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Detection of the antimicrobials triclocarban and triclosan in agricultural soils following land application of municipal biosolids

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ABSTRACT

The occurrence of the antimicrobials triclocarban (TCC) and triclosan (TCS) was investigated in agricultural soils following land application of biosolids using liquid chromatography–tandem mass spectrometry (LC–MS–MS) with negative ion multimode ionization. The method detection limits were 0.58 ng TCC/g soil, 3.08 ng TCC/g biosolids, 0.05 ng TCS/g soil and 0.11 ng TCS/g biosolids and the average recovery from all of the sample matrices was >95%. Antimicrobial concentrations in biosolids from three Michigan wastewater treatment plants (WWTPs) ranged from 4890 to 9280 ng/g, and from 90 to 7060 ng/g, for TCC and TCS respectively. Antimicrobial analysis of soil samples, collected over two years, from ten agricultural sites previously amended with biosolids, indicated TCC was present at higher concentrations (1.24–7.01 ng/g and 1.20–65.10 ng/g in 2007 and 2008) compared to TCS (0.16–1.02 ng/g and from the method detection limit, <0.05–0.28 ng/g in 2007 and 2008). Soil antimicrobial concentrations could not be correlated to any soil characteristic, or to the time of last biosolids application, which occurred in either 2003, 2004 or 2007. To our knowledge, our data represent the first report of TCC, and the first comparison of TCC and TCS concentrations, in biosolids-amended agricultural soils. Such information is important because approximately 50% of US biosolids are land applied, therefore, any downstream effects of either antimicrobial are likely to be widespread.

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1. Introduction

Triclocarban (3,4,4′-trichlorocarbanilide) (TCC) and triclosan (2,4,4′-trichloro-2′-hydroxydiphenyl ether) (TCS) (Fig. 1) are antimicrobials currently added to a large number of consumer products. In particular, they are commonly added to household soaps; in a retail survey these chemicals were found in 76% of liquid soaps and 29% of bar soaps (Perencevich et al., 2001). This widespread use, reported at 0.6–10 million kg yr^{-1} (Miller et al., 2008; TSCA, 2003), is a cause for concern because of recent reports of incomplete TCC and TCS removal during wastewater treatment (Chu and Metcalfe, 2007; Heidler et al., 2006; Sapkota
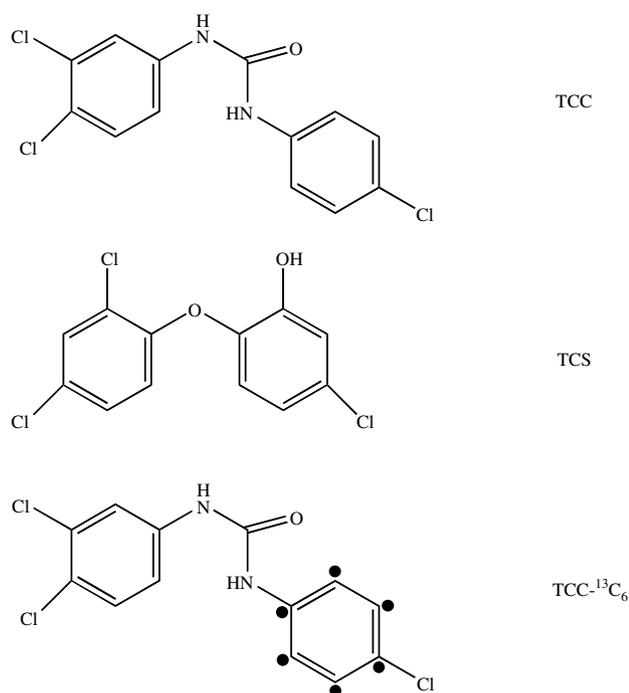
et al., 2007) and the detection of these chemicals in surface waters (Coogan et al., 2007; Halden and Paull, 2004, 2005; Kolpin et al., 2002; Lindstrom et al., 2002; Sapkota et al., 2007; Young et al., 2008). Such release of TCC and TCS has the potential to cause a number of environmental and human health problems, including: the bioaccumulation of TCC, TCS and methyltriclosan (a lipophilic metabolite of TCS) in algae and snails (Coogan and La Point, 2008; Coogan et al., 2007); algal growth inhibiting effects (Yang et al., 2008); the potential of TCC to act as an endocrine disrupting compound (Ahn et al., 2008; Chen et al., 2008); the formation of toxic degradation products (Aranami and Readman, 2007; Chhabra et al., 1991; Gledhill, 1975);

Fig. 1 – Chemical structures of triclocarban (TCC), triclosan (TCS) and triclocarban-$^{13}C_6$ (TCC-$^{13}C_6$).

and the development of microbial resistance (Heath et al., 2000, 1998, 1999; Hoang and Schweizer, 1999; McMurry et al., 1998a,b). Therefore, to protect aquatic ecosystems, as well as drinking water supplies, there is a clear need to examine the fate of TCC and TCS following release to the environment.

The occurrence of TCC and TCS in wastewater treatment plant (WWTP) influent, effluent and surface waters has been well documented (Coogan et al., 2007; Halden and Paull, 2004, 2005; Heidler et al., 2006; Sapkota et al., 2007; Trenholm et al., 2008; Young et al., 2008). However, less information is available on the concentrations of these chemicals in biosolids. Although there have been several reports of TCS accumulation in WWTP activated sludge and biosolids (Kinney et al., 2006; McAvoy et al., 2002; Morales et al., 2005; Singer et al., 2002; Ying and Kookana, 2007), only two groups have investigated the occurrence of TCC in biosolids or primary sludge (Chu and Metcalfe, 2007; Heidler et al., 2006; Sapkota et al., 2007). The first report, published in 2006, involved a TCC mass balance on a full scale wastewater treatment plant, and the finding that approximately three quarters of the initial mass of TCC were still detectable in sludge, reaching concentrations up to $51,000 \pm 15,000$ ng/g dry weight (Heidler et al., 2006). TCC concentrations in the ppm range have also been documented for biosolids and activated sludge samples from four WWTPs in Canada (Chu and Metcalfe, 2007), and for primary sludge samples from five WWTPs in the United States (Sapkota et al., 2007). Such reports are a concern because 50% of all biosolids produced in the United States are land applied (USEPA, 2007), and the fate of TCC following land application to agricultural areas has yet to be addressed.

