Fate and mass balance of contaminants of emerging concern during wastewater treatment determined using the fractionated approach

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Abstract

Contaminants of emerging concern (CECs) are often poorly removed from wastewater using conventional treatment technologies and there is limited understanding of their fate during treatment. Inappropriate sampling strategies lead to inaccuracies in estimating removals of CECs. In this study, we used the "fractionated approach" that accounts for the residence time distribution (RTD) in treatment units to investigate the fate of 26 target CECs in a municipal wastewater treatment plant (WWTP) that includes primary, secondary and tertiary treatment steps. Prior hydraulic calibration of each treatment unit was performed. Wastewater and sludge samples were collected at different locations along the treatment train and the concentrations of target CECs were measured by liquid chromatography mass spectrometry (LC-MS). The most substantial aqueous removal occurred during activated sludge treatment (up to 99%). Removals were <50% in the primary clarifier and tertiary rotating biological contactors (RBCs) and up to 70% by sand filtration. Mass balance calculations demonstrated that (bio)degradation accounted for up to 50% of the removal in the primary clarifier and 100% in activated sludge. Removal by sorption to primary and secondary sludge was minimal for most CECs. Analysis of the selected metabolites demonstrated that negative removals obtained could be explained by transformations between the parent compound and their metabolites. This study contributes to the growing literature by applying the fractionated approach to calculate removal of different types of CECs across each wastewater treatment step. An additional level of understanding of the fate of CECs was provided by mass balance calculations in primary and secondary treatments.

Keywords: Adsorption, Fractionated approach, Hydraulics, Micropollutants, Pharmaceuticals, Tertiary treatment

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41 # 1. Introduction:

42 Contaminants of emerging concern (CECs), including pharmaceuticals and personal care products
43 (PPCPs), drugs of abuse (DOAs), hormones and stimulants are present in the aquatic environment
44 (Blair et al., 2013a; Hughes et al., 2013; Rodayan et al., 2015), and these CECs were found to have
45 adverse effects on aquatic organisms (Gay et al., 2015; Kidd et al., 2007; Kümmerer, 2008; Purdom
46 et al., 1994). Discharges of wastewater treatment plants (WWTPs) have been identified as the
47 primary source for CECs introduced into surface waters (Luo et al., 2014). Therefore, accurate
48 determination of the fate of CECs during wastewater treatment is required in order to facilitate risk
49 assessments and to identify strategies to improve their removal.
50
51 Unexplained temporal variations in the removals of CECs in activated sludge units and in some cases
52 negative removals (i.e. higher concentration in the effluents than in the influents) have often been
53 observed in WWTPs, which has raised questions about the appropriateness of the protocols for
54 sampling wastewater (Majewsky et al., 2011; Ort et al., 2010). In a critical review, Ort et al. (2010)
55 suggested that sampling strategies that fail to account for the residence time distribution (RTD),
56 which affects the transport of CECs in treatment units, could result in a mismatch between the
57 sampled influent and effluent. This mismatch results in biased and unreliable data on removal
58 efficiencies. Majewsky et al. (2011) proposed a strategy for sampling and removal calculation
59 referred to as the "fractionated approach" to account for the RTD within the units of the WWTPs and
60 match the mass loads of the influent with the effluent. This approach requires prior hydraulic
61 modelling of the WWTP followed by a tailored sampling strategy where composite samples are
62 collected on several consecutive days. The fractionated approach has shown promise for evaluating
63 the removal of pharmaceuticals, pesticides and drugs of abuse during activated sludge treatment
64 (Majewsky et al., 2013; Rodayan et al., 2014a). However, the fractionated approach has not yet been
65 applied to a complete WWTP treatment train to identify and quantify the mechanisms of CEC
66 removal at each stage of treatment.
67
68 To date, most studies on the removal of CECs did not investigate the contribution of different
69 removal mechanisms, as they ignored the distribution of CECs between the aqueous and the
70 particulate compartments (Petrie et al., 2015). A limited number of previous studies have taken a
71 mass balance approach to determine removals of CECs in conventional primary and secondary
72 treatment steps through the processes of adsorption and (bio)degradation that were the major removal
73 mechanisms for the CECs investigated (Carballa et al., 2007; Gao et al., 2012; Heidler & Halden,
74 2008; Jelic et al., 2011; Petrie et al., 2014; Wick et al., 2009; Winkler et al., 2007). The majority of
75 these studies were based on analysis of CECs in grab or 24-h composite wastewater samples
76 collected simultaneously from different treatment stages, while a small number of studies accounted
77 for the hydraulic retention time in the sampling campaign but did not consider the wastewater
78 residence time distribution.
79
80 In a recent review, Petrie et al. (2015) identified inadequate sampling approaches, a lack of
81 understanding of adsorption of CECs onto particulates in WWTPs, as well as a lack of data on the
82 fate of the metabolites of pharmaceuticals as information gaps that lead to misrepresentative data of
83 CEC removals. In the present study, advanced sampling strategies that account for the hydraulic
84 behaviour of treatment units were combined with mass balance analysis to monitor the fate of 26
85 target compounds in a WWTP in order to gain insights into the mechanisms of removal of CECs. The

target CECs were contaminants that were reported to be present in surface water or persistent during wastewater treatment. The list included pharmaceuticals and some of their metabolites, hormones, drugs of abuse, a stimulant (i.e. caffeine), an artificial sweetener (i.e. sucralose) and an antibacterial agent (i.e. triclosan). The WWTP monitored in this study employs primary treatment, secondary treatment by activated sludge, tertiary treatment by both rotating biological contactors (RBCs) and sand filtration and disinfection by chlorination/dechlorination. This study contributes to the literature on the use of the fractionated approach for reliable determination of CEC removals, and for the first time, uses this approach to estimate removals during the tertiary treatment steps with RBCs and sand filtration. Further, the predominant removal mechanisms during primary and secondary treatment were identified by the mass balance analysis.

2. Materials and Methods

2.1. Chemicals and other supplies

The target CECs included several pharmaceuticals identified by Dickenson et al. (2011) in a study that illustrated the value of monitoring a small number of CECs in WWTPs in the USA. Sucralose was added to the list as a tracer of wastewater contamination due to its persistence and ubiquitous presence at high concentrations (Mawhinney et al., 2011). The drugs of abuse were selected based upon our previous studies indicating their presence in wastewaters in Canada (Metcalfe et al., 2010; Rodayan et al., 2014a). Androstenedione was selected as a model androgen because of the lack of data in the literature investigating its fate (Esperanza et al., 2007), and estrone was selected as a model estrogen because of its widespread occurrence in wastewater (Servos et al., 2005). To illustrate the importance of including metabolites in mass balance calculations, two metabolites of carbamazepine, rac trans-10,11-dihydro-10,11-dihydroxy carbamazepine (CBZ-DiOH) and carbamazepine 10,11-epoxide (CBZ-EP) were monitored because these compounds have been previously detected in wastewater and sludge (Hummel et al., 2006; Miao & Metcalfe, 2003; Miao et al., 2005). Other metabolites monitored in this study included EDDP, the primary metabolite of methadone, and benzoylecgonine, the primary metabolite of cocaine.

The target CECs, along with their physicochemical properties and the suppliers from which they were purchased are listed in Table 1. The analytes were classified as Class A and B compounds (Table 1) according to the extraction and analysis procedures. Class B compounds are drugs of abuse and some of their metabolites, which are mainly weak bases, in addition to carbamazepine and its two metabolites. Class A compounds are all other target compounds that are weak acids, neutral or phenolic compounds. The internal standards used for each compound are listed in Table 1. 10,11-dihydrocarbamazepine was used as a surrogate for CBZ-DiOH, as performed in previous studies (Leclercq et al., 2009; Miao & Metcalfe, 2003). All analytical standards and stock solutions were stored in amber glass vials at -20 °C.

Methanol, acetonitrile, and water of liquid chromatography–mass spectrometry (LC–MS) grade, as well as other chemicals used for sample preparation were purchased from Fisher Scientific (Ottawa, ON, Canada). Ultrapure water was generated using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Study site

The study site is a municipal WWTP located in Guelph, ON, Canada, serving a population of approximately 134,894, having a design capacity of 64,000 m^3/d and receiving an average incoming flow rate of 50,755 m^3/d of domestic, commercial and industrial wastewater. The WWTP (Figure 1)

provides preliminary treatment by screening and aerated grit removal, after which the load is split into 4 activated sludge lines. Each of the lines consists of 2 primary clarifiers, 2 aeration tanks and 2 secondary clarifiers in parallel. The WWTP provides tertiary treatment for the recombined effluent of the lines by rotating biological contactors (RBCs), followed by sand filtration. The tertiary treatment step was added to the treatment sequence in order to meet regulations concerning the levels of TSS (i.e. 10 mg/L) and ammonia nitrogen (i.e. 3.4 mg/L) in the effluent. For disinfection, chlorine is added to wastewater after the RBCs as 12% sodium hypochlorite at an average dosage of 1760 kg/d. The disinfected de-chlorinated final effluent is discharged into the Speed River. Table 2 summarizes the main characteristics of line 1 in the WWTP since only line 1 was investigated in the present study.

Table 1: Target CECs and their chemical and physical characteristics, internal standards, class (determining the corresponding extraction and analysis methods), LODs and LOQs in aqueous and biosolids samples and the supplier of the compounds and their surrogates

Type	Subtype	Compound	Log K_{ow}[1]	pKa[1]	Internal standard (surrogate)	Class	Aqueous LOD, LOQ (ng/L)[3]	Biosolids LOD, LOQ (ng/L)[3]	Company[4] Compound surrogate
Pharmaceuticals	Antibiotics	Trimethoprim	0.91	6.8	Trimethoprim-13C3	A	7,22	3,10	S,I
		Sulfamethoxazole	0.89	5.7	Sulfamethoxazole-13C6	A	4,14	2,9	S,I
	Analgesics	Codeine	1.14	8.2	Codeine-d3	B	6,18	3,9	C,C
		Ibuprofen	3.97	4.9	Ibuprofen-13C3	A	6,21	10,32	S,I
		Naproxen	3.18	4.2	Naproxen-13c1-d3	A	4,14	6,19	S,I
	Antiepileptic and metabolites	Carbamazepine	2.45	3.2	Carbamazepine-d10	B	2,5	2,6	C,C
		rac trans-10,11-Dihydro-10,11-dihydroxy Carbamazepine (CMZ-DiOH)	0.13	N.A.	10,11 dihydro carbamazepine	B	4,13	3,9	T,T
		Carbamazepine 10,11-Epoxide (CMZ-EP)	1.26	N.A.	Carbamazepine 10,11-Epoxide-d8	B	2,7	1,5	T,T
Drugs of abuse	Cocaine and metabolite	Benzoylecgonine (cocaine's metabolite)	-1.32	N.A.	Benzoylecgonine-d3	B	4,14	2,5	C,C
		Cocaine	2.3	8.6	Cocaine-d3	B	26,84	11,36	C,C
	Amphetamines	Amphetamine	1.76	10.1	Amphetamine-d5	B	4,13	1,4	C,C
		Methamphetamine	2.07	10.2	Methamphetamine-d9	B	7,23	1,4	C,C
		EDDP (methadone's metabolite)	4.94	9.6	EDDP-d3	B	5,18	4,15	C,C
		Ephedrine	1.13	9.7	Ephedrine-d3	B	7,25	3,9	C,C
	Opioids	Dihydrocodeine	1.49	8.8	Dihydrocodeine-d6	B	8,22	5,14	C,C
		Fentanyl	4.05	8.6	Fentanyl-d5	B	6,20	3,8	C,C
		Ketamine	2.18	7.5	Ketamine-d4	B	8,27	2,8	C,C
		Methadone	3.93	8.9	Methadone-d9	B	4,13	1,4	C,C
		Morphine (codeine metabolite)	0.89	9.9	Morphine-d3	B	5,17	10,32	C,C
		Oxycodone	0.66	8.3	Oxycodone-d3	B	10,22	3,7	C,C
		Tramadol	2.63	9.4	Tramadol-d3	B	7,24	1,3	C,C
Personal Care Products	Antibacterial	Triclosan	4.76	7.9	Triclosan-13C12	A	6,19	6,19	K,M
Steroid hormones		Androstenedione	2.75	NA	Androstene-3,17-dione-2,3,4-13C3	A	2,5	7,25	S,C
		Estrone	3.13	10.3	Estrone-3,4-13C2	A	2,5	6,19	S,I
Nervous stimulant		Caffeine	-0.07	14	Caffeine-13C3	A	4,14	5,16	S,I
Artificial sweetener		Sucralose	-1[2]	11.8[3]	Sucralose-d6	A	7,22	9,30	S,T

