CONTAMINANTS OF EMERGING CONCERN IN EFFLUENTS FROM WASTEWATER TREATMENT PLANTS IN THE LAKE SIMCOE WATERSHED

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1. Introduction

Contaminants of emerging concern (CECs), including personal care products, pharmaceuticals, and steroid hormones are known to be discharged in the effluents of wastewater treatment plants into receiving waters in Canada (Metcalfe et al., 2003; Lishman et al., 2006; Chen et al., 2006; Yang and Metcalfe, 2006; Lajeunesse et al., 2008; Schuerer et al., 2010; Metcalfe et al., 2010). Studies conducted in Canada have also reported CECs of wastewater origin in drinking water (Servos et al., 2007; Garcia-Ac et al., 2009; Kleywegt et al., 2011; Metcalfe et al., 2014). For this reason, CECs were identified as a key public health concern in the Lake Simcoe Protection Plan. Therefore, the objective of this study was to determine the concentrations of CECs in the effluents discharged from wastewater treatment plants (WWTPs) in the Lake Simcoe watershed.

CECs were measured in the influent and effluent of six WWTPs situated in the Lake Simcoe watershed. The WWTPs were selected to evaluate CEC discharges from WWTPs with different types of wastewater treatment,. The CECs monitored included selected “indicator compounds” that included personal care products, pharmaceuticals, steroid hormones, and an artificial sweetener. These target compounds were selected according to the criteria identified by Dickenson et al. (2011) in a study that illustrated the value of monitoring a selected number of CECs in wastewater treatment facilities in the U.S. The artificial sweetener, sucralose has been proposed as a tracer of wastewater contaminants in water samples (Mawhinney et al., 2011).

This study was performed using passive sampler technologies for *in situ* monitoring of CECs in wastewater. The Polar Organic Contaminants Integrative Sampler (POCIS) and Semi-Permeable Membrane Devices (SPMD) are designed, respectively, to sample water-soluble (polar or hydrophilic) and water-insoluble (hydrophobic) organic chemicals from aqueous environments. These integrative samplers provide estimates on time-weighted average concentrations of chemicals over the deployment period of several weeks. In comparison, traditional “grab” samples only provide data for the time of sampling and “composite” samples only monitor over a period of hours. In addition, because of the rapid temporal changes in the concentrations of PPCPs in wastewater, it is difficult to estimate removals of chemicals using data from “grab” samples or even composite samples (Ort et al., 2011). Sampling rates (Rs) for individual chemicals determined in the laboratory are used in conjunction with theoretical uptake models for the passive samplers to provide estimates of the ambient water concentrations of chemicals of concern.

2. Methods and Materials

2.1. Analytes and passive samplers

The indicator compounds selected for monitoring are summarized in Table 1, along with notation on whether they were monitored in POCIS or SPMD passive samplers. With the exception of HHCB, the target compounds were all matched to stable isotope surrogates (i.e. either deuterated or ^{13}C labelled compounds) that were spiked into the samples prior to analysis as internal standards. HHCB and AHTN were both matched to the surrogate, AHTN-d3.

The POCIS samplers were purchased from Environmental Sampling Technologies (St. Joseph, MO, USA). However, POCIS samplers were also prepared in the lab from HLB sorbent and membranes purchased from Environmental Sampling Technologies. These POCIS were spiked with performance reference compounds (PRCs) consisting of deuterated surrogates of the beta-blocker drugs, metoprolol, propranolol and atenolol. One of these spiked POCIS was included in each sampling cage with two other unspiked samplers.

The SPMDs were prepared in the lab from layflat polyethylene tubing containing triolein, as described previously (Helm et al., 2012). The triolein in the SPMDs was spiked with PRCs that were used to refine the R_S that vary according to the temperature and flow rates in the water and biofouling (Huckins et al., 2002). The PRCs were PCB congener 14 (3,5-dichlorobiphenyl) and PCB congener 32 (2,4',6-trichlorobiphenyl).