Studies examining the degradation potential of TCC are limited in number, but all indicate TCC will likely persist in the

environment. These reports include a one-soil laboratory microcosm experiment, for which the aerobic TCC half life was estimated to be 108 days, whereas under anaerobic conditions little TCC biodegradation was found within 70 days (Ying et al., 2007). Further, using quantitative structure–activity relationship analysis, the predicted TCC half-lives in soils and sediments were 120 and 540 days, respectively (Halden and Paull, 2005). In addition, a recent study documented persistence of TCC in estuarine sediment cores taken near wastewater treatment plants (New York), with peak levels being on the order of 24,000 ng/g (Miller et al., 2008). Although not specifically investigated in these studies, the data suggest that TCC will likely persist in agricultural soils following land application of biosolids.

A complete understanding of the concentration and fate of TCC and TCS in agricultural soils following land application of biosolids is needed because of concerns over bioaccumulation and movement to surface waters. For example, biosolids derived compounds have been identified in earthworm tissue with bioaccumulation factors ranging from 0.05 to 27 (Kinney et al., 2008). Further, two recent studies illustrated the movement of several pharmaceuticals and personal care products from agricultural areas amended with biosolids to tile drainage water (Lapen et al., 2008) and to runoff (Topp et al., 2008). All three studies included TCS, but not TCC, in their analyses.

In this study, we describe a rapid analytical method for the determination of TCC and TCS in biosolids and agricultural soils using pressurized liquid extraction (PLE) and liquid chromatography–tandem mass spectrometry (LC–MS–MS) with negative ion multimode ionization. We determined antimicrobial concentrations in biosolids produced from three WWTPs in Michigan and quantified TCC and TCS concentrations, over two years, in ten agricultural soils following biosolid land application. To our knowledge, these data represent the first investigation of TCC concentrations, and the first comparison of TCC and TCS concentrations, in agricultural soils following application of biosolids.

2. Experimental procedures

2.1. Chemicals

Triclocarban (3,4,4′-trichlorocarbanilide) (>99%) (TCC) and triclosan (2,4,4-trichloro-2-hydroxydiphenyl ether) (>97%) (TCS) were obtained from Sigma–Aldrich (St. Louis, MO, USA) and triclocarban-$^{13}C_6$ was purchased from Cambridge Isotope Laboratories (Andover, MA, USA) (Fig. 1). Methanol, acetone, and acetonitrile were HPLC grade (Sigma–Aldrich, St. Louis, MO, USA). Ottawa sand used for pressurized liquid extraction was purchased from EMD (Gibbstown, NJ, USA). Individual stock solutions of TCC and TCS were prepared monthly by dissolving each compound in acetonitrile at a concentration of 100 mg/L. All stock solutions were stored at $-20\,^{\circ}C$ in the dark. Working solutions were prepared daily by diluting the individual stock solution with the same solvent and were stored at $4\,^{\circ}C$ in the dark. The internal standard (triclocarban-$^{13}C_6$) working solutions (0.2 mg/L) were prepared daily by diluting the standard solution with solvent.

2.2. Sample collection and preparation

Soil samples were collected from ten agricultural sites within 100 miles of Grand Rapids (MI) in August 2007 and 2008. All sites had previously been amended with biosolids (from WWTP 1, see below), with the time of last application ranging from 2003 to 2007. The biosolid application rate was estimated at 0.73 dry Mg per 1000 m^2 or 3.25 dry tons per acre. The soil samples, consisting of 10–14 subsamples, were taken in the plow depth, or 6–8 inch, as the biosolids were applied 6–12 inch deep. The subsamples were combined in the field and following transport to the laboratory, samples were manually sorted to remove gravels and plant residues, homogenized using a laboratory blender, air-dried, and sieved through a 2 mm screen. Soil samples were analyzed for a range of soil properties by A & L Analytical Laboratories (Memphis, TN). Grab samples of biosolids were collected in 2007 and 2008 from three wastewater treatment plants (WWTPs) in Michigan. The WWTPs cover catchment areas from populations of 80,000 (WWTP 1), 13,000 (WWTP 2) and 23,000 (WWTP 3). WWTP 1 and WWTP 3 use activated sludge for their secondary treatment process, and WWTP 2 uses rotating biological contactors.

Pressurized liquid extraction (PLE) was performed using a Dionex ASE 200 accelerated solvent extractor (Sunnyvale, CA, USA). Portions of 0.2 g oven-dried biosolids and 5 g air-dried soil samples, mixed with Ottawa sand, were placed into 11 mL stainless steel extraction cells. Before loading the samples, a glass fiber filter was placed in the outlet of the cell, and 20 ng of triclocarban-^{13}C$_6$ was spiked into the sample. One cycle of extraction with acetone was carried out with the following PLE conditions: oven temperature, 100 °C; extraction pressure, 1500 psi; static time, 5 min and flush volume, 100%. The extracts were evaporated to dryness under nitrogen gas, resuspended in 200 μL of acetonitrile, and transferred to 2 mL amber autosampler vials.

