FINAL REPORT

Occurrence of Pharmaceuticals and Personal Care Products (PPCPs) at an effluent-dominated wastewater application site: Estrogens, Triclosan, and Caffeine

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Abstract

Pharmaceuticals and personal care products (PPCPs) have recently been identified in the environment; their potential effects on ecosystems are of increasing concern. These contaminants can reach the soil and aquatic environment through land application of wastewater effluent and agricultural runoff. The objective of this work was to assess the fate of PPCPs at field scale. PPCPs were measured systematically in a wastewater treatment plant (WWTP), and in soil and groundwater receiving the treated effluent from the WWTP. The occurrence of target PPCPs was evaluated to determine PPCP transfer from the WWTP to soil and groundwater. The Lubbock Land Application Site (LLAS) was used as the study site, which has received treated wastewater effluent for more than 70 years in order to remove additional nutrients and irrigate non-edible crops. The site was ideal for investigating the long-term fate of PPCPs in the environment above drinking water sources. Target compounds (e.g., estrone, 17β-estradiol, estriol, 17α-ethynylestradiol, triclosan, and caffeine) in wastewater, sewage sludge, soil, and groundwater were determined using HPLC/UV as the primary mode of analysis with qualitative confirmatory analyses using GC-MS on a portion (10%) of the samples. Samples were collected quarterly over twelve months for wastewater and sludge samples and over nine months for soil and groundwater samples. The results indicated that concentrations of PPCPs in the influent, effluent, sludge solid phase, and sludge liquid phase were in the range of not detected (ND)-127 μg/L, ND-83 μg/L, ND-19 μg/g, and ND-50 μg/L, respectively. Concentrations in soil and groundwater samples from the LLAS were in the range of ND-136 ng/g and ND-1,745 ng/L. Overall, data suggested that PPCPs in the effluent from the wastewater treatment plant could be transported both

vertically and horizontally in the soil, and eventually transported to groundwater via land application of the effluent.

Problem and Research Objectives

Pharmaceuticals and personal care products (PPCPs), which are identified in the environment, have prompted an important concern on their ecotoxicity and persistence in the environment (Daughton and Ternes 1999). Some natural estrogens such as estriol, estradiol and estrone are considered to be potent endocrine disruptors (Gross et al. 2004; Ying et al. 2004). However, the fate and persistence of these compounds in the environment are still unclear (Daughton and Ternes 1999; Gross et al. 2004; Kolpin et al. 2002; Ankley et al. 2007). Other antimicrobial compounds (for example, triclosan is used in many personal care products) are believed to lead to the development of antibiotic resistance and are considered as persistent chemicals in the environment (Ying and Kookana 2007). These PPCPs transport to municipal wastewater treatment plants (WWTPs) and eventually are discharged into aquatic environments or continued to exist in surface water, groundwater, and soil (Chu and Metcalfe 2007; Allaire et al. 2006).

Hundreds of tons of PPCPs are estimated to be produced and consumed annually in the developed countries (Scheytt et al. 2006; Polar 2007). The effluent from WWTPs is the primary route of these PPCPs being introduced into the environment. Since wastewater treatment processes are designed to remove pathogens and nutrients from sewage, PPCPs can only be incidentally removed and the elimination is variable (Daughton and Ternes 1999; Heberer 2002a). Most PPCPs consumed by humans enter the wastewater system; they can be excreted completely unmetabolized, rinsed off of the body, or disposed as unused medications. Some PPCPs are conjugated in the body prior to excretion. These conjugated forms are often broken during the wastewater treatment process and transformed back to the parent compound. The PPCPs are not typically persistent, but are constantly released into the environments and hence, PPCPs have the potential for continual environmental entry (Heberer 2002a; Kümmerer 2004; Gielen et al. 2009). Several studies have determined that PPCPs exist in effluents in the range of high ng/L to low μg/L concentrations, and can be detected in stream surveys in the United States (Gross et al. 2004; Haggard et al. 2006; Waltman et al. 2006; Glassmeyer et al. 2008). Although PPCPs occur at relatively low concentrations, their continual long-term release may result in significant environmental concentrations.

Effluents from WWTPs are increasingly applied to irrigate crops and public areas in arid regions in the United States, as well as other countries to reduce the demand on water supplies (Pedersen et al. 2005; Kinney et al. 2006). The effluent is also applied to lands for the natural treatment of wastewater as the effluent moves through the natural filter provided by soil and plants (Davis and Cornwell 1998; Overcash et al. 2005). Such application to lands is considered as the oldest method for the treatment and disposal of wastes. There are around 600 communities in the United States reusing the effluent from municipal wastewater treatment plants for surface irrigation (Davis and Cornwell 1998). However, the application of wastewater to lands is also a route of PPCPs transfer to soil (Oppel et al. 2004). Various PPCPs in the effluent, such as estrogens, can sorb to soil once the soil is exposed to these compounds (Casey et al. 2005; Drillia et al. 2005; Hildebrand et al. 2006). These compounds can be transported from the soil to other

aquatic systems such as surface water and groundwater, the extent of which is dependent on various factors including the solubility, sorption behavior, and persistence of the contaminant as well as climatic conditions and physicochemical properties of the soil (Boxall 2008). Since PPCPs remaining in treated wastewater can leach or percolate through the soil to groundwater supplies during runoff events or subsurface flow, concerns about these compounds in the effluent entering potential drinking water resources and the environment are increasing. There are several reports indicating that PPCPs such as estrone, ibuprofen, diclofenac, and chlofibric acid can be detected in groundwater and drinking water (Ternes et al. 2001; Heberer 2002b, Rodriguez-Mozaz et al. 2004).

