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Detection of triclocarban and two co-contaminating chlorocarbanilides in US aquatic environments using isotope dilution liquid chromatography tandem mass spectrometry[☆]

Amir Sapkota[1], Jochen Heidler, Rolf U. Halden*

Department of Environmental Health Sciences, Johns Hopkins University, Bloomberg School of Public Health, Johns Hopkins University Center for Water and Health, Baltimore, MD 21205-2103, USA

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Abstract

The antimicrobial compound triclocarban (TCC; 3,4,4′-trichlorocarbanilide; CAS# 101-20-2) is a high-production-volume chemical, recently suggested to cause widespread contamination of US water resources. To test this hypothesis, we developed an isotope dilution liquid chromatography electrospray ionization tandem mass spectrometry method for ultratrace analysis of TCC (0.9 ng/L detection limit) and analyzed low-volume water samples (200 mL) along with primary sludge samples from across the United States. All river water samples (100%) collected downstream of wastewater treatment plants had detectable levels of TCC, as compared to 56% of those taken upstream. Concentrations of TCC (mean ± standard deviation) downstream of sewage treatment plants (84 ± 110 ng/L) were significantly higher ($P < 0.05$; Wilcoxon rank sum test) than those of samples taken upstream (12 ± 15 ng/L). Compared to surface water, mean TCC concentrations found in dried, primary sludge obtained from municipal sewage treatment plants in five states were six orders of magnitude greater (19,300 ± 7100 μg/kg). Several river samples contained a co-contaminant, identified based on its chromatographic retention time, molecular base ion, and MS/MS fragmentation behavior as 4,4′-dichlorocarbanilide (DCC; CAS# 1219-99-4). In addition to TCC and DCC, municipal sludge contained a second co-contaminant, 3,3′,4,4′-tetrachlorocarbanilide (TetraCC; CAS# 4300-43-0). Both newly detected compounds were present as impurities ($0.2\%_{w/w}$ each) in technical grade TCC (99%). Application of the new method for chlorocarbanilide analysis yielded TCC occurrence data for 13 US states, confirmed the role of sewage treatment plants as environmental inputs of TCC, and identified DCC and TetraCC as previously unrecognized pollutants released into the environment alongside TCC.

Keywords: Antimicrobials; Disinfectant; Phenyl urea compounds; Emerging contaminants; LC-MS/MS

1. Introduction

Triclocarban (3,4,4′-trichlorocarbanilide; TCC) is an antimicrobial compound commonly added for its sanitizing properties to a wide range of household and personal care products including bar soaps, detergents, body washes, cleansing lotions, and wipes. In 1998, TCC production for the US market was estimated to approach one million pounds or about 454 metric tons per year, according to a summary report submitted by the industry to the US Environmental Protection Agency (EPA) in support of the ongoing risk evaluation for the antimicrobial compound; the maximum current use is estimated at 750 metric tons

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*Corresponding author. Fax: +1 410 955 9334.
E-mail address: rhalden@jhsph.edu (R.U. Halden).

[1] Current address: International Agency for Research on Cancer, Lyon, France.

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per year (TCC Consortium, 2002a). Despite extensive production of TCC over the past 49 yrs, its fate in wastewater and the environment has received only limited attention. For the past three decades, a study by Gledhill was the only peer-reviewed report focusing on the fate of TCC in US wastewater (Gledhill, 1975). Using two different ^{14}C-labeled analogs, TCC was observed to persist in raw sewage for up to 2 weeks without any significant biotransformation. In fresh activated sludge, less than 10% of the antimicrobial was mineralized over a period of six weeks (Gledhill, 1975). However, following long-term acclimation of activated sludge cultures, final $^{14}CO_2$ evolution values reached 80–90%, indicating that essentially complete biodegradation is possible under optimized laboratory conditions, at least for the more biodegradable monochlorinated aromatic ring of TCC (Gledhill, 1975). One additional study, published in 1991, reported the tentative identification of TCC in effluent samples from two out of three examined wastewater treatment plants (WWTPs) in New Jersey using a particle beam liquid chromatography/mass spectrometry method (Clark et al., 1991).

Recently, peer-reviewed quantitative environmental monitoring data for TCC in the US were produced using a liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS) method (Halden and Paull, 2004). Analysis of water samples from six urban streams in the Greater Baltimore area (Maryland, USA) demonstrated the presence of TCC in surface water at concentrations ranging from nondetect (<30 ng/L) to 5600 ng/L. All TCC detections were linked to leakages of raw wastewater into urban streams, none of which were receiving treated WWTP effluent. In a follow-up study, even higher TCC concentrations of up to 6750 ng/L were detected in Baltimore streams heavily impacted by raw wastewater from leaking sewer lines (Halden and Paull, 2005). A statistical analysis of 42 samples from various environmental compartments revealed a strong correlation between the occurrence of TCC and the structurally related antimicrobial compound triclosan. Using an empirical model and published triclosan concentrations (Kolpin et al., 2002) as input, the study suggested that TCC is a previously unrecognized environmental pollutant, likely present at detectable concentrations in about 60% of US streams nationwide (Halden and Paull, 2005).

