Fate of triclosan in tertiary wastewater treatment: chlorination

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ABSTRACT

Behavior of the antimicrobial triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol) was investigated under laboratory chlorination conditions and in a wastewater treatment plant discharging 380 million liters daily to the Delaware River, USA. Reactions of triclosan with chlorine were investigated using concentrations and exposure time typical of municipal wastewater treatment plants, i.e., 1 h contact time and average 1–2 mg/L residual chlorine. In reagent water, triclosan reacted quickly, transforming into mono- and dichlorinated species and further into dichlorophenol and trichlorophenol. However, triclosan remained stable for up to 2 h in wastewater samples chlorinated under these conditions. To confirm observed behavior under field conditions, a liquid chromatography tandem mass spectrometry-based analytical method capable of monitoring triclosan and its transformation products in wastewater was developed. Qualitative and quantitative wastewater characterization before and after chlorination are presented. Triclosan was present at the same concentration ($P > 0.05$) in pre-chlorination and post-chlorination aqueous wastewater samples (mean 368 ng/L). This finding is consistent with the non-detection of specific triclosan transformation products above sample reporting limits (30.0–100 ng/L), but contrasts markedly with detection of chlorination transformation products reported in reagent water. These data suggest the importance of influent matrix components in chlorination reactions of triclosan in contaminated wastewater under treatment plant conditions.

Key words | chlorination, Delaware River, pharmaceutical and personal care product, transformation, triclosan, wastewater

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INTRODUCTION

Triclosan, 5-chloro-2-(2,4-dichlorophenoxy)phenol, is an antimicrobial agent categorized as a pharmaceutical and personal care product (PPCP). First introduced in medical settings, triclosan is widely used in many consumer products including soaps, fabric, cosmetics, and toothpaste, typically at concentrations of 0.1–0.3% of the product weight (Dann & Hontela 2011). Many such personal care products ultimately enter public sewage systems under normal usage. Consequently, inputs of triclosan to wastewater treatment plants have been reported in the range of 0.01–4.01 μg/L concentrations (Lishman *et al.* 2006). Due to the incomplete removal of triclosan via wastewater treatment processes, point source discharge of this chemical into the aqueous environment is observed at concentrations of hundreds of ng/L (Fatta-Kassinos *et al.* 2011; Kookana *et al.* 2011). The typical range of triclosan concentrations in discharge wastewater effluent passing though activated sludge treatment has been estimated at 42–1,100 ng/L, averaging 216 ng/L (Wilson *et al.* 2009). In systems where only limited dilution is present, elevated levels of triclosan reaching μg/L concentrations have been observed (Ricart *et al.* 2010).

Triclosan removal efficiency in wastewater treatment varies depending on the specific treatments employed but is routinely >90% (Kumar *et al.* 2010; Buth *et al.* 2011;

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Krishnakumar *et al.* 2011) with sorption as a significant mechanism (Heidler & Halden 2007). This removal however, does not necessarily indicate the complete mineralization of triclosan. Even when transformation occurs, it is possible that different forms of the parent chemical, as yet unmonitored, are discharged in wastewater effluents (Burkhardt-Holm 2010). With a long history of disinfection by-products, chlorination is a relevant treatment for consideration. While wastewater chlorination has never been specifically intended for removal of chemical contaminants from water, chlorine's oxidation potential of 1.36 V may oxidize sites on organic compounds. The oxidation of certain wastewater contaminants by chlorine treatment may result in conversion of the parent compound to product(s) more toxic than the precursor molecule (Dann & Hontela 2011; El Najjar *et al.* 2013).

The reactions of triclosan with chlorine have been reported under drinking water treatment conditions (0–2.4 mg/L residual chlorine). These studies identified two tetraclosan isomers (4,5-dichloro-2-(2,4-dichlorophenoxy) phenol, 5,6-dichloro-2-(2,4-dichlorophenoxy)phenol) and pentaclosan (4,5,6-trichloro-2-(2,4-dichlorophenoxy)phenol), as well as the more stable 2,4-dichlorophenol and 2,4,6-trichlorophenol, as chlorination products (Canosa *et al.* 2005; Rule *et al.* 2005). Under photolytic conditions, triclosan may also be transformed into 2,8-dichlorobenzo-*p*-dioxin (2,8-DCDD) in fresh and seawater (Aranami & Readman 2007), as well as wastewater (Mezcua *et al.* 2004) resulting in aquatic and terrestrial biota exposure (Chalew & Halden 2009). Additional dioxin congeners produced from triclosan's chlorinated transformation products, such as 2,3,7-trichlorobenzo-*p*-dioxin (2,3,7-TCDD) and 1,2,3,8-tetrachlorobenzo-*p*-dioxin (1,2,3,8-TeCDD), have been identified (Buth *et al.* 2009). These triclosan transformation products may produce congeners with elevated toxicity due to the increased substitution on the lateral positions of the triclosan rings (Buth *et al.* 2011). Biodegradation of triclosan results in different transformation products than by chemical means and of particular concern in this category due to its elevated potential for bioaccumulation is methyl-triclosan (Fernandes *et al.* 2011). Therefore, understanding the environmental chemical fate of widely used personal care additives, such as triclosan (and/or its metabolites), is of importance in assessing potential risks to biological systems and associations with current waste disposal and treatment technologies (Bedoux *et al.* 2012).

