OCCURRENCE OF TRICLOSAN AND TRICLOCARBON IN WATER AND SEDIMENT COLLECTED FROM THREE RIVERS IN SAVANNAH, GEORGIA, USA

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Abstract
The antibacterial agents such as triclosan (TCS) and triclocarbon (TCC) are used in various consumer and personal care products. Earlier research has revealed considerable levels of these antibacterial agents in wastewater, river water, aquatic wildlife and humans. TCS and TCC are reported to be toxic and produce adverse health effects to wildlife and humans. Consequently, in this study we measured TCS and TCC in river water and sediment samples collected from three rivers in Savannah, Georgia (USA) for the first time. Vernon river showed to contaminate with TCS and TCC comparatively higher than Savannah River and Ogeechee River. On the whole concentration ranges of TCS in Savannah River water and sediment were 0.9 to 9.3 ng/L (water), 2.2 to 16 ng/g wet weight (sediment). Concentrations of TCC in Savannah River water and sediment were 3.3 to 75 ng/L (water), 11 to 52 ng/g wet weight (sediment).

Introduction
The antibacterial triclosan (TCS) is a diphenyl ether (bis-phenyl) derivative known as 2,4,4'-trichloro-2'-hydroxydiphenyl ether[1]. Because of its chemical structure similar to polychloro phenoxy phenol, it is possible that dioxin can be found in TCS as an impurity[2]. TCS is hydrophobic and lipophilic in nature. It is hydrolytically stable, relatively non-volatile, has low water solubility, relatively short half-life. Its partition coefficient (K_{oc}) in sludge solids is 47, 500 and its octanol-water partition coefficient is indicative of its tendency to accumulate in sludge and sediments. In the 1970's it was demonstrated that TCS, like hexachlorophene used in soaps, can impact the nervous system of children[2]. An assessment of liquid soaps available on the market in the USA showed that 76% of 395 soaps from different brands contained TCS. 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 similar amount in skin care products[3].

From 1957, triclocarbon "TCC" (N-(4-chlorophenyl)-N'-(3,4-dichlorophenyl) urea) has been used for industrial and domestic purposes. Like TCS, TCC is also used as an antimicrobial agent. TCC has been estimated to be released into wastewater in the U.S. at rates of 500,000-1,000,000 pounds/year[4-8]. TCC has an octanol-water partition cofficient (log K_{ow}) of 4.2 at 22.6 °C, low water solubility, a low vapor pressure and estimated half-lives in soil and sediment of 120 and 540 days, respectively. Despite its extensive use over several decades, environmental occurrence data on TCC are scarce. TCC is toxic to humans and other animals[4] by triggering methemoglobinemia, a reduction in mammal birth/survival rate, and low body weight.

The majority of TCS and TCC is used and disposed of through municipal or on-site wastewater treatment plants (WWTPs)[9]. Even with large fraction of TCS and TCC removed in WWTP, significant quantities of these compounds enter U.S. surface waters (rivers)[9]. 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. Consequently, we anticipate that TCS and TCC compounds may be contaminated in rivers in Savannah and therefore, in this study we propose to determine these compounds in three important rivers from in Savannah city in Georgia State USA.

Materials and Methods

One liter of river water (from Savannah River [six sampling stations], Ogeechee River [three sampling stations] and Vernon River [three sampling stations]) and sediment samples at same water sampling locations were collected during September to October 2008. For river water, six random samples were collected at each stations in each river. Sediment samples were stored in pre-cleaned I-CHEM bottles, while river were collected in polar solvent and methanol rinsed bottles. Aqueous samples were analyzed within one day after collection, while sediment samples were analyzed few days after collection and samples were stored in freezer in dark condition.

Known amount of internal standards of $^{13}C_6$-TCS and $^{13}C_6$-TCC were spiked into 1-L of river water samples. Solid phase extraction (SPE) was employed with Oasis HLB cartridge (Waters, Milliford, USA). Approximately 5g sediment samples were loaded with Na_2SO_4 in a pressurized fluid extractor (ASE 200; Dionex, Sunnyvale, CA) and extracted after adding known amount of $^{13}C_6$-TCS and $^{13}C_6$-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, 6-mL 20% dichloromethane in hexane was eluted which is discarded. 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 pure water were delivered at 0.2-μL/min with the Agilent HPLC. 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 pure water 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 $^{13}C_6$-TCS and $^{13}C_6$-TCC spiked into aqueous samples was 92-98%. Recoveries of $^{13}C_6$-TCS and $^{13}C_6$-TCC spiked into sediment was 82-88%. 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. $^{13}C_6$-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. $^{13}C_6$-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 we found no impurity of TCS and TCC in any of the samples. Concentrations of analytes in water and sediment expressed on ng/L and ng/g dry wt, respectively.

