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Triclosan, chlorinated triclosan derivatives, and hydroxylated polybrominated diphenyl ethers (OH-BDEs) in wastewater effluents†

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Various halohydroxydiphenyl ethers, including triclosan, chlorinated triclosan derivatives (CTDs), and hydroxylated polybrominated diphenyl ethers (OH-BDEs), are present in aquatic systems. While it is well established that wastewater effluents are a source of triclosan and CTDs, the evidence for OH-BDEs being in wastewater is limited. In this work, pre- and post-disinfection effluent samples were taken from four activated sludge plants, two using chlorine and two using ultraviolet (UV) disinfection. Triclosan levels ranged from 36–465 ng L^{-1} and CTD levels were non-detect to 27 ng L^{-1}. While CTDs were generally higher in the plants using chlorine, they were also present in the UV plants, likely due to chlorine residual in the drinking water. Of the five target OH-BDE congeners (selected because they produce dioxins upon photolysis), three were detected. When detected the levels were generally 1–10 ng L^{-1}, but some samples had levels as high as 100 ng L^{-1}. Three different analytical methods were used to quantify OH-BDEs, and the levels were comparable using the different methods. Results were inconclusive as to the effect of disinfection method on OH-BDE levels. This study confirms that wastewater is a source of selected OH-BDEs to surface waters, but the overall loading is likely small. Further experiments and analyses are required to determine if the OH-BDEs are formed during the wastewater treatment process.

Water impact

Treated wastewater is a source of emerging contaminants to aquatic systems, and these compounds may have adverse environmental impacts. This work reveals that halohydroxydiphenyl ethers, such as triclosan and selected hydroxylated polybrominated diphenyl ethers (OH-BDEs) are present in wastewater effluents. The OH-BDE levels approach those of chlorinated triclosan derivatives. Wastewater effluent serves a source of OH-BDEs, and thus potentially brominated dioxins, to aquatic systems.

Introduction

Halohydroxydiphenyl ethers are a class of emerging contaminants that contains both the antimicrobial agent triclosan and hydroxylated polybrominated diphenyl ethers (OH-BDEs). Triclosan (2,4,4′-trichloro-2′-hydroxydiphenylether) contained in personal care products, such as antibacterial liquid handsoap and toothpaste, is flushed into sewer systems and enters wastewater treatment plants (WWTPs).[1] While much of the incoming triclosan is removed *via* biodegradation and sorption to biosolids during the treatment process,[2–10] triclosan concentrations have been detected in the effluent of WWTPs around the world ranging from 0.04–18.6 nM (0.011–5.4 μg L^{-1}).[6,9,11–15] From measurements of wastewater effluent, it has been estimated that approximately 11 metric tons of triclosan per year flows into the surface waters of the US.[6,11,12]

Three chlorinated triclosan derivatives (CTDs) are known to form from the chlorination of triclosan: 4,5-chloro-2-(2,4-dichlorophenoxy)phenol (4-Cl-TCS), 5,6-chloro-2-(2,4-dichlorophenoxy)phenol (6-Cl-TCS), and 4,5,6-chloro-2-(2,4-dichlorophenoxy)phenol (4,6-Cl-TCS).[6,11,16] Chlorination of wastewater can increase concentrations of total CTDs in WWTP effluent up to 30% of the concentration of triclosan.[11]

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WWTPs with UV disinfection do not see this effect, although CTDs may still be present at low concentrations from reactions with bleach or residual chlorine in tap water during transport to the WWTPs.[6,11,16] A recent report demonstrated that both CTDs and brominated triclosan derivatives are present in biosolids samples from WWTPs.[17] Additionally, CTDs have been detected in several sediment cores from wastewater impacted lakes, indicated that CTDs are present in wastewater impacted surface waters.[18]

Since the 1970's brominated flame retardants have been used in polyurethane foams, textiles, carpets, and electronics to prevent fires and the spread of fire.[19,20] Due to the extent that polybrominated diphenyl ethers (PBDEs) are found in environmental matrices, transformation products of PBDEs have also become a concern. OH-BDEs derived from the transformation of PBDEs are suggested to arise from metabolism of PBDEs by animals, oxidation of PBDEs by hydroxyl radicals in the atmosphere, and biological processing during wastewater treatment.[21–29] OH-BDEs are of environmental concern because they have been shown to be endocrine disruptors and neurotoxins with potency equivalent to or greater than PBDEs.[30,31] Toxic effects that have been reported include uncoupling of oxidative phosphorylation,[32] indirect estrogenic effects in rats,[33] and effects on hormone transport.[34] In addition, OH-BDEs are produced by marine organisms.[28,35–38] Whether natural or anthropogenic sources are more important contributors to environmental levels of OH-BDEs continues to be an open question.[28,29,39]

Reports of OH-BDEs in wastewater systems are sparse. While looking for triclosan in wastewater from a WWTP on the Detroit River, one study reported other peaks near the internal standard, 2′-OH-BDE-28, with the same mass fragmentation pattern, but the compounds were not identified.[40] 6-OH-BDE-47 and 5-OH-BDE-47 were recently detected at ~1 pg L^{-1} in wastewater effluent.[41] Six OH-BDE congeners were found in sewage sludge samples, with 6-OH-BDE-47 and 2′-OH-BDE-68 comprising the majority of the OH-BDE mass,[42] and 6-OH-BDE-47 has been found in water impacted by sewage from a seafood processing facility.[43]

