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1 2	Impact of approach used to determine removal levels of drugs of abuse during wastewater treatment
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13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32	Abstract In this study the levels of 19 drugs of abuse were estimated throughout a wastewater treatment plant using polar organic chemical integrative samplers (POCIS), 24 h composite samples and grab samples. Overall removal efficiencies and removals in between each treatment unit were calculated using load data for each sampling technique as well as removals that take into account the hydraulic residence time distribution of the treatment plant (time-shifted mass balancing approach). Amphetamine-type stimulants, cocaine and its major metabolite, benzoylecgonine and opioid levels determined with 24 h composite samples were generally comparable to those obtained with POCIS and grab samples. Negative mass balancing of 24 h composite and grab sample data did not occur when the hydraulic retention time (HRT) distributions of the plant were taken into account for calculation. Among the compounds investigated, cocaine exhibited the highest overall removal (90%) while codeine had the lowest with (13%), respectively. Sampling between the treatment units revealed that highest removal occurs during biological treatment as compared to primary or secondary clarification. Methylenedioxyamphetamine (MDA), fentanyl, dihydrocodeine and heroin were not detected in wastewater at any of the sampling locations at the treatment plant regardless of the sampling technique. The study demonstrates the benefits of applying the time-shifted mass balancing approach to the calculation of removals of drugs of abuse during wastewater treatment.
33	Keywords
34	Cocaine
35	Amphetamine-type stimulants
36	Opioids
37	Passive sampling
38	Removal efficiency
39	Time shifted mass balancing
40 41	1. Introduction

42 Illicit drugs are used worldwide and in fact the United Nations Office on Drugs and Crime

43 (UNODC) reported that between 149 and 272 million people used illicit drugs at least once in

44 2008 (UNODC, 2008). Under the category of drugs of abuse (DOAs) are both illicit drugs and

45 prescribed drugs that are abused. Human consumption and excretion of these compounds leads to

46 their (or their metabolite's) presence in wastewater and have therefore been detected in

47 wastewater and surface waters in several countries (Boleda et al., 2007; Bones et al., 2007;

48 Castiglioni et al., 2006a; Gheorghe et al., 2008; Huerta-Fontela et al., 2007; Hummel et al., 2006;

49 Irvine et al., 2011; Kasprzyk-Hordern et al., 2009; Metcalfe et al., 2010; Nefau et al., 2013;

50 Pedrouzo et al., 2011; Postigo et al., 2011; Zuccato and Castiglioni, 2009).

51

While environmental fate assessment for a variety of pharmaceuticals and personal care products has been accomplished and rests upon a broad data base of studies investigating their fate during the last decade, the current picture of DOAs is still ambiguous in this regard. With wastewater treatment plants being the major pathway to surface waters and given the significant uncertainties about their amounts of consumption, the need for reliable data about their levels and removals in wastewater treatment plants (WWTPs) is apparent.

58

59 Reported WWTP removal efficiencies vary to a large extent and also negative removal 60 efficiencies have been observed in some studies. In the latter case, the level of the investigated 61 compound is higher in the effluent that in the influent to one of the treatment units or over the 62 entire WWTP (Blair et al., 2013). Reasons for this can include the release of fecal particles 63 during wastewater treatment, significant desorption from the return activated sludge thereby 64 increasing their concentration throughout the treatment process (Göbel et al., 2007; Salgado et 65 al., 2012) or the transformation of conjugate compounds to the parent compound during 66 biological treatment (Monteiro and Boxall, 2010; Salgado et al., 2012). Negative removals for 67 compounds that are not excreted from the human body, such as pesticides, pharmaceuticals and 68 personal care products have also been reported, making the latter reason less likely. The principal 69 source of error is most likely the applied sampling strategy. Although it has been recognized that 70 loads can vary greatly from day to day or over time during a day and that sampling can be a 71 dominant source of uncertainty in many applications (Ramsey and Thompson, 2007), usually 72 little attention is given to the set-up of adequate sampling strategies. Consequently, since

advanced analytical methodologies for the detection of emerging micropollutants are now

available, unsuitable sampling increasingly represents a major source for inaccuracy (Ort et al.,2010b).

76

Ort et al. (2010a) assessed how sampling strategies were addressed in numerous studies and found that only few provided justification for the choice of sampling technique, duration, mode and frequency. Most studies that have monitored the levels of DOAs in wastewater used grab samples or isolated 24-h composite samples of the influent and effluent of the WWTP (Bones et al., 2007; Mazzella et al., 2007; Postigo et al., 2008b) and did not consider for the HRT or mixing in the treatment tanks for the estimation of full-scale removal efficiencies.

83

84 Grab sampling does not capture short or long-term concentration variation since it takes an 85 instantaneous picture of the concentration of the contaminants in the water at the time of 86 sampling. In addition, in most cases the sampling frequency for composite sampling is longer 87 than one hour and since pollutant peaks generally extend over only a few minutes, the error can 88 be quite large. It is therefore recommended to keep the sampling interval as short as possible 89 given the constraints of the automated sampler. In fact, time proportional as opposed to flow 90 proportional sampling can over or underestimate the contaminant concentration but it is 91 acknowledged that it is not always feasible to carry out a flow proportional sampling campaign 92 (Ort et al., 2010b). In addition, over a short time period the discharge flow from a WWTP is 93 generally quite stable and therefore flow proportional sampling may not significantly reduce 94 sampling error.