2.3. Sample analysis

Liquid chromatography (LC) was conducted with a Waters ACQUITY Ultra Performance LC (UPLC) system, which consisted of an autosampler and a binary pump. TCC and TCS were separated by using a Waters ACQUITY UPLC BEH C$_{18}$ column (1.7 μm, 2.1 × 50 mm) at 50 °C. Mobile phase A (10 mM ammonium acetate) and phase B (acetonitrile) were used to produce a binary elution gradient with a flow rate of 0.40 mL/ min for TCC and TCS. The separation of TCC and TCS was achieved with the following linear mobile phase gradient program: at 0 min A/B = 50:50 (v/v); 0.5 min A/B = 50:50; 3.5 min A/B = 30:70; 3.51 min A/B = 50:50; and 4 min A/ B = 50:50. A one-minute post-separation time allowed re-equilibration of the column.

Each ionization method was optimized independently using separate source-tuning parameters. Ionization sources used were the electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and a new combined ESI–APCI source (ESCi). MASSLYNX version 4.1 software was used to control the mass spectrometric conditions. The optimized tune page settings were: 3.0 kV for capillary voltage in ESI, 3.0 μA for corona current for APCI, 120 °C for the source temperature, 350 °C for the desolvation temperature, 697 L/h for the desolvation gas flow, and 49 L/h for the cone gas flow.

2.4. Method validation study

The product ions producing the highest intensity used for Multiple Reaction Monitoring (MRM) and quantification to increase analytical sensitivity and selectivity in LC–MS–MS mode for the targeted TCC, TCS and TCC-^{13}C$_6$ (Internal Standard, or IS) are listed in Table 1. Quantification was based on the detector response defined as the ratio of peak area of the base peak ion (the specific product ion of interest) to peak area of the base peak ion for the IS. Calibration curves were constructed with biosolids spiked at TCC and TCS concentrations of 100, 500, 1000, 5000, and 10,000 ng/g before extraction. The working solutions (0.1, 1, 5, 10 mg/L) of TCC and TCS were added to achieve a spiking concentration from 100 to 10,000 ng/g in 0.2 g dried biosolids. Calibration curves for TCC and TCS spiked into soil before extraction were constructed at 10, 50, 100, 300, 500 and 1000 ng/g. In five grams of soils, working solutions (1, 5, 10 mg/L) of target compounds were added to achieve a spiking concentration from 10 to 1000 ng/g, and spiked soils were then mixed and analyzed. Because the biosolids and soils when used as a matrix already contained TCC and TCS, calibration curves for these antimicrobials in these matrices were constructed by subtracting the background concentration from the spiked concentration. The method detection limit (MDL) was determined using two methods: (1) a signal-to-noise (S/N) ratio, which can be measured directly using the instrument software, and (2) the methodology recommended by the U.S. Environmental Protection Agency (USEPA) (Berthouex and Brown, 2002), based on the variability of multiple analyses of seven soil and biosolids extracts spiked at a concentration of 5 ng/g and 20 ng/g for TCC and TCS, respectively.

3. Results and discussion

3.1. Method development and validation

A number of extraction and analytical methods have been reported for the quantification of TCC and/or TCS from various solid samples (Table 2). Here, we developed the rapid and sensitive analytical methods to facilitate the quantification of both TCC and TCS in biosolids and soil samples. Each ionization method (ESI, APCI and ESCi) was optimized independently by using separate source-tuning parameters.

Table 1 – Summary of the ESCi MS optimization results for antimicrobial compounds.

	Ionization mode	Precursor ion > product ion (m/z)	Collision voltage (V)
Triclocarban-^{13}C$_6$	ESI-	319 > 160	15
Triclocarban	ESI-	313 > 160	15
Triclosan	APCI-	253 > 217	25

Table 2 – Triclocarban and triclosan in various solid WWTP and environmental samples along with detection methods.

Sample	Triclocarban (ng/g)	Triclosan (ng/g)	Extraction method	Analytical method	Detection limit (ng/g)	Ref.
Digested sludge		500–15,600	Supercritical fluid extraction	GC–MS	LOQ: 70[a]	(McAvoy et al., 2002)
Sludge		No data	Pressurized liquid extraction (PLE)	GC–MS–MS		(Singer et al., 2002)
Lake sediment		37–53			LOQ: 5	
Marine sediment		0.27–130.7	PLE-solid phase extraction (SPE)	LC–ES-MS–MS Ion trap	LOD: 3.50[b]	(Aguera et al., 2003)
Sludge		400–8800	Soxhlet extraction-SPE	GC–MS	LOQ: 4	(Bester, 2003)
Sludge		418–5400	Microwave extraction-SPE	GC–MS–MS	LOQ: 0.8	(Morales et al., 2005)
River sediment		4.4–35.7			LOQ: 0.4	
Marine sediment		<0.4			LOQ: 0.4	
Digested sludge	51,000		PLE	LC–ESI-MS–MS Triple quadrupole		(Heidler et al., 2006)
Biosolids		3000–32,900	PLE–SPE	GC–MS	MDL: 49.6[c]	(Kinney et al., 2006)
Biosolids		90–16,790	Liquid–liquid extraction (LLE)–SPE	GC–MS	LOQ: 5	(Ying and Kookana, 2007)
Digested sludge		20,000–55,000	PLE	LC–ESI-MS Single quadrupole	MDL: 1000	(Heidler and Halden, 2007)
Sludge	7500–25,900		PLE	LC–ESI-MS–MS Triple quadrupole		(Sapkota et al., 2007)
Biosolids	3050–5970	680–11,550	PLE–SPE	LC–ESI-MS–MS Triple quadrupole	Biosolid LOQ: 0.5 (TCC), 5.0 (TCS)	(Chu and Metcalfe, 2007)
Activated sludge	2170–4820	620–1450				
Biosolids		10,500	PLE–SPE	GC–MS	MDL: 49.6	(Kinney et al., 2008)
Soil		69–833				
River sediment	1620–23,910	310–800	LLE	LC–ESI-MS Triple quadrupole	LOQ: 0.5 (TCC), 50 (TCS)	(Miller et al., 2008)
Biosolids		320	Ultrasonic extraction-SPE	LC–ESI-MS–MS Triple quadrupole	LOQ: 57	(Chenxi et al., 2008)
Biosolids	4890–9280	90–7060	PLE	LC–ESCi-MS–MS Triple quadrupole	LOQ: 0.05 (TCC & TCS) Biosolids MDL:3.08(TCC), 0.1 (TCS)	This study
Soil	1.20–65.10	<0.05–1.02			Soil MDL: 0.58 (TCC), 0.05 (TCS)	

