Pennsylvania Sea Grant College Program
Final Report

Evaluating the Sources and Fate of Triclosan in a Watershed Traversing a Rural to Urban Gradient in Southeastern Pennsylvania

Amanda M Grannas, Professor, Chemistry & Associate Vice Provost for Research, PI
Villanova University, amanda.grannas@villanova.edu, 610-519-4881

Steven Goldsmith, Associate Professor, Geography and the Environment, co-PI
Villanova University, steven.goldsmith@villanova.edu, 610-519-3589

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Executive Summary: *Summarize the project, including the objectives, methods, results, conclusions, and recommendations.*

The environmental fate of pharmaceuticals and personal care products (PPCP) in regional waterways is of growing concern. One such product, an antimicrobial agent known as triclosan (TCS), has been the focus of recent investigation. Once released into waterways, TCS can photochemically degrade to form even more toxic contaminants like dioxins. The focus of this research is to identify both traditional sources (i.e., wastewater treatment plants) and non-traditional sources (i.e., non-point sources like leach fields associated with septic systems) of TCS release to the environment and to understand environmental conditions that can enhance TCS degradation. In order to address these questions, both natural and model water samples were prepared and analyzed. Natural stream water samples were collected from 16 locations along the East Branch of the Brandywine Creek located in southeast Pennsylvania. Each sample was chemically characterized and underwent a chemical extraction process to determine their TCS concentration. Additionally, model solutions containing TCS with varying pH's, salinities and dissolved organic matter were irradiated to determine how these three variables affected TCS degradation rates. Our work confirms that non-traditional sources of wastewater, such as low volume public and privately-operated waste water treatment plants, leach fields associated with septic tanks, and leaching from biosolids; etc. are all viable sources of TCS. This indicates that aquatic environments other than those downstream of major waste water treatment facilities could potentially be exposed to TCS and its breakdown products. Nitrate concentrations could be used as a lower cost alternative for approximating TCS delivery in rural to urban watersheds. It was also determined that higher pH's, salinity and the presence of dissolved organic material can affect the photochemical degradation rate of TCS, the latter of which has the greatest affect. Therefore, streams with greater amounts of organic material inputs, either natural or from waste waters, could experience greater rates of TCS breakdown and byproduct production. On a positive note, there appeared to be a decrease in TCS concentration between the 2016 and 2017 sampling events indicating that the 2016 FDA ban on TCS in topical antiseptic wash products is helping to reduce its exposure to the environment.

Introduction:

Over the last two decades, there has been a growing urgency to determine both the source and fate of emerging contaminants, such as pharmaceuticals and personal care products (PPCP) in our waterways and/or drinking water (Kolpin et al., 2002; Lindström et al., 2002; Benotti et al., 2008; Reif et al., 2012). Many studies have focused on the widely used antimicrobial agent triclosan (TCS) due to its use in a wide array of personal care products (e.g. toothpaste, mouthwash, cosmetics, deodorant, etc.), hand sanitizers and cleaning disinfectants, and as a commercial microbicide (Bedoux et al., 2012). This ubiquitous use of TCS, coupled with its incomplete degradation during the wastewater treatment process (Lindström et al., 2002; Kanda et al. 2003; Heidler and Halden 2007), has drawn frequent attention due to its link with antimicrobial resistant bacteria in aquatic communities (Drury et al., 2013; Carey and McNamara, 2014), and its toxicity (Orvos et al., 2002; Dann and Hontela, 2011; Ajao et al. 2015; Vincze et al. 2015) and potential role as an endocrine disruptor (Ishibashi et al., 2004; Matsumura et al., 2005; Gee et al., 2008; Zorilla et al., 2009; Dann and Hontela, 2011) to both terrestrial and aquatic species. These notable ecosystem impacts contributed to the issuance of a mandatory phase out of TCS in topical

antiseptic wash products by the U.S. Food and Drug Administration beginning in 2016 (U.S. FDA, 2016).

To date, several studies have focused on evaluating the concentrations of TCS in waste water treatment plant (WWTP) effluent or in streamwaters immediately downstream of municipal WWTPs and/or large metropolitan areas. TCS concentrations in U.S. WWTP effluent has been found to range over several orders of magnitude (50 to 5,370 ng/L) (Lindström et al., 2002; Tixier et al, 2002; Halden and Paull, 2005; Bedoux et al., 2012) with an equally pronounced range of values in downstream waters (~2.5 to 1600 ng/L; Kolpin et al. 2002; Lindström et al., 2002; Halden and Paull, 2005; Bedoux et al., 2012). Similar to other PPCPs, large variations of TCS concentrations in WTTP effluent have been attributed to factors such as the size of population served by the facility, TCS concentration in the influent, the sophistication of the WWTP (e.g. activated sludge with or without tertiary treatment), and residence time of water in the WWTP (i.e., storm versus non-storm conditions) (Miège et al 2009; Bedoux et al., 2012; WHO, 2012; Luo et al. 2014). Upon delivery to freshwater systems, TCS concentrations has shown affinity for particulate sorption due to its hydrophobicity (Tixier et al, 2002, Halden and Paull, 2005; Behera et al., 2010). In addition, photodegradation of TCS in the water column to potentially harmful and persistent products, such as chlorophenols and dioxins has been widely documented (Latch et al., 2003; Mezcua et al., 2004; Sanchez-Prado, 2006). Despite these avenues for removal and/or degradation, TCS was one of the seven most frequently identified PPCPs in U.S. waterways tested as part of nationwide reconnaissance in the early 2000s (Kolpin et al., 2002).

Yet little to no data exists with regards to TCS inputs to streamwater from non-point sources (e.g. leach fields associated with septic tanks, leachate from landfills, biosolids applied to agricultural fields), and/or low-volume, privately-operated WWTPs (hereafter referred to as non-traditional sources). TCS is found in concentrations up to 30 mg kg^{-1} in wastewater biosolids (Heidler and Halden, 2007; Bedoux et al., 2012), which are either applied to agricultural fields or disposed of in landfills. Small-scale empirical studies have shown that TCS delivery via surface runoff (Topp et al., 2008) and tile drainage (Lapen et al., 2008; Edwards et al. 2009) are viable pathways to streamwater. Studies have also documented the presence of TCS in shallow groundwater beneath areas of biosolid application (Karnjanapiboonwong et al., 2011) as well as in leach fields associated with high input septic systems (i.e., park campsite) (Carrara et al., 2008). This documented occurrence of TCS in shallow groundwater, coupled with its predicted half-life of ~540 days in sediment (Halden and Paull, 2005), suggests that TCS contaminated groundwater can be exported to surface water bodies through baseflow. Finally, the lower complexity and water residence times associated with small privately-operated WWTPs, suggests these systems can be sources of TCS to streamwater, regardless of whether they are overwhelmed during a high-intensity rainfall event.

This study sought to address these knowledge gaps by evaluating the input of TCS from both municipal and non-municipal sources in the Upper East Branch of the Brandywine Creek (EBBC), which traverses a rural to urban gradient in southeast Pennsylvania. Sampling above and below the only WWTP in the watershed allowed for the determination of relative TCS loading in the EBBC from traditional (i.e., municipally-operated WWTPs) and non-traditional sources (i.e., low volume, privately-operated WWTPs; leach fields associated septic tanks; leaching from biosolids; etc.). Samples were collected from several locations in the EBBC both during summer baseflow

conditions as well as during and following a 1cm/24 hr period fall storm event to evaluate the role of seasonality on TCS concentrations within the watershed and elucidate preferential delivery pathways of TCS from "non-traditional" sources. Additionally, controlled laboratory photochemistry experiments were conducted to investigate the influence of varying surface water characteristics on photochemical fate of TCS in water (specifically as a function of pH, salinity, and impact of dissolved organic matter (DOM)).

The specific hypotheses tested were:

H1: Non-traditional sources (small sewage treatment facilities and/or septic systems) are a significant contribution to TCS loading in the East Branch of the Brandywine Creek

H2: Changing surface water characteristics (pH and DOM concentration/composition) will impact TCS photochemical degradation across a rural to urban surface water gradient

H3: Bacteria can be used as a low-cost testing alternative for assessing TCS stream-water inputs from non-traditional sources