95

Basing the estimation of full-scale removal estimations on influent-effluent load mass balances
requires considering the hydraulic retention time and mixing of the water parcel in the tanks. As
treatment tanks can usually be regarded as well-mixed systems, an influent-effluent mass balance
of daily loads does not provide an adequate capture of the influent load and is therefore not valid.
As a consequence, erroneous removal efficiencies are obtained from the comparison of two
largely different water volumes (Majewsky et al., 2011).

102

To design the sampling campaign for the WWTP for the present study, prior hydraulic calibration is necessary using an artificial tracer or a routinely taken measurement such as the water's electrical conductivity (EC) (Anhert et al., 2010; Olivet et al., 2005). Once calibrated, the model can be used to determine an optimal sampling strategy as well as the influent fraction captured by effluent sampling, which takes into account the hydraulic mixing regimes of the WWTP under study to yield reliable mass balances. To our knowledge, no studies have implemented time-shifted mass balancing for the measurement of DOAs in wastewater to date.

110

111 Moreover, among sampling techniques applied for micropollutant sampling, passive sampling 112 recently has gained popularity providing a cost effective way to monitor time-weighted average 113 concentrations of organic chemicals in aquatic systems (Greenwood et al., 2007). Polar organic 114 chemical integrative samplers (POCIS) developed by Alvarez et al. (2004) have been shown to 115 be suitable for measuring compounds such as pharmaceuticals, in wastewater (Mazzella et al., 116 2007; Mills et al., 2007; Petty et al., 2004; Zhang et al., 2008) but few studies have used POCIS 117 for monitoring DOAs (Harman et al., 2011; Jones-Lepp et al., 2012; Yargeau et al., 2014). An 118 advantage of using POCIS is that since the samplers are deployed over extended periods of time, 119 the quantification of residues from random events and the identification of contaminants that are 120 at ultra-low levels can be carried out, both of which are generally missed when grab sampling is 121 employed. A current drawback of these samplers is that to measure the amount of chemical 122 adsorbed, the sampler must first be calibrated for the target compounds by determining their 123 sampling rates (R_s in Ld⁻¹). Since the technology is still developing, many uptake rates have yet 124 to be determined and R_s obtained in the laboratory can be quite different from those in the field 125 (Bailly et al., 2013). In addition, fouling and clogging of POCIS, especially in untreated 126 wastewater can limit its deployment time.

127

The behavior of DOAs during wastewater treatment is only partially understood and must therefore be further considered in order to control their release into the environment and avoid any potential adverse effects on aquatic ecosystems. A better understanding of the occurrence and removal of DOAs to control their release into the environment and avoid any potential adverse effects on aquatic ecosystems can only be achieved on the basis of reliable empirical data being inherently connected to well-defined sampling strategies.

135	As a consequence, the goals of this study were to determine the levels of the selected DOAs in the
136	influent and effluent as well in between each treatment unit at a WWTP in Quebec, Canada using
137	POCIS and 24 h composite (for four consecutive days) sampling. In addition, grab samples were
138	taken at each sampling point as a way to confirm the inadequacy of this sampling mode in obtaining
139	reliable results. Removal efficiencies of each DOA throughout the treatment process were
140	calculated by conventional input-output mass balancing for POCIS and 24 h composites.
141	Additionally, correction for HRT and mixing over the whole plant as well as between the units was
142	considered to confirm or reject the applicability and reliability of the three sampling approaches.
143	
144	2. Methods
145	
146	2.1 Chemicals, reagents and supplies
147	
148	The DOAs used in this study were from several classes of drugs and are listed in Table 1.
149	Analytical standards of all target compounds and their deuterated analogs were obtained from
150	Cerilliant (Round Rock, TX, USA) at a purity higher than 99%. The deuterated stable isotope
151	surrogates were used to correct for extraction recoveries and matrix effects. Working solutions
152	and calibration standards prepared by appropriate dilution of stock solutions were stored in
153	amber glass bottles at -20°C and were used directly from the freezer since they were in pure
154	solvent or in a mixture of solvent and water.
155	
156	Optima liquid chromatography-mass spectrometry (LC-MS) grade methanol, acetonitrile, and
157	water were used for LC-MS analysis and were purchased from Fisher Scientific (Ottawa, ON,
158	Canada). High performance liquid chromatography (HPLC grade or equivalent) acetone,
159	acetonitrile, methanol, ACS reagent grade dichloromethane (DCM), hydrochloride acid (37%),
160	sulphuric acid (96%), formic acid (88%), trace metal grade ammonium hydroxide (88%) and
161	sodium sulfate were purchased from Fisher Scientific (Ottawa, ON, Canada). Ultrapure water
162	was obtained using a Milli-Q water purification system from Millipore (Bedford, MA, USA).
163	Pharmaceutical POCIS containing 200mg Waters Oasis (R) Hydrophilic-Lipophilic Balance
164	sorbent were purchased from Environmental Sampling Technologies (EST Labs, St. Joseph,