The objective of this work was to study the fate of PPCPs at field scale. The PPCPs were measured systematically in a WWTP, and in soil and groundwater receiving effluent from the WWTP. The occurrence of target PPCPs were evaluated to obtain the overall view of PPCPs transfer from the WWTP to soil and groundwater. The unique study site "the Lubbock Land Application Site (LLAS)" selected for the project is a wastewater land application site used for nutrient removal and non-edible crop production. The LLAS has received wastewater effluent for over 70 years, and is the ideal site to determine the long-term fate of PPCPs in the environment above drinking water sources. Target PPCPs included estrone (E1), 17β-estradiol (E2), estriol (E3), 17α-ethynyl estradiol (EE2), caffeine, and triclosan. Target PPCPs were determined using HPLC/UV as the primary mode of analysis; qualitative confirmatory analyses utilized GC-MS on a portion (10%) of the samples. The GC-MS technique was applied for the confirmatory analyses because of its ease of convenience over the LC-MS.

Materials and Methods

Study Area

The Lubbock Water Reclamation Plant (LWRP**)** located in Lubbock, Texas, served as the test facility for the fate of PPCPs in a full-scale WWTP. Wastewater is delivered to the plant through 900 miles of collection lines and 21 lift stations. Lubbock's water consumers can be characterized as residential (85%), small commercial (10%), municipal (4%); other user classes (1%) include industrial, schools, wholesale, and irrigation. The LWRP treats approximately 21 million gallons of wastewater per day and has an average daily flow design capacity of 31.5 million gallons. There are three process streams for the plant including one bio tower process and two activated sludge processes (Fig.1). The primary treatment of the influent to the plant consists of screening and grit removal. After primary treatment, the flow streams are split before secondary treatment. The plant applies activated sludge in Plants 3 and 4 for secondary biological treatment. Plant 2 uses biotowers for secondary treatment. Without tertiary removal, treated effluent is reused; nearly two-thirds of wastewater produced each day are reused by agricultural irrigation at land application sites and as industrial cooling water. Some effluents are also disposed by discharge to streams. Sludge from secondary treatment is thickened, digested in anaerobic digesters, dewatered, and landfilled.