Methodology

1. H1: Identification of non-traditional TCS sources

A. Sampling:

Streamwater samples for TCS analysis were collected from 16 locations along the EBBC on June 19, 2016 and November 18-19, 2017 (Figure 1; Tables S1 & S2). The June 2016 sampling campaign occurred under baseflow conditions, while the November 2017 event occurred during and/or immediately following a rain event. The 16 locations were located either above or below eight National Pollutant Discharge and Elimination Sites (NPDES) affiliated with low-volume public or privately-operated WWTPs. Sampling under different hydrologic conditions allowed for the evaluation of the role of runoff on TCS concentration. An additional 13 samples were collected from both tributaries and the mainstem of the EBBC on December 2-3, 2017. Samples were collected in pre-combusted 1-liter amber glass bottles, stored on ice in a cooler while in the field, and immediately refrigerated
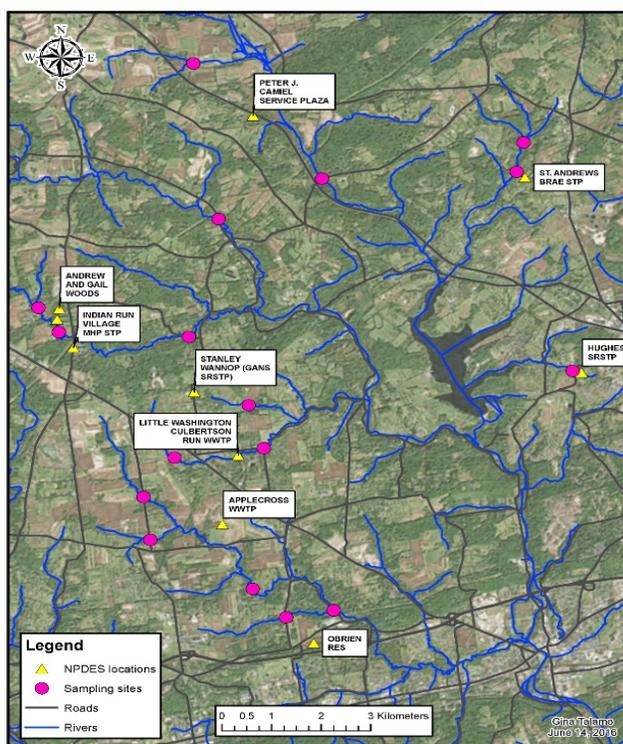

Figure 1. View of NPDES sites and corresponding sample locations on the East Branch of the Brandywine Creek.

upon return to Villanova. Blanks were prepared using Millipore Direct-Q ultrapure water (>18.2 MΩ•cm, <5 ppb TOC).

Instantaneous discharge measurements were collected from each location using either a Teledyne RDI Stream Pro Acoustic Doppler Stream Profiler and/or a FP101-FP201 Global Flow Probe. For three of the sample locations, discharge measurements were obtained from existing USGS gauging stations.

B. *Extraction and quantification of TCS in surface water:*

TCS was extracted from whole water samples using the method of Ramaswamy et al. (2011). $^{13}C_{12}$-labelled TCS (300 ng) was spiked into a 1 L sample to serve as a recovery standard. The solution was made basic by adding NaOH and mixing well in a separatory funnel. The solution was then washed with 50 mL of hexane with shaking for 10 min. The aqueous phase remained in the separatory funnel, was adjusted to pH 2, and extracted twice with 50 mL of hexane. The hexane extracts were combined. The resultant solution was removed and concentrated to 1 mL using a rotary evaporator. HyperSep silica columns (Thermo Scientific) were used to remove any additional impurities from the 1 mL extract. The silica columns were first conditioned with a 1:1 hexane:acetone mixture. Afterward, the extract was added to the silica column and eluted with 15 mL of the hexane and acetone mixture. The eluate was concentrated to 1 mL and stored in an autosampler vial at 4^0C until GC–MS analysis. GC-MS was performed on a Thermo ISQ in EI ionization mode, using full scan from 50 – 500 m/z. A 30m HP-5ms GC column with 0.25μm film thickness and 0.25mm ID was used with helium as the carrier gas (1.0 mL/min). One μL injections were introduced in split mode at 280^0C. Initial column temperature was 70^0C, ramped at 10^0C/min to 280^0C and held for 5 minutes. TCS is quantified using m/z 288 and using m/z 218 as a confirmation ion. Where necessary due to matrix effects, reported data are corrected for loss of the ^{13}C-substituted TCS internal standard. Sample results that were corrected using ^{13}C labeled TCS are indicated in the results. Laboratory and field blanks typically resulted in minimally detected TCS. Blanks were analyzed with each batch of samples and the blank values were subtracted from all measured values.

C. *Evaluation of non-traditional sources:*

A digital elevation model (DEM) for the EBBC watershed was prepared to evaluate the control of land-use/land-cover (LULC) on triclosan concentrations, loads and yields above each sampling location. The ArcGIS™10.3.1 based DEM incorporates existing 30 m resolution USGS (created in 2011) LULC maps to classify the watershed using the following 13 categories: low-, medium- and high-density development, deciduous forest, evergreen forest, mixed forest, cultivated land, hay, grassland, open space, open water, wetlands, and shrubs. A summary of the LULC practices located above each of the sampling locations can be found in Supplementary Table S3.

A search for all NPDES permit sites located throughout the watershed was conducted to determine their potential as a TCS source. Initially, all zip codes falling within the watershed boundary were entered into the USEPA's Permit Compliance System (PCS) and Integrated Compliance Information System (ICIS) databases using the Envirofacts website (https://www3.epa.gov/enviro/facts/pcs-icis/search.html). Of the initial 110 NPDES sites

identified, only 77 were found to be within the watershed boundary. The remaining sites were further narrowed using the following criteria: 1) the NPDES permit was expired or terminated over 3 years prior to the initial sampling event, 2) the type of effluent would not likely contribute triclosan loading to the watershed (i.e. not sewage related), and 3) the permit was associated with a municipal storm sewer system. For the eight remaining sites, the name of the permittee, the date of expiration and the total of was recorded (Table S4). A similar search was performed using the Pennsylvania's Environment Facility Application Compliance Tracking System (*e*FACTS) (http://www.ahs.dep.pa.gov/eFACTSWeb/default.aspx/default.aspx) to identify additional privately WWTPs that directly discharge treated wastewater via surface application. Sites meeting the above-referenced criteria were subsequently geo-referenced into our existing DEM using either the lat/long data and/or site address provided on the permit. A subsequent visual verification for each of these sites was conducted using either Google Earth™ imagery or a site visit.

D. *Determination of the total percentage of TCS loading in the EBBC from non-traditional sources.*

Using the data obtained from our sampling sites located immediately above and below the WWTPs, we were able to determine the relative percentage of TCS loading from traditional and non-traditional sources (Instantaneous TCS loads were determined by multiplying the TCS concentrations for each sampling period by the calculated stream discharge data). Understanding how TCS loading varies by season and/or flow regime allows for the prediction of future values, which might be of particular importance to consumers, such as drinking water plants.

E. *Isotopic characterization of the dissolved organic matter (DOM) in the EBBC.*

Streamwater and effluent samples (from the SevernTrent WWTP) will also be analyzed for total DOC concentration and $^{13/12}C$ ratios in an effort to better characterize their differences. This information will be valuable for both our laboratory experiments quantifying TCS degradation rates and possible identification of localized input of wastewater from non-point sources in the watershed. As of preparation of this final report, we are still waiting upon the associated analytical results.

Sample prep (Total DOC):

Samples for DOC analysis were taken from the glass amber bottles and vacuum filtered directly into a 20 ml amber glass vials through a Millipore 0.7 μm nominal pore size glass fiber filter. Filters, vials, and filter funnel were combusted before use at 450°C for 3 hours and rinsed with 10% v/v HCl in DI water. After filtration, samples were acidified to pH 2 with concentrated HCl and stored at 4°C. A Shimadzu 5050A Total Organic Carbon analyzer equipped with both low and high sensitivity catalyst was used for DOC determinations. The high sensitivity catalyst allows for quantification to approximately 0.1 mg/L of DOC. Samples were sparged with hydrocarbon-free air for up to 7 minutes to ensure all purgeable organic carbon and inorganic carbon was removed. The remaining non-purgeable organic carbon in the sample was calculated using 3–4 injections per sample. Multiple injections of individual samples were conducted in order to determine sample measurement precision. Blanks were also analyzed using the procedures outlined above.

Sample prep ($^{13/12}$C of DOC):
A separate aliquot of sample was vacuum filtered into a 40 ml amber glass vial using the procedures outlined above. Samples were subsequently sent to the Ohio State University School of Earth Sciences to be analyzed using an OI Analytical Aurora TIC-TOC analyzer interfaced to a Picarro G2131-*i* Analyzer.
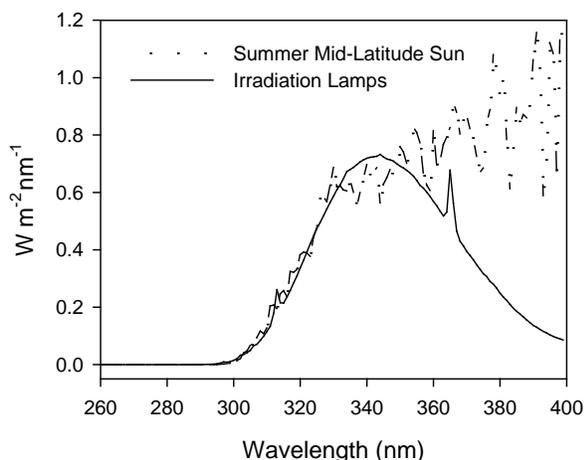

2. H2: Evaluation of the role of changing surface water characteristics on TCS degradation rates.

The photochemical fate of TCS was studied in the laboratory under controlled conditions, using a combination of laboratory-prepared aqueous samples as well as TCS-spiked whole water samples collected from up and downstream of a WWTP.