165	MO, USA). The sorbent was held between two polyethersulfone membranes which were
166	compressed between two stainless steel washers. Whatman 1.5 μ m glass microfiber filters were
167	purchased from Fisher Scientific (Ottawa, ON, Canada) and Oasis MCX cation exchange
168	cartridges (6 mL/150 mg) were purchased from Waters Corporation (Milford, MA, USA).
169	
170	2.2 Sample collection and WWTP data
171	
172	All samples were obtained from the WWTP in Gatineau, QC, Canada. The WWTP employs
173	activated sludge treatment (See Figure 1 for plant schematic). Sampling was conducted for all
174	three approaches at the exit of the grit removal chamber (Untreated WW), primary clarifiers
175	(Influent to Aeration) and aeration basins (Influent to secondary clarifier) and in the effluent
176	(Treated WW), as denoted in Figure 1.
177	
178	The average flow received by the plant is 127,400 $m^3d^{-1}(2011)$ and it serves a population of
179	approximately 235,000 habitants. The concentration of suspended solids in the treated water was
180	measured to be 6.4 mgL ⁻¹ using Standard Method #2540D. The chemical oxygen demand was
181	measured to be 36 mg/L using a HACH Digital Reactor Block 200 (DRB 200) and a HACH
182	spectrophotometer DR/2500. Average data for biochemical oxygen demand (BOD ₅) and total
183	phosphorus were obtained by plant personnel and were 3.9 and 0.41 mgL ⁻¹ , respectively.
184	
185	2.2.1 Grab and composite sample collection
186	
187	Grab samples were collected in 1 L pre-cleaned amber bottles on August 13 and 27, 2012.
188	Composite samples were taken over a four day period of August 13 to 17, 2012 using Isco 6712
189	samplers equipped with a 24 plastic bottle collection system packed with ice (Avensys, St-
190	Laurent, QC, Canada). Samples were taken in 50 mL volumes every 45 minutes over the four
191	day period. Flow data at a 10 minute time-scale were provided by plant operators but the average
192	flow into the treatment plant over the composite sampling period was 5718 ± 948 m ³ h ⁻¹ and the
193	mean HRT during dry weather was 12 h.
194	

195 2.2.2 POCIS deployment

190	
197	POCIS were deployed on August 13, 2012 for 14 days at each of the sampling locations
198	identified in Figure 1 and debris accumulating on the samplers were removed daily. POCIS disks
199	were kept in -tight canisters prior to deployment and three disks were transferred to stainless
200	steel cages upon arrival at each location at the WWTP and deployed shortly after. POCIS field
201	blanks were exposed to ambient air during the deployment and retrieval of the POCIS samplers.
202	Once removed from the cages each POCIS disk was wrapped in aluminum foil and stored in a
203	sealable plastic bag. All aqueous samples and POCIS were stored in a cooler at 4 $^{\circ}$ C during
204	transportation to the laboratory where they were then stored at -20 $^{\circ}$ C until extraction, which was
205	carried out within three weeks of retrieval. The average flow rate to the WWTP over the
206	deployment period was 5796 m ³ h ⁻¹ .
207	
208	2.2.3 Electrical Conductivity (EC) measurement
209	
210	Electrical wastewater conductivity and temperature were measured at intervals of 30 seconds
211	with HOBO conductivity loggers (Hoskin Scientific, St-Laurent, QC, Canada) at the four
212	sampling locations over a four week period in April, 2012 for hydraulic model calibration and
213	during the composite sampling campaign between August 13 and 17, 2012 for further validation
214	of the calibration. A universal optic-USB base station and Onset HOBOware Pro Version 3.2.2
215	software (Hoskin Scientific, St-Laurent, QC, Canada) were used for data transfer and read out,
216	respectively.
217	
218	2.3 Plant layout, calibration & residence time distribution
219	
220	Model calibration and determination of HRT distributions were based on the method proposed
221	by Majewsky et al. (2011). Briefly, the Gatineau WWTP plant layout was modeled using the
222	wastewater modeling software GPS-X from Hydromantis (Hamilton, Canada). Volumes, tank
223	operation and sequencing were chosen according to the data supplied by the WWTP staff. For
224	hydraulic calibration, untreated wastewater EC was fed to the model and iteratively fit to the

- 225 measured effluent data to determine the best model in terms of the number of completely mixed
- 226 tanks-in-series and the number and type of secondary clarifiers. The EC varied sufficiently over

227 both data collection periods to be able to use the data for the hydraulic calibration of the WWTP. 228 Of course, if large enough fluctuations of EC were not observed over the deployment period, the 229 data could not be used to accurately calibrate the WWTP. Considering that the data of the 230 untreated wastewater were strongly biased due to high ionic strength, clogging or biofouling, the 231 EC data from the Influent to aeration sampling point was fed to the model as input. This 232 approach did not result in significant difference in the modelling, suggesting that the mixing in 233 the primary clarifier does not significantly impact the accuracy of the overall modeling results. 234 Both Chi square and Pearson's correlation coefficient were used as criteria to decide which 235 model was best suited to describe the mixing behavior of the plant. The calibrated model was 236 then used to determine the residence time distribution of an analyte within the plant by running a 237 simulation based on a single influent pulse with a duration of 24 h.

238

239 <u>2.4 Aqueous sample extraction</u>

240

241 Grab and composite samples were vacuum filtered using 1.5 µm glass fibre filters, adjusted to a pH of 2.5 using sulphuric acid and spiked with 100 µL of 500 ngmL⁻¹ surrogate standard mixture 242 243 prior to pre-concentration using solid phase extraction (SPE). Automated SPE was carried out using a Gilson GX-271 ASPECTM instrument with 6 mL-150g Oasis MCX cartridges (Waters, 244 245 Milford, USA). The extraction method was based on a previously published method for illicit 246 drugs (Metcalfe et al., 2010) that was modified to include opioid drugs (Yargeau et al., 2014). 247 Briefly, the cartridges were pre-conditioned with 6mL of acetone, 6 mL methanol and 6 mL 248 milli-Q water adjusted to pH 2.5. The samples (200 mL for WWTP effluent and 100 mL for all 249 other sampling points) were loaded onto the cartridges at a rate of 5 mLmin⁻¹, aspired to dryness 250 and eluted with 8 mL of 5% ammonium hydroxide in methanol. The eluent was evaporated to 251 almost dryness using a Savant SPD 131DDA Speed Vac Concentrator connected to a RVT4104 252 refrigerated vapor trap (Thermo Fisher Scientific, Waltham, MA). The samples were 253 reconstituted to a volume of 0.4 mL in 25% water/75% methanol and a 25 μ L aliquot of the 254 sample extract was injected directly into the LC-MS system for analysis. All samples were 255 extracted in triplicate. Procedural blanks were also pre-concentrated to measure the SPE 256 recoveries of the target compounds and their deuterated analogs. All recoveries were >80% and 257 are listed in Table 1.

