# **Electronic Supplementary Information** for Triclosan, Chlorinated Triclosan Derivatives, and Hydroxylated Polybrominated Diphenyl Ethers (OH-BDEs) in Wastewater Effluents

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*Environmental Science: Water Research & Technology*

## Synthesis of 6-OH-BDE-90

The 6-OH-BDE-90 congener was synthesized and purified using methods developed by Hofsløkken and Skattebøl (1999) for step 2 and Marsh et al (2003) for steps 1 and 3-5. The relevant reactions are shown in Figure S1.

(1): The synthesis of 2,2’,4,4’-tetrabromodiphenyliodonium chloride (**A**) from 1,3-dibromobenzene was previously described in the supporting information of Steen et al. (2009).

(2): Bromophenol (2.3 mL), MgCl (2.86 g) and triethylamine (10.5 mL) were added to a flask containing 100 mL of anhydrous ACN under nitrogen. Next, paraformaldehyde (3.7 g) was added and the mixture was refluxed for 3.5 hrs. The reaction mixture was cooled, and 5% HCl was added until an acidic pH was reached. The mixture was washed three times with diethyl ether, and the organic phases were combined and dried with $Na_2SO_4$. The solvent was removed *in vacuo*, where eventually two layers formed again. The bottom, dark layer was separated and pumped to dryness, yielding a dark solid. This crude product was cleaned by silica gel flash column chromatography (90% hexane 10% dioxane) and finally by sublimation yielding yellow crystals of 3-bromosalicylaldehyde (**B**; 3 g, 70%). $^1$H NMR (400 MHZ) $\delta$ = 11.612 (s, 1H, CHO), 9.864 (s, 1H, OH), 7.786 (d, 7.8 Hz, 1H, CH) 7.552 (d, 7.8 Hz, 1H, CH) 6.952 (t, 7.8 Hz 1H, CH)

(3): Sodium hydroxide (12 mmol) and **B** (10 mmol) were dissolved in dioxane (16 mL) and water (30 mL). To this yellow mixture, **A** (12 mmol) was added. The mixture was heated to 80 $^oC$ and allowed to stir (90 min). The solution was then cooled to room temperature and diluted with water (60 mL) and methylene chloride (60 mL). The layers were allowed to separate and the aqueous layer was extracted with methylene chloride (100 mL, 3×). The organic fractions were combined, washed with aqueous sodium hydroxide (1 M, 100 mL, 2×), water (100 mL, 2×) and dried over sodium sulfate. The solvents were removed under vacuum. Flash chromatography (3:2 hexanes: $CH_2Cl_2$; $R_f$= 0.375) gave the desired product 3-bromo-2-(2,4-dibromophenoxy) benzaldehyde (**C**) in Figure S1 (1.7952 g, 65%) as a white solid. $^1$H NMR (500 NMR, $CDCl_3$) $\delta$ = 10.136 (s, 1H, CHO), 7.97 (dd, 1H, CH)

(4): **C** (2.6830 mmol) and monopotassium phosphate (53.2 mmol, 7.24 g) were suspended in methylene chloride (25 mL). In another flask, hydrogen peroxide (30%) and methylene chloride (2 mL) were cooled to 0 $^oC$. Trifluoroacetic acid anhydride was added dropwise (20.04 mmol). The mixture stirred for 1 h at 0 $^oC$. The aldehyde mixture was cooled and added dropwise to the peroxide solution. The mixture was stirred for 2.25 hours, after which brine (32 mL) and aqueous $Na_2SO_3$ (20 %) were added to quench the reaction. The solution stirred overnight. The layers were separated and the aqueous layer was extracted with methylene chloride (30 mL, 3×) The organic layers were removed under vacuum. The residue was re-dissolved in methanol (32 mL) with two drops of concentrated hydrochloric acid. Solvents were removed *in vacuo*. Flash chromatography (5:2 $CH_2Cl_2$:hexanes; $R_f$= 0.5) gave the desired product 3-bromo-2-(2,4-dibromophenoxy)phenol (**D)** in Figure S1, (0.611 g, 58%) as a clear and colorless oil.

(5): **D** (0.8379 mmol) and calcium carbonate (6.62 mmol, 0.662 g) were suspended in methylene chloride (100 mL) and methanol (20 mL). Benzyltrimethylammonium tribromide (14.66 mmol, 3.991 g) was added in small increments over a time span of two hours. The solution then stirred for an additional two hours. The reaction mixture was filtered and the supernant was added to aqueous $Na_2SO_3$ (5%, 80 mL). The layers separated and the aqueous layer was extracted with methylene chloride (50 mL, 3×) and dried over sodium sulfate. Solvents were removed with under vacuum. Flash chromatography 6-OH-BDE-90 (**E**) in Figure S1 (4:1 $CH_2Cl_2$: hexanes, $R_f$= 0.5) gave desired product (0.815 g, 32%) as a yellow solid.