A. *Sample prep:*

The kinetics of photochemical loss of TCS in various whole water samples was compared using established methods successfully used in our lab in other photochemistry studies (e.g. Grannas et al., 2007; Rowland et al., 2011; Grannas et al., 2014; Fede and Grannas, 2015). Aqueous TCS solutions were made using Millipore Direct-Q ultrapure water (>18.2 MΩ•cm, <5 ppb TOC) for control samples and field-collected whole waters. To avoid exceeding the maximum solubility of TCS, but to remain at concentrations above our instrumental detection limit, aqueous solutions of TCS were prepared at a concentration of 1 mg/L. Solutions of aqueous dissolved-phase TCS were prepared by plating an aliquot of TCS stock solution (in hexane) onto the walls of a volumetric flask. The hexane was evaporated and the desired amount of ultrapure water or whole water sample was added. The solution equilibrated at room temperature in the dark for approximately four days until the solute fully dissolved (confirmed by replicate extractions of the aqueous solution followed by gas chromatography-electron capture detector analysis). For pH studies, solution pH was adjusted by adding HCl or NaOH and monitored by pH meter until desired pH was attained. For salinity studies, inorganic salts were added to equilibrated TCS solutions (gravimetrically) to obtain desired salt concentration. We also conducted comparison studies using commercially available DOM surrogates (Suwannee River Fulvic Acid). In this case, an appropriate amount of DOM was added to the equilibrated TCS solution from a stock aqueous DOM solution. The TCS with DOM solution equilibrated overnight. In all cases, samples for irradiation were prepared by transferring 500µL of the solution into pre-combusted (overnight at 450°C) borosilicate ampules which were then flame-sealed. Samples were stored overnight at room temperature in the dark prior to use.

Figure 2. Comparison of irradiation lamp output with natural sunlight.

A home-built irradiation apparatus was used for sample irradiation. Q-Panel UV 340 lamps were used to simulate the profile of natural sunlight (Figure 2). An Eppley TUVR radiometer was used to measure any variations in light intensity over the course of all experiments (<5% measured light intensity fluctuation is typically observed over several months usage time). Samples were placed in the irradiation apparatus on a black surface at room temperature. Dark controls are kept at room temperature in a closed cabinet. At defined intervals for each experiment a triplicate set of irradiated samples and dark controls are removed, acidified (to ensure efficient extraction of TCS) and extracted with 500 µL of hexane spiked with a hexachlorobenzene internal standard. For each extraction the sides of the ampule were rinsed several times and the hexane/water mixture was transferred from the ampules to 2 mL amber vials. The mixture was shaken using a vortex mixer, then 200 µL of the hexane layer was removed, placed into a pre-combusted micro-insert in a clean, amber autosampler vial and used for immediate GC-ECD analysis.

C. *Sample Analysis:*

Samples were analyzed via HP 5890 Series II GC equipped with an ECD detector. The column used was a 30m RTX-5 (Restek Corporation) capillary column with a 0.25 mm internal diameter and a 0.25 µm coating thickness. The GC method parameters were as follows: initial oven temperature: 70°C, hold for 1 minute; ramp 20.0°C/min to the final oven temperature of 280°C hold for 6 minutes; make up gas is N_2 at 45 psi; inlet temperature: 230°C; inlet pressure: 60 psi; ECD base temperature: 300°C; ECD temperature: 350°C. TCS and hexachlorobenzene calibration curves were obtained with each batch of samples analyzed. The ratio of TCS to hexachlorobenzene in each sample was obtained (to account for potential analytical variability between samples/injections) and a ratio was taken of average irradiated samples to average dark control samples for each time point in order to account for any possible observed dark loss of TCS. The dark loss of TCS was typically less than 10% over a three day experiment time. Uncertainties in data points were obtained via propagation of error of the triplicate analyses. TCS degradation profiles were generated as plots of time versus the irradiated/dark ratio ($[TCS]_L/[TCS]_D$) at time=t relative to the irradiated/dark ratio at time t=0 (Equation 1). To perform comparative statistical significance tests for different experimental conditions, data were linearized by plotting the natural log of the irradiated/dark ratio versus time. The linearized slopes (and 95% confidence intervals) were used to obtain rate constant values for TCS degradation and were compared using analysis of regression statistics for each experimental condition.

$$\textit{Equation 1} \quad \text{TCS Degradation Ratio} = \frac{\left({[TCS]_L}\big/{[TCS]_D}\right)_{t=t}}{\left({[TCS]_L}\big/{[TCS]_D}\right)_{t=0}}$$

3. H3: Evaluation of bacteria as a low cost testing alternative for assessing TCS streamwater inputs from non-traditional sources.

Measurements of PPCP products such as TCS can be of considerable expense due to time intensive sample processing procedures. Therefore, it would be preferable to have a low cost diagnostic tool to further pinpoint non-traditional sources of PPCPs to watersheds, such as the EBBC. Along these lines, we collected streamwater samples for total fecal coliform bacteria, total suspended solids, and nitrate from each sample location during all of the sampling campaigns. . Fecal coliform bacterial pollution can also result from the incomplete treatment of wastewater (Young and Thackston 1999; George et al. 2002; Kelsey et al. 2004), particularly in WWTPs that do not have the secondary and tertiary treatment steps (George et al. 2002). Similarly, WWTP effluent can be a major source of nitrate to natural waters (Puckett 1995; Berner and Berner 2012).

Water samples for total fecal coliform bacterial analysis were collected in deionized water washed 500 ml low density polyethylene (LDPE) bottles. Samples were stored on ice in a cooler while in the field and processed immediately upon return to Villanova. Samples for bacteria analysis were placed on a 3M Petrifilm E. Coli/Coliform Count Plate, and incubated in an oven at approximately 20°C for 24 hours. Total fecal coliform colonies were counted using a binocular wide-field dissecting microscope (EPA Method 9222). Triplicate analysis of each sample will be performed. Total fecal coliform colonies were counted using a binocular wide-field dissecting microscope (EPA Method 9222).

Water samples for TSS analysis were collected in a separate 500-ml LDPE bottle and measured by weight differentiation (EPA 1971). 47mm Whatman™ glass fiber filters were dried at 70°C for 24 hours in a drying oven and a dry filter weight will be recorded. Water samples were then filtered using the dried filters and a vacuum filtration apparatus. The sediment laden filter was placed in the drying oven at 70°C for at least 24 hours and a final filter weight was recorded. TSS was determined as the difference between the initial and final filter weights divided by the total sample volume.

Samples for nitrate analysis were syringe filtered in the field using 0.45 µm nylon filters, directly into a dedicated DI washed 60-ml LDPE bottles. The samples were analyzed for nitrate using a Dionex ICS-1000 ion chromatograph (IC) and the methods of Welch et al. (1996).

Results and Discussion

1. H1: Identification of non-traditional TCS sources

Detectable TCS concentrations were observed in 7 of the 16 sites sampled during the June 2016 (70 to 230 ng/l), while detectable concentrations of TCS were identified in 15 of the 16 sites during

the November 2017 (0.33 to 3.17 ng/l) sampling events (Tables S1 & S2) (Note: TCS concentrations from the December 2018 sampling event were not able to be determined due to instrument error). The concentrations from the June 2016 sampling event are amongst the highest reported within the literature (Bedoux et al. 2012) and ranged anywhere between 35 to ~595x greater than their respective counterpart in November 2017. A subsequent two-tailed t-test (α = 0.05) assuming unequal variance confirmed statistical difference between the seasonal means (p = <0.002). Interestingly, a two-tailed t-test (α = 0.05) between the seasonal mean instantaneous discharge values did not reveal any statistical differences (p = <0.002), suggesting seasonal differences in concentration were not the result of a dilution effect. These results suggest that the 2016 FDA phase out in topical antiseptically products may be playing a role in lowering streamwater TCS concentrations in rural to urban watersheds, such as the EBBC.

A Pearson correlation analysis did not identify any statistical relationship between 2016 TCS yields with either the cumulative permitted NPDES-related discharge (Table 1). However, strongly positive and nearly significant relationships were observed between 2016 TCS yields
and percent medium intensity developed land (r = 0.66, p = 0.11) and high-intensity developed land (r = 0.66, p = 0.07) in the upstream sampling areas. The role of municipal sewage leaks in PPCP contributions to stream and or groundwater has been documented in previous watershed scale studies (Wolf et al 2012) and is likely playing a similar role in portions of the EBBC. Alternatively, these results could be explained by the fact that higher TCS concentrations/yields were identified downstream of NPDES sites located in the southern and more developed portion of the watershed.

Table 1: Pearson correlation coefficients for TCS concentrations and yields

	2016 2016 Yields		2017 Yields	
	r	p	r	p
Permitted NPDES volume	---	---	0.04	0.68
TSS Yield	0.57	0.16	0.31	0.36
NO3 Yield	**0.80**	**0.03**	0.40	0.23
Developed, Open Space	0.28	0.54	-0.16	0.74
Developed, Low Intensity	0.36	0.43	-0.12	0.73
Developed, Medium Intensity	0.66	0.11	-0.06	0.86
Developed High Intensity	0.72	0.07	0.29	0.39
Forest (total)	0.48	0.28	-0.37	0.52
Grassland/Herbaceous	0.45	0.52	-0.32	0.34
Pasture/Hay	0.37	0.32	0.05	0.88
Cultivated Crops	0.13	0.77	0.42	0.20

--- = not enough data

Generally weaker correlations between TCS yields and percent land use in upstream areas were observed for the 2017 dataset, with the exception of percent cultivated crops (r = 0.42, p = 0.20).

