Linking drugs of abuse in wastewater to contamination of surface and drinking water

Angela Rodayan[†], Shadi Afana[†], Pedro Alejandro Segura[†], Tamanna Sultana[‡], Chris D. Metcalfe[‡], Viviane Yargeau[†*]

[†]*Department of Chemical Engineering, McGill University, Montreal, QC, Canada*

[‡]*Water Quality Centre, Trent University, Peterborough, ON, Canada*

* Address correspondence to viviane.yargeau@mcgill.ca

Abstract

The concentrations of 17 drugs of abuse (DOAs), including cocaine, several amphetamine, opioid drugs and two metabolites, benzoylecgonine (BE) a metabolite of cocaine and 2-Ethylidene-1,5-Dimethyl-3,3-Diphenylpyrolidine (EDDP) a metabolite of methadone, were investigated in an urban watershed that is heavily impacted by discharges of municipal wastewater. The artificial sweetener, sucralose was also monitored as a persistent tracer of contamination from municipal wastewater. Monitoring was conducted in a municipal wastewater treatment plant (WWTP) and at sites upstream and downstream of the WWTP discharge, as well as in a drinking water treatment plant (DWTP) located 19 km downstream of the WWTP discharge that withdraws raw water from the river. Drug concentrations were monitored with Polar Organic Chemical Integrative Samplers (POCIS) deployed for 2 weeks in the river and in the WWTP and DWTP. Several of the investigated compounds exhibited a decrease in concentration with distance downstream from the wastewater discharge into the river, but there was little attenuation of sucralose, cocaine, BE, morphine, acetylmorphine (a-morphine),

Rodayan, A., Afana, S., Segura, P.A., Sultana, T., Metcalfe, C., Yargeau, V., **Linking drugs of abuse in wastewater to contamination of surface and drinking water**, *Environmental Toxicology and Chemistry*, Volume 35, Issue 4, 843-849 (April 2016)

acetylcodeine (a-codeine) and oxycodone. Heroin and methadone were not detected at any sample locations. Amphetamine (AMP), methamphetamine (METH), 3,4-methylenedioxy-methamphetamine (MDMA), and EDDP were not detected in the samples collected at the drinking water intake. Many of the DOAs were not removed effectively in the DWTP, including cocaine, BE, methylenedioxyamphetamine (MDA), ephedrine, and several prescription opioids, most probably because the DWTP was operating at or above its rated treatment capacity. These data indicate that there can be transport of DOAs from wastewater sources into drinking water in urban watersheds.

Keywords: Cocaine, amphetamines, opioids, POCIS, drugs of abuse

1 INTRODUCTION

Drugs of abuse (DOAs), including both illicit drugs and prescription drugs are contaminants of emerging concern (CECs) that have been detected in wastewater and surface waters [1]. After excretion as either the parent compound or as metabolites, these classes of drugs make their way to wastewater treatment plants (WWTPs). Many studies have investigated the presence of DOAs in wastewater and it is clear that many of these compounds are not completely removed during conventional wastewater treatment and that they are released into surface waters via effluent discharges [2-9]. DOAs have potent biological activities and they may impact aquatic organisms, either alone or as mixtures with other pharmaceuticals [10, 11]. Pharmaceuticals can be transformed through biological, chemical or photochemical processes or can be adsorbed, depending on the nature of the compound [12, 13]. In areas where surface

Rodayan, A., Afana, S., Segura, P.A., Sultana, T., Metcalfe, C., Yargeau, V., **Linking drugs of abuse in wastewater to contamination of surface and drinking water**, *Environmental Toxicology and Chemistry*, Volume 35, Issue 4, 843-849 (April 2016)

47 waters are used as sources of drinking water, DOAs may also contaminate drinking water [14,
48 15]. Wastewater reuse is becoming more common and there is concern about the presence of
49 DOAs in water supplies [16].

50

51 Since concentrations of CECs in the aquatic environment are subject to large temporal
52 variations, passive sampling has been used to provide estimates of the time weighted average
53 (TWA) concentrations of compounds over several days or weeks of sampler deployment [17].
54 The Polar Organic Chemical Integrative Sampler (POCIS) was developed by Alvarez et al. [18]
55 to sequester hydrophilic organic chemicals from aquatic matrices, and TWA concentrations can
56 be calculated using sampling rates (R_S in L d^{-1}) measured experimentally for each target analyte.
57 POCIS have been shown to be effective at sampling pharmaceuticals and pesticides from water
58 [19, 20] but relatively few studies have used POCIS to estimate the concentrations of DOAs in
59 water and wastewater [16, 21-23].

60

61 The objective of this study was to evaluate the transport of DOAs from wastewater to
62 drinking water in an urban watershed. A range of DOAs, including cocaine and its primary
63 metabolite (BE), EDDP a metabolite of methadone, amphetamines and opioid compounds were
64 monitored as they were transported through a WWTP, carried downstream in a river from the
65 point of wastewater discharge, and subsequently taken up and treated in a downstream drinking
66 water treatment plant (DWTP). The study area was the Grand River in southern Ontario, Canada,
67 which receives effluents from several municipal WWTPs, and also serves as a source of drinking
68 water for several communities [24, 25]. Our hypothesis is that these compounds are persistent

Rodayan, A., Afana, S., Segura, P.A., Sultana, T., Metcalfe, C., Yargeau, V., **Linking drugs of abuse in wastewater to contamination of surface and drinking water**, *Environmental Toxicology and Chemistry*, Volume 35, Issue 4, 843-849 (April 2016)

enough in aquatic systems and may potentially impact the quality of drinking water. To test this hypothesis, we compared the concentrations of DOAs to the concentrations of an artificial sweetener, sucralose that is known to be a persistent tracer of wastewater contamination [26, 27] and has been detected previously at high concentrations in the Grand River [28].

2 MATERIALS AND METHODS

2.1 Chemicals, reagents and supplies

Analytical standards of all DOAs and their deuterated analogs (list of compounds provided in the Supplemental Data, Table S1) were obtained from Cerilliant at a purity higher than 99%. Sucralose and its deuterated surrogate were purchased from Sigma-Aldrich and Toronto Research Chemicals, respectively. Stock solutions were made up in methanol and stored in the refrigerator (4^0C) until required. Working standard solutions were prepared from stock solutions through serial dilution.