1: (National Center for Biotechnology Information, 2004), 2: (Subedi & Kannan, 2014a), 3: (Busetti et al., 2015)
3: LODs and LOQs were obtained based on standard deviation of y-intercept of measured concentrations of serial dilutions
4: Companies: **S:** Sigma-Aldrich Canada (Oakville, ON, Canada), **I:** C/D/N Isotopes (Pointe-Claire, QC, Canada), **C:** Cerilliant Corporation (Round Rock, Tex, USA), **M:** Cambridge isotope Laboratories (Tewksbury, MA, USA), **T:** Toronto Research Chemicals (North York, ON, Canada), **K:** KICTeam (Langely, BC, Canada)

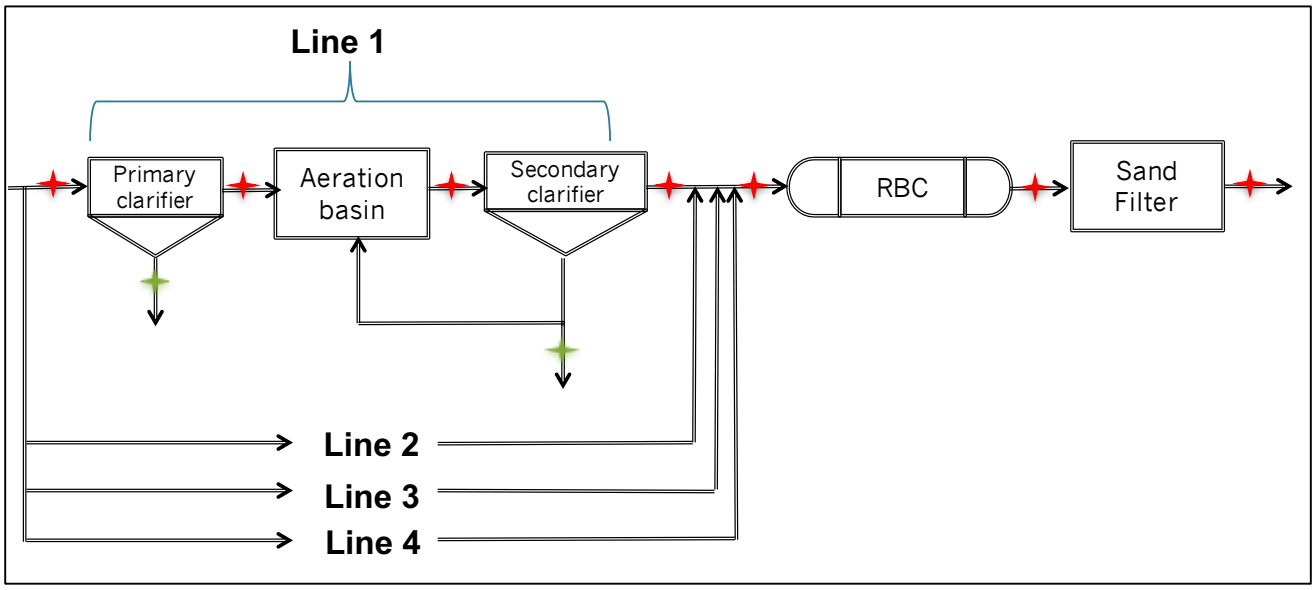

Figure 1: Schematic of the WWTP. Plants 1-4 correspond to the four lines of primary and secondary treatment. Red marks represent locations where conductivity probes were deployed and aqueous samples were collected. Green marks represent locations where sludge samples were collected

Table 2: Main characteristics of the WWTP, including hydraulic retention time (HRT), solids retention time (SRT) of treatment units in line 1, average temperature and pH during sampling campaigns

Characteristic		
HRT (h)	Primary clarifier (line 1)	3.72
	Aeration tanks (line 1)	6.3
	Secondary clarifier (line 1)	2.9
	RBCs	0.8
	Sand filter	0.4
SRT (days)[1]	(line 1)	7.76
Average T (°C)		20
Average pH		8.03

[1] The SRT was calculated by dividing total solids in the activated sludge reactors of line 1 (volume times sludge concentration) by the wasted sludge (waste flow times waste sludge concentration)

2.3. Hydraulic model

Electrical conductivity was utilized as a tracer for the investigation of the residence time distribution as proposed earlier (Ahnert et al., 2010; Majewsky et al., 2011). HOBO conductivity loggers (Hoskin Scientific, St-Laurent, QC, Canada) were used to collect electrical conductivity and temperature data (one reading per minute) for hydraulic model calibration. The probes were deployed over 3 weeks (June 12 – July 8, 2014) before and after each treatment unit (red marks in Figure 1). A universal optic-USB base station and Onset HOBOware Pro Version 3.2.2 software (Hoskin Scientific, St-Laurent, QC, Canada) were used for data transfer and read out, respectively.

The hydraulic model was created in the simulation software WEST (Mike Powered by DHI, Hørsholm, Denmark). Each part of the treatment plant was modelled separately, using the measured electrical conductivity at the entrance of each treatment step as tracer input for the respective hydraulic model, along with the actual flow conditions and tank volumes. A good fit between the

simulated and measured electrical conductivities at the exit of each treatment step was obtained by varying the number of tanks in series (aeration tanks) and the number of layers and feed layer (clarifiers) which determine the flow regime (i.e. from plug flow to fully mixed). The best-fit model was determined on the basis of minimizing the root mean square error (RMSE) and visual inspection of the output graphs. Simulations were run with the calibrated hydraulic model of each treatment unit employing a 24-h step increase of inert tracer as input, along with the actual flow rates during the sampling period. The output was used to obtain the load fractions that describe how the material in the effluent of each treatment stage on a certain day is composed out of fractions of the influent to that treatment step over several days, as illustrated by Majewsky et al. (2011).

2.4. Wastewater sampling

Wastewater was collected before and after each treatment unit at the locations shown in Figure 1 (red marks). The number of sampling days was based on the best-fit hydraulic model that indicated that the effluent of the activated sludge unit on a given day is composed of influent material entering the WWTP over four days, which will be elaborated upon in the results section. The samples collected during the first sampling campaign (July 21-25, 2014) were analyzed for the Class A compounds, while the samples collected during the second campaign (June 16-20, 2015) were analyzed for the Class B compounds. The samples were collected as 24-h composite using onsite Hach Sigma samplers that collect flow-proportional samples and refrigerate them at 4° C. This is with the exception of the effluent of the RBC, where ISCO 6712 samplers (Avensys, St-Laurent, QC, Canada) equipped with 24 bottles and packed with ice replaced daily were used to collect 24-h time-proportional composite samples. Both samplers collected samples every 15 minutes. Primary and secondary waste sludge samples were collected as grab samples over the four days (green marks in Figure 1). At the end of each day of the sampling campaign, the collected aqueous and sludge samples were transferred into 1-L amber HDPE bottles (Fisher Scientific) and stored at -20 °C until extraction was performed (within 3 weeks).

2.5. Sample preparation

2.5.1. Wastewater samples

Wastewater samples were thawed and filtered using 1-μm glass-fiber filter (Fisher Scientific) prior to extraction. Volumes of 100 mL of raw wastewater influent and 200 mL of all other sample matrixes were spiked with the appropriate internal standards (listed in Table 1). Solid phase extraction (SPE) was performed using two different methods. Class A compounds were extracted with Oasis MAX anion exchange cartridges (Waters Corporation), as described by Metcalfe et al. (2014). Class B compounds were extracted with Oasis MCX cation exchange cartridges, as described by Yargeau et al. (2014). Both methods and instruments used are summarized in the supplementary material (Table S1). SPE recoveries of target compounds ranged from 71% to 130% for Class A compounds (average recovery of 75%) and from 60% to 100% for Class B compounds (average recovery of 78%). Glass containers pre-washed with hexane and acetone were used for all sample preparation and analysis experiments.

2.5.2. Sludge samples

Approximately 1 g of freeze-dried sludge was placed in accelerated solvent extraction (ASE) stainless steel cells and spiked with internal surrogates (Table 1, 100 ng/g) before extraction. Extraction of sludge was conducted by pressurized liquid extraction using a Dionex ASE 350 accelerated solvent extraction system (Thermo Fisher Scientific, Waltham, MA, USA), followed by SPE clean up based on the methods summarized in the supplementary materials (Table S1). For the Class A compounds, the two extraction methods used were described by Edwards et al. (2009) as the neutral drug method and the acidic drug method. However, the neutral drugs method with acetone

and water (3:7) as the ASE extraction solvent (Table S1) gave the highest recoveries in the sludge matrix for this study. For class B compounds (mainly drugs of abuse), the extraction method that achieved the highest recoveries was the one suggested for the beta-blocker atenolol in the same study by Edwards et al. (2009) and is also summarized in Table S1. All sludge samples were extracted in triplicates and the extraction efficiencies of target compounds were >70%.

2.6. Analysis

Analysis of the Class A compounds was performed by liquid chromatography with tandem mass spectrometry (LC-MS/MS) using an Agilent 1100 HPLC (Mississauga, ON, Canada) coupled to a Q-Trap 5500 instrument (AB Sciex, Concord, ON, Canada) operated with a turbospray ionization source. The Class A target compounds of all samples were separated chromatographically using the method described by Metcalfe et al. (2014). The analytes were measured in either negative or positive ion mode, depending on the compound. Acquisition was performed using the precursor and product ion transitions for multiple reaction monitoring (MRM) of the target compounds and their corresponding deuterated surrogates. The MRM transitions for the target compounds are listed elsewhere (Metcalfe et al., 2014; Thompson et al., 2011).

Analysis of the Class B compounds was conducted by liquid chromatography with high-resolution mass spectrometry (LC-HRMS) using an Accela LC system coupled to a LTQ Orbitrap XL (Thermo Fisher Scientific, Waltham, MA, USA). Chromatographic separation and analysis in positive ion mode was achieved using the methods described by Rodayan et al. (2014b). Acquisition was performed in full scan mode (50–400 m/z) at high resolution (RFWHM = 41,000). The ion of interest was extracted using an m/z window of ±0.01. Linear calibration curves of nine points were used for quantification of the concentrations of the target compounds of both classes. Recoveries of the internal standards were used to adjust the concentrations of all target analytes.

2.7. CEC removals

The equations used for the calculations of CEC removals from the aqueous phase are presented in Eqs. 1-3 and Figure S1 in the supplementary material. Calculation of the CEC removals was first based on the CEC load in the aqueous phase only of the input and output streams of each treatment unit, as described in Eq. 1. To account for the residence time distribution, a fractionated input load of the CEC that corresponds to the output load on the fourth day of sampling was calculated (using Eq. 2), as proposed by Majewsky et al. (2011). The term "input reference load" will be used throughout the paper to refer to the incoming fractionated load of each treatment unit. The input reference load was compared to the output load to obtain estimates of the CEC removals per treatment unit (Eq. 3).

$$L_{aq} = Q * C_{aq} \quad (1)$$

$$L_{aq,\,ref} = \sum_{i=1}^{i=4} f_i * L_{aq,\,in,\,i} \quad (2)$$

$$R = \frac{L_{aq,ref} - L_{aq,out}}{L_{aq,ref}} * 100\% \quad (3)$$

Where L_{aq}: Load of the contaminant in the aqueous phase of a specific stream (mg/d), C_{aq}: Concentration of the contaminant in the aqueous phase of a specific stream (mg/L), Q: Flow rate of the corresponding stream (L/d), $L_{aq,\,ref}$ (mg/d): Reference mass load of the contaminant in the aqueous phase of the input stream based on several days of sampling, $L_{aq,\,in,\,i}$ (mg/d): Mass load of the contaminant in the input stream on the ith day of sampling, f_i : Fraction of incoming contaminant load on the ith day of sampling that is contained in the outgoing load on the last day of sampling, $L_{aq,out}$ (mg/d): Mass load of the CEC in the aqueous phase of the output stream on the last day of

sampling, R (%): Removal of CEC from the aqueous phase in a specific treatment unit using the fractionated approach.