TCS has been found at concentrations up to 30 mg kg^{-1} in wastewater biosolids (Heidler and Halden, 2007; Bedoux et al., 2012), and small-scale empirical studies have shown that TCS delivery via surface runoff (Topp et al., 2008) and tile drainage (Lapen et al., 2008; Edwards et al. 2009) are viable pathways to streamwater. The viability of applied biosolids runoff as a delivery mechanism for TCS to the EBBC is confirmed by the sampling location with both the second highest TCS yields and the greatest percentage of agricultural land in its upstream area (Service Station 1). This site also had no NPDES sites in its upstream areas. The lack of correlations between TCS and land use could also be explained by the presence of septic tank related leach fields in upstream areas.

The lack of a statistical relationship between total NPDES permitted flow and TCS yields also suggests that other factors influence TCS yield, such as the type of treatment processes employed at an individual WWTP. For example, the sampling location downstream of the Applecross WWTP (Applecross 3) had the second highest TCS yield in 2016 and third highest yield in 2017. The permit is associated with a small one-step WWTP in conjunction with a private subdivision, which is permitted to either spray its treated wastewater on its associated golf course or discharge directly into Beaver Creek. Interestingly, elevated TCS yields in the location of one of the associated upstream sites (Applecross 1) receives runoff from the area of the golf course.

2. H2: Evaluation of the role of changing surface water characteristics on TCS degradation rates.
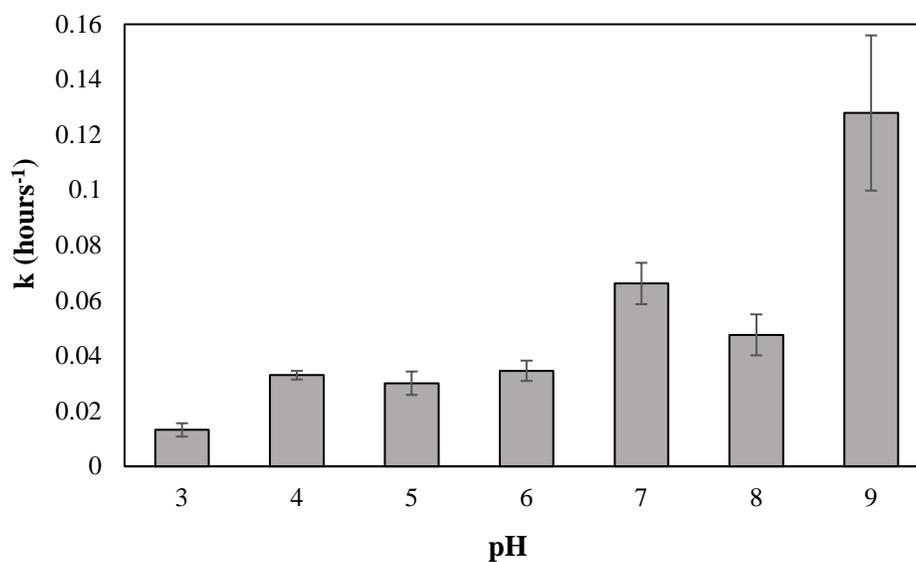
A. *Effect of pH on TCS Photochemical Degradation Rates*

First order rate constants for the photochemical degradation of TCS were calculated as a function of pH (Table 2, Figure 3). The solution pH influences the TCS decomposition with degradation increasing with decreasing acidity. A 10x decrease in the half-life of TCS was observed between a pH of 3 and 9. This behavior is congruent with previous literature that reported increasing rates of TCS degradation with increasing pH (Dhillon et al 2015). TCS exists in its deprotonated phenolate form when the pH > 8.1 and in its phenolic state when the pH < 8.1 (Reaction 1). In its deprotonated state, it has a higher absorption cross section than its phenolic form, as shown in Figure 3, making it is less photostable as supported by our findings (Lindstrom et al, 2002; Tixier et al, 2002; Mezcua et al, 2004). Although it was previously understood that the stability of TCS was pH dependent, this work shows that pH alone in the absence of other dissolved species is able to influence the rate at which TCS photodegrades.

Reaction 1

Phenolic Form *Phenolate Form*
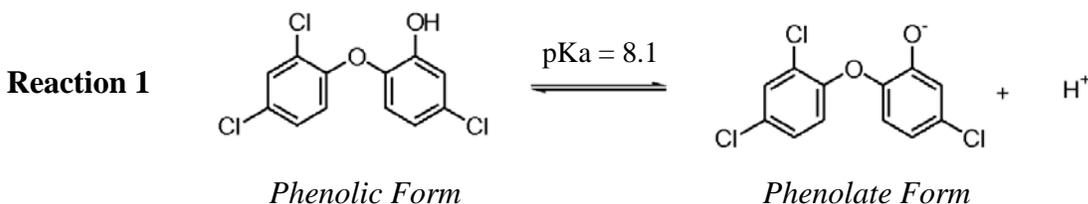
Table 2. TCS photochemical degradation rate constant and half-life as a function of pH.

pH	k (hour^{-1})	t$_{1/2}$ (hours)
3	0.013 ± 0.002	53
4	0.033 ± 0.002	21
5	0.030 ± 0.004	23
6	0.035 ± 0.004	20
7	0.066 ± 0.008	11
8	0.048 ± 0.007	14
9	0.13 ± 0.03	5.3

Figure 3: Rate constant for photochemical degradation of TCS as a function of pH.
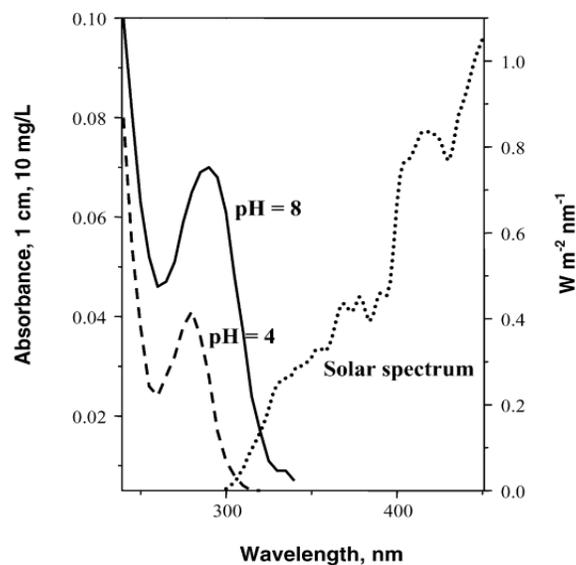
Figure 4: UV absorbance spectrum for TCS at pH = 8 (solid line), pH = 4 (dashed line) relative to the solar spectrum (dotted line). Source: Mezcua et al, 2004.

B. *Effect of Ionic Strength on TCS Photochemical Degradation Rates*

In addition to pH, there was also observed differences in the rate of TCS degradation in solutions of varying ionic strength (Figure 5; Table 3). Previous work also revealed a difference in degradation rates among freshwater and seawater samples (Aranami and Readman, 2017). Aranami and Readman (2017) determined that TCS in seawater with a salinity of 28,000 ppm (pH = 8) and TCS in freshwater (pH = 7.5) had a half-life of 4 and 8 days, respectively. However, this differs from our work in that we did not observe statistically different degradation rates between our 25,000 ppm solution and our pure water sample. This might indicate that the observed differences in TCS degradation due to salinity as reported by Aranami and Readman were due to pH differences rather than salinity differences. In our work, there appeared a bimodal distribution in which TCS degradation was most rapid at very high NaCl concentrations and also when no NaCl was added. The slowest degradation was observed when the NaCl concentration was 10,000 ppm. However, the rate of degradation at both 0 and 35,000 ppm NaCl was similar to TCS degradation rates in a solution adjusted to a pH of 6, which was also the pH of both solutions. This indicates that high salinity did not enhance the degradation rate but rather a concentration of 10,000 ppm NaCl suppressed TCS photosensitivity. This is most likely due to indirect effects that require further exploration.
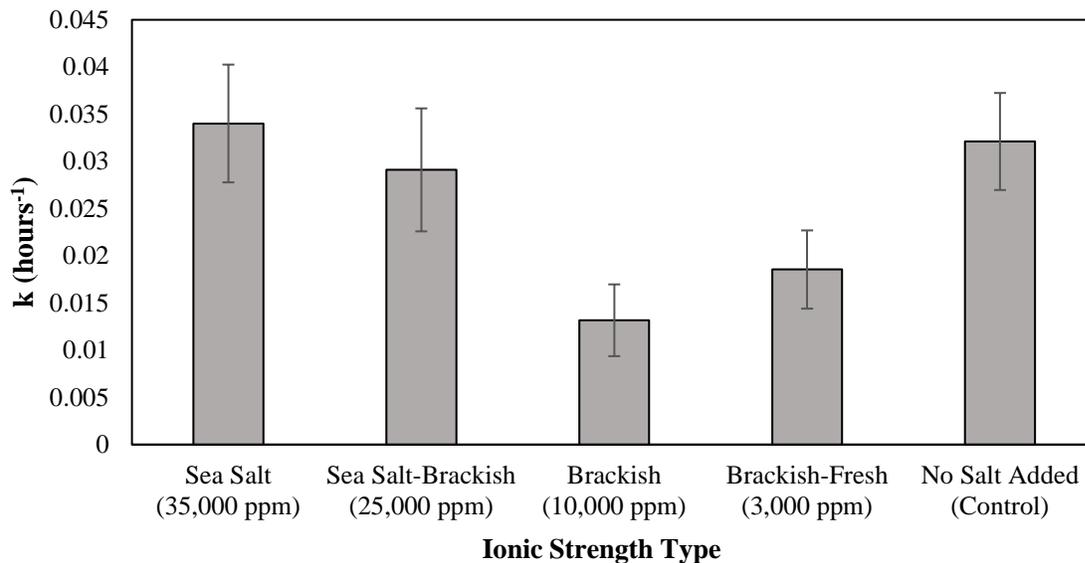
Table 3. Observed photochemical degradation rate constant and half-life as a function of ionic strength.

| Sample Type	[NaCl] (ppm)	k (hour^{-1})	$t_{1/2}$ (hours)
Sea Salt	35,000	0.034 ± 0.006	20
Sea Salt - Brackish	25,000	0.029 ± 0.007	24
Brackish	10,000	0.013 ± 0.004	53

Figure 5: Rate constant for photochemical degradation of TCS as a function of ionic strength.