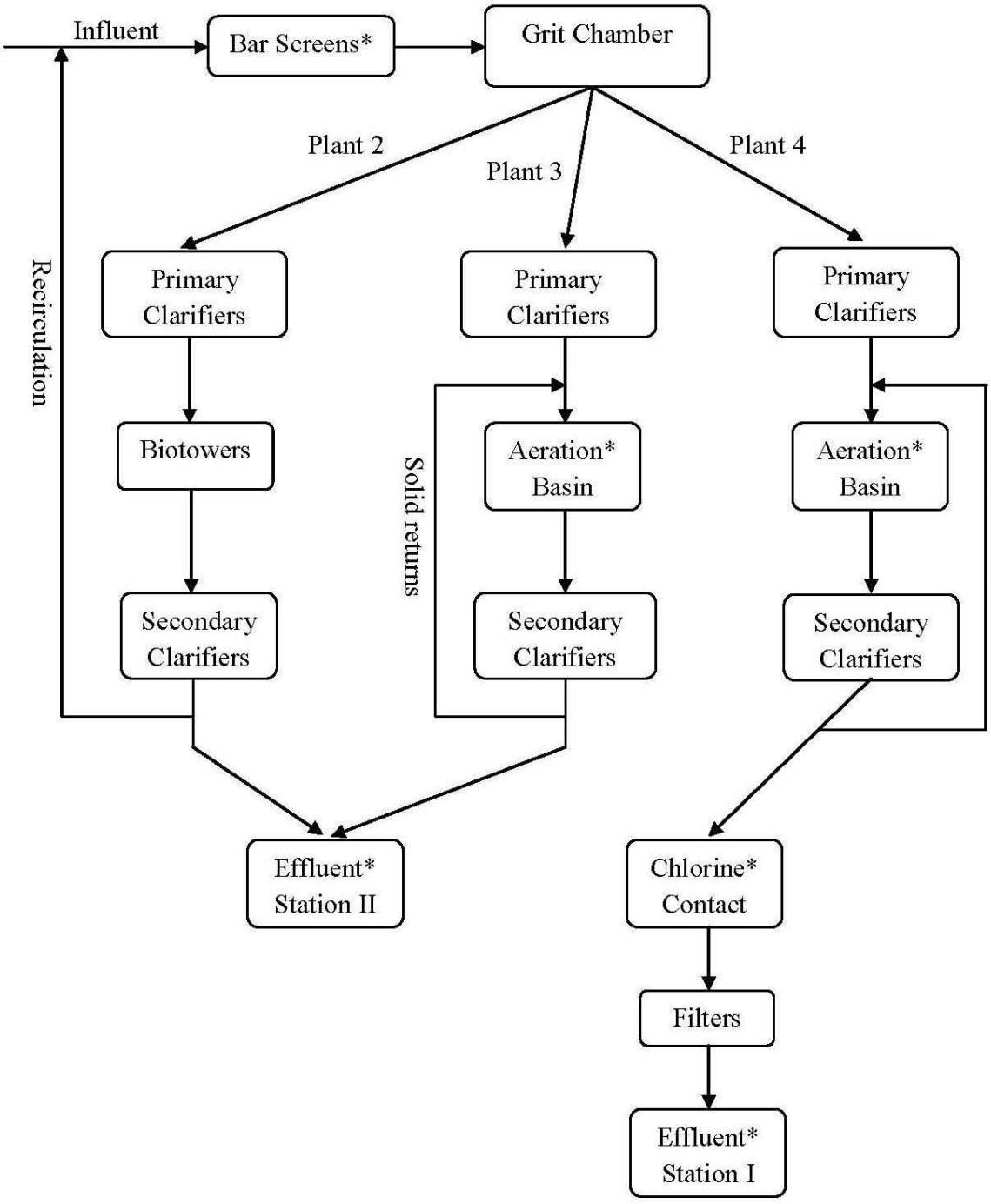

Fig. 1 Process schematic of the Lubbock Water Reclamation Plant (LWRP). The asterisk (*) indicates sampling locations.

The Lubbock Land Application Site (LLAS) is the 6,000-acre irrigated farmland used by the city of Lubbock as a site of secondarily-treated wastewater effluent application. The LLAS is seeded with grasses, cotton and legume plant species that absorb and utilize the high amount of nitrogen compounds present in the effluent. The site has been in use for this purpose since 1937, starting with 200 acres with additional land purchased over time. Since then, monitoring wells have been constructed and used to determine the amount of pollutants, especially nitrate concentrations in the groundwater at various locations. Pivot irrigation systems are employed to apply the effluent to 31 treatment plots comprising a total land area of 2,538 acre. A storage reservoir of 412 million gallon enables the farm to store and distribute treated effluent to the treatment plots as needed. On a daily basis, approximately 13 million gallons of effluent from the LWRP are applied to treatment plots. Prairie dogs occupy approximately 700 acres of the 6,000-acre site; however, only about 30% of the occupied area is under the center pivot points.

Sampling points inside pivot irrigation at the LLAS

Sample Collection

Wastewater and sludge. Grab samples of wastewater and sludge were collected from various sampling points at the LWRP (Fig. 1) quarterly from December, 2008 through September, 2009 to determine the fate of target compounds in the plant. As the LWRP contains three independent process trains referred to as Plant 2, 3, and 4, samples were collected from Plants 3 and 4 in order to compare water quality between these plants that attain different effluent water quality. Approximately 50 percent of the plant flow was sent to Plant 4. Since the removal of PPCPs from the wastewater stream may indicate the chemicals present in the sludge, it was important to obtain the data of PPCPs concentration in both wastewater and sludge for determining the phase in which the chemical persists, if it was not degraded. Wastewater samples were collected at bar rack, aeration basin, chlorine contact chamber, and effluent station. Sludge samples were collected from feed sludge, which was the wasted sludge from the secondary treatment, and anaerobic digester. All samples were collected in 1-L amble jars stored on ice during transport to the laboratory and refrigerated at 4°C until extraction.

Groundwater and soil. Groundwater and soil samples were collected at the LLAS to determine whether PPCPs accumulate in the soil and/or transport into the groundwater. There were four sampling points named after the code of monitoring wells: CL-11, CL-29, CL-43 and CL-48. The CL-29 and CL-48 were the wells located outside the area of pivot irrigation, while the CL-11 and CL-43 were under the center pivot points. At all sampling points, both groundwater and soil samples were collected quarterly in the same days from March, 2009 through September, 2009. Groundwater samples were collected from a tap above the wells, stored in 1-L amble glass bottles on ice during transport to the laboratory, and refrigerated at 4°C until processed prior to analysis. Soil cores were collected from each sampling point at a depth of 0-30 inch to cover target soil depths of 0-6 inch, 12-18 inch, and 24-30 inch. A soil core sampler with diameter of 4.5 cm was used for soil sampling. Soil cores were stored at 4°C until further use for PPCPs analysis.