a LOQ: Limit of quantitation.
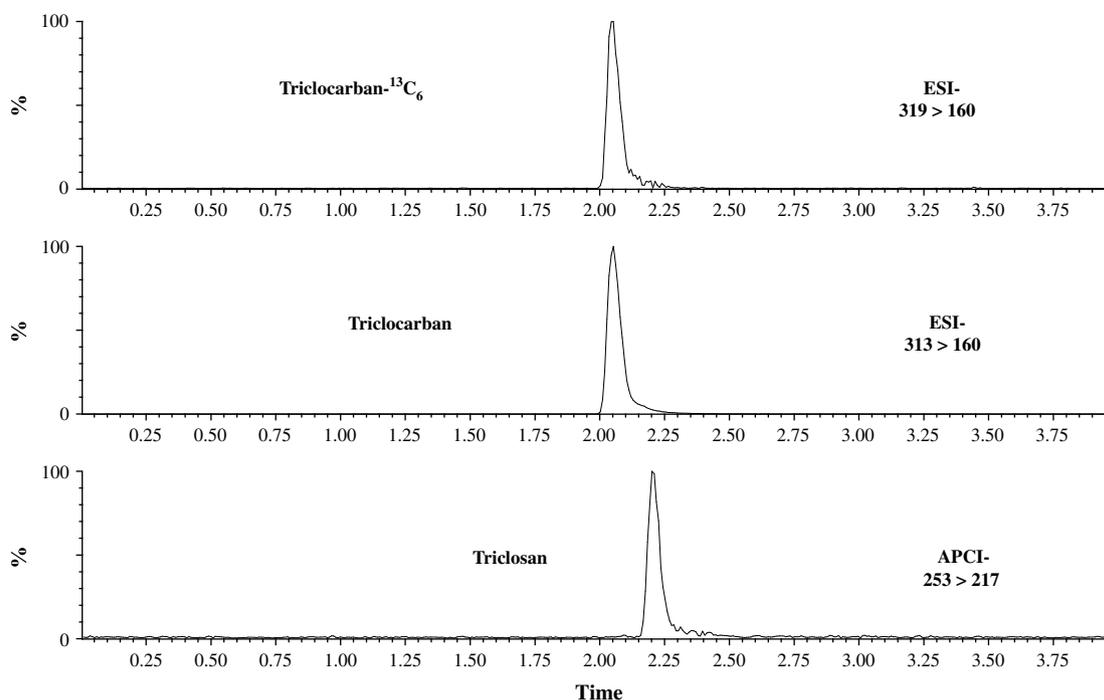b LOD: Limit of detection.
c MDL: Method detection limit.

Fig. 2 – Representative ESCi MRM chromatograms for triclocarban-$^{13}C_6$, triclocarban and triclosan.

Compared with the peak area at same concentration, TCC is approximately 12 times more sensitive to ESI negative than to APCI negative, and TCS is approximately two times more sensitive to APCI negative than to ESI negative. The mass peaks corresponding to TCC and TCS appeared on the total-ion chromatograms (TICs) monitored at the selected product ion (Fig. 2). The data were processed by creating the reconstructed total-ion chromatograms (RTICs) for each analyte. The product molecular ion of TCC was observed at m/z 160 at a retention time of 2.08 min. TCS was produced at m/z 217 at a retention time of 2.23 min. These results indicate that efficient separation of triclocarban and triclosan was achieved in only 2.23 min by the short C_{18} column using column temperature (50 °C), a volumetric flow rate of 0.40 mL/min and mobile phases in a binary solvent system, meaning fast analysis of the investigated antimicrobials. Therefore, the LC method employing a binary gradient sequence combined with ESCi(−)-MS–MS allowed the rapid, sensitive, selective, and reliable determination of the investigated TCC and TCS.

Concentrations of TCC and TCS were calculated reproducibly by using the matrix-matched internal standard calibration curves to correct, to a certain extent, for the minimal matrix effects. Calibration curves were constructed for TCC and TCS extracts spiked into 0.2 g of dried biosolid before extraction within range of 100–10,000 ng/g. Calibration curves also were constructed for TCC and TCS extracts spiked into soil samples before extraction at levels from 10 to 1000 ng/g. The resulting calibration curves were linear with correlation coefficients (R^2) >0.99 for the MS–MS procedure.

The recoveries of TCC and TCS were measured by extracting analytes from soils and biosolids, respectively, spiked at levels from 50 to 300 ng/g and from 500 to 10,000 ng/g. The recoveries of TCC and TCS were specifically based on matrix spike samples that are extracted by PLE and sample extracts spiked after PLE of the matrix. Because TCC and TCS were detected at the soil and biosolid samples using the developed method, recovery in these matrixes was determined using a concentration calculated by subtracting the measured background concentration from the spiked concentration. Recovery determinations were calculated as the average of analyses of duplicate soil samples spiked at 50 and 300 ng/g and biosolid samples at 500, 5000 and 10,000 ng/g before and after extraction. The average recovery of TCC and TCS including the internal standard (TCC-$^{13}C_6$) from all the sample matrices was generally higher than 95% (Table 3). No concentration dependence was observed.

The method detection limit (MDL) was determined using a signal-to-noise (S/N) ratio and the US EPA-recommended method (Berthouex and Brown, 2002). The resulting MDLs (statistical method) for TCC and TCS in soil and biosolids samples, along with the percent recoveries, are summarized in

Table 3 – Percent recoveries (±S.D.) ($n = 2$) and method detection limits (MDL) ($n = 7$) for antimicrobials spiked into soil and biosolids samples.

