OCCURRENCE AND FATE OF ENDOCRINE DISRUPTORS THROUGH THE SAN MARCOS WASTEWATER TREATMENT PLANT

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OCCURRENCE AND FATE OF ENDOCRINE DISRUPTORS

THROUGH THE SAN MARCOS WASTEWATER

TREATMENT PLANT

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In the past decade, the scientific community has become increasingly concerned that humans and wildlife are harmed by exposure to chemicals that interact with the endocrine (hormonal) system, known as endocrine disrupting compounds (EDCs). Wastewater treatment plants (WWTPs) have been shown to be major point sources of these compounds to the environment because they are not completely removed by traditional treatment processes. This study was designed to investigate the removal efficiencies of 23 known or suspected EDCs through the San Marcos WWTP and to determine which treatment process was the most effective at removal. Of the 23 compounds monitored, the most frequently detected in the WWTP influent were acetaminophen, nonylphenol, coprostanol, caffeine, benzophenone, triethyl citrate, DEET, bisphenol A, tris(2-chloroethyl)phosphate (TCEP), and triclosan. Comparison of influent and effluent concentrations showed that the San Marcos WWTP is effectively removing (>92%) of these compounds, with the exception of carbamazepine and TCEP. Within the treatment plant, results indicate that the aeration process (biological treatment) was the most effective at removal. When compounds were not completely removed from the wastewater, they were detected in waters downstream of the effluent discharge in the San Marcos River. This study also investigated the occurrence of these compounds in wastewaters from the San Marcos Hospital and the finished water of the San Marcos water treatment facility (WTF). Results from samples collected at the hospital indicate that the hospital discharge is contributing to the concentration of these compounds in the San Marcos wastewater collection system. Analyses of samples collected from the WTF indicate that there is no significant amount of these compounds in the finished drinking water supply. TCEP was only detected once at trace amounts.

CHAPTER I

INTRODUCTION

In the past decade, the scientific community has become increasingly concerned that humans and wildlife are affected by exposure to chemicals that interact with the endocrine (hormonal) system. Chemicals that interfere with the body's natural hormones are called endocrine disrupting chemicals (EDCs). A broad range of environmental contaminants, including some pesticides and industrial pollutants, are either known or suspected EDCs. In 1996, research on EDCs was identified as a high-priority topic in the EPA's Office of Research and Development Strategic Plan (USEPA 1996).

In humans and other mammals, hormones aid in development, growth, reproduction, and behavior. Hormones are also responsible for many bodily functions including: food storage, blood pressure, and blood glucose levels. EDCs can interfere with the ordinary functioning of hormones in several different ways. Normally, a hormone will bind exclusively to a receptor, which in turn sets off a chain of events that result in a biological response. EDCs can interfere with this process by binding to receptors, causing unwanted responses. These responses include negative feedback, positive feedback, and blocking.

Negative feedback is a biological response which is less than normal. Positive feedback is a biological response which is greater than normal. Blocking occurs when

EDCs bind to receptors and there is no subsequent biological response. EDCs may also alter the rate at which hormones are broken down and excreted.

Despite the fact that significant quantities of EDCs have been found in surface water and ground water globally, scientists know very little about what concentrations induce effects on aquatic ecosystems and their organism populations. There has been some evidence that suggests that at parts per trillion some EDCs can have an impact on reproductive organs in fish and frogs (Tyler et al., 2001 and Berrill et al., 2003). At higher concentrations, EDCs may cause birth defects, altered immune functions, sexual dysfunction, cancer, and possibly heart disease (Nadal et al., 2006).

Some compounds, such as industrial pollutants, are released into the environment through industrial waste discharges. Pesticides and herbicides are knowingly released into the environment and make their way to ponds, streams, and rivers. Other chemicals, such as household chemicals, pharmaceuticals, synthetic hormones, and other consumables are released into the environment through Wastewater Treatment Plants (WWTPs) of cities and other municipalities. Research has shown that EDCs are not totally removed or degraded by conventional WWTPs and that after entering the environment many EDCs will disperse and eventually accumulate in river sediment where they can persist for years.

Once EDCs are in the environment, they can pose problems for municipalities whose water treatment facilities (WTFs) treat surface water and groundwater from locations that have been effected by WWTP effluents.

It is important to determine how effectively WWTPs are removing EDCs. The main goals of this study were to:

1. monitor the influent and effluent waters of the San Marcos WWTP to determine the efficiency of the plant in removing EDCs from the water,
2. to determine what process within the plant is the most efficient at removing EDCs.

The San Marcos WWTP uses primary, secondary, and tertiary treatment processes. The primary treatment uses bar screens, grit removers, and primary clarifiers. The secondary treatment uses aeration and activated sludge processes followed by secondary clarifiers. The tertiary treatment uses a sand/granular activated charcoal (GAC) (anthrafilt) filtration process and ultraviolet (UV) disinfection.

The WWTP is permitted to receive an average daily flow (ADF) of 9 million gallons per day (MGD) and peak wet weather flow of 18 MGD, but the treatment process has been rated for a capacity of 7.6-MGD-ADF. In 2004, the WWTP treated 1,923 million gallons of wastewater with an Annual Average Daily Flow of 4.9MGD.

Due to time, technology, and funding, 23 known or suspected EDCs (Table 1) were chosen for this study. These compounds represent a large range of general use categories, including: personal care products, pesticides, pharmaceuticals, natural and synthetic hormones, and industrial pollutants. The 23 EDCs were chosen based on several factors: relevance in the San Marcos community, time and cost required in running the analysis, availability of required instrumentation, and finally, which EDCs pose the greatest threat to local fish and wildlife in the San Marcos River. Once we

Table 1. Target compounds, Chemical Abstract Service registry number (CAS), and Use/Source

Compound; CAS #	Use/Source
Nonylphenol; 104-40-5	Detergent Metabolite
Octylphenol; 1806-26-4	Detergent Metabolite
Acetaminophen; 103-90-2	Antipyretic
Benzophenone; 119-61-9	Fragrance Fixative
Bisphenol A; 80-05-7	Plasticizer
Caffeine; 58-08-2	Stimulant
Carbamazepine; 298-46-4	Anti-Epileptic
Codeine; 76-57-3	Analgesic
Coprostanol; 360-68-9	Fecal Steroid
Cotinine; 486-56-6	Nicotine Metabolite
Diazinon; 333-41-5	Pesticide
Diltiazem; 42399-41-7	Antihypertensive
Estradiol; 50-28-2	Natural Female Hormone
Ethynylestradiol; 57-63-6	Synthetic Estrogen
Fluoranthene; 206-44-0	Polycyclic Aromatic Hydrocarbon (PAH)
Fluoxetine; 54910-89-3	Anti-Depressant
N,N-diethyl-meta-toluamide (DEET); 134-62-3	Insecticide
Sulfamethoxazole; 723-46-6	Antibiotic
Tributyl Phosphate; 126-73-8	Fire Retardant
Triclosan; 3380-34-5	Anti-Microbial
Triethyl Citrate; 77-93-0	Cosmetics
Trimethoprim; 738-70-5	Antibiotic
Tris(2-chloroethyl)phosphate (TCEP); 115-96-8	Fire Retardant

understand how effectively the plant is removing the EDCs, then proper steps can be taken to address whatever problems are identified.

Other goals of this study were to determine how effectively the San Marcos WTF is removing these 23 compounds from surface water from Lake Dunlap before being distributed in the San Marcos water drinking supply, and to determine what concentration of these compounds the hospital is contributing to the San Marcos wastewater collections system.

CHAPTER II

METHODS AND MATERIALS

Chemicals. Coprostanol, DEET, diltiazem, ethyl citrate, ethynylestradiol, sulfamethoxazole, tributylphosphate, triclosan, and TCEP were purchased from Sigma-Aldrich Chemicals (St. Louis, MO). Acetaminophen, caffeine, carbamazepine, codeine, cotinine, estradiol, and fluoxetine were purchased from Cerilliant (Round Rock, TX). Bisphenol A that was purchased from Aldrich Chemical Company and then purified further via sublimation at 145 $^{\circ}$C under vacuum was graciously donated by Dr. Chad Booth (Texas State University-San Marcos). Trimethoprim was purchased from Fluka (Steinheim, Germany). Fluoranthene was purchased from the National Bureau of Standards (Gaithersburg, MD). Diazinon was purchased from Protocol (Metuchen, NJ). Nonylphenol (technical mixture) was purchased from Riedel-de Haen (Seelze, Germany). Octylphenol and benzophenone were purchased from Supelco (Bellefonte, PA). Internal standard caffeine-$^{13}C_3$ was purchased from ISOTEC (Miamisburg, OH). Surrogate standards fluoranthene-d_{10} and bisphenol A-d_{16} were purchased from Supelco (Bellefonte, PA); Codeine-d_6, and acetaminophen-d_4 were purchased from Cerilliant (Round Rock, TX). Internal standard chrysene-d_{12} was purchased from Ultra Scientific (North Kingston, RI).

A primary standard solution of 1,000 μg/mL of each compound was prepared in an appropriate solvent. Compounds that were analyzed by LC were prepared in methanol (MeOH) and compounds that were analyzed by GC were prepared in dichloromethane (DCM). These solutions were stored in the refrigerator at 4ºC. All working standards were prepared by combining the standards with solvents and then subsequent serial dilutions.

Reagent water, methyl tert-butyl ether (MTBE), MeOH, ethyl acetate and DCM were purchased from EM Science (Darmstadt, Germany). All solvents were HPLC grade. Sulfuric acid was purchased from VWR International (West Chester, PA). Formic acid was purchased from Fluka (Steinheim, Germany)

Sample Collection. Samples were collected at the three sampling locations during periods of normal operation from October 2006 to March 2007 using pre-cleaned 1-L, amber glass bottles (VWR, West Chester, PA). Samples were kept on ice and brought back to the Bio Assay Lab at the Edwards Aquifer Research and Data Center (EARDC) within 2 hours of collection. Once in the lab, samples were immediately preserved by adjusting to pH 2 with concentrated sulfuric acid and then stored in the refrigerator at 4ºC until extraction. Sulfuric acid has previously been shown to effectively preserve samples without degradation of the majority of these analytes (Vanderford et al., 2003). Samples were extracted within 7 days of collection.

Sample Sites. At the San Marcos Hospital, samples were taken from two discharge ports. One port services the main facility (designated discharge #1) and the other port services the emergency room (designated discharge #2).

At the San Marcos WTF, grab samples were collected from the raw untreated water from Lake Dunlap and from the final treated water that is pumped into the drinking water supply for San Marcos.

At the San Marcos WWTP, grab samples were collected from the influent, from primary and secondary clarifier effluents, from the aeration basis effluent, after the sand/GAC filtration, after UV disinfection, and finally from the reaeration basin effluent (Figure 1). Samples were also taken from points approximately thirty yards upstream and downstream of the WWTP effluent discharge in the San Marcos River.

Quality Assurance. Lab blanks, field blanks, surrogate spikes, laboratory-reagent spikes, matrix spikes, and matrix spike duplicates were run to assure proper quality control. Sampling also included taking regular samples and duplicates to indicate reproducibility.

Fluoranthene-d_{10}, bisphenol A-d_{16}, and caffeine-$^{13}C_3$ were used as surrogates in the GC-MS experiments, and acetaminophen-d_4 and codeine-d_6 were used as surrogates in the LC-MS experiments. Known amounts of surrogates were spiked into each sample before extraction to indicate individual sample extraction efficiency.

At least one field blank and one lab blank were analyzed for target compounds with each sampling event. Field blanks were made by filling pre-cleaned 1-L amber glass bottles with 1000 mL laboratory-grade organic-free water, spiking the water with a known amount of surrogate, and then taking them out into the field while sampling. Lab blanks were made in a similar fashion but without taking them into the field. Lab blanks and field blanks were then processed with the environmental samples. Field blanks were used to indicate whether sampling procedures, sampling equipment, field conditions, or

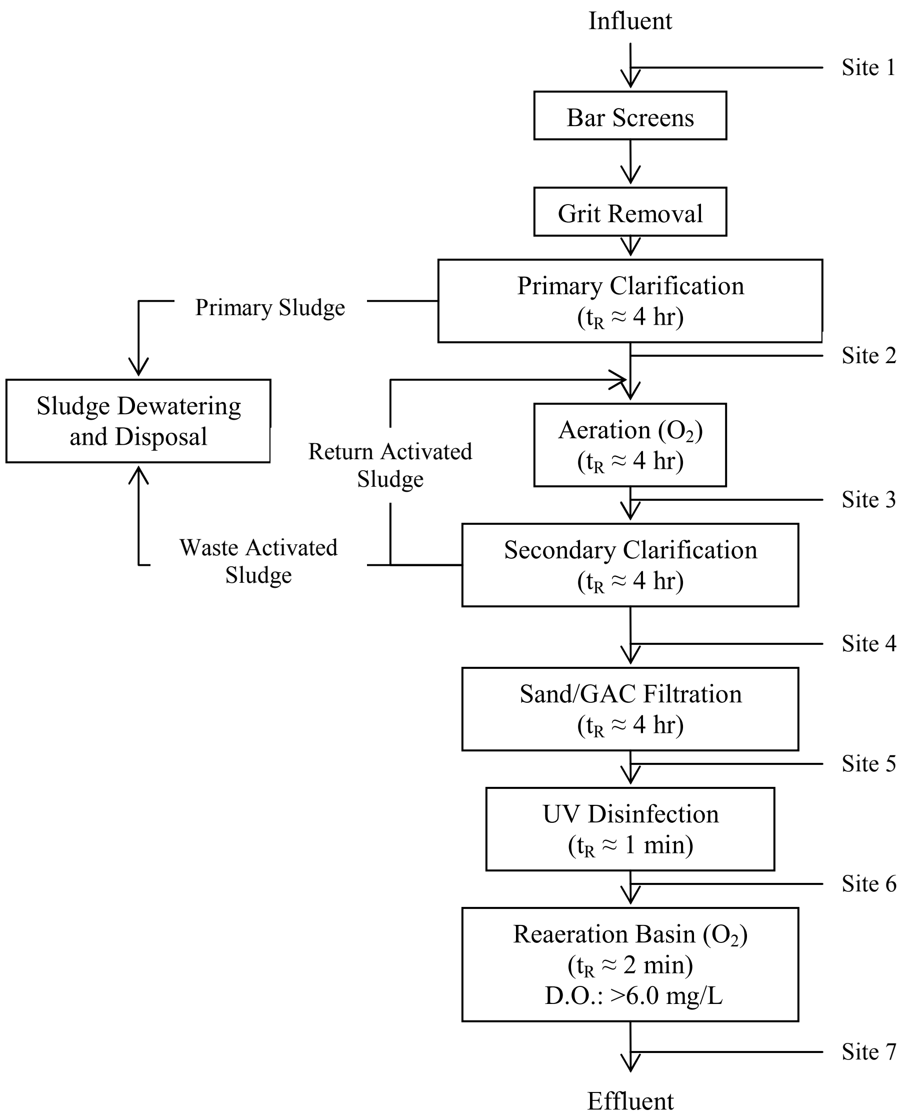

Figure 1. Schematic diagram of San Marcos WWTP and the sample-site locations. D.O., dissolved oxygen; t_R, retention time.

sample-shipment procedures introduced target compounds into environmental samples. Lab blanks were used to assess the potential for sample contamination in the laboratory.

Only one compound (fluoranthene) was detected in blanks. Therefore, environmental concentrations within two times the concentrations reported in the blanks were censored to less than the reporting level.

At least one laboratory-reagent spike was processed with each sampling event. This indicates that the extraction method for these compounds was sufficient. One matrix spike and one matrix spike duplicate were processed with each sampling event. This indicates that the extraction method for these compounds was sufficient even in the presence of complex interferences.

One compound (cotinine) was not detected in the three spikes for the January sampling event. Therefore, all environmental concentrations for cotinine for that sampling event were reported as "not applicable".

GC-Method. Samples were extracted following USGS (Zaugg et al., 2002) methods. Analytes were extracted in batches of six samples using 6 mL/200 mg hydrophilic-lipophilic balance (HLB) cartridges from Waters Corp. (Millford, MA). All extractions were performed on a BAKER SPE-12G Column Processor (Phillipsburg, NJ). The SPE cartridges were sequentially preconditioned with 5 mL of ethyl acetate, 5 mL of MeOH, and 5 mL of reagent water. One thousand-milliliter samples were spiked with surrogates fluoranthene-d_{10}, bisphenol A-d_{16}, and caffeine-$^{13}C_3$. The samples were then loaded onto the cartridges at ~15 mL/min, after which the cartridges were rinsed with 5 mL reagent water and then dried with a stream of nitrogen for a minimum of 60 minutes.

Next, the cartridges were eluted with 10 mL of ethyl acetate and 10 mL of DCM at a flow rate of ~1 mL/min into 60-mL glass vials. Ten grams of sodium sulfate was then added to each vial to remove any excess water. The dried extracts were then transferred to 60 mL glass vials, and concentrated with a gentle stream of nitrogen to a volume of ~750 µL. The extracts were then transferred to 2 mL vials with Teflon® lined septa using a Pasteur pipet and brought to ~1 mL with DCM. Each vial had 10 µL of a 100 µg/mL solution of Chrysene-d_{12} added as an internal standard.