Mass balances were then carried out across the primary and biological treatment steps based on the total contaminant load in both aqueous and particulate phases, according to Eq. 4-6. The total load of the CEC on each day was obtained in the input stream ($L_{in,tot}$), output stream ($L_{out,tot}$) and sludge stream ($L_{sludge,tot}$) by summing particulate and dissolved loads. In both the primary and biological treatment stages, the primary mechanisms of removal of the studied CECs are biodegradation and sorption to solids (Andersen et al., 2005; Li & Zhang, 2010; Radjenović et al., 2009; Verlicchi et al., 2012). Abiotic removal due to hydrolysis or photolysis was previously investigated for some of the studied CECs and reported to be negligible (Li & Zhang, 2010; Pèrez et al., 2005). Volatilization is also expected to be limited, due to the low Henry constants of the studied CECs (Gao et al., 2012). Therefore, the difference between the total incoming load and the outgoing load (in the output stream or sorbed to sludge) was assumed to be the load that was lost due to (bio)degradation (L_{deg}), as shown in Eq. 7. It should be kept in mind that this load could also include experimental or modelling errors that could cause a bias in the results. *t*

$$L_{tot,\,ref} = \sum_{i=1}^{i=4} f_i * L_{tot,\,in,\,i} \quad (4)$$

$$L_{tot} = L_{aq} + L_s \quad (5)$$

$$L_s = Q * C_s * TSS \quad (6)$$

$$L_{deg} = L_{tot,ref} - L_{tot,out} - L_{tot,sludge} \quad (7)$$

Where L_{tot}: Mass load of the contaminant in the liquid and particulate phase of a certain stream (mg/d), L_s: Mass load of contaminant measured in the particulate phase of a stream (mg/d), C_s: Concentration of contaminant measured in the particulate phase of a stream on a dry weight basis (mg/g), TSS: Total suspended solids in the corresponding stream (g/L), L_{aq} is computed using Eq. 1, except in the sludge stream where the volume fraction of solids is more significant, so $L_{aq,\,sludge} = Q_{sludge} * C_{aq,\,sludge} * f_w$, f_w: Volume fraction of water in sludge, L_{deg}: Mass load of the contaminant that was (bio)degraded (mg/d).

The measured CEC concentrations were used to calculate the K_d value, defined by Eq. 8, to represent the partitioning of CECs between the dry solids and the aqueous phase for primary and secondary waste sludge, separately. Error bars for loads, removals and K_d values were obtained based on standard deviations from lab triplicates using the propagation of error formulas (Ku, 1959). Investigating the statistical significance of the difference between the incoming and the outcoming loads (Section 3.7) was performed using the unequal-variance two-sample t-test with a confidence level of 95%.

$$Log\ Kd = \log\left(\frac{C_s}{C_{aq}}\right) \quad (8)$$

3. Results and Discussion

3.1. Calibration of hydraulic model and load fractions

The hydraulic mixing of the aeration tanks in line 1 was best described by three continuous-stirred tanks (CSTs) in series, all having equal volume and each representing perfect mixing. This is consistent with previous studies where the hydrodynamics of the aeration tanks in the activated sludge process were represented by a number of perfectly mixed tanks in series (Majewsky et al., 2011; Rodayan et al., 2014a). The primary and secondary clarifiers were both best modelled with a 10-layer settling tank and the 5th layer as feed layer. Figure S2 illustrates that the simulated and the

measured output conductivity profiles were in good agreement for all treatment steps, verifying the goodness of the fit of the obtained hydraulic model. The RBC hydraulic behaviour was modelled by a single CST and the sand filter as a series of 2 CSTs. The volumes for the RBCs and sand filters were adjusted until the simulated effluent matched the actual hydraulic behaviour, as it was not possible to estimate the actual hydraulic volume. The obtained volume was small, leading to low retention times, consistent with data obtained from operators at the WWTP. The absence of significant shifts or damping in the dynamics (Figure S2) demonstrates the minimal mixing occurring in both the RBCs and the sand filters due to their very short HRTs (see Table 2).

The obtained load fractions for the removal calculations are summarized in Table 3. For a selected treatment unit, load fractions represent the fractions of CEC incoming load on different days that make their way to the output on the last day of sampling, assuming no removal. Hence, these fractions do not necessarily add up to 100%. For both the primary and secondary clarifier the effluent was comprised of 92% of CEC load in the influent of the last day of sampling and only 8% of the day before. By contrast, the effluent of the aeration tank was composed of 73% of the CEC load on the same day and 12% from the day before, which can be related to the higher degree of mixing that occurs in the aeration tanks. The effluent from the activated sludge unit had even more distributed load fractions (64% from the last day and 36% from the previous day, 1% of three days ago and 0.04% of four days ago) due to the recycling of sludge. The load fractions of the activated sludge unit indicated the need for four days of sampling. For the RBCs and sand filters, only 4% and 3% respectively of CEC load on a previous day was present in the effluent on a given day, which is attributed to their low HRTs (Table 2), resulting in low mixing.

Table 3: Load fractions for each treatment unit (fi) describing the fraction of incoming CEC load on day i that is contained in the output of day 4 (last day of sampling) assuming no removal (including hydraulics effect only)

Treatment unit	Load fractions (%)			
	f1	f2	f3	f4
Primary clarifier	0	0	8	92
Aeration tanks	0	0	12	73
Secondary clarifier	0	0	8	92
Activated sludge (combination)	4E-02	1	36	64
RBCs	0	0	4	96
Sand filter	0	0	3	97

3.2. Concentrations of CECs in wastewater

The concentrations of the CECs at the different treatment steps are shown in Table 4. Most of the target compounds were detected in the influent to the primary clarifier, except for the prescription opioid drugs, fentanyl and ketamine. The lowest concentration of a target compound detected in the influent was observed for estrone (7 ng/L) and the highest mean concentration was for caffeine (28,960 ng/L). Among all target pharmaceuticals, ibuprofen, was present at the highest levels in influent wastewater. Selected metabolites of cocaine, methadone and carbamazepine were monitored as well. In the case of cocaine, only 1-9% is excreted unchanged from the human body, while 35-54% is excreted as benzoylecgonine (Ratola et al., 2012). The ratio between benzoylecgonine and cocaine was calculated to be 1.5. Despite the fact that the majority of studies reported values in the range of 3.1-3.5, lower values similar to the present study were reported in some studies (Bones et al., 2007), suggesting that some cocaine is being discharged into the sewage system without consumption (Karolak et al., 2010; Ratola et al., 2012).

Table 4 shows that the CEC concentrations in the combined secondary effluent (i.e. combined output from all the lines, Figure 1) were generally in the same ranges as the concentrations in the secondary effluent (i.e. from line 1), indicating that in terms of CEC removal, line 1 had a similar efficiency at removing CECs as the other three lines combined. In the final effluent, some target compounds, nine out of 26 CECs were not detected at concentrations above the limits of detection (LODs) or limits of quantification (LOQs). The highest effluent levels were observed for sucralose (3476 ng/L) followed closely by caffeine (2015 ng/L), unlike in the influent where caffeine had levels one order of magnitude higher than sucralose.

Table 4: Concentrations (ng/L ± standard deviation) of target CECs in line 1 of the WWTP at the influent to the primary clarifier (primary influent), effluent of the primary clarifier (primary effluent), effluent of the secondary clarifier (secondary effluent), as well as the combined secondary effluents of all lines (1-4), and effluent of RBCs (RBCs effluent) and sand filter effluent. Standard deviation was based on 3 replicates of sample preparation and analysis.

Compound	Primary influent	Primary effluent	Secondary effluent (Line 1)	Combined secondary effluent	RBCs effluent	Sand filter effluent
Androstenedione	92 ± 5	65 ± 2	42 ± 2	41 ± 2	40 ± 1	44 ± 1
Estrone	7 ± 3	8 ± 2	5 ± 1	7 ± 3	5 ± 1	6 ± 2
Trimethoprim	25 ± 2	25 ± 3	37 ± 3	29 ± 3	22 ± 2	<LOD
Sulfamethoxazole	33 ± 5	31 ± 5	43 ± 16	47 ± 21	37 ± 6	<LOQ
Ibuprofen	3644 ± 105	1690 ± 90	<LOQ	<LOQ	<LOQ	<LOQ
Naproxen	471 ± 36	504 ± 35	36 ± 5	20 ± 3	18 ± 2	<LOQ
Triclosan	166 ± 26	132 ± 11	50 ± 2	31 ± 4	42 ± 3	29 ± 2
Caffeine	28960 ± 4658	28123 ± 4750	1756 ± 226	2148 ± 127	2245 ± 194	2015 ± 270
Sucralose	2437 ± 220	2635 ± 166	4591 ± 291	3178 ± 84	3175 ± 209	3476 ± 129
Cocaine	361 ± 18	287 ± 30	111 ± 26	97 ± 21	<LOQ	<LOQ
Benzoylecgonine	524 ± 14	415 ± 18	83 ± 5	58 ± 4	50 ± 2	47 ± 3
Amphetamine	101 ± 2	77 ± 3	<LOQ	<LOD	<LOD	<LOD
Methamphetamine	300 ± 7	213 ± 10	47 ± 5	43 ± 2	23 ± 0.2	<LOD
EDDP	249 ± 29	299 ± 21	231 ± 55	156 ± 7	169 ± 15	156 ± 4
Ephedrine	1616 ± 62	1053 ± 61	111 ± 8	86 ± 8	72 ± 1	72 ± 3
Codeine	2116 ± 92	1204 ± 40	1281 ± 109	901 ± 16	529 ± 15	130 ± 5
Dihydrocodeine	324 ± 23	459 ± 23	22 ± 1	39 ± 3	28 ± 1	27 ± 1
Methadone	123 ± 3	98 ± 3	48 ± 4	54 ± 2	44 ± 1	42 ± 2
Morphine	295 ± 12	338 ± 61	68 ± 19	24 ± 3	23 ± 4	21 ± 5
Oxycodone	126 ± 10	137 ± 24	64 ± 3	46 ± 3	35 ± 3	27 ± 7
Tramadol	174 ± 6	116 ± 8	177 ± 11	105 ± 5	125 ± 7	105 ± 5
Ketamine	<LOD	<LOD	<LOQ	<LOQ	<LOQ	<LOQ
Fentanyl	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Carbamazepine	591 ± 24	416 ± 20	606 ± 28	588 ± 17	532 ± 8	519 ± 13
CMZ-DIOH	1074 ± 54	702 ± 40	553 ± 51	650 ± 26	703 ± 21	648 ± 49
CMZ-EP	192 ± 36	258 ± 40	295 ± 41	315 ± 44	385 ± 62	119 ± 32

3.3. Removals by primary clarifier

3.3.1. Aqueous phase

The CEC removals (%) based on aqueous phase data at each treatment step were estimated using the fractionated approach, as well as concentrations at the input and output streams (Figure 2). The

results obtained in the present study are generally comparable to results from the literature summarized in the Supplemental Material (Table S2). Poor removals from the aqueous phase of wastewater (<40%) were observed for all target CECs in the primary clarifier, with the highest removals observed for tramadol and codeine (Figure 2). Negative removals in the primary clarifier were observed for a number of CECs, namely oxycodone, naproxen, estrone, EDDP and dihydrocodeine (Figure 2). Some of these CECs were previously reported to have negative removals (Table S2).

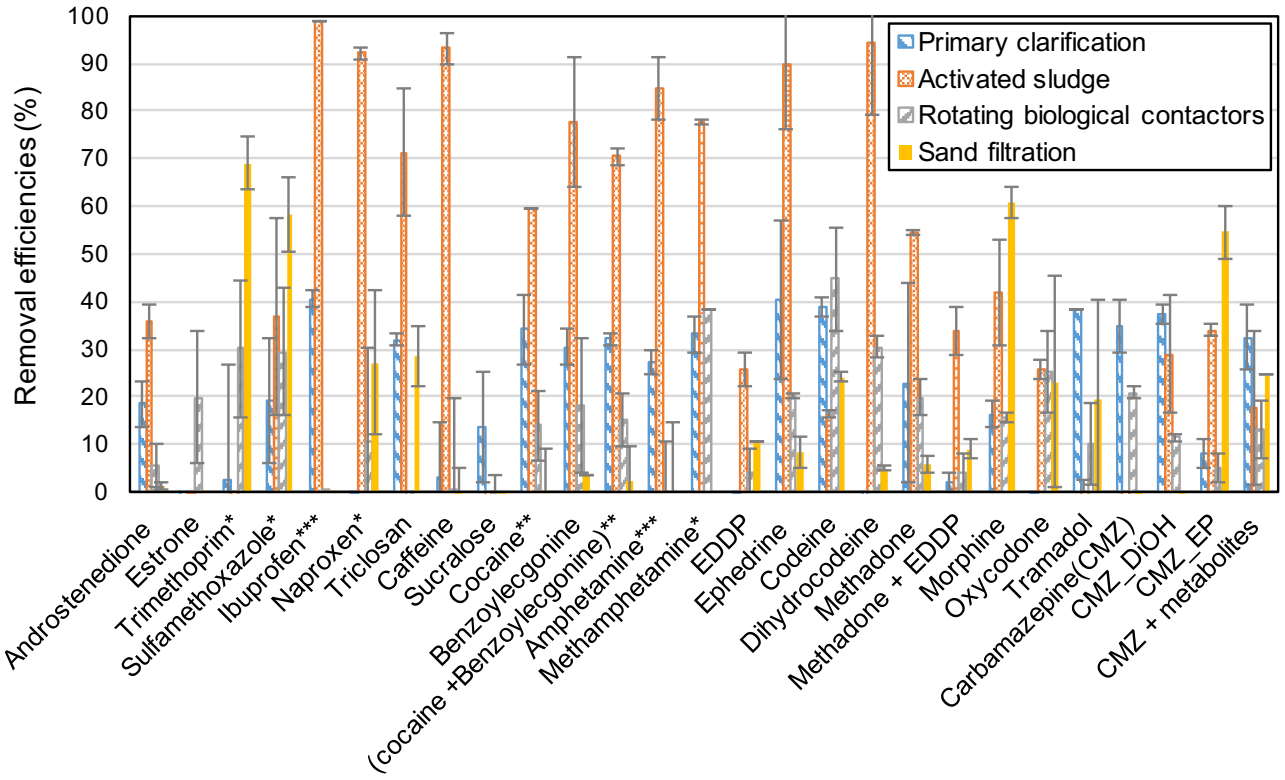

Figure 2: Estimated removal efficiencies (%) of target CECs. The removal efficiency was based on the incoming load to each treatment unit. (*, **, * denote compounds that were <LOD or <LOQ in the effluent of the activated sludge, RBC and sand filter, respectively, so removal was based on LOD (for <LOD) or LOQ (for <LOQ) and may be higher than reported here. Absence of a column indicates that either the removal was negative or the compound was <LOD or <LOQ in the influent to the treatment unit).**