HPLC or equivalent grades of methanol, acetone, acetonitrile, ACS reagent grade dichloromethane (DCM), hydrochloride acid (37%), sulphuric acid (96%), formic acid (88%), and trace metal grade ammonium hydroxide (88%) and sodium sulfate were purchased from Fisher Scientific. Optima grade methanol, acetonitrile, and water were also purchased from Fisher Scientific. All other water was obtained from a Milli-Q water purification system purchased from Millipore. Whatman 1.5 μm glass microfiber filters were purchased from Fisher Scientific. Oasis MCX cation exchange cartridges (6 mL/150 mg) were purchased from Waters

Rodayan, A., Afana, S., Segura, P.A., Sultana, T., Metcalfe, C., Yargeau, V., **Linking drugs of abuse in wastewater to contamination of surface and drinking water**, *Environmental Toxicology and Chemistry*, Volume 35, Issue 4, 843-849 (April 2016)

90 Corporation. Pharmaceutical POCIS containing Oasis HLB (200 mg) sequestration medium were
91 purchased from Environmental Sampling Technologies.

92

93 *2.2 Samples*

94 POCIS samplers were deployed over a two week period from July 10-24, 2012 at locations
95 in the Grand River watershed in southern, Ontario, Canada. POCIS monitoring was conducted at
96 a WWTP in the treated wastewater stream, at sites in the Grand River upstream (2 locations) and
97 downstream (3 locations) of the WWTP discharge, and in the raw and treated water streams of a
98 DWTP that uses the river as a source of water. POCIS (n=3 per location) were deployed on July
99 10 and retrieved two weeks later. Grab samples were collected in 1 L pre-cleaned amber glass
100 bottles on the days of POCIS deployment and retrieval in order to compare the concentrations of
101 target compounds detected using both methods. The WWTP treats wastewater for an urban
102 population of approximately 94,000 using conventional activated sludge treatment and has a
103 maximum flow capacity of 81,800 $m^3 d^{-1}$. Surface water samples were collected and POCIS were
104 deployed in the Grand River at two points approximately 2 km apart located upstream of the
105 WWTP discharge and at three downstream sites approximately 40 m downstream of the
106 discharge, and at 10 km and 18 km further downstream. The furthest downstream site was only
107 20 m from the intake for the DWTP. The small DWTP serves a population of approximately
108 1,800 people and the design flow is 1.6×10^6 L d^{-1}. The plant uses a limited treatment train
109 consisting of coagulation, flocculation, filtration and chlorine disinfection. POCIS were deployed
110 in the treatment stream for both raw and treated drinking water.

111

112 POCIS samplers were kept in air-tight canisters prior to deployment. A total of 6 POCIS
113 were deployed at each site in a stainless steel sampling cage; three POCIS for monitoring DOAs
114 and three POCIS for monitoring sucralose. POCIS field blanks were exposed to ambient air
115 during the deployment and retrieval of the POCIS samplers. Upon retrieval, each POCIS disk
116 was individually wrapped in aluminum foil and stored in a sealable plastic bag and placed on ice
117 during transportation. Following collection, samples were stored in a cooler at 4°C for transport
118 to the laboratory, where they were then stored at -20°C until extraction, which was carried out
119 within one month of collection.

120

121 *2.3 Extraction*

122 POCIS samplers were extracted according to methods previously described by Li et al.
123 [29]. Briefly, frozen samplers were removed from storage and allowed to thaw, then rinsed with
124 water to remove debris and biofouling material. The sorbent in the POCIS was transferred
125 manually to a glass chromatography column previously packed to 1/3 full with granular Na_2SO_4.
126 Deuteurated analogs were then added to the column as surrogates. Elution from the column was
127 performed with 100 mL methanol. After evaporation, the samples were made up to their final
128 volume (0.4 mL) with methanol. The DOAs and sucralose were sampled in individual POCIS
129 that were extracted separately. Extraction efficiencies of all compounds in the three matrices
130 were >80% (Supplemental Data, Table S1).

131

132 Grab samples were extracted in triplicate by solid phase extraction (SPE) with Oasis MCX
133 cartridges using previously developed methods. The DOAs and sucralose were extracted

separately using two different methods. Prior to SPE, all aqueous samples were vacuum filtered through 1.5 μm glass fibre filters. For untreated wastewater samples, 100 mL volumes were filtered, whereas for treated wastewater, surface water, raw and treated drinking water samples, a volume of 200 mL was used. All samples were acidified to a pH of 2.5 using 3.5 M sulphuric acid and then spiked with 100 μL of 500 ng mL^{-1} surrogate standard mixture. For DOAs, SPE was carried out using a Gilson GX-271 ASPEC^{TM} automated extraction system. The extraction method was based on our previously published method for illicit drugs [25] that was modified to include opioid drugs [22]. Sucralose was extracted by SPE using the manual SPE method previously described by [30]. Extracts were evaporated and reconstituted to a volume of 0.4 mL in 25% water/75% methanol. SPE recoveries for all compounds in each of the three matrices were >80% (Supplemental Data, Table S1).

2.4 POCIS sampling rates

Sampling rates (Rs) for the most DOA target compounds were previously reported by Yargeau et al. [22] and are listed in the Supplemental Data, Table S1. Note that R_s values for acetylcodeine, acetylmorphine and heroin could not be determined, which is probably due to the highly polar nature of these compounds [22]. The sampling rate for sucralose was previously described by Metcalfe et al. [30]. The POCIS sampling rates for ketamine (0.197±0.007 Ld^{-1}) and fentanyl (0.390±0.051 Ld^{-1}) are reported here for the first time.

The sampling rates (R_s) were determined as previously described by Li et al. [29] using bench scale experiments with static exposure conditions where the decrease in concentration of the compounds in water was monitored over time. Briefly, the static experiments were

Rodayan, A., Afana, S., Segura, P.A., Sultana, T., Metcalfe, C., Yargeau, V., **Linking drugs of abuse in wastewater to contamination of surface and drinking water**, *Environmental Toxicology and Chemistry*, Volume 35, Issue 4, 843-849 (April 2016)

conducted in triplicate in containers with 3 L of water placed in a temperature controlled environmental chamber at 25°C. For each replicate, the water was spiked with the compounds of interest at a nominal concentration of 3 μg L^{-1} and a single POCIS was placed in the water for a period of 3 days. A magnetic stirrer was used to gently mix the water. Aliquots of the exposure water (40 mL) were removed from the bottles every 24 h to monitor the decrease in water concentration over time. The water was extracted by SPE according to the methods described above. Control experiments containing only fortified water without the POCIS (i.e. positive control) were run along with the calibration to correct for sorption, volatilization or degradation during exposure. As a negative control, one POCIS was exposed to 3 L of water without spiking of pharmaceuticals. The R_s of the POCIS was calculated from the slope of the line describing the decline in concentration over time. As in previous experiments to determine sampling rates with this static exposure method [19, 29], the sampling rates were confirmed by comparing estimates of the mass of the target compounds accumulated on the sorbent over the 3 day experiment to the measured mass of the compound extracted from the POCIS at the end of the experiment. The estimates of the mass accumulated on the POCIS over time, $m_s(t)$ were determined by rearranging the equation presented by Allan et al. [32] to yield:

$$m_s(t) = m_s(0) + C_w R_s t$$

Where: The amount of the analyte on the sorbent at time 0, $m_s(0)$, which was determined from control experiments to be zero, C_w is the initial concentration in the water, R_s is the estimated sampling rate, and t = 3 days. These data for fentanyl and ketamine are illustrated in Supplemental Data, Figure S1. The results indicate good agreement between the estimated and measured amounts of the target compounds retained by the POCIS sorbent. Further, these data

indicate that the amounts of the target compounds retained by the POCIS membrane (not extracted) are negligible relative to the amount retained by the solid sorbent.