## References

N. U. Hofsløkken; L. Skattebøl; Convenient Method for the *ortho*-Formylation of Phenols. *Acta Chemica Scandinavica*. **1999**, 53, pp. 258-262

G. Marsh, R.; Stenutz, Å.; Bergman Synthesis of hydroxylated and methoxylated polybrominated diphenyl ethers – Natural products and potential polybrominated diphenyl ether metabolites. *Eur. J. Org. Chem.* **2003**, pp. 2566–2576

P. Steen; M. Grandbois; K. McNeill; W. Arnold; Photochemical Formation of Halogenated Dioxins from Hydroxylated Polybrominated Diphenyl Ethers (OH-PBDEs) and Chlorinated Derivatives (OH-PBCDEs). *Environmental Science and Technology*. **2009**, 43, pp. 4405-4411.

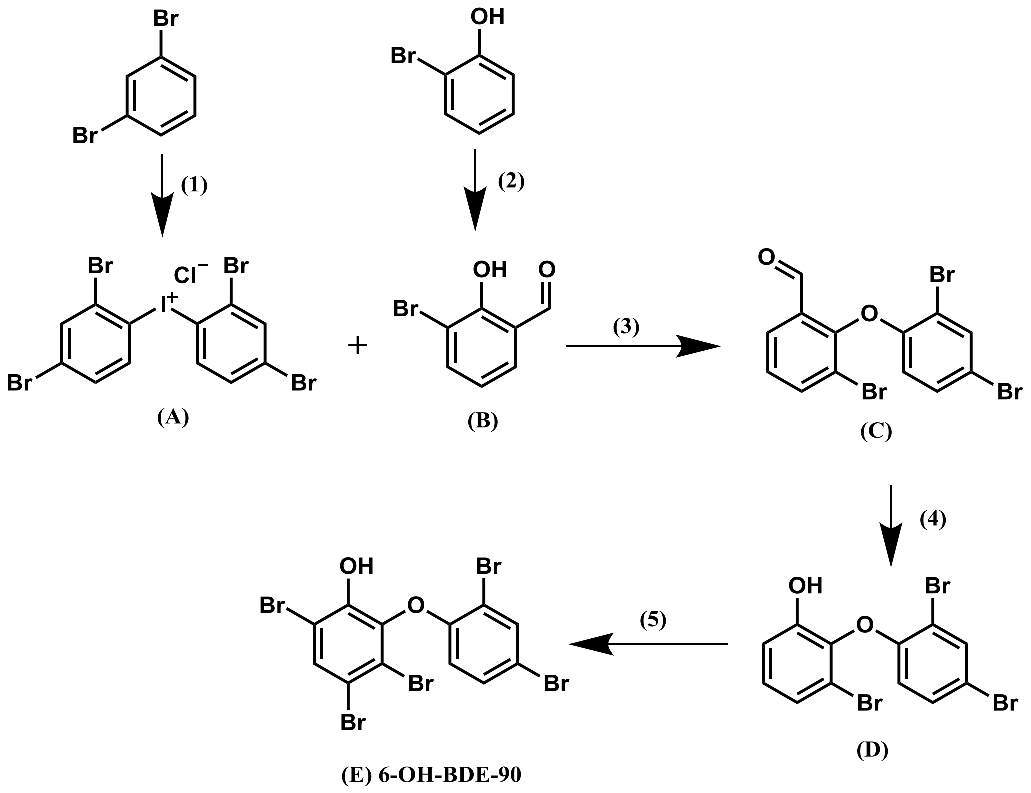

**Figure S1:** The synthesis procedure of 6-OH-BDE-90
(A) 2,2’,4,4’-tetrabromodiphenyliodonium chloride; (B) 3-bromosalicyaldehyde (C) 3-bromo-2-(2,4-dibromophenoxy) benzaldehyde; (D) 3-bromo-2-(2,4-dibromophenoxy)phenol.