In the present work, a two-dimensional mass spectrometry method was developed and applied to the analysis of TCC in river water and primary sludge samples from WWTPs located in 13 US states. Liquid chromatography tandem mass spectrometry (LC-MS/MS) is the preferred method for environmental trace analysis of pharmaceuticals and personal care products or PPCPs (Kolpin et al., 2002). In Europe, where TCC usage is more limited due to public health concerns, Hancock et al. (Hancock et al., 1998) previously used TCC as an internal standard for the analysis of the pesticides sulcofuron and flucofuron in river water, thereby demonstrating that the antimicrobial is amenable to liquid chromatography negative electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS). We built on this methodology by expanding the method to include TCC and other chlorocarbanilides as target analytes, and by adding the isotope dilution technique to the protocol to prevent the potential underestimation of environmental concentrations due to incomplete recovery of TCC from complex sample matrices such as river water, wastewater, and sludge.

The isotope dilution technique has been used extensively for the detection and quantification of analytes in complex environmental and biological matrixes (Eljarrat et al., 2003; Li et al., 2005). It involves the addition of a stable isotope-labeled analog of the target compound to the sample at the very beginning of the sample preparation process. Since this surrogate standard and the analyte are expected to behave identically, except for a slight isotope effect that is without consequence in most analytical applications, incomplete recovery of the analyte due to sorption, hydrolysis, degradation, volatilization, and similar mechanisms can be accounted for by normalizing the obtained result to the amount of surrogate recovered.

Using two TCC analogs of distinct mass as surrogate and internal standards ($^{13}C_6$-TCC and TCC-d_7), we developed an isotope dilution liquid chromatography electrospray ionization tandem mass spectrometry (ID-LC-ESI-MS/MS) method and applied it to the analysis of river water and sludge samples that originated in 13 different US states. To determine the importance of WWTPs as a source of TCC inputs into US streams, river sampling locations were chosen strategically to reflect the water quality upstream and downstream of discharge locations of treated wastewater. Finally, the method was applied to monitor for chlorocarbanilide congeners other than TCC in the environment.

2. Methods

Chemicals: TCC (99% purity; CAS♯ 101-20-2) was obtained from Aldrich, Milwaukee, WI. Isotope-labeled $^{13}C_6$-TCC and TCC-d_7 were custom synthesized by Cambridge Isotope Laboratories, Inc., Andover, MA. The 3,3′,4,4′-tetrachlorocarbanilide congener (TetraCC; 99%; CAS♯ 4300-43-0) was obtained from Dr. Ehrenstorfer, Augsburg, Germany, and 4,4′-dichlorocarbanilide (DCC; 99%; CAS♯ 1219-99-4) was obtained from Oakwood Products, West Columbia, SC. HPLC grade water and MS grade methanol (Burdick & Jackson) were obtained from VWR International, West Chester, PA.

Sample collection: Samples originated in 13 US states (Fig. 1). Duplicate grab samples of river water were collected manually immediately under the water surface along the riverbanks about 1000 ft upstream and downstream of WWTPs that contributed $<50\%$ of the total flow volume measurable at downstream locations. Sample locations were selected at random from a pool of sampling volunteers belonging to the Groundwater Foundation. Samples were collected in the fall/winter of 2004 on weekdays during weather conditions deemed to be typical for the sampling locations. Participants used sampling equipment that was shipped to the various locations by our laboratory. The sampling kits were accompanied by detailed instructions and protective equipment to prevent sample contamination. Sample containers for surface waters consisted of precleaned, disposable 500-mL polypropylene bottles.

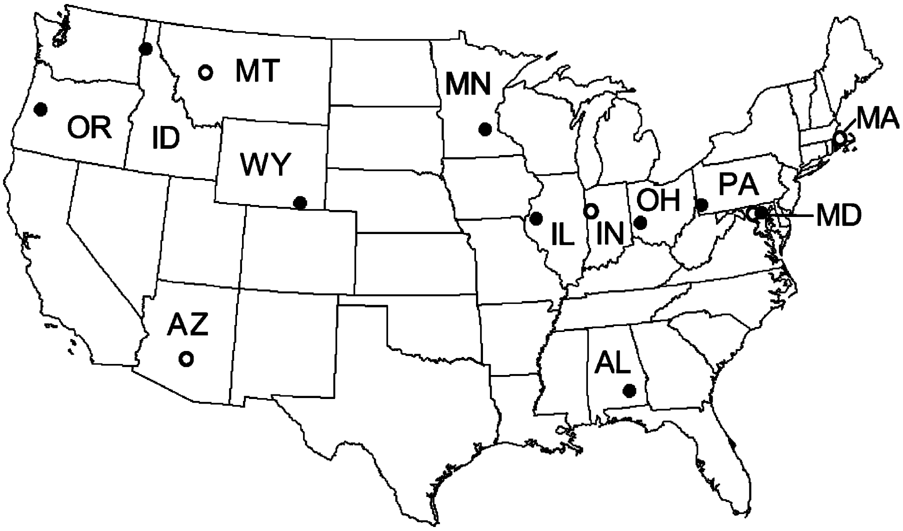

Fig. 1. Geographic map showing the locations of wastewater treatment plants in 13 US states from which surface water ($n = 18$ upstream and downstream samples; solid circles) or primary sludge samples ($n = 5$; open circles) were obtained and analyzed for the presence and concentration of chlorinated carbanilides.

