l).

Table 2. Concentrations of triclocarbon (ng/L) in four WWTP samples from Savannah, Georgia.

Plant Names	Dissolved Phase		Particulate Phase		Total TCC		Total	Sludge (ng/g)	Clearance Rate (%)
	Influent	Effluent	Influent	Effluent	Influent	Effluent			
President St.,	5812	1372	20166	920	25978	2292	28270	897	92
Travis Field	6798	670	2527	1686	9325	2356	11681	NA	80
Wilshire	3108	857	14249	2188	17357	3045	20402	NA	85
Wilshire Pond	1602		1134				0	NA	
George Town	19626	157	16595	124	36221	281	36502	NA	99
Blank	32		8.7		41	0	41	ND	

Concentrations of TCC were greater than TCS in President Street and Georgetown plants (Table 1-2). TCC clearance rate were comparatively less than TCS in Travis Field and Wilshire plants. Structural similarities of TCS and TCC have been illustrated in Figure 1. TCS microbially degraded and converted into Me-TCS. Therefore, the clearance rate was found to be 94-99% as it readily converted by microbes in sewage water as well as chlorinated water in WWTPs. The trichlorinated aromatic structure of TCC suggests potential resistance

to both chemical and biological transformation processes and they would form chlorinated anilines, which are hematotoxic and carcinogenic.

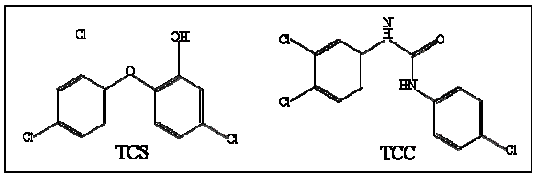

Figure 1. Structure and similarities of TCS and TCC.

Mass loadings were calculated by multiplying the observed TCS and TCC concentrations determined by the daily flow values for influent, effluent, and solid wastes produced at plants. For this purpose, daily influent, effluent, and sludge data were obtained from the individual WWTPs and used for the calculation of mass loading of TCS and TCC on a daily basis. Based on this information, the total water treated was 27, 1.5, 4.5 and 2.45 MGD (million gallons a day) for President Street, Travis Field, Wilshire and Georgetown plants, respectively. President Street's plant discharges water to Savannah River. Georgetown's plant discharges treated water into Ogeechee River. Information about the discharge of effluent water from Travis Field and Wilshire plants was not studied. Except Travis Field plant, all other plants treat water from domestic origins, while the Travis Field plant treats water from both industrial and domestic origins. This information and the calculated release of TCS and TCC were shown in Table 3. Based on the loading estimate it is apparent that President Street WWTP discharges 28 and 62 g/day TCS and TCC, respectively. Sludge was also analyzed from this plant. Average daily production of sludge is 5559 kg/day. Therefore total TCS and TCC from sludge alone equals 2.4 and 5.0 g/kg of TCS and TCC, respectively. However, the sludge from this plant gets incinerated at 400-700°C before slicing the ash into the settling ponds. Therefore, efficient removal of TCS and TCC after incineration analysis may provide the exact removal rate. For accurate loading estimate, not only effluent but also sludge data is necessary. Currently, we're analyzing sludge samples from each treatment plant and also sludge after incineration from President Street's plant. The results will be discussed during the presentation of this paper at Dioxin 2008 meeting. TCS and TCC was analyzed from pond water from Wilshire plant (Tables 1 & 2). TCS (dissolved: 2364 and particulate: 606 ng/L) and TCC (dissolved: 1602 and particulate: 124 ng/L) were occurred at high concentrations. The wastewater water would have leached or discharged to the nearby pond and thus TCS and TCC. Further study is going on regarding TCS and TCC in collaborative research with Skidaway Institute of Oceanography.

Table 3. Loading estimate (g/day) of TCS and TCC in Savannah WWTPs.

	Influent	Effluent	Total	Influent	Effluent	Total
WWTPs	TCS			TCC		
President St.,	509	28	537	701	62	763
Travis	21	0.27	21	14	3.5	18
Wilshire	388	24	412	78	14	92
Georgetown	80	0.67	81	89	0.69	89

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