GS-MS-MS Analysis. A ThermoFinnigan (Austin, TX) TRACE GC 2000 gas chromatograph equipped with a Polaris Q MS^n ion trap mass spectrometer and an AS 2000 autosampler was used for all GC-MS analysis. Excalibur software from ThermoFinnigan was used for data processing. All analytes were separated on a Restek (Bellefonte, Pa) RTX®-XLB 30 m by 0.25 mm id capillary column with 25 µm film thickness. A Restek IP Deactivated guard column (5m by 0.53 mm) was also installed in front of the analytical column via a Valco (Houston, TX) internal union. The column temperature program was as follows: initial temp, 40ºC held for 0.5 minutes; ramp, 100ºC/min to 150ºC; ramp, 5ºC/min to 300ºC; hold at 300ºC for 1 minute. Total run time was 33 minutes. Helium was used as the carrier gas at a constant flow rate of 1.2 mL/min. Using cool on-column injection; the injection port tracked the column oven with no additional heating. An injection volume of 1.0 µL was used for all analyses. Internal standard calibration was used for quantitation. Calibration standards for most compounds were 5, 10, 25, 50, 500, and 2500 ng/mL. Calibration standards for coprostanol, ethynylestradiol, and estradiol were four times higher, 20 – 10,000 ng/mL. Calibration

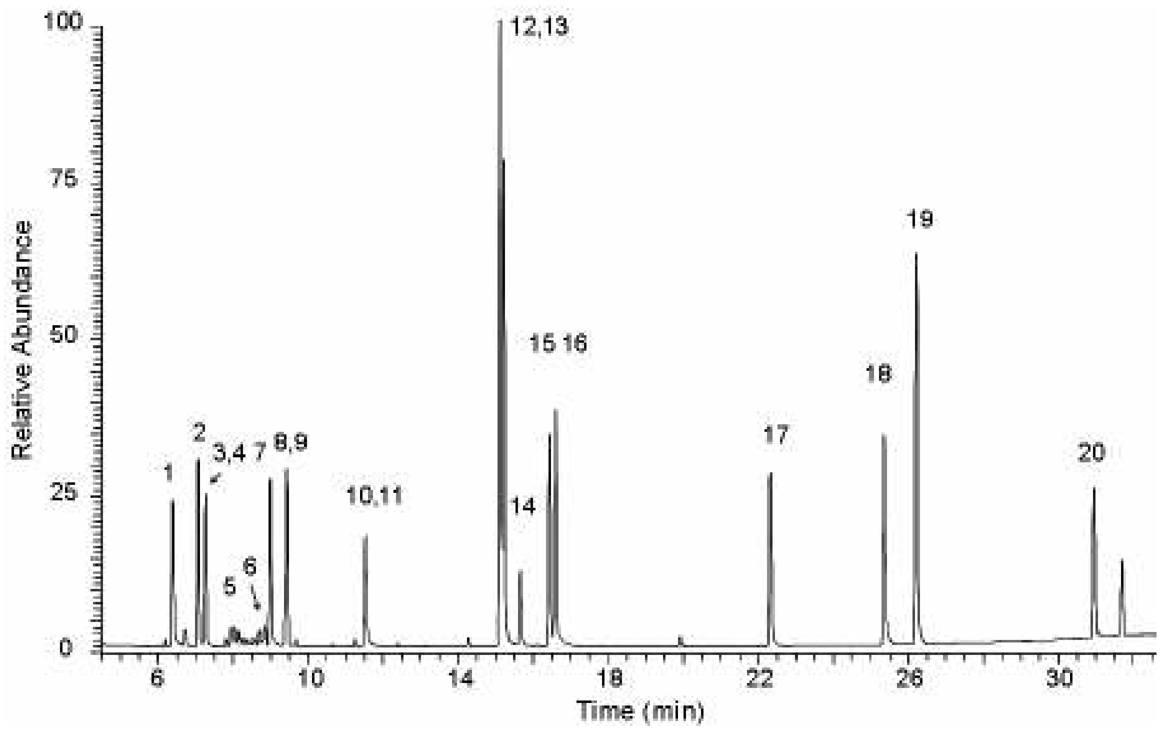

Figure 2. GC-MS total ion chromatograph generated from analysis of the 2500 ng/L standard mix. Peak identifications: (1) DEET, (2) tributylphosphate, (3) triethyl citrate, (4) benzophenone, (5) nonylphenol, (6) cotinine, (7) octylphenol, (8) TCEP, (9) diazinon, (10) caffeine, (11) caffeine-$^{13}C_3$, (12) fluoranthene, (13) fluoranthene-d_4, (14) triclosan, (15) bisphenol A, (16) bisphenol A-d_{12}, (17) (IS) chrysene-d_{12}, (18) estradiol, (19) ethynylestradiol, (20) coprostanol.

standards for Nonylphenol were 8 times higher 40 – 20,000 ng/mL. This was suggested by Zaugg et al. (2002). One continuing calibration verification was run for every ten environmental samples to ensure the instrument performance. Figure 2 is a representative chromatogram of the 2500 ng/L standard mix under the conditions listed above.

The MS analyses were performed by electron impact ionization and operated in tandem MS mode. The source temperature was set at 250ºC. Ions were selected and fragmented with collision-induced dissociation helium gas collision in the ion trap using a range of collision excitation voltages. Isolation and fragmentation conditions were optimized for each analyte and shown in Table 2. In general, scan intervals ranged from 30 m/z greater than precursor ions to 30 m/z less than the product ions. The precursor and product ions used for confirmation and quantitation for each compound are listed in Table 2.

GC-MS-MS peak assignment was determined by comparison of peak retention times and mass spectra produced by the known standards while using Chrysene-d_{12} as the internal standard. When confirming compounds, a signal to noise (S/N) of three was used for each ion.

Table 2. General Conditions used for GC-MS and GC-MS-MS analysis.

Compound	MW	GC-MS² Precursor Ions	Product Ions	MS² full scan interval
DEET	191.27	190, M^+	145, 162,175,	75-200
Tributylphosphate[a]	266.32	211, $[M-C_4H_7]^+$	155 –> 99	50-300
Triethyl citrate	276.29	157, $[M-COOCH2CH3-OCH2CH3]^+$	111, 115, 129	52-210
Benzophenone	182.21	182, M^+	105, 152	91-200
Nonylphenol[b]	220.35		107, 121, 135, 149	100-240
Cotinine[a]	176.21	176, M^+	147 –> 118, 132	50-200
Octylphenol	206.33	206, M^+	77, 107	68-220
Diazinon	304.36	304, M^+	162, 179	101-310
TCEP	285.49	249, $[M-Cl]^+$	125, 143, 187	83-265
Caffeine-$^{13}C_3$ (SS)	197.19	197, M^+	140, 168	70-210
Caffeine	194.19	194, M^+	138, 165	70-211
Fluoranthene-d_{10} (SS)[b]	212.26		212	106-220
Fluoranthene[b]	202.26		202	106-210
Triclosan	289.55	290, M^+	148, 218, 255	100-300
Bisphenol A	228.29	213, $[M-CH_3]^+$	91, 119	74-250
Bisphenol A-d_{16} (SS)	244.29	224, $[M-CD_3]^+$	97, 125	74-250
Chrysene-d_{12} (IS)[b]	240.00		240	120-400
Estradiol	272.37	272, M^+	172, 188, 213	90-280
Ethynylestradiol	296.41	296, M^+	133, 157, 171, 184	100-300
Coprostanol	388.65	215, $[M-C_{12}H_{29}]^+$	131, 145, 159, 173	107-250

SS, surrogate standard; IS, internal standard; MW, molecular weight
[a] determined by MS^3
[b] determined by Fullscan

LC-Method. Samples were extracted following previously developed analytical methods (Vanderford et al., 2003). Analytes were extracted in batches of six samples using 6 mL/200 mg hydrophilic-lipophilic balance (HLB) cartridges from Waters Corp. (Millford, MA). All extractions were performed on a BAKER SPE-12G Column Processor (Phillipsburg, NJ). The SPE cartridges were sequentially preconditioned with 5 mL of MTBE, 5 mL of MeOH, and 5 mL of reagent water. One thousand-milliliter samples were spiked with surrogates acetaminophen-d_4 and codeine-d_6. The samples were then loaded onto the cartridges at ~15 mL/min, after which the cartridges were rinsed with 5 mL reagent water and then dried with a stream of nitrogen for a minimum of 60 minutes. Next, the cartridges were eluted with 5 mL of 10/90 (v/v) MeOH/MTBE followed by 5 mL of MeOH at a flow rate of ~1 mL/min into 60-mL glass vials. The resulting extracts were concentrated with a gentle stream of nitrogen to a volume of ~750 µL. The extracts were then transferred to 2 mL vials with Teflon® lined septa using a Pasteur pipet and brought to ~1 mL with MeOH. Each vial had 10 µL of a 100 µg/mL solution of caffeine-$^{13}C_3$ added as an internal standard.

LC-MS-MS Analysis. A ThermoFinnigan (Austin, TX) Surveyor LC 2000 liquid chromatograph equipped with a LC Q MS^n advantage ion trap mass spectrometer and a surveyor 2000 autosampler was used for all LC-MS analysis. Excalibur software from ThermoFinnigan was used for data processing. All analytes were separated using a 150 m by 4.6 mm Restex (Bellefonte, Pa) C18 Allure column with a 5 µm particle size. A binary gradient consisting of 0.1% formic acid (v/v) in HPLC grade water and 100% HPLC grade MeOH at a flow rate of 400 µL/min was used. The gradient was as follows: 30% MeOH held for 1 minute, increased linearly to 100% by 10 minutes, and held for 12 minutes. There was a 5 minute equilibration step at 30% MeOH in between each sample run. An injection volume of 20 µL was used for all analyses. The column temperature and tray temperature were maintained at 30ºC. Internal standard calibration was used for quantitation. Calibration standards for all compounds were 50, 100, 250, 500, and 2500 ng/mL. Figure 3 is representative chromatograms of the 2500 ng/L standard mix under the conditions listed above.

All MS analyses were performed by positive electrospray ionization (ESI +) operating in the tandem MS mode. The ESI source conditions were as follows: source temperature, 250 ºC; sheath gas flow, 9 mL/min; source voltage, 4.5 kV; capillary voltage, 30 V. To determine optimal MS-MS transitions for target analytes, each compound was infused individually into the mass spectrometer at a concentration of 5 µg/L in methanol at a flow rate of 10 µL/min. The most intense precursor ion was selected and then collision energy was varied while the quadrupole was scanned from m/z 50 to [M+50]. This enabled identification of the most intense product ions for each compound. Isolation and fragmentation conditions were optimized for each analyte and shown in Table 3.

LC-MS-MS peak assignment was determined by comparison of peak retention times and mass spectra produced by the known standards while using caffeine-$^{13}C_3$ as the internal standard. When confirming compounds, a S/N of three was used for each ion.

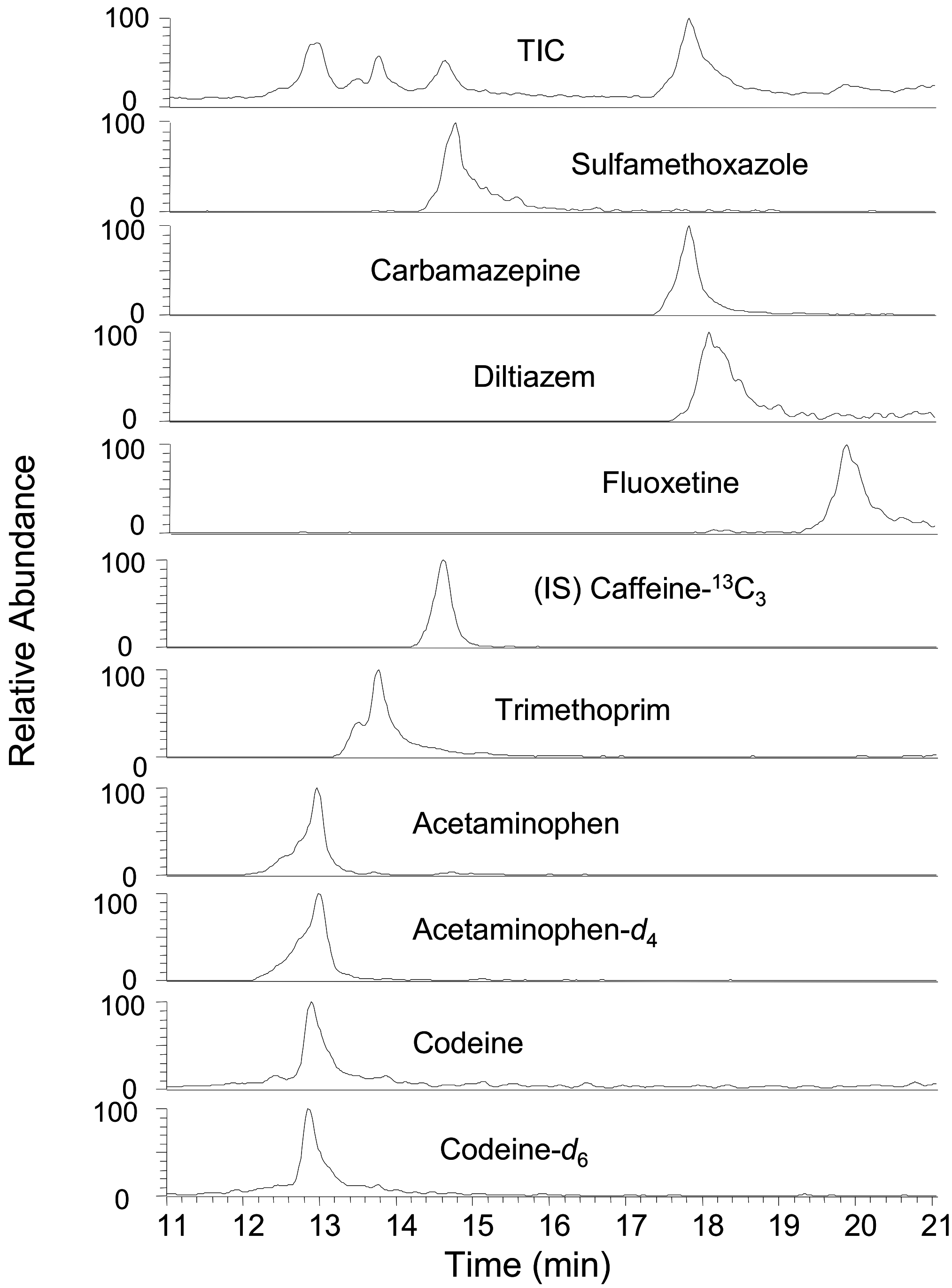

Figure 3. Representative LC-MS ion chromatograms of 10 compounds at 2500 ng/L generated under optimized conditions. The top trace is the TIC of a scan from 50 – 430 m/z. The others are generated from the extracted mass spectral ion option.

Table 3. General conditions used for LC-MS-MS analysis.

Compound	MW	LC-MS-MS Precursor Ions	CE (%)	Product Ions	MS-MS full scan interval
Codeine	299.36	300, $[M+H]^+$	30	215, 243	75-325
Codeine-d_6	305.36	306, $[M+H]^+$	33	218	50-325
Acetaminophen	151.17	152, $[M+H]^+$	25	110	52-200
Acetaminophen-d_4	155.17	156, $[M+H]^+$	25	114	52-200
Trimethoprim	290.32	291, $[M+H]^+$	40	230, 258, 276	70-300
Sulfamethoxazole	253.28	254, $[M+H]^+$	30	148, 156, 188	50-275
Carbamazepine	236.27	237, $[M+H]^+$	30	194	65-300
Diltiazem	414.52	415, $[M+H]^+$	28	178, 370	110-450
Fluoxetine	309.30	310, $[M+H]^+$	30	148	85-350

CE, collision energy

CHAPTER III

RESULTS AND DISCUSSION

Replicates. Differences in individual paired replicates were evaluated by relative standard deviation (RSD). RSD is defined as:

$$\mathrm{RSD} = 100\left(\mathrm{S}/\overline{\mathrm{x}}\right) \qquad \text{(eq. 1)}$$

where S is the standards deviation of the two concentration, and $\overline{\mathrm{x}}$ is the average of the two concentrations.

For the 16 GC compounds, 13 samples sites on 3 sampling events yielded 624 instances to compare concentrations. Of those 624 instances, there were 21 occasions when one of the replicate samples had a detectable amount of analyte and the other one did not. That left 603 instances where the replicates could be evaluated. Of those 603 instances, the average RSD was 13.7%, with greater than eighty percent of those having an RSD lower than 20%.

For the 7 LC compounds, 13 samples sites on 2 sampling events yielded 182 instances to compare concentrations. Of those 182 instances, there were 13 occasions when one of the replicate samples had a detectable amount of analyte and the other one did not. That left 169 instances where the replicates could be evaluated. Of those 169 instances, the average RSD was 29.9%.

Detection Limits. The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with a 99% confidence that the compound concentration is greater than zero. The limit of quantitation (LOQ) is defined as the level above which quantitative results may be obtained. LOQ is used to define the lower limit of the useful range of the measurement technology in use.

The MDLs and LOQs in this study (Table 4) were determined according to US Environmental Protection Agency guidelines. Seven 1-L water samples were collected from the San Marcos River, behind the A.E. Wood fish hatchery, and were spiked with 200 ng of each compound. Analytes were then extracted and analyzed as previously described. The MDL for each compound was determined from the standard deviation of replicate analysis multiplied by the Student's t-value for the 99% confidence level with n-1 degrees of freedom (Equation 2).

$$MDL = t_{0.99} \times S \text{ (for 7-1 = 6 degrees of freedom)} \qquad \text{(eq. 2)}$$

The LOQ for each compound was determined from the standard deviation of replicate analysis multiplied by ten (Equation 3).

$$LOQ = 10 \times S \qquad \text{(eq. 3)}$$

A number of detections discussed in this report include concentrations reported below the MDL. Because both methods identify compounds by mass spectrometry (S/N > 3) and use replicates, results are not censored at the MDL. When concentrations were detected below MDLs and they did not replicate, those values were censored. Furthermore, a number of concentrations were found at or below the LOQ**.** When concentrations were found below the LOQ they are estimates and therefore reported with

Table 4. Method detection limits (MDL) and limit of quantification (LOQ) for EDCs in matrix matched standards (n=7).

Compounds	MDL (ng/L)	LOQ (ng/L)
GC		
DEET	14.5	48.2
Tributylphosphate	9.3	31.0
Triethyl citrate	8.4	27.8
Benzophenone	11.2	37.2
Nonylphenol	10.0	33.4
Cotinine	15.2	50.6
Octylphenol	16.8	56.0
Diazinon	16.0	53.5
TCEP	8.4	28.1
Caffeine	10.9	36.2
Caffeine-$^{13}C_3$	14.9	49.5
Fluoranthene	109.1	363.8
Fluoranthene-d_{10}	92.6	308.5
Triclosan	8.3	27.8
Bisphenol A-d_{16}	10.8	36.1
Bisphenol A	15.8	52.5
Estradiol	18.2	60.7
Ethynylestradiol	18.4	61.3
Coprostanol	15.0	50.1
LC		
Codeine	117.1	390.3
Codeine-d_6	83.2	277.3
Acetaminophen	120.0	400.0
Acetaminophen-d_4	87.5	291.7
Trimethoprim	54.1	180.3
Sulfamethoxazole	46.3	154.3
Diltiazem	64.4	214.7
Carbamazepine	35.7	119.0
Fluoxetine	141.4	471.3

an asterisk. When compounds were not detected, they were assigned a value of <20 (ng/L) for GC analysis and <50 (ng/L) for LC analysis.

Recoveries. Recovery results are shown in Appendix 1. For GC-MS, analytical recoveries ranged from 51 to 154%. All RSDs were less than 25%, except for tributylphosphate and triclosan on the January sampling event, which had RSDs of 34% and 26%, respectively. The concentrations of compounds were not corrected by the recoveries.