The objectives of this study were to develop a chromatographic method that is capable of detecting and quantifying triclosan and its possible chlorination degradation products in wastewater treatment samples in a treatment plant serving a major metropolitan area and discharging to the Delaware River, USA. The behavior of triclosan under chlorination procedures consistent with this plant was investigated in a laboratory setting using reagent water and wastewater samples, allowing the identification of the transformation products.

MATERIALS AND METHODS

Experimental overview

The experimental design applied the following approach: (i) a field reconnaissance of PPCPs in pre-chlorination influent; (ii) a reagent water investigation of the chlorinated transformation products of triclosan over a 2 h time period (conditions specific to the publicly owned treatment works (POTW) under investigation); (iii) a reactivity comparison study undertaken in reagent water and wastewater; (iv) development of an analytical method to quantify triclosan and its transformation products employing liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS); (v) application of the analytical method to pre- and post-chlorination wastewater samples from the selected POTW.

Reagents/materials

Ultra pure reagent water and hydrochloric acid (34–37% HCl) were obtained from Seastar Chemicals Inc. (Sidney, BC, Canada). All solvents used were high-performance liquid chromatography (HPLC) grade; methanol was purchased from Honeywell (Morristown, NJ, USA). Acetone and Burdick & Jackson methanol were purchased from VWR International Ltd (Lutterworth, Leicestershire, UK). Sodium hypochlorite (5%) was obtained from Caledon Laboratory Chemicals (Georgetown, ON, Canada). Ascorbic acid, ammonium acetate and ethylenediaminetetraacetic acid, tetrasodium salt dihydrate (EDTA) were purchased from Sigma-Aldrich (Oakville, ON, Canada). Glacial acetic acid

was purchased from Fisher Scientific (Ottawa, ON, Canada). Free and total chlorine were measured employing HACH AquaChek Water Quality Test Strips (Loveland, CO, USA) and/or a HACH Pocket Colorimeter II (Loveland, CO, USA) utilizing diethyl-*p*-phenylenediamine (DPD) reagent. Wide range pH paper (Panpeha, Whatman, Aldrich) was used for measuring pH. Glass fiber filters were purchased from VWR International (Edmonton, AB, Canada).

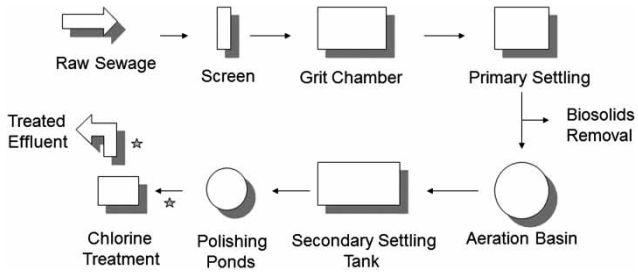

Figure 1 | Schematic diagram of the treatments used by the publicly owned treatment works investigated. Sampling points were located directly prior to and following chlorination treatment as denoted by asterisk.

Analytical standards

Native triclosan, 2,4-dichlorophenol, 2,4,5-trichlorophenol and 2,4,6-trichlorophenol as well as labeled standards $^{13}C_{12}$-triclosan, $^{13}C_6$-2,4-dichlorophenol, $^{13}C_6$-2,4,5-trichlorophenol, $^{13}C_6$-2,4,6-trichlorophenol, and $^{13}C_6$-2,4,5-trichlorophenoxyacetic acid were obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Tetraclosan and pentaclosan standards were prepared in our laboratory as they were not commercially available at the time the investigation was performed. Native triclosan (89.3 μg) was added into Seastar ultra pure water (26.5 mL) and allowed to equilibrate. Dilute sodium hypochlorite (3.00 mL) was added for an initial triclosan concentration of 3.03 μg/mL. The reaction was quenched to neutralize residual chlorine after 5 min using 2.0 mg of ascorbic acid yielding a mixture of tetraclosan (0.992 μg/mL) and pentaclosan (0.651 μg/mL). This solution was used as a chromatographic retention time marker in the LC-MS/MS analysis of the degradation products.