2.5 Analysis

The concentrations of DOAs were measured by liquid chromatography with high resolution mass spectrometry (LC-HRMS) using an Accela LC system coupled to a hybrid linear ion trap-orbital trap instrument, LTQ Orbitrap XL (Thermo Fisher Scientific, Waltham, MA). Chromatographic separation of the target compounds was achieved using a Hypersil Gold column (50 x 2.1 mm, 1.9 μm) with an in-line Direct-Connection UHPLC 0.2 μm filter held at a temperature of 30 °C. An optimized gradient of methanol in water (solvent A) and acetonitrile (Solvent B) both with 0.1% acetic acid at a flow of 0.3 $mLmin^{-1}$ was used. The percentage of organic (B) was changed as follows: 0 min (10%), 1.75 min (10%), 3.05min (25%), 5.55 min (97%), 7.55 min (97%), 7.75 min (10%), 16.00 min (10%). Ionization was done in positive mode using a heated electrospray ionization (HESI) source with the following parameters: sheath gas flow = 45 arbitrary units, auxiliary sheath gas flow = 10 arbitrary units, capillary temperature = 375°C, capillary voltage = 5 V, tube lens = 100 V. Acquisition was performed in full scan mode (*m/z* 50-400) at high resolution (R_{FWHM} = 41 000) and analyte quantification was carried out by extracting the ion of interest from the orbital trap total ion current chromatogram using an *m/z* window of ±0.01. Confirmation of the presence of the target analyte was done by collision-induced mass spectra using a data dependent tandem mass spectrometry experiment in the linear ion trap portion of the instrument. Quantification was done using an eight-point calibration curve generated for each compound in the range of 3 to 150 μg L^{-1}, corresponding to the range of concentration obtained after preconcentration, as well as the linear correlation coefficients. The

200 deuterated stable isotope surrogates were used as internal standards at a constant concentration of
201 100 μg L^{-1} to correct for mass losses during sample preparation, as well as matrix effects during
202 analysis.

203

204 Analysis of sucralose was conducted by LC coupled with tandem mass spectrometry (LC-
205 MS/MS) in negative ion mode using an AB Sciex Q-Trap 5500 instrument with a turbospray
206 ionization source, and equipped with an Agilent 1100 series (Mississauga, ON, Canada)
207 separation system. Sucralose was separated chromatographically using a Genesis C-18 column
208 and a guard column of the same stationary phase (Chromatographic Specialties, Brockville, ON,
209 Canada). The LC mobile phases for gradient elution were described previously by Metcalfe et al.
210 [30]. Multiple reaction monitoring (MRM) was performed using the precursor and product ion
211 transitions of 395→359 and 395→35 for sucralose, and 403→367 and 403→35 for the
212 corresponding labelled surrogate, sucralose-d6. An external standard method with a five-point
213 calibration curve was used for quantification, and the data were adjusted according to response
214 for the surrogate internal standard.

215 *2.6 Method validation*

216 The limits of detection (LODs) and limits of quantification (LOQs) for the target
217 compounds in wastewater, surface water and drinking water were defined as the analyte
218 concentration that produced a peak with a signal to noise ratio of 3 and 10, respectively. All
219 method validation was done using spiked samples of each matrix. The LODs and LOQs of the
220 target compounds in each matrix are listed in the Supplemental Data, Table S2. The repeatability
221 between runs on the same day and between days was always <15%. Quality control samples in

Rodayan, A., Afana, S., Segura, P.A., Sultana, T., Metcalfe, C., Yargeau, V., **Linking drugs of abuse in wastewater to contamination of surface and drinking water**, *Environmental Toxicology and Chemistry*, Volume 35, Issue 4, 843-849 (April 2016)

each matrix were included in sample runs and the relative error between the measured concentration and the expected concentration was always <20% for all target compounds. Field blank POCIS were processed exactly as described for the deployed POCIS and none of these samples contained residues of the analytes at levels above the limits of detection.

3 RESULTS AND DISCUSSION

3.1 Overview of the results

The concentrations of the analytes in the Grand River at sites upstream and downstream of the WWTP discharge, as well as the concentrations in treated wastewater in the WWTP and in raw and treated drinking water in the DWTP are summarized in Figure 1. These data are the estimated TWA concentrations from the POCIS (n=3). The values of the average concentrations and corresponding standard deviations are provided in the Supplemental Data, Table S3 along with the concentrations measured in grab samples collected at deployment and retrieval. The concentrations measured in grab samples were used only to provide a comparison point to determine if POCIS was an effective monitoring technique, despite the potential for biofouling of the samplers or blockage of the deployment cages. Concentrations of some analytes measured in grab samples were lower than TWA concentrations estimated from POCIS, consistent with the results of a study by Jones-Lepp et al. [16]. In some cases, analytes were detected or quantifiable in POCIS but not in the corresponding grab samples, which illustrates the value of passive sampling for concentrating trace contaminants to detectable levels, and the importance of effective sampling strategies. Overall, the results are in good agreement and no significant variations were observed in the grab samples collected at deployment and retrieval.

Rodayan, A., Afana, S., Segura, P.A., Sultana, T., Metcalfe, C., Yargeau, V., **Linking drugs of abuse in wastewater to contamination of surface and drinking water**, *Environmental Toxicology and Chemistry*, Volume 35, Issue 4, 843-849 (April 2016)

244 Concentration observed in upstream samples can be explained by the presence of upstream
245 WWTPs on discharging their treated effluent in the same river.

246

247 Considering that our previous studies showed that POCIS underestimated the
248 concentrations of DOAs in untreated wastewater (Metcalfe et al., 2010), we did not deploy
249 POCIS in the untreated wastewater stream and so, did not attempt to calculate removals of DOAs
250 within the WWTP. However, our previous studies of monitoring for pharmaceuticals in DWTPs
251 using POCIS showed that this monitoring technique provides reliable estimates of time-weighted
252 average concentrations in treated and untreated drinking water that are free of the biases from
253 grab sample monitoring caused by the residence time in the DWTP (Metcalfe et al., 2014). Table
254 1 presents the average removals (%) of each compound during drinking water treatment. The
255 statistical significance of the values, identified by an asterisk, was determined using the Software
256 Prism 6 and unpaired t-tests. This small DWTP was operating above its designed capacity during
257 the sampling campaign and so therefore, cannot be considered as representative of the drinking
258 water treatment in most developed countries. However, these results indicate that, under
259 conditions of sub-optimal treatment, contamination of surface water with DOAs of wastewater
260 origin can impact the quality of treated drinking water due to low removals of these compounds.