Samples and trip blanks, containing reagent water in identical containers, were returned to the laboratory overnight for analysis. Samples of raw municipal sludge were obtained from the primary clarifiers of activated sludge wastewater treatment plants from across the continental United States using 250-mL polypropylene bottles. Samples were frozen at minus 20 °C and processed as described below.

Sample preparation: Aliquots (200 mL) of thawed water samples were extracted as follows. An aliquot (5 μL) of a methanol stock solution containing 1 μg/mL of the surrogate standard $^{13}C_6$-TCC was added to all samples. Following thorough mixing and equilibration, samples were extracted using Oasis HLB (60 mg) cartridges (Waters Corp; Milford MA) that were pre-equilibrated with 2 mL methanol in acetone (50%) followed by 2 mL methanol and 6 mL water. Following loading of the sample, cartridges were washed with 1 mL water containing 5% methanol by volume, and analytes were eluted with 3 mL of methanol in acetone (50%) at a flow rate of <0.5 mL/min. Extracts were dried under nitrogen at 40 °C and reconstituted in 800 μL methanol. A 400-μL aliquot of this sample was transferred to an autosampler vial to which 5 μL of internal standard (200 μg/L TCC-d_7 in methanol) was added. This was followed by 95 μL of water, so that the water content in the sample was similar to that of the mobile phase. Primary municipal sludge samples were homogenized individually, and extracted with organic solvents as described previously (Halden and Paull, 2004).

Sample Analysis: Purified, concentrated organic extracts were placed in a Surveyor HPLC and autosampler system (Thermo Electron, San Jose, CA) coupled to a Quantum Ultra triple quadruple tandem mass spectrometer (Thermo Electron; San Jose, CA). Using the autoinjector, 15 μL of the sample was injected into the HPLC system and analytes were separated on a BetaBasic-18 column (5 m particle size, 100 × 2 mm ID; Thermo Hypersil-Keystone, Bellefonte, PA) with a precolumn filter (4 × 2 mm ID; Phenomenex, Torrance CA). The isocratic mobile phase maintained at 35 °C consisted of 80% methanol flowing at a rate of 200 μL/min with a total runtime of 10 min. Analytes were introduced into the mass spectrometer using an electrospray ionization probe operating in negative mode. Selected reaction monitoring (SRM) was conducted as described in the *Results* section. Nine-point calibration curves ranging from 0 to 250 ng/mL (TCC) and 0 to 50 ng/mL (DCC and TetraCC) were constructed for each batch of analysis. The smallest nonzero calibration standard was 0.01 ng/mL for TCC and 0.002 ng/mL for DCC and TetraCC. All measured concentrations were adjusted for recovery of the surrogate standard $^{13}C_6$-TCC to obtain a best estimate of the true concentration using the isotope dilution technique.

QA/QC and data analysis: For quality assurance/quality control (QA/QC) purposes, all river samples were collected in duplicate accompanied by trip blanks (20%). All duplicate samples were extracted and analyzed independently. To prevent potential carryover from one run to the next, a blank injection (methanol–water 80/20 mixture) was made after each field sample was analyzed. In addition, spiked check standards were included throughout the analysis to monitor for potential shifts in instrument response.

The method detection limit (MDL) was determined according to the Code of Federal Regulations, Part 136, Appendix B (1993), by analyzing seven 200-mL aliquots of reagent water spiked to a level of 5 ng/L with the three analytes. Samples were extracted using HLB cartridges, dried, reconstituted, and analyzed as described above. The MDL was calculated by multiplying the standard deviation of the seven spiked samples with the Student's t-value associated with the 99% confidence interval at six degrees of freedom. Where applicable, experiments were repeated until the calculated MDL value was at least 1/10 of the spiking level. For field samples, non-detect observations were assigned a concentration equivalent to one-half of the MDL according to established practices (Clarke, 1998; Hornung and Reed, 1990).

The extraction efficiency for sludge samples was assessed separately. Aliquots of municipal sludge were fortified with nonlabeled TCC at a level approximately equivalent to 50% and 100% of the TCC mass detected prior to spiking. Following equilibration overnight, samples were extracted and analyzed. Average recoveries for low (50%) and high (100%) spiking levels were $91 \pm 8\%$ and $93 \pm 17\%$.

Acquisition and automated processing of data were conducted using Xcalibur software Version 1.3 for the Quantum Ultra. Statistical analyses were performed after logarithmic transformation of the concentration data using Intercooled Stata, Version 7.0 for Windows (Stata Corporation, College Station, TX). Nonparametric analyses were conducted without any such transformations. The Student two-sample t-test (log transformed data) and the Wilcoxon rank sum test (nontransformed data) were used to test for statistical differences in the occurrence of TCC upstream and downstream of WWTPs.

Chemical property estimation: Select physical and chemical properties of chlorocarbanilide congeners and their respective behaviors in the environment were estimated using standard quantitative structure–activity relationship (QSAR) models. Analyses were performed on personal computers using the following software: PBT profiler available online at http://www.pbtprofiler.net/, ECOSAR, K_{OW}–WIN, and the Advanced Chemistry Development (SCD/Labs) software Solaris V4.67.