Photodegradation of triclosan and CTDs has been shown to produce specific dioxin congeners with a yield of 0.5–2.5% with a potential upper limit of 3%.[44–46] Friedman *et al.*[47] measured an efflux of 2,7/8-DCDD from Newark Bay to the surrounding atmosphere, which was attributed to the photolysis of triclosan, and studies of lake sediment cores have shown that the levels of triclosan, CTDs, and their photoproduced dioxins correlate temporally.[18,48] OH-BDEs also undergo photolysis to form PBDDs.[49–51] Because the toxicity of PBDDs is equal or greater to their chlorinated analogues,[52,53] potential sources of PBDDs to the environment need to be understood.

The focus of this research was to measure triclosan, CTDs, and OH-BDEs in wastewater effluents and to compare the levels in systems using different modes of disinfection. The OH-BDEs chosen for study are among those capable of forming dioxins upon photolysis (*i.e.*, with OH and Br substituents *ortho* to the ether linkage on opposing rings). Because data from different sampling campaigns were combined, this also gave the opportunity to compare different analytical methods.

Materials and methods

Chemicals

Triclosan (>97%) was purchased from Sigma Aldrich. Isotopically labeled triclosan ($^{13}C_{12}$-triclosan, >99%) was purchased from Wellington Laboratories as a solution in methanol. Three CTDs (4-Cl-TCS, 6-Cl-TCS, and 4,6-Cl-TCS) and 6-hydroxy-2,2′,4,4′-tetrabromodiphenyl ether (6-OH-BDE-47) were synthesized for previous studies.[44,49] The 6-OH-BDE-47, 6-OH-BDE-99, 6′-OH-BDE-100 and 6′-OH-BDE-118 were from stocks synthesized as previously described.[51] 6-OH-BDE-90 was synthesized as described in the ESI.†

Stock solutions of each compound were prepared gravimetrically in methanol. Sulfuric acid (ACS grade, BDH) silica gel (60 Å, BDH), ammonium acetate (Mallinckrodt AR), methanol (HPLC grade, >99%, Sigma-Aldrich), methyl *t*-butyl ether (MTBE, >99.0% Sigma-Aldrich), and ethyl acetate (>99.5%, Macron Chemicals) were purchased from commercial suppliers. Ultrapure water (18.2 MΩ cm) was obtained from a Millipore Simplicity UV purification system. A Thermo-Orion Ross Ultra Semi-Micro pH meter was used to make pH measurements.

Collection and preparation of samples

Time-composited samples (24 hour) from three WWTPs, Metropolitan Wastewater Treatment Plant (MWP; 251 MGD; chlorine disinfection), Palo Alto Regional Water Quality Control Plant (PAWP; 21 MGD; UV disinfection) and Saint John's University Wastewater Treatment Plant (SJWP; 0.23 MGD; UV disinfection) were collected. Additionally, grab samples from a fourth WWTP (Western Lake Superior Sanitation District, WLSSD; 40 MGD; chlorination when fecal coliforms exceed 100 MPN/100 mL) were collected. Further details about the WWTPs and their disinfection practices are in the ESI.† At MWP, PAWP, and SJWP, pre- and post-disinfection effluent, offset to represent the same wastewater stream, were collected in solvent rinsed glass containers. At WLSSD, samples were collected using a small watercraft at the discharge point in the St. Louis River. Samples were transported on ice. Samples were filtered within a day of arrival through pre-combusted glass fiber filters (47 mm; Fisher Scientific). The pH of each sample was recorded and then adjusted to 3–4 with sulfuric acid. At pH < 4, all of the analytes will be >98% in their hydrophobic, neutral forms allowing high recovery from solid-phase extraction.[44,54] Samples were then stored in the dark at 4 °C until further processing, which was usually carried out within 72 hours.

Solid phase extraction

A previously developed method[11] was slightly modified for analysis of the compounds of interest. Three or four 500 mL replicates were prepared in Erlenmeyer flasks by spiking 0.5 nM $^{13}C_{12}$-triclosan as a surrogate for the compounds of interest. Another 500 mL sample of wastewater or deionized water was prepared in the same manner, but was also spiked with 1.5 nM triclosan and 0.3 nM of the other analytes (CTDs and OH-BDEs) to verify that the other compounds partition as triclosan does throughout the extraction method. All the flasks were then shaken and stored overnight in the dark to allow for equilibration of the analytes.

Solid phase extraction (SPE) cartridges (Oasis HLB) were loaded onto a vacuum manifold and preconditioned with consecutive 5 mL aliquots of MTBE, methanol, and pH 3 ultrapure water. Wastewater replicates were loaded onto the SPE cartridges at a flow rate of 15 mL min^{-1}. Samples spiked with all analytes were processed after the replicates to minimize cross contamination. After loading the samples, cartridges were flushed with 3 consecutive aliquots of 50 : 50 methanol : H_2O (v/v) under slight vacuum (~5 mL min^{-1}) and dried under vacuum for at least 15 min. Cartridges were eluted with 10 mL of methanol and 5 mL of 90 : 10 MTBE : methanol (v/v). Eluents were then blown down with a gentle stream of nitrogen to ~500 μL.