Quality assurance and quality control were maintained during entire research program. For example, we avoid using solid and liquid soap, antibacterial coated papers as maximum as possible. Soap washed glass wares other analytical materials were rinsed with acetone and methanol and dried using autoclave. The blank checks were run for all particulate and dissolved phase samples as well as sludge and sediment. High purity internal as well as external (calibration standard) was used for the research. Calibration standards were freshly prepared for each set analysis in which relative response for each analysis was (r^2=0.99) agreeable. The data obtained were employed to statistical analysis where ever it is possible.

Results and Discussion

Mean concentrations of triclosan in river water and sediment were 4.6, 3.5 and 5.3 ng/L and 8.8, 5.1 and 11 ng/g wet wt, orderly for Savannah River, Ogeechee River and Vernon River. Overall, Vernon river contained greater concentrations (however not significantly different) of TCS in water and sediment followed by

Savannah River and Ogeechee river. During early 2000's a survey conducted from 139 streams across 30 United States found to have detectable levels of TCS in over 55% of 85 sites examined with a median concentration of 0.14 μg/L and maximum of 2.3 μg/L[10]. Similar levels (0.01 - 0.09 μg/L) are also reported in river and lake systems in Europe[11-12]. In comparison, concentrations of TCS found in the Savannah estuarine waters (1.1 to 5.6 ng/L) are lower than reported values for freshwater systems[9]. However, there is little data available on TCS concentrations in coastal and estuarine waters from the United States. Only one study based on three estuarine water samples collected in Charleston in South Carolina Harbor during 2006 found an average TCS concentration of 627 pg/L[13], which was much lower than the Savannah estuary with the average 5.2 ng/L (Peck unpublished data) as well as toxicity threshold for grass shrimp[13]. There is no report available for the TCS concentration in sediment samples. Concentrations of TCS in sludge samples from Savannah wastewater treatment plants were in between 423-1547 ng/g and 574 ng/g in Wilshire Plant pond sediment[9]. These results also comprehend that TCS sorbed to the solid particles rather than aqueous phase.

Table 1. Concentrations of triclosan in river water and sediment samples collected from Savannah, Georgia.

River Names	Water (ng/L)	Sediment (ng/g)
Savannah River (n=6)	4.6 (1.2-7.3)	8.8 (2.5-12)
Ogeechee River (n=3)	3.5 (0.9-5.9)	5.1 (2.2-8.7)
Vernon River (n=3)	5.3 (2.1-9.3)	11 (4.8-16)

The bactericide TCS an environmental transformation product which is also reported in lakes and in a river in Switzerland at concentrations of up to 74 ng/L. Laboratory experiments showed that TCS in the dissociated form was rapidly decomposed in lake water when exposed to sunlight (half-life less than 1 h in August at 47° latitude).

Mean concentrations of triclocarbon in river water and sediment were 30, 24 and 49 ng/L and 27, 24 and 37 ng/g wet wt, for Savannah River, Ogeechee River and Vernon River, respectively. Similar to TCS, Vernon river contained greater concentrations (however not significantly different) of TCC in water and sediment followed by Savannah River and Ogeechee river. Concentration of TCC in sludge sample was between 462-1479 ng/g in WWTP sample and 689 ng/g in Wilshire Plant pond sediment[9].

In the US, 9 out of 26 urban stream samples tested showed negative TCC levels with a estimated detection limit of 25-30 ng/L[5]. Seventeen river samples, obtained from various locations along six urban streams, tested positive for TCC. Measured concentrations spanned more than 2 orders of magnitude, ranging from 33 to 5600 ng/L. In Savannah River estuary TCC concentrations recorded to be 7.3-48 ng/L with an average concentration of 27 + 20 (Peck Unpublished data). These values are similar to those reported from river water and urban stream water from other parts of US. Environmental concentrations of TCC summarized by Halden and Paull[5] range from non detectable in U.S. drinking water (<10 ng/L), to 240 ng/L in United States surface waters, to 50 000 ng/L in wastewater[4]. Compared to previously reported data for surface waters in the United States[4], the maximum TCC concentrations detected in surface waters of the Greater Baltimore area are 20-fold higher. This discrepancy may be due to a number of factors including (i) increased usage of TCC in recent years, (ii) underestimation of concentrations in previous studies resulting from the use of less selective techniques, (iii) regional differences in TCC usage translating into geographically distinct environmental concentrations, (iv) sampling in locations that were less impacted by inputs of raw and treated wastewater, and (v) underestimation of true concentrations due to the analysis of filtered water, thereby disregarding the particle associated mass of TCC[5].

Table 2. Concentrations of triclocarbon in river water and sediment samples from Savannah, Georgia.

River Names	Water (ng/L)	Sediment (ng/g)
Savannah River (n=6)	30 (4.6-55)	27 (16-42)
Ogeechee River (n=3)	24 (3.3-41)	24 (11-37)
Vernon River (n=3)	49 (8.8-75)	37 (16-52)

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