For LC-MS, analytical recoveries ranged from 46 to 224%. All RSDs were less than 40%, except for diltiazem and fluoxetine, which had RSDs of 76% and 74%, respectively. The wider range of recoveries and higher RSD values is most likely due to LC-MS being more susceptible to matrix effects than GC-MS. Matrix effects are defined as the effects of co-eluting residual matrix components on the ionization of the target analyte, typically resulting in either signal suppression or enhancement.

Shewhart QC charts (Figure 4 - Figure 16) (also known as Levey-Jennings charts) were constructed using surrogate data from each sampling event. These charts were used to indicate whether or not the results were "in control". The 1*s*, 2*s*, and 3*s* lines in the charts correspond to one, two, and three standard deviations of the pooled recovery data away from the average.

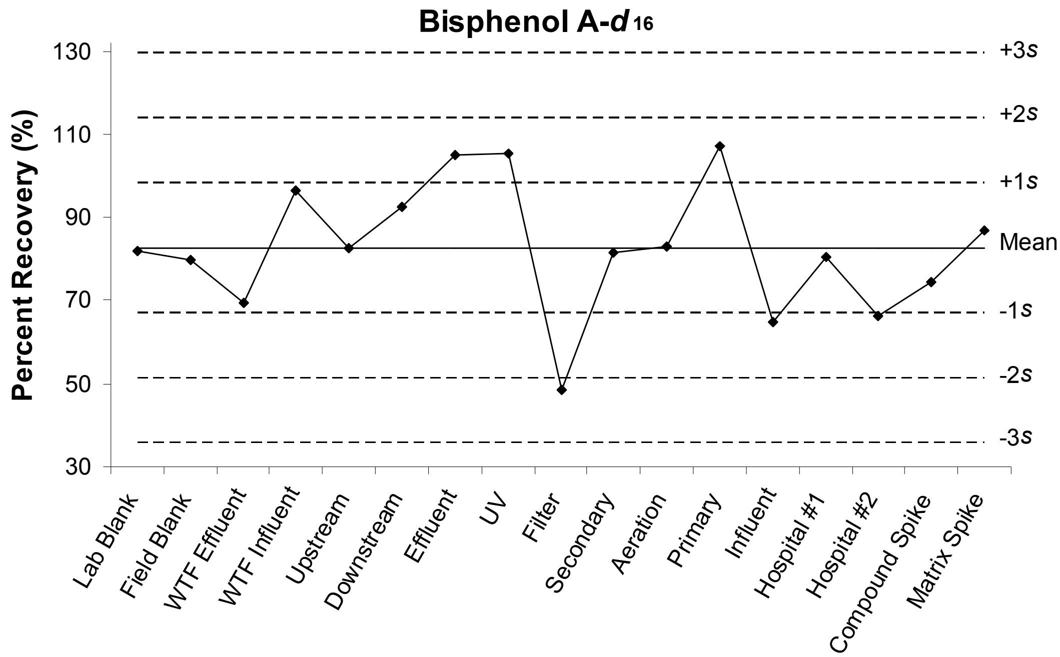

Figure 4. Shewhart QC chart of bisphenol A-d_{16} for the October sampling event.

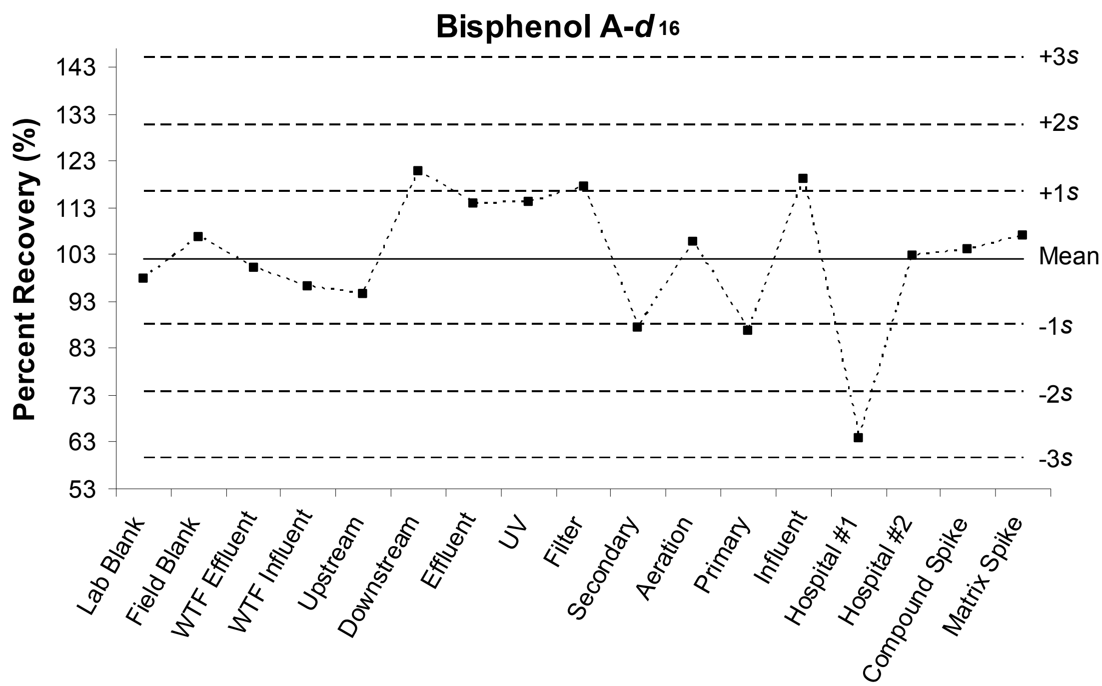

Figure 5. Shewhart QC chart of bisphenol A-d_{16} for the January sampling event.

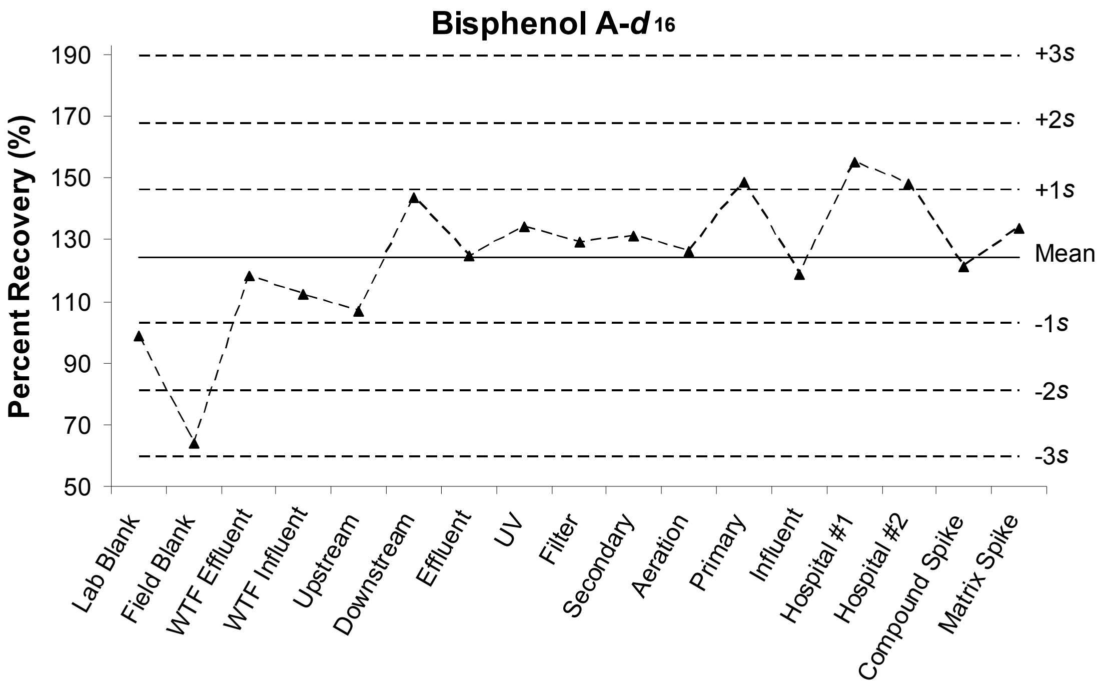

Figure 6. Shewhart QC chart of bisphenol A-d_{16} for the March sampling event.

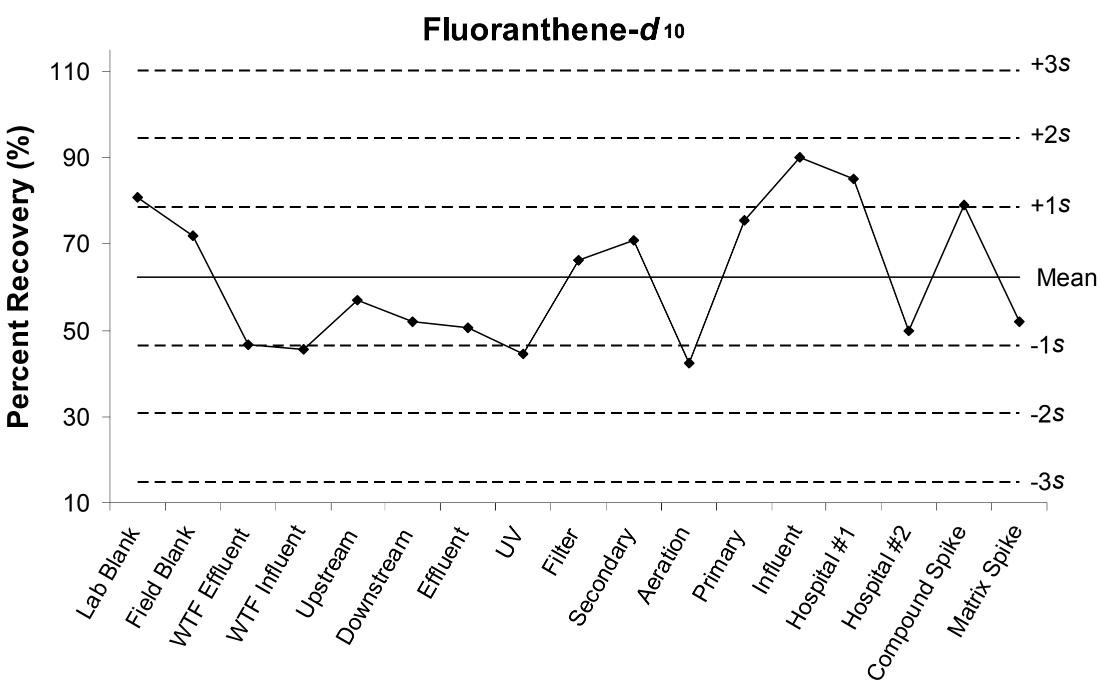

Figure 7. Shewhart QC chart of fluoranthene-d_{10} for the October sampling event.

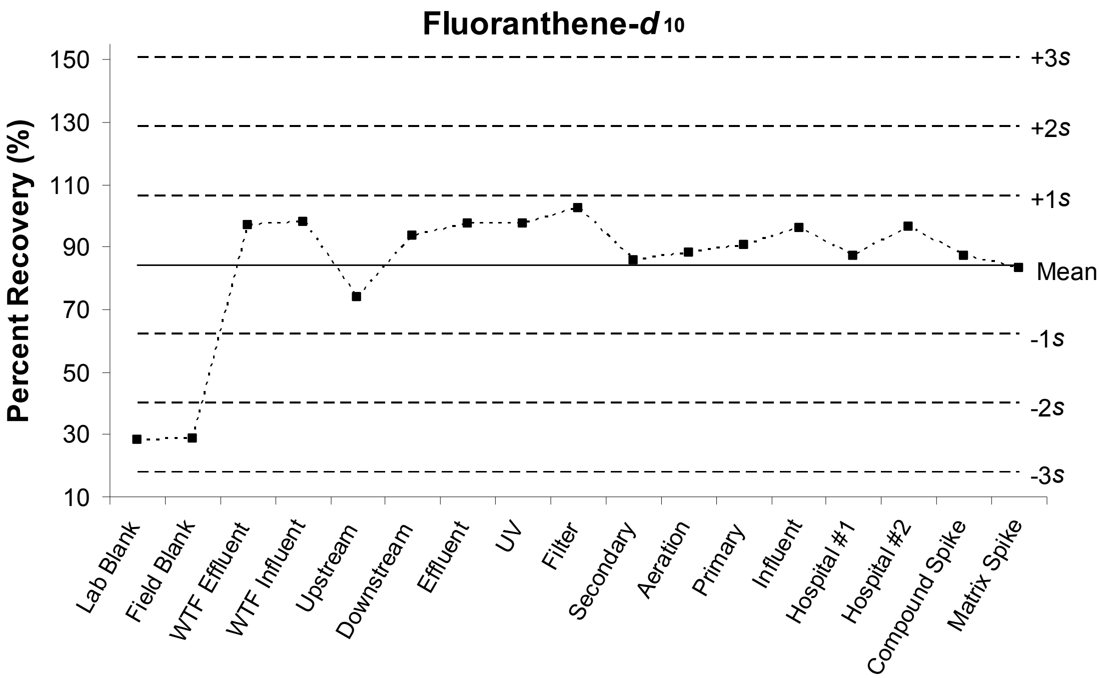

Figure 8. Shewhart QC chart of fluoranthene-d_{10} for the January sampling event.

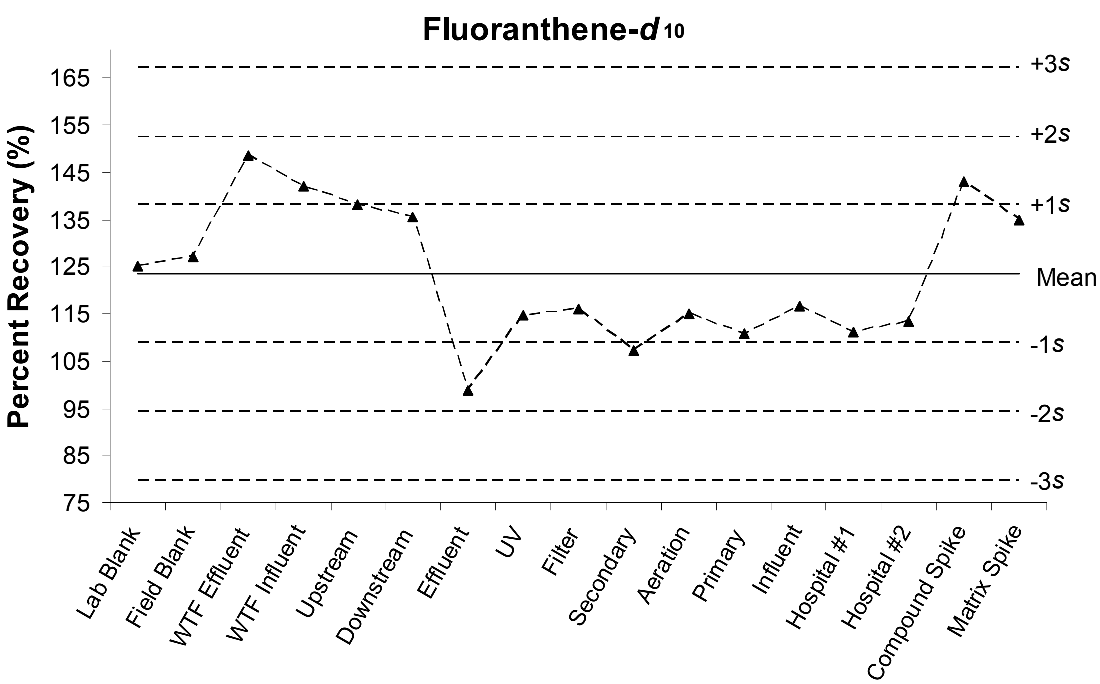

Figure 9. Shewhart QC chart of fluoranthene-d_{10} for the March sampling event.

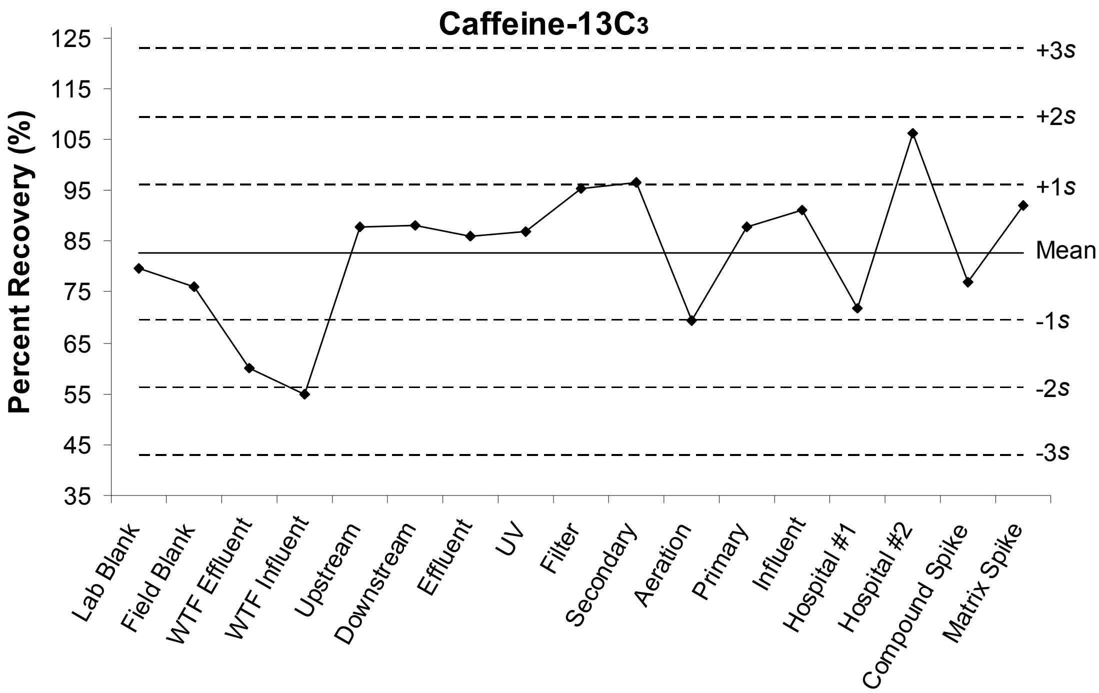

Figure 10. Shewhart QC chart of caffeine-$^{13}C_3$ for the October sampling event.