Silica column clean-up

The eluent from the SPE step was loaded onto a silica column (comprised of glass wool, a thin layer of sand, 2 g silica gel, and a second thin layer of sand in a 6 mL plastic Luer tip syringe), as were three 1 mL aliquots of ethyl acetate used to rinse the centrifuge tube containing the SPE eluent.[11] After the rinses were loaded, the column was eluted with ~11 mL of ethyl acetate. This collected ethyl acetate was blown down with nitrogen to ~300 μL. The final extracts were transferred to amber glass vials with 350 μL conical inserts. The extract

Table 1 Comparison of the three analytical methods used. The APCI method and ESI method 2 were used to quantify pentabrominated OH-BDEs

	ESI method 1	APCI method	ESI method 2
Chromatography			
HPLC	Agilent 1100 series	Agilent 1100 series	Waters nanoAcquity
Column type	Phenomenex synergi RP-Max	Phenomenex synergi polar-RP	Thermo hypersil gold
Size (mm × mm)	150 × 0.5	150 × 2	100 × 0.32
Particle sizes (μm)	4	4	1.9
Pore size (Å)	80	80	100
Injection volume (μL)	8	20	8
Mobile phase A	10 mM NH_4OAc buffer	2 mM NH_4OAc buffer (10% MeOH)	5 mM NH_4OAc (40% MeOH)
Mobile phase B	CH_3CN	MeOH	CH_3CN
Flow rate (μL min^{-1})	10	200	10
Gradient	50% A for 10 min	55% B for 3 min	25% B initial; 40% B by 5 min
	100% B by 20 min	86% B by 15 min	55% B by 25 min
	50% A by 23 min	86% B from 15–27 min	80% B from 27–30 min
	50% A until 35 min	55% B for 29–36 min	25% B from 32–45 min
Divergance to waste	First and last 10 min	First 10 min	First 5 min
Mass Spectrometer			
Triple quadrupole MS	Thermo scientific TSQ vantage	Thermo electron quantum discovery max	Thermo scientific TSQ ultra AM
Source	Negative mode ESI	Negative mode APCI	Negative mode ESI
6-OH-BDE-47 precursor and product ions			
First SRM (quantification)	500.7 → 79	500.6 → 79[a]	500.6 → 79
Second SRM (confirmation)	498.7 → 79	502.6 → 81[a]	502.6 → 81
OH-pentaBDE precursor and product ions			
First SRM (q)	—	578.6 → 79[b]	578.6 → 79
Second SRM (confirmation)	—	580.6 → 81[b]	580.6 → 81
$^{13}C_{12}$-triclosan precursor and product ions			
SRM	299 → 35.1	299 → 35	299 → 35.2
Tuning Parameters			
Tuning compound	$^{13}C_{12}$-triclosan	2′-OH-BDE-118	$^{13}C_{12}$-triclosan
Spray voltage (*V*)	2800	—	3200
Sheath gas pressure (psi)	45	20	35
Capillary temperature (°C)	250	250	300
Collision energy	10	10	12
Skimmer offset (*V*)	8	10	5
Collision gas pressure (mTorr)	0.8	2	0.9
Q1	0.1	0.05	0.7
Q3	0.1	0.05	0.7
Discharge current (kV)	—	25	—
Vaporizer temperature (°C)	—	250	—
Scan time (s)	0.13	—	0.15

[a] Includes 6′-OH-BDE-100 and 6′-OH-BDE-118. [b] Does not include 6′-OH-BDE-100 and 6′-OH-BDE-118.

was allowed to dry overnight in the vial and resuspended in 40–50 μL of 50 : 50 acetonitrile : H_2O (v/v). Spiked samples were diluted 5–10 times to lessen suppression effects of $^{13}C_{12}$-triclosan.

Mass spectrometry methods

Extracts were analyzed by HPLC-tandem mass spectrometry (LC-MS/MS). A previously published method for triclosan and CTDs using electrospray ionization (ESI), was initially used for analysis of processed samples,[11] but of the OH-BDEs, only 6-OH-BDE-47 could be detected *via* this method. Thus, two additional methods, one using atmospheric pressure chemical ionization (APCI; based on ref. 55) and one using ESI[56] were developed. A comparison of the chromatography and mass spectrometer settings is given in Table 1. SRM transitions for triclosan, the CTDs, and OH-BDEs are in the ESI.† Calibration curves using more than five points were constructed by plotting the analyte peak area to internal standard peak area ratio (*y*-axis) *versus* the analyte concentration (*x*-axis). Triclosan concentrations in standards ranged from 0.001–4.3 mg L^{-1}, while the concentrations of CTDs and 6-OH-BDE-47 ranged from 0.0003–1.5 mg L^{-1}. In most cases, two calibrations curves were plotted for each analyte, one for low ranges and one for high ranges. The concentrations of the spiked samples determined the endpoints of the high range calibration curve, while the concentration of the unspiked and blank samples determined the endpoints of the low range calibration curve. At higher concentrations, the $^{13}C_{12}$-triclosan signal became suppressed by triclosan (*i.e.*, ion suppression), thus changing the slope of the calibration curve.