3.3.2. Primary sludge

Concentrations of CECs in primary sludge, along with estimated log K_d values for the CECs in primary sludge are summarized in Table 5. The average concentration of the selected CECs in the solids of the primary sludge was the highest for caffeine, followed by triclosan and codeine; however, triclosan was found to have the highest log K_d value (Table 5). High concentrations in primary sludge can be attributed to high incoming concentrations (i.e. codeine and caffeine) in the wastewater, or high partitioning to sludge because of high hydrophobicity (i.e. triclosan, Table 1). Some CECs (i.e. naproxen, sucralose and ketamine) were not detected in the solids of primary sludge (Table 5), which is consistent with previous studies (Brorström-Lunden, 2008b; Jelic et al., 2011; Radjenović et al., 2009). The data reported in the present study on the levels drugs of abuse in primary and secondary waste sludge are valuable additions to the limited research in this area (Subedi & Kannan, 2015). The estimated log K_d values for drugs of abuse indicate that these compounds are poorly sorbed to

particulates, and the concentrations detected in the aqueous phase of untreated wastewater can thus be used to estimate community drug consumption using the "sewage epidemiology" approach (Castiglioni et al., 2006).

The total input reference mass load of the CECs to the primary clarifier was assigned to different fate pathways, and the percentage of each pathway was calculated (Figure 3). According to data obtained from operators at the WWTP, the primary clarifier removes 84% of TSS on average. This is higher than normal and can be attributed to the high HRT of 3.72 h as opposed to the usual HRT of 2-3 h in primary clarifiers under dry weather conditions. Also, the chemical addition of ferric chloride for phosphorus removal enhances the settling of solids, contributing to the high TSS removal. Despite the high TSS reduction, the fraction of CECs that is removed with sludge was less than 5% of the total incoming load for 15 out of 22 compounds (Figure 3) owing to their hydrophilic properties (Table 1). This fraction was previously reported to be less than 0.1% for ibuprofen, naproxen, sulfamethoxazole and caffeine in primary treatment (Carballa et al., 2007; Gao et al., 2012). Ternes et al. (2004) suggested that compounds with K_d < 500 L/Kg or log K_d < 2.70 often have a negligible fraction sorbed to sludge. In the present study, the estimated log K_d values for androstenedione, trimethoprim, triclosan and carbamazepine were above this threshold (Table 5), and these compounds were observed to be the only four compounds with more than 10% of their incoming load sorbed to primary sludge.

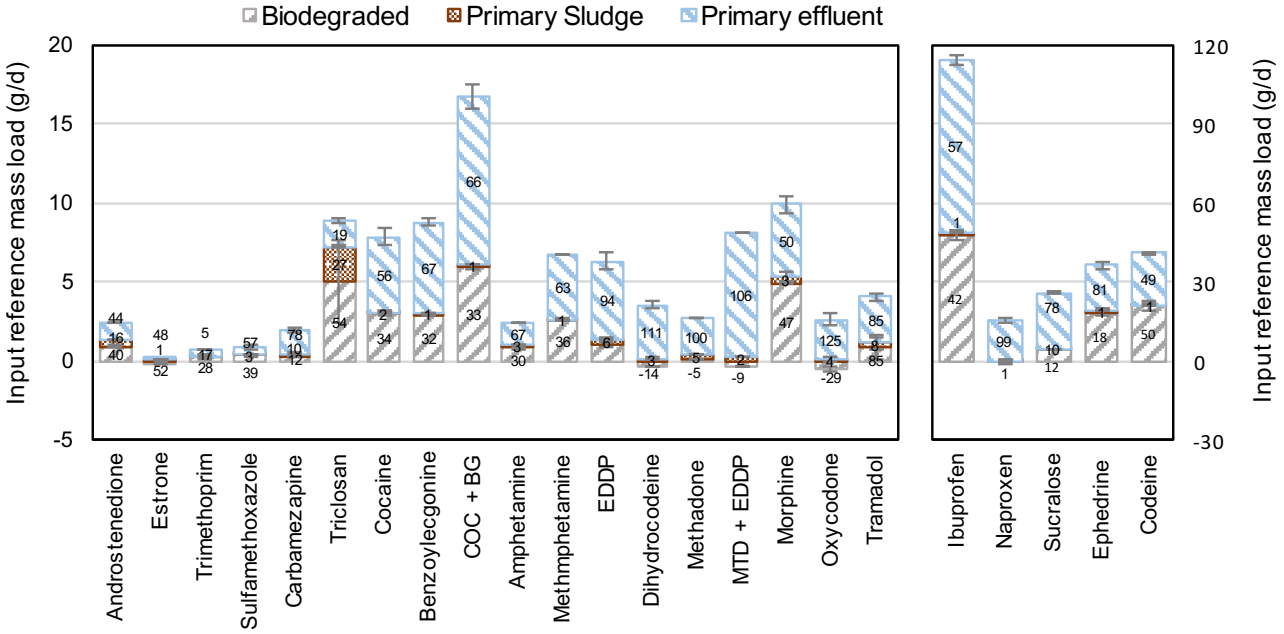

Figure 3: Input reference mass loads of target CECs into the primary clarifier assigned into three main fate pathways: (bio)degraded, discharged with primary sludge and discharged with the primary effluent with % of each pathway in column (COC: cocaine, BG: benzoylecgonine, MTD: methadone). For caffeine (not shown due to the high mass loads): 14% biodegraded, 2% sorbed to primary sludge and 84% in the effluent.

Table 5: Average concentrations of target CECs in primary and activated sludge and the range of concentrations on the four days of sampling represented as average (lowest –highest), along with the estimated Log K_d ± standard deviation in primary and activated sludge

Compound	Average Concentrations (Lowest-highest) (ng/L)		Log K_d ± standard deviation (Log L/Kg)	
	Primary sludge	Activated sludge	Primary clarifier	Secondary clarifier

Androstenedione	98 (85-113)	39 (15-65)	3.20 ± 0.19	2.92 ± 0.37
Estrone	<LOD	28 (<LOD-53)	<LOD	3.65 ± 0.32
Trimethoprim	31 (24-43)	154 (92-198)	3.07 ± 0.19	3.59 ± 0.18
Sulfamethoxazole	12 (6-17)	<LOQ	2.15 ± 0.49	<LOQ
Ibuprofen*	196 (73-496)	39 (21-59)	2.27 ± 0.64	3.36 ± 0.38
Naproxen	<LOD	23 (<LOQ-25)	<LOD	2.95 ± 0.32
Triclosan	599 (340-909)	1335 (1199-1377)	3.68 ± 0.11	4.49 ± 0.27
Caffeine	2828 (1536-4716)	776 (674-953)	2.10 ± 0.37	2.73 ± 0.31
Sucralose	<LOD	152 (110-218)	<LOD	1.53 ± 0.19
Cocaine	27 (16-35)	<LOD	1.93 ± 0.18	<LOD
Benzoylecgonine	5 (<LOD-8)	<LOQ	1.40 ± 0.07	<LOQ
Amphetamine*	20 (7-37)	13 (5-20)	2.29 ± 0.38	3.09 ± 0.33
Methamphetamine	14 (11-17)	4 (<LOD-4)	1.83 ± 0.02	1.92
EDDP	89 (73-109)	65 (44-95)	2.46 ± 0.1	2.44 ± 0.17
Ephedrine	47 (22-69)	26 (<LOQ-55)	1.60 ± 0.48	2.16 ± 0.5
Codeine	202 (77-305)	36 (<LOQ-53)	2.17 ± 0.26	1.36 ± 0.4
Dihydrocodeine	29 (10-44)	41 (18-56)	1.71 ± 0.08	3.20 ± 0.31
Methadone	49 (7-125)	46 (<LOQ-125)	2.57	2.55 ± 0.71
Morphine	96 (40-171)	76 (37-117)	2.61 ± 0.4	2.99 ± 0.29
Oxycodone	25 (9-42)	8 (<LOD-13)	2.09 ± 0.45	1.97 ± 0.44
Tramadol	92 (21-232)	7 (<LOD-11)	2.58 ± 0.5	1.49 ± 0.36
Ketamine	<LOQ	<LOQ	<LOQ	<LOQ
Fentanyl	18 (11-27)	23 (15-31)	N.A.	N.A.
Carbamazepine	49 (32-78)	27 (22-34)	2.75 ± 0.21	2.29 ± 0.04
CMZ-DiOH	<LOD	<LOD	<LOD	<LOD
CMZ-EP	<LOD	<LOD	<LOD	<LOD

*Denotes compounds that were <LOD or LOQ in the secondary effluent, indicating that the obtained Log K_d in the secondary clarifier might not be accurate as it was based on the LOD or LOQ of the compound.
Carbamazepine solids analysis was based on the 2014 sampling campaign.

Despite the generally low biological activity of primary clarifiers, some of the removal during primary clarification can be attributed to (bio)degradation, as shown in Figure 3. The percentage of incoming load degraded during primary clarification was as high as 40-54% for a number of the investigated CECs (i.e. ibuprofen, morphine, codeine, triclosan and ephedrine). Considering the small error bars of the mass loads in Figure 3 (except for triclosan), this high degradation in the primary clarifier is unlikely to be due to measurement bias caused by sample preparation and analysis. McCall et al. (2016) showed in a review article that several studies reported the formation of transformation products of drugs of abuse in the sewers (HRT 30 min to 12 h) under different conditions, and Heuett et al. (2015) reported the detection of transformation products of drugs of abuse in influent sewage to a WWTP. The degradation of some pharmaceuticals and formation of transformation products in a real sewer pipe was also reported by Jelic et al. (2015). Having similar conditions as in sewers, primary clarifiers could also allow (bio)degradation of CECs. In addition, the primary clarifier under study has a higher HRT (3.72 h) than usual, which could explain the high observed CEC (bio)degradation. In fact, most of the compounds with the highest (bio)degradation in the primary clarifier also showed high (bio)degradation potential in the activated sludge (Figure 4). It should be noted that the sum of the percentages removed by (bio)degradation and removed with sludge (Figure 3) is not equivalent to the percentage removal from the aqueous phase calculated previously (Figure 2). This is explained by the fact that the input reference mass load used in the mass balance calculations includes not only CEC loads in the aqueous phase, but also in the

particulate phase. The CEC concentrations in the suspended particulate phase of primary inlet load were assumed to be equal to those in primary sludge, making the assumption that equilibrium partitioning of CECs between the particulate and aqueous phases takes place before the primary clarifier.

3.4. Removals by activated sludge

3.4.1. Aqueous phase

The estimated removals of CECs (%) during the activated sludge treatment step are illustrated in Figure 2. In general, for the majority of the compounds, most of the removal takes place in the activated sludge treatment stage when compared to other treatment steps. Amongst the target compounds, the removals varied from >80% for six target CECs, namely ibuprofen, naproxen, amphetamine, ephedrine, dihydrocodeine and caffeine to negative removals for carbamazepine, tramadol, estrone, sucralose and trimethoprim. The variable data on CEC removal by activated sludge treatment reported in the literature is summarized in the supplementary material (Table S2).