	Soil		Biosolid	
	Recovery X ± S.D. (%)	MDL (ng/g)	Recovery X ± S.D. (%)	MDL (ng/g)
Triclocarban	96.0 ± 1.2	0.58	99.3 ± 4.4	3.08
Triclosan	97.4 ± 3.7	0.05	98.4 ± 4.3	0.11
Triclocarban-$^{13}C_6$ (IS)	98.2 ± 2.9		95.7 ± 5.1	

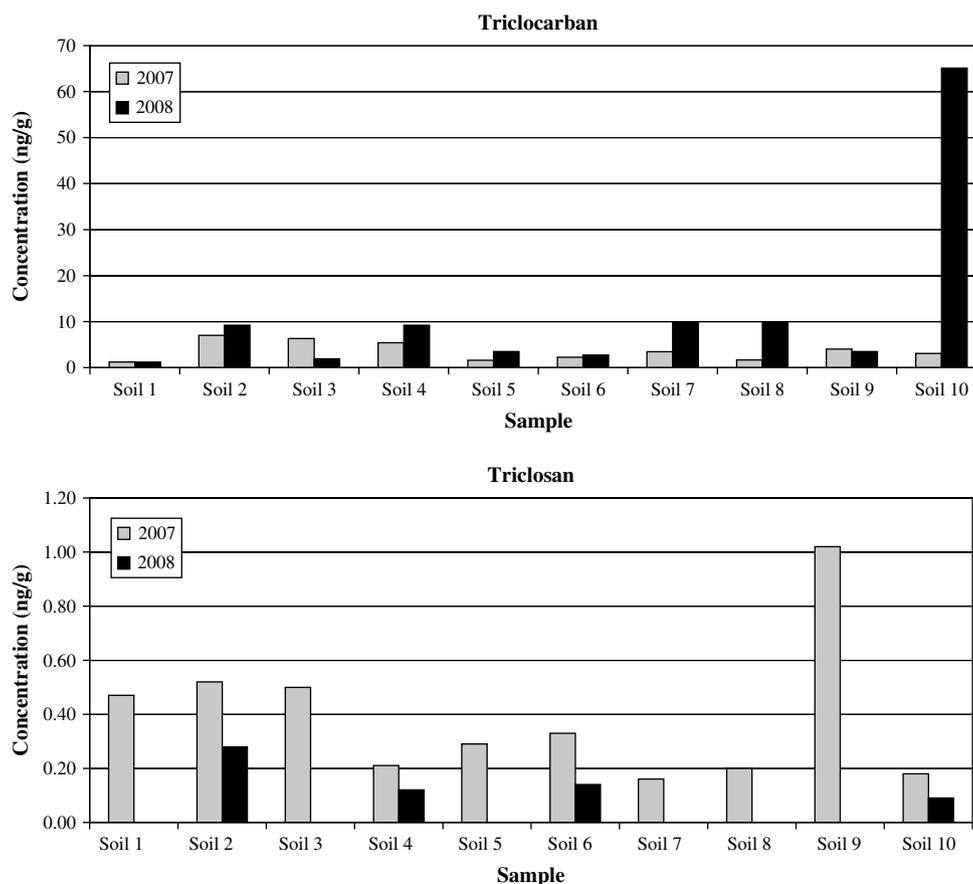| Table 4 – Concentration (ng/g d.w.) of triclocarban and triclosan in biosolids samples from wastewater treatment plants (WWTPs).			
Location	Sample	Triclocarban (ng/g d.w.)	Triclosan (ng/g d.w.)
WWTP 1	Biosolid 1	9280	7060
WWTP 1	Biosolid 2	7550	200
WWTP 2	Biosolid 3	7020	90
WWTP 3	Biosolid 4	4890	110

Table 3. The MDL based on a S/N greater than 10 (limit of quantitation or LOQ) was 0.05 ng/g in soil and biosolids for TCC and TCS compounds. Chu and Metcalfe (2007) obtained a LOQ of 0.5 ng/g and 5.0 ng/g (S/N > 10) for TCC and TCS, respectively, in biosolids employing LC–MS–MS, and Miller et al. (2008) reported a LOQ of 0.5 ng/g and 50 ng/g for TCC and TCS, respectively, in sediment employing LC–MS. The calculation of MDL using the US EPA method was based on the variability of multiple analyses of seven soil and biosolids extracts spiked at a concentration of 5 ng/g and 20 ng/g for TCC and TCS, respectively. The MDL was specifically determined by multiplying the sample standard deviation calculated from each group of extracts spiked by the Student's t-variate for a one-sided t-test at the 99% confidence level with $n - 1$ degrees of freedom. The MDL for TCC and TCS compounds ranged from 0.05 to 0.58 ng/g in soil and 0.11 to 3.08 ng/g in biosolids.

3.2. Triclocarban and triclosan in biosolids and soils

TCC and TCS concentrations were determined in both biosolid samples and in biosolids-impacted soil samples. In general, TCC was found at higher biosolids concentrations, ranging from 4890 to 9280 ng/g, compared to TCS, which ranged from 90 to 7060 ng/g (Table 4). The concentrations determined here agree well with data obtained by others (Table 2), confirming the importance of TCC and TCS accumulation in biosolids produced by WWTPs. Our data contribute to the growing body of literature illustrating the common occurrence of organic waste contaminants (OWC) in the solid materials produced during wastewater treatment (Chu and Metcalfe, 2007; Heidler et al., 2006; Kinney et al., 2006; Sapkota et al., 2007; Ying and Kookana, 2007). This issue deserves continued attention since the magnitude of biosolids application nationwide is considerable.