Results and discussion

Chromatography and limits of detection and quantification

Each LC method effectively separated the analytes and provided satisfactory peak shapes without processing through peak fitting. Example chromatograms for ESI method 1 are in the ESI.† The separation for the OH-BDEs *via* the APCI method and ESI method 2 are shown in Fig. 1. Note that in the APCI method, the 6′-OH-BDE-100 and 6′-OH-BDE-118 were detected with the 500.6 → 79 transition rather than the expected 578.6 → 79 transition.

For ESI method 1 analyses with only one method blank (early stage of the sampling campaign), the limit of quantitation (LOQ) for each analyte was defined as 10 times the analyte concentration determined in a single method blank. Later multiple method blanks were used, and the LOQ was the concentration determined in the method blanks plus 10 times the standard deviation of the method blanks. The limit of detection (LOD) was calculated as 3 times the method blank or the average method blank plus 3 times the standard deviation of the method blanks. Using multiple method blanks allowed for lower LODs and LOQs as the standard deviation of the analyte concentrations in the method blanks were much lower than the analyte concentrations in the method blanks. The reported limits with only one method blank (Table S2†) are, therefore, conservative.

For the APCI method and ESI method 2, the LOQ and LOD were obtained by different means, due to insufficient sample volume for the additional analyses. The analytes were quantified if: (1) the analyte was above 80% of the lowest calibration point (the LOQ), and (2) the analyte was above a 10 signal-to-noise ratio within the water matrix. The LODs are 0.3 × LOQs, and the analyte must have been above a 3 signal-to noise ratio within the water matrix.

Most calibration curves were of high quality, with $R^2 > 0.93$. A detailed summary of LOQ and LOD information for each method is located in the ESI.† Because these were based on the method blank (*i.e.*, MilliQ water put through the extraction process), these values varied depending on the date the analyses were run. Briefly, for ESI method 1 the LOQs ranged from 2.3–29 ng L^{-1} for triclosan, 0.003–2.8 ng L^{-1} for the CTDs, and 0.22–3 ng L^{-1} for 6-OH-BDE-47. The OH-BDE LOQs for the APCI method ranged from 0.10–3.23 ng L^{-1} for 6-OH-BDE-47, 6-OH-BDE-90, and 6-OH-BDE-99. In this method, no limits are reported for 6′-OH-BDE-100 and 6′-OH-BDE-118 because they were not detected in any samples, but the lowest calibration point was 2.9 ng L^{-1}. For ESI method 2, the LOQs ranged from 0.46–1.09 ng L^{-1} for 6-OH-BDE-47, 6-OH-BDE-90, 6-OH-BDE-99, and 6′-OH-BDE-118. No limits were reported for 6′-OH-BDE-100 for this method because it was not detected in any of the water samples or method blanks, but the lowest calibration point was 1.74 ng L^{-1}. The chromatographic peak area for every reported concentration was greater than 10 times the peak area of the corresponding instrument and method blanks.

Recoveries

The absolute recovery of $^{13}C_{12}$-triclosan was 59 ± 31% in ESI method I, 36 ± 28% ESI method II, and 67 ± 34% in the APCI method (average ± standard deviation), see Table S3–S5† for more detailed results as a function of date/location. Accurate results are confirmed by the relative recovery of each analyte, rather than the absolute recovery, based on isotope dilution methodology. Spiked samples were used to determine the relative recovery as compared with triclosan. The average relative recoveries of all spiked samples for each analyte are shown in Table 2. Equations used to calculate the absolute and relative recoveries are located in the ESI.† All reported concentrations are recovery corrected values using the relative recoveries for wastewater samples processed at the same time (see Tables S3–S5†).

Triclosan and CTD concentrations in wastewater

Triclosan and the CTDs were detected in all wastewater samples analyzed, except for 4-Cl-TCS in the SJWP samples in April 2012, as compiled in Table 3. Triclosan concentrations varied from 36–465 ng L^{-1}. Concentrations of total CTDs ranged from below the LOD to 27.2 ng L^{-1}.