Wastewater treatment study site

Wastewater samples were obtained from a POTW discharging over 380 million liters daily to the tidal portion of the Delaware River in the eastern USA. This high capacity minimized fluctuation impacts on the treatment plant influent characteristics leading to better overall system stability than a smaller plant would present. The POTW under investigation employs grit and primary settling chambers at which point biosolids are collected, dewatered, and reclaimed. The filtered portion passes through aeration basins for contact with activated sludge and further settling tanks (see Figure 1). Polishing ponds and chlorine contact basins are utilized to finish the wastewater prior to its discharge of the final effluent to the Delaware River, a major waterway in the USA, resulting in a mean system retention time of 89.1 h (3.71 days). In the discharged effluent, the average total suspended solids (TSS) is 7 mg/L with pH = 7.0 and the average minimum total residual chlorine is 1.0 mg/L. The 5 day biochemical oxygen demand (20 °C) decreases from an average of 200 mg/L in the raw sewage/influent to 15 mg/L in effluent after treatment through the facility.

Reconnaissance study sampling

Analysis of 1 L samples of the pre-chlorination wastewater was undertaken in the summer of 2009 using appropriate US Environmental Protection Agency (EPA) Method 1694 protocols (U.S. EPA 2007) to confirm the presence of triclosan and provide an overview of additional PPCP present (see 'Reconnaissance study' section). Triplicate grab sampling occurred on 3 non-consecutive days: August 17th, 19th, and 21st, 2009.

Qualitative bench study procedure

Triclosan was chlorinated in reagent water to determine potential chlorinated transformation products through a qualitative bench study procedure similar to that described by both Pinkston (Pinkston & Sedlak 2004) and Canosa (Canosa *et al.* 2005). A 1.0 mg/L chlorine residual was used for the first reaction, followed by a second reaction using a 1.7 mg/L chlorine residual (initial chlorination increased 10×) after 2 h. The potential impact of interactions between chlorination and wastewater matrix were also

investigated by assessing transformation products formed following bench chlorination of wastewater samples.

To study the reactions of triclosan with chlorine, a bench study was conducted using aqueous solutions of triclosan and sodium hypochlorite. Seastar ultra pure water, to give a final volume of 5,000 μL, was added to a 20 mL clear glass vial. A 50.0 μL quantity of diluted bleach solution at 2,000 mg/L, prepared from 5% sodium hypochlorite the day of the chlorination, was then added. The vial was shaken for 10 s and allowed to equilibrate for a minimum of 2 min. This provided a solution at a chlorination level equivalent to 1.0 mg/L chlorine. AquaChek water quality test strips were used to confirm the chlorination level was between 1.0 and 2.0 ppm total chlorine in a separate solution prepared in parallel. Triclosan, prepared in methanol at 119 mg/L, was then added (at a volume of 125 μL), providing an analyte concentration of 2.98 mg/L. The reaction was sampled using an autopipette to withdraw a 250 μL subsample at 2, 10, 30, 60, and 120 min time intervals. Each subsample was then combined with 750 μL of methanol providing a final solution with a 3:1 methanol:water composition with the analyte present at 744 μg/L prior to reaction. In addition, a non-chlorinated sample prepared by the same method was sampled to represent 0 min.

To investigate transformation product formation in matrix, chlorination studies were done using a pre-chlorination, unquenched (i.e., no ascorbic acid added) wastewater sample fortified with triclosan and diluted sodium hypochlorite solution (~0.02%) prepared fresh daily. The reactions were subsampled by autopipette at 2, 60, and 120 min intervals. Initially a pre-chlorination, unquenched wastewater sample was chlorinated to allow the estimation of the degree of chlorination required to produce a free chlorine residual in the unfortified sample matrix.

For the bench study, identification of degradation products was conducted by infusing test solutions directly into a Micromass Q-ToF *II*™ quadrupole/time-of-flight mass spectrometer running manufacturer's MassLynx v.4.0 software. An optimized cone voltage of 25–30 V, collision energy of 5 V and capillary setting of 2,900 V were applied. The cone and desolvation gas flows were set from 50 to 75 L/h and to 250 L/h, respectively, while desolvation and source temperatures were maintained at 200 and 80 °C. Both high and low mass resolution settings were 15.0 and the ion energy applied was 2.0 V. A syringe pump set at 10 μL/min was used for the infusions and the scans were acquired in continuum mode, storing individual scans, over a 2 min time period. The spectra for each time interval were then summed and evaluated.

Quantitative method development and validation

A quantitative method suitable for extraction, clean up, and analysis of triclosan and its chlorinated transformation products, tetraclosan, pentaclosan, 2,4-dichlorophenol, and trichlorophenol was developed and validated using laboratory reagent water, as well as wastewater samples collected from the study site. The method's detection limits, accuracy, and precision were characterized as described in the sections entitled 'Method validation', 'Method detection limits (MDLs)', and 'Accuracy and precision'.