261 *3.2 Cocaine, BE and sucralose*

262 Cocaine and its major metabolite BE were detected at all monitoring locations with both
263 sampling techniques (Figure 1a). These compounds were detected at the two stations upstream of
264 the WWTP discharge, which illustrates that the Grand River is impacted by discharges of CECs
265 from several upstream WWTPs. A decrease in the level of cocaine was observed between the

two upstream sites. Since there was not a significant decline in the concentrations of sucralose at these two upstream locations (Figure 1b), the loss of cocaine may be explained by microbial or photolytic degradation of cocaine to transformation products [15, 33]. The ratios of BE to cocaine were approximately 1 at the two upstream locations.

The ratios of BE to cocaine in surface water were between 2.6 and 4.2 downstream of the WWTP discharge (Figure 2), which is within the range of values observed by others (Van Nuijs et al. 2009b). There was a trend to declining ratios of BE to cocaine with distance downstream in the river for data generated from both the grab samples and the POCIS (Figure 2). Since the ratios were similar for both POCIS and grab sample data, we can rule out that this trend was due to preferential adsorption of one of the two compounds onto the POCIS sorbent. These ratio data suggest that cocaine might be more persistent relative to BE. Since it has been shown that cocaine is more susceptible to microbial degradation than BE in aqueous matrices [6, 34], it may be that BE is lost through other fate processes, such as sorption to particulates, volatilization or photodegradation. For example Bijlsma et al. [9] showed that BE is removed in WWTPs slightly more efficiently than cocaine (90% for BE compared to 79% for cocaine); possibly through transport to sludge. Further studies are required to investigate the processes responsible for these changes in the ratios of cocaine and BE during wastewater treatment and in natural surface waters. The concentrations of BE and cocaine remained stable with distance downstream of the WWTP discharge (Figure 1a). This is not consistent with the data for sucralose, which showed a decline in concentrations between the first (561 ng L^{-1}) and second (260 ng L^{-1}) downstream sites, but then stable concentrations further downstream (Figure 1b).

The concentrations of cocaine and BE observed in all surface water samples were lower than the maximum concentrations reported in Europe. For instance, cocaine and BE were detected in surface water at concentrations between 10 and 111 ng L^{-1} in Spain [35], at maximum concentrations of 115 and 520 ng L^{-1}, respectively in Belgium [36], and at maximum concentrations of 78 to 92 ng L^{-1}, respectively in south Wales [35-37]. The concentrations of sucralose in the Grand River (i.e. >100 ng L^{-1}) are consistent with previous studies [26, 28, 38].

Considering that the concentrations of cocaine and BE (4.2 and 10 ng L^{-1}, respectively) in the treated drinking water were not significantly different than the influent concentrations, removals in the DWTP could not be calculated. In contrast, a significant removal of 49% was found for sucralose in the DWTP (Table 1). A study conducted at a DWTP in Spain equipped with chlorination, ozonation and granular activated carbon filtration reported overall removal efficiencies for cocaine and BE of 100% and 89%, respectively, and BE was detected in the treated drinking water at mean concentrations of 45 ng L^{-1}, with a maximum of 130 ng L^{-1} [35]. However, Boleda et al. [14] investigated tap water from DWTPs with different treatment technologies in several countries and found that BE and cocaine were present in treated drinking water at maximum concentrations of 15 and 2.9 ng L^{-1}, respectively and that the removal of these compounds is a strong function of the treatment technologies available. The DWTP monitored in the present study was operating at flows close to or greater than the maximum design capacity of the plant and it did not employ advanced treatment technologies, such as ozonation and activated carbon filtration. Therefore, it is not surprising that removals of sucralose, cocaine and BE were relatively low. In addition, van der Aa et al. [39] reported that only BE was detected in finished

Rodayan, A., Afana, S., Segura, P.A., Sultana, T., Metcalfe, C., Yargeau, V., **Linking drugs of abuse in wastewater to contamination of surface and drinking water**, *Environmental Toxicology and Chemistry*, Volume 35, Issue 4, 843-849 (April 2016)

311 drinking water in a study preformed in the Netherlands, at a concentration below its limit of
312 detection.

313

314 *3.3 Amphetamines*

315 Amphetamine, MDA and ephedrine were detected at concentrations >LOQ in surface
316 water upstream (4 to 24 ng L^{-1}) and downstream (6 to 49 ng L^{-1}) of the WWTP discharge (Figure
317 1c). There is a trend of declining concentrations of ephedrine with distance downstream of the
318 WWTP discharge (Figure 1c), indicating possible degradation of these compounds in surface
319 waters or sorption to river sediments, considering that no such decline in concentration was
320 observed for sucralose. The levels of ephedrine measured in the present study are in agreement
321 with those reported by Valcárcel et al. [15], who detected ephedrine at concentrations between
322 1.76 and 14.70 ng L^{-1} in a river in Spain. Postigo et al. [5] reported an ephedrine concentration of
323 145 ng L^{-1} in another river in Spain. The higher levels of ephedrine in surface water, compared to
324 other amphetamine stimulants are probably due to its widespread use as a decongestant and
325 possibly in herbal medicines, high rate of excretion of the parent compound from the human
326 body (22-99%) and incomplete removal during wastewater treatment [5]. Amphetamine was
327 detected in surface waters at 6.8 and 3.38 ng L^{-1} in Spain (Vazquez-Roig et al. 2010), but was not
328 detected in a study in the USA [5, 40, 41], which is consistent with the data obtained in the
329 present study. MDMA and methamphetamine were not detected in treated wastewater and in
330 surface water in the present study, which may be due to low usage of Ecstasy as an illicit drug in
331 this urban area.

332

Rodayan, A., Afana, S., Segura, P.A., Sultana, T., Metcalfe, C., Yargeau, V., **Linking drugs of abuse in wastewater to contamination of surface and drinking water**, *Environmental Toxicology and Chemistry*, Volume 35, Issue 4, 843-849 (April 2016)

For samples collected at the DWTP, amphetamine, methamphetamine and MDMA were not detected in any samples (untreated or treated). MDA and ephedrine were detected above their respective LOQs. Removals from drinking water during treatment could not be estimated because there was no significant difference in the concentrations in raw and treated drinking water. Few studies have investigated the presence of amphetamine stimulants in drinking water, but one study conducted in Europe showed that all amphetamines studied were completely removed during drinking water treatment [35].