3. Results

ID-LC-ESI-MS/MS method development: Optimal ionization and fragmentation of TCC occurred under the conditions specified in Table 1. Nonlabeled TCC and its two isotope-labeled analogs, $^{13}C_6$-TCC and TCC-d_7, exhibited similar fragmentation behaviors (Fig. 2A). The dominant fragmentation pattern observed for TCC was via cleavage in the urea moiety of the nitrogen–carbon (N–C) bond closest to the ring carrying two chlorine atoms (Fig. 2A). Both TCC and $^{13}C_6$-TCC gave rise to the same daughter ion of m/z 160 since all six heavy carbon-13 isotopes resided in the *p*-chloroaniline ring of $^{13}C_6$-TCC, whose signal was lost following fragmentation (Fig. 2A; Panels 1 and 2). The deuterated TCC-d_7 serving as the internal standard yielded a daughter ion of m/z 163 due to the presence of three heavy deuterium atoms in the 3,4-dichloroaniline moiety (Fig. 2A; Panel 3).

Method sensitivity and reproducibility: The ID-LC-ESI-MS/MS method yielded a method detection limit (MDL) for TCC in the sub-part-per-trillion range of 0.9 ng/L, despite the use of a relatively small sample volume of only 200 mL. Similarly, analysis by LC-ESI-MS/MS of low-level standards of DCC and TetraCC yielded MDLs of 0.3 and 0.5 ng/L, respectively. Unweighted nine-point calibration curves were linear over a range of 0–250 ng/mL (TCC) and 0–50 ng/mL (DCC and TetraCC), and routinely yielded coefficients of determination (R^2) of greater than 0.998. Results obtained for spiked check samples had a coefficient of variation of less than 10%, indicating good overall precision. Field samples collected, processed, and analyzed in duplicate were statistically indistinguishable ($P<0.05$) and overall showed a high degree of correlation (slope = 1.02, $R = 0.97$). In addition, the efficiency of TCC recovery from sludge was determined in spiking experiments where aliquots of previously analyzed sludge samples were fortified with nonlabeled TCC to final concentrations equivalent to 150% and 200% of the original TCC mass detected. Following equilibration overnight, extraction and analysis of these samples indicated average recovery rates of 91 ± 8% and 93 ± 17% for the lower and higher spiking level, respectively.

Environmental monitoring: The method was applied to the analysis of TCC in river water collected upstream and downstream of WWTP discharge locations in nine different states: Alabama (AL), Idaho (ID), Illinois (IL), Maryland (MD), Minnesota (MN), Ohio (OH), Oregon (OR), Pennsylvania (PA) and Wyoming (WY; Fig. 1). Additional samples of primary municipal sludge were obtained from five different states: Arizona (AZ), Indiana (ID), Maryland (MD), Massachusetts (MA), and Montana (MT). Analytical results are presented in Table 2. All surface waters (9 duplicate samples total; 100%) collected downstream of WWTPs had detectable levels of TCC compared to 56% (5 of 9) of samples obtained upstream of treated wastewater discharge locations. The distribution of TCC varied by state and by sampling location, i.e., upstream versus downstream of WWTPs. Across the nine states studied, TCC levels upstream of WWTP ranged from non-detectable (0.45 ng/L; one half MDL) to 45 ng/L, whereas

Table 1
Analytical parameters for LC-ESI-MS/MS analysis of 3,4,4′-trichlorocarbanilide (TCC), 4,4′-dichlorocarbanilide (DCC), and 3,3′,4,4′-tetrachlorocarbanilide (TetraCC)

HPLC parameter	Instrument setting		
Injection volume	15 µL		
Column temperature	35 °C		
Runtime	10 min		
MS/MS parameter			
Ion source	Negative electrospray ionization		
Spray voltage	−2900 V		
Sheath gas pressure	35[a]		
Auxiliary gas pressure	14[a]		
Capillary temperature	275 °C		
Source CID	10 V		
Collision pressure	0.9 mTorr		
Peak widths	$Q1 = 0.5\,m/z$; $Q3 = 0.7\,m/z$		
Selective reaction monitoring (SRM)			
Analyte	Parent ion (m/z)	Daughter ion (m/z)	Collision energy[a]
TCC	313	160	16
$^{13}C_6$-TCC	319	160	16
TCC-d_7	320	163	17
DCC	279	126	19
TetraCC	347	160	19

[a] Arbitrary instrument unit.