## Wastewater treatment plants

Metropolitan Wastewater Treatment Plant (MWP) in St. Paul, MN has a capacity of 251 million gallons/day (MGD) serving 1.8 million people. MWP chlorinates their effluent from April through October with a dosage of 1.25 µg/L Cl as $Cl_2$ for > 30 min, aiming for a residual of 0.20 µg/L Cl as $Cl_2$. Effluent is dechlorinated with sodium bisulfite at 0.95 µg/L. MWP discharges directly into the Mississippi River. Composite samples from MWP were obtained on three separate dates. On two dates in the fall, pre- and post-chlorination samples were obtained, and one non-chlorinated effluent sample was obtained during the winter. A pre-chlorination grab sample was also obtained to determine the effects of ozonation on the analytes.

Two activated sludge WWTPs, Palo Alto Regional Water Quality Control Plant (PAWP) and Saint John's University Wastewater Treatment Plant (SJWP), were each sampled (24-hour composite, pre- and post-UV) during two dates to measure the effect of UV disinfection on triclosan and CTDs in wastewater. PAWP treats on average 21.8 MGD serving 220,000 people with ~5% industry wastewater. PAWP disinfects year round using a system of Trojan UV 3000 Plus assemblies with an energy output of 35 mW-s $cm^{-2}$. The average ultraviolet transmittance of the wastewater is 62% with a contact time of 3.8 seconds. PAWP discharges effluent directly into the southern San Francisco Bay.

St. John's University uses groundwater for their potable water supply and does not chlorinate prior to use. The SJWP treats the used water serving a population of 2600 during the academic year and 1200 in summer. SJWP is licensed to process a maximum flow of 0.23 MGD and treats about 0.16 MGD on an average day. After filtration through sand, secondary effluent is disinfected using a Package Treatment UV-3000 system containing six modules. Each module has four 162.6-cm lamps which provide 190 µW $cm^{-2}$ at 1 m with radiation centered at 254 nm. The contact time in the disinfection tank is between 2 and 4 minutes depending on flow conditions. SJWP discharges into East Lake Gemini which then drains into the North Fork of the Watab River and eventually into the Mississippi River.

Effluent grab samples from Western Lake Superior Sanitation District (WLSSD) were collected by boat on two occasions. Approximately half of the wastewater that WLSSD treats originates from industries. WLSSD filters the secondary effluent through mixed media beds and disinfects with chlorine, but is only required to chlorinate when a fecal coliform analysis of treatment plant intake exceeds 100 MPN/100 mL. The chlorine dosages were not available for the dates that sampling took place. WLSSD discharges into the St. Louis Bay which flows into Lake Superior.

**Table S1:** Quantification and confirmation SRM transitions for analyte detection for ESI Method I and II and APCI method.

| Analyte | Precursor ion m/z | Product ion m/z | Purpose[a] |
|---|---|---|---|
| | *ESI Method I* | | |
| Triclosan | 287 | 35.1 | Q |
| | 289 | 37.1 | C |
| 4-Cl-TCS | 321 | 35.1 | Q |
| | 323 | 37.1 | C |
| 6-Cl-TCS | 321 | 35.1 | Q |
| | 323 | 37.1 | C |
| 4,6-Cl-TCS | 355 | 35.1 | Q |
| | 357 | 37.1 | C |
| 6-OH-BDE-47 | 500.7 | 79 | Q |
| | 498.7 | 79 | C |
| $^{13}C_{12}$-Triclosan | 299 | 35.1 | Q |
| | *ESI Method II* | | |
| 6-OH-BDE-47 | 500 | 79 | Q |
| | 502 | 81 | C |
| 6-OH-BDE-90 | 578 | 79 | Q |
| | 580 | 81 | C |
| 6-OH-BDE-99 | 578 | 79 | Q |
| | 580 | 81 | C |
| 6’-OH-BDE-100 | 578 | 79 | Q |
| | 580 | 81 | C |
| 6’-OH-BDE-118 | 578 | 79 | Q |
| | 580 | 81 | C |
| $^{13}C_{12}$-Triclosan | 299 | 35 | Q |
| | *APCI Method* | | |
| 6-OH-BDE-47 | 500 | 79 | Q |
| | 502 | 81 | C |
| 6-OH-BDE-90 | 578 | 79 | Q |
| | 580 | 81 | C |
| 6-OH-BDE-99 | 578 | 79 | Q |
| | 580 | 81 | C |
| 6’-OH-BDE-100 | 500 | 79 | Q |
| | 502 | 81 | C |
| 6’-OH-BDE-118 | 500 | 79 | Q |
| | 502 | 81 | C |
| $^{13}C_{12}$-Triclosan | 299 | 35 | Q |

[a]Quantification SRM denoted as “Q” and confirmation SRM denoted as “C”