3.4 Opioids

Methadone was not present at concentrations >LOQs in any samples and therefore, this compound was not included in Figure 1d. Heroin was not detected at concentrations above either its LOQ or LOD. As we previously did not detect heroin in 24-h composite samples from two WWTPs in Canada [22], it is likely that the results are not due to low uptake by the passive samplers, but rather due to a rapid rate of transformation. Low concentrations of this compound may be explained by its biotransformation to 6-acetylmorphone and morphine [42].

Fentanyl was detected in all samples (Figure 1d), despite its high excretion in humans as norfentanyl (26-55%) and its tendency to partition into the sludge phase [4, 43]. The low concentration of oxycodone and the high levels of fentanyl and ketamine in wastewater are significant from the perspective of drug abuse in the province of Ontario, Canada. The Oxycontin® formulation of oxycodone was banned in Ontario in 2012 because of concerns that this form of the drug, which can be easily crushed and dissolved in water for intravenous

Rodayan, A., Afana, S., Segura, P.A., Sultana, T., Metcalfe, C., Yargeau, V., **Linking drugs of abuse in wastewater to contamination of surface and drinking water**, *Environmental Toxicology and Chemistry*, Volume 35, Issue 4, 843-849 (April 2016)

injection, was contributing to high overdose deaths from abuse of this prescription pain killer [44]. Subsequent to this ban, drug enforcement agencies in Ontario reported an increase in the use of ketamine and fentanyl, and indeed there has been a rise in deaths as a result of fentanyl overdoses, as reported in 2012 by the Office of the Coroner for Ontario.

The concentrations of the opioid target compounds in surface water did not show any clear patterns between sampling points (Figure 1d). The concentrations of fentanyl, codeine and ketamine decreased downstream of the WWTP discharge, while the concentrations of morphine, acetylmorphine, acetylcodeine, dihydrocodeine, tramadol and oxycodone did not show any clear trends. These data may indicate that the latter compounds are not rapidly degraded in surface water. Of particular interest are the data for tramadol, which showed declining concentrations in surface water downstream of the WWTP discharge (Figure 1d). This was also observed in a study conducted in German surface waters where levels of tramadol were <25 to 381 ng L^{-1} and declined with distance from the WWTP discharge [45]. Indirect photodegradation has been shown to be the main process responsible for the removal of tramadol in surface water under ambient conditions [46]. EDDP was not detected in any surface water samples in the present study, although these compounds have been detected in river water samples in other studies [2, 15].

Several of the opioids were not detected, or were detected at concentrations below the LOQ in the untreated and treated drinking water. Removals could not be calculated for these compounds, including amphetamine, methamphetamine, MDMA, methadone and EDDP.

377 Several other opioids were detected in POCIS deployed in both untreated and treated drinking
378 water, including fentanyl, ketamine, oxycodone, tramadol, morphine and codeine and
379 dihydrocodeine. Using the POCIS data, removals could be calculated for compounds where there
380 were statistically significant differences in the TWA concentrations in raw and treated drinking
381 water; specifically for codeine (25%) and ketamine (49%). In studies conducted in Europe on
382 opioids in drinking water, only methadone and EDDP were detected at concentrations >LOQs [2,
383 14]. The high number of opioid compounds detected in the current studies using POCIS
384 demonstrates the value of using passive sampling techniques to monitor for trace quantities of
385 CECs in drinking water.

386

387

388 # 4 CONCLUSIONS

389 The results of the present study demonstrate for the first time that drugs of abuse are
390 present in Canadian surface waters impacted by wastewater discharges. The data also provide
391 evidence that there is downstream transport of these compounds, such that wastewater discharges
392 into surface waters have the potential to contaminate downstream sources of drinking water with
393 drugs of abuse; albeit at relatively low concentrations.

394

395 Analysis of surface water samples indicated that the concentrations of cocaine, BE,
396 morphine, acetylmorphine, dihydrocodeine and acetylcodeine and oxycodone did not decline in
397 the Grand River with distance downstream from the WWTP discharge, which was consistent
398 with the persistence of sucralose in the watershed. Overall, we accept our original hypothesis that

Rodayan, A., Afana, S., Segura, P.A., Sultana, T., Metcalfe, C., Yargeau, V., **Linking drugs of abuse in wastewater to contamination of surface and drinking water**, *Environmental Toxicology and Chemistry*, Volume 35, Issue 4, 843-849 (April 2016)

399 at least some of these DOAs are persistent in aquatic systems and surface water contamination
400 associated with wastewater discharges may impact the quality of drinking water in urban
401 watersheds.

402 5 SUPPLEMENTAL DATA

403 **Figure S1.** Comparison of estimated and measured amounts of the target compounds retained by
404 the POCIS sorbent

405 **Table S1.** Chemical formulae, corresponding deuterated analogs, SPE recoveries, POCIS
406 extraction efficiencies and POCIS sampling rates of target compounds

407 **Table S2.** Limits of detection (LODs) and limits of quantification (LOQs) in wastewater, surface
408 water and drinking water

409 **Table S3.** Mean concentration (n=3, ±SD) in ngL^{-1} estimated from amounts accumulated in
410 POCIS, or determined from analysis of grab samples collected at the time of deployment and
411 retrieval of the POCIS, respectively.

412

413 6 ACKNOWLEDGEMENTS

414 We gratefully acknowledge the assistance of the staff at the wastewater and drinking water
415 treatment plants for aid in collecting the samples. We also acknowledge the help of G. Tetreault
416 from the University of Waterloo for field work assistance and B. McIlwain of Trent University
417 for her help with sample preparation. This work was supported by the Natural Sciences and
418 Engineering Council (NSERC) of Canada through a Strategic Grant (PI: V. Yargeau) and the

Ministry of Research and Innovation for the Province of Ontario for the work on artificial sweeteners (PI: C. Metcalfe). We also thank NSERC, Fonds québécois de la recherche sur la nature et les technologies (FRQ-NT) and the Eugenie Ulmer Lamothe Fund scholarships awarded to A. Rodayan, P.A. Segura and S. Afana.