Soil samples collected from both years (2007 and 2008) from ten different agricultural sites contained detectable levels of TCC and TCS (Fig. 3). In both 2007 and 2008, TCC was present at higher concentrations compared to TCS. TCC concentrations ranged from 1.24 to 7.01 ng/g in 2007 and from 1.20 to 65.10 ng/g in 2008. TCS was detected in soil samples at levels from 0.16 to 1.02 ng/g in 2007 and from the method detection limit (<0.05 ng/g) to 0.28 ng/g in 2008. TCC concentrations were similar for both years except for soil ten which increased from 3.10 to 65.10 ng/g from 2007 to 2008 (the high

Fig. 3 – Concentration of triclocarban and triclosan in soil samples, over two years, from ten agricultural sites previously amended with biosolids.

Table 5 – Soil properties and time since last application of biosolids.

	Crop	Organic matter %	Sand %	Silt %	Clay %	Textural Classification	Soil pH	Calcium (Ca) mg/kg	Magnesium (Mg) mg/kg	Potassium (K) mg/kg	Phosphorus (P) mg/kg	CEC meq/100 g	Time of last application
Soil 1	Soybean	1.7	72	22	6	Sandy Loam	6.6	881	93	141	143	4.8	April 04
Soil 2	Light red kidney bean	1.6	76	20	4	Loamy Sand	5.9	1005	91	177	295	6.4	May 07
Soil 3	Corn	1.2	70	24	6	Sandy Loam	4.9	489	68	151	46	5.0	June 04
Soil 4	Alfalfa	1.6	66	26	8	Sandy Loam	6.1	1328	192	174	57	8.3	August 04
Soil 5	Corn	1.0	76	22	2	Loamy Sand	5.5	787	42	108	79	5.0	May 07
Soil 6	Corn	1.2	72	24	4	Sandy Loam	5.7	755	119	136	156	5.3	June 04
Soil 7	Corn	1.5	50	44	6	Sandy Loam	5.9	1293	159	207	154	8.6	April 07
Soil 8	Alfalfa	1.5	80	20	0	Loamy Sand	6.4	811	74	173	106	4.6	May 03
Soil 9	Corn	1.2	74	26	0	Loamy Sand	5.7	739	102	148	186	5.1	April 07
Soil 10	Soybean	2.1	56	32	12	Sandy Loam	7.7	3746	492	125	51	18.9	August 04

CEC: Calculated Cation Exchange Capacity.

TCC concentration was confirmed analytically on five separate soil samples). The large TCC increase in this sample possibly reflects an unmonitored biosolid application in 2007, or perhaps the inclusion of subsamples containing primarily biosolids residue. In contrast, TCS concentration decreased in all soil samples from 2007 to 2008. TCC or TCS concentrations could not be correlated to any soil characteristic, or to the time of last biosolids application, which occurred in either 2003, 2004 or 2007 (Table 5).

The measured soil antimicrobial concentrations were compared to estimated concentrations based on the biosolids application rate (0.73 dry Mg per 1000 m^2 or 3.25 dry tons per acre) and the measured TCC and TCS biosolids concentrations (Table 6). The estimated concentrations were calculated using the measured higher and lower concentrations of TCC and TCS in the biosolids (Table 4), an assumed soil bulk density (1.3 g/cm^3) and a 5 inch depth of incorporation. Based on biosolids concentration for TCC (9280 and 4890 ng/g) and TCS (7060 and 90 ng/g), the estimated soil concentrations were 21.6 and 41.0 ng/g for TCC, and 0.4 and 31.2 ng/g for TCS. The average measured concentrations for TCC (3.6 ± 2.1 ng/g and 11.6 ± 19.2 ng/g for 2007 and 2008, respectively) and TCS (0.39 ± 0.26 ng/g and 0.16 ± 0.08 ng/g for 2007 and 2008, respectively) were lower than these estimated concentrations, indicating some removal likely occurred.

The literature contains one other report of TCS in agricultural soil following land application of biosolids (Kinney et al., 2008). These researchers found TCS concentrations of 160 and 96 ng/g in samples collected 31 and 156 days following biosolids application, respectively. These values are significantly higher than those obtained in the current investigation (0.39 ± 0.26 ng/g and 0.16 ± 0.08 ng/g for 2007 and 2008, respectively). This result may be a reflection of differences in TCS biosolids concentrations, and/or in the rate or method of biosolid application between the two studies. In our investigation, both the TCS biosolids concentrations (7060 and 200 ng/g compared to 10,500 ng/g) and the biosolids application rate (0.73 Mg/ 1000 m^2 compared to 1.8 Mg/1000 m^2) were lower than those reported above (Kinney et al., 2008). However, these data seem insufficient to account for the differences in reported TCS soil concentrations between the two studies. These results indicate further research is needed to definitively establish the factors controlling residual TCS concentrations in soils following biosolids land application.

To our knowledge, our data represent the first report of TCC concentrations in agricultural soils and the first comparison of TCC and TCS soil concentrations following land application of biosolids. We found higher soil concentrations of TCC compared to TCS. A similar trend, of higher TCC concentrations compared to TCS, was observed recently in estuarine sediments (Miller et al., 2008). In addition, TCC was noted to persist to a greater extent than TCS (76 ± 30 vs 50 ± 19%, respectively) during wastewater treatment (Heidler and Halden, 2007; Heidler et al., 2006). Others have reported faster aerobic TCS degradation compared to TCC, with soil half-lives of 18 and 108 days, respectively (Ying et al., 2007), which likely contributes to the above trends. In addition, TCC likely sorbs stronger to soil, compared to TCS (log K_{ow} values of 6.0 and 4.5 for TCC and TCS, respectively) (Chu and

Table 6 – Estimated (see text for all assumptions) and measured triclocarban (TCC) and triclosan (TCS) soil concentrations (ng/g d.w.).