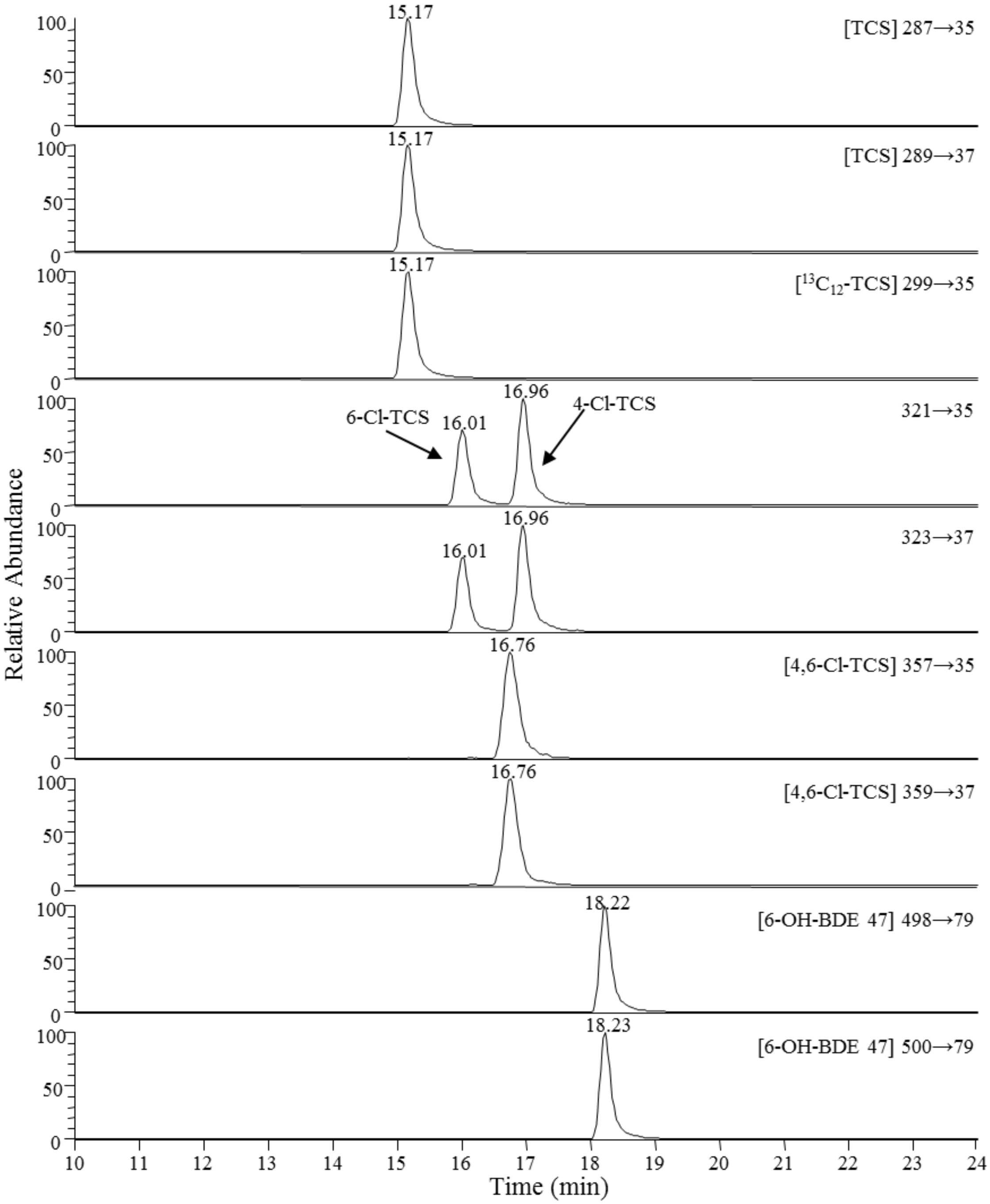

**Figure S2:** ESI Method I Standard Chromatograph
Representative of a standards (normalized to the highest peak) for ESI Method I. Retention times (RT) are noted for known analytes. The SRM transitions are indicated to the right of each chromatogram.