2.2. Sampling sites

The six WWTPs monitored in this study are all located in the Lake Simcoe watershed and discharge treated wastewater either directly into Lake Simcoe, or into surface waters that flow into Lake Simcoe. The sampling schedule, temperatures in treated wastewater over the sampling period and treatment processes for each WWTP are summarized in Table 2. The passive samplers were deployed for a period of 14 days in the treated wastewater stream prior to the discharge point. The passive samplers were placed in stainless steel sampling cages with capacity to accommodate three POCIS and three SPMD passive samplers; so each cage gave triplicate measurements. The sampling cages were deployed at the six WWTPs in either December 2012 or March, 2013, according to the dates provided in Table 2. WWTP 1 was sampled in both December and March.

2.3. Sample preparation

Extraction from POCIS samplers was performed in the lab according to the procedures described elsewhere (Li et al., 2010). Briefly, frozen samplers were removed from storage and allowed to thaw, then rinsed with water to remove debris and biofouling materials. The sorbent in the POCIS was transferred to a glass chromatography column (1 cm ID x 30 cm length) and 0.1 mL mixture of an internal standard (500 ng/mL) containing the stable isotope labelled surrogates of the analytes was then added to the column. Elution from the column was performed with 100 mL methanol. The eluate was collected and then evaporated in a rotary

evaporator to a volume of ~ 1 mL. Final evaporation to 0.1 mL was conducted using a vacuum centrifuge evaporator, and then the samples were made up to their final volume (0.4 mL) with methanol.

SPMD samples were prepared according to the procedures previously described by Helm et al. (2012). Briefly, the SPMD was cleaned and then dialyzed into hexane for 24 hrs. Labelled surrogates were added to the extract at this point. Co-extractives in the hexane extract were then removed by gel permeation chromatography (GPC) using BioBeads S-X3 (BioRad Laboratories, Mississauga, Ontario, Canada) with a mobile phase of hexane and ethyl acetate. The eluate was split into two aliquots. One aliquot was solvent exchanged into hexane for analysis of the synthetic musks and PRCs (i.e., Aliquot A) and the other aliquot was exchanged into methanol for analysis of triclosan (i.e., Aliquot B). Aliquot A was further fractionated by silica gel column chromatography as described by O'Toole and Metcalfe (2006) to yield Fraction 1 containing the PCB congeners 14 and 32 (i.e., PRCs) and Fraction 2 containing the synthetic musks, HHCB and AHTN. These fractions were solvent exchanged into iso-octane (0.150 mL final volume) prior to analysis by GC-MS.

2.4. Analysis

Carbamazepine, trimethoprim, sulfamethoxazole and androstenedione extracted from POCIS were analyzed by liquid chromatography and tandem mass spectrometry (LC-MS/MS) in positive ion mode using an API 3000 instrument purchased from Applied Biosystems Sciex (Concord, Ontario, Canada) equipped with an electrospray ionization (ESI) source. This system was equipped with a Series 200 autosampler from Perkin Elmer (Waltham, MA, USA), and pumps (LC-10AD), degasser (DGU-14A) and system controller (SCL-10A) from Shimadzu (Columbia, MD, USA).

For analysis of ibuprofen, gemfibrozil, naproxen, sucralose and estrone accumulated in the POCIS and for triclosan and bisphenol A accumulated in the SPMDs, LC-MS/MS analysis was conducted in negative ion mode using an AB Sciex Q-Trap 5500 instrument with an ESI source. The beta-blocker surrogates were also analyzed in negative ion mode using this instrument. This system was equipped with an Agilent 1100 series (Mississauga, ON, Canada) LC systems.

For LC-MS/MS analysis, the analytes were separated chromatographically using a Genesis C18 column that was 150 mm long, 2.1 mm ID and 4 µm particle size (Chromatography Specialities Inc., Brockville, Ontario, Canada) with a guard column (Genesis C18, 10 x 2.1 mm and 4 µm). MS detection was performed using multiple reactions monitoring (MRM). For quantification, an internal standard method with a five-point calibration graph covering the range of anticipated analyte concentration in the samples, using a weighted (1/concentration) linear regression. Internal standards (i.e. stable isotope labelled compounds) were used to correct for analyte recovery and matrix effects.