259 <u>2.5 POCIS extraction and sampling rates</u>

260

261 POCIS samplers were extracted according to methods previously described by Li et al. (2010). 262 Briefly, the POCIS were extracted by placing the sorbent into the glass column, washing it with 263 methanol and spiking it with a mixture of the deuterated analogs of the compounds of interest, 264 followed by elution with methanol, drying and preparation for analysis. Extraction efficiencies 265 (>80% for all compounds) and the sampling rates (R_s) used in the calculations are listed in Table 266 1. POCIS data were not obtained for acetylcodeine, acetylmorphine and heroin because of the 267 missing Rs values for these compounds.

268

269 <u>2.6 Analysis</u>

270

271 Liquid chromatography-high resolution mass spectrometry (LC-HRMS) using an Accela LC 272 system coupled to a LTO Orbitrap XL (Thermo Fisher Scientific, Waltham, MA) was used to 273 measure analyte concentrations. Chromatographic separation of the target compounds was 274 achieved using the method described in (Rodayan et al., 2014). Ionization was done in positive 275 mode using a heated electrospray ionization (HESI) source with the following parameters: sheath 276 gas flow = 45 arbitrary units, auxiliary sheath gas flow = 10 arbitrary units, capillary temperature 277 = 375 °C, capillary voltage = 5 V, tube lens = 100 V. Acquisition was performed in full scan 278 mode (50-400 m/z) at high resolution ($R_{\rm FWHM} = 41\ 000$) and analyte quantification was carried 279 out by extracting the ion of interest using an m/z window of ±0.01 and confirmation by MS/MS 280 spectra using a data dependent MS/MS experiment. An eight-point calibration curve generated for each compound in the range of 3-150 μ gL⁻¹ was used for quantification with a constant 281 282 deuterated stable isotope surrogate concentration of 100 µgL⁻¹. The linear correlation coefficients 283 were at least 0.993 for all analytes studied.

284

285 The limits of detection (LODs) and limits of quantification (LOQs) for the analytes in

wastewater were in the range of 0.14 to 22 ngL⁻¹ and 0.48 to 74 ngL⁻¹, respectively (see Table 1).

287 These were determined by a signal to noise ratio of >3 for the LODs and >10 for the LOOs and

288 method validation was done using spiked samples of wastewater. The analytical methodology

289	was validated in terms of repeatability between runs on the same day and measured as the
290	relative standard deviation (RSD) of three injections in wastewater was found to be $\leq 13\%$. In
291	addition, reproducibility between days was measured as the RSD (%, n=3) and was $\leq 17\%$.
292	Quality controls were included in every sample series to control the performance of the
293	analytical instrumentation. The relative error between the measured concentration and the
294	expected concentration was always <10% for all target compounds. Field blank POCIS were
295	processed the same as described for the sample POCIS and no analyte residues were detected.
296	
297	3. Results and Discussion
298	
299	3.1 Model calibration, residence time distribution & sampling scenario
300	
301	Minimizing the Chi square between modeled and measured treated wastewater EC values
302	resulted in a model consisting of 4 lanes with $n = 4$ completely mixed tanks-in-series and 3
303	slopping bottom secondary clarifiers to best describe the mixing behavior at the Gatineau
304	WWTP.
305	
306	Once the model was calibrated, the transition time and hydraulic mixing behaviour of a 24 h
307	influent load through the plant was simulated by use of a pulse injection and dry weather diurnal
308	flow data. The simulated effluent loads (Treated WW) allowed the estimation of the fraction of
309	an influent load (Untreated WW) that is captured by a given effluent sampling duration. The
310	detailed description of method and an experimental case study can be found elsewhere
311	(Majewsky et al., 2013; Majewsky et al., 2011). For the Gatineau plant, it was found that a
312	sampling duration of 24 h captures 52% of the influent load that entered the WWTP the same
313	day when starting the autosamplers simultaneously. This demonstrates that a daily water volume
314	is distributed over more than one day when discharged in the effluent. Based on this, the
315	sampling strategy can be designed to account for the transition time and mixing and to allow for
316	adequate calculation of the mass balances using these fractions. As a result, a sampled 24 h
317	effluent load was composed of 2% of the load that entered the plant two days ago, 46% of the
318	load that entered one day ago and 52% of the load that entered the same day. It was therefore

determined that sampling one more day in the influent than in the effluent with a time lag of 24
hours between the start of the influent and effluent sampling was the optimal sampling strategy.

To calculate the removals for the single treatment units, the captured fractions were analogously calculated for the biological treatment unit, which resulted in 23% (of the load that entered the plant one day ago) and 77% (same day). The removal of each analyte across the secondary clarifiers was then calculated by subtracting the removal until the secondary clarifier (after biological treatment) from the overall removal.