Chemical and Reagent

Anhydrous caffeine (purity > 99%) and estrogenic compounds, including E1 (purity > 99%), E2 (purity > 98%), E3 (purity > 99%), EE2 (purity > 98%), β-estradiol-17acetate (purity > 99%), and triethylamine (purity > 99%) were obtained from Sigma-Aldrich (St. Louis, MO). Triclosan (purity > 97) was purchased from Fluka Chemie GmbH (Buchs, Switzerland). Relevant chemical properties of the test compounds are shown in Table 1. HPLC-grade acetonitrile was obtained from Fisher Scientific (Fair Lawn, NJ). Ultra-pure water (> 18MΩ) was prepared by a Barnstead NANOpure infinity ultrapure water system (Dubuque, IA). Standard solutions of test compounds were prepared in 1:1 (v/v) acetonitrile:water for estrogens and 100% acetonitrile for caffeine and triclosan.

Table 1 Chemical properties of the test compounds

Compound	Water solubility (mg/L at 20 ºC)	log K_{ow}	K_d (mL/g)	Vapor pressure (mm Hg)
Estrone (E1)	13[b]	2.95[a]	67.7[d]	1.41 x 10^{-7} [c]
17β-estradiol (E2)	13[b]	3.86[a]	115.8[d]	1.26 x 10^{-8} [c]
Estriol (E3)	13[b]	2.45[a]	8.6[d]	1.97 x 10^{-10} [c]
17α-ethynylestradiol (EE2)	4.8[b]	3.67[a]	176.2[d]	2.64 x 10^{-9} [c]
Triclosan	10[c]	4.76[c]	256.8[d]	6.45 x 10^{-7} [c]
Caffeine	2.16 x 10^{4} [c,*]	-0.07[c]	18.5[d]	15[c]

* at 25 ºC

[a] Machatha and Yalkowsky (2005)

[b] Ying and Kookana (2005)

[c] National Library of Medicine Toxnet (http://toxnet.nlm.nih.gov)

[d] Karnjanapiboonwong et al. (2010)

Sample Preparation

Wastewater. Wastewater samples were first filtered through a 10-cm P5 filter paper (Fisher Scientific, PA, USA) to remove suspended solids. Solid phase extraction (SPE) was applied for target PPCP analysis. The extraction procedure of target compounds (E1, E2, E3, EE2, triclosan, and caffeine) was modified partially based on methods reported in the literature (Kvanli et al, 2008) using C18 SPE cartridge. β-estradiol-17acetate (EA) was also used as an internal standard for QA/QC purpose. The 200 mL of sample was passed through an SPE cartridge (Honeywell Burdick & Jackson, MI, USA, Product No.9008), which was first conditioned with 3 mL of acetonitrile followed by 3 mL of Milli-Q water. Then, samples were extracted through SPE cartridges at a flow rate < 5 mL min^{-1} and were subsequently eluted with 3×1 mL of acetonitrile. The eluate was then analyzed using HPLC/UV or derivatized for GC-MS analysis on a portion (10%) of samples. The recovery of this extraction method in both clean (Milli-Q) water and wastewater matrices were shown in Table 2. The method applied also provided adequate detection limits (Table 3) using the U.S. EPA guidelines (U.S. EPA, 2000).

Sludge. Sludge samples (200 mL) were filtered by using 10-cm P5 filter papers to separate the solid phase from the liquid phase. Filtrate was extracted with the same procedure as described in wastewater samples extraction. The solid phase of sludge samples was air-dried and the dry weight was noted. In 250-mL FEP centrifuge bottles, the air-dried sludge samples were extracted for the determination of estrogens, caffeine, and triclosan by 30 mL of acetonitrile. The EA was also used as an internal standard for QA/QC purpose. The samples were then agitated on an orbital shaker for 2 hours and

centrifuged for 10 min (4,000 rpm). The supernatant was collected, evaporated to about 500 μL under nitrogen stream, and made up to 3 mL with acetonitrile.

Sludge supernatants collected were analyzed to determine target PPCPs by using HPLC/UV. A portion of samples (10%) were derivatized for the qualitative confirmatory analyses using GC-MS. The recovery and detection limit of sludge extraction methods are shown in Tables 2 and 3, respectively.

Groundwater. Groundwater samples were first filtered through a 10-cm P5 filter paper to remove suspended solids. The extraction of PPCPs was performed by the same procedures as described in wastewater samples extraction. The 500 mL of groundwater sample was passed through C18 SPE cartridge which was conditioned with 3 mL of acetonitrile followed by 3 mL of water. Samples were then eluted with 3×1 mL of acetonitrile, evaporated to about 100 μL under nitrogen stream, made up to 1 mL by acetonitrile, and analyzed by using HPLC/UV. Detection limits of these methods based on the U.S. EPA guidelines are presented in Table 3.