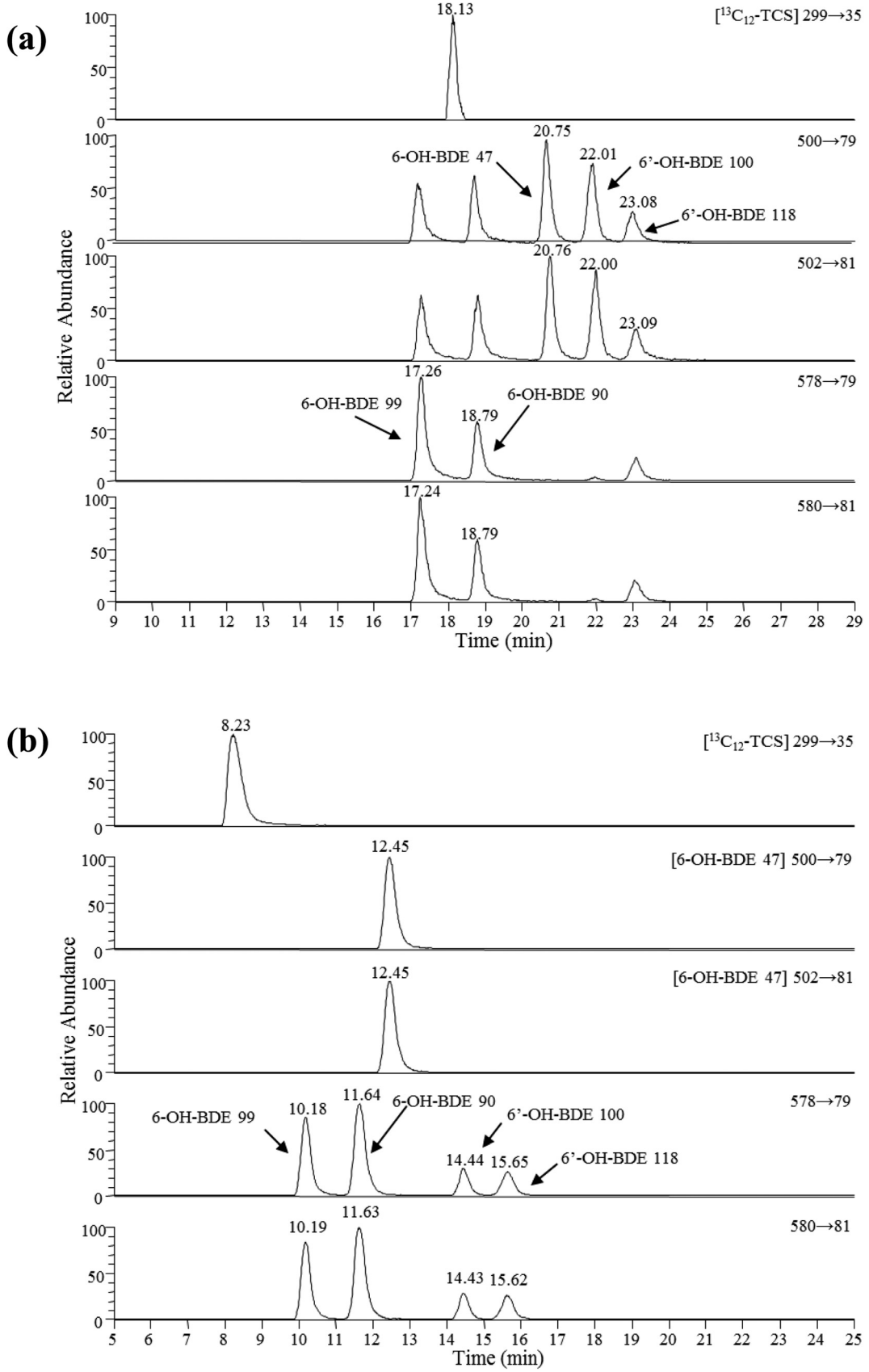

Fig. 1 Representative chromatogram of the OH-BDEs, using the (a) APCI-LC-MS/MS method and (b) ESI-LC-MS/MS method 2. Details of these methods are provided in Table 1.

Table 2 The absolute recovery of $^{13}C_{12}$-triclosan and relative recoveries of analytes of interest to $^{13}C_{12}$-triclosan for three LC-MS/MS methods

	Absolute recovery (%)		
Compound	ESI method I	APCI method	ESI method II
$^{13}C_{12}$-triclosan	59 ± 31	67 ± 34	36 ± 28
	Relative recovery (%)		
Triclosan	93 ± 18	—	—
4-Cl-TCS	84 ± 18	—	—
6-Cl-TCS	75 ± 31	—	—
4,6-Cl-TCS	59 ± 15	—	—
6-OH-BDE-47	54 ± 15	66 ± 12	52 ± 0
6-OH-BDE-90	—	54 ± 14	25 ± 2
6-OH-BDE-99	—	48 ± 13	24 ± 2
6′-OH-BDE-100	—	73 ± 32	96 ± 7
6′-OH-BDE-118	—	48 ± 10	17 ± 7

The results for MWP are comparable to those previously reported by Buth *et al.*[11] The chlorination of the effluent in the September samples leads to production of CTDs. In the October samples, the triclosan and CTD levels are similar (and CTDs are elevated in the prechlorination sample), which is inconsistent with the September sample and previous findings at MWP.[11] This suggests either that is a balance between CTD removal and formation at this time period or that the sample timing offset was not correct. In the November 2011 sample, seasonal chlorination had ceased, and only small levels of CTDs were detected, which are ascribed to influent CTDs that had persisted through the treatment process. The WLSSD plant only chlorinates occasionally, but the presence of CTDs in the collected samples, along with the ratio of CTDs to triclosan in the final effluent, which were similar to those at the MWP, indicated that the chlorination was active at WLSSD during the sample collection.