3.4.2. Secondary sludge

The measured concentrations of CECs in the solids of secondary sludge and estimates of the log K_d values for secondary sludge are summarized in Table 5. Triclosan followed by caffeine and trimethoprim were shown to have the highest average concentrations in secondary sludge. Similar to the analysis of the primary sludge, triclosan had the highest estimated log K_d value (i.e. 4.49) for secondary sludge. Caffeine and sucralose (log K_{ow} -0.07 and -1, respectively), on the other hand, had high concentrations in secondary waste sludge owing to their high loads to the biological treatment (Table 5). The differences in the estimated log K_d values between primary and secondary waste sludge for each of the CECs can be explained by variable sludge composition (Ternes et al., 2004).

Figure 4 displays the CEC input reference load to the activated sludge unit assigned to different fate pathways. The reference mass loads were calculated based on the assumption that the CEC concentrations in the suspended solids contained in the output of the primary and secondary clarifiers were the same as those measured in the primary and secondary sludge, respectively. This involves making the assumption that the CEC particulate concentration is uniform throughout the primary and secondary clarifiers. Discharge with the secondary waste sludge was found to be the predominant removal mechanism for trimethoprim, triclosan and estrone, with 55%, 51% and 33% of their input reference load, respectively ending up in the secondary waste sludge. For all the remaining compounds, a low fraction (i.e. <5%) of the input reference load was discharged with secondary waste sludge (Figure 4), due to their hydrophilic nature (Log K_{ow}, Table 1). This is in good agreement with previous research for a number of the target compounds whose fate in activated sludge units has been previously investigated through mass balance (i.e. ibuprofen, naproxen, carbamazepine, sulfamethoxazole and caffeine) (Gao et al., 2012; Joss et al., 2005; Petrie et al., 2014).

(Bio)degradation was the predominant removal mechanism for 17 out of the 22 target CECs. More than 90% of the incoming load was removed by (bio)degradation in the case of ibuprofen, naproxen, ephedrine, dihydrocodeine, as well as caffeine (not shown in Figure 4). Substantial (bio)degradation of ibuprofen, naproxen and caffeine was also reported by other authors (Carballa et al., 2007; Gao et al., 2012; Joss et al., 2005). For the majority of the compounds studied, the mass fraction removed by (bio)degradation (Figure 4) and the data on removals from the aqueous phase (Figure 2) were comparable. This indicates that (bio)degradation is responsible for the majority of the removal of the

studied CECs from the aqueous phase in the activated sludge unit, while removal by adsorption is of insignificant importance. By contrast, the fraction of biodegraded material was negative for several CECs, such as carbamazepine, sucralose, trimethoprim and tramadol, which indicated that the mass loads in the secondary output were higher than the incoming loads, consistent with their negative removal from the aqueous phase (Figure 2). The use of the fractionated approach accounting for RTD of the treatment units limited the bias associated with the sampling strategy and removal calculations but some negative removals were still observed. These negative removals are, therefore, likely due to the presence of conjugated forms of the compound that transform into the parent compound during treatment, as well as desorption from particulate matter (Jelic et al., 2011; Ternes & Joss, 2006). For carbamazepine and trimethoprim, the absence of removal by biodegradation obtained in this study is in agreement with previous studies (Gao et al., 2012; Joss et al., 2005; Li & Zhang, 2010; Petrie et al., 2014).

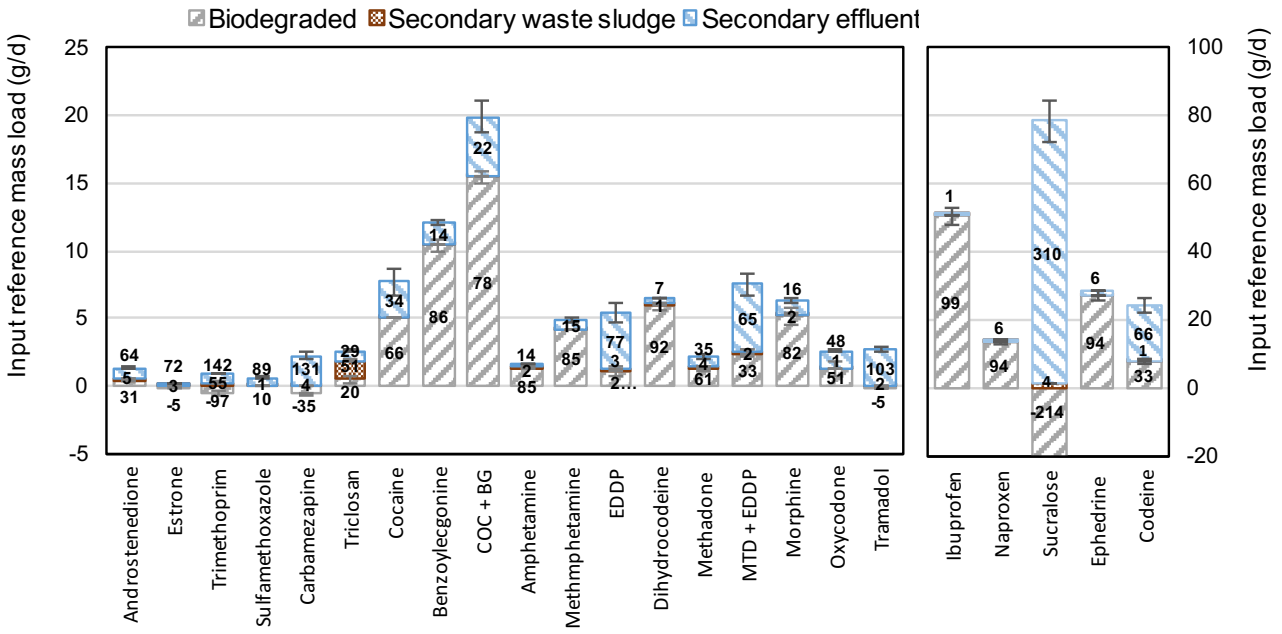

Figure 4: Input reference mass loads of target CECs into activated sludge assigned into three main fate pathways: (bio)degraded, discharged with waste activated sludge and discharged with the secondary effluent with % of each pathway in columns (COC: cocaine, BG: benzoylecgonine, MTD: methadone). For ibuprofen and amphetamine, the concentration in the effluent was <LOQ, so the LOQ was used for calculations. For caffeine (not shown due to the high mass loads): 92% biodegraded and 8% in the effluent.

3.5. Removals by RBCs

The WWTP was upgraded to include RBCs in order to meet regulations with regards to the ammonia nitrogen concentration in the effluent (i.e. maximum concentration of 3.4 mg/L). Although the ammonia nitrogen in the secondary effluent was reported to be <1 mg/L during the summer, indicating full nitrification by the activated sludge process, levels as high as 6.6 mg/L were observed over some days during the winter. The RBCs removed ammonia nitrogen and achieved effluent levels below 2.41 mg/L throughout the year. In spite of their capability to remove ammonia nitrogen, the RBCs were generally ineffective at removing most of the target CECs (i.e. <30%), except for methamphetamine, codeine and dihydrocodeine, as shown in Figure 2. Additionally, the CECs whose removal calculation had to be based on LOQ or LOD due to the fact that they were below these limits (marked with two stars) could also have high removals. Among the target CECs, low average removals of <15% were observed for 11 of the 26 target CECs. Negative removals at this treatment stage were observed for sucralose and triclosan, indicating the possible de-conjugation of their

conjugates during tertiary treatment, as in the activated sludge treatment stage or possible sampling bias. The fate of a limited number of the target compounds (i.e. trimethoprim, sulfamethoxazole, triclosan, ibuprofen, carbamazepine and caffeine) has been investigated before in RBCs, and their obtained removals were higher than in the present study (Batt et al., 2007; Kanda et al., 2003; Thompson et al., 2005; Vasiliadou et al., 2014). This can be explained by the fact that the RBCs are used as tertiary treatment in this study, with a low HRT (i.e. 0.8 h) and little organic material available for biofilm growth, while in the previous studies, RBCs were used for secondary treatment as an alternative to activated sludge treatment. The contribution of the particulate CEC load to the total load in the RBCs and sand filter is deemed insignificant, since the TSS load entering these units is low (<20 mg/L). Hence, a detailed mass balance was not performed for these units.

3.6. Removals by sand filtration

Similar to the RBCs, the average removal of CECs by sand filtration and chlorination was based on the incoming load in the aqueous phase only, due to the low TSS load. The removal was greater than 50% for only 4 CECs (i.e. trimethoprim, sulfamethoxazole, morphine and CBZ-EP). The incoming loads of trimethoprim and sulfamethoxazole were reduced by more than 69% and 58%, respectively by sand filtration. Data from previous studies reported considerable removal (over 60%) of trimethoprim in sand filters (Göbel et al., 2007; Nakada et al., 2007; Sui et al., 2010), but lower removals (i.e. 20-30%) were reported for sulfamethoxazole (Gao et al., 2012; Göbel et al., 2007; Nakada et al., 2007). With the exception of the four compounds listed above, sand filtration and chlorination were inefficient at removing most of the other target CECs, as less than 30% of their incoming load was removed (Figure 2). The observed removal for triclosan was similar to that observed by Nakada et al. (2007) (i.e. 29%). Negative removals (or no removal) were obtained for estrone, sucralose, carbamazepine and CBZ-DiOH during the sand filtration and chlorination steps. For estrone and carbamazepine, the negative removals are in agreement with previous studies (Gao et al., 2012; Nakada et al., 2007; Sui et al., 2010), indicating possible desorption of these compounds during sand filtration. Removal from the aqueous phase in sand filters is attributed to adsorption to solid particles that are retained by the sand filter. However, previous studies also indicated that (bio)degradation may contribute to CEC removal due to the formation of biofilm on sand particles (Göbel et al., 2007). In the WWTP investigated, chlorine was added right before sand filtration, which makes the growth of a biofilm unlikely.

3.7. Metabolites

The combined loads of parent compounds and their metabolite(s) were considered for some target CECs when calculating removal efficiencies for each treatment stage (Figure 5) to account for possible inter-transformations. Cocaine and its major metabolite had comparable removals in all treatment units. Although EDDP (methadone's major metabolite) alone exhibited negative removal of -17% in the primary clarifier, the removal efficiency based on combined concentrations of EDDP and its parent compound methadone was above zero, indicating that some of the methadone was possibly converted into EDDP. Similarly, removals based on combined loads were obtained for carbamazepine and its two investigated metabolites, CBZ-DiOH and CBZ-EP. A limited number of studies have investigated the removals of carbamazepine and its major metabolites in WWTPs (Hummel et al., 2006; Leclercq et al., 2009; Miao et al., 2005). Only the study by Miao et al. (2005) examined the concentrations of carbamazepine and its metabolites at each treatment step of the WWTP and observed a decline in the concentration of carbamazepine and its two metabolites in the primary clarifier and an increase in carbamazepine and CBZ-DiOH concentrations by activated sludge. In the present study, the calculation was further refined by considering the input and output mass loadings of carbamazepine and its two major metabolites in each treatment unit taking the RTD into account, as shown in Figure 5. It should be noted that in the present study, due to use of the

fractionated approach, the input reference load a treatment step is not equivalent to the output load of the previous treatment step (Figure 5).

A decrease was observed in the loads of carbamazepine and CBZ-DiOH during primary clarification ($p<0.05$), whilst the variations in the loads of CBZ-EP across the primary clarifier were not statistically different ($p=0.26$). Similarly, activated sludge was not shown to cause a decrease in the loads of carbamazepine and CBZ-EP ($p=0.57$ and 0.07, respectively), but it decreased the loads of CBZ-DIOH ($p<0.05$) and, thus, the combined load of carbamazepine and its metabolites. This is possibly explained by partial transformation of CBZ-DiOH back into carbamazepine during both primary clarification and activated sludge causing the persistence of carbamazepine by compensating for the biodegraded load. Tertiary treatment in the form of RBCs diminished the load of carbamazepine and CBZ-DiOH significantly ($p<0.05$), but not that of CBZ-EP ($p=0.25$). Sand filtration and chlorination, on the other hand, resulted in a decrease in the average load of only CBZ-EP, accompanied with a load increase of both carbamazepine and CBZ-DiOH ($p<0.05$ for all the compounds). The combined load of carbamazepine and the two metabolites was also increased ($p=0.03$). This might suggest the transformation of CBZ-EP to both CBZ-DiOH and carbamazepine during the sand filtration step. The two metabolites of carbamazepine were not quantified in sludge samples in the present study. However, Miao et al. (2005) concluded that these compounds are present at low concentrations in biosolids (<8 ng/g).