327

328 <u>3.2 Levels of DOAs in wastewater</u>

329

330 Table 2 summarize the data for the selected compounds. Table 2 presents the mean 331 concentrations (ngL⁻¹, \pm one standard deviation) of each analyte at the four sampling points at the 332 Gatineau WWTP for POCIS (n = 3), traditional 24 h composite samples (average of triplicate 333 samples taken on four consecutive days) and grab samples (n = 6, triplicate samples taken on the 334 same days that the POCIS were deployed and picked up). For all sampling techniques, MDA, 335 heroin, fentanyl and dihydrocodeine were below their respective LODs or LOQs. In addition, 336 heroin, acetylcodeine and acetylmorphine were omitted for POCIS data since their Rs are not 337 available.

338

In untreated wastewater, the highest levels were observed for BE followed by cocaine and
codeine respectively while in treated wastewater, codeine levels were highest followed by BE

341 and ephedrine. In general, the levels of the target compounds observed in this study are

342 consistent with those reported in studies conducted in Europe (Bijlsma et al., 2012; Boleda et al.,

343 2009; Pedrouzo et al., 2011; Van Nuijs et al., 2011) and the U.S.A. (Pal et al., 2013) as well as

the only two other studies conducted in Canada (Metcalfe et al., 2010; Yargeau et al., 2014).

345

346 All of the ATS included in this study were detected in untreated wastewater with the exception

of MDA which was not detected using any sampling technique at any of the sampling locations

348 at the WWTP. This is unlike another Canadian study which detected MDA in 32 of 40 samples

of untreated wastewater (Metcalfe et al., 2010). The levels of ATS in untreated wastewater were

350 slightly higher than those reported by Metcalfe et al. (2010) where levels of these compounds

351 were less than 70 ngL⁻¹. Methamphetamine levels were higher than those reported in Europe

352 where they are generally <10 ngL⁻¹ but lower than those that have been reported in the U.S.A.

353 (Chiaia et al., 2008).

354

355 Although the levels of cocaine and BE were in the range of those reported in other studies, the 356 ratios of cocaine to BE in untreated wastewater were not. Ratios of cocaine to BE were in the 357 range of 0.49 to 0.52 which are high compared to those reported in Europe and the U.S.A. where 358 the ratio is typically much less than 0.50 (Castiglioni et al., 2006a; Gheorghe et al., 2008; Mari et 359 al., 2009; Metcalfe et al., 2010; Postigo et al., 2010; Zuccato et al., 2005). However, in a study 360 conducted in Ireland, higher concentrations of cocaine relative to BE were also observed (Bones 361 et al., 2007). Since it is expected that only 9% of a cocaine dose is excreted as the parent 362 compound in urine, it is possible that the higher ratios observed here are due to direct disposal of 363 cocaine in the sewage system (Kasprzyk-Hordern et al., 2009; Phillips et al., 2010).

364

365 In the case of opioids, dihydrocodeine and fentanyl were below their LOQs in all samples. This 366 is to be expected for fentanyl since it has a tendency to be found in the sludge phase as opposed 367 to the aqueous phase (log octanol water partitioning coefficient (log K_{ow}) of 4.05), 26 to 55% of 368 the parent compound is excreted in urine as norfentanyl and only up to 6% is excreted as the 369 parent compound (Baselt, 2004; Huerta-Fontela et al., 2008a; Thompson et al., 2011). Heroin 370 was not detected in any samples which is consistent with other studies (Boleda et al., 2009; 371 Yargeau et al., 2014). It is known to be excreted mainly as morphine and acetylmorphine 372 (Trescot et al., 2008) and may therefore contribute to it not being detected in this study. Finally, 373 acetylmorphine was detected in 24 h composite and grab samples in spite of its tendency to be 374 hydrolyzed to morphine. 375

376 <u>3.3 Comparison of DOA levels obtained by POCIS, 24 h composite and grab sampling</u> 377

As can be seen from Table 2, analytes were consistently detected using POCIS, 24 h composite
and grab samples (100% frequency in all cases). Those not detected in grab samples were also

not detected in composite samples and POCIS, with the exception of those compounds for which
 R_s are not available and for which comparison is not possible.

382

Analyte levels were generally comparable between POCIS and grab samples, and values for MDMA and methamphetamine are lower in POCIS as reported in a study conducted by Jones-Lepp et al. (2012). In addition, the levels of the target compounds in composite samples were generally comparable to those obtained using POCIS and grab sampling. The lower values obtained with POCIS may be explained by the fact that R_s are determined at the lab scale in pure water and at ambient temperature which adds a potential source of error to the measurement of target compound levels when used in a heavy matrix such as wastewater.

390

391 One of the advantages of POCIS however, is the ability to detect target compounds that are often 392 not detected using other sampling techniques since the analytes are accumulated over time in the 393 POCIS sorbent. In this study however, MDA, dihydrocodeine and fentanyl were not quantifiable 394 in POCIS as was the case in other samples. Therefore, the use of POCIS did not allow for 395 additional compounds to be quantified in wastewater and in fact, since some R_s were not 396 available, fewer analytes could be quantified. In addition, it may not be possible to obtain 397 sampling rates for certain compounds due to low uptake rates which may be attributed to the 398 polarity of the compounds. This was the case for heroin, acetylcodeine and acetylmorphine for 399 which sampling rates could not be determined (Yargeau et al., 2014). It is important to note that 400 although the data obtained via grab samples and POCIS were quite similar in this study, this is 401 not always the case nor should it necessarily be (Jones-Lepp et al., 2012). In the case of POCIS, 402 the integrated concentration of the analytes over the entire deployment period is obtained and 403 often presents a better view of the concentration over time due to the potential of mixing or 404 changes in human activities (e.g. drug consumption patterns that change as a function of the day 405 of the week) (Gerrity et al., 2011; Managaki et al., 2008; Ort et al., 2005).