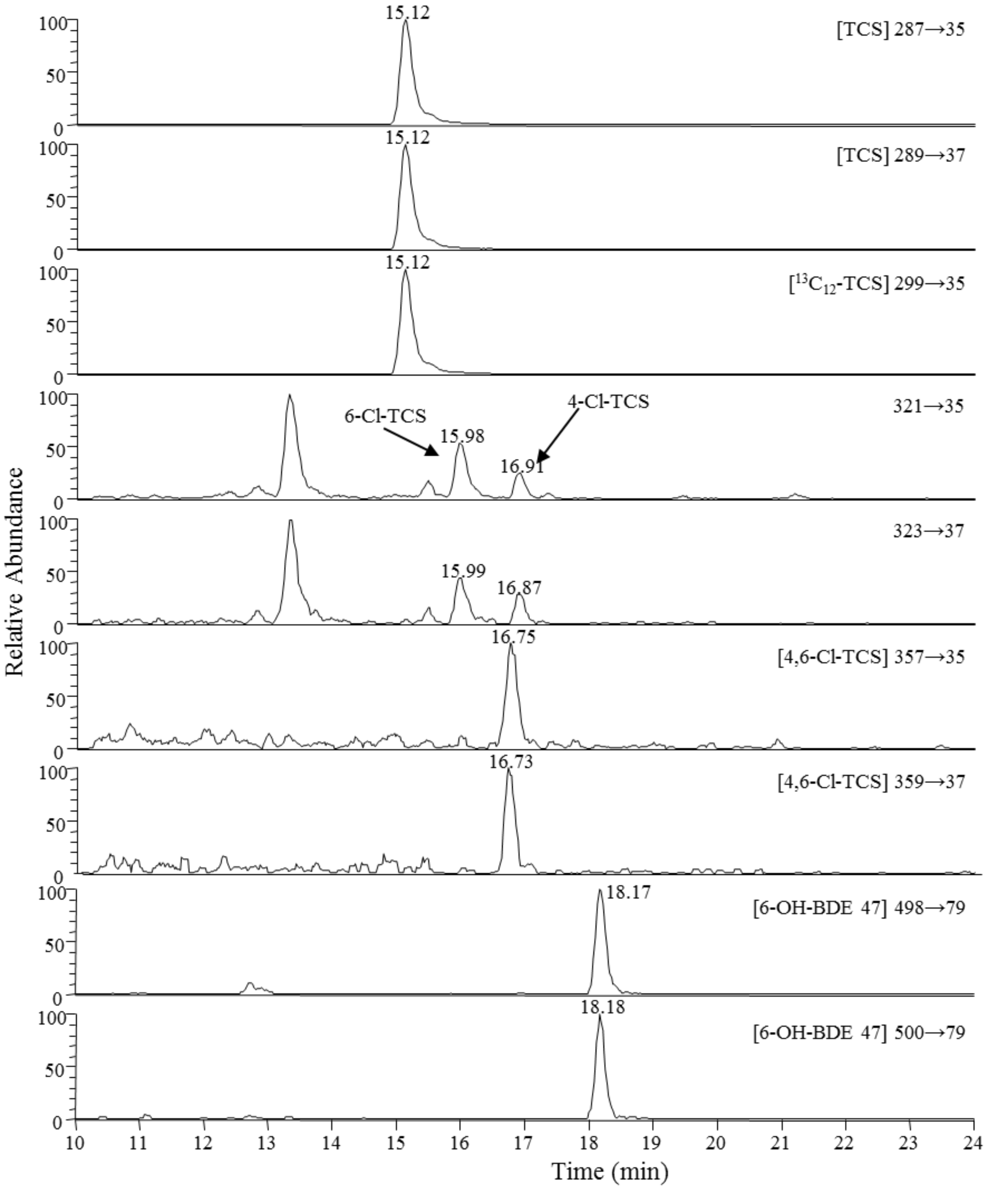

**Figure S3:** ESI Method I chromatograph of PAWP pre-UV sample

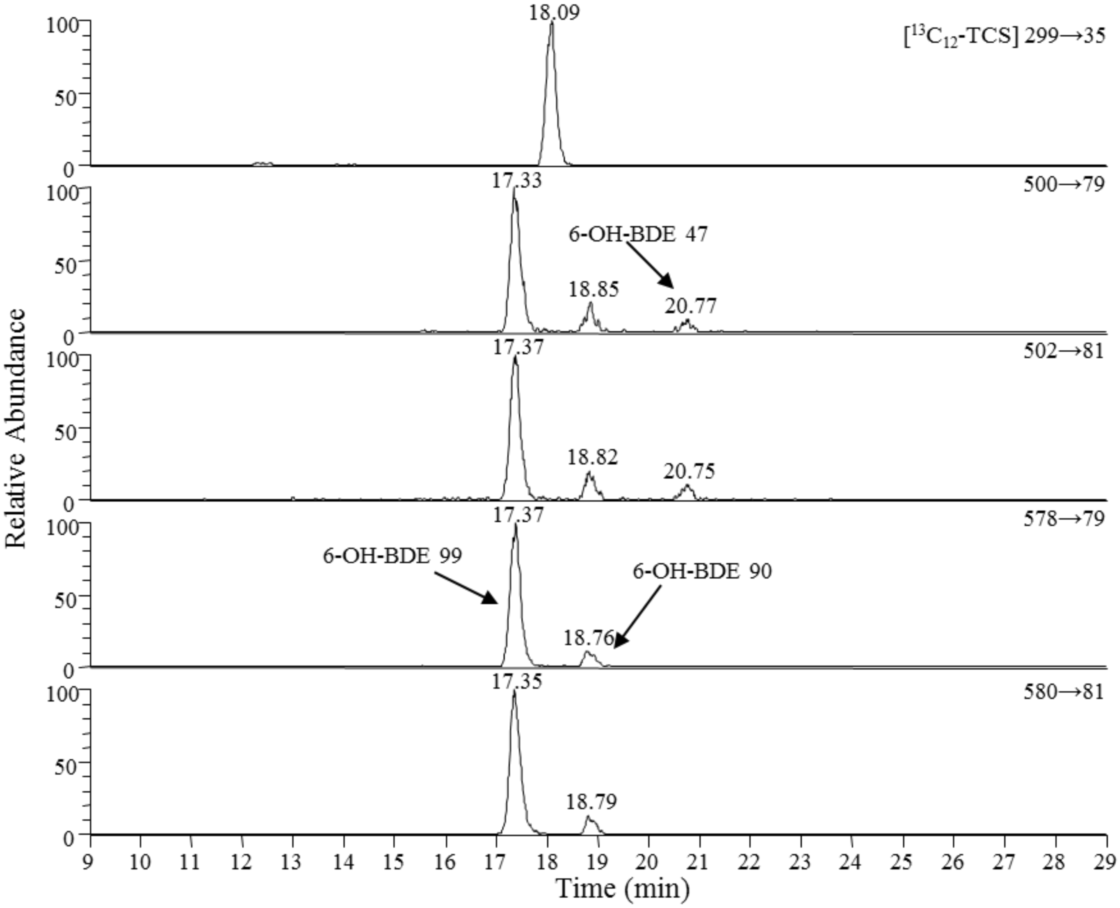

**Figure S4:** APCI Method chromatograph of PAWP pre-UV sample

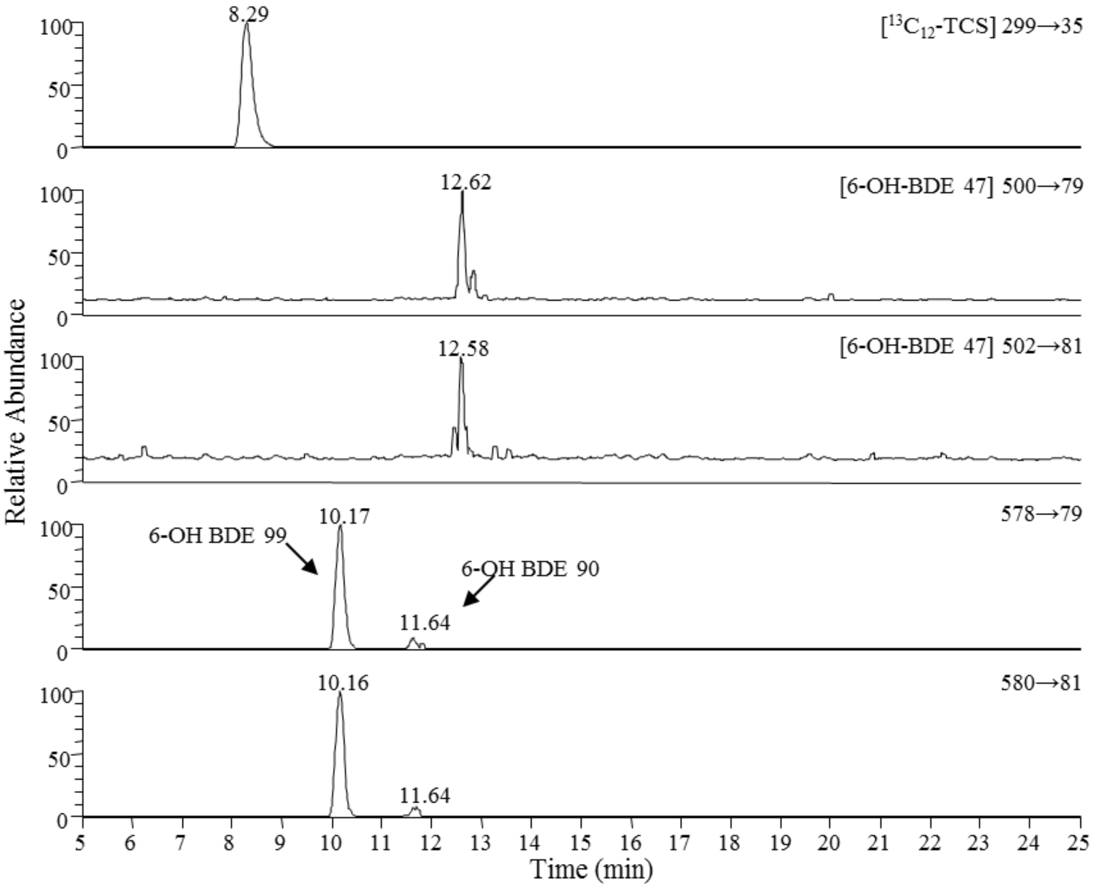

**Figure S5:** ESI Method II chromatograph of PAWP pre-UV sample

**Table S2:** LOQs for ESI Method I, APCI Method, and ESI Method II

| | LOQ* Ranges (ng/L) | | |
|---|---|---|---|
| | ESI Method I[a] | APCI Method[b] | ESI Method II[b,c] |
| Triclosan | 3.9 - 29.1 | - | - |
| 4-Cl-TCS | 0.02 - 0.24 | - | - |
| 6-Cl-TCS | 0.1 - 2.4 | - | - |
| 4,6-Cl-TCS | 0.1 - 2.6 | - | - |
| 6-OH-BDE-47 | 0.2 - 1.8 | 0.2 - 3.0 | 0.5 |
| 6-OH-BD- 90 | - | 0.1 - 2.7 | 0.6 |
| 6-OH-BDE-99 | - | 0.1 - 3.2 | 0.7 |
| 6'-OH-BDE-100 | - | 2.9[d] | 1.74[d] |
| 6'-OH-BDE-118 | - | 2.9[d] | 1.09 |

[*] LODs may be calculated from LOQ by multiplying by 0.3

[a] LOQs defined as 10 times the analyte concentration determined in the method blank