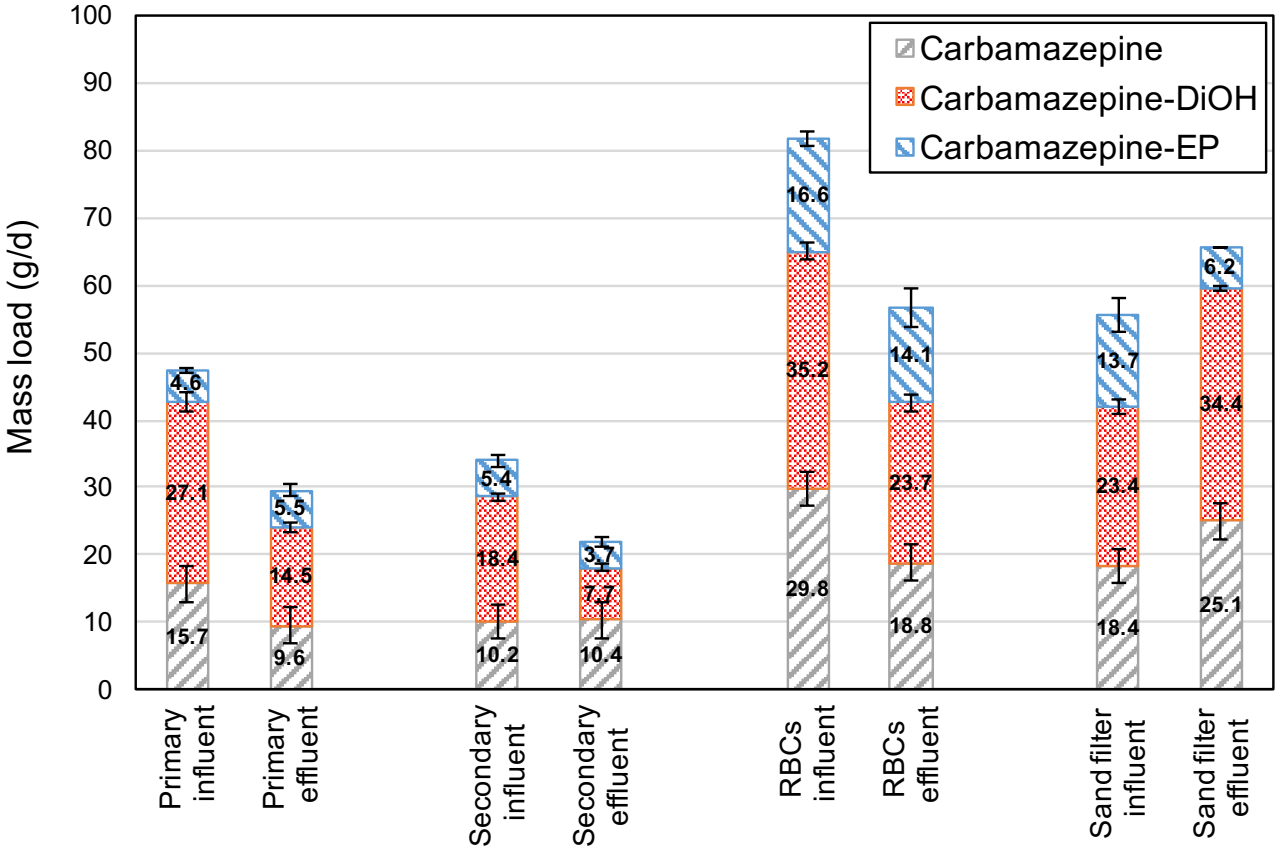

Figure 5. Mass loads of carbamazepine and its two investigated metabolites (CMZ-DiOH & CMZ-EP) in both the input (reference load) and the output of the primary clarifier, secondary treatment, RBCs and sand filter.

4. Conclusions

Removal data for the aqueous phase showed that most CECs were poorly removed (i.e. <40%) in the primary clarifier. The greatest removals typically occurred in the activated sludge, while removals were <50% in the RBCs and ranged from no removal to 70% removal during sand filtration. The mass balances, which looked at dissolved as well as adsorbed CECs provided further insight into the predominant removal mechanisms for CECs during primary and secondary treatment. It showed that not only sorption to the primary sludge, but also (bio)degradation contribute to the removal of some of the target CECs in the primary clarifier. In activated sludge, (bio)degradation was found to be the predominant removal mechanism, with sorption accounting for <5% for most CECs. The estimated log K_d values (1.40 to 3.68 in primary sludge and 1.36 to 4.49 in activated sludge) also indicated that most CECs are not significantly removed by partitioning onto sludge, with the exception of triclosan. Accounting for the levels of metabolites of the selected CECs (i.e. carbamazepine, methadone and cocaine) explained some of the negative removals that were observed. The current study expands the understanding of the removal pathways of CECs at different treatment steps, for which limited data is available in the literature. It also takes into account the hydraulics following a novel approach applied for the first time to primary clarification, RBCs and sand filtration to provide reliable data of the CEC removals. Reliable data on the fate of CECs are valuable for the calibration of mathematical fate models that can be used to optimize treatment technologies and reduce discharges of CECs into the aquatic environment.

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Supplementary Material

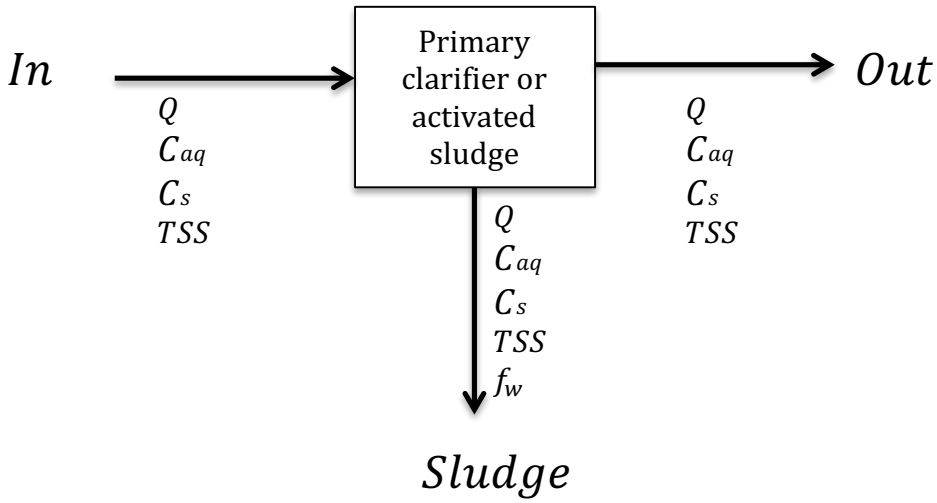

Figure S1: Schematic illustrating the streams information used for mass balance calculations

Table S1: Extraction methods of wastewater and biosolids samples for Class A and Class B compounds using SPE (aqeuous) and ASE-SPE (biosolids) methods

Sample type	Method	Extraction step	Class A	Class B
Aqueous	Solid-phase extraction (SPE) (aqueous samples)	Instrument	Manual manifold	Gilson GX-271 ASPECTM automated instrument
		Cartridge[a]	6 mL-500 mg Oasis MAX cartridges	6 mL-150 mg Oasis MCX cartridges
		Sample pH	pH 8.0 using sodium hydroxide	pH 2.5 using sulfuric acid
		Cartridge conditioning	6 mL of methanol, 6 mL of 0.1 M ammonium hydroxide and 6 mL of water (pH 8.0)	6 mL of acetone and 6 mL of water (pH 2.5)
		Elution	2 mL methanol and then 3x3 mL of 2% formic acid in methanol, 1 mL/min	3x3 mL of 5% ammonium hydroxide in methanol, 1 mL/min
		Reconstitution	50% methanol/50% water to a total volume of 0.4 mL	25% methanol/75% water to a total volume of 0.4 mL
Biosolids	Accelerated solvent extraction (ASE) (Biosolids samples)	Instrument	Dionex ASE 350	Dionex ASE 350
		Conditions	Temperature =80 °C Static cycle= 3	Temperature =50 °C Static cycles= 2
		Elution solvent	Acetone: water (3:7)	Methanol:water:acetic acid (49:49:2)
	Solid-phase extraction (SPE) (aqueous samples)	Instrument	Manual manifold	Gilson GX-271 ASPECTM automated instrument
		Cartridge[a]	6 mL-500 mg Oasis HLB cartridges	6 mL-150 mg Oasis MCX cartridges
		Sample pH	pH 7.5 using sodium hydroxide	pH 2.5 using sulfuric acid
		Cartridge conditioning	6 mL acetone, 6 mL methanol, and 6 mL	6 mL of acetone and 6 mL of water (pH 2.5)

	Milli-Q water (pH 7.5).	
Elution	3x3 mL of methanol, 1 mL/min	3x3 mL of 5% ammonium hydroxide in methanol, 1 mL/min
Reconstitution	50% methanol/50% water to a total volume of 0.1 mL	25% methanol/75% water to a total volume of 0.4 mL

Table S2: Average estimated removal efficiencies of target CECs in activated sludge and primary clarifier, separately from previous studies that investigated the removal from the aqueous phase

Compound	Primary clarifier	Activated sludge
Androstenedione	-8% [1]	<100%[1]
Estrone	40%[2]; - 8%[3]; -59%[4]	40%[2]; -49% to 99%[15]; -40% to 20%[5]; 70%[6]; 85%[3]
Trimethoprim	15%[3]	70%[3]; 7[5]; 40[16]
Sulfamethoxazole	-10%[2]	65%[2]; 56%[7]; -138% to 60%[5]; 90%[6]; 40%[3]
Ibuprofen	-7%[2]; 5%[3]; 88%[4]	75%[8]; 70%[2]; 83%[7]; 90%[6]; 100%[3]
Naproxen	3%[2]; -10%[3]; <0%[4];	78%[8]; 45%[2]; 85%[7]; 80%[6]; 90%[3]; 72[16]
Triclosan	53%[9]; 32%[4]	75%[3]
Caffeine	17%[3]	100%[3]
Sucralose	N.A.	-40-10%[10]
Cocaine	70%[11]; 0%[12]	90%[12]; 40%[11]
Benzoylecgonine	50%[11]; 2%[12]	83%[12]; 40%[11]
Amphetamine	60%[11]; 5%[12]	40%[11]
Methamphetamine	-120%[11]; 3%[12]	79%[12]; -20%[11]
EDDP	-40%[11]; 0%[12]	26%[12]
Ephedrine	9%[12]	25%[12]
Codeine	4%[12]	45%[6]; 85%[13]; 9%[12]
Dihydrocodeine	N.A.	50%[13]
Methadone	14%[12]; -110%[11]	20%[13]; -5%[11]
Morphine	0%[3]; 5%[12]; 25%[11]	95%[13]
Oxycodone	-4%[12]	28%[12]
Tramadol	21%[12]	35%[6]
Ketamine	-	-
Fentanyl	-	-
Carbamazepine	26%[14]; -10%[3]	0%[7]; -20%[6]; 15%[13]; 25%[3]

1: (Esperanza et al., 2007), 2: (Carballa et al., 2004), 3**:** (Behera et al., 2011), 4: (Blair et al., 2013b), 5: (Göbel et al., 2007), 6: (Kasprzyk-Hordern et al., 2009), 7: (Radjenovic et al., 2007), 8: (Stumpf et al., 1999),9: (Winkler et al., 2007), 10: (Brorström-Lunden, 2008a), 11: (Subedi & Kannan, 2014b), 12: (Rodayan et al., 2014a), 13: (Wick et al., 2009), 14: (Zhou et al., 2009), 15: (Joss et al., 2004), 16:(Radjenović et al., 2009)

In (Rodayan et al., 2014a; Subedi & Kannan, 2014b), the removal by activated sludge was not calculated by authors, but the value in this table is an estimation obtained as the difference between the total removal and removal by the primary clarifier since both removals (calculated on the basis of Eq. 3) were based on the same input load in the denominator (i.e. primary influent load).