Wastewater collection

Grab samples were collected in June on three non-consecutive days (June 25th, 28th, and 29th, 2010) to minimize the likelihood of non-routine plant events at points directly pre- and post-chlorination. Sample collection was conducted following the appropriate US EPA Method 1694 protocols (U.S. EPA 2007). Both sets of pre- and post-chlorination triplicate samples were quenched with 50 mg/L ascorbic acid to minimize extraneous effects of residual chlorine or the quenching process itself on the analyte concentrations (Vanderford & Snyder 2006). The samples were then shipped to the laboratory and stored frozen (−20 °C) in amber glass containers prior to extraction and analysis.

Wastewater sample extraction procedure

Each aqueous sample was filtered through GF/A filter papers using Millipore apparatus and the solids discarded. A 500 mL aliquot of the effluent was acidified to pH ~ 2.5 using concentrated hydrochloric acid and ^{13}C-labeled quantification standards were added. Ethylenediaminetetraacetic acid, tetrasodium salt dihydrate (EDTA, 500 mg) was used to bind metals, common in wastewater. The sample pH was re-adjusted (as required) after the addition of the EDTA to bring the sample into the pH range 3–4 prior to

the solid phase extraction (SPE) procedure. The SPE cartridge (HLB, 500 mg) was conditioned with 3×6 mL of 1:1 methanol:acetone followed by 2×6 mL of pH 2 Seastar ultra pure water. The samples were loaded on the SPE cartridge and rinsed with 2×6 mL Seastar ultra pure water followed by 3 mL of 5% methanol in Seastar ultra pure water. The cartridge was dried for 2 min under vacuum, then eluted with 8 mL of 1:1 methanol:acetone. This extract was reduced to 300–500 μL under nitrogen, the instrumental standard ($^{13}C_6$-2,4,5-trichlorophenoxyacetic acid) was added and the final extract was diluted to a volume of 1 mL with methanol for a final composition of ~3:1 methanol:water. The sample extracts were then analyzed by LC-MS/MS.

Instrumental analysis

Positive identifications of the analytes (i.e., triclosan and its chlorinated transformation products), labeled quantification standard and recovery standards were based on relative retention times (RRT). The compounds that had exact isotope-labeled quantification standards were required to elute within 0.1 min of the isotope-labeled standard and those without exact isotope labels for the labeled quantification standard were required to elute within 0.4 min of the RRT predicted from the initial calibration. In addition, each of the target analytes were identified with two product ions formed from the parent ion with specific mass to charge ratios. A signal for each of the analytes was considered quantifiable if the signal was greater than or equal to three times the background noise. Absolute recovery of the analytes was corrected for by the use of the labeled standards. The labeled quantification standards were corrected against the instrumental standard to account for matrix suppression. Transformation products without available standards (i.e., tetraclosan, pentaclosan) were quantified assuming a relative response factor equivalent to that of triclosan.

Chromatographic separation was conducted using a C_{18} stationary phase (4.6×30 mm, 3.5 μm, Sunfire, Waters) column. A 15 μL portion of the final extract was injected onto the column (maintained at 30 °C). The mobile phase consisted of 1:1 methanol:acetonitrile (solvent A) and 0.1% ammonium hydroxide/0.1% acetic acid in reagent water buffer, pH = 4 (solvent B). The starting mobile phase composition was 40% solvent A which was held for 0.5 min at a flow rate of 200 μL/min. This was increased to 70% solvent A by 5 min at a flow rate of 150 μL/min and then to 100% solvent A by 10 min. Flow rate was held constant until 18.5 min before being returned to the starting conditions. A 4.5 min column reconditioning period was allowed after the elution of the analytes for optimal peak shape.

Analysis of the sample extracts was performed using a Waters 2795 *Separations Module* (HPLC)/Micromass *Quattro Ultima*™ triple quadrupole mass spectrometer, running manufacturer's MassLynx v.4.1 software. The mass spectrometer used a capillary setting of 3,200 V and the ion energies applied were 1.2 and 2.1 V. The cone and desolvation gas flows were set to 75 and 425 L/h, respectively, while the desolvation and source temperatures were maintained at 400 and 120 °C. High and low mass resolutions were set at 14.5 for the first mass analyzer and 9.0 for the second mass analyzer. Instrumental calibration was performed using a series of calibration solutions (7 points) covering the working concentration range of the instrument (5.0–2,500 ng/mL for all analytes and 50.0–2,500 ng/mL for 2,4-dichlorophenol). The mass spectrometer was operated monitoring parent ion/daughter ion transitions in the negative ionization mode and quantification performed by recording the peak areas of the applicable parent ion/daughter ion transitions (see Table 1).

Method validation

Method detection limits (MDLs)

A MDL study was conducted for eight replicate aqueous samples following generally accepted protocol (Electronic Code of Federal Regulations 2002). In brief, eight replicate reagent water samples were spiked with triclosan and trichlorophenol (10 ng/L reagent water) and dichlorophenol (250 ng/L reagent water). MDL samples underwent the same method analysis as subject samples. The results (see 'Method validation') were analyzed statistically using a one-tailed Student's *t*-value and 99% confidence limit.