Soil. Each soil core sample was subdivided into five 6-inch segments. Only soil samples at the depths of 0-6 inch, 12-18 inch, and 24-30 inch were applied to determine the concentration of target PPCPs. Each sample was air dried and mixed well for homogeneity. The extraction of PPCPs was done by the same procedures as those for sludge samples. 30 g of soil was extracted in a 250-mL FEP centrifuge bottle with 30 mL of acetonitrile for the extraction of E1, E2, EE2, and triclosan, and with 30 mL of 3:1 acetonitrile:water (v/v) for the extraction of E3 and caffeine. EA was also used as an internal standard for QA/QC purpose. Then, samples were agitated on an orbital shaker for 2 hours and centrifuged for 10 min (4,000 rpm). The supernatant was collected, evaporated to about 500 μL under nitrogen stream, and made up to 3 mL with acetonitrile.

Soil supernatants collected were analyzed to determine target PPCPs by using HPLC/UV. A portion of samples (10%) were derivatized for the qualitative confirmatory analyses using GC-MS. The recovery and detection limit of sludge extraction methods are shown in Tables 2 and 3, respectively.

Derivatization.

Prior to GC-MS determination, samples were derivatized using N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA) following methods of Ternes et al. (2002) and the U.S. EPA Method 1698. Derivatized samples were analyzed by GC-MS in the selected ion monitoring (SIM) mode using the respective parent and 1-2 daughter ions for each compound.

Table 2 The recovery obtained from extraction methods and HPLC/UV analysis applied for PPCPs in different types of matrices (n=3).

Compound	Recovery (%)			
	Milli-Q water[a]	Wastewater[a]	Sludge[b]	Soil[b]
E1	105.9 ± 1.1	102.5 ± 5.7	51.1 ± 3.2	98.5 ± 0.2
E2	109.8 ± 7.9	106.0 ± 0.5	38.9 ± 4.2	94.7 ± 1.7
E3	104.9 ± 3.9	105.7 ± 2.7	38.0 ± 0.4	103.7 ± 1.4
EE2	114.4 ± 5.1	106.3 ± 5.4	45.5 ± 6.6	99.8 ± 0.9
EA	90.7 ± 11.9	105.8 ± 3.3	28.3 ± 3.8	98.7 ± 1.6
Caffeine	84.0 ± 5.1	101.8 ± 4.1	72.1 ± 2.5	90.6 ± 0.7
Triclosan	82.9 ± 1.0	79.1 ± 0.4	79.6 ± 4.7	93.0 ± 1.0

[a] Prepared from spiking each compound at 100 µg/L into sample.
[b] Prepared from spiking each compound at 0.1 µg/g dry weight into sample.

Table 3 Calculated detection limits for target PPCPs obtained from HPLC/UV analysis of spiked samples.

Compound	Method detection limit*			
	Wastewater (µg/L)	Groundwater (ng/L)	Sludge (ng/g dry weight)	Soil (ng/g dry weight)
E1	0.12	8.08	6.42	0.96
E2	0.12	3.19	6.42	0.96
E3	0.12	17.73	6.42	0.40
EE2	0.12	4.78	6.42	0.96
EA	0.12	17.34	6.42	0.96
Caffeine	0.10	9.95	6.39	0.30
Triclosan	0.12	14.09	5.87	1.04

*Determined using U.S.EPA guideline (2000) where MDL = SD × t (99%; n-1) and assuming 1L of water and 1g of sludge/soil were extracted.

Instrumental Analysis

HPLC. The HPLC with UV detection was used for the determination of target PPCPs. An Alltech Prevail C18 column (25 cm × 4.6 mm i.d., 5 µm) was used for PPCPs separation. Mobile phase characteristics varied depending on the analyte of interest. For estrogens, the mobile phase was acetonitrile:water (gradient, flow rate = 0.8 mL/min) which was set at 60:40 (v/v) initially. The mobile phase was changed to 65:35 at 1.0 min, and to 100% acetonitrile at 11.5 min. Then, the mobile phase was maintained at 100% acetonitrile until 15.0 min, changed to 60:40 at 15.5 min, and maintained at 60:40 until 21.0 min. For caffeine separation, the mobile phase was 50:50 acetonitrile:water (isocratic; flow rate = 0.8 mL/min). Triclosan was chromatographed using a mobile phase containing acetonitrile:water (isocratic; 80:20 v/v; flow rate = 0.8 mL min^{-1}). Detection wavelengths were at 200 nm for estrogens and triclosan, and 254 nm for caffeine.

Principal Findings

PPCPs in Wastewater

The concentrations of target PPCPs in wastewater samples collected from the LWRP are presented in Table 4. All target PPCPs were detected in the process at a range of not detected (ND) -126.53 μg/L with the observed concentrations fluctuated among quarters. This fluctuation in PPCPs concentrations may be attributed to the use of PPCPs that likely varied daily, let alone quarterly. In general, concentrations of PPCPs in effluents at both stations were less than those in influents from bar rack or aeration basin in the same quarter except for EE2 in the first and the third quarters. This indicated that target PPCPs can be removed during the treatment process. Among PPCPs studied, E3 had the highest concentrations in the effluent from both chlorine contact chamber and effluent stations at a range of ND-86.71μg/L. In some quarters, E1, E2, E3, and EE2 appeared to have lower concentrations in samples collected from bar rack than those collected from aeration basins, chlorine contact chamber, or effluent stations. This indicated that these compounds may not be easily degraded, or the inactive conjugates of estrogens may be deconjugated during the wastewater treatment process resulting in the release of the active parent compounds that produce higher effluent concentrations. Another possible reason of the higher concentrations of PPCPs in effluents than those of their input may be the daily variations of these compounds in the inlet since influent samples at bar rack were collected between 2 pm and 4 pm, which might not be during the peak load. Although wastewater samples at bar rack were collected at the same period as other wastewater sampling points, samples at other points were proportional samples in 24 hours, in which their concentrations may be affected by the previous load.