In the two plants using UV disinfection, PAWP and SJWP, the triclosan and CTD levels are essentially constant through the disinfection step, indicating that the UV dose was not enough to cause significant triclosan transformation. The higher levels of CTDs in the PAWP samples is explained by the fact that this plant serves a community that has residual chlorine in their drinking water, while SJWP serves a community that does not. This is consistent with the findings of formation of CTDs upon chlorination in tap water by Rule *et al.*[16] and previous detections of CTDs in influents to wastewater treatment plants attributed to reaction with residual chlorine.[6,11] The concentrations of CTDs in PAWP final effluent are still less than chlorinated MWP effluent and similar to WLSSD and non-chlorinated MWP effluents. In our prior study,[11] CTDs were not detected in the SJWP effluents, but the LOQs in the current study were, in general, 2–10 times lower than those in our prior study.

OH-BDEs in wastewater effluent

While triclosan is expected to be in wastewater given its use in soap and toothpaste, it is less obvious whether OH-BDEs should also be found in wastewater samples. There are some precedents that indicate OH-BDEs might be expected. 6-OH-BDE-47 has been previously detected in wastewater,[41] several OH-BDEs have been detected in biosolids,[42] and elevated levels of OH-BDEs were detected near a WWTP.[26]

In the present work, an APCI method and two ESI methods were used to determine the concentration of 6-OH-BDE-47 in the samples. A comparison of the results are found in Table 4, which highlights the samples from this study that had detectable amounts of 6-OH-BDE-47 in at least one replicate extract. A grab sample from MWP had by far the highest

Table 3 Triclosan and CTD concentrations in wastewater effluent samples before and after disinfection

Wastewater sample	*n*	Concentration ± SD ng L^{-1}			
Metropolitan Plant (MWP)		Triclosan	4-Cl-TCS	6-Cl-TCS	4,6-Cl-TCS
Pre-chlorination effluent, September 2011	3	239 ± 42	0.5 ± 0.1	1.9 ± 0.1	1.9 ± 0.2
Post-chlorination effluent, September 2011	4	425 ± 51	4 ± 0.7	9.8 ± 1.2	13.4 ± 1.6
Pre-chlorination effluent, October 2011	4	112 ± 4	0.73 ± 0.02	8.5 ± 0.7	11.3 ± 0.4
Post-chlorination effluent, October 2011	4	144 ± 12	1.9 ± 0.2	7.9 ± 0.1	11.7 ± 1.1
Final effluent with no chlorination, November 2011	4	465 ± 90	<0.9[c]	<2.4[c]	<2.8[c]
Western Lake Superior Sanitary District (WLSSD)					
Grab sample, June 2011	4	94 ± 20	0.3 ± 0.1	3.0 ± 0.6	4.7 ± 0.6
Grab sample, April 2012	4	108 ± 4	0.16 ± 0.03	1.9 ± 0.1	3.9 ± 0.3
Palo Alto Regional Water Quality Control Plant (PAWP)					
Pre UV effluent, July 2011	4	390 ± 32	1.2 ± 0.2[a]	4.2 ± 0.3	7.8 ± 0.9
Post UV effluent, July 2011	4	313 ± 72	1.2 ± 0.3[b]	3.1 ± 0.7[b]	5.8 ± 0.8
Pre UV effluent, January 2012	4	51 ± 11	0.3 ± 0.1	2 ± 0.4	4.1 ± 0.9
Post UV effluent, January 2012	4	58 ± 4	0.42 ± 0.04	2.4 ± 0.1	4.3 ± 0.4
St. John's University (SJWP)					
Pre UV effluent, January 2012	4	48 ± 3	0.27 ± 0.04	0.6 ± 0.1	1.4 ± 0.2
Post UV effluent, January 2012	4	48 ± 7	0.18 ± 0.04	0.5 ± 0.1	1.2 ± 0.3
Pre UV effluent, February 2012	3	57 ± 2	<0.2[c]	<0.2[c]	0.7 ± 0.1
Post UV effluent, February 2012	3	36 ± 1	ND	<0.2[c]	1.1 ± 0.2

*LODs and LOQs of analytes for each sample analyzed are summarized in the appendix; ND – not detected (<LOD). If a replicate is >LOD but <LOQ, the LOQ is shown. [a] One replicate between LOD and LOQ, while other replicates above LOQ. [b] Two replicates between LOD and LOQ, while other replicate(s) above LOQ. [c] All replicates between LOD and LOQ.