Accuracy and precision

To determine method accuracy and precision, analyses of four replicate blank samples, fortified with target analytes

Table 1 | Parent and daughter ions monitored, mass spectrometry conditions, and typical retention times

Analyte	Parent (m/z)	Daughter (m/z)	CV[a] (V)	CE[b] (eV)	RT[c] (min)
Triclosan 1	287	35	25	30	15.8
Triclosan 2	289	37	25	30	15.8
Tetraclosan 1	323	37	25	45	16.1
Tetraclosan 2	321	35	25	45	16.1
Pentaclosan 1	357	37	25	30	17.2
Pentaclosan 2	355	35	25	30	17.2
2,4-Dichlorophenol 1	161	35	25	40	12.1
2,4-Dichlorophenol 2	161	125	25	20	12.1
Trichlorophenol 1	195	35	35	45	14.0
Trichlorophenol 2	195	95	35	35	14.0
$^{13}C_{12}$-Triclosan 1	299	35	25	30	15.8
$^{13}C_{12}$-Triclosan 2	301	37	25	30	15.8
$^{13}C_6$-2,4-Dichlorophenol 1	167	131	25	20	12.1
$^{13}C_6$-2,4-Dichlorophenol 2	167	35	25	40	12.1
$^{13}C_6$-Trichlorophenol 1	201	35	35	45	14.0
$^{13}C_6$-Trichlorophenol 2	201	165	35	20	14.0
$^{13}C_6$-2,4,5-T	259	201	22	11	9.1

[a]Cone voltage.
[b]Collision energy.
[c]HPLC retention time.

was conducted. The suite of analytes was added near the mid-point instrument calibration level (triclosan and trichlorophenol at 100 ng/L, 2,4-dichlorophenol at 1,000 ng/L). To determine accuracy and precision in the presence of sample matrix pre- and post-chlorination samples collected from the POTW were fortified with the compounds of interest and analyzed.

RESULTS AND DISCUSSION

Reconnaissance study

Concentrations of triclosan and a variety of common PPCPs including carbamazepine and valsartan are presented (see Table 2) based on the initial reconnaissance of pre-chlorination wastewater. Due to the antimicrobial properties of triclosan, broad consumer use, frequent environmental presence, and known reactivity to chlorination, triclosan was the PPCP selected as a viable candidate for further study. Triclosan concentrations of 291, 206 and 344 ng/L were found in the pre-chlorination wastewater samples collected on August 17th, 19th, and 21st, 2009, respectively. The expected range of analyte concentration variability was low (relative standard deviation (RSD) <25%) and indicated steady-state conditions. These concentrations were sufficient to allow investigation into potential transformation products following the chlorination process.

Bench study: chlorination of triclosan in reagent water

To investigate the potential transformation of triclosan under chlorination conditions representative of the wastewater treatment plant under consideration (average 1.2–2.2 mg/L

Table 2 | Pharmaceutical and personal care products detected in three pre-chlorination influent samples (August 17th, 19th, 21st, 2009)

Analyte	Use	Max (ng/L)	Mean, $n = 3$ (ng/L)	Standard deviation (ng/L)
Azithromycin	Antibiotic	481	373	94.0
Carbamazepine	Anticonvulsant	339	303	34.2
Diphenhydramine	Antihistamine	420	398	18.9
Gemfibrozil	Antilipidemic	999	831	152
Meprobamate	Antianxiety	756	639	120
Naproxen	Anti-inflammatory	751	412	299
Triclocarban	Antimicrobial	129	108	32.0
Triclosan	Antimicrobial	344	280	69.6
Trimethoprim	Antibiotic	332	326	8.72
Valsartan	Antihypertensive	7,160	5,070	1,840

residual total chlorine, and a contact time of approximately 1 h), a bench study was undertaken in reagent water. Triclosan (2.98 mg/L) was allowed to react with 1.0 mg/L equivalent of free chlorine and within 2 min produced pentaclosan and tetraclosan (see Figure 2) in the negative ionization mode. Triclosan was still present at the 2 h limit in addition to the transformation products in reagent water. When identifying the transformation products of triclosan, indicators such as the addition of chlorine (and subsequent change in the isotope pattern), stability of the parent peak, and overall changes in the mass spectra were examined. No transformation products were identified under the positive ionization mode.