There was the difference of PPCPs concentrations between plant 3 and plant 4, specifically both between aeration basins and between effluent stations of each plant. In addition, in some quarters, concentrations of PPCPs in effluents from effluent station were higher than those collected from chlorine contact chamber of the same plant. The reason may be explained by the fact that samples collected were proportional samples in 24-hour flow and conditions between these two basins or effluent stations were not exactly the same. At detectable concentrations, E2, triclosan, and caffeine were detected in effluents at lower concentrations than in the influent during the entire study period, suggesting that these compounds can be removed efficiently from wastewater by the LWRP.

In general, there is no wastewater treatment process particularly responsible for the removal of PPCPs. However, several studies indicated that these compounds can be reduced or eliminated in biological wastewater treatment systems using the activated sludge (aeration basin) process where sorption to particles and biotransformation are potential mechanisms of PPCPs removal (Sedlak and Pinkston 2001; Giger et al. 2003; Andersen et al. 2005; Bester 2005; Thomas and Foster 2005; Thompson et al. 2005; Nakada et al. 2006; Kim et al. 2008). Some of the PPCPs in this study were probably also removed from wastewater via chlorination. Snyder et al. (2008) suggested that a majority of PPCPs in wastewaters such as estrogens and triclosan can be effectively oxidized using chlorination. In our study, data obtained can be supported by Snyder et al. (2008) since concentrations of PPCPs in the wastewater collected from bar rack were generally higher than in the samples collected from aeration basins, chlorine contact chamber, and

effluent stations. However, because the conjugated form of estrogens may be deconjugated by microbial processes without further degradation effluent, concentrations of estrogens can be higher than those in the corresponding influent samples (Kirk et al. 2002; Andersen et al. 2003; D'Ascenzo et al. 2003).

PPCPs in Sludge

The concentrations of target PPCPs in sludge samples collected from the LWRP are presented in Table 5. All target PPCPs were detected in solid phase of sludge at a range of ND-18.62 μg/g. In sludge liquid phase, target PPCPs were detected at a range of ND-50.14 μg/L except for EE2, which was not detected during the entire study period. Concentrations of estrogens in sludge solid phase might be underestimated due to the low recovery of these compounds (<51.1± 3.2%). In sludge solid phase, target PPCPs in samples from anaerobic digester generally had less concentration than those from feed sludge chamber, except for E1 in the second quarter, E2 in the second and the third quarters, E3 in the fourth quarter, and triclosan in the third quarter. The less PPCPs concentration in the solid phase of digested sludge may be due to the desorption of PPCPs from the solid phase into the liquid phase. In sludge liquid phase, EE2 was the only compound that was not detected from both feed sludge chamber and anaerobic digester during the entire study period, while caffeine was not detected in any samples from anaerobic digester. For other compounds in sludge liquid phase, compared to those in anaerobic digested sludge, the tendency of PPCPs concentrations in feed sludge was hard to predict. In some quarters, PPCPs had less concentration in sludge liquid phase from feed sludge than those from anaerobic digester, but this fashion did not occur in other quarters. E1, E2, E3, and triclosan were detected in sludge liquid phase from either feed sludge or anaerobic digester, except for some quarters in which they were not detectable in both sampling points. From the result explained earlier, the unpredictable concentrations in sludge liquid phase along the treatment train (from feed sludge chamber to anaerobic digester) together with the fluctuated amount of sorbed PPCPs in sludge solid phase of same samples indicated the slow sorption kinetics. No equilibrium may occur between the sorbed and dissolved PPCPs in sludge during the treatment system at the LWRP.

Sorption to sludge is considered to be an important mechanism for the removal of hydrophobic organic chemicals from wastewater (Harrison et al. 2006). Therefore, it is necessary to know the phase of sludge at which PPCPs may present, including their concentrations at the phase. Among estrogens studied, E3 had the lowest octanol-water partition coefficient (log K_{ow}) with high water solubility (Table 1); therefore, it had less tendency to sorb into sludge particles. This explained our findings that compared to other estrogens E3 had generally higher concentrations in the effluent from wastewater treatment plant (Table 4) and sludge liquid phase (Table 5). EE2 had a high log K_{ow} with the lowest water solubility among the studied estrogens and could have a high tendency to sorb into sludge. However, it was rarely detected in both solid and liquid phase of sludge in this study. This may be due to the consequences of non-detectable or very low concentrations of EE2 presented during the wastewater treatment, which was the input of sludge treatment. E1 and E2 were detected in both phases of sludge more frequently than other estrogens. This indicated that these compounds readily sorbed or desorbed in sludge, probably due to a high value of Log K_{ow} with high water solubility of E1, and a

moderate value of Log K_{ow} with high water solubility of E2 compared to other estrogens. The lower concentrations of estrogens in the anaerobic digester may also be the result of biodegradation during sludge treatment process. Studies also reported that estrogens can be biodegraded during anaerobic sludge digestion (Holbrook et al. 2002; Kreuzinger et al. 2004a).