[b] LOQs defined as 80% of lowest concentration on calibration curve

[c] No range because all samples were analyzed in a single run

[d] Analyte not detected; LOQ defined as lowest concentration on calibration curve

**Table S3:** The relative recoveries of analytes of interest to $^{13}C_{12}$-triclosan for ESI Method I and absolute recovery of $^{13}C_{12}$-triclosan for *n* number of replicates.

ESI Method I

| **Wastewater Sample** | | **Relative Recovery (%)** | | | | | | **Absolute Recovery (%)** |
|---|---|---|---|---|---|---|---|---|
| *Metropolitan plant (MWP)* | n | Triclosan | 4-Cl-TCS | 6-Cl-TCS | 4,6-Cl-TCS | 6-OH-BDE 47 | n | $^{13}C_{12}$-Triclosan |
| September 2011 | 2 | 103 ± 48 | 96 ± 55 | 109 ± 72 | 72 ± 40 | 54 ± 38 | 7 | 37 ± 8 |
| October 2011 | 2 | 99 ± 3 | 81 ± 1 | 80 ± 4 | 56 ± 1 | 50 ± 4 | 8 | 78 ± 5 |
| November 2011 | 1 | 71.2 | 75.2 | 60.2 | 52.9 | 39.5 | 4 | 46 ± 4 |