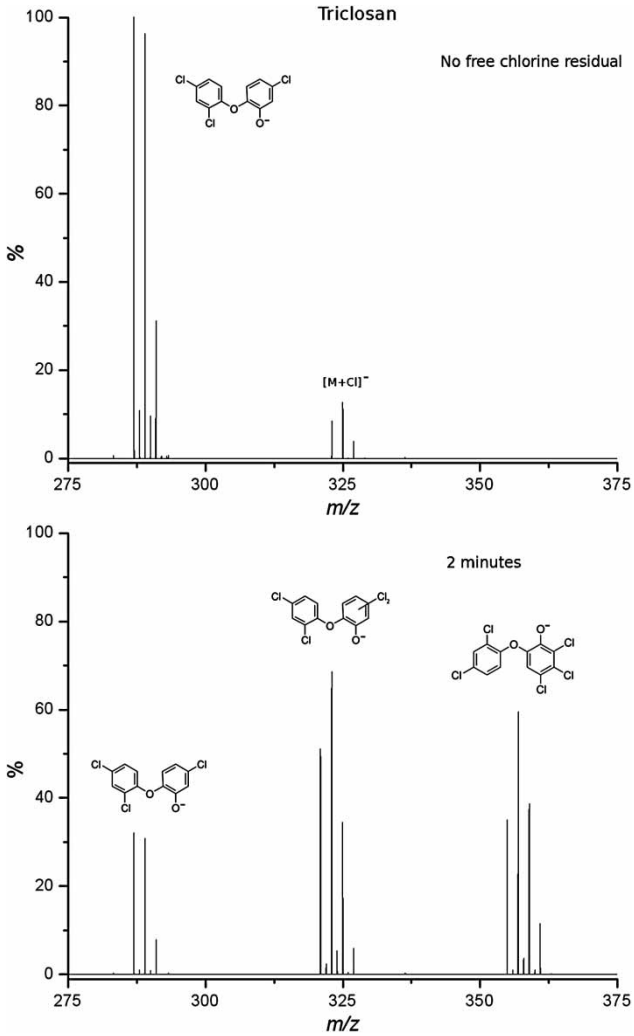

Figure 2 | Quadrupole/time-of-flight mass spectra demonstrating triclosan speciation prior to chlorination (top) and after 2 min of exposure to 1.0 mg/L free chlorine in reagent water (bottom) where the tetraclosan and pentaclosan transformation products are present. Multiple *m/z* peaks are due to isotopic patterns produced from multiple chlorine atoms in triclosan and transformation product structures.

When the chlorination was increased 10 times using the same concentration of triclosan a 1.7 mg/L free chlorine residual was present after 2 h (improved experimental replication of the treatment plant under investigation). Examined under the negative ionization mode, triclosan was consumed producing tetraclosan and pentaclosan as well as the additional transformation products dichlorophenol and trichlorophenol within 2 min. The rate of consumption of triclosan and formation of the various products is depicted in Figure 3. The peak height for tetraclosan and pentaclosan decreased over the reaction period from 30 min onward corresponding with increased trichlorophenol, the most abundant transformation product present at the 2 h experimental limit. Dichlorophenol was present at a low but consistent concentration after the 10 min point of the reaction. These reaction products have all been observed in their derivatized forms previously by gas chromatography–mass spectrometry (Canosa *et al.* 2005; Rule *et al.* 2005) verifying the results for the system under study. Subsequently, the bench experiment was repeated using wastewater samples collected from the selected POTW.

Bench study: chlorination of triclosan in wastewater

Using an unquenched, analyte fortified wastewater and the increased initial level of chlorination (10×) from the second experiment above, triclosan appeared stable in wastewater up to the 2 h experimental limit despite a low 0.30 mg/L free chlorine residual – potentially an artifact of the high total chlorine residual (>8.8 mg/L) indicating the presence of less reactive, combined chlorine species. This finding contrasted markedly with the predicted reaction of triclosan to chlorination, observed as expected in the reagent water when prepared in parallel. Despite chlorination being increased seven-fold, the same null result was observed. At this point, the chlorination level was increased to the breakpoint (an overall 55× increase over the initial chlorination) resulting in 1.8 mg/L free chlorine and 22 mg/L total chlorine residuals after a 1 h reaction time at which point the transformation products tetraclosan, pentaclosan dichlorophenol and trichlorophenol were observed.