Among all PPCPs, triclosan had the highest log K_{ow} at 4.76 with the low water solubility at 10 mg/L. Although triclosan was detected at moderate concentrations in wastewater samples compared to other compounds, it was the only compound detected in all sludge samples both in solid and liquid phases. This finding indicated that triclosan may readily sorb into sludge solid phase, but may not be easily biodegraded in anaerobic digester. Our results can be also supported by other studies reporting that only little or no biodegradation of triclosan occurred under anaerobic sludge digestion (McAvoy et al. 2002; Chenxi et al. 2008). Compared to other PPCPs, caffeine had a very high water solubility at 2.16×10^4 mg/L with the lowest log K_{ow} at -0.07. Therefore, caffeine was not likely to sorb to sludge and it was rarely detected in sludge solid phase. Studies also reported that caffeine may be readily biodegraded during wastewater treatment system (Ternes et al. 2001; Buerge et al. 2003; Thomas and Foster 2005). The biodegradation of caffeine may result in the lower or non-detectable concentrations in both solid phase and liquid phases of sludge along the treatment process.

PPCPs in Soil

The concentrations of target PPCPs in soil samples collected from the LWRP are presented in Table 6. PPCPs can be detected at the range of ND- 34.52 ng/g in the soil inside pivot irrigation (CL-11 and CL-43) and ND-135.92 ng/g in the soil outside pivot irrigation (CL-29 and CL-48). Except for caffeine, target PPCPs were detected in both inside and outside pivot areas indicating that PPCPs may transport via runoff. Among PPCPs studied, caffeine was the only compound which was not detected in all soil samples. The observed concentrations of other target PPCPs in soil fluctuated among quarters, but were unpredictable among soil depths. EE2 was the only compound which was not detected in any samples at the depth of 24-30 inch although it was detected at the upper soil depths at the same sampling point. This indicated that EE2 may not be easily leached through the soil and hence, the vertical transport of EE2 might be low.

The fluctuated concentrations of PPCPs in soil among quarters were more likely due to the application of various concentrations of PPCPs in the effluent on the site. The PPCPs to the land through the application of irrigation were subject to volatilization at soil surfaces and vegetation, chemical and biological degradation, sorption by soil organic matter, and plant uptake (Cordy et al. 2004; Cardoza et al. 2005; Boxall 2008; Xu et al. 2009). These factors also affected the concentrations of PPCPs in the soil. In the soil environment, while the sorption is considered as an important process governing the mobility of organic compounds including PPCPs (Drillia et al. 2005, Boxall 2008), the volatilization and degradation processes govern the elimination of these compounds from the soil. In this study, E1, E2, E3, and triclosan were detected in the soil sampled at the depth of 24-30 inch indicating that these compounds were mobile and persistent enough to undergo percolation through the soil. However, the extent of concentrations of each compound was variable among soil depths. In this study, the tendency of PPCPs concentrations along soil depth was hard to be predicted or generalized. This is because

of the consequences of various biodegradation rates of PPCPs along soil depth as degradation of PPCPs can be affected by environmental complexities and conditions such as soil temperature, pH, moisture content, soil organic carbon, presence of specific microorganisms, and presence/absence of oxygen (Colucci et al. 2001; Boxall 2008; Monteiro and Boxall 2009). For instance, Ying and Kookana (2005) found that the degradation of E2 and EE2 were different in non-sterile aerobic soil with half-lives at 3 and 4.5 days, respectively, but no degradation of both compounds occurred in the sterile soil within 70 days. In anaerobic soil, E2 was degraded slowly with a half-life of 24 days, while no significant degradation of EE2 was observed within 70 days. Hence, studies suggested that the degradation, which affects the concentration of PPCPs in soil, was influenced by the presence of microorganisms and oxygen that could vary along soil depths. In this study, the volatilization was not likely to be a pathway of PPCPs elimination in soil since all target PPCPs had very low vapor pressures ($\leq 1.41 \times 10^{-7}$ mm Hg) except for caffeine. Caffeine had the highest vapor pressure (15 mm Hg) among target PPCPs and the biodegradation of caffeine in soil could occur rapidly both in aerobic and anaerobic conditions (Topp et al. 2006), which might explain the reasons why caffeine was not detectable in all soil depths in the study.