High TCC estimate[a]	Low TCC estimate[b]	Measured TCC	High TCS estimate[c]	Low TCS estimate[d]	Measured TCS
41.00	21.60	3.6 ± 2.1 (2007); 11.6 ± 19.2 (2008)	31.19	0.40	0.39 ± 0.26 (2007); 0.16 ± 0.08 (2008)

a Based on biosolids concentration of 9280 ng TCC/g d.w.
b Based on biosolids concentration of 4890 ng TCC/g d.w.
c Based on biosolids concentration of 7060 ng TCC/g d.w.
d Based on biosolids concentration of 90 ng TCS/g d.w.

Metcalfe, 2007; Kinney et al., 2006; Sapkota et al., 2007) and thus may be less available for biodegradation. Further, phototransformation of TCS in the environment has previously been reported (Tixier et al., 2002). Whatever the mechanism, it appears that, compared to TCS, TCC may be a more persistent compound in soil systems.

4. Conclusions

In this study, a method was developed and used to quantify TCC and TCS concentrations in soils and biosolids. Specifically, a rapid analytical method for the determination of TCC and TCS using pressurized liquid extraction (PLE) and liquid chromatography–tandem mass spectrometry (LC–MS–MS) with negative ion multimode ionization is described. The method was used to determine antimicrobial concentrations in biosolids produced from three WWTPs in Michigan and in ten agricultural soils, over two years, following biosolid land application. The fate of TCC and TCS in the environment is important because of concerns over bioaccumulation and movement to surface waters coupled with the widespread use of biosolids land application (approximately 50% of US generated biosolids are land applied). This study represents the first examination of TCC concentrations, and the first comparison of TCC and TCS concentrations, in agricultural soils following application of biosolids. We found higher soil concentrations of TCC compared to TCS, indicating that TCC may be a more persistent compound in soil systems. Such information is important because, to date, the literature has focused primarily on the effects (toxicity, bioaccumulation) of TCS in the environment. Our data, and the studies discussed above (Heidler and Halden, 2007; Heidler et al., 2006; Miller et al., 2008; Ying et al., 2007), now indicate that attention should also be directed towards the downstream effects of TCC persistence.

Acknowledgements

R E F E R E N C E S

Aguera, A., Fernandez-Alba, A.R., Piedra, L., Mezcua, M., Gomez, M.J., 2003. Evaluation of triclosan and biphenylol in marine sediments and urban wastewaters by pressurized liquid extraction and solid phase extraction followed by gas chromatography mass spectrometry and liquid chromatography mass spectrometry. Analytica Chimica Acta 480, 193–205.

Ahn, K.C., Zhao, B., Chen, J., Cherednichenko, G., Sanmarti, E., Denison, M.S., Lasley, B., Pessah, I.N., Kultz, D., Chang, D.P.Y., Gee, S.J., Hammock, B.D., 2008. In vitro biologic activities of the antimicrobials triclocarban, its analogs, and triclosan in bioassay screens: receptor-based bioassay screens. Environmental Health Perspectives 116, 1203–1210.

Aranami, K., Readman, J.W., 2007. Photolytic degradation of triclosan in freshwater and seawater. Chemosphere 66, 1052–1056.

Berthouex, P.M., Brown, L.C., 2002. Statistics for Environmental Engineers, second ed. Lewis Publisher.

Bester, K., 2003. Triclosan in a sewage treatment process – balances and monitoring data. Water Research 37, 3891–3896.

Chen, J.G., Ahn, K.C., Gee, N.A., Ahmed, M.I., Duleba, A.J., Zhao, L., Gee, S.J., Hammock, B.D., Lasley, B.L., 2008. Triclocarban enhances testosterone action: a new type of endocrine disruptor? Endocrinology 149, 1173–1179.

Chenxi, W., Spongberg, A.L., Witter, J.D., 2008. Determination of the persistence of pharmaceuticals in biosolids using liquid-chromatography tandem mass spectrometry. Chemosphere 73, 511–518.

Chhabra, R.S., Huff, J.E., Haseman, J.K., Elwell, M.R., Peters, A.C., 1991. Carcinogenicity of p-chloroaniline in rats and mice. Food and Chemical Toxicology 29, 119–124.

Chu, S., Metcalfe, C.D., 2007. Simultaneous determination of triclocarban and triclosan in municipal biosolids by liquid chromatography tandem mass spectrometry. Journal of Chromatography A 1164, 212–218.

Coogan, M.A., La Point, T.W., 2008. Snail bioaccumulation of triclocarban, triclosan, and methyltriclosan in a North Texas, USA, stream affected by wastewater treatment plant runoff. Environmental Toxicology and Chemistry 27, 1788–1793.

Coogan, M.A., Edziyie, R.E., La Point, T.W., Venables, B.J., 2007. Algal bioaccumulation of triclocarban, triclosan, and methyl-triclosan in a North Texas wastewater, treatment plant receiving stream. Chemosphere 67, 1911–1918.

Gledhill, W.E., 1975. Biodegradation of 3,4,4′-trichlorocarbanilide, TCC, in sewage and activated-sludge. Water Research 9, 649–654.

Halden, R.U., Paull, D.H., 2004. Analysis of triclocarban in aquatic samples by liquid chromatography electrospray ionization mass spectrometry. Environmental Science & Technology 38, 4849–4855.

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

Heath, R.J., Li, J., Roland, G.E., Rock, C.O., 2000. Inhibition of the Staphylococcus aureus NADPH-dependent enoyl-acyl carrier protein reductase by triclosan and hexachlorophene. Journal of Biological Chemistry 275, 4654–4659.

Heath, R.J., Yu, Y.T., Shapiro, M.A., Olson, E., Rock, C.O., 1998. Broad spectrum antimicrobial biocides target the FabI component of fatty acid synthesis. Journal of Biological Chemistry 273, 30316–30320.

Heath, R.J., Rubin, J.R., Holland, D.R., Zhang, E.L., Snow, M.E., Rock, C.O., 1999. Mechanism of triclosan inhibition of bacterial fatty acid synthesis. Journal of Biological Chemistry 274, 11110–11114.