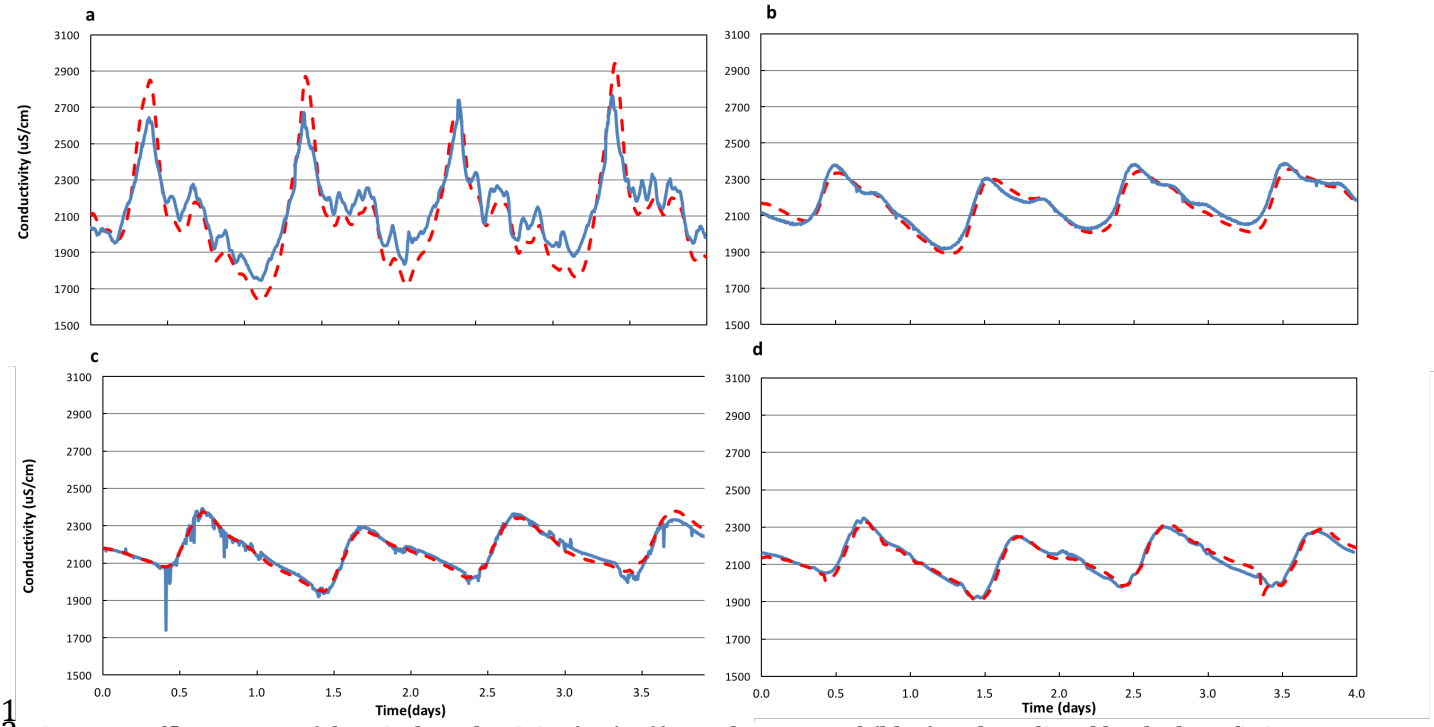

Figure S2: Effluent tracer (electrical conductivity (µS/cm)) trends measured (blue) and predicted by the best-fitting hydraulic model (dashed red) throughout four consecutive days for: a) primary clarifier, b) aeration tanks, c) RBCs and d) sand filter

REFERENCES:

Ahnert, M., Kuehn, V., & Krebs, P. (2010). Temperature as an alternative tracer for the determination of the mixing characteristics in wastewater treatment plants. *Water Res, 44*, 1765-1776. doi: http://dx.doi.org/10.1016/j.watres.2009.11.047

Andersen, H. R., Hansen, M., Kjølholt, J., Stuer-Lauridsen, F., Ternes, T., & Halling-Sørensen, B. (2005). Assessment of the importance of sorption for steroid estrogens removal during activated sludge treatment. *Chemosphere, 61*, 139-146. doi: http://dx.doi.org/10.1016/j.chemosphere.2005.02.088

Batt, A. L., Kim, S., & Aga, D. S. (2007). Comparison of the occurrence of antibiotics in four full-scale wastewater treatment plants with varying designs and operations. *Chemosphere, 68*, 428-435. doi: http://dx.doi.org/10.1016/j.chemosphere.2007.01.008

Behera, S. K., Kim, H. W., Oh, J.-E., & Park, H.-S. (2011). Occurrence and removal of antibiotics, hormones and several other pharmaceuticals in wastewater treatment plants of the largest industrial city of Korea. *Science of The Total Environment, 409*, 4351-4360. doi: http://dx.doi.org/10.1016/j.scitotenv.2011.07.015

Blair, B. D., Crago, J. P., Hedman, C. J., & Klaper, R. D. (2013a). Pharmaceuticals and personal care products found in the Great Lakes above concentrations of environmental concern. *Chemosphere, 93*, 2116-2123. doi: http://dx.doi.org/10.1016/j.chemosphere.2013.07.057

Blair, B. D., Crago, J. P., Hedman, C. J., Treguer, R. J. F., Magruder, C., Royer, L. S., & Klaper, R. D. (2013b). Evaluation of a model for the removal of pharmaceuticals, personal care products, and hormones from wastewater. *Science of The Total Environment, 444*, 515-521. doi: http://dx.doi.org/10.1016/j.scitotenv.2012.11.103

Bones, J., Thomas, K. V., & Paull, B. (2007). Using environmental analytical data to estimate levels of community consumption of illicit drugs and abused pharmaceuticals. *Journal of Environmental Monitoring, 9*, 701-707. doi: 10.1039/B702799K

Brorström-Lunden, E., Svenson, A., Viktor, T., Woldegiorgis, A., Remberger, M., Kaj, L., Dye, C., Bjerke, A., Schlabach, M. (2008a). Measurements of Sucralose in the Swedish Screening Program 2007, PART I: Swedish Environmental Research Institute Ltd.

Brorström-Lunden, E., Svenson, A., Viktor, T., Woldegiorgis, A., Remberger, M., Kaj, L., Dye, C., Bjerke, A., Schlabach, M. (2008b). Measurements of sucralose in the Swedish screening program 2007, Part II: Swedish Environmental Research Institute Ltd.

Busetti, F., Ruff, M., & Linge, K. L. (2015). Target screening of chemicals of concern in recycled water. *Environmental Science: Water Research & Technology, 1*, 659-667. doi: 10.1039/C4EW00104D

Carballa, M., Omil, F., & Lema, J. M. (2007). Calculation methods to perform mass balances of micropollutants in sewage treatment plants. Application to pharmaceutical and personal care products (PPCPs). *Environmental Science and Technology, 41*, 884-890. doi: http://dx.doi.org/10.1021/es061581g

Carballa, M., Omil, F., Lema, J. M., Llompart, M. a., García-Jares, C., Rodríguez, I., Gómez, M., & Ternes, T. (2004). Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant. *Water Res, 38*, 2918-2926. doi: http://dx.doi.org/10.1016/j.watres.2004.03.029

Castiglioni, S., Zuccato, E., Crisci, E., Chiabrando, C., Fanelli, R., & Bagnati, R. (2006). Identification and measurement of illicit drugs and their metabolites in urban wastewater by Liquid Chromatography−Tandem Mass Spectrometry. *Analytical Chemistry, 78*, 8421-8429. doi: http://dx.doi.org/10.1021/ac061095b

Dickenson, E. R. V., Snyder, S. A., Sedlak, D. L., & Drewes, J. E. (2011). Indicator compounds for assessment of wastewater effluent contributions to flow and water quality. *Water Res, 45*, 1199-1212. doi: http://dx.doi.org/10.1016/j.watres.2010.11.012

Edwards, M., Topp, E., Metcalfe, C. D., Li, H., Gottschall, N., Bolton, P., Curnoe, W., Payne, M., Beck, A., Kleywegt, S., & Lapen, D. R. (2009). Pharmaceutical and personal care products in tile drainage following surface spreading and injection of dewatered municipal biosolids to an agricultural field. *Science of The Total Environment, 407*, 4220-4230. doi: http://dx.doi.org/10.1016/j.scitotenv.2009.02.028

Esperanza, M., Suidan, M. T., Marfil-Vega, R., Gonzalez, C., Sorial, G. A., McCauley, P., & Brenner, R. (2007). Fate of sex hormones in two pilot-scale municipal wastewater treatment plants: Conventional treatment. *Chemosphere, 66*, 1535-1544. doi: http://dx.doi.org/10.1016/j.chemosphere.2006.08.020

Gao, P., Ding, Y., Li, H., & Xagoraraki, I. (2012). Occurrence of pharmaceuticals in a municipal wastewater treatment plant: Mass balance and removal processes. *Chemosphere, 88*, 17-24. doi: http://dx.doi.org/10.1016/j.chemosphere.2012.02.017

Gay, F., Ferrandino, I., Monaco, A., Cerulo, M., Capasso, G., & Capaldo, A. (2015). Histological and hormonal changes in the European eel (Anguilla anguilla) after exposure to environmental cocaine concentration. *Journal of Fish Diseases*, n/a-n/a. doi: http://dx.doi.org/10.1111/jfd.12362

Göbel, A., McArdell, C. S., Joss, A., Siegrist, H., & Giger, W. (2007). Fate of sulfonamides, macrolides, and trimethoprim in different wastewater treatment technologies. *Science of The Total Environment, 372*, 361-371. doi: http://dx.doi.org/10.1016/j.scitotenv.2006.07.039

Heidler, J., & Halden, R. U. (2008). Meta-Analysis of Mass Balances Examining Chemical Fate during Wastewater Treatment. *Environ Sci Technol, 42*, 6324-6332. doi: http://dx.doi.org/10.1021/es703008y

Heuett, N. V., Batchu, S. R., & Gardinali, P. R. (2015). Understanding the magnitude of emergent contaminant releases through target screening and metabolite identification using high resolution mass spectrometry: Illicit drugs in raw sewage influents. *Journal of Hazardous Materials, 282*, 41-50. doi: http://dx.doi.org/10.1016/j.jhazmat.2014.08.009

Hughes, S. R., Kay, P., & Brown, L. E. (2013). Global synthesis and critical evaluation of pharmaceutical data sets collected from river systems. *Environmental Science and Technology, 47*, 661-677. doi: http://dx.doi.org/10.1021/es3030148

Hummel, D., Löffler, D., Fink, G., & Ternes, T. A. (2006). Simultaneous determination of psychoactive drugs and their metabolites in aqueous matrices by Liquid

Chromatography Mass Spectrometry. *Environmental Science and Technology, 40*, 7321-7328. doi: http://dx.doi.org/10.1021/es061740w

Jelic, A., Gros, M., Ginebreda, A., Cespedes-Sánchez, R., Ventura, F., Petrovic, M., & Barcelo, D. (2011). Occurrence, partition and removal of pharmaceuticals in sewage water and sludge during wastewater treatment. *Water Res, 45*, 1165-1176. doi: http://dx.doi.org/10.1016/j.watres.2010.11.010

Jelic, A., Rodriguez-Mozaz, S., Barceló, D., & Gutierrez, O. (2015). Impact of in-sewer transformation on 43 pharmaceuticals in a pressurized sewer under anaerobic conditions. *Water Res, 68*, 98-108. doi: http://dx.doi.org/10.1016/j.watres.2014.09.033

Joss, A., Andersen, H., Ternes, T., Richle, P. R., & Siegrist, H. (2004). Removal of Estrogens in Municipal Wastewater Treatment under Aerobic and Anaerobic Conditions: Consequences for Plant Optimization. *Environ Sci Technol, 38*, 3047-3055. doi: 10.1021/es0351488

Joss, A., Keller, E., Alder, A. C., Göbel, A., McArdell, C. S., Ternes, T., & Siegrist, H. (2005). Removal of pharmaceuticals and fragrances in biological wastewater treatment. *Water Res, 39*, 3139-3152. doi: http://dx.doi.org/10.1016/j.watres.2005.05.031

Kanda, R., Griffin, P., James, H. A., & Fothergill, J. (2003). Pharmaceutical and personal care products in sewage treatment works. *Journal of Environmental Monitoring, 5*, 823-830. doi: http://dx.doi.org/10.1039/B306355K

Karolak, S., Nefau, T., Bailly, E., Solgadi, A., & Levi, Y. (2010). Estimation of illicit drugs consumption by wastewater analysis in Paris area (France). *Forensic Science International, 200*, 153-160. doi: http://dx.doi.org/10.1016/j.forsciint.2010.04.007

Kasprzyk-Hordern, B., Dinsdale, R. M., & Guwy, A. J. (2009). The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. *Water Res, 43*, 363-380. doi: http://dx.doi.org/10.1016/j.watres.2008.10.047

Kidd, K. A., Blanchfield, P. J., Mills, K. H., Palace, V. P., Evans, R. E., Lazorchak, J. M., & Flick, R. W. (2007). Collapse of a fish population after exposure to a synthetic estrogen. *Proceedings of the National Academy of Sciences, 104*, 8897-8901. doi: http://dx.doi.org/10.1073/pnas.0609568104

Ku, H. H. (1959). Journal of research of the National Bureau of Standards: Section C. Engineering and instrumentation. *Engineering and instrumentation*, 14 v.