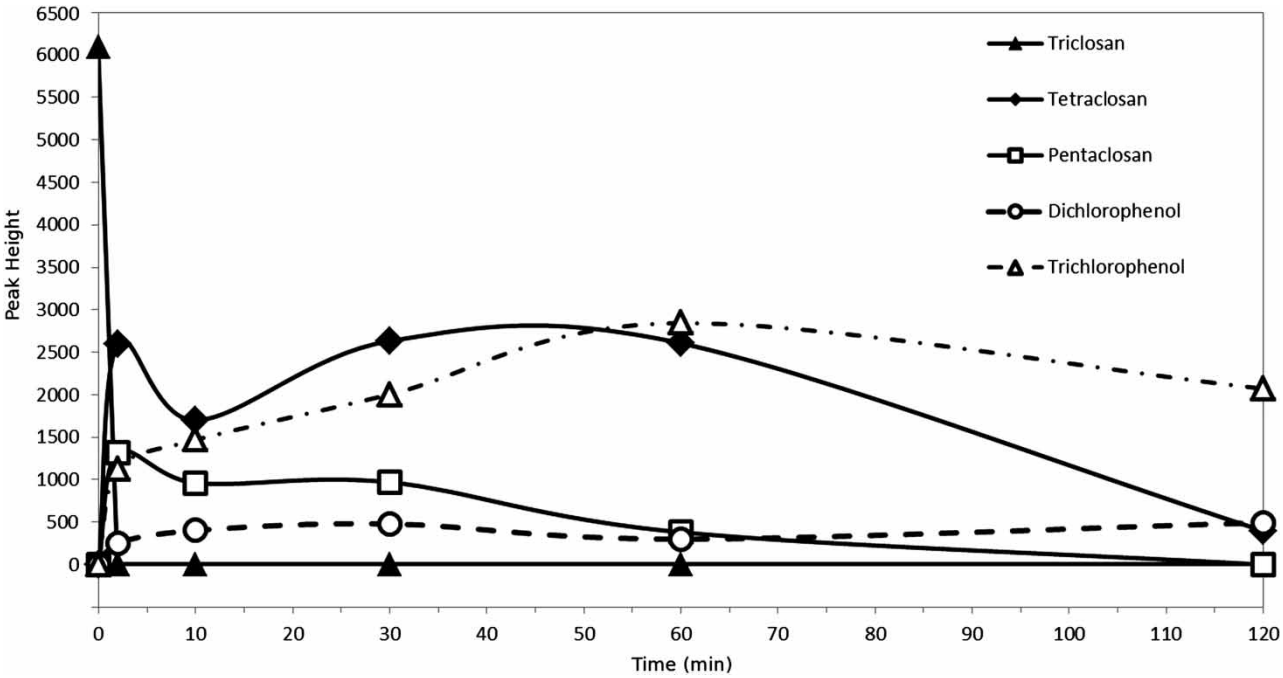

Figure 3 | A 10-fold increased chlorination of triclosan in ultra pure water resulting in the subsequent production of transformation products: tetraclosan, pentaclosan, dichlorophenol, and trichlorophenol.

As demonstrated above, bench studies that investigate solely the effect of free chlorine do not properly take the system complexity introduced by matrix into account and this may drastically impact the resulting data. Combined chlorine in the form of chloramines is easily produced from the ammonia present in wastewater exerting a chlorine demand and contributes to the total chlorine residual. Monochloramine has proven to be an acceptable disinfectant when allowed suitable contact time and triclosan has been shown to react in chloraminated water (provided a nine-fold excess of monochloramine), though two to four orders of magnitude slower than the reaction with free chlorine (Greyshock & Vikesland 2006).

Quantitative method validation and application

Method validation

MDL values of 4.94, 99.7 and 5.38 ng/L were experimentally determined for triclosan, 2,4-dichlorophenol, and trichlorophenol respectively. The MDL values for tetraclosan and pentaclosan are equivalent to that of triclosan based on the quantification procedures. Based on the concentrations of triclosan observed in the reconnaissance study, the determined detection limits were deemed adequate to meet the objectives of the project. The method accuracy is acceptable with a mean recovery of 74.9–102% (see Table 3). Percent RSD values of less than 15 were observed for all target analytes demonstrating good method precision. No background levels were present above the MDLs in the procedural blank. Percent recoveries of the transformation products which did not have exact match analytical standards and were not included in the linearity preparation were calculated compared to a non-extracted aliquot of the retention time marking standard at the same analyte level.

Quality control

Method control was monitored throughout the analysis. A minimum five-point calibration ($R^2 > 0.985$, linear quantification, 1/x weighting, origin excluded) was verified at the beginning and end of each analysis run using the mid-level calibration solution. The acceptance criteria of 70–130% was used for the calibration verification. Each batch processed through the laboratory contained a method blank

Table 3 | Method precision and accuracy study

Analyte	IPR 1 (%)	IPR 2 (%)	IPR 3 (%)	IPR 4 (%)	Mean (%)	RSD (%)
Triclosan	105	107	92.5	105	102	6.50
Tetraclosan	99.4	87.7	88.2	90.0	91.3	6.02
Pentaclosan	85.0	75.2	69.1	70.2	74.9	9.72
2,4-Dichlorophenol	88.4	103	117	96.4	101	12.0
Trichlorophenol[a]	93.0	96.2	110	94.9	98.4	7.64
$^{13}C_{12}$-Triclosan	80.0	98.7	93.1	88.0	90.0	8.83
$^{13}C_6$-2,4-Dichlorophenol	26.6	60.8	64.3	78.3	57.5	38.2
$^{13}C_6$-Trichlorophenol	55.4	92.3	83.9	91.7	80.8	21.5
$^{13}C_6$-2,4,5-T	93.9	92.2	101	101	97.1	4.85