7 REFERENCES

1. Daughton, CG, 2011. Illicit drugs: Contaminants in the environment and utility in forensic epidemiology. *Rev Environ Contam T*, 210: p. 59-110.
2. Boleda, MR, MT Galceran, and F Ventura, 2009. Monitoring of opiates, cannabinoids and their metabolites in wastewater, surface water and finished water in catalonia, spain. *Water Res*, 43(4): p. 1126-1136.
3. Chiaia, AC, C Banta-Green, and J Field, 2008. Eliminating solid phase extraction with large-volume injection lc/ms/ms: Analysis of illicit and legal drugs and human urine indicators in us wastewaters. *Environ Sci Technol*, 42: p. 8841-8848.
4. Huerta-Fontela, M, MT Galceran, J Martin-Alonso, and F Ventura, 2008a. Occurrence of psychoactive stimulatory drugs in wastewaters in north-eastern spain. *Sci Total Environ*, 397(1-3): p. 31-40.
5. Postigo, C, MJ Lopez de Alda, and D Barcelo, 2010. Drugs of abuse and their metabolites in the ebro river basin: Occurrence in sewage and surface water, sewage treatment plants removal efficiency, and collective drug usage estimation. *Environ Pollut*, 36(1): p. 75-84.
6. Zuccato, E, S Castiglioni, R Bagnati, C Chiabrando, P brassi, and R Fanelli, 2008. Illicit drugs, a novel group of environmental contaminants. *Water Res*, 42: p. 961-968.
7. Loganathan, B, M Phillips, H Mowery, and TL Jones-Lepp, 2009. Contamination profiles and mass loadings of macrolide antibiotics and illicit drugs from a small urban wastewater treatment plant. *Chemosphere*, 75(1): p. 70-77.
8. Terzic, S, I Senta, and M Ahel, 2010. Illicit drugs in wastewater of the city of zagreb (croatia) – estimation of drug abuse in a transition country. *Environmental Pollution*, 158(8): p. 2686-2693.
9. Bijlsma, L, E Emke, F Hernández, and P de Voogt, 2012. Investigation of drugs of abuse and relevant metabolites in dutch sewage water by liquid chromatography coupled to high resolution mass spectrometry. *Chemosphere*, 89(11): p. 1399-1406.
10. Brodin, T, J Fick, M Jonsson, and J Klaminder, 2013. Dilute concentrations of a psychiatric drug alter behavior of fish from natural populations. *Science*, 339(6121): p. 814-815.

11. De Lange, HJ, W Noordoven, AJ Murk, M Lürling, and ETHM Peeters, 2006. Behavioural responses of gammarus pulex (crustacea, amphipoda) to low concentrations of pharmaceuticals. *Aquatic Toxicology*, 78(3): p. 209-216.

12. Castiglioni, S, R Bagnati, R Fanelli, F Pomati, D Calamari, and E Zuccato, 2006. Removal of pharmaceuticals in sewage treatment plants in italy. *Environ Sci Technol*, 40: p. 357-363.

13. Tixier, C, HP Singer, S Oellers, and SR Müller, 2003. Occurrence and fate of carbamazepine, clofibric acid, diclofenac, ibuprofen, ketoprofen, and naproxen in surface waters. *Environ Sci Technol*, 37(6): p. 1061-1068.

14. Boleda, MR, M Huerta-Fontela, F Ventura, and MT Galceran, 2011. Evaluation of the presence of drugs of abuse in tap waters. *Chemosphere*, 84: p. 1601-1607.

15. Valcárcel, Y, F Martínez , S González-Alonso, Y Segura, M Catalá, R Molina, JC Montero-Rubio, N Mastroianni, M López de Alda, C Postigo, and D Barcelo, 2012. Drugs of abuse in surface and tap waters of the tagus river basin: Heterogeneous photo-fenton process is effective in their degradation. *Environ Int*, 41: p. 35-43.

16. Jones-Lepp, TL, C Sanchez, DA Alvarez, DC Wilson, and R-L Taniguchi-Fu, 2012. Point sources of emerging contaminants along the colorado river basin: Source water for the arid southwestern united states. *Sci Total Environ*, 430: p. 237-245.

17. Kot-Wasik, A, B Zabiegala, M Urbanowicz, E Dominiak, A Wasik, and J Namiesnik, 2007. Advances in passive sampling in environmental studies. *Anal Chim Acta* 602: p. 141-163.

18. Alvarez, DA, JD Petty, JN Huckins, TL Jones-Lepp, DT Getting, JP Goddard, and SE Manahan, 2004. Development of a passive, in situ, integrative sampler for hydrophilic organic contaminants in aquatic environments. *Environ Toxicol Chem*, 23(7): p. 1640-1648.

19. MacLeod, SL, EL McClure, and CS Wong, 2007. Laboratory calibration and field deployment of the polar organic chemical integrative sampler for pharmaceuticals and personal care products in wastewater and surface water. *Environ Toxicol Chem*, 26(12): p. 2517-2729.

20. Mazzella, N, JF Dubernet, and F Delmas, 2007. Determination of kinetic and equilibrium regimes in the operation of polar organic chemicals integrative samplers - application to the passive sampling of the polar hebicides in aquatic environments. *J Chromatogr A*, 1154: p. 42-51.

21. Harman, C, M Reid, and KV Thomas, 2011. In situ calibration of a passive sampling device for selected illicit drugs and their metabolites in wastewater, and subsequent year-long assessment of community drug usage. *Environ Sci Technol*, 45(13): p. 5676-5682.

22. Yargeau, V, B Taylor, H Li, A Rodayan, and CD Metcalfe, 2014. Analysis of drugs of abuse in wastewater from two canadian cities. *Sci Total Environ*, 487: p. 722-730.

23. Jones-Lepp, TL, DA Alvarez, JD Petty, and JN Huckins, 2004. Polar organic chemical integrative sampling and liquid chromatography-electrospray/ion-trap mass spectrometry

for assessing selected prescription and illicit drugs in treated sewage effluents. *Arch Environ Contam Toxicol*, 47(4): p. 427-39.

24. Servos, M, M Smith, R McInnis, K Burnison, B-H Lee, and P Seto, 2007. The presence of selected pharmaceuticals and personal care products in drinking water in ontario, canada. *Wat Qual Res J Canada*, 42(2): p. 130-137.

25. Metcalfe, C, K Tindale, H Li, A Rodayan, and V Yargeau, 2010. Illicit drugs in canadian municipal wastewater and estimates of community drug use. *Environ Pollut*, 158: p. 3179-3185.

26. Scheurer, M, H-J Brauch, and F Lange, 2009. Analysis and occurrence of seven artificial sweeteners in german waste water and surface water and in soil aquifer treatment (sat). *Analytical and Bioanalytical Chemistry*, 394(6): p. 1585-1594.

27. Mawhinney, DB, RB Young, BJ Vanderford, T Borch, and SA Snyder, 2011. Artificial sweetener sucralose in u.S. Drinking water systems. *Environmental Science & Technology*, 45(20): p. 8716-8722.

28. Spoelstra, J, S Schiff, and S Brown, 2013. Artificial sweeteners in a large canadian river reflect human consumption in the watershed. *PLoS ONE*, 8(12).

29. Li, H, PA Helm, and C Metcalfe, 2010. Sampling in the great lakes for pharmaceuticals, personal care products and endocrine disrupting substances using the passive polar organic chemical integrative sampler. *Environ Toxicol Chem*, 29(4): p. 751-762.