C. *Effect of DOM on TCS Photochemical Degradation Rates*

TCS in the presence of 0.5 ppm Suwannee River DOM showed the greatest rate of degradation relative to all other conditions tested (Figure 6). Other work has also shown that the presence of organic material enhances the photosensitivity of TCS (Mezcua et al 2004; Aranami and Readman, 2007; Dhillon et al, 2015). The half-life of TCS in the presence of DOM was ten times shorter than both TCS in a solution of an equivalent pH, pH = 5, and the control TCS solution indicating the importance of DOM as either a photosensitizer or scavenger of reactive intermediates affecting the kinetics of TCS degradation (Canonica et al, 1995).

As proposed, we also compared the effects of this DOM surrogate (Suwanneee River Fulvic Acid) on the photosensitivity of TCS to that of natural DOM and potential wastewater effluent (EfDOM) found in actual watershed samples. Previous work found that EfDOM had greater quantum yields for singlet oxygen generation relative to IHSS reference materials thus enhancing the oxidation of organic wastewater contaminants like triclosan (Canonica et al, 1995; Mostafa and Rosario-Ortiz, 2013). However, the greater production of reactive transients like ^{3}DOM and $^1O_2$ was also found to be offset by self-quenching mechanisms thus resulting in negligible change in TCS degradation rates (Bodhipaksha et al., 2015). We found that the half-life of TCS in solution with DOM was twice as short as the half-life of TCS spiked into a natural watershed sample. This indicates that while the presence of effluent and natural DOM found in these waterways enhances the rate of

TCS degradation relative to the other conditions tested, the presence of other dissolved species in the natural sample may be acting to quench reactive intermediates like ^3DOM and $^1O_2$ thus reducing their ability to react with TCS.
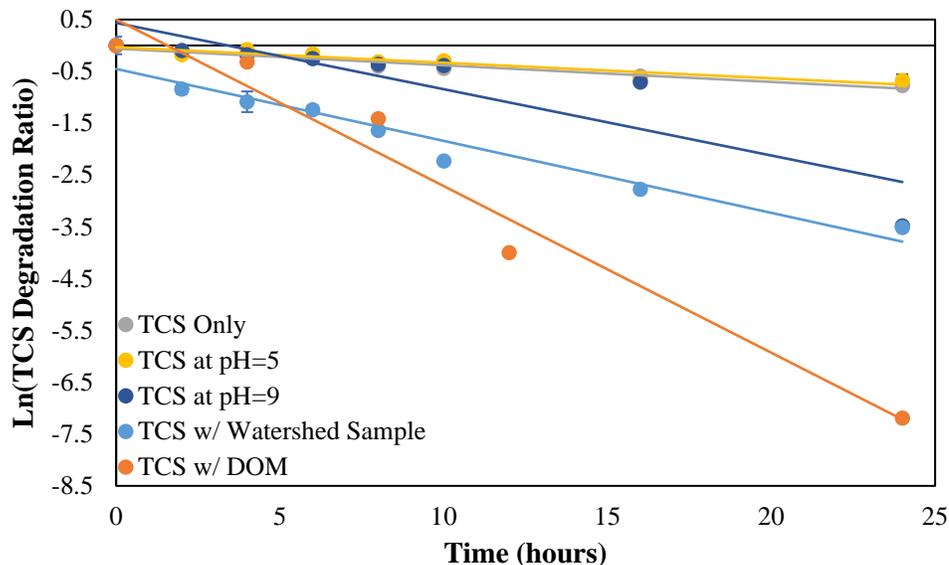
Figure 6: Rate constant for photochemical degradation of TCS under varying conditions including TCS in solution adjusted to a pH of 5, a pH of 9, in the presence of DOM (pH=5) and in a natural watershed sample. A control plot of the photochemical degradation of TCS is also shown as 'TCS Only' (pH=6).

Table 4. Observed photochemical degradation rate constant and half-life under various conditions.

Condition	k (hour^{-1})	t$_{1/2}$ (hours)
TCS Only	0.032 ± 0.003	22
TCS at pH=5	0.030 ± 0.004	23
TCS at pH=9	0.13 ± 0.03	5.3
TCS w/ Watershed Sample	0.14 ± 0.01	5.0
TCS w/ DOM	0.32 ± 0.04	2.3

3. **H3: Evaluation of bacteria as a low-cost testing alternative for assessing TCS streamwater inputs from non-traditional sources**.

A Pearson correlation between 2016 total fecal coliform bacteria and TCS concentrations did not reveal a statistical relationship between the two parameters ($r = 0.04$, $p = 0.66$) (Table 1) (Note: a

similar statistical comparison with the 2017 dataset was not possible due to a laboratory error). Given this lack of statistical relationship, we subsequently compared TCS yields to those of two other parameters potentially indicative of wastewater effluent, such as nitrate and TSS. A strongly positive, statistically significant relationship was observed between 2016 nitrate and TSS yields ($r = 0.80$, $p = 0.03$), while a strongly-positive, albeit non-statistically significant relationship was observed between 2016 TSS and TCS yields ($r = 0.57$, $p = 0.16$). Similarly, positive non-statistically significant relationships were observed between 2017 TCS and nitrate yields ($r = 0.40$, $p = 0.23$) and TCS and TSS yields ($r = 0.31$, $p = 0.36$). Although a statistical relationship was not observed between TCS and total coliform bacteria in the EBBC, the study results suggest that the relative increase in nitrate loading observed between sites located upstream and downstream of non-traditional sources, could also be indicative of a proportional increase in TCS loading. This finding is not surprising as the incomplete treatment of wastewater has been linked to their prevalence in receiving water bodies.

Conclusion:

The results of this study confirm that non-traditional sources of wastewater, such as low volume public and privately-operated WWTPs, leach fields associated with septic tanks, and leaching from biosolids; etc. are all viable sources of TCS delivery to the EBBC. The positive statistical relationship between developed land and 2016 TCS yields suggest that leaks from sewage pipe networks may also be a source. These results coupled with the lack of a statistical relationship between total permitted NDPES related discharge and TCS yields suggests that not all low volume WWTPs achieve a desired efficacy. In summary, the results suggest that local municipalities should consider more robust minimum standards for WWTP systems in lower population density areas. In agricultural areas, farmers should consider timing the field application of biosolids with lower precipitation/runoff periods. Alternatively, increases in both the extent and width of riparian buffers could increase the residence time of TCS in land application areas well beyond its half-life of ~540 days in sediment. Furthermore, the study results suggest TCS could be use a tracer of sewage system leaks in urban areas.

The significant decrease in TCS concentrations between the 2016 and 2017 sampling events, despite a corresponding increase in discharge, suggests that the 2016 FDA ban on TCS in topical antiseptic wash products is having a positive impact on overall water quality in the EBBC. This result suggests that public information campaigns focused on consumer awareness of products which contain TCS could further lower its concentration in waterways.

The observed correlation between TCS and nitrate yields, with varying statistical significance, suggests streamwater nitrate concentrations could be used as a lower cost alternative for approximating TCS delivery in rural to urban watersheds. In particular, increases in nitrate yields between sites located upstream and downstream of non-traditional sources, could be used to approximate the relative increase in TCS loading.

Additionally, it was determined that TCS photosensitivity is dependent on the pH, salinity and presence of DOM in water with the latter having the greatest impact on the TCS longevity. Acting as a photosensitizer, excited triplet state DOM, ^{3}DOM, exhibited a ten-fold increase in degradation of TCS relative to the rate of TCS degradation in pure water adjusted to the same pH. Degradation

of TCS in the presence of DOM was attributed to either direct or indirect photooxidation through increased production of excited state $^1O_2$. TCS half-life in the presence of DOM was twice as short as its half-life in a natural water sample, indicating that while natural and effluent DOM enhance the degradation of TCS, the presence of other species in the natural sample may act to quench TCS photooxidation mechanisms.