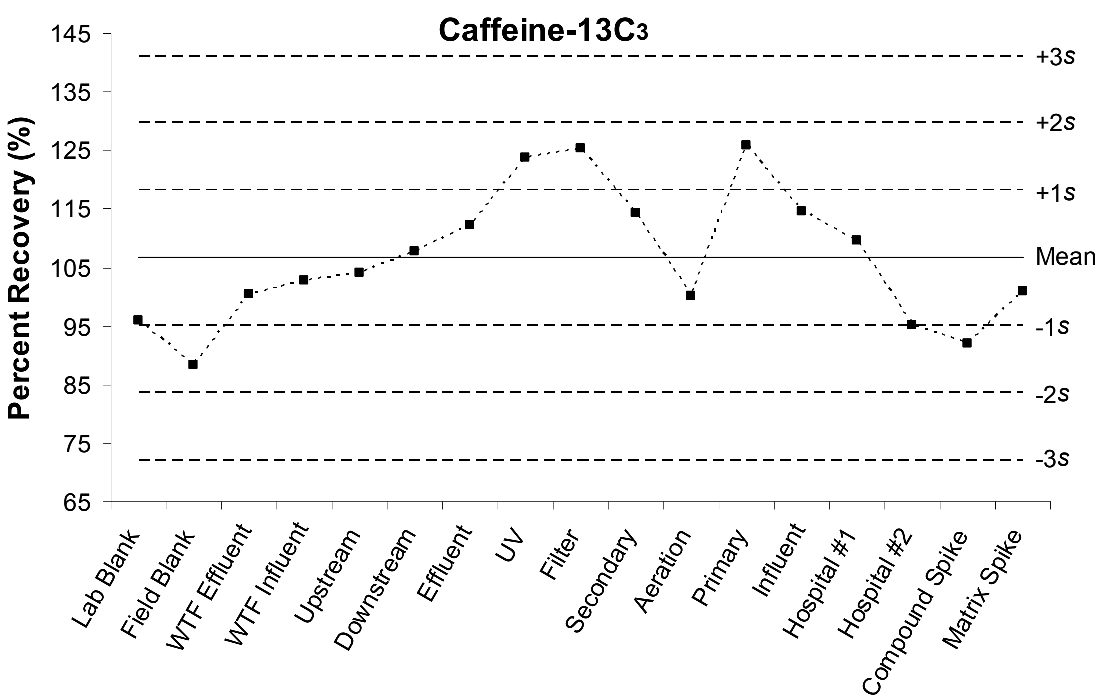

Figure 11. Shewhart QC chart of caffeine-$^{13}C_3$ for the January sampling event.

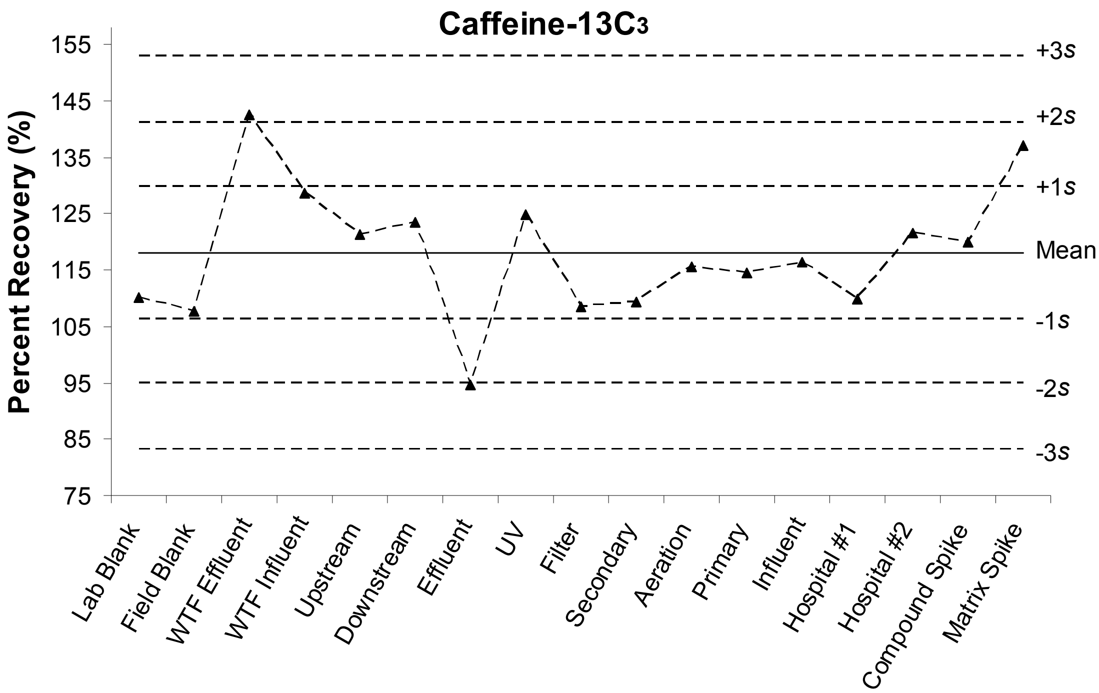

Figure 12. Shewhart QC chart of caffeine-$^{13}C_3$ for the March sampling event.

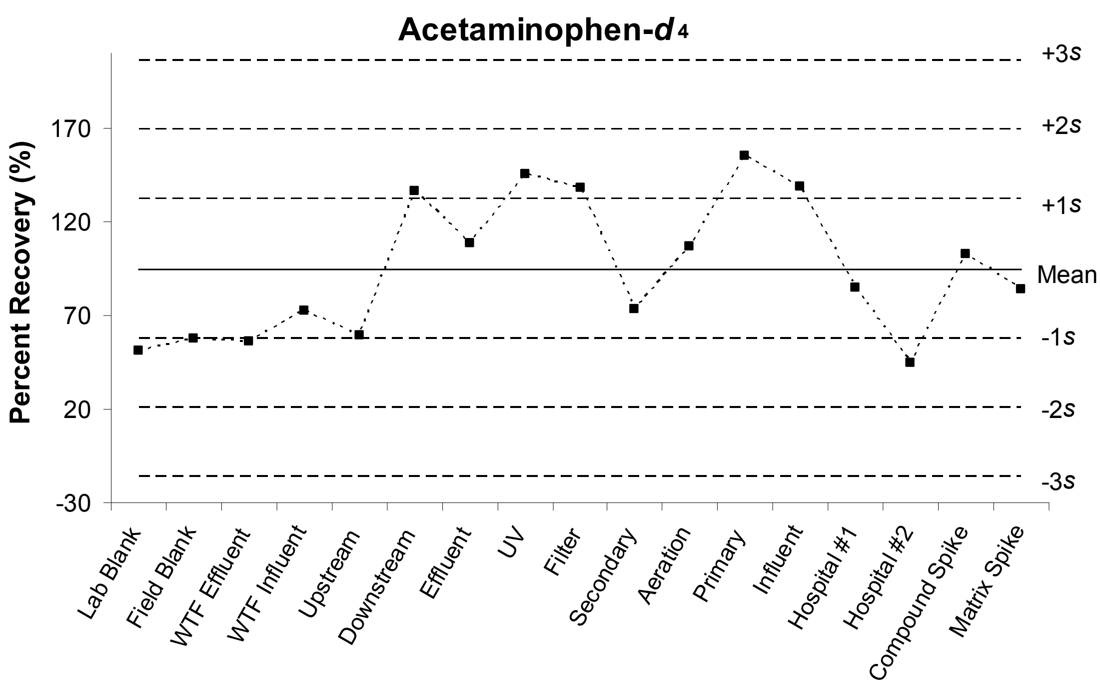

Figure 13. Shewhart QC chart of acetaminophen-d_4 for the January sampling event.

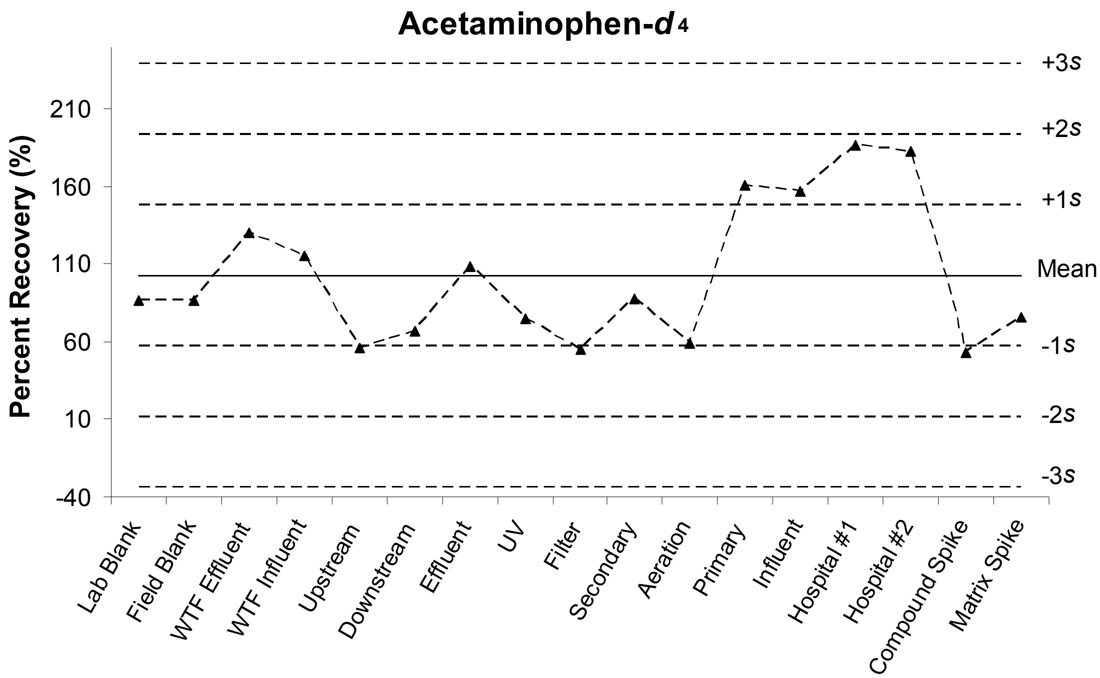

Figure 14. Shewhart QC chart of acetaminophen-d_4 for the March sampling event.

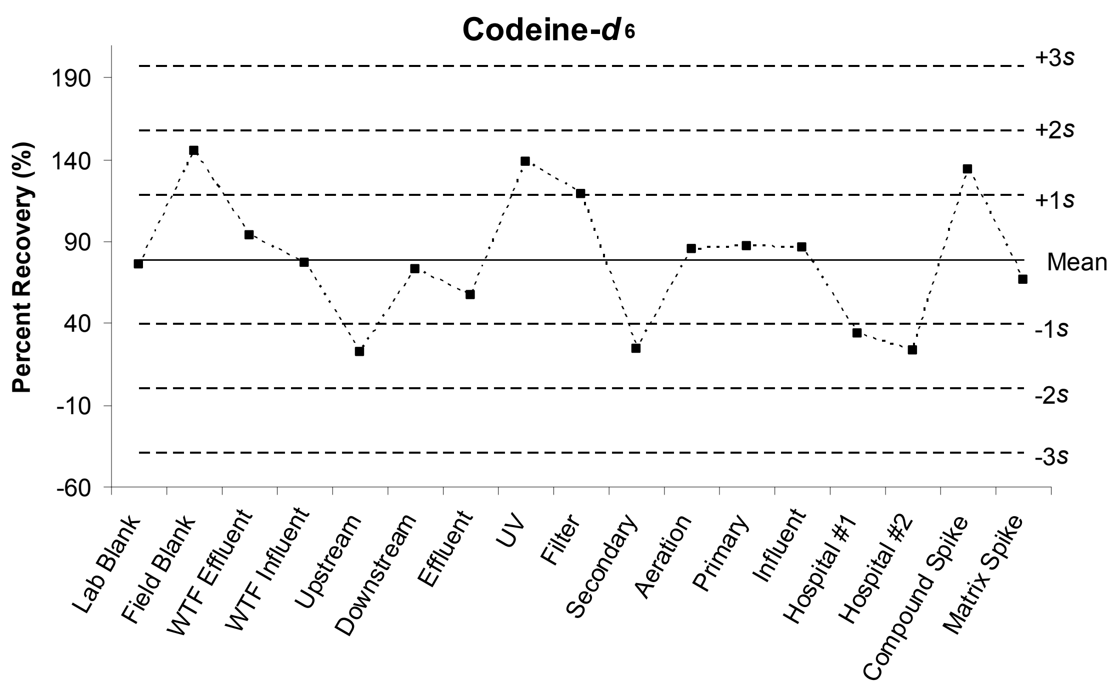

Figure 15. Shewhart QC chart of codeine-d_6 for the January sampling event.

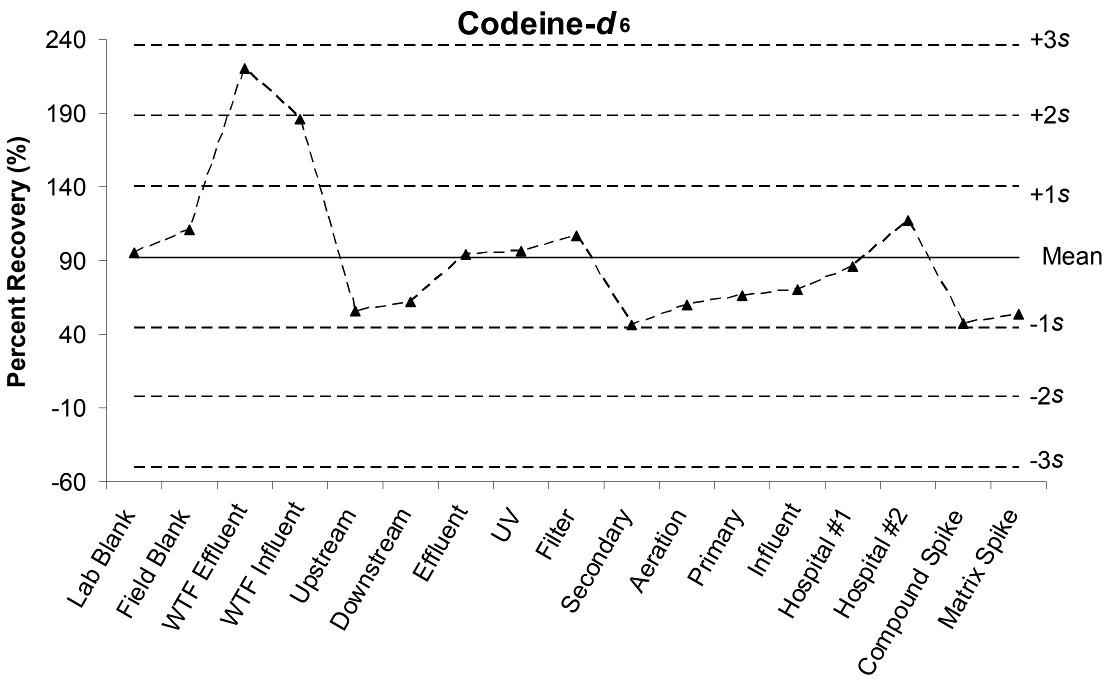

Figure 16. Shewhart QC chart of codeine-d_6 for the March sampling event.

Hospital Discharge. The occurrence of 23 organic wastewater compounds (OWCs) was investigated in the two discharge ports of the San Marcos Hospital. One port accesses the wastewater from the main facility (discharge #1) and the other port accesses the wastewater from the emergency room (discharge #2). Appendix 2 compares the differences in the two discharges and shows the combined results. At the time of this study, there was no pretreatment of the water before combining with the San Marcos wastewater collection system.

Of the 23 compounds monitored, 12 were detected at least once in the waters coming from the hospital. The total frequency of detection was 33% at both sampling sites. Compound concentrations ranged from near detection limits to hundreds of thousands of ng/L. The compounds that were detected the most frequently (>60%) were acetaminophen, caffeine, coprostanol, DEET, TCEP, and triclosan. Of these compounds, acetaminophen had the highest average concentration at 140,000 ng/L (n = 8), followed by caffeine 73,000 ng/L (n = 12), triclosan 57,000 ng/L (n = 12), coprostanol 12,000 ng/L (n = 11), DEET 110 ng/L (n = 8), and TCEP 53 ng/L (n = 8).

Benzophenone, diltiazem, tributyl phosphate, triethyl citrate, nonylphenol, and codeine were detected in less than 60 percent of the hospital samples. When detected, both codeine and nonylphenol had high average concentrations at 50,000 ng/L (n = 1) and 19,000 ng/L (n = 4), respectively. The codeine concentration was only found in one of the replicates on the January sampling date, and therefore is reported as a single grab sample. Cotinine, octylphenol, diazinon, fluoranthene, bisphenol A, estradiol, ethynylestradiol, trimethoprim, sulfamethoxazole, carbamazepine, and fluoxetine were not detected in any hospital discharge samples.

Data suggest there is no real difference in chemicals coming from each side of the hospital, except for codeine and tributylphosphate. Codeine was detected in waters coming out of the main hospital and wasn't detected in waters coming from the emergency room, and vice versa for tributylphosphate.

WWTP Effluent. The occurrence of 23 OWCs was investigated in several locations in the San Marcos River, including upstream and downstream of the WWTP effluent as well as the effluent itself. Appendix 3 compares the differences in the three sampling locations.

Only two compounds (caffeine and bisphenol A) were detected in waters upstream from the effluent discharge. Both were found below their LOQs. The overall frequency of detection was 5%. This was expected since the upstream sample site was approximately two miles downstream of the source of the San Marcos River, the San

Marcos Springs. Furthermore, the San Marcos River receives no WWTP effluent nor any other major discharges in that two mile segment.

The effluent, on the other hand, had detectable amounts of 13 OWCs. The overall frequency of detection was 32%. Sulfamethoxazole had the highest average concentration and the highest single grab sample concentration at 560 ng/L (n = 2) and 580 ng/L, respectively. The compounds that were detected the most frequently (>60%) were caffeine, triethyl citrate, TCEP, carbamazepine, and triclosan. Of the most frequently detected compounds in the effluent, carbamazepine had the highest average concentration and the highest single grab sample concentration at 330 ng/L (n = 3) and 490 ng/L, respectively. Average concentrations for other frequently detected compounds were as follows: Caffeine 22 ng/L (n = 6), triclosan 15 ng/L (n = 5), triethyl citrate 16 ng/L (n = 6), and TCEP 140 ng/L (n = 6). Similar results were found in a US nationwide effluent study (Glassmeyer et al., 2005), effluent studies in Germany (Ternes, 1999), and effluent studies in South Korea (Snyder et al., 2007). Similar results for triclosan were found in a North Texas WWTP effluent (Venables et al., 2006) and effluent studies in Switzerland (Singer et al., 2002).

Sulfamethoxazole, coprostanol, DEET, nonylphenol, diltiazem, and estradiol were detected in less than 60 percent of the effluent samples. Cotinine, benzophenone, tributylphosphate, octylphenol, diazinon, fluoranthene, bisphenol A, ethynylestradiol, codeine, acetaminophen, trimethoprim, and fluoxetine were not detected in any of the effluent samples.