406

407 <u>3.4 Removal of DOAs by wastewater treatment</u>

408

Table 3 presents the removal efficiencies estimated by load data using different approaches. The removal efficiencies for the 24 h composites were calculated by mass balancing daily loads of

411 influent and effluent, and then by using the captured fractions of the sampled influent by effluent

412 sampling accounting for time-shift and hydraulic mixing. Most of the removal of the target

413 compounds was observed during biological treatment which is generally the case for organic

414 contaminants in wastewater (Blair et al., 2013; Joss et al., 2006; Khan and Ongerth, 2002). In

415 some cases however, comparable levels of removal were also noted during primary and

416 secondary clarification.

417

During primary clarification, the highest removals observed were those for tramadol (21% using composite sample data) while all other compounds exhibited removals $\leq 15\%$. In addition, 11 of the 15 quantified analytes in grab samples exhibited negative removals while only one negative removal was observed using POCIS and traditional 24 h composite data. The removals of the target analytes were not calculated using the time-shifted mass balancing approach since primary clarifiers could not be included in the model used to calculate the load fractions captured by the autosamplers.

425

426 Removals during biological treatment were generally lower using the time-shifted mass 427 balancing approach as compared to other mass balances. Considering the time-shifted removal 428 data, the highest removal was observed for cocaine where 73% of the compound that entered 429 aeration tanks was removed. The lowest removal was observed for codeine of which only 3% 430 was removed, followed by ephedrine (7%), methadone (10%), oxycodone (10%), tramadol 431 (11%), and EDDP (11%). Although, the majority of organic contaminant removal takes place 432 during biological treatment, the persistence of some of these compounds during wastewater 433 treatment is primarily due to their resistance to biodegradation (Joss et al., 2006; Khan and 434 Ongerth, 2002). All of the analytes that exhibited negative removals during this treatment step 435 using grab (6 analytes), POCIS (5 analytes) and 24 h composite sample (2 analytes) data turned 436 into positive removals using the time-shifted approach.

437

438 During secondary clarification the most significant removal was observed for methamphetamine

439 according to POCIS, traditional and time-shifted composite data. All other compounds exhibited

440 removals of $\leq 21\%$ including 3 negative removals for POCIS and 5 for grab samples. Removals

calculated using the traditional 24 h composite approach reveal 3 negative values whereas thosecalculated using the same data but with fractionation generated no negative removal values.

443

444 Overall removals of cocaine, benzoylecgonine and amphetamine-type stimulants were generally 445 in the range of those reported by others even though the latter are known to be very sensitive to 446 different treatment technologies and often exhibit negative removals during treatment (Bijlsma et 447 al., 2012; Boleda et al., 2007; Bones et al., 2007; Loganathan et al., 2009; Metcalfe et al., 2010; 448 Postigo et al., 2008a; Postigo et al., 2008b; Postigo et al., 2010; Valcárcel et al., 2012; Yargeau 449 et al., 2014). The discrepancies with the removals reported in literature and those observed in this 450 study may be due to various factors such as the type of treatment process used at the treatment 451 plant under investigation (Joss et al., 2006; Kanda et al., 2003), the solids and hydraulic retention 452 times (Clara et al., 2005; Kreuzinger et al., 2004; Tauxe-Wuersch et al., 2005), the dilution of 453 influent wastewater by rain events during the sampling period (Joss et al., 2006; Tauxe-Wuersch 454 et al., 2005; Ternes, 1998) and very likely by the calculation used to obtain the removals 455 themselves.

456

In the case of opioids, removals of ketamine and acetylmorphine were in the range of those 457 458 reported in other studies (Bijlsma et al., 2012; Huerta-Fontela et al., 2008b). Overall codeine 459 removal was negative except in the case of the time-shifted removal where it was only 13%. It 460 remains unclear, if this is due to the cleavage of codeine-6-glucuronide to free codeine during the 461 treatment process (Boleda et al., 2009) or a bias of the sampling scheme. Negative removals of 462 this compound were also observed using POCIS and traditional 24 h composite sampling in a 463 study conducted by Yargeau et al. (2014). The average overall removal of morphine was 39% 464 using the time-shifted approach which is lower than that reported by Boleda et al. (2009) where 465 73% overall removal was observed. However, the cleavage of morphine glucuronide conjugates 466 to form morphine can account for low or negative removals of the drug during wastewater 467 treatment (Baselt, 2004). POCIS and grab data reveal that EDDP exhibit -22 and -55% overall 468 removals which are consistent with another study (Yargeau et al., 2014). Other studies have 469 observed negligible or no removal of EDDP (Boleda et al., 2009; Castiglioni et al., 2006b) 470 whereas time-shifted and traditional composite sample removals were 26 and 18%, respectively 471 for this study.

473 Negative removals using grab sampling data are not uncommon since this sampling technique 474 does not capture short or long-term concentration variation and as expected some grab sample 475 removals were negative. For the removals using traditional 24 h composite sampling, even 476 though a sampling frequency of 32 times per day (equivalent to every 45 minutes over a 24 h 477 period) was used, it was not sufficient to avoid negative removals. This may be due to the fact 478 that time proportional as opposed to flow proportional sampling was used which has been known 479 to over or underestimate the target compound concentration (Ort et al., 2010b). In addition, the 480 negative removals may be true negative values due to the release of these compounds from fecal 481 particles or the transformation of conjugate compounds to the parent compound during the 482 biological treatment step (Göbel et al., 2007; Monteiro and Boxall, 2010; Salgado et al., 2012; 483 Ternes, 1998). However, the removals obtained using the time-shifted approach demonstrate that 484 negative overall removal values and removals for each treatment unit were no longer obtained. 485 This suggests that the most significant reason for the observed negative removals obtained when 486 using grab or 24 h composite sampling is that the two largely different loads (water volumes) are 487 used to calculate the removals.