Heidler, J., Halden, R.U., 2007. Mass balance assessment of triclosan removal during conventional sewage treatment. Chemosphere 66, 362–369.

Heidler, J., Sapkota, A., Halden, R.U., 2006. Partitioning, persistence, and accumulation in digested sludge of the topical antiseptic triclocarban during wastewater treatment. Environmental Science & Technology 40, 3634–3639.

Hoang, T.T., Schweizer, H.P., 1999. Characterization of Pseudomonas aeruginosa enoyl-acyl carrier protein reductase (FabI): a target for the antimicrobial triclosan and its role in acylated homoserine lactone synthesis. Journal of Bacteriology 181, 5489–5497.

Kinney, C.A., Furlong, E.T., Zaugg, S.D., Burkhardt, M.R., Werner, S.L., Cahill, J.D., Jorgensen, G.R., 2006. Survey of organic wastewater contaminants in biosolids destined for land application. Environmental Science & Technology 40, 7207–7215.

Kinney, C.A., Furlong, E.T., Kolpin, D.W., Burkhardt, M.R., Zaugg, S.D., Werner, S.L., Bossio, J.P., Benotti, M.J., 2008. Bioaccumulation of pharmaceuticals and other anthropogenic waste indicators in earthworms from agricultural soil amended with biosolid or swine manure. Environmental Science & Technology 42, 1863–1870.

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, 1202–1211.

Lapen, D.R., Topp, E., Metcalfe, C.D., Li, H., Edwards, M., Gottschall, N., Bolton, P., Curnoe, W., Payne, M., Beck, A., 2008. Pharmaceutical and personal care products in tile drainage following land application of municipal biosolids. Science of the Total Environment 399, 50–65.

Lindstrom, A., Buerge, I.J., Poiger, T., Bergqvist, P.A., Muller, 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, 2322–2329.

McAvoy, D.C., Schatowitz, B., Jacob, M., Hauk, A., Eckhoff, W.S., 2002. Measurement of triclosan in wastewater treatment systems. Environmental Toxicology and Chemistry 21, 1323–1329.

McMurry, L.M., Oethinger, M., Levy, S.B., 1998a. Triclosan targets lipid synthesis. Nature 394, 531–532.

McMurry, L.M., Oethinger, M., Levy, S.B., 1998b. Overexpression of marA, soxS, or acrAB produces resistance to triclosan in laboratory and clinical strains of Escherichia coli. FEMS Microbiology Letters 166, 305–309.

Miller, T.R., Heidler, J., Chillrud, S.N., Delaquil, A., Ritchie, J.C., Mihalic, J.N., Bopp, R., Halden, R.U., 2008. Fate of triclosan and evidence for reductive dechlorination of triclocarban in estuarine sediments. Environmental Science & Technology 42, 4570–4576.

Morales, S., Canosa, P., Rodriguez, I., Rubi, E., Cela, R., 2005. Microwave assisted extraction followed by gas chromatography with tandem mass spectrometry for the determination of triclosan and two related chlorophenols in sludge and sediments. Journal of Chromatography A 1082, 128–135.

Perencevich, E.N., Wong, M.T., Harris, A.D., 2001. National and regional assessment of the antibacterial soap market: a step toward determining the impact of prevalent antibacterial soaps. American Journal of Infection Control 29, 281–283.

Sapkota, A., Heidler, J., Halden, R.U., 2007. Detection of triclocarban and two co-contaminating chlorocarbanilides in US aquatic environments using isotope dilution liquid chromatography tandem mass spectrometry. Environmental Research 103, 21–29.

Singer, H., Muller, S., Tixier, C., Pillonel, L., 2002. Triclosan: occurrence and fate of a widely used biocide in the aquatic environment: field measurements in wastewater treatment plants, surface waters, and lake sediments. Environmental Science & Technology 36, 4998–5004.

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

Topp, E., Monteiro, S.C., Beck, A., Coelho, B.B., Boxall, A.B.A., Duenk, P.W., Kleywegt, S., Lapen, D.R., Payne, M., Sabourin, L., Li, H.X., Metcalfe, C.D., 2008. Runoff of pharmaceuticals and personal care products following application of biosolids to an agricultural field. Science of the Total Environment 396, 52–59.

Trenholm, R.A., Vanderford, B.J., Drewes, J.E., Snyder, S.A., 2008. Determination of household chemicals using gas chromatography and liquid chromatography with tandem mass spectrometry. Journal of Chromatography A 1190, 253–262.

TSCA, 2003. Chemical Substances Inventory. U.S. Environmental Protection Agency, Washington, D.C.

USEPA, 2007. http://www.epa.gov/owm/mtb/biosolids/genqa.htm (accessed November 2008).

Yang, L.H., Ying, G.G., Su, H.C., Stauber, J.L., Adams, M.S., Binet, M.T., 2008. Growth-inhibiting effects of 12 antibacterial agents and their mixtures on the freshwater microalga Pseudokirchneriella subcapitata. Environmental Toxicology and Chemistry 27, 1201–1208.

Ying, G.G., Yu, X.Y., Kookana, R.S., 2007. Biological degradation of triclocarban and triclosan in a soil under aerobic and anaerobic conditions and comparison with environmental fate modelling. Environmental Pollution 150, 300–305.

Ying, G.G., Kookana, R.S., 2007. Triclosan in wastewaters and biosolids from Australian wastewater treatment plants. Environment International 33, 199–205.

Young, T.A., Heidler, J., Matos-Perez, C.R., Sapkota, A., Toler, T., Gibson, K.E., Schwab, K.J., Halden, R.U., 2008. Ab initio and in situ comparison of caffeine, triclosan, and triclocarban as indicators of sewage-derived microbes in surface waters. Environmental Science & Technology 42, 3335–3340.