The synthetic musks (HHCB, AHTN and AHTN-d3) and the PRC compounds were analyzed with a GC-MS purchased from Agilent Technologies (Mississauga, Ontario. Canada) consisting of a Model 3800 GC and a Model 4000 Ion Trap MS. The GC-MS method using

electron impact (EI) ionization was similar to the method described by Yang and Metcalfe (2006). MS detection was performed using selected ion monitoring (SIM). Chromatographic separation was performed using a 30 m VF5 capillary column (0.25 mm ID). The injector, transfer line and ion trap temperatures were 275^{o}C, 250^{o}C and 200^{o}C, respectively. The temperature program for the column oven consisted of an initial hold for 1.5 min. at 50°C, then an increase to 150°C at 10°C/min, then to 190°C at 2°C/min, and finally to 290°C at 100°C/min, where the temperature was held for 4 min.

2.5. Time weighted average concentrations

The estimated concentrations of the target compounds accumulated in POCIS were calculated using the sampling rate (R_S) values listed in Table 3, which were previously determined in laboratory experiments at 15^{o}C (Li et al., 2010). The R_S of sucralose was recently reported by Metcalfe et al. (2014). The concentrations of the personal care products accumulated in SPMDs were calculated using the R_S values (Table 3) previously determined in laboratory experiments at 15^{o}C (Helm et al., 2012).

Table 1. Indicator compounds analyzed in POCIS and SPMD passive samplers.

	Compound	Class	Sampler
1	Carbamazepine – neutral drug	pharmaceutical	POCIS
2	Ibuprofen – acidic drug	pharmaceutical	POCIS
3	Gemfibrozil – acidic drug	pharmaceutical	POCIS
4	Naproxen – acidic drug	pharmaceutical	POCIS
5	Trimethoprim – antibiotic	pharmaceutical	POCIS
6	Sulfamethoxazole – antibiotic	pharmaceutical	POCIS
7	Estrone – estrogen	steroid hormone	POCIS
8	Androstenedione – androgen	steroid hormone	POCIS
9	Sucralose	artificial sweetener	POCIS
10	HHCB – synthetic musk	personal care product	SPMD
11	AHTN – synthetic musk	personal care product	SPMD
12	Triclosan – antibacterial	personal care product	SPMD
13	Bisphenol A– plasticizer	household product	SPMD

3. Results and Discussion

The estimated time weighted average concentrations (ng/L) of the indicator compounds in treated wastewater are summarized in Table 3. These data show that the estimated concentrations lof the CECs are generally lower in comparison to previously published data on the concentrations of these compounds in treated wastewater discharged from other Canadian WWTPs (Metcalfe et al., 2003; Lishman et al., 2006; Chen et al., 2006; Yang and Metcalfe, 2006). Indeed, many of the indicator compounds would not have been detectable in the treated wastewater (i.e. <2 ng/L) using conventional grab sampling or composite sampling techniques.

Table 2. Sampling dates, untreated wastewater (effluent) temperatures over the sampling period, and the treatment process for the six WWTPs monitored in this study. The WWTPs are listed according to the highest to lowest rated capacity in millions of litres per day.

WWTP #	Sampling Dates	Effluent Temp (^{o}C)	Treatment Process
1	Dec 5 to19 and Mar 21 to Apr 4	13.7 to 14.7 and 12.0 to 13.6	Oxygen activated sludge for secondary treatment, tertiary nitrifying rotating biological contactors, tertiary filtration by automatic backwash sand filters, and UV disinfection; phosphorus removal involves multi-point alum addition
2	Mar 21 to Apr 4	9.9 to 10.5	Conventional activated sludge for secondary treatment followed by UV disinfection; phosphorus removal involves dual-point alum addition
3	Mar 21 to Apr 4	15.7 to 17.1	Extended aeration and sequencing batch reactor processes configured in parallel for secondary treatment, followed by continuous backwash sand filters for tertiary treatment, and UV disinfection; phosphorus removal involves dual-point addition of alum as well as polymer
4	Dec 5 to19	12.2 to 13.4	Sequencing batch reactor for secondary treatment followed by continuous backwash sand filters for tertiary treatment, and UV disinfection; phosphorus removal involves dual-point alum addition
5	Dec 5 to19	13.2 to 13.6	Extended aeration for secondary treatment, followed by continuous backwash sand filters for tertiary treatment, and UV disinfection; phosphorus removal involves dual-point alum addition as well as polymer addition.
6	Dec 5 to 19	8.6 to 11.2	Extended aeration, followed by secondary clarifiers and tertiary clarifier, and UV disinfection.