Table 4 6-OH-BDE-47 concentrations in wastewater effluent samples before and after disinfection

Wastewater sample	6-OH-BDE-47 concentration ± SD ng L^{-1}					
Metropolitan Plant (MWP)	*n*	ESI method 1	*n*	APCI method	*n*	ESI method 2
Pre-chlorination effluent, September 2011	3	<1.8[a]	2	0.72[a]	1	<0.46[c]
Post-chlorination effluent, September 2011	4	3.4 ± 2.2[b]	2	<3.04	2	0.50[a]
Final effluent with no chlorination, November 2011	4	<0.4[c]	3	ND	2	ND
Effluent grab sample, April 2012	3	16.9 ± 3.1		N/A		N/A
Western Lake Superior Sanitary District (WLSSD)						
Grab sample, June 2011	3	1.8 ± 0.2		N/A		N/A
Palo Alto Regional Water Quality Control Plant (PAWP)						
Pre UV effluent, July 2011	4	1.4 ± 0.3	3	0.83[b]	3	<0.46[c]
Post UV effluent, July 2011	4	<0.4[d]	3	ND	3	ND
Pre UV effluent, January 2012	4	ND	3	ND	2	ND
Post UV effluent, January 2012	4	ND	3	ND	1	ND
St. John's University (SJWP)						
Pre UV effluent, January 2012	4	ND	3	<0.48[d]		N/A
Post UV effluent, January 2012	4	ND	3	ND		N/A
Pre UV effluent, February 2012	3	ND	3	ND		N/A
Post UV effluent, February 2012	3	ND	3	<0.96[d]		N/A

*LODs and LOQs of analytes for each sample analyzed are summarized in the appendix; ND – not detected (<LOD). If a replicate is >LOD but <LOQ, the LOQ is shown. N/A was used when not enough sample was left to be analyzed. [a] One replicate between LOD and LOQ, while other replicates above LOQ. [b] Two replicates between LOD and LOQ, while other replicate(s) above LOQ. [c] All replicates between LOD and LOQ. [d] One replicate between LOD and LOQ, while other replicates below LOD.

amount of 6-OH-BDE-47 (analyzed by ESI method 1), while lower levels were detected in the composite samples. The ~17 ng L^{-1} concentration for 6-OH-BDE-47 detected in the April 2012 grab sample of MWP wastewater is the highest wastewater concentration reported to date. From comparison of pre and post disinfection extracts, 6-OH-BDE-47 may be susceptible to UV light (>71% removal; PAWP July 2011). The differing analyte concentrations were near or below the LOQ, and these slight differences are likely due to the high uncertainty at these low concentrations. It is unclear why there is a higher frequency and abundance of 6-OH-BDE-47 in MWP effluents compared to PAWP or SJWP, but it could be due to the larger population served.

An APCI and additional ESI method were developed to analyze pentabrominated OH-BDEs, which were not measureable with ESI method 1. The highest concentration of 6-OH-BDE-90 (109 ng L^{-1}) was detected using the APCI method at SJWP. The differences in the concentrations, shown in Table 5, for the two analytical methods are likely caused by (1) the 10-fold dilution of samples prior to the ESI method 2 analysis (but not APCI); and (2) the variation of sensitivity between analyses. The differences may have also been reduced if a $^{13}C_{12}$-OH-BDE was used as the surrogate/internal standard rather than $^{13}C_{12}$-triclosan. The samples underwent a 10-fold dilution to increase the sample volume in order to undergo ESI method 2 analysis. Thus, analytes with low levels prior to the dilution went undetected by the ESI method 2. A comparison of samples with sufficient analyte levels by the two analytical methods showed similar results. At MWP, 6-OH-BDE-90 levels were statistically unaffected ($p > 0.05$) by chlorination in September 2011. Levels of 6-OH-BDE-90, however, increased ($p \approx 0 < 0.05$) during UV

Table 5 6-OH-BDE-90 concentrations in wastewater effluent samples before and after disinfection

Wastewater sample	6-OH-BDE-90 concentration ± SD ng L^{-1}			
Metropolitan Plant (MWP)	*n*	APCI method	*n*	ESI method 2
Pre-chlorination effluent, September 2011	2	2.3 ± 1.2	1	ND
Post-chlorination effluent, September 2011	2	4.8 ± 1.2	2	ND
Final effluent with no chlorination, November 2011	3	1.6 ± 0.2	2	<0.7[d]
Palo Alto Regional Water Quality Control Plant (PAWP)				
Pre UV effluent, July 2011	3	3.7 ± 0.2	3	0.79 ± 0.04[a]
Post UV effluent, July 2011	3	39.4 ± 1.4	3	24.1 ± 6.3
Pre UV effluent, January 2012	3	ND	2	<0.7[c]
Post UV effluent, January 2012	3	ND	1	<0.7[c]
St. John's University (SJWP)				
Pre UV effluent, January 2012	3	109.4 ± 33.1		N/A
Post UV effluent, January 2012	3	0.5 ± 0.2[b]		N/A

*LODs and LOQs of analytes for each sample analyzed are summarized in the appendix; ND – not detected (<LOD). If a replicate is >LOD but <LOQ, the LOQ is shown. N/A was used when not enough sample was left to be analyzed. [a] One replicate between LOD and LOQ, while other replicates above LOQ. [b] Two replicates between LOD and LOQ, while other replicate(s) above LOQ. [c] All replicates between LOD and LOQ. [d] One replicate between LOD and LOQ, while other replicates below LOD.