30. Metcalfe, C, M Hoque, T Sultana, C Murray, P Helm, and S Kleywegt, 2014. Monitoring for contaminants of emerging concern using pocis passive samplers in drinking water from ontario, canada. *Environ Sci: Processes & Impacts*, Accepted.

31. Li, H, PA Helm, and CD Metcalfe, 2010. Sampling in the great lakes for pharmaceuticals, personal care products, and endocrine-disrupting substances using the passive polar organic chemical integrative sampler. *Environmental Toxicology and Chemistry*, 29(4): p. 751-762.

32. Allan, IJ, J Knutsson, N Guigues, GA Mills, AM Fouillac, and R Greenwood, 2208. Chemcatcher and dgt passive sampling devices for regulatory monitoring of trace metals in surface water. *Journal of Environmental Monitoring*, 10: p. 821-829.

33. Gheorghe, A, A van Nuijs, B Pecceu, L Bervoets, PG Jorens, R Blust, H Neels, and A Covaci, 2008. Analysis of cocaine and its principal metabolites in waste and surface water using solid-phase extraction and liquid chromatography-ion trap tandem mass spectrometry. *Anal Bioanal Chem*, 391: p. 1309-1319.

34. Van Nuijs, ALN, B Pecceu, L Theunis, N Dubois, C Charlier, PG Jorens, L Bervoets, R Blust, H Meulemans, H Neels, and A Covaci, 2009. Can cocaine use be evaluated through analysis of wastewater? A nation-wide approach conducted in belgium. *Addiction*, 104(5): p. 734-741.

35. Huerta-Fontela, M, MT Galceran, and F Ventura, 2008b. Stimulatory drugs of abuse in surface waters and their removal in a conventional drinking water treatment plant. *Environ Sci Technol*, 42(18): p. 6809-6816.

533 36. Van Nuijs, ALN, B Pecceu, L Theunis, N Dubois, C Charlier, PG Jorens, L Bervoets, R
534 Blust, H Neels, and A Covaci, 2009. Cocaine and metabolites in waste and surface water
535 across belgium. *Environ Pollut*, 157: p. 123-129.

536 37. Kasprzyk-Hordern, B, RM Dinsdale, and AJ Guwy, 2008. Multiresidue methods for the
537 analysis of pharmaceuticals, personal care products and illicit drugs in surface water and
538 wastewater by solid-phase extraction and ultra performance liquid chromatography-
539 electrospray tandem mass spectrometry. *Anal Bioanal Chem*, 39: p. 1293-1308.

540 38. Loos, R, BM Gawlik, K Boettcher, G Locoro, S Contini, and G Bidoglio, 2009. Sucralose
541 screening in european surface waters using a solid-phase extraction-liquid
542 chromatography-triple quadrupole mass spectrometry method. *J. Chromatogr. A*,
543 1216(7): p. 1126-1131.

544 39. van der Aa, M, L Bijlsma, E Emke, E Dijkman, ALN van Nuijs, B van de Ven, F
545 Hernández, A Versteegh, and P de Voogt, 2013. Risk assessment for drugs of abuse in
546 the dutch watercycle. *Water Research*, 47(5): p. 1848-1857.

547 40. Bartelt-Hunt, SL, DD Snow, T Damon, J Shockley, and K Hoagland, 2009. The
548 occurrence of illicit and therapeutic pharmaceuticals in wastewater effluent and surface
549 waters in nebraska. *Environ Pollut*, 157(3): p. 786-791.

550 41. Vazquez-Roig, P, V Andreu, C Blasco, and Y Picó, 2010. Spe and lc-ms/ms
551 determination of 14 illicit drugs in surface waters from the natural park of l'albufera
552 (València, spain). *Anal Bioanal Chem*, 397(7): p. 2851-2864.

553 42. Trescot, AM, S Datta, M Lee, and H Hansen, 2008. Opioid pharmacology. *Pain*
554 *Physician 2008: Opioid Special Issue*, (11): p. S133-S153.

555 43. Baselt, RC, *Disposition of toxic drugs and chemicals in man*. 3rd ed. 2004, Foster City,
556 CA: Biomedical Publications.

557 44. Fischer, B, N Nakamura, B Rush, J Rehm, and K Urbanoski, 2010. Changes in and
558 characteristics of admissions to treatment related to problematic prescription opioid use
559 in ontario, 2004–2009. *Drug and Alcohol Dependence*, 109(1–3): p. 257-260.

560 45. Rua-Gomez, PC and W Puttmann, 2012. Impact of wastewater treatment plant discharge
561 of lidocaine, tramadol, venlafaxine and their metabolites on the quality of surface waters
562 and groundwater. *J Environ Monitor*, 14(5): p. 1391-1399.

563 46. Rúa-Gómez, PC and W Püttmann, 2013. Degradation of lidocaine, tramadol, venlafaxine
564 and the metabolites o-desmethyltramadol and o-desmethylvenlafaxine in surface waters.
565 *Chemosphere*, 90(6): p. 1952-1959.