Surprisingly, there were low estimated concentrations of bisphenol A (Table 3), which may reflect phasing out of this estrogenic pasticizer in many domestic and personal care products. The artificial sweetener, sucralose is a persistent compound that has been found at high concentrations (i.e. >100 ng/L) in treated wastewater from WWTPs in Germany (Schuerer et al., 2009). Our recent study indicates that this compound can be used as a tracer of wastewater contamination in sources of drinking water (Metcalfe et al., 2014). Carbamazepine is a persistent pharmaceutical that is known to show low removals in WWTPs (Miao et al., 2005). The relatively high concentrations of the antibiotic, trimethoprim may have been due to the winter sampling schedule during the season for the highest incidence of communicable diseases. HHCB and AHTN are the two synthetic musks that are typically found at the highest concentrations in treated wastewater (Yang and Metcalfe, 2006). The steroid hormones, estrone (estrogen) and androstenedione (androgen) were not detected in POCIS deployed in the treated wastewater (Table 3).

There was a trend of declining concentrations of some CECs in treated wastewater (e.g. HHCB, TCS, TMP) with the rated capacity of the treatment plant, but this trend was not observed with other compounds (e.g. carbamazepine, sucralose).

Table 3 : Estimated time weighted average concentrations (ng/L) of the indicator compounds in treated wastewater from the WWTPs monitored in the Lake Simcoe watershed. These concentrations were estimated from the mean amounts (n=3) accumulated in POCIS and SPMD passive samplers using sampling rates (Rs = L/d)) previously determined in laboratory experiments at 15^{o}C. IBP = Ibuprofen; GMF = Gemfibrozil; CBZ = Carbamazepine; NAP = Naproxen; TMP = Trimethoprim; SMX = Sulfamethoxazole; SCL = Sucralose; EST = Estradiol; ADS = Androstenedione; TCS = Triclosan; BPA = Bisphenol A.

Compound	Rs (15^{o}C)	WWTP1 (Dec)	WWTP1 (March)	WWTP2	WWTP3	WWTP4	WWTP5	WWTP6
IBP	0.254	4.4	2.6	0.4	2.8	1.3	0.6	1.3
GMF	0.306	0.8	1.2	0.8	2.4	ND	ND	0.2
CBZ	0.397	7.5	6.5	12.9	29.5	23.4	13.7	22.8
NAP	0.298	7.7	9.8	0.3	0.9	1.3	0.2	0.7
TMP	0.411	45.6	38.8	98.4	74.7	42.8	36.4	7.3
SMX	0.348	0.8	0.4	4.3	1.4	4.0	1.4	3.2
SCL	0.163	118.6	188.4	123.6	258.6	151.2	170.3	201.6
EST	0.636	ND	ND	ND	ND	ND	ND	ND
ADS	0.415	ND	ND	ND	ND	ND	ND	ND
TCS	4.9	7.9	7.4	7.3	1.6	1.2	0.3	1.3
BPA	5.2	<0.1	<0.1	ND	<0.1	ND	<0.1	<0.1
HHCB	4.3	1.3	2.1	2.4	1.9	0.9	0.8	0.8
AHTN	7.9	0.1	0.1	0.1	0.1	0.2	0.1	0.2

Overall, these data indicate that the WWTPs in the Lake Simcoe watershed are discharging relatively low concentrations of CECs in treated wastewater, which may reflect the fact that these treatment plants have installed advanced treatment technologies, primarily for the removal of phosphorus and other conventional pollutants. However, these data indicate that these technologies designed to improve wastewater quality for conventional parameters do not entirely remove contaminants of emerging concern.

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