| April 2012 | 2 | 105 ± 1 | 83 ± 24 | 102 ± 28 | 76 ± 19 | 65 ± 5 | 6 | 99 ± 7 |
| *Western Lake Superior Sanitary District (WLSSD)* | | | | | | | | |
| June 2011 | 1 | 93.73 | 80.80 | 47.82 | 50.55 | 64.96 | 4 | 73 ± 8 |
| April 2012 | 1 | 110.57 | 108.53 | 104.26 | 79.64 | 71.22 | 4 | 79 ± 7 |
| *Palo Alto Regional Water Quality Control Plant (PAWP)* | | | | | | | | |
| July 2011 | 2 | 71 ± 4 | 93 ± 1 | 90 ± 2 | 67 ± 0 | 56 ± 2 | 8 | 33 ± 7 |
| January 2012 | 2 | 94 ± 5 | 80 ± 11 | 58 ± 9 | 54 ± 10 | 47 ± 9 | 8 | 36 ± 3 |
| *St. John's University (SJWP)* | | | | | | | | |
| January 2012 | 2 | 99 ± 3 | 70 ± 20 | 49 ± 15 | 52 ± 25 | 51 ± 27 | 8 | 74 ± 18 |
| February 2012 | 2 | 106 ± 3 | 93 ± 3 | 102 ± 7 | 67 ± 5 | 66 ± 2 | 6 | 119 ± 2 |

**Table S4**: The relative recoveries of analytes of interest to $^{13}C_{12}$-triclosan for ESI Method II and absolute recovery of $^{13}C_{12}$-triclosan for *n* number of replicates.