488

489 It is important to note however, that negative POCIS removals cannot be explained by mixing 490 regime characteristics since POCIS levels represent time-weighted average concentrations of the 491 target compounds. Moreover, the long exposure time of 14 days is supposed to average out day-492 to-day concentration variability. However, as mentioned in Section 3.3, the R_s are determined in 493 the lab and are then used to calculate analyte levels in both the influent and effluent, which are 494 quite different matrices, and where in reality the R_s are surely different due to the heavy matrix 495 of the influent. In the influent there is likely to be increased inhibition due to clogging, fouling, 496 biofilm accumulation, etc and improved calibration strategies, such as the in-situ calibration 497 proposed by (Harman et al., 2011) would certainly improve the reliability of the removal levels 498 determined using POCIS data.

499

500 4. Conclusions

502 The levels of the target compounds obtained were consistent with estimates reported in other

- 503 studies and further confirm the ubiquitous presence of DOAs in wastewater of urban areas. The
- 504 results also contribute to expanding the data available for these compounds in wastewater which
- 505 to date are scarce and add to the database required for environmental fate and risk assessment. In
- 506 addition, the low removal of the targeted DOAs indicates that the potential exists for adverse
- 507 effects on the aquatic organisms in surface waters.
- 508
- 509 The removal efficiency results obtained in this study demonstrate for the first time the
- 510 application of a time-shifted mass balancing approach to reliably match influent loads to effluent
- 511 loads in the calculation of DOA removals during wastewater treatment. The results indicate that
- 512 negative removals obtained using other sampling and calculation methods can be successfully
- 513 corrected when the time-shifted approach is applied. It further demonstrates the importance of
- using adequate sampling and calculation approaches for proper evaluation of removal
- 515 efficiencies of target compounds during water treatment.
- 516

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- 526

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Figure 1: Schematic of the Gatineau wastewater treatment plant where numbers denote the sampling locations, 1=Untreated WW, 2=Influent to aeration, 3=Influent to secondary clarifier and 4=Treated WW.

Table 1: List of target compounds, corresponding deuterated analogs, molecular weights, LODs and LOQs, SPE

 recoveries, POCIS extraction efficiencies and POCIS sampling rates

Target Compound (Surrogates)	Target Compound MW	LOD, LOQ (ngL ⁻¹)	SPE Recovery (%)	POCIS Extraction Efficiency (%)	POCIS Sampling Rate ^a (R _s ±SD)							
Cocaine and its metabol	lite		1	1	1							
Cocaine (cocaine-d3)	303.4	1.3, 4.2	90	89	0.230±0.036							
Benzoylecgonine (Benzoylecgonine-d3)	289.3	1.3, 4.2	96	85	0.134±0.011							
Amphetamine-type stimulants												
Amphetamine (Amphetamine-d5)	135.2	1.1, 3.5	98	93	0.201±0.038							
Methamphetamine (Methamphetamine-d9)	149.2	2.1, 6.9	99	92	0.231±0.025							
MDA (MDA-d5)	179.2	1.0, 3.2	95	91	0.288±0.021							
MDMA (MDMA-d5)	193.2	2.5, 8.4	95	90	0.222±0.013							
Ephedrine (Ephedrine-d3)	165.2	1.7, 5.6	88	82	0.123±0.039							
Opioid drugs												
Codeine (Codeine-d3)	299.4	0.91, 3.0	87	83	0.394±0.049							
Acetylcodeine (Acetylcodeine-d9)	341.4	1.5, 4.9	86	85	NA ^b							
Dihydrocodeine (Dihydrocodeine-d6)	301.4	0.42, 1.4	86	82	0.110±0.041							
Morphine (Morphine-d3)	285.3	0.27, 0.89	90	88	0.261±0.036							
Acetylmorphine (Acetylmorphine-d3)	327.4	0.14, 0.48	85	81	NA ^b							
Methadone (Methadone-d9)	309.4	21, 69	90	85	0.408±0.147							
Heroin (Heroin-d9)	369.4	1.8, 6.1	87	83	NA ^b							
Tramadol (Tramadol-d6)	263.4	1.8, 5.8	83	81	0.241±0.062							
Ketamine (Ketamine-d4)	237.7	0.51, 1.7	82	83	0.590±0.021							
Oxycodone (Oxycodone-d3)	315.4	1.4, 4.8	88	84	0.152±0.039							
EDDP (EDDP-d3)	277.1	22, 74	86	83	0.532±0.193							
Fentanyl (Fentanyl-d5)	336.5	1.1, 3.6	81	81	1.17±0.160							

^aTaken from Yargeau et al. 2014 with the exception of the R_s of ketamine and fentanyl ^bSampling rate not available

Table 2: Average concentrations (\pm one standard deviation) of drugs of abuse at the wastewater treatment plant for grab, POCIS and 24 hour composite samples. MDA, fentanyl, dihydrocodeine and heroin are not included in the table since they were either not detected in any sample or were detected below their LOQs.