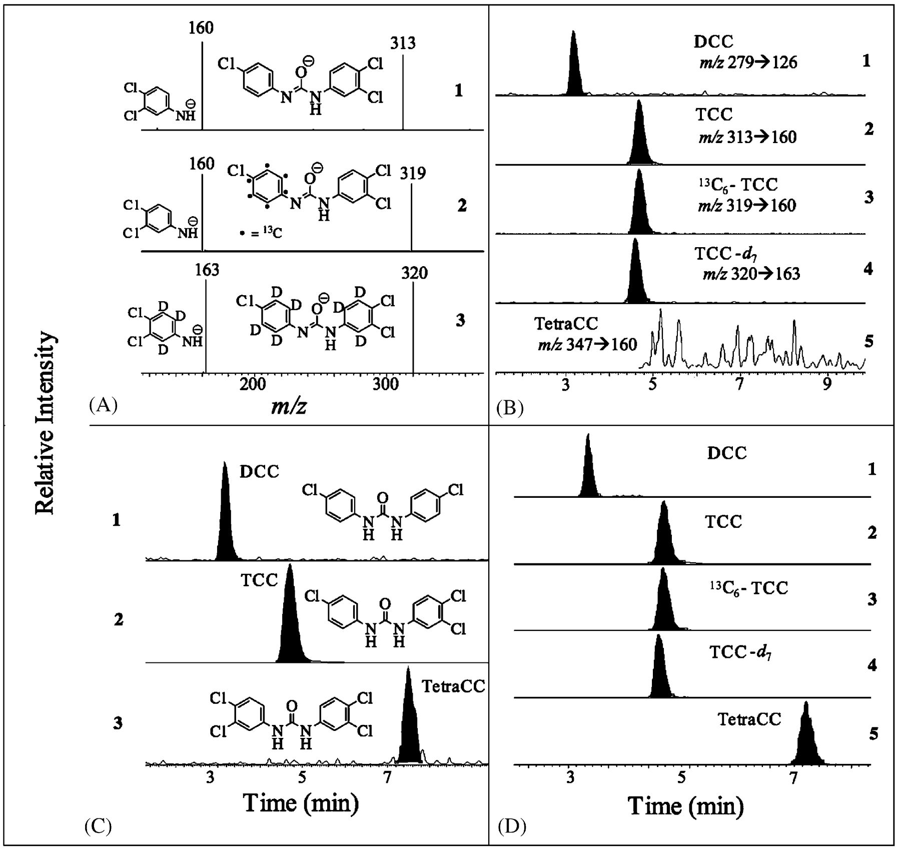

Fig. 2. Mass spectra and chromatograms of chlorocarbanilide congeners analyzed by LC-ESI-MS/MS. Obtained mass spectra (**A**) indicate consistent fragmentation behaviors of triclocarban (TCC; Panel 1) and its stable isotope-labeled analogs $^{13}C_6$-TCC (Panel 2), and TCC-d_7 (Panel 3); the locations of intramolecular proton loss are hypothetical. Selected reaction monitoring (SRM) chromatograms (**B**) demonstrate the presence of 4,4′-dichlorocarbanilide (DCC) and triclocarban (TCC), but absence of 3,3′,4,4′-tetrachlorocarbanilide (TetraCC) in river water collected downstream of a wastewater treatment plant. Analysis of technical grade triclocarban in methanol (300 μg/L) yielded SRM chromatograms (**C**), demonstrating the presence of DCC (m/z 279 → 126; Panel 1), TCC (m/z 313 → 160; Panel 2), and TetraCC (m/z 347 → 160; Panel 3). Analysis of primary municipal sludge (**D**) yielded representative SRM chromatograms, demonstrating the presence of TetraCC in the organic extract, detectable in addition to DCC and TCC.

the downstream concentrations ranged from 2 to 250 ng/L, as determined by averaging the results of duplicate samples (Table 2). Concentrations of TCC (mean ± standard deviation) downstream of WWTPs (84 ± 110 ng/L) were significantly higher ($P<0.05$; Wilcox rank sum test) than those detected upstream (12 ± 15 ng/L). Median (and 5th and 95th percentile) TCC concentrations upstream and downstream of WWTPs were 7 (0.5 and 38) ng/L and 20 (3 and 250) ng/L, respectively (Table 3). Compared to upstream samples, all downstream samples had higher concentrations of TCC, with the mean difference and standard deviation equaling 72 and 102 ng/L, respectively.

In addition to TCC, some river water samples also had detectable levels of another halogenated diaryl urea compound, identified by chromatographic retention time, ionization behavior and MS/MS fragmentation pattern as 4,4′-dichlorocarbanilide (DCC). For example, in a characteristic selected reaction monitoring (SRM) chromatogram obtained for a surface water sample collected downstream of the WWTP at location SW-8, the presence of DCC and TCC is evidenced by peaks with characteristic retention times and molecular transitions (Fig. 2B; Panels 1 and 2). However, none of the surface water samples had detectable levels of 3,3′,4,4′-tetrachlorocarbanilide (TetraCC) (Fig. 2B; Panel 5).

Origin of co-contaminating chlorocarbanilides: To elucidate the origin of the novel surface water contaminant, DCC, technical grade TCC (99%) was analyzed for the presence of lower and higher chlorinated carbanilide congeners representing potential manufacturing impurities. Screening of technical grade TCC solutions using SRM channels programmed for the molecular transitions for diochlorocarbanilide (DCC; m/z 279 → 126) and tetrachlorocarbanilide (TetraCC; m/z 347 → 160) resulted in the chromatograms shown in Fig. 2C. Results obtained from analysis of authentic standards showed that technical grade TCC contains the manufacturing impurities DCC and TetraCC at an estimated level of 0.2% each on a weight-by-weight basis.