[a]Trichlorophenol consists of a 1:1 mixture of the 2,4,5 and 2,4,6 substituted isomers.

sample to determine background levels (not detected above the reporting limits). A clean water matrix fortified with native analytes, the method spike, was used to evaluate accuracy through the analytical procedure and resulting native recoveries were 75–100%. A secondary, non-extracted standard mix containing the same amounts of native analytes, labeled quantification standards and injection (instrumental) standards was prepared alongside each analysis batch and used as a control on the standards and no issues were observed. The sample workgroups contained a duplicate post-chlorination sample (June 25th) processed in parallel to further evaluate method precision. Triclosan and 2,4-dichlorophenol were detected with relative percent differences calculated against the mean of 6.68 and 21.0%, respectively. Native analytes were added into one pre-chlorination and one post-chlorination wastewater matrix (June 29th) to evaluate the role of matrix on analyte recovery. Percent recoveries ranged from 93.5 to 140%. An unquenched pre- and post-chlorination sample (June 29th) was analyzed to evaluate the impact of the selected quenching agent, ascorbic acid. Triclosan remained relatively consistent with the quenched result (a mean value of 364 ng/L versus 410 ng/L, respectively) while elevated results (approximately 10-fold higher) were found for 2,4-dichlorophenol indicating the accuracy of the triclosan concentrations determined may be considered unaffected by the quenching agent. In addition, trip blanks, one for each day sampled, were included with the field sample collection and analyzed, and all were found to be non-detectable at the reporting limits. Recovery values of the isotope-labeled standards were used as general indicators of method performance for individual samples. $^{13}C_6$-2,4-dichlorophenol recoveries were at least 20% lower than the other labeled quantification standards used (see Table 3).

Field study: results

Summary of the pre- and post-chlorination concentrations of triclosan are presented in Table 4. The precursor analyte, triclosan, was detected at an average concentration of 368 ng/L and no change ($P > 0.05$) in concentrations across the chlorination treatment was found. The results of $n = 3$ determinations for each of the pre- and post-chlorination measurements were tested for statistical significance using the *F*-test followed by Student's *t*-test ($\alpha = 0.05$). The data were contained within the 95% confidence interval and this is consistent with the fact that transformation products at levels above the reporting limit of the developed method were not observed. Concentrations of triclosan observed in the current study fall within the range expected based on previous studies of 42–1,100 ng/L (Wilson *et al.* 2009) indicating that the behavior of the wastewater system under investigation is typical. Observation of 2,4-dichlorophenol in the pre-chlorination samples is not unusual as this analyte may be present in wastewater from alternate sources (e.g., 2,4-dichlorophenol is a degradation product of the commonly used herbicide 2,4-dichlorophenoxyacetic acid (Daugherty & Karel 1994; Crespin *et al.* 2001)) and is also used as an intermediate in herbicide and industrial production (Radwan & Ramanujam 1997;

Table 4 | Pre- and post-chlorination mean concentration of $n = 3$ filtered wastewater samples

Analyte	Pre-chlorination mean (ng/L)	Post-chlorination mean (ng/L)	R. L.[a] (ng/L)	Significant difference?
Triclosan	394	343	30.0	No
2,4-Dichlorophenol	143	319	100	No
Trichlorophenol	N.D.[b]	N.D.	30.2	N/A[c]
Tetraclosan	N.D.	N.D.	30.0	N/A
Pentaclosan	N.D.	N.D.	30.0	N/A

[a]Reporting limit.
[b]Not detected.
[c]Not applicable.

Yadav & Jadhav 2002). To our knowledge, concentrations of the transformation products tetraclosan and pentaclosan above the reporting limit in the current study have not been previously reported in effluents of a similar tertiary chlorination system. However, concentrations that were close to or below the reporting limit of the current study have been reported (McAvoy *et al*. 2002; Buth *et al*. 2011).

CONCLUSIONS

The POTW effluent samples analyzed demonstrate that chlorination as practiced in the treatment plant under investigation does not significantly contribute to the production of chlorinated transformation products of triclosan in wastewater. For the precursor analyte triclosan, detected at an average of 368 ng/L, no significant change in concentration across the chlorination treatment was found. This observation is consistent with the fact that transformation products at concentrations above the reporting limit of the developed method were not observed. These results are supported by the two-part bench study where, provided the same level of chlorination, triclosan reacted rapidly in reagent water while simultaneously maintaining its stability in the wastewater matrix. It is critical to assess these matrix effects when extrapolating 'clean-water' results from laboratory studies to field wastewater conditions. Overall, transformation products for triclosan have been demonstrated to form in a variety of instances, including a wastewater matrix. By extension, further investigation of potential transformation in commonly used PPCP during wastewater treatment, particularly in situations promoting free chlorine or elevated contact time with chloramines, would contribute to the overall understanding of the occurrence and fate of PPCP in the environment at large.

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