Future Research

Our results point to the need for additional work evaluating TCS removal efficacy from low-volume WWTPs; especially given the total number of these types of systems has increased with urbanization. These systems should be evaluated both with respect to the level of treatment as well as the overall residence time of wastewater. The results also suggest that open air, low volume WWTPs could be quickly overwhelmed during large storm events. Therefore, additional studies focused on streamwater TCS inputs during a variety of hydrological events is warranted. In addition, field studies evaluating the effectiveness of riparian buffers on TCS removal from applied biosolids runoff will provide guidance for minimizing streamwater impacts.

Additional work investigating the effect of salinity on the photochemical behavior of TCS is needed, principally to determine why certain concentrations of dissolved inorganic species suppress its photooxidation. It would also be valuable to determine how the concentration and chemical composition of DOM affect the rate of TCS degradation. While, this work focused on quantitating dissolved TCS concentrations, it may be more informative to measure TCS concentrations in sediment considering its low solubility. We had attempted to extract TCS from sediment collected at several locations within the watershed, but our extraction technique required further development as matrix effects were a major issue.

Citations

Ajao, C., Andersson, M. A., Teplova, V. V., Nagy, S., Gahmberg, C. G., Andersson, L. C., Salkinoja-Salonen, M. (2015). Mitochondrial toxicity of triclosan on mammalian cells. *Toxicology Reports*, *2*, 624-637.

Aranami, K., Readman, J. (2007) Photolytic degradation of triclosan in freshwater and seawater. *Chemosphere, 66,* 1052-1056.

Bedoux, G., Roig, B., Thomas, O., Dupont, V., & Le Bot, B. (2012). Occurrence and toxicity of antimicrobial triclosan and by-products in the environment. *Environmental Science and Pollution Research*, *19*(4), 1044-1065.

Behera, S. K., Oh, S. Y., & Park, H. S. (2010). Sorption of triclosan onto activated carbon, kaolinite and montmorillonite: effects of pH, ionic strength, and humic acid. *Journal of hazardous materials*, *179*(1-3), 684-691.

Benotti, M. J., Trenholm, R. A., Vanderford, B. J., Holady, J. C., Stanford, B. D., & Snyder, S. A. (2008). Pharmaceuticals and endocrine disrupting compounds in US drinking water. *Environmental Sscience & Technology*, *43*(3), 597-603.

Berner E.K. and Berner R. A. (2012). Global Environment: Water, Air, and Geochemical Cycles, Second Edition. Princeton University Press. ISBN: 978-0-691-13678-3.

Bernot, M. J., Smith, L., & Frey, J. (2013). Human and veterinary pharmaceutical abundance and transport in a rural central Indiana stream influenced by confined animal feeding operations (CAFOs). *Science of the total environment*, *445*, 219-230.

Bodhipaksha, L., Sharpless, C. M., Chin, Y. P., MacKay, A. A. (2017) Role of eluent organic matter in the photochemical degradation of compounds of wastewater origin. *Water Research, 110(1),* 170-179.

Carey, D. E., & McNamara, P. J. (2015). The impact of triclosan on the spread of antibiotic resistance in the environment. *Frontiers in microbiology*, *5*, 780.

Carrara, C., Ptacek, C. J., Robertson, W. D., Blowes, D. W., Moncur, M. C., Sverko, E. D., & Backus, S. (2008). Fate of pharmaceutical and trace organic compounds in three septic system plumes, Ontario, Canada. *Environmental science & technology*, *42*(8), 2805-2811.

Canonica, S., Jans, U., Stemmler, K., Hoigne, J. (1995) Transformation kinetics of phenols in water: photosensitization by dissolved natural organic material and aromatic ketones. *Environmental Science and Technology, 29,* 1822-1831.

Dann AB, Hontela A (2011) Triclosan: environmental exposure, toxicity and mechanisms of action. J Appl Toxicol 31:285–311.

Dhillon, G., Kaur, S., Pulicharla, R., Brar, S. K., Cledon, M., Verma, M., Surampalli, R. Y. (2015) Triclosan: current status, occurrence, environmental risks and bioaccumulation potential. *International Journal of Environmental Research and Public Health, 12*, 5657-5684.

Drury, B., Scott, J., Rosi-Marshall, E. J., & Kelly, J. J. (2013). Triclosan exposure increases triclosan resistance and influences taxonomic composition of benthic bacterial communities. *Environmental science & technology*, *47*(15), 8923-8930.

Gee, R. H., Charles, A., Taylor, N., & Darbre, P. D. (2008). Oestrogenic and androgenic activity of triclosan in breast cancer cells. *Journal of Applied Toxicology*, *28*(1), 78-91.

George, I., Crop, P., & Servais, P. (2002). Fecal coliform removal in wastewater treatment plants studied by plate counts and enzymatic methods. *Water Research*, *36*(10), 2607–2617. https://doi.org/10.1016/S0043-1354(01)00475-4

Gray, J. L., Borch, T., Furlong, E. T., Davis, J. G., Yager, T. J., Yang, Y. Y., & Kolpin, D. W. (2017). Rainfall-runoff of anthropogenic waste indicators from agricultural fields applied with municipal biosolids. *Science of the Total Environment*, *580*, 83-89.

Halden, R. U. and Paull, D. H. (2005) Co-occurrence of triclocarban and triclosan in US water resources. *Environmental Science & Technology*, *39*(6), 1420-1426.

Heidler, J., & Halden, R. U. (2007). Mass balance assessment of triclosan removal during conventional sewage treatment. *Chemosphere*, *66*(2), 362-369.

Ishibashi H, Matsumura N, Hirano M, Matsuoka M, Shiratsuchi H, Ishibashi Y, Takao Y, Arizono K (2004) Effects of triclosan on the early life stages and reproduction of medaka Oryzias latipes and induction of hepatic vitellogenin. Aquat Toxicol 67:167–179.

Kanda R, Griffin P, James HA, Fothergill J (2003) Pharmaceutical and personal care products in sewage treatment works. J Environ Monit 5:823–830.

Karnjanapiboonwong, A., Suski, J. G., Shah, A. A., Cai, Q., Morse, A. N., & Anderson, T. A. (2011). Occurrence of PPCPs at a wastewater treatment plant and in soil and groundwater at a land application site. *Water, Air, & Soil Pollution*, *216*(1-4), 257-273.

Kolpin, D. W., Furlong, E. T., Meyer, M. T., Thurman, E. M., Zaugg, S. D., Barber, L. B., & Buxton, H. T. (2002). Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999− 2000: A national reconnaissance. *Environmental science & technology*, *36*(6), 1202-1211.

Lapen DR, Topp E, Metcalfe CD, Li H, Edwards M, Gottschall N (2008) Pharmaceutical and personal care products in tile drainage following land application of municipal biosolids. Sci Total Environ 399:50–65.

Latch, D. E., Packer, J. L., Arnold, W. A., & McNeill, K. (2003). Photochemical conversion of triclosan to 2, 8-dichlorodibenzo-p-dioxin in aqueous solution. *Journal of Photochemistry and Photobiology A: Chemistry*, *158*(1), 63-66

Lindström, A., Buerge, I. J., Poiger, T., Bergqvist, P. A., Müller, M. D., & Buser, H. R. (2002). Occurrence and environmental behavior of the bactericide triclosan and its methyl derivative in surface waters and in wastewater. *Environmental science & technology*, *36*(11), 2322-2329.

Luo, Y., Guo, W., Ngo, H. H., Nghiem, L. D., Hai, F. I., Zhang, J., ... & Wang, X. C. (2014). A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Science of the Total Environment*, *473*, 619-641.

Matsumura N, Ishibashi H, Hirano M, Nagao Y, Watanabe N, Shiratsuchi H, Kai T, Nishimura T, Kashiwagi A, Arizono K. (2005) Effects of nonylphenol and triclosan on production of plasma vitellogenin and testosterone in male South African clawed frogs (Xenopus laevis). Biol Pharm Bull 28:1748–1751.

Mezcua, M., Gómez, M. J., Ferrer, I., Aguera, A., Hernando, M. D., & Fernández-Alba, A. R. (2004). Evidence of 2, 7/2, 8-dibenzodichloro-p-dioxin as a photodegradation product of triclosan in water and wastewater samples. *Analytica Chimica Acta*, *524*(1-2), 241-247.

Miege, C., Choubert, J. M., Ribeiro, L., Eusèbe, M., & Coquery, M. (2009). Fate of pharmaceuticals and personal care products in wastewater treatment plants–conception of a database and first results. *Environmental Pollution*, *157*(5), 1721-1726.

Mostafa, S., Rosario-Ortiz, F. L. (2013) Singlet oxygen formation from wastewater organic matter. *Environmental Science and Technology, 47(15),* 8179-8186.

Nebot, C., Falcon, R., Boyd, K. G., & Gibb, S. W. (2015). Introduction of human pharmaceuticals from wastewater treatment plants into the aquatic environment: a rural perspective. *Environmental Science and Pollution Research*, *22*(14), 10559-10568

Orvos DR, Versteeg DJ, Inauen J, Capdevielle M, Rothenstein A, Cunningham V (2002) Aquatic toxicity of triclosan. Environ Toxicol Chem 21:1338–1349.