The downstream samples showed similar results with effluent samples. The overall frequency of detection was 26%. The compounds detected most frequently (>60%) were caffeine, triethyl citrate, TCEP, and carbamazepine.

Benzophenone, sulfamethoxazole, coprostanol, DEET, nonylphenol, diltiazem, and triclosan were detected in less than 60 percent of the samples. As in the effluent samples, sulfamethoxazole had the highest average concentration and the highest single grab sample concentration at 1200 ng/L (n = 2) and 1800 ng/L, respectively. Cotinine, tributylphosphate, octylphenol, diazinon, fluoranthene, bisphenol A, estradiol, ethynylestradiol, codeine, acetaminophen, trimethoprim, and fluoxetine were not detected in any of the downstream samples.

In general, results indicate low to undetectable levels of compounds in upstream samples, higher frequency and maximum concentrations in the effluent, and lower concentrations in downstream samples. These trends have been observed by others (Glassmeyer et al., 2005 and Zhou et al., 2004).

WWTP Influent. Appendix 4 shows the concentrations of the 23 compounds monitored in WWTP influent waters.

Ten of the 23 compounds (caffeine, coprostanol, acetaminophen, DEET, benzophenone, nonylphenol, triethyl citrate, triclosan, TCEP, and bisphenol A) were detected in 100% of the influent samples. Acetaminophen had the highest average concentration and the highest single grab sample concentration at 44,000 ng/L (n = 4) and 80,000 ng/L, respectively. Because of the high recovery of acetaminophen-d_4 during the analytical process (148%), this value might be overestimated. Nonylphenol had the second highest average concentration at 31,000 ng/L (n = 6), which was similar to other research done in New York (Phillips et al., 2005). Also, influent concentration of nonylphenol varied widely from date to date (9,000 to 63,000 ng/L) which was seen by others in Kansas (Keller et al., 2003). Personal care products, DEET, benzophenone, triethyl citrate, and TCEP had average concentrations of 1,700 (n = 6), 2,500 (n = 6), 2,300 (n = 6), and 260 (n = 6) ng/L, respectively. Triclosan had an average concentration of 2,200 ng/L (n = 6), which was similar to influent concentrations in other studies (Venables et al., 2006; McAvoy et al., 2002; and Lindstrom et al., 2002). The average caffeine concentration was 17,000 ng/L (n = 6). This is one order of magnitude lower than other studies (Heberer et al., 2002).

Diltiazem, octylphenol, tributylphosphate, fluoranthene, estradiol, codeine, and carbamazepine were detected in less than 60 percent of the influent samples. The estradiol concentration was 3,000 ng/L, which is two orders of magnitude higher than other studies (Joss et al., 2005; Joss et al., 2004; and Ternes et al., 2003). Octylphenol was detected in the January influent samples at 4,100 ng/L, which is similar to influent studies in Tokyo, Japan (Takada et al., 2006).

Cotinine, diazinon, ethynylestradiol, sulfamethoxazole, trimethoprim, and fluoxetine were not detected in any of the WWTP influent samples.

San Marcos WTF. The treatment efficiency of the San Marcos WTF for the compounds in this study was investigated. Overall, source waters from Lake Dunlap did not contain detectable amounts of the 23 compounds monitored. TCEP was detected once at trace amounts (8 ng/L) by mass spectrometry. Therefore, no real conclusions can be drawn from the WTF data.

San Marcos WWTP. Appendix 5 summarizes the removal of the 23 compounds through the San Marcos WWTP. The San Marcos WWTP showed effective removal (>92% efficiency) of all detectable compounds, with the exceptions of TCEP and carbamazepine. This is similar to results seen by researchers in New York (Phillips et al., 2005) and in Spain (Carballa et al., 2004), which showed excellent removal of a broad range of organic wastewater contaminants in activated sludge treatment facilities. Moreover, seven compounds (fluoranthene, bisphenol A, codeine, acetaminophen, benzophenone, tributylphosphate, and octylphenol) had 100% removal, and seven had ~99% removal when detected in the influent waters.

TCEP and carbamazepine showed low average removal rates of 33.0% and 24.8%, respectively. Removal efficiencies for TCEP varied widely (-19.4 – 67.7%) among the three sampling trips. Research in Germany (Heberer et al., 2002 and Ternes et al., 1999) and in South Korea (Snyder et al., 2007) showed similar removal rates for these two compounds.

Among the personal care products, DEET had a significantly higher average removal rate (99.2% efficiency) than other studies in Tokyo (<45%, Takada et al., 2006). The fragrances, benzophenone and triethyl citrate, had average removal efficiencies of 100.0% and 98.8%, respectively.

Caffeine and triclosan showed greater than 99% removal efficiencies on all three sampling dates. This is similar to other studies that determined removal efficiencies of caffeine (99%, Snyder et al., 2007; 99.9 – 100.0%, Foster et al., 2005; and 100%, Philips et al., 2005) and triclosan (97%, Venables et al., 2006; 97.7 – 99.2%, Foster et al., 2005;

94%, Philips et al., 2005; and 96%, McAvoy et al., 2002;) through WWTPs that use activated sludge.

Among the pharmaceuticals, codeine and acetaminophen were removed with 100% efficiency, whereas diltiazem had lower removal efficiency, 91.8% when detected in the influent.

When detected in the influent, the natural female hormone estradiol was removed with 97% efficiency. This is similar to removal efficiencies of activated sludge plants in Germany (Ternes et al., 2003 and Joss et al., 2004) and Tokyo (Takada et al., 2006).

Detergent metabolites were removed efficiently: 100.0% for octylphenol and 99.7% for nonylphenol on average. This is comparable to the results of previous studies (Philips et al., 2005 and Bennie et al., 1998).

Among the different processes at the San Marcos WWTP, the aeration process (biological treatment) was the most effective at removing the compounds in the study (Figure 17 - Figure 32). For all compounds, the average percent removal during the aeration process was 93.4%. There were several instances were concentrations were higher after primary clarification than in the influent. This trend has been observed by other researchers and has been attributed to analytical deviations caused by the different characteristics of the wastewaters and plug-flow timing of sample collection (Snyder et al., 2007 and Carballa et al., 2004).

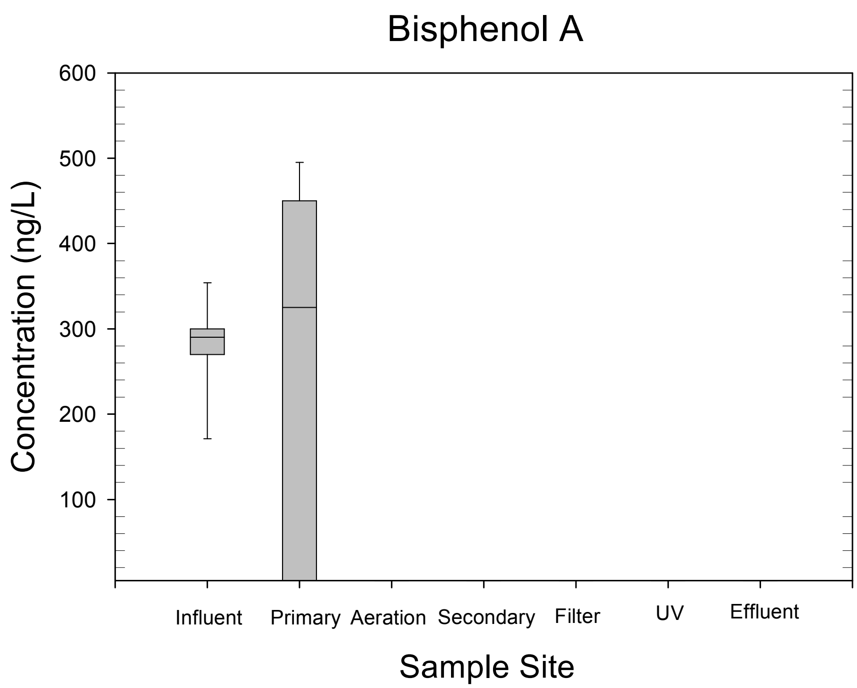

Figure 17. Concentrations (ng/L) of bisphenol A found at each site on three different sampling dates.

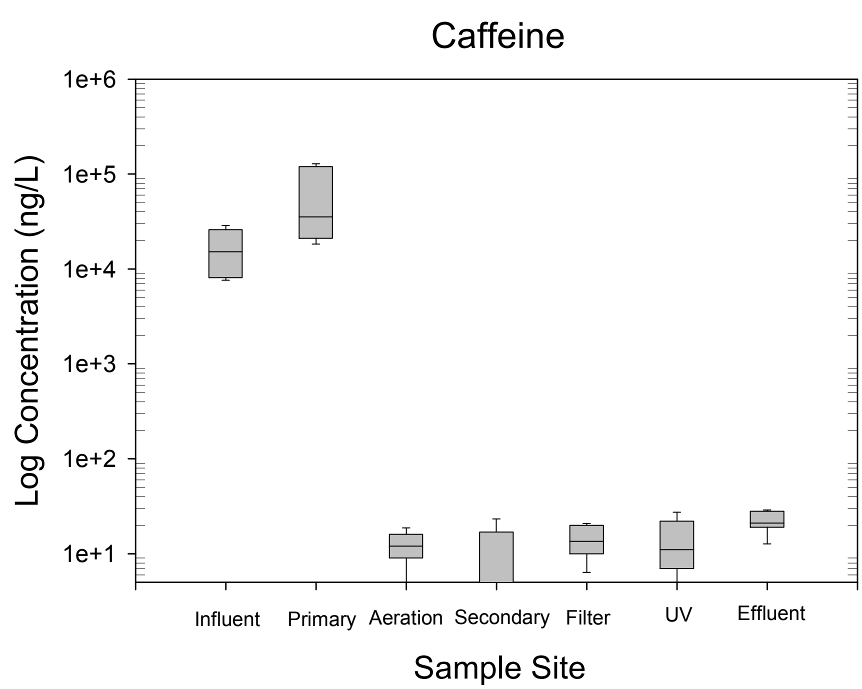

Figure 18. Concentrations (ng/L) of caffeine found at each site on three different sampling dates.

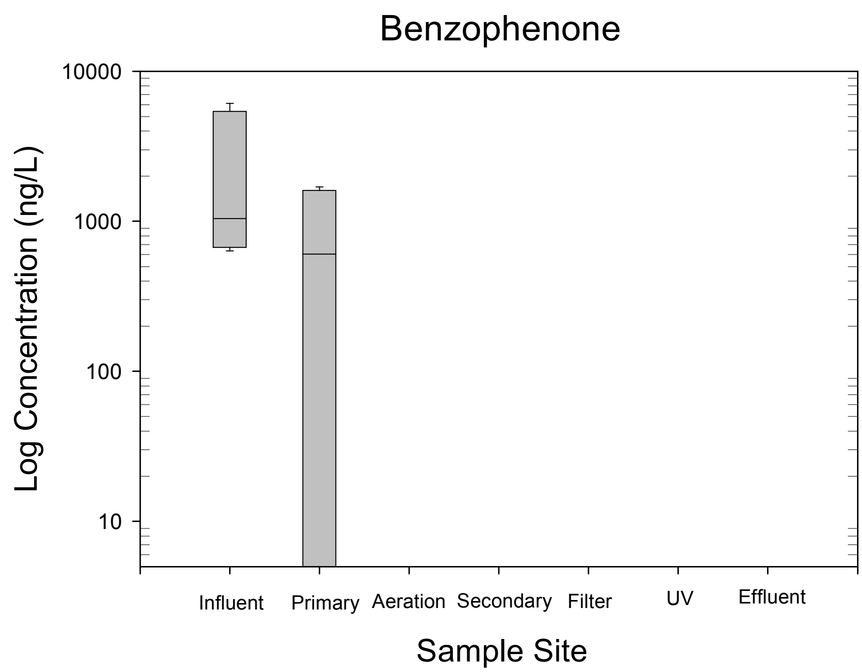

Figure 19. Concentrations (ng/L) of benzophenone found at each site on three different sampling dates.

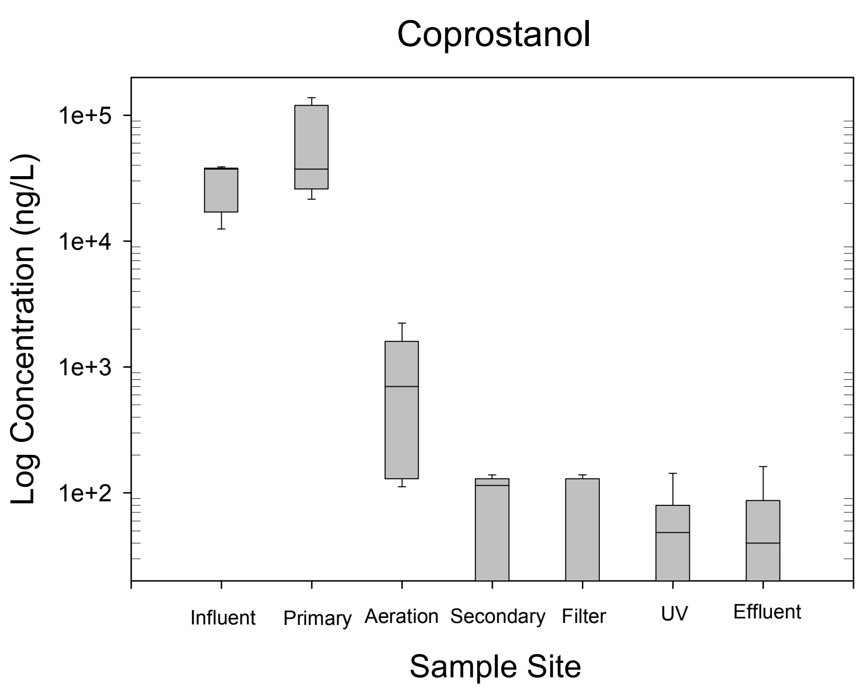

Figure 20. Concentrations (ng/L) of coprostanol found at each site on three different sampling dates.

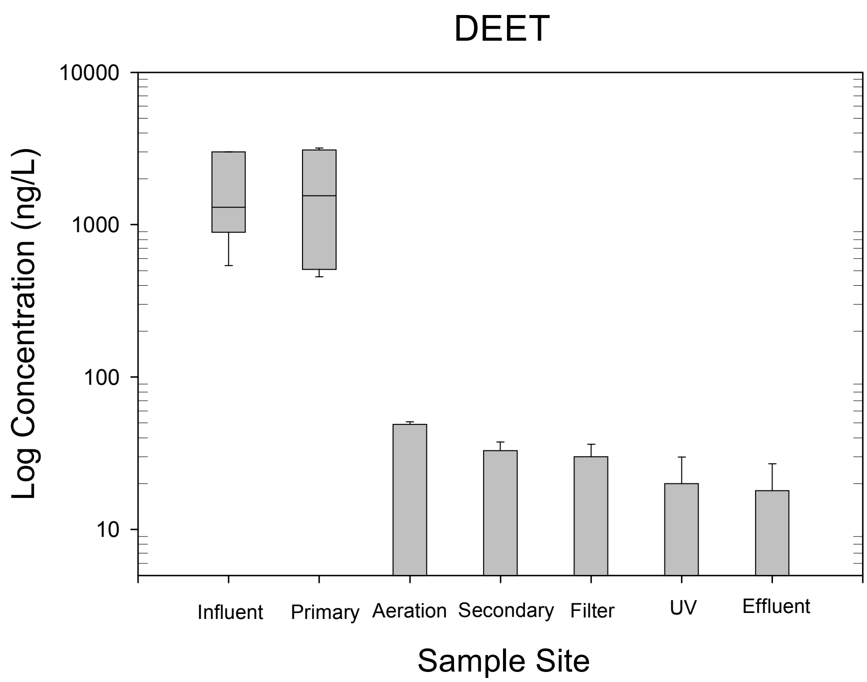

Figure 21. Concentrations (ng/L) of DEET found at each site on three different sampling dates.

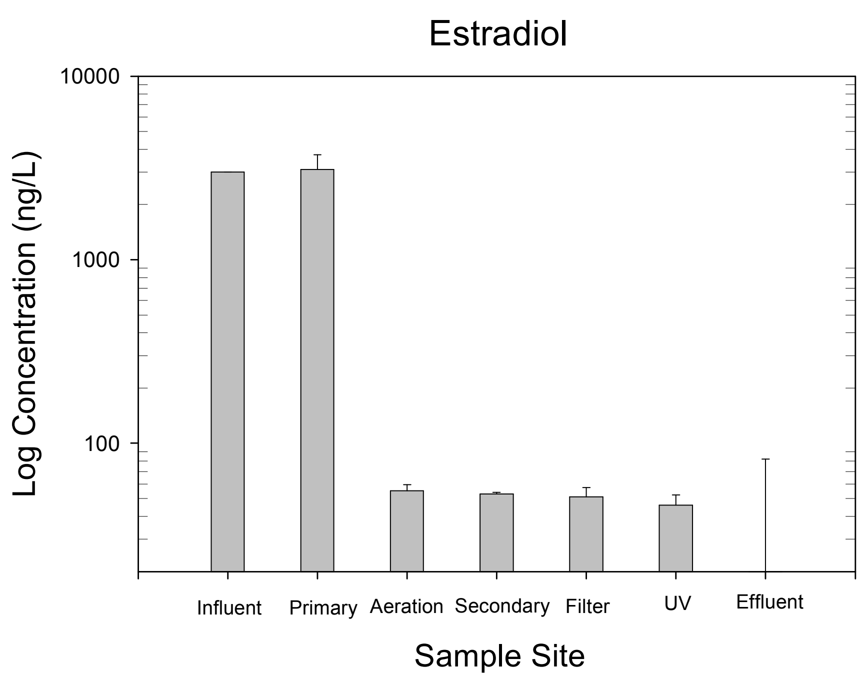

Figure 22. Concentrations (ng/L) of estradiol found at each site on three different sampling dates.