Table 6 6-OH-BDE-99 concentrations in wastewater effluent samples before and after disinfection

Wastewater sample	6-OH-BDE-99 concentration ± SD ng L^{-1}			
Metropolitan Plant (MWP)	*n*	APCI method	*n*	ESI method 2
Pre-chlorination effluent, September 2011	2	<0.8[b]	1	<0.6[b]
Post-chlorination effluent, September 2011	2	<3.2[b]	2	1.8 ± 1.6
Final effluent with no chlorination, November 2011	3	1.4 ± 0.1	2	<0.6[c]
Palo Alto Regional Water Quality Control Plant (PAWP)				
Pre UV effluent, July 2011	3	16.1 ± 0.2	3	11.9 ± 2.5
Post UV effluent, July 2011	3	24.9 ± 0.8	3	23.7 ± 9.0
Pre UV effluent, January 2012	3	ND	2	1.1[a]
Post UV effluent, January 2012	3	4.2 ± 4.2	1	<0.6[b]
St. John's University (SJWP)				
Pre UV effluent, January 2012	3	4.5 ± 2.1		N/A
Post UV effluent, January 2012	3	<0.5[c]		N/A
Pre UV effluent, February 2012	3	ND		N/A
Post UV effluent, February 2012	3	1.5 ± 0.9		N/A

*LODs and LOQs of analytes for each sample analyzed are summarized in the appendix; ND – not detected (<LOD). If a replicate is >LOD but <LOQ, the LOQ is shown. N/A was used when not enough sample volume was left to be analyzed. [a] One replicate between LOD and LOQ, while other replicates above LOQ. [b] All replicates between LOD and LOQ. [c] One replicate between LOD and LOQ, while other replicates below LOD.

disinfection at PAWP on July 2011. On the other hand, UV disinfection at SJWP removed 99.6% (p = 0.005) of the 6-OH-BDE-90 from the effluent stream in January 2012.

The highest concentration of 6-OH-BDE-99 detected was approximately 24 ng L^{-1} at PAWP using both the APCI method and ESI method 2. Overall, both analytical methods determined similar concentrations (seen in Table 6), but again slight differences were observed due to varying sensitivities between analyses. Similar trends were observed between 6-OH-BDE-90 and 6-OH-BDE-99. The July 2011 UV disinfection at PAWP resulted in an increase of 6-OH-BDE-99 using the APCI method and the ESI method 2. A different trend was seen in Jan 2012 at PAWP. 6-OH-BDE-99 increased after UV disinfection according to the APCI method, but ESI method 2 showed a slight decrease. Similar to 6-OH-BDE-90, UV disinfection at SJWP removed the 6-OH-BDE-99 (88.9% removal ratio) in Jan. 2012. But one month later, disinfection appeared to slightly increase the levels of 6-OH-BDE-99. Therefore, these results are inconclusive in determining whether UV disinfection is effective at removing OH-BDEs, while showing clearly that chlorination is ineffective at removing 6-OH-BDE-90 and 6-OH-BDE-99.

6-OH-BDE-100 was not detected in any of the effluents. 6′-OH-BDE-118, however, was detected in only one sample using the ESI method 2 at MWP on September 2011 in the post chlorination effluent at 1.77 ng L^{-1} (other replicate was in between LOD and LOQ). An unknown peak at an earlier retention time with the same quantification and confirmation ions as the pentabrominated (Br_5-) OH-BDEs was also detected in the same sample as 6′-OH-BDE-118. The unknown compound may be another Br_5-OH-BDE or could be a dihydroxylated polybrominated biphenyl,[51] which also has the same formula as Br_5-OH-BDEs.

This study confirms that wastewater can be a source of selected OH-BDEs to surface waters. Further experiments and analyses are required to determine if the OH-BDEs are formed during the wastewater treatment process. Whether wastewater is the most important source of OH-BDEs, and consequently PBDDs *via* photolysis, in freshwater environments remains to be seen. Natural production of OH-BDEs in freshwater is unlikely, owing to lack of bromide ions available needed for construction of these compounds. Photolysis of brominated phenols contributes to formation OH-BDEs in fresh waters. Sustained levels of 2′-OH-BDE-68 were formed from photolysis of 2,4-dibromophenol.[57] Like PBDEs, brominated phenols are present in dust[58] and may also be present in wastewater. If brominated phenols are present in wastewater, they could be contributing to levels of OH-BDEs and PBDDs in fresh water environments.

Conclusions

The method of wastewater disinfection affects levels of CTDs in the final effluent. Chlorination can significantly increase all three CTDs. Even in the case where CTDs did not increase after chlorination, CTDs are still detected in higher amounts than other non-chlorinating plants. UV disinfection has little, if any, effect on triclosan and CTDs in wastewater.

Overall, the concentrations of OH-BDEs are of similar levels as CTDs. Although the loadings to surface water are small, the confirmation of 6-OH-BDE-47, 6-OH-BDE-90, and 6-OH-BDE-99 in WWTP effluent is of concern for the same reasons as triclosan and CTDs. PBDDs may form from OH-BDEs *via* photolysis. The presence of 6-OH-BDE-47, 6-OH-BDE-90, and 6-OH-BDE-99, which are not directly manufactured, in wastewater provides evidence that these compound are formed *via* metabolism of PBDEs, which are also present in wastewater. Whether the OH-BDEs are present in the influent (as the result of human metabolism) or bacterial metabolism from the activated sludge process is unknown.

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