To determine whether the incongruent occurrence of DCC and TetraCC in river water may be linked to their contrasting environmental partitioning behaviors (Table 4), primary sludges from WWTPs located in five different US states (Fig. 1) were analyzed for the presence of TCC

Table 2
Chlorocarbanilide concentrations detected in surface waters impacted by wastewater treatment plant effluent and in primary sludges obtained from various municipal wastewater treatment plants in the United States

Sample ID	Surface water upstream of WWTPs (ng/L)			Surface water downstream of WWTPs (ng/L)		
	TCC	DCC	TetraCC	TCC	DCC	TetraCC
SW-1	7	ND (0.15)	ND (0.25)	10	ND (0.15)	ND (0.25)
SW-2	17	ND (0.15)	ND (0.25)	20	ND (0.15)	ND (0.25)
SW-3	ND (0.45)	ND (0.15)	ND (0.25)	2	ND (0.15)	ND (0.25)
SW-4	27	0.3	ND (0.25)	250	5.5	ND (0.25)
SW-5	ND (0.45)	ND (0.15)	ND (0.25)	22	0.6	ND (0.25)
SW-6	ND (0.45)	ND (0.15)	ND (0.25)	3	ND (0.15)	ND (0.25)
SW-7	ND (0.45)	ND (0.15)	ND (0.25)	5	ND (0.15)	ND (0.25)
SW-8	9	ND (0.15)	ND (0.25)	240	2.2	ND (0.25)
SW-9	45	2.2	ND (0.25)	200	1.7	ND (0.25)
Mean ± SD	12 ± 15	0.4 ± 0.7	—	84 ± 110	1.2 ± 1.8	—
	Primary sludges obtained from select US wastewater treatment plants (μg/kg dry weight)					
	TCC	DCC	Tetra TCC			
PS-1	7500	17	80			
PS-2	22,600	30	310			
PS-3	25,900	10	500			
PS-4	21,800	13	240			
PS-5	18,500	7	253			
Mean ± SD	19,300 ± 7,100	16 ± 9	275 ± 150			

Abbreviations: WWTPs, wastewater treatment plants; SW, surface water; PS, primary sludge; TCC, 3,4,4′-trichlorocarbanilide; DCC, 4,4′-dichlorocarbanilide; TetraCC, 3,3′,4,4′-tetrachlorocarbanilide; Nondetect observations (ND) are reported as one-half the method detection limit. All reported concentrations represent two independent measurements of duplicate samples that were averaged and rounded to the closest integer.

Table 3
Descriptive statistics of environmental monitoring data for TCC obtained via analysis of river water samples taken upstream and downstream of WWTP discharge locations in nine US states

Parameter	Upstream	Downstream
Method detection limit (MDL), (ng/L)	0.9	0.9
Number of samples	18	18
Number of samples above MDL, (%)	56	100
Mean, (ng/L)	12	84
Standard deviation (SD), (ng/L)	15	110
Median, (ng/L)	7	20
5th Percentile, (ng/L)	0.5	3
95th Percentile, (ng/L)	38	250
Average difference, (ng/L) (SD) (downstream conc. minus upstream conc.)	72 (102)	

Note: All calculated values were rounded to the closest integer.

manufacturing impurities. Representative SRM chromatograms presented in Fig. 2D show that, in contrast to river water samples, both DCC and TetraCC were detected consistently in primary sludge from all municipal treatment plants surveyed (see also Table 2).

4. Discussion

Reliable environmental monitoring data for the emerging pollutant TCC are still scarce. Prior to the present study, peer-reviewed data were available only for six urban streams and selected sludge samples from the Greater Baltimore area (Halden and Paull, 2004). These previously published data were obtained using liquid chromatography negative electrospray ionization mass spectrometry in conjunction with the isotope dilution technique via monitoring of adducts of deprotonated molecular base ions. The two-dimensional mass spectrometry technique introduced here overcomes some of the limitations inherent to the single-quadrupole MS method and also provides the first peer-reviewed data on environmental occurrences of TCC in US water resources nationwide.

Benefits of the ID-LC-ESI-MS/MS method: Use of tandem mass spectrometry provided some significant improvements over previously available approaches that were reviewed elsewhere (Halden and Paull, 2004). The

Table 4
Selected physical-chemical properties of three chlorocarbanilides obtained from the literature or calculated using quantitative structure–activity relationship (QSAR) analyses

Parameters reviewed	DCC	TCC	TetraCC
Number of chlorines	2	3	4
Molecular weight	281.14	315.58	350.03
Percent in each medium			
Water	8	7	2
Soil	87	77	70
Sediment	5	16	28
Air	0	0	0
$\log_{10} K_{OW}$[a]	4.3	4.9	5.6
$\log_{10} K_{OW}$[b]	4.84	5.74	6.65
pK_A[c]	13.14±0.7	12.77±0.7	12.16±0.7
Water solubility[c] (mg/L)	3.7	0.65	0.11
Bioconcentration factor, method 1[d]	380	1200	3700
Bioconcentration factor, method 2[e]	2810	13,700	66,500

Note: DCC, 4,4′-dichlorocarbanilide; TCC, 3,4,4′-trichlorocarbanilide; TetraCC, 3,3′,4,4′-tetrachlorocarbanilide.

[a] Octanol–water partitioning coefficient (K_{OW}) calculated using K_{OW} WIN.