Puckett, L. J. (1995). Identifying the Major Sources of Nutrient Water Pollution: A national watershed-based analysis connects nonpoint and point sources of nitrogen and phosphorus with regional land use and other factors. *Environmental Science and Technology*, *29*(9), 408A–414A. https://doi.org/10.1021/es00009a001

Reif, A. G., Crawford, J. K., Loper, C. A., Proctor, A., Manning, R., & Titler, R. (2012). *Occurrence of pharmaceuticals, hormones, and organic wastewater compounds in Pennsylvania waters, 2006-09*. US Department of the Interior, US Geological Survey.

Sanchez-Prado, L., Llompart, M., Lores, M., García-Jares, C., Bayona, J. M., & Cela, R. (2006). Monitoring the photochemical degradation of triclosan in wastewater by UV light and sunlight using solid-phase microextraction. *Chemosphere*, *65*(8), 1338-1347.

Tixier, C., Singer, H. P., Canonica, S., Muller, S. (2002) Phototransformation of triclosan in surface waters: a relevant elimination process for this widely used biocide – laboratory studies, field measurements, and modeling. *Environmental Science and Technology, 36,* 3482-3489.

Topp E, Monteiro SC, Beck A, Coelho BB, Boxall AB, Duenk PW (2008). Runoff of pharmaceuticals and personal care products following application of biosolids to an agricultural field. Sci Tot Environ 396:52–59.

US FDA (2016) FDA issues final rule on safety and effectiveness of antibacterial soaps, https://www.fda.gov/newsevents/newsroom/pressannouncements/ucm517478.htm

Vincze, K., Scheil, V., Kuch, B., Köhler, H. R., & Triebskorn, R. (2015). Impact of wastewater on fish health: a case study at the Neckar River (Southern Germany) using biomarkers in caged brown trout as assessment tools. *Environmental Science and Pollution Research*, *22*(15), 11822-11839. Welch, K.A., Lyons, W.B., Graham, E., Neumann, K., Thomas, J.M., Mikesell, D. (1996). *Journal of Chromatography*, 739, 257-263.

Wolf, L., Zwiener, C., & Zemann, M. (2012). Tracking artificial sweeteners and pharmaceuticals introduced into urban groundwater by leaking sewer networks. *Science of the Total Environment*, *430*, 8-19.

World Health Organization. (2012). Pharmaceuticals in drinking-water.

Young, K.D..Thackston E. L. (1999). Housing density and Bacterial loading in urban streams. *Journal of Environmental Engineering, 125(12): 1177-1180.*

Zorrilla, L. M., Gibson, E. K., Jeffay, S. C., Crofton, K. M., Setzer, W. R., Cooper, R. L., & Stoker, T. E. (2008). The effects of triclosan on puberty and thyroid hormones in male Wistar rats. *Toxicological Sciences*, *107*(1), 56-64.

Appendix A: Metrics

- **Undergraduate and Graduate Student Support**:

  o **Abigail Roselli** – graduate researcher, MS Chemistry, May 2018 (paid as temporary research assistant via grant funds)
  o **Matthew Petrie –** undergraduate researcher, BS Biochemistry, May 2017 (summer stipend was provided as part of required cost-share)
  o **Garrett Waligroski** – graduate researcher, MS Chemistry, thesis in progress (stipend was paid via a teaching assistantship)
  o **Gina Talamo –** undergraduate researcher, BS Environmental Science, May 2018 (academic year researcher, no stipend support required)
  o **Opeyemi Famakinwa –** undergraduate researcher, BS Chemistry, anticipated May 2019 (summer stipend was provided as part of required cost-share)
  o **Romeo Tiu -** undergraduate researcher, BS Chemistry anticipated May 2020 (academic year researcher, no stipend support required)
  o **Julia Martiner** - undergraduate researcher, BS Mathematics anticipated May 2021 (academic year researcher, no stipend support required)

- **Faculty and Staff Support**:
  o **Amanda Grannas** (PI), full-time faculty, 0.131 FTE
  o **Steven Goldsmith** (co-PI), full-time faculty, 0.083 FTE
  o **Vanessa Boschi,** full time visiting research assistant faculty, 0.083 FTE – note: Dr. Boschi did not receive compensation from grant funds, but assisted with the project as part of her 12-month appointment as a Research Assistant Professor in the Department of Chemistry

- **Publications:** *Include journal publications (submit full reference and copy of publication, where possible – we will respect all copyright laws), thesis or dissertations, reports, papers presented at conferences, poster presentations specifically resulting from Sea Grant-funded research.*

Talamo, G.D., Waligroski, G.J., Barr, M.F., Grannas, A.M., and Goldsmith, S.T., 2016, Identifying controls on triclosan loading to the East Branch of the Brandywine Creek, *Abstracts with Programs – Geological Society of America*, 48, 7, p. 398, doi: 10.1130/abs/2016AM-285197.

Waligroski, G. Hanley, K., Grannas, A.M., Goldsmith, S.J. Environmental influences and fate of triclosan in a Southeastern Pennsylvania watershed: Sources in the East Branch of the Brandywine Creek. *American Chemical Society, National Meeting*. August 2016

Petrie, M., Waligroski, G., Grannas, A.M. Investigating the photochemical fate of triclosan as a function of water quality parameters. *American Chemical Society, National Meeting*. August 2016

- **Volunteer Hours:** *Include the number of individual volunteers and hours.*
  o **Melanie Vile,** full time faculty, 20 hours – Dr. Vile assisted with 2017 sampling events

- **Public and professional presentations, and attendees:** *Include a title of the presentation, event title (meeting, workshop, or conference title), location, date of presentation, and the number attendees.*
  See above in Publications

- **Project Collaborators**: *None*

Appendix B: Impact and/or Accomplishment Statement(s)

Our work confirms that non-traditional sources of wastewater, such as low volume public and privately-operated waste water treatment plants, leach fields associated with septic tanks, and leaching from biosolids, etc. are all viable sources of triclosan (TCS) to waterways. This indicates that aquatic environments other than those downstream of major waste water treatment facilities could potentially be exposed to TCS and its breakdown products. Nitrate concentrations could be used as a lower cost alternative for approximating TCS delivery in rural to urban watersheds. It was also determined that higher pH's, salinity, and the presence of dissolved organic material can affect the photochemical degradation rate of TCS, the latter of which has the greatest affect. Therefore, streams with greater amounts of organic material inputs, either natural or from waste waters, could experience greater rates of TCS breakdown and byproduct production. On a positive note, there appeared to be a decrease in TCS concentration between the 2016 and 2017 sampling events indicating that the 2016 FDA ban on TCS in topical antiseptic wash products is helping to reduce its exposure to the environment.

Table S1: Triclosan (TCS), total suspended solids (TSS), and nitrate concentrations and total coliform and E.Coli bacteria counts in select locations of the EBBC on June 19, 2016

Site Name	Location with respect to NPDES Site	NPDES Permitted Flow (MGD)[1,2]	Date	Upstream Area (km²)	Discharge (m3/s)	TCS (ng/L)	TSS (g L⁻¹)	NO₃ (mg/l)	Fecal Coliform bacteria count[3]	E. Coli bacteria
Service Station 1	Upstream		6/19/2016	3.95	0.015		0.005	23.5	390	5
Service Station 2	Downstream	0.05	6/19/2016	4.68	0.075		0.005	2.15	243	2
St. Andrews 1	Upstream		6/19/2016	3.95	0.023	90	0.002	11.0	559	2
St. Andrews 2	Downstream	0.004	6/19/2016	4.68	0.31		0.002	9.64	301	1
Hughes	Downstream	0.005	6/19/2016	0.45	0.0005		0.006	5.44	517	2
Indian Run/McKenna/Woods 1	Upstream		6/19/2016	3.51	0.008		0.005	15.1	413	5
Indian Run/McKenna/Woods 2	Upstream		6/19/2016	5.01	0.017		0.004	14.9	330	4
Indian Run/McKenna/Woods 3	Downstream	0.375	6/19/2016	9.74	0.062		0.003	9.56	302	2
Stanley Wannop	Downstream	0.0004	6/19/2016	1.64	0.006	200	0.002	7.87	145	2
Little Washington Culbertson Run 1	Upstream		6/19/2016	1.84	0.004	73	0.013	5.50	385	1
Little Washington Culbertson Run 2	Downstream	0.0053	6/19/2016	4.26	0.017	90	0.002	11.5	425	11
Applecross 1	Upstream		6/19/2016	3.01	0.005	110	0.005	20.5	1105	5
Applecross 2	Upstream		6/19/2016	2.04	0.005		0.008	24.7	561	7
Applecross 3	Downstream	0.137	6/19/2016	11.53	0.058	230	0.002	11.7	455	1
O'Brien 1	Upstream		6/19/2016	2.26	0.022		0.002	9.93	484	3
O'Brien 2	Downstream		6/19/2016	3.06	0.064	70	0.004	10.43	317	0

[1]Permitted flow of upstream NPDES site in million gallons per day (MGD)

[2]Blanks cells indicate TCS value not determined due to instrument replicate error

[3]Bacteria counts are presented as the average of three replicates

Table S2: Triclosan (TCS), total suspended solids (TSS), and nitrate concentrations and total coliform and E.Coli bacteria counts in select locations of the EBBC in November 18, 2017