Kümmerer, K. (2008). *Pharmaceuticals in the environment sources, fate, effects, and risks*. Berlin: Springer.

Leclercq, M., Mathieu, O., & Gomez, E. (2009). Presence and fate of carbamazepine, oxcarbazepine, and seven of their metabolites at wastewater treatment plants. *Archives of Environmental Contamination & Toxicology, 56*, 408-415. doi: http://dx.doi.org/10.1007/s00244-008-9202-x

Li, B., & Zhang, T. (2010). Biodegradation and adsorption of antibiotics in the activated sludge process. *Environmental Science and Technology, 44*, 3468-3473. doi: http://dx.doi.org/10.1021/es903490h

Luo, Y., Guo, W., Ngo, H. H., Nghiem, L. D., Hai, F. I., Zhang, J., Liang, S., & Wang, X. C. (2014). A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Science of*

The Total Environment, 473-474, 619-641. doi: http://dx.doi.org/10.1016/j.scitotenv.2013.12.065

Majewsky, M., Farlin, J., Bayerle, M., & Galle, T. (2013). A case-study on the accuracy of mass balances for xenobiotics in full-scale wastewater treatment plants. *Environmental Science: Processes & Impacts, 15*, 730-738. doi: http://dx.doi.org/10.1039/C3EM30884G

Majewsky, M., Galle, T., Bayerle, M., Goel, R., Fischer, K., & Vanrolleghem, P. A. (2011). Xenobiotic removal efficiencies in wastewater treatment plants: residence time distributions as a guiding principle for sampling strategies. *Water Res, 45*, 6152-6162. doi: http://dx.doi.org/10.1016/j.watres.2011.09.005

Mawhinney, D. B., Young, R. B., Vanderford, B. J., Borch, T., & Snyder, S. A. (2011). Artificial sweetener sucralose in U.S. drinking water systems. *Environmental Science and Technology, 45*, 8716-8722. doi: http://dx.doi.org/10.1021/es202404c

McCall, A.-K., Bade, R., Kinyua, J., Lai, F. Y., Thai, P. K., Covaci, A., Bijlsma, L., van Nuijs, A. L. N., & Ort, C. (2016). Critical review on the stability of illicit drugs in sewers and wastewater samples. *Water Res, 88*, 933-947. doi: http://dx.doi.org/10.1016/j.watres.2015.10.040

Metcalfe, C., Hoque, M. E., Sultana, T., Murray, C., Helm, P., & Kleywegt, S. (2014). Monitoring for contaminants of emerging concern in drinking water using POCIS passive samplers. *Environmental Science: Processes & Impacts, 16*, 473-481. doi: http://dx.doi.org/10.1039/C3EM00508A

Metcalfe, C., Tindale, K., Li, H., Rodayan, A., & Yargeau, V. (2010). Illicit drugs in Canadian municipal wastewater and estimates of community drug use. *Environmental Pollution, 158*, 3179-3185. doi: http://dx.doi.org/10.1016/j.envpol.2010.07.002

Miao, X.-S., & Metcalfe, C. D. (2003). Determination of carbamazepine and its metabolites in aqueous samples using Liquid Chromatography−Electrospray Tandem Mass Spectrometry. *Analytical Chemistry, 75*, 3731-3738. doi: http://dx.doi.org/10.1021/ac030082k

Miao, X.-S., Yang, J.-J., & Metcalfe, C. D. (2005). Carbamazepine and its metabolites in wastewater and in biosolids in a municipal wastewater treatment plant. *Environmental Science and Technology, 39*, 7469-7475. doi: http://dx.doi.org/10.1021/es050261e

Nakada, N., Shinohara, H., Murata, A., Kiri, K., Managaki, S., Sato, N., & Takada, H. (2007). Removal of selected pharmaceuticals and personal care products (PPCPs) and endocrine-disrupting chemicals (EDCs) during sand filtration and ozonation at a municipal sewage treatment plant. *Water Res, 41*, 4373-4382. doi: http://dx.doi.org/10.1016/j.watres.2007.06.038

National Center for Biotechnology Information. (2004). PubChem Compound Database. Available from National Center for Biotechnology Information, U.S. National Library of Medicine PubChem Compound Retrieved 1/8/2016 https://pubchem.ncbi.nlm.nih.gov

Ort, C., Lawrence, M. G., Rieckermann, J., & Joss, A. (2010). Sampling for pharmaceuticals and personal care products (PPCPs) and illicit drugs in wastewater systems: Are your conclusions valid? A Critical Review. *Environmental Science and Technology, 44*, 6024-6035. doi: http://dx.doi.org/10.1021/es100779n

Pèrez, S., Eichhorn, P., & Aga, D. S. (2005). Evaluating the biodegradability of sulfamethazine, sulfamethoxazole, sulfathiazole, and trimethoprim at different stages of sewage treatment. *ETC Environmental Toxicology and Chemistry, 24*, 1361-1367.

Petrie, B., Barden, R., & Kasprzyk-Hordern, B. (2015). A review on emerging contaminants in wastewaters and the environment: Current knowledge, understudied areas and recommendations for future monitoring. *Water Res, 72*, 3-27. doi: http://dx.doi.org/10.1016/j.watres.2014.08.053

Petrie, B., McAdam, E. J., Lester, J. N., & Cartmell, E. (2014). Obtaining process mass balances of pharmaceuticals and triclosan to determine their fate during wastewater treatment. *Science of The Total Environment, 497–498*, 553-560. doi: http://dx.doi.org/10.1016/j.scitotenv.2014.08.003

Purdom, C. E., Hardiman, P. A., Bye, V. V. J., Eno, N. C., Tyler, C. R., & Sumpter, J. P. (1994). Estrogenic effects of effluents from sewage treatment works. *Chemistry and Ecology, 8*, 275-285. doi: http://dx.doi.org/10.1080/02757549408038554

Radjenovic, J., Petrovic, M., & Barceló, D. (2007). Analysis of pharmaceuticals in wastewater and removal using a membrane bioreactor. *Analytical and Bioanalytical Chemistry, 387*, 1365-1377. doi: 10.1007/s00216-006-0883-6

Radjenović, J., Petrović, M., & Barceló, D. (2009). Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment. *Water Res, 43*, 831-841. doi: http://dx.doi.org/10.1016/j.watres.2008.11.043

Ratola, N., Cincinelli, A., Alves, A., & Katsoyiannis, A. (2012). Occurrence of organic microcontaminants in the wastewater treatment process. A mini review. *Journal of Hazardous Materials, 239–240*, 1-18. doi: http://dx.doi.org/10.1016/j.jhazmat.2012.05.040

Rodayan, A., Afana, S., Segura, P. A., Sultana, T., Metcalfe, C. D., & Yargeau, V. (2015). Linking drugs of abuse in wastewater to contamination of surface and drinking water. *Environ Toxicol Chem, 35*, 843-849. doi: http://dx.doi.org/10.1002/etc.3085

Rodayan, A., Majewsky, M., & Yargeau, V. (2014a). Impact of approach used to determine removal levels of drugs of abuse during wastewater treatment. *Sci Total Environ, 487*, 731-739. doi: http://dx.doi.org/10.1016/j.scitotenv.2014.03.080

Rodayan, A., Segura, P. A., & Yargeau, V. (2014b). Ozonation of wastewater: Removal and transformation products of drugs of abuse. *Science of The Total Environment, 487*, 763-770. doi: http://dx.doi.org/10.1016/j.scitotenv.2013.11.023

Servos, M. R., Bennie, D. T., Burnison, B. K., Jurkovic, A., McInnis, R., Neheli, T., Schnell, A., Seto, P., Smyth, S. A., & Ternes, T. A. (2005). Distribution of estrogens, 17β-estradiol and estrone, in Canadian municipal wastewater treatment plants. *Science of The Total Environment, 336*, 155-170. doi: http://dx.doi.org/10.1016/j.scitotenv.2004.05.025

Stumpf, M., Ternes, T. A., Wilken, R.-D., Silvana Vianna, R., & Baumann, W. (1999). Polar drug residues in sewage and natural waters in the state of Rio de Janeiro, Brazil. *Science of The Total Environment, 225*, 135-141. doi: http://dx.doi.org/10.1016/S0048-9697(98)00339-8

Subedi, B., & Kannan, K. (2014a). Fate of Artificial Sweeteners in Wastewater Treatment Plants in New York State, U.S.A. *Environ Sci Technol, 48*, 13668-13674. doi: http://dx.doi.org/10.1021/es504769c

Subedi, B., & Kannan, K. (2014b). Mass Loading and Removal of Select Illicit Drugs in Two Wastewater Treatment Plants in New York State and Estimation of Illicit Drug Usage in Communities through Wastewater Analysis. *Environ Sci Technol, 48*, 6661-6670. doi: http://dx.doi.org/10.1021/es501709a

Subedi, B., & Kannan, K. (2015). Occurrence and fate of select psychoactive pharmaceuticals and antihypertensives in two wastewater treatment plants in New York State, USA. *Science of The Total Environment, 514*, 273-280. doi: http://dx.doi.org/10.1016/j.scitotenv.2015.01.098

Sui, Q., Huang, J., Deng, S., Yu, G., & Fan, Q. (2010). Occurrence and removal of pharmaceuticals, caffeine and DEET in wastewater treatment plants of Beijing, China. *Water Res, 44*, 417-426. doi: http://dx.doi.org/10.1016/j.watres.2009.07.010

Ternes, T. A., Herrmann, N., Bonerz, M., Knacker, T., Siegrist, H., & Joss, A. (2004). A rapid method to measure the solid–water distribution coefficient (Kd) for pharmaceuticals and musk fragrances in sewage sludge. *Water Res, 38*, 4075-4084. doi: http://dx.doi.org/10.1016/j.watres.2004.07.015

Ternes, T. A., & Joss, A. (2006). *Human Pharmaceuticals, Hormones and Fragrances: The Challenge of Micropollutants in Urban Water Management*. New York: IWA Publishing.

Thompson, A., Griffin, P., Stuetz, R., & Cartmell, E. (2005). The Fate and Removal of Triclosan during Wastewater Treatment. *Water Environment Research, 77*, 63-67.

Thompson, K., Zhang, J., & Zhang, C. (2011). Use of fugacity model to analyze temperature-dependent removal of micro-contaminants in sewage treatment plants. *Chemosphere, 84*, 1066-1071. doi: http://dx.doi.org/10.1016/j.chemosphere.2011.04.063

Vasiliadou, I. A., Molina, R., Martínez, F., & Melero, J. A. (2014). Experimental and modeling study on removal of pharmaceutically active compounds in rotating biological contactors. *Journal of Hazardous Materials, 274*, 473-482. doi: http://dx.doi.org/10.1016/j.jhazmat.2014.04.034

Verlicchi, P., Al Aukidy, M., & Zambello, E. (2012). Occurrence of pharmaceutical compounds in urban wastewater: removal, mass load and environmental risk after a secondary treatment--a review. *Science of The Total Environment, 429*, 123-155. doi: http://dx.doi.org/10.1016/j.scitotenv.2012.04.028

Wick, A., Fink, G., Joss, A., Siegrist, H., & Ternes, T. A. (2009). Fate of beta blockers and psycho-active drugs in conventional wastewater treatment. *Water Res, 43*, 1060-1074. doi: http://dx.doi.org/10.1016/j.watres.2008.11.031

Winkler, G., Thompson, A., Fischer, R., Krebs, P., Griffin, P., & Cartmell, E. (2007). Mass flow balances of triclosan in small rural wastewater treatment plants and the impact of biomass parameters on the removal. *Engineering in Life Sciences, 7*, 42-51. doi: 10.1002/elsc.200620158

Yargeau, V., Rodayan, A., Taylor, B., Li, H., & Metcalfe, C. D. (2014). Analysis of drugs of abuse in wastewater from two Canadian cities. *Science of The Total Environment, 487*, 722-730. doi: http://dx.doi.org/10.1016/j.scitotenv.2013.11.094

Zhou, J. L., Zhang, Z. L., Banks, E., Grover, D., & Jiang, J. Q. (2009). Pharmaceutical residues in wastewater treatment works effluents and their impact on receiving river water. *Journal of Hazardous Materials, 166*, 655-661. doi: http://dx.doi.org/10.1016/j.jhazmat.2008.11.070