[b] K_{OW} calculated using Solaris V4.67.

[c] Negative log of the acid ionization constant (pK_A) and water solubility calculated using Solaris V4.67.

[d] Calculated using ECOSAR.

[e] Calculated using Solaris V4.67.

most sensitive previously available method employed ID-LC-ESI single quadrupole mass spectrometry; however, coelution of interferences along with TCC required time-consuming gradient chromatography with run times of 30 min, while also necessitating monitoring of adducts of TCC and acetic acid to boost sensitivity and selectivity (Halden and Paull, 2004). These inconvenient modifications are not necessary with the technique described here, which employs the targeted fragmentation of molecular base ions after loss of one proton ($[M–H]^-$) via collision-induced dissociation followed by detection of their characteristic daughter ions in MS/MS mode. Using isocratic elution, TCC and related compounds were detected and quantified successfully even in challenging samples using 10-min runs (Fig. 2). Compared to the original ID-LC-ESI-MS method, this approach increased the practical sample throughput per unit time by more than 300%. Time savings were realized not only by shortened runs but also by minimal downtime between injections, since isocratic chromatography did not require the column equilibration period typical of gradient chromatography. Owing to the selectivity and sensitivity of the triple quadrupole mass spectrometer, a method detection limit in the sub-part-per-trillion range (0.9 ng/L) was achieved that was 33 times lower than that of the single quadrupole method (30 ng/L) when applied to river water (Halden and Paull, 2004). This improvement was accomplished despite a reduction by 80% in the sample volume required for analysis (200 mL versus 1000 mL). Thus, the rapidity and sensitivity of the assay and its reduced sample volume requirements made it a convenient tool for the analysis of more economical, low-volume water samples shipped to the laboratory from across the United States.

TCC concentrations in the US environment: Application of the method to the analysis of stream samples provided experimental evidence for the widespread contamination of US surface waters with TCC predicted earlier using an empirical relationship (Halden and Paull, 2005). All samples collected downstream of WWTPs had higher concentrations of TCC than corresponding samples taken upstream. These increases must be attributed mostly to discharge of treated wastewater that is known to contain residual amounts of TCC ranging in concentration from 110±10 ng/L (Halden and Paull, 2005) to as much as 6000 ng/L (Clark et al., 1991); more recent and extensive monitoring of raw and treated wastewater and sludges at the plant in the State of Maryland supports this conclusion (Heidler et al., 2006). Four effluent-receiving streams (SW-3, -5, -6, and -7) showed nondetectable TCC levels upstream (<0.9 ng/L) and low concentrations downstream. These low TCC levels determined downstream may have resulted either from a high removal efficiency of the respective treatment plant or, more likely, from significant dilution of the effluent in receiving streams. However, since data on plant effluent concentrations and river flow volumes were unavailable, neither scenario could be affirmed. At the time of sampling, the intermittently operating WWTP located near SW-1 reported no discharge of effluent to the stream which helps to explain the very similar TCC levels found upstream and downstream of this plant (7 vs 10 ng/L).

The detection frequency found in this study is higher than previously reported, which may be due to at least two factors: first, a recent increase in the usage of antimicrobial personal care products and, second, the low MDL of the method applied. Sampling at 108 sites conducted by the soap and detergent industry in 1979 and 1982 showed a detection frequency for TCC of 10% with a method detection limit of 50 ng/L (TCC Consortium, 2002a). More recent data from 1999 showed an increase in detection frequency to a level of 30%; however, the significance of this increase is difficult to judge, since the type of method used and its MDL were not reported (TCC Consortium, 2002b). An empirical model that uses triclosan concentrations as the input parameter for estimating the occurrence of TCC in various environmental compartments suggested a likely detection frequency of 58% in 85 US streams sampled in 1999–2000 at an estimated detection limit for TCC of 33 and 42 ng/L (Halden and Paull, 2005). In the present study, the observed detection frequency of 78% (14/18) is significantly higher than those reported previously, whereas the absolute values are much lower (TCC Consortium, 2002a). This result is not surprising given a comparatively lower and therefore superior detection limit of the ID-LC-ESI-MS/MS method of 0.9 ng/L and a random selection of sampling locations instead of a targeted analysis of polluted streams (Kolpin et al., 2002).

Mean TCC levels observed in the present study in river samples taken in nine states upstream and downstream (12 and 84 ng/L) of known wastewater discharge locations were lower than the mean TCC level (213 ng/L) predicted for 28 states using the previously mentioned empirical model (Halden and Paull, 2005). Calculated TCC concentrations were derived from the 1999–2000 national reconnaissance of pharmaceuticals, hormones, and other organic wastewater contaminants conducted by the US Geological Survey (USGS) (Kolpin et al., 2002). Since the sampling strategy used in the USGS study was aimed toward streams susceptible to contamination (e.g., downstream of intense urbanization and livestock production) (Kolpin et al., 2002), the resultant estimate for TCC was known to represent an environmentally unfavorable, high-exposure scenario. The sampling strategy employed in the present study was not specifically targeted at potentially problematic locations. While an assessment of average concentrations of TCC present in US streams would be desirable, the data set presented here may be too small to provide a definitive answer as to the frequency and level of nationwide contamination of water resources with TCC. Additional monitoring studies are needed to provide more comprehensive information.