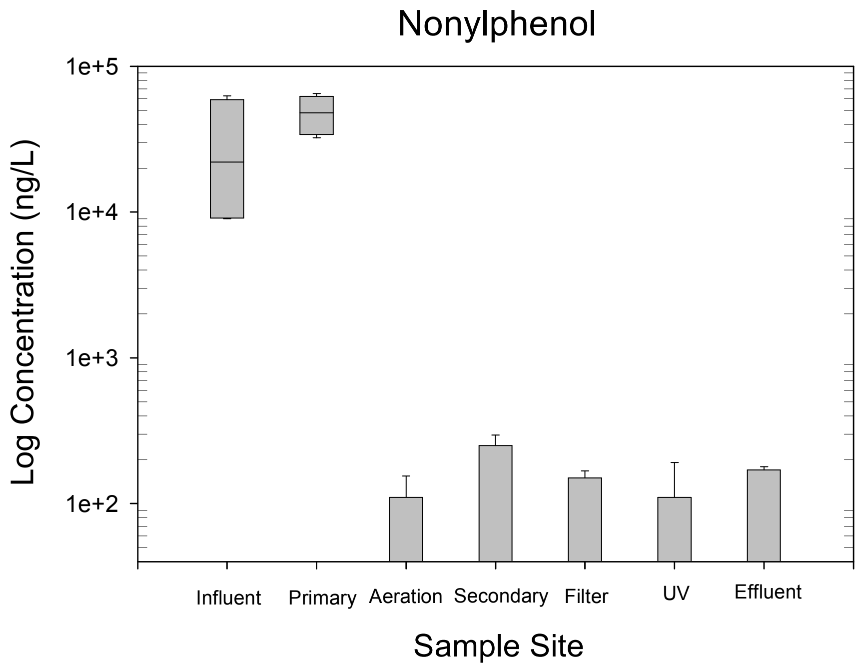

Figure 23. Concentrations (ng/L) of nonylphenol found at each site on three different sampling dates.

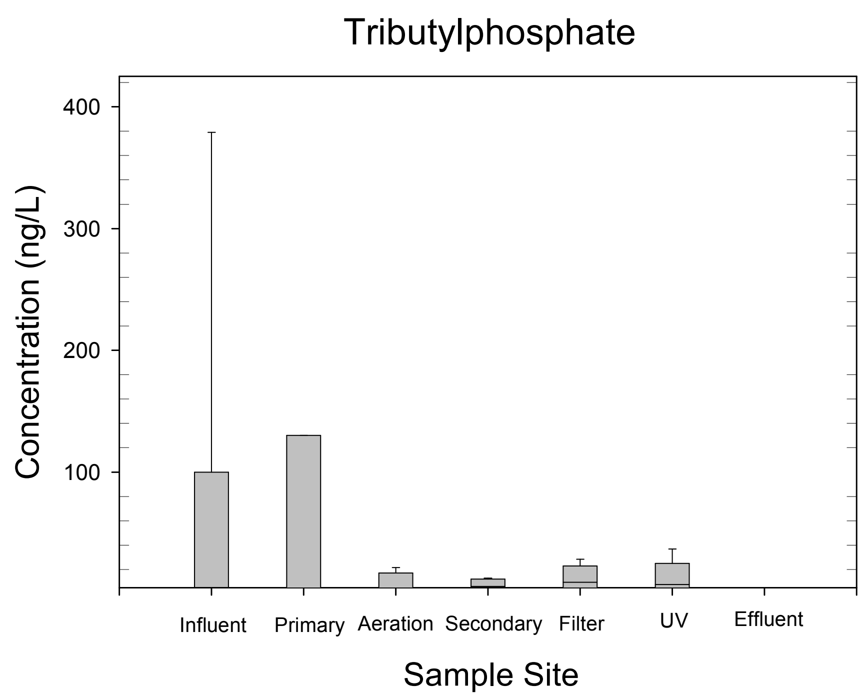

Figure 24. Concentrations (ng/L) of tributylphosphate found at each site on three different sampling dates.

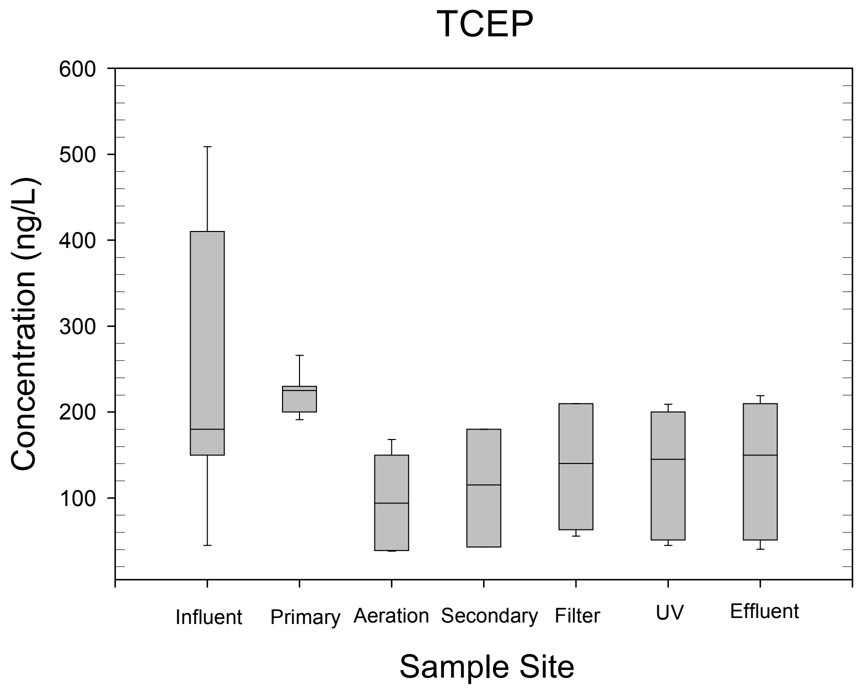

Figure 25. Concentrations (ng/L) of TCEP found at each site on three different sampling dates.

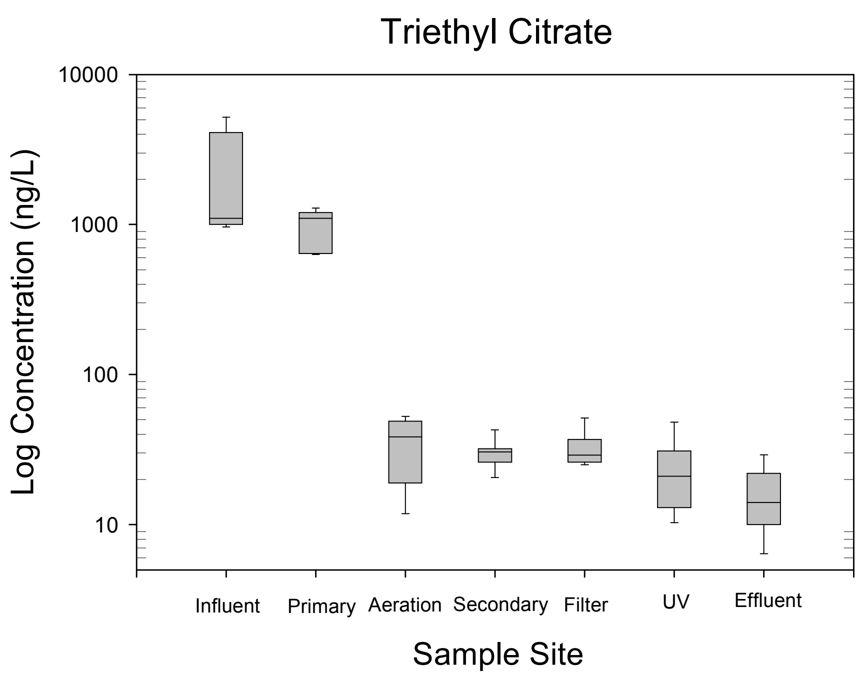

Figure 26. Concentrations (ng/L) of triethyl citrate found at each site on three different sampling dates.

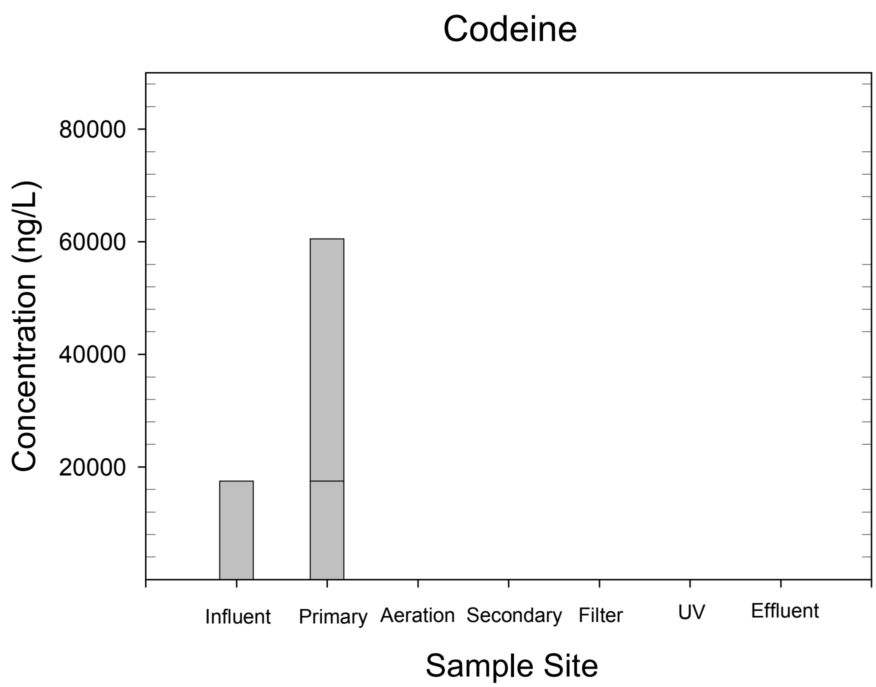

Figure 27. Concentrations (ng/L) of codeine found at each site on three different sampling dates.

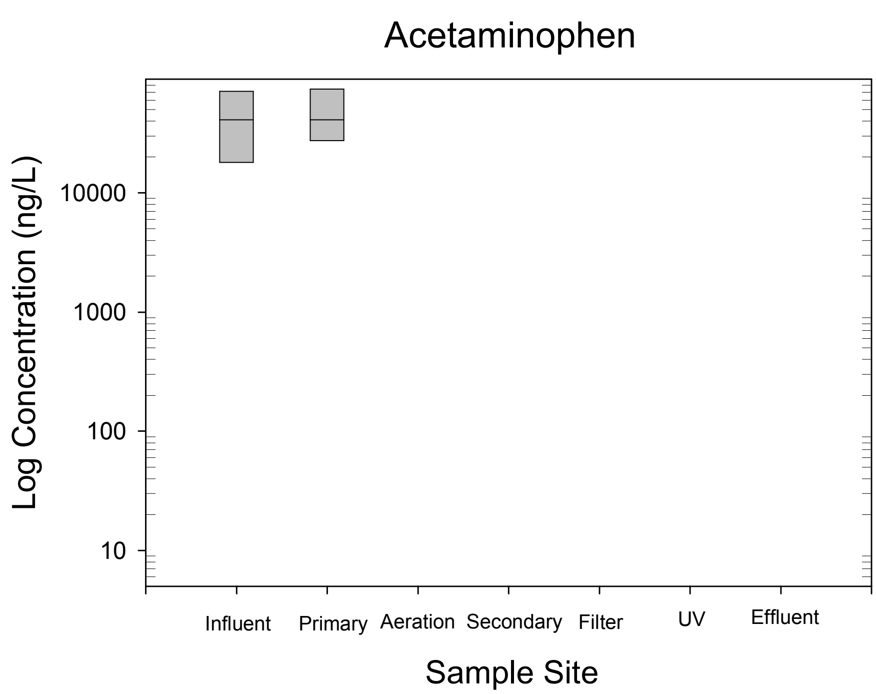

Figure 28. Concentrations (ng/L) of acetaminophen found at each site on three different sampling dates.

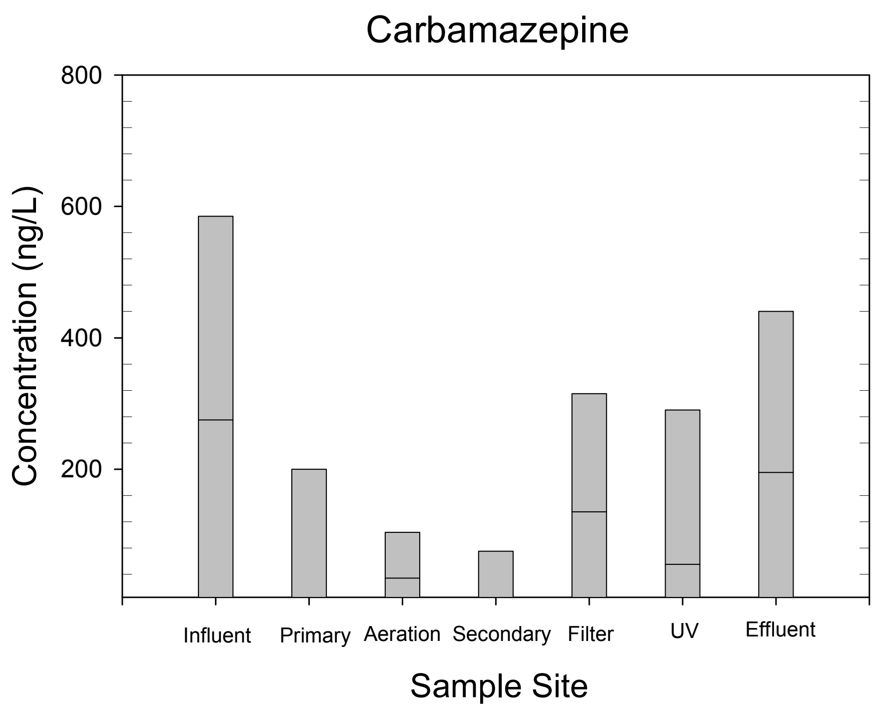

Figure 29. Concentrations (ng/L) of carbamazepine found at each site on three different sampling dates.

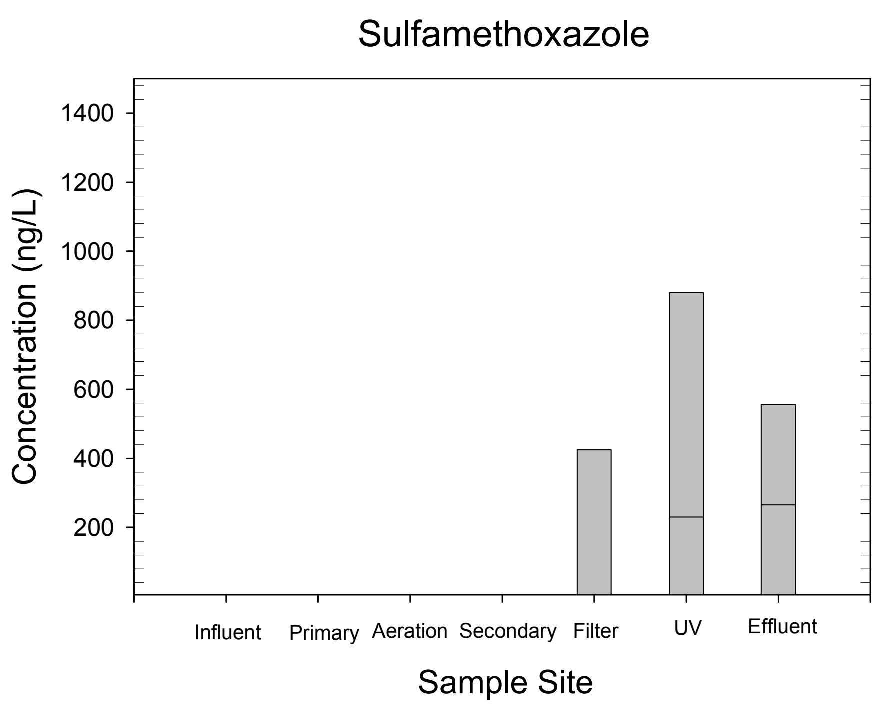

Figure 30. Concentrations (ng/L) of sulfamethoxazole found at each site on three different sampling dates.

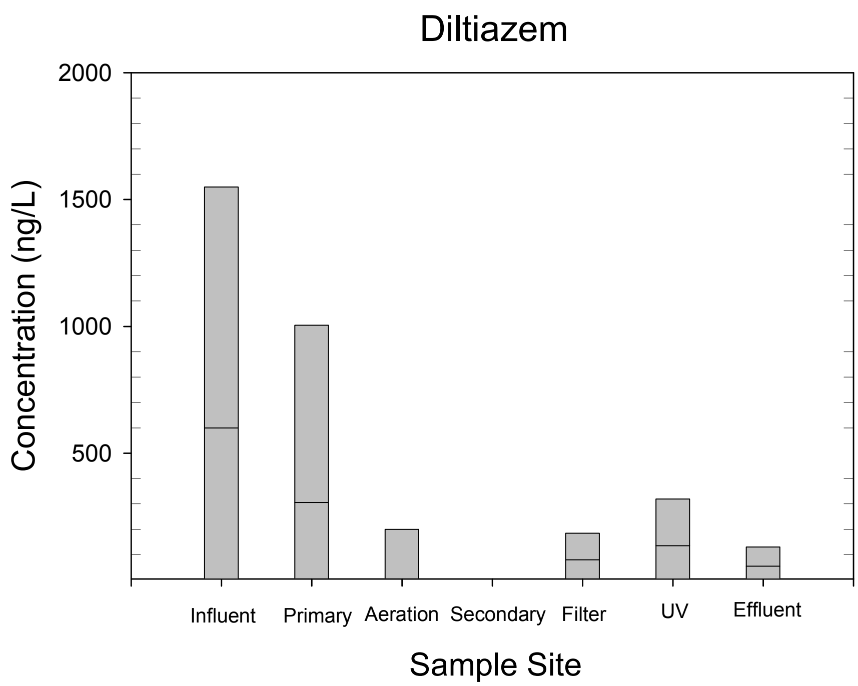

Figure 31. Concentrations (ng/L) of diltiazem found at each site on three different sampling dates.