Site Name	Date Sampled	Location with respect to NPDES Site	NPDES Permitted Flow (MGD)[1,2]	Upstream Area (km²)	Discharge (m³/s)	TCS (ng/L)	TSS (g L⁻¹)	NO₃ (mg/l)
Service Station 1	11/18/2017	Upstream		3.95	0.06	1.10	0.06	
Service Station 2	11/18/2017	Downstream	0.05	4.68	0.11	0.43	0.11	3.91
St. Andrews 1	11/18/2017	Upstream		3.95	0.01	0.34	0.01	13.8
St. Andrews 2	11/18/2017	Downstream	0.004	4.68	0.01	0.52	0.01	11.5
Hughes	11/18/2017	Downstream	0.005	0.45		0.78		9.92
Indian Run/McKenna/Woods 1	11/18/2017	Upstream		3.51	0.01	0.33	0.01	14.6
Indian Run/McKenna/Woods 2	11/18/2017	Upstream		5.01	0.03		0.03	
Indian Run/McKenna/Woods 3	11/18/2017	Downstream	0.375	9.74	0.04	0.99	0.04	10.0
Stanley Wannop	11/18/2017	Downstream	0.0004	1.64	0.03	0.44	0.03	9.29
Little Washington Culbertson Run 1	11/19/2017	Upstream		1.84	0.07	0.52	0.07	2.73
Little Washington Culbertson Run 2	11/19/2017	Downstream	0.0053	4.26	0.12	0.36	0.12	6.50
Applecross 1	11/19/2017	Upstream		3.01	0.07	3.17	0.07	8.51
Applecross 2	11/19/2017	Upstream		2.04		0.52		13.7
Applecross 3	11/19/2017	Downstream	0.137	11.53	0.34	0.39	0.34	14.4
O'Brien 1	11/19/2017	Upstream		2.26		0.88		5.78
O'Brien 2	11/19/2017	Downstream		3.06	0.23	0.45	0.23	10.4

[1]Permitted flow of upstream NPDES site in million gallons per day (MGD)

[2]Blanks cells indicate TCS value not determined due to instrument replicate error

Table S3: Percent land use above sampling location[1]. Dev = Developed; D = Deciduous; E = Evergreen; M = Mixed;

Site Name	Dev. Open Space	Dev. Low Intensity	Dev. Medium Intensity	Dev. High Intensity	Barren Land (Rock/ Sand/ Clay)	Forest (D)	Forest (E)	Forest (M)	Shrub/ Scrub	Grassland/ Herbaceous	Pasture/ Hay	Cultivated Crops	Woody Wetlands	Emergent Herbaceous Wetlands
Service Station 1	0.16	0.02	0.00	0.00	0.00	0.13	0.00	0.00	0.05	0.00	0.18	0.42	0.02	0.00
Service Station 2	0.11	0.01	0.00	0.00	0.00	0.34	0.01	0.00	0.05	0.01	0.12	0.25	0.09	0.00
St. Andrews 1	0.16	0.04	0.02	0.00	0.00	0.28	0.00	0.00	0.08	0.00	0.25	0.13	0.03	0.00
St. Andrews 2	0.16	0.03	0.02	0.00	0.00	0.28	0.00	0.00	0.10	0.00	0.21	0.14	0.04	0.00
Hughes	0.25	0.19	0.07	0.00	0.00	0.14	0.00	0.00	0.07	0.00	0.07	0.19	0.03	H.00
Indian Run/McKenna/Woods 1	0.14	0.02	0.00	0.00	0.00	0.27	0.00	0.00	0.05	0.01	0.17	0.25	0.08	0.00
Indian Run/McKenna/Woods 2	0.16	0.03	0.01	0.00	0.00	0.28	0.00	0.00	0.05	0.01	0.16	0.24	0.06	0.00
Indian Run/McKenna/Woods 3	0.14	0.03	0.01	0.00	0.00	0.42	0.00	0.00	0.06	0.01	0.11	0.16	0.05	0.00
Stanley Wannop	0.23	0.02	0.00	0.00	0.00	0.28	0.00	0.00	0.09	0.00	0.26	0.11	0.01	0.00
Little Washington Culbertson Run 1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Little Washington Culbertson Run 2	0.36	0.11	0.01	0.00	0.00	0.15	0.00	0.00	0.07	0.00	0.17	0.08	0.03	0.00
Applecross 1	0.15	0.03	0.01	0.00	0.00	0.15	0.00	0.00	0.02	0.00	0.19	0.41	0.04	0.00
Applecross 2	0.43	0.06	0.01	0.00	0.00	0.09	0.01	0.00	0.03	0.00	0.19	0.14	0.05	0.00
Applecross 3	0.20	0.05	0.01	0.00	0.01	0.18	0.00	0.00	0.03	0.01	0.17	0.30	0.04	0.00
O'Brien 1	0.21	0.10	0.05	0.01	0.00	0.15	0.00	0.00	0.05	0.01	0.20	0.19	0.03	0'.00
O'Brien 2	0.21	0.08	0.04	0.01	0.00	0.22	0.00	0.00	0.06	0.00	0.20	0.16	0.03	0.00

[1]determined using the 2010 USGS data set

Table S4: Summary of NPDES sites in the East Branch of the Brandywine Creek (EBBC) evaluated in this study

Site Name	Latitude	Longitude	Receiving Waters	Permitted Flow (MGD)	Type of Permit Issued	Permit Number	SIC Code	Type of ownership	Activity Status
Peter J. Camiel Service Plaza	40º07'3.99" N	75º46'9.99" W	Unnamed Tributary to Marsh Creek	0.05	NPDES Individual Permit	PA0027987	5541= Gasoline Service Station	Corporation	Effective
St. Andrews Brae STP	40º06'14.00" N	75º42'20.00" W	Black Horse Creek-tributary to Marsh Creek	0.004	NPDES Individual Permit	PA0058378	4952= Sewerage Systems	Municipality	N/A
Hughes SRSTP	40º03'51.99" N	75º41'38.99" W	Unnamed Tributary to Marsh Creek	0.0005	NPDES Individual Permit	PA0057274	8811= Private Households	Corporation	Effective
Indian Run Village MHP STP	40º04'13.79" N	75º48'35.70" W	Indian Run	0.0375	NPDES Individual Permit	PA0050547	6515= Mobile Home Site Operators	Corporation	Effective
Stanley Wannop (GANS SRSTP)	40º03'38.99" N	75º47'5.99" W	Culberston Run	0.0004	NPDES Individual Permit	PA0058785	6514= Dwelling Operators, Except Apartments	Corporation	Effective
Little Washington Culberston Run WWTP	40º02'50.99" N	75º46'30.00" W	Culberston Run	0.053	NPDES Individual Permit	PA0050458	4952= Sewerage System	Corporation	Effective
Apple Cross WWTP	40º01'59.99" N	75º46'45.00" W	Beaver Creek	0.137	NPDES Individual Permit	PA0244333	4952= Sewerage System	Municipality	Effective
Obrein Res	40º0'29.99" N	75º45'29.99" W	Unit to Beaver Creek	N/A	General Permit Covered Facility	PAG040113	8811= Private Households	N/A	Expired (4-Feb-2014)

N/A = Not available

Table S5: Instantaneous TCS, TSS, and nitrate yields in select locations of the EBBC on June 2016 and November 2017

Site Name	Location with respect to NPDES Site	Upstream Area (km²)	Jun-16			Nov-17		
			TCS (ng/s/km²)	TSS (g/s/km²)	NO$_3$ (mg/s/km²)	TCS (ng/s/km2)	TSS (g/s/km²)	NO3 (mg/s/km²)
Service Station 1	Upstream	3.95		0.017	89.1	16.4	0.88	
Service Station 2	Downstream	4.68		0.084	34.5	9.86	2.42	88.8
St. Andrews 1	Upstream	3.95	524	0.012	63.9	0.52	0.01	20.9
St. Andrews 2	Downstream	4.68		0.119	639	0.89	0.01	19.7
Hughes	Downstream	0.45		0.006	6.05	0.00		
Indian Run/McKenna/Woods 1	Upstream	3.51		0.012	34.4	0.70	0.02	31.3
Indian Run/McKenna/Woods 2	Upstream	5.01		0.014	50.6		0.13	
Indian Run/McKenna/Woods 3	Downstream	9.74		0.022	60.9	4.09	0.17	41.4
Stanley Wannop	Downstream	1.64	732	0.007	28.8	6.97	0.41	147
Little Washington Culbertson Run 1	Upstream	1.84	159	0.028	12.0	19.7	2.66	104
Little Washington Culbertson Run 2	Downstream	4.26	359	0.008	45.8	9.96	3.21	179
Applecross 1	Upstream	3.01	183	0.009	34.0	75.0	1.68	201
Applecross 2	Upstream	2.04		0.020	60.5	0.00		
Applecross 3	Downstream	11.53	1157	0.008	58.8	11.4	10.05	426
O'Brien 1	Upstream	2.26		0.021	96.6			
O'Brien 2	Downstream	3.06	1464	0.075	218	33.8	17.09	777

[1]Permitted flow of upstream NPDES site in million gallons per day (MGD)

[2]Blanks cells indicate TCS value not determined due to instrument replicate error

[3]Bacteria counts are presented as the average of three replicates