Discovery of additional chlorocarbanilide congeners in the environment: Application of the LC-ESI-MS/MS protocol to analytes other than TCC resulted in the discovery of two novel chlorocarbanilide compounds in river water (DCC) and in municipal sludge (DCC and TetraCC). Prior to this study, DCC had been identified only once as a contaminant of the US environment in sediments and tar balls collected downstream of a specialty chemicals manufacturing plant from a brackish river on the East Coast. However, quantitative data for DCC were not obtained in that study, which employed fractionation of organic solvent extracts by HPLC followed by introduction of samples into a quadrupole mass spectrometer using a direct insertion probe (Jungclaus et al., 1978). In the same investigation, monitoring of DCC in river water and wastewater yielded nondetect values for both matrices. The present study demonstrates the occurrence of DCC in river water as well as DCC and TetraCC in municipal sludge, albeit at low concentrations.

When compared to TCC, even less information is available regarding the environmental fate of structurally related chlorocarbanilides. A recent investigation into the transport of anthropogenic, nonionic organic chemicals in laboratory soil columns demonstrated that DCC sorbs strongly to soil constituents due to its hydrophobic properties (Xu et al., 2002a, b). This observed chemical behavior is consistent with the QSAR results presented in Table 4, indicating a significant hydrophobicity of DCC (K_{OW} of ~20,000–69,000). Although sorption of TCC (K_{OW} of ~80,000–500,000) and TetraCC (K_{OW} of ~400,000–4,470,000) was not investigated in these studies, the QSAR data presented in Table 4 suggest that both compounds will sorb to soil and sediment materials even more strongly than DCC due to their nonionic character at ambient pH ($pK_A > 12$) and their comparatively greater hydrophobicity. Results of the QSAR analysis also suggest that all three chlorocarbanilide congeners potentially may accumulate in biota. Estimates of bioconcentration factors ranged from 380 for DCC to 66,500 for TetraCC (Table 4).

Conceptual application of the QSAR results to sorption and fugacity models also can provide insights into why DCC, but not TetraCC, was detectable in some surface water samples. Relative to TetraCC, DCC is less particle-active and 34-times more water-soluble (3.7 versus 0.11 mg/L at 25 °C). Thus, given an entry of equal masses of both impurities into the environment, the mass fraction of DCC in the dissolved state was calculated to be four-fold greater than that of TetraCC (8% versus 2%) (Table 4). In addition, owing to stronger partitioning into sludge, TetraCC is likely to be removed more effectively by sorption in WWTPs than is DCC. Assuming identical source strengths and limited biodegradation of chlorocarbanilides during wastewater treatment (Gledhill, 1975; Halden and Paull, 2005), one would expect TetraCC concentrations to be lower in effluent-receiving streams and higher in municipal sludge relative to DCC. Indeed, when normalized to the mass of TCC found in dried primary sludge, levels of TetraCC (1.35±0.35% of TCC) were observed to be 13-fold higher than those of DCC (0.10±0.08% of TCC).

The confirmed presence of DCC and TetraCC as manufacturing impurities in technical grade TCC at equal weight percentages (0.2% each) suggests personal care products as one of multiple potential sources of environmental release of these compounds. As stated earlier, the upper range of TCC use in the US is estimated at 750 metric tons per year (TCC Consortium, 2002a). Thus, when extrapolated to the national scale, the newly found impurities DCC and TetraCC are expected to enter the environment from TCC usage alone at maximum rates of 1.5 metric tons or 3300 pounds each per year. Environmental transformation of TCC to lower or, less likely, higher chlorinated congeners also must be considered as a potential source term.

Aside from this demonstrated source, additional unintentional sources of chlorocarbanilides may have contributed to the levels of contamination found in river water and municipal sludge. For example, TetraCC is known to be present as an impurity in the herbicide diuron at estimated levels of 1–5% by weight (Newman et al., 2001). Based on available herbicide production numbers (Newman et al., 2001), we calculated unintentional inputs of TetraCC into the US environment from usage of diuron alone to equal 4.7–23.6 metric tons per year.

Since DCC and TetraCC also have potential uses in industry, additional sources of intentional release may have contributed to the occurrence of these compounds in US surface waters and municipal sludges. Both DCC and TetraCC can serve as electrophotographic photoreceptors in photocopying processes (Argiriadi et al., 1999; Horie et al., 1990). The DCC congener also may serve as an electrophotographic printing plate precursor, a charge-controlling agent in electrostatographic toner (Argiriadi et al., 1999), and an enzyme inhibitor (McElroy et al., 2003). In addition, TetraCC has been proposed as a bacteriostatic and bactericidal additive to polymers (Leeming, 1969) and as an enzyme inhibitor (Kroetz et al., 2003; Proctor et al., 2002; Salituro et al., 1999). However, none of these proposed uses could be confirmed and chemical production numbers for the targeted synthesis of both DCC and TetraCC are unknown.

The present work identified chlorocarbanilide compounds as widespread environmental contaminants that—despite decades of mass production, usage, and environmental dispersal—historically have attracted little scientific attention due in part to the difficulty of detecting these compounds with traditional monitoring tools.

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