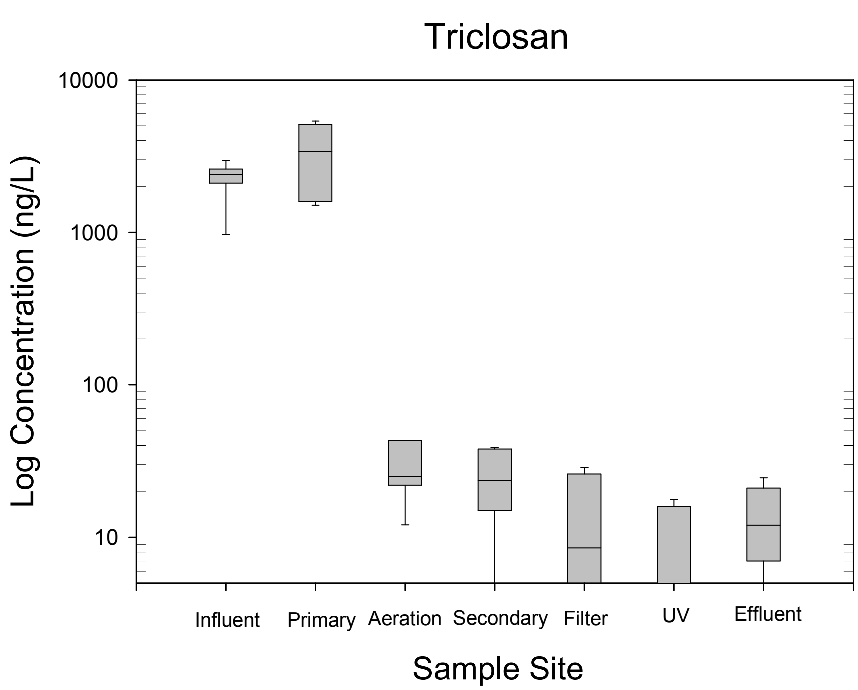

Figure 32. Concentrations (ng/L) of triclosan found at each site on three different sampling dates.

CHAPTER IV

CONCLUSION

Twenty three known or suspected EDCs were monitored in surface waters and wastewaters in the San Marcos area during a six month period from October 2006 to March 2007. Sample locations included: two sewage discharge ports of the hospital, influent and effluent of the WTF, influent and effluent of the WWTP (as well as five locations within the plant), and locations upstream and downstream of the WWTP effluent in the San Marcos River. Of the 23 EDCs monitored, 17 were detected at least once (cotinine, diazinon, ethynylestradiol, sulfamethoxazole, trimethoprim, and fluoxetine were never detected).

The most frequently detected compounds in the WWTP influent were acetaminophen, nonylphenol, coprostanol, caffeine, benzophenone, triethyl citrate, DEET, bisphenol A, TCEP, and triclosan. Comparison of influent and effluent concentrations showed that the San Marcos WWTP is effectively removing (>92%) of these compounds, with the exception of carbamazepine and TCEP. Within the treatment plant, results indicate that the aeration process was the most effective at removal. When compounds were not completely removed from the wastewater, they were found in waters downstream of the effluent discharge.

Results from samples collected at the hospital indicate that the hospital discharge is contributing to the concentration of these compounds in the San Marcos wastewater collection system. In the future, it might be beneficial for the City of San Marcos and the hospital to pre-treat the wastewater from the hospital before discharging it into the collections system.

APPENDIX

Appendix 1. Percent recoveries and relative standard deviations of spiked compounds (250 ng for GC-MS and 500 ng for LC-MS) from solid-phase extraction compared to long-term recoveries of compounds determined in environmental samples.

Compound	October				January				March			
	S	MS	MSD	%RSD	S	MS	MSD	%RSD	S	MS	MSD	%RSD
GC-MS												
DEET	69	68	68	0.0	132	122	119	1.8	127	125	114	6.5
Tributylphosphate	63	98	77	17.0	83	62	102	34.0	90	91	87	3.2
Triethyl citrate	69	63	67	4.4	88	93	107	9.9	107	92	78	11.6
Benzophenone	69	62	57	5.9	124	89	99	7.5	139	131	115	9.2
Nonylphenol	65	66	62	4.4	113	145	110	19.4	108	138	154	7.7
Cotinine	83	77	68	8.8	n/a	n/a	n/a	n/a	51	82	78	4.0
Octylphenol	51	82	78	3.5	94	89	79	8.4	58	61	68	7.7
TCEP	77	75	74	0.9	101	104	112	5.2	108	120	112	4.9
Diazinon	66	62	60	2.3	89	101	77	19.1	110	115	114	0.6
Caffeine	69	101	92	6.6	92	90	81	7.4	115	124	112	7.2
Fluoranthene	57	61	61	0.0	88	89	85	3.3	105	105	103	1.4
Bisphenol A	69	90	83	5.7	92	105	99	4.2	81	84	87	2.4
Triclosan	78	82	82	0.0	96	74	108	26.4	72	72	76	3.8
Estradiol	60	74	70	3.9	81	82	110	20.4	52	58	60	2.4
Ethynylestradiol	53	75	87	10.4	99	100	120	13.0	92	94	100	4.4
Coprostanol	57	66	71	5.2	82	90	88	1.6	73	81	82	0.9
Average	64	73	70	5.0	96	95	98	11.4	95	98	95	5.4
LC-MS												
Codeine					106	104	91	9.4	60	61	82	20.8
Acetaminophen					64	79	112	24.4	47	77	57	21.1
Trimethoprim					133	46	70	29.3	57	93	140	28.5
Sulfamethoxazole					78	149	86	37.9	57	195	177	6.8
Carbamazepine					128	89	100	8.2	105	170	224	19.4
Diltiazem					121	50	165	75.6	68	114	146	17.4
Fluoxetine					188	89	113	16.8	143	61	194	73.8
Average					117	87	105	28.8	77	110	146	26.8

GC-MS, gas chromatography – mass spectrometry; LC-MS, liquid chromatography – mass spectrometry; n/a, not applicable; S, spike made with reagent-water; MS, matrix spike; MSD, matrix spike duplicate; %RSD, percent relative standard deviation.

Appendix 2. Summary of detection frequencies (%) and concentration (ng/L) for 23 OWC in the San Marcos Hospital discharges.

Compound	Detection Frequency (%)			Mean Concentration (ng/L)			Minimum Concentration (ng/L)			Maximum Concentration (ng/L)		
	#1	#2	Total	#1	#2	Average	#1	#2	Total	#1	#2	Total
Acetaminophen	100	100	100	110,000	180,000	140,000	89,000	5,400	5,400	150,000	370,000	370,000
Caffeine	100	100	100	61,000	86,000	73,000	19,000	14,000	14,000	120,000	200,000	200,000
Triclosan	100	100	100	77,000	36,000	57,000	9,000	9,700	9,000	210,000	63,000	210,000
Coprostanol	100	83	92	11,000	14,000	12,000	880	3,000	880	31,000	27,000	31,000
DEET	67	67	67	110	110	110	57*	90	57*	170	140	170
TCEP	50	83	67	43*	63*	53*	22*	31*	22*	56*	95	95
Benzophenone	67	50	58	380	600	490	70*	170	70*	720	1,000	1,000
Diltiazem	50	50	50	710	1,600	1,100	580	1,300	580	840	1,800	1,800
Triethyl citrate	67	33	50	230	300	260	110	210	110	380	390	390
Nonylphenol	33	33	33	29,000	8,900	19,000	27,000	8,700	8,700	30,000	9,100	30,000
Tributylphosphate	0	67	33	<80	190	190	<80	110	110	<80	320	320
Codeine	25	0	13	50,000	<200	50,000	50,000	<200	50,000	50,000	<200	50,000
Cotinine	0	0	0	<80	<80	<80	<80	<80	<80	<80	<80	<80
Octylphenol	0	0	0	<80	<80	<80	<80	<80	<80	<80	<80	<80
Diazinon	0	0	0	<80	<80	<80	<80	<80	<80	<80	<80	<80
Fluoranthene	0	0	0	<80	<80	<80	<80	<80	<80	<80	<80	<80
Bisphenol A	0	0	0	<80	<80	<80	<80	<80	<80	<80	<80	<80
Estradiol	0	0	0	<80	<80	<80	<80	<80	<80	<80	<80	<80
Ethynylestradiol	0	0	0	<80	<80	<80	<80	<80	<80	<80	<80	<80
Trimethoprim	0	0	0	<200	<200	<200	<200	<200	<200	<200	<200	<200
Sulfamethoxazole	0	0	0	<200	<200	<200	<200	<200	<200	<200	<200	<200
Carbamazepine	0	0	0	<200	<200	<200	<200	<200	<200	<200	<200	<200
Fluoxetine	0	0	0	<200	<200	<200	<200	<200	<200	<200	<200	<200

#1, discharge from the main hospital; #2, discharge from the emergency room; Total, combined results of the two discharges; *, below LOQ but confirmed as trace by mass spectrum.

Appendix 3. Summary of concentrations (ng/L) for 23 OWCs in the WWTP effluent as well as in the San Marcos River upstream and downstream of the effluent.

Compound	Detection Frequency (%)			Mean Concentration (ng/L)			Minimum Concentration (ng/L)			Maximum Concentration (ng/L)		
	US	Effluent	DS	US	Effluent	DS	US	Effluent	DS	US	Effluent	DS
Triethyl citrate	0	100	67	<20	16*	12*	<20	6*	5*	<20	30	20
TCEP	0	100	100	<20	140	130	<20	39	35	<20	220	220
Caffeine	67	100	83	7*	22	15*	5*	12*	6*	8*	29	23
Triclosan	0	83	0	<20	15*	<20	<20	7*	<20	<20	25	<20
Carbamazepine	0	75	75	<50	330	290	<50	120	140	<50	490	410
Diltiazem	0	50	50	<50	130	100	<50	100	97	<50	150	110
Coprostanol	0	50	33	<20	110	84	<20	60	70	<20	170	98
Sulfamethoxazole	0	50	50	<50	560	1,200	<50	530	660	<50	580	1,800
DEET	0	33	33	<20	23*	9*	<20	18*	8*	<20	28	10*
Nonylphenol	0	33	33	<40	180	210	<40	170	200	<40	180	220
Benzophenone	0	0	33	<20	<20	11*	<20	<20	11*	<20	<20	11*
Estradiol	0	17	0	<20	89	<20	<20	89	<20	<20	89	<20
Bisphenol A	33	0	0	7*	<20	<20	5*	<20	<20	8*	<20	<20
Tributylphosphate	0	0	0	<20	<20	<20	<20	<20	<20	<20	<20	<20
Cotinine	0	0	0	<20	<20	<20	<20	<20	<20	<20	<20	<20
Octylphenol	0	0	0	<20	<20	<20	<20	<20	<20	<20	<20	<20
Diazinon	0	0	0	<20	<20	<20	<20	<20	<20	<20	<20	<20
Fluoranthene	0	0	0	<20	<20	<20	<20	<20	<20	<20	<20	<20
Ethynylestradiol	0	0	0	<20	<20	<20	<20	<20	<20	<20	<20	<20
Codeine	0	0	0	<50	<50	<50	<50	<50	<50	<50	<50	<50
Acetaminophen	0	0	0	<50	<50	<50	<50	<50	<50	<50	<50	<50
Trimethoprim	0	0	0	<50	<50	<50	<50	<50	<50	<50	<50	<50
Fluoxetine	0	0	0	<50	<50	<50	<50	<50	<50	<50	<50	<50

US, Upstream; DS, Downstream; *, below LOQ but confirmed as trace by mass spectrum.

Appendix 4. Summary of concentrations (ng/L) for 23 OWCs in the WWTP influent.

Compound	Influent			
	Detection Frequency (%)	Mean Concentration (ng/L)	Minimum Concentration (ng/L)	Maximum Concentration (ng/L)
Acetaminophen	100	44,000	16,000	80,000
Nonylphenol	100	31,000	9,000	63,000
Coprostanol	100	30,000	12,000	39,000
Caffeine	100	17,000	7,600	29,000
Benzophenone	100	2,500	630	6,200
Triethyl citrate	100	2,300	960	5,300
Triclosan	100	2,200	840	3,000
DEET	100	1,700	500	3,000
Bisphenol A	100	280	160	360
TCEP	100	260	33*	590
Diltiazem	50	1,600	1,200	1,900
Carbamazepine	50	590	550	620
Octylphenol	33	4,100	3,900	4,300
Estradiol	33	3,000	3,000	3,000
Fluoranthene	33	360	290	430
Tributylphosphate	33	260	100	410
Codeine	25	35,000	35,000	35,000
Cotinine	0	n/a	n/a	n/a
Diazinon	0	n/a	n/a	n/a
Ethynylestradiol	0	n/a	n/a	n/a
Trimethoprim	0	n/a	n/a	n/a
Sulfamethoxazole	0	n/a	n/a	n/a
Fluoxetine	0	n/a	n/a	n/a

n/a, not applicable

Appendix 5. Summary of concentrations (ng/L) for 23 OWCs through the WWTP.

Compound	WWTP Process							% Removal	
	Influent	Post Primary	Post Aeration	Post Secondary	Post Filter	Post UV	Effluent	Total	Average
DEET									
October	3000	3200	<20	<20	<20	<20	<20	100.0	99.2
January	1000	480	<20	<20	<20	<20	<20	100.0	
March	1000	1600	50	36*	34*	26*	23*	97.7	
Tributyl phosphate									
October	<80	<80	<20	12*	22*	38	<20	n/a	100.0
January	410	<80	<20	<20	<20	<20	<20	100.0	
March	100	130	20*	13*	29*	20*	<20	100.0	
Triethyl citrate									
October	1100	1300	51	26*	31	12*	8*	99.3	98.8
January	4700	640	15*	35	42	37	17*	99.6	
March	980	1100	39	31	27*	25*	24*	97.6	
Benzophenone									
October	650	600	<20	<20	<20	<20	<20	100.0	100.0
January	5800	<80	<20	<20	<20	<20	<20	100.0	
March	1000	1700	<20	<20	<20	<20	<20	100.0	
Nonylphenol									
October	48000	9100	<40	<40	<40	<40	<40	100.0	99.7
January	61000	64000	<40	<40	<40	<40	<40	100.0	
March	22000	33000	140	280	160	160	180	99.2	
Cotinine									
October	<80	<80	<20	<20	<20	<20	<20	n/a	n/a
January	<80	<80	<20	<20	<20	<20	<20	n/a	
March	<80	<80	<20	<20	<20	<20	<20	n/a	
Octylphenol									
October	<80	<80	<20	<20	<20	<20	<20	n/a	100.0
January	4100	<80	<20	<20	<20	<20	<20	100.0	
March	<80	<80	<20	<20	<20	<20	<20	n/a	
TCEP									
October	180	210	160	180	210	210	220	-19.4	33.0
January	470	240	94	120	140	150	150	67.7	
March	92	230	39	43	59	48	45	50.8	

Appendix 5 - Continued

Compound	WWTP Process							% Removal	
	Influent	Post Primary	Post Aeration	Post Secondary	Post Filter	Post UV	Effluent	Total	Average
Diazinon									
October	<80	<80	<20	<20	<20	<20	<20	n/a	n/a
January	<80	<80	<20	<20	<20	<20	<20	n/a	
March	<80	<80	<20	<20	<20	<20	<20	n/a	
Caffeine									
October	7900	130000	18*	21*	21*	19*	24*	99.7	99.8
January	26000	23000	11*	<20	10*	18*	21*	99.9	
March	17000	33000	12*	<20	12*	7*	21*	99.9	
Fluoranthene									
October	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	100.0
January	360	<80	<20	<20	<20	<20	<20	100.0	
March	<80	<80	<20	<20	<20	<20	<20	n/a	
Triclosan									
October	2300	5300	43	39	28	17*	23*	99.0	99.3
January	2700	1600	17*	15*	<20	<20	9*	99.7	
March	1700	3400	25*	24*	9*	9*	14*	99.2	
Bisphenol A									
October	280	480	<20	<20	<20	<20	<20	100.0	100.0
January	330	<80	<20	<20	<20	<20	<20	100.0	
March	230	330	<20	<20	<20	<20	<20	100.0	
Estradiol									
October	<80	<80	<20	<20	<20	<20	<20	n/a	97.0
January	<80	<80	<20	<20	<20	<20	<20	n/a	
March	3000	3500	58*	54*	55*	50*	89	97.0	
Ethynylestradiol									
October	<80	<80	<20	<20	<20	<20	<20	n/a	n/a
January	<80	<80	<20	<20	<20	<20	<20	n/a	
March	<80	<80	<20	<20	<20	<20	<20	n/a	
Coprostanol									
October	38000	130000	700	130	<20	80	74	99.8	99.5
January	39000	24000	2000	<20	<20	<20	<20	100.0	
March	15000	38000	120	130	100	110	170	98.8	

Appendix 5 - Continued

Compound	WWTP Process							% Removal	
	Influent	Post Primary	Post Aeration	Post Secondary	Post Filter	Post UV	Effluent	Total	Average
Codeine									
January	<200	<200	<50	<50	<50	<50	<50	n/a	100.0
March	35000	61000	<50	<50	<50	<50	<50	100.0	
Acetaminophen									
January	18000	28000	<50	<50	<50	<50	<50	100.0	100.0
March	71000	75000	<50	<50	<50	<50	<50	100.0	
Trimethoprim									
January	<200	<200	<50	<50	<50	<50	<50	n/a	n/a
March	<200	<200	<50	<50	<50	<50	<50	n/a	
Sulfamethoxazole									
January	<200	<200	<50	<50	850	1300	<50	n/a	n/a
March	<200	<200	<50	<50	<50	460	560	n/a	
Carbamazepine									
January	590	<200	100*	150	320	290	440	24.8	24.8
March	<200	400	<50	<50	<50	<50	120	n/a	
Diltiazem									
January	1600	1000	400	<50	190*	320	130*	91.9	91.9
March	<200	<200	<50	<50	<50	<50	<50	n/a	
Fluoxetine									
January	<200	<200	<50	<50	<50	<50	<50	n/a	n/a
March	<200	<200	<50	<50	<50	<50	<50	n/a	

n/a, not applicable; *, below LOQ but confirmed as trace by mass spectrum.

Appendix 6. Results from GC-MS-MS analysis.