566

567

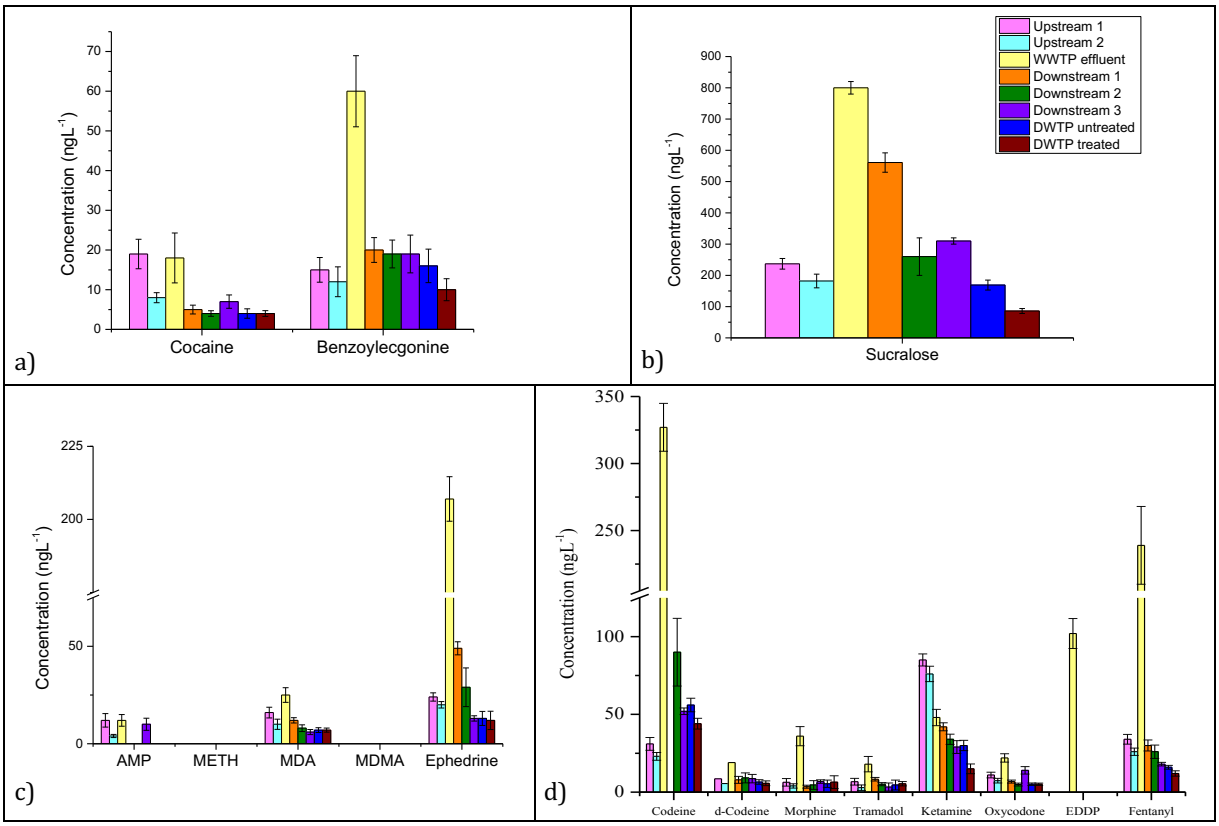


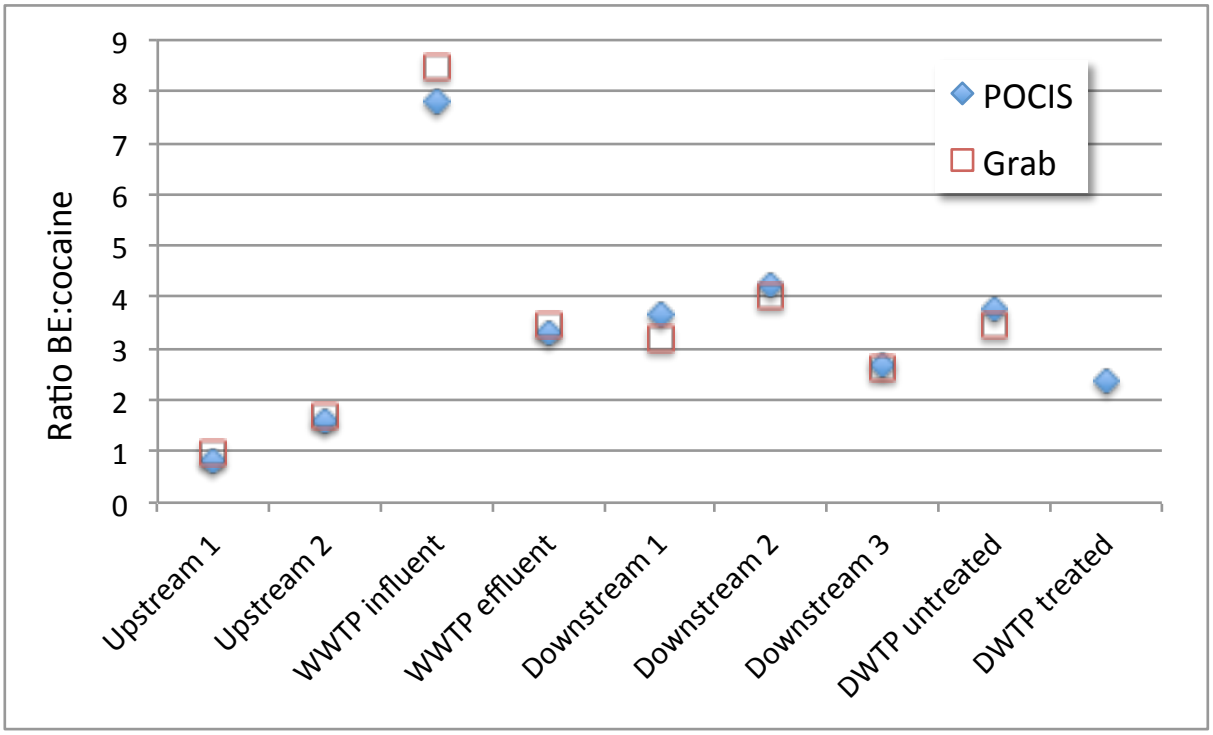
Figure 1. Average trends of concentrations of (a) cocaine and benzoylecgonine, (b) sucralose, (c) amphetamine-type stimulants and (d) other DOAs, in the Grand River, at the WWTP and DWTP (error bars represent one standard deviation). d-Codeine = dihydrocodeine. Heroin, acetylcodeine and acetylmorphine are not included since no sampling rate was available. Methadone is not included since it was not detected in any sample.

568

569

Rodayan, A., Afana, S., Segura, P.A., Sultana, T., Metcalfe, C., Yargeau, V., **Linking drugs of abuse in wastewater to contamination of surface and drinking water**, *Environmental Toxicology and Chemistry*, Volume 35, Issue 4, 843-849 (April 2016)

570



571

572 **Figure 2.** Ratio of average benzoylecgonine (BE) concentration to average cocaine

573 concentration at a given sample location in POCIS and grab samples (each data point

574 represents the average concentration of n = 3 for POCIS and n = 6 for grab).

575

576 **Table 1.** Mean removal efficiencies of analytes at the DWTP (n=3)

Compounds	DWTP Removal Efficiencies
Cocaine & its metabolite	
Cocaine	-18
Benzoylecgonine	14
Sucralose	**49***
Amphetamine-type stimulants	
Amphetamine	< LOQ
Methamphetamine	< LOQ
MDA	7
MDMA	< LOQ
Ephedrine	10
Opioids	
Codeine	**25***
Acetylcodeine	NA
Dihydrocodeine	-10
Morphine	-2
Acetylmorphine	NA
Methadone	< LOQ
Heroin	NA
Tramadol	-39
Ketamine	**49***
Oxycodone	-3
EDDP	< LOQ
Fentanyl	0

577 < LOQ Removal level not calculated due to concentrations < LOQ
578 NA Removal level not calculated due to unavailable sampling rates
579 * Statistically significant based on t-test ($p<0.05$)
580

Rodayan, A., Afana, S., Segura, P.A., Sultana, T., Metcalfe, C., Yargeau, V., **Linking drugs of abuse in wastewater to contamination of surface and drinking water**, *Environmental Toxicology and Chemistry*, Volume 35, Issue 4, 843-849 (April 2016)