		Gr	ab			PO	CIS		24 h composite				
	Untreated WW	Influent to aeration	Influent to secondary clarifier	Treated WW	Untreated WW	Influent to aeration	Influent to secondary clarifier	Treated WW	Untreated WW	Influent to aeration	Influent to secondary clarifier	Treated WW	
Cocaine and its me	etabolite												
Cocaine	869±20	871±20	83±5	70±9	1841 ± 28	1735±14	367±4	333±8	903±6	875±9	83±5	72±5	
Benzoylecgonine	1594 ± 58	1616±66	367±21	356±15	78±35	76±28	19±10	18±25	1855±24	1750±18	330±12	298±11	
Amphetamine-type	e stimulants												
Amphetamine	68±8	74±9	17±2	18±2	78±7	76±6	19±3	18±3	85±5	78±6	13±3	10±2	
Methamphetamine	76±8	79±2	17±2	16±2	92±9	83±3	12±2	9±1	85±5	80±3	19±2	14±2	
MDMA	177±12	192±10	148 ± 10	140±2	196±11	192±10	101±8	96±5	216±6	210±4	109±4	99±6	
Ephedrine	114±5	136±10	164±27	141±15	139±10	136±10	167±27	165±24	158±5	140±5	129±3	118±4	
Opioid drugs													
Codeine	405±26	464±15	508±10	477±33	459±20	460±24	508±10	497±9	494±7	456±3	478±8	519±4	
Acetylcodeine	45±8	46±11	48±7	55±3	_ ^a	-	-	-	70±6	60 ± 8	43±3	40±3	
Morphine	42±5	48±5	45±3	45±4	56±6	56±6	43±4	43±5	76±4	70±3	54±3	46±2	
Acetylmorphine	137±12	144±8	98±7	99±8	-	-	-	-	142±4	132±3	91±3	88±2	
Methadone	40±5	36±4	55±5	62±4	40±5	36±4	55±5	62±4	63±4	52±4	59±3	63±2	
Tramadol	68±12	58±6	128±8	125±10	50±5	41±4	68±7	73±4	62±4	48±5	44±5	67±3	
Ketamine	67±9	71±7	22±7	20±2	84±7	78±8	22±3	20±2	90±5	78±3	21±5	18±2	
Oxycodone	65±6	55±5	52±4	52±4	69±6	62±2	52±4	52±3	78±4	79±3	65±5	58±2	
EDDP	93±6	103±9	131±12	144±5	107±5	103±9	125±5	130±5	107±4	103±2	94±3	87±5	

^aDashes represent values that were not obtained for compounds whose sampling rates are not available.

Table 3: Average removal efficiencies across the primary clarifiers, aeration tanks and secondary clarifiers and overall removal efficiencies of the target compounds at the Gatineau wastewater treatment plant, QC, Canada

		Removal Efficiencies (%)														
	Primary clarifiers				Aeration tanks				Secondary clarifiers				Overall			
Compound	Grab	POCIS	24 hr comp.	Time- shifted mass balance ^a	Grab	POCIS	24 hr comp.	Time- shifted mass balance	Grab	POCIS	24 hr comp.	Time- shifted mass balance	Grab	POCIS	24 hr comp.	Time- shifted mass balance.
Cocaine and its me	tabolite			-		-			-	-			-			
Cocaine	0	0	0	N.A.	90	91	91	73	15	13	14	17	92	92	92	90
Benzoylecgonine	-1	6	2	N.A.	77	79	81	66	3	9	10	17	78	82	84	85
Amphetamine-type	e stimula	ants														
Amphetamine	-8	2	5	N.A.	76	75	84	66	-2	7	18	23	74	77	88	90
Methamphetamine	-4	10	3	N.A.	78	86	77	60	6	26	25	19	78	91	83	82
MDA ^b	-	-	-	N.A.	-	-	-	-	-	-	-	-	-	-	-	-
MDMA	-8	2	-1	N.A.	23	48	49	45	6	5	8	8	21	51	53	54
Ephedrine	-19	2	9	N.A.	-20	-22	9	7	14	1	8	18	-24	-19	24	34
Opioid drugs																
Codeine	-15	0	4	N.A.	-10	-11	-3	3	6	2	-9	4	-18	-8	-7	13
Acetylcodeine ^c	-3	-	11	N.A.	-4	-	28	35	-15	-	6	8	-23	-	41	53
Dihydrocodeine ^b	-	-	-	N.A.	-	-	-	-	-	-	-	-	-	-	-	-
Morphine	-15	-1	5	N.A.	7	24	24	25	0	0	14	8	-7	23	38	39
Acetylmorphine ^c	-5	-	4	N.A.	32	-	32	27	-1	-	3	9	28	-	37	40
Methadone	10	10	14	N.A.	-53	-53	-11	10	-13	-13	-8	4	-56	-56	-3	25
Heroin ^{b,c}	-	-	-	N.A.	-	-	-	-	-	-	-	-	-	-	-	-
Tramadol	14	17	21	N.A.	-121	-64	7	11	3	-7	-51	3	-84	-46	-10	37
Ketamine	-6	6	9	N.A.	69	71	73	64	8	10	13	17	70	76	80	84
Oxycodone	15	11	-4	N.A.	5	15	19	10	1	0	9	21	21	24	24	28
EDDP	-10	4	0	N.A.	-28	-21	11	11	-10	-4	8	12	-55	-22	18	26
Fentanyl ^b	-	-	-	N.A.	-	-	-	-	-	-	_	-	-	-	-	_

^aThe removal efficiencies of the target compounds across the primary clarifiers were not calculated using the time-shifted mass balancing approach since the latter could not included in the model

^bBelow the LOD or LOQ

^cPOCIS sampling rate not available