	DEET						Tributylphosphate					
	October		January		March		October		January		March	
	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)
Lab Blank	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
Field Blank	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
WTF Effluent	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
WTF Influent	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
Upstream	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
Downstream	<20	n/a	<20	n/a	9*	16	<20	n/a	<20	n/a	<20	n/a
Effluent	<20	n/a	<20	n/a	23*	31	<20	n/a	<20	n/a	<20	n/a
UV	<20	n/a	<20	n/a	26*	31	38	n/a	<20	n/a	20*	35
Filter	<20	n/a	<20	n/a	34*	15	22*	16	<20	n/a	29*	n/a
Secondary	<20	n/a	<20	n/a	36*	10	<20	n/a	<20	n/a	13*	6
Aeration	<20	n/a	<20	n/a	50*	3	<20	n/a	<20	n/a	20*	18
Primary	3200	2	480	9	1600	5	<80	n/a	<80	n/a	130*	0
Influent	3000	0	1000	15	1000	71	<80	n/a	410	n/a	100*	n/a
Hospital #1	60*	6	<80	n/a	170*	4	<80	n/a	<80	n/a	<80	n/a
Hospital #2	91*	2	<80	n/a	130*	17	250	43	<80	n/a	130*	22
Compound Spike	69%	n/a	132%	n/a	127%	n/a	63%	n/a	83%	n/a	90%	n/a
Matrix Spike	68%	0	121%	2	120%	7	88%	17	82%	34	89%	3

Appendix 6 - Continued

	Triethyl Citrate						Benzophenone					
	October		January		March		October		January		March	
	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)
Lab Blank	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
Field Blank	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
WTF Effluent	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
WTF Influent	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
Upstream	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
Downstream	7*	35	<20	n/a	17*	30	11*	0	<20	n/a	<20	n/a
Effluent	8*	29	17*	47	24*	39	<20	n/a	<20	n/a	<20	n/a
UV	11*	19	37	52	25*	34	<20	n/a	<20	n/a	<20	n/a
Filter	31	27	42	37	27*	3	<20	n/a	<20	n/a	<20	n/a
Secondary	26*	33	35	36	31	2	<20	n/a	<20	n/a	<20	n/a
Aeration	51	6	15*	38	39	9	<20	n/a	<20	n/a	<20	n/a
Primary	1300	6	640	1	1100	0	610	4	<80	n/a	1700	4
Influent	1100	0	4700	18	980	3	650	4	5800	10	1000	21
Hospital #1	340	17	<80	n/a	120	6	71	2	<80	n/a	680	8
Hospital #2	<80	n/a	<80	n/a	300	42	170	n/a	<80	n/a	820	31
Compound Spike	69%	n/a	88%	n/a	107%	n/a	69%	n/a	124%	n/a	139%	n/a
Matrix Spike	65%	4	100%	10	85%	12	60%	6	94%	8	123%	9

Appendix 6 - Continued

	Nonylphenol						Cotinine					
	October		January		March		October		January		March	
	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)
Lab Blank	<40	n/a	<40	n/a	<40	n/a	<20	n/a	n/a	n/a	<20	n/a
Field Blank	<40	n/a	<40	n/a	<40	n/a	<20	n/a	n/a	n/a	<20	n/a
WTF Effluent	<40	n/a	<40	n/a	<40	n/a	<20	n/a	n/a	n/a	<20	n/a
WTF Influent	<40	n/a	<40	n/a	<40	n/a	<20	n/a	n/a	n/a	<20	n/a
Upstream	<40	n/a	<40	n/a	<40	n/a	<20	n/a	n/a	n/a	<20	n/a
Downstream	<40	n/a	<40	n/a	210	7	<20	n/a	n/a	n/a	<20	n/a
Effluent	<40	n/a	<40	n/a	180	4	<20	n/a	n/a	n/a	<20	n/a
UV	<40	n/a	<40	n/a	160	41	<20	n/a	n/a	n/a	<20	n/a
Filter	<40	n/a	<40	n/a	160	9	<20	n/a	n/a	n/a	<20	n/a
Secondary	<40	n/a	<40	n/a	280	13	<20	n/a	n/a	n/a	<20	n/a
Aeration	<40	n/a	<40	n/a	140	26	<20	n/a	n/a	n/a	<20	n/a
Primary	1000	1	64000	3	33000	4	<80	n/a	n/a	n/a	<80	n/a
Influent	1700	4	61000	5	22000	6	<80	n/a	n/a	n/a	<80	n/a
Hospital #1	29000	7	<160	n/a	<160	n/a	<80	n/a	n/a	n/a	<80	n/a
Hospital #2	8900	3	<160	n/a	<160	n/a	<80	n/a	n/a	n/a	<80	n/a
Compound Spike	65%	n/a	113%	n/a	108%	n/a	83%	n/a	0%	n/a	51%	n/a
Matrix Spike	64%	4	128%	19	146%	8	73%	9	0%	n/a	80%	4

Appendix 6 - Continued

	TCEP						Octylphenol					
	October		January		March		October		January		March	
	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)
Lab Blank	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
Field Blank	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
WTF Effluent	8*	0	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
WTF Influent	7*	10	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
Upstream	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
Downstream	220	0	150	5	37	8	<20	n/a	<20	n/a	<20	n/a
Effluent	220	3	150	0	45	19	<20	n/a	<20	n/a	<20	n/a
UV	210	3	150	5	48	10	<20	n/a	<20	n/a	<20	n/a
Filter	210	0	140	0	59	10	<20	n/a	<20	n/a	<20	n/a
Secondary	180	0	120	18	43	0	<20	n/a	<20	n/a	<20	n/a
Aeration	160	9	94	5	39	2	<20	n/a	<20	n/a	<20	n/a
Primary	210	10	240	21	230	0	<80	n/a	<80	n/a	<80	n/a
Influent	180	8	470	17	92*	90	<80	n/a	4100	7	<80	n/a
Hospital #1	54*	7	<80	n/a	<80	n/a	<80	n/a	<80	n/a	<80	n/a
Hospital #2	71*	19	95*	n/a	39*	28	<80	n/a	<80	n/a	<80	n/a
Compound Spike	77%	n/a	101%	n/a	108%	n/a	51%	n/a	94%	n/a	58%	n/a
Matrix Spike	75%	1	108%	5	116%	5	80%	4	84%	8	65%	8

Appendix 6 - Continued

	Diazinon						Caffeine					
	October		January		March		October		January		March	
	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)
Lab Blank	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
Field Blank	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
WTF Effluent	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
WTF Influent	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
Upstream	<20	n/a	<20	n/a	<20	n/a	7*	15	<20	n/a	5*	5
Downstream	<20	n/a	<20	n/a	<20	n/a	21*	17	15*	78	<20	n/a
Effluent	<20	n/a	<20	n/a	<20	n/a	24*	27	21*	59	21	13
UV	<20	n/a	<20	n/a	<20	n/a	19*	27	17*	88	<20	n/a
Filter	<20	n/a	<20	n/a	<20	n/a	21*	3	9*	55	12*	26
Secondary	<20	n/a	<20	n/a	<20	n/a	21*	24	<20	n/a	<20	n/a
Aeration	<20	n/a	<20	n/a	<20	n/a	18*	12	10*	21	<20	n/a
Primary	<80	n/a	<80	n/a	<80	n/a	130000	6	23000	9	33000	63
Influent	<80	n/a	<80	n/a	<80	n/a	7900	5	26000	19	17000	72
Hospital #1	<80	n/a	<80	n/a	<80	n/a	120000	6	19000	0	49000	29
Hospital #2	<80	n/a	<80	n/a	<80	n/a	83000	8	15000	9	160000	35
Compound Spike	66%	n/a	89%	n/a	110%	n/a	69%	n/a	92%	n/a	115%	n/a
Matrix Spike	61%	2	89%	19	115%	1	97%	7	86%	7	118%	7

Appendix 6 - Continued

	Fluoranthene						Triclosan					
	October		January		March		October		January		March	
	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)
Lab Blank	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
Field Blank	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
WTF Effluent	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
WTF Influent	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
Upstream	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
Downstream	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
Effluent	<20	n/a	<20	n/a	<20	n/a	23*	12	8*	19	<20	n/a
UV	<20	n/a	<20	n/a	<20	n/a	17*	8	<20	n/a	<20	n/a
Filter	<20	n/a	<20	n/a	<20	n/a	28	8	<20	n/a	9*	11
Secondary	<20	n/a	<20	n/a	<20	n/a	39	2	<20	n/a	24*	3
Aeration	<20	n/a	<20	n/a	<20	n/a	43	0	17*	47	25*	6
Primary	<80	n/a	<80	n/a	<80	n/a	5300	4	1600	5	3400	4
Influent	<80	n/a	360	27	<80	n/a	2300	12	2700	19	1700	72
Hospital #1	<80	n/a	<80	n/a	<80	n/a	210000	3	18000	4	9500	7
Hospital #2	<80	n/a	<80	n/a	<80	n/a	61000	5	11000	15	38000	40
Compound Spike	57%	n/a	88%	n/a	105%	n/a	78%	n/a	96%	n/a	72%	n/a
Matrix Spike	61%	0	87%	3	104%	1	82%	0	91%	26	74%	4

Appendix 6 - Continued

	Bisphenol A						Estradiol					
	October		January		March		October		January		March	
	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)
Lab Blank	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
Field Blank	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
WTF Effluent	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
WTF Influent	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
Upstream	7*	30	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
Downstream	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
Effluent	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	89	n/a
UV	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	50*	10
Filter	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	55*	9
Secondary	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	54*	1
Aeration	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	58*	6
Primary	480	7	<80	n/a	330	11	<80	n/a	<80	n/a	3500	14
Influent	280	5	330	15	230	43	<80	n/a	<80	n/a	3000	0
Hospital #1	<80	n/a	<80	n/a	<80	n/a	<80	n/a	<80	n/a	<80	n/a
Hospital #2	<80	n/a	<80	n/a	<80	n/a	<80	n/a	<80	n/a	<80	n/a
Compound Spike	69%	n/a	92%	n/a	81%	n/a	60%	n/a	81%	n/a	52%	n/a
Matrix Spike	87%	6	102%	4	86%	2	72%	4	97%	20	59%	2

Appendix 6 - Continued

	Ethynylestradiol						Coprostanol					
	October		January		March		October		January		March	
	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)
Lab Blank	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
Field Blank	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
WTF Effluent	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
WTF Influent	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
Upstream	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a
Downstream	<20	n/a	<20	n/a	<20	n/a	84	24	<20	n/a	<20	n/a
Effluent	<20	n/a	<20	n/a	<20	n/a	74	26	<20	n/a	170	n/a
UV	<20	n/a	<20	n/a	<20	n/a	80	n/a	<20	n/a	120	45
Filter	<20	n/a	<20	n/a	<20	n/a	<20	n/a	<20	n/a	100	n/a
Secondary	<20	n/a	<20	n/a	<20	n/a	130	6	<20	n/a	130	17
Aeration	<20	n/a	<20	n/a	<20	n/a	700	46	2000	25	120	12
Primary	<80	n/a	<80	n/a	<80	n/a	130000	11	24000	15	38000	2
Influent	<80	n/a	<80	n/a	<80	n/a	38000	2	39000	2	15000	24
Hospital #1	<80	n/a	<80	n/a	<80	n/a	1100	7	31000	2	1300	49
Hospital #2	<80	n/a	<80	n/a	<80	n/a	26000	8	11000	n/a	3600	22
Compound Spike	53%	n/a	99%	n/a	92%	n/a	57	n/a	82	n/a	73	n/a
Matrix Spike	81%	10	110%	13	97%	4	69	5	89	2	82	1

Appendix 6 - Continued

	Caffeine-$^{13}C_3$						Fluoranthene-d_{10}					
	October		January		March		October		January		March	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Lab Blank	80	n/a	96	n/a	110	n/a	81	n/a	28	n/a	125	n/a
Field Blank	76	n/a	88	n/a	108	n/a	72	n/a	28	n/a	127	n/a
WTF Effluent	60	9	100	2	142	2	47	4	97	7	149	0
WTF Influent	55	10	103	3	128	4	46	0	98	4	142	3
Upstream	88	2	104	1	121	0	57	6	74	2	138	2
Downstream	88	2	108	3	124	8	52	2	93	2	135	4
Effluent	86	7	112	3	94	17	51	7	97	1	99	6
UV	87	0	124	5	125	10	44	1	97	9	115	1
Filter	95	10	125	8	108	8	66	7	103	9	116	2
Secondary	96	13	114	4	109	2	71	8	86	2	107	1
Aeration	69	1	100	7	116	3	42	2	88	2	115	1
Primary	88	9	126	14	114	0	76	2	90	1	111	1
Influent	91	3	115	1	116	12	90	9	96	8	117	18
Hospital #1	72	6	110	7	110	3	85	6	87	11	111	2
Hospital #2	106	4	95	4	122	4	50	3	97	6	114	2
Compound Spike	77	n/a	92	n/a	120	n/a	79	n/a	87	n/a	143	n/a
Matrix Spike	92	2	101	10	137	0	52	1	83	4	135	1

Appendix 6 - Continued

	Bisphenol A-d_{16}					
	October		January		March	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Lab Blank	82	n/a	98	n/a	99	n/a
Field Blank	80	n/a	107	n/a	64	n/a
WTF Effluent	69	6	100	7	118	1
WTF Influent	96	7	96	11	112	2
Upstream	83	1	94	15	107	5
Downstream	92	8	121	2	144	4
Effluent	105	4	114	6	125	1
UV	105	0	114	6	134	13
Filter	49	41	118	7	129	1
Secondary	82	7	87	16	131	0
Aeration	83	2	106	2	126	2
Primary	107	5	87	28	149	9
Influent	65	6	119	0	119	2
Hospital #1	80	14	64	12	155	1
Hospital #2	66	21	103	7	148	5
Compound Spike	74	n/a	104	n/a	121	n/a
Matrix Spike	87	9	107	9	134	1

n/a, not applicable; *, below LOQ but confirmed as trace by mass spectrum; %RSD, percent relative standard deviation.

Appendix 7. Results from LC-MS-MS analysis.

	Codeine				Acetaminophen				Trimethoprim			
	January		March		January		March		January		March	
	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)
Lab Blank	<50	n/a	<50	n/a	<50	n/a	<50	n/a	<50	n/a	<50	n/a
Field Blank	<50	n/a	<50	n/a	<50	n/a	<50	n/a	<50	n/a	<50	n/a
WTF Effluent	<50	n/a	<50	n/a	<50	n/a	<50	n/a	<50	n/a	<50	n/a
WTF Influent	<50	n/a	<50	n/a	<50	n/a	<50	n/a	<50	n/a	<50	n/a
Upstream	<50	n/a	<50	n/a	<50	n/a	<50	n/a	<50	n/a	<50	n/a
Downstream	<50	n/a	<50	n/a	<50	n/a	<50	n/a	<50	n/a	<50	n/a
Effluent	<50	n/a	<50	n/a	<50	n/a	<50	n/a	<50	n/a	<50	n/a
UV	<50	n/a	<50	n/a	<50	n/a	<50	n/a	<50	n/a	<50	n/a
Filter	<50	n/a	<50	n/a	<50	n/a	<50	n/a	<50	n/a	<50	n/a
Secondary	<50	n/a	<50	n/a	<50	n/a	<50	n/a	<50	n/a	<50	n/a
Aeration	<50	n/a	<50	n/a	<50	n/a	<50	n/a	<50	n/a	<50	n/a
Primary	<200	n/a	61000	60	28000	3	75000	39	<200	n/a	<200	n/a
Influent	<200	n/a	35000	n/a	18000	16	71000	18	<200	n/a	<200	n/a
Hospital #1	<200	n/a	50000	n/a	120000	30	94000	7	<200	n/a	<200	n/a
Hospital #2	<200	n/a	<200	n/a	6100	15	350000	10	<200	n/a	<200	n/a
Compound Spike	106%	n/a	60%	n/a	64%	n/a	47%	n/a	133%	n/a	57%	n/a
Matrix Spike	98%	9	72%	21	96%	24	67%	21	58%	29	117%	29

Appendix 7 - Continued

	Sulfamethoxazole				Carbamazepine				Diltiazem			
	January		March		January		March		January		March	
	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)
Lab Blank	<50	n/a	<50	n/a	<50	n/a	<20	n/a	<50	n/a	<50	n/a
Field Blank	<50	n/a	<50	n/a	<50	n/a	<50	n/a	<50	n/a	<50	n/a
WTF Effluent	<50	n/a	<50	n/a	<50	n/a	<50	n/a	<50	n/a	<50	n/a
WTF Influent	<50	n/a	<50	n/a	<50	n/a	<50	n/a	<50	n/a	<50	n/a
Upstream	<50	n/a	<50	n/a	<50	n/a	<50	n/a	<50	n/a	<50	n/a
Downstream	<50	n/a	1200	66	370	17	140	n/a	100*	9	<50	n/a
Effluent	<50	n/a	560	6	440	16	120	n/a	130*	28	<50	n/a
UV	1300	n/a	460	n/a	290	88	<50	n/a	320	22	<50	n/a
Filter	850	n/a	<50	n/a	320	20	<50	n/a	190*	19	<50	n/a
Secondary	<50	n/a	<50	n/a	150	n/a	<50	n/a	<50	n/a	<50	n/a
Aeration	<50	n/a	<50	n/a	100*	49	<50	n/a	<50	n/a	<50	n/a
Primary	<200	n/a	<200	n/a	<200	n/a	400	n/a	1000	56	<200	n/a
Influent	<200	n/a	<200	n/a	590	8	<200	n/a	1600	32	<200	n/a
Hospital #1	<200	n/a	<200	n/a	<200	n/a	<200	n/a	710	26	<200	n/a
Hospital #2	<200	n/a	<200	n/a	<200	n/a	<200	n/a	1600	23	<200	n/a
Compound Spike	78%	n/a	57%	n/a	128%	n/a	105%	n/a	121%	n/a	68%	n/a
Matrix Spike	118%	38	186%	7	95%	8	197%	19	108%	76	130%	17

Appendix 7 - Continued

	Fluoxetine				Codeine-d_6				Acetaminophen-d_4			
	January		March		January		March		January		March	
	Conc. (ng/L)	RSD (%)	Conc. (ng/L)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Lab Blank	<50	n/a	<50	n/a	76	n/a	95	n/a	51	n/a	87	n/a
Field Blank	<50	n/a	<50	n/a	145	n/a	110	n/a	58	n/a	87	n/a
WTF Effluent	<50	n/a	<50	n/a	94	46	221	14	56	62	131	21
WTF Influent	<50	n/a	<50	n/a	77	15	186	27	72	52	116	11
Upstream	<50	n/a	<50	n/a	23	9	55	20	59	2	56	19
Downstream	<50	n/a	<50	n/a	73	46	62	23	136	47	67	55
Effluent	<50	n/a	<50	n/a	58	3	94	48	108	9	108	33
UV	<50	n/a	<50	n/a	138	2	96	59	145	4	75	20
Filter	<50	n/a	<50	n/a	119	17	107	39	138	5	55	78
Secondary	<50	n/a	<50	n/a	24	43	46	7	73	30	88	3
Aeration	<50	n/a	<50	n/a	85	32	60	20	107	3	59	13
Primary	<200	n/a	<200	n/a	87	44	66	16	155	36	161	35
Influent	<200	n/a	<200	n/a	86	4	70	38	139	8	157	82
Hospital #1	<200	n/a	<200	n/a	34	5	86	30	84	4	187	7
Hospital #2	<200	n/a	<200	n/a	24	29	118	14	45	21	182	44
Compound Spike	188%	n/a	143%	n/a	134	n/a	47	n/a	103	n/a	53	n/a
Matrix Spike	101%	17	128%	74	67	57	54	6	82	n/a	76	44

n/a, not applicable; *, below LOQ but confirmed as trace by mass spectrum; %RSD, percent relative standard deviation.

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