ESI Method II

| **Wastewater Sample** | | **Absolute Recovery (%)** |
|---|---|---|
| *Metropolitan plant (MWP)* | n | $^{13}C_{12}$-Triclosan |
| September 2011 | 3 | 53 ± 56 |
| October 2011 | 1 | 39 |
| November 2011 | 2 | 16 ± 7 |
| *Palo Alto Regional Water Quality Control Plant (PAWP)* | | |
| July 2011 | 6 | 41 ± 8 |
| January 2012 | 3 | 47 ± 27 |
| **Compound** | | **Relative Recovery (%)** |
| 6-OH-BDE-47 | 2 | 52 ± 0 |
| 6-OH-BDE-90 | 2 | 25 ± 2 |
| 6-OH-BDE-99 | 2 | 24 ± 2 |
| 6'-OH-BDE-100 | 2 | 96 ± 7 |
| 6'-OH-BDE-118 | 2 | 17 ± 7 |

**Table S5:** The relative recoveries of analytes of interest to $^{13}C_{12}$-triclosan for APCI Method and absolute recovery of $^{13}C_{12}$-triclosan for *n* number of replicates.

APCI Method

| **Wastewater Sample** | | **Absolute Recovery (%)** |
|---|---|---|
| *Metropolitan plant (MWP)* | n | $^{13}C_{12}$-Triclosan |
| September 2011 | 4 | 60 ± 19 |
| November 2011 | 3 | 68 ± 6 |
| *Palo Alto Regional Water Quality Control Plant (PAWP)* | | |
| July 2011 | 6 | 65 ± 11 |
| January 2012 | 6 | 22 ± 15 |
| *St. John's University (SJWP)* | | |
| January 2012 | 3 | 64 ± 32 |
| February 2012 | 3 | 135 ± 24 |
| **Compound** | | **Relative Recovery (%)** |
| 6-OH-BDE-47 | 3 | 66 ± 12 |
| 6-OH-BDE-90 | 3 | 54 ± 14 |
| 6-OH-BDE-99 | 3 | 48 ± 13 |
| 6'-OH-BDE-100 | 3 | 73 ± 32 |
| 6'-OH-BDE-118 | 3 | 48 ± 10 |

## Equations Used to Calculate Environmental Concentrations and Absolute and Relative Recoveries

The water concentrations above LOQ were calculated using isotope dilution analysis, an example is shown below for chemical ‘X’ using peak areas (PA) in standards (std) and samples. The response factor (RF) was calculated using:

$$RF = \frac{1}{[{}^{13}C_{12}X_{std}] \cdot m}$$

where ${}^{13}C_{12}X_{std}$ is the concentration of isotope labeled compound in standards and *m* is the calibration slope defined by:

$$m = \frac{X_{sample}^{PA}}{{}^{13}C_{12}X_{std}^{PA} \cdot [X_{std}]}$$

The concentration (μM) of X was calculated using:

$$X_{sample}(\mu M) = RF \times \frac{X_{sample}^{PA}}{{}^{13}C_{12}X_{sample}^{PA}} \times {}^{13}C_{12}X_{sample}(\mu M)$$

where ${}^{13}C_{12}X_{sample}$ is the amount of isotope labeled chemical spiked in the sample matrix. The relative recovery for each compound was calculated using the following equation:

$$RelativeRecovery = \frac{X_{spiked,sample} - X_{sample,avg}}{X_{spiked}} \times 100$$

Were $X_{spiked,sample}$ is the concentration in the spiked sample after SPE and silica column clean-up, $X_{sample,avg}$ is the average concentration in the sample, and $X_{spiked}$ is the concentration in the sample after being spiked. Water concentrations were corrected for relative recovery. The absolute recovery (AbsRec) of ${}^{13}C_{12}$-triclosan was calculated using:

$$AbsRec = \frac{{}^{13}C_{12}X_{sample}^{PA}}{{}^{13}C_{12}X_{std}^{PA}(avg)} \times \frac{[{}^{13}C_{12}X_{std}]}{[{}^{13}C_{12}X_{sample}]} \times 100$$

where ${}^{13}C_{12}X^{PA}_{std}$ (avg) is the average peak area of isotope labeled compound in standards.