Among estrogens studied, E1 was detected in soil at the LLAS more frequently compared to other estrogens, and was detected at the highest concentrations although it presented at the same level as E2 and at less concentration than E3 in the effluent applied from the LWRP. Several studies reported that E2 was biotransformed to E1 rapidly under both aerobic and anaerobic soil conditions (Colucci et al. 2001; Jacobsen et al. 2005; Ying and Kookana 2005; Xuan et al. 2008). The high concentrations of E1 in all soil depths in our study might be caused by biotransformation of E2 into E1. For E3, it was detected in the soil at concentrations lower than E1 although it presented at higher concentrations than E1 in the effluent from the LWRP which was applied to the land. This might be attributed to a higher mobility of E3 compared to that of E1 in the soil as E3 had lower log K_{ow} and K_d than those of E1, resulting in less concentration of E3 sorbed into the soil.

PPCPs in Groundwater

The concentrations of target PPCPs in groundwater samples collected from the LLAS are presented in Table 7. Concentrations of PPCPs in groundwater were in the range of ND-1,744.62 ng/L. All target PPCPs can be detected in groundwater samples in both inside and outside pivot areas indicating that these compounds can move via runoff, which eventually leach or percolate through the soil to groundwater. Compared to other compounds, E3 was detected in groundwater at the highest concentrations except for the last quarter in which E3 was not detectable. This suggested that E3 had a higher mobility in the soil than other compounds since it was the most compound detected in groundwater but not much detected in soil. In the third quarter (late summer), most PPCPs studied were non-detectable except for EE2 and caffeine that were detected at low concentrations, i.e., 10.87 and 16.03 ng/L, respectively. This can be a result of high degradation rates of PPCPs in soil and groundwater that may occur during summer. Although caffeine was not detected in any soil samples, it was detected in some effluent and groundwater samples. This suggested that caffeine did not readily sorb to the soil or had a higher degradation rate than other target PPCPs in the subsurface environment.

PPCPs contaminated in groundwater may be originated from these compounds persisted in the soil. Since the field runoff and subsurface transport are the important processes for the movement of PPCPs and other organic compounds from soil to groundwater (Mansell and Drewes 2004; Overcash et al. 2005; Sangsupan et al. 2006). PPCPs in the soil at the site applied with the effluent may transport to groundwater through these processes. The extent of PPCPs in groundwater can be affected by sorption and biodegradation of these compounds during the soil-aquifer treatment (Kreuzinger et al. 2004b; Mansell et al. 2004; Snyder et al. 2004; Osenbrück et al. 2007). Sorption is considered as an important process governing mobility and transport of hydrophobic organic compounds in the soil-water environment (Lai et al. 2000; Cardoza et al. 2004; Casey et al. 2005; Mansell et al. 2004; Oppel et al., 2004; Scheytt et al. 2005; Sangsupan et al. 2006). Therefore, the tendency of PPCPs to persist in soil or remobilize to groundwater may be indicated by log K_{ow} and sorption coefficient (K_d) of these compounds. The less the coefficient, the more the tendency of PPCPs to move from soil into groundwater. Among PPCPs studied, E3 was detected at the highest concentrations in groundwater. This may be due to a low K_d of E3 at 8.6 mL/g compared to other target PPCPs. Caffeine was detected in groundwater samples, whereas it was not detected in any soil samples. This may be caused by a very low log K_{ow} at -0.07 and a high water solubility of caffeine.

Significance

PPCPs can be detected in wastewater, sludge, soil, and groundwater at the LWRP and LLAS. PPCPs can be removed from wastewater during the treatment process with aeration basin (activated sludge); however, E3 and EE2 can be occasionally detected in the effluent at higher concentrations than in the influent. All PPCPs studied can be detected in both sludge solid phase and sludge liquid phases except for EE2 which was not detected in sludge liquid phase. Regardless of season, concentrations of PPCPs in wastewater, sludge, and at each soil depth (0-6 inch, 12-18 inch, and 24-30 inch) varied with an unpredictable extent. Only groundwater tended to have less occurrence of PPCPs during summer. PPCPs had both vertical and horizontal (via runoff) transports at the study sites, which were detected along soil depth, and in soil and groundwater both inside and outside pivot irrigation, respectively. Caffeine was detected in effluent and groundwater, but not detected in soil, suggesting that caffeine may not readily sorb to the soil or degradation rate of caffeine was high in the soil during the study period. E3 was the most compound detected in groundwater, but not the most detected in soil, indicating that E3 may have a higher mobility in the soil than other target PPCPs. Overall, findings of this study indicated that PPCPs in the effluent from the wastewater treatment plant was eventually transported to groundwater via the land application of effluent, which is essentially an important concern for the possible long-term effects due to the contamination of PPCPs in the groundwater if it is used for drinking-water purposes. The result presented in this study may provide useful information for the wastewater treatment system to be upgraded or for other effective measures to be adopted to reduce these PPCP concentrations in soil at the LLAS.

Table 4 Concentrations (µg/L) of PPCPs in wastewater

Compound	Date	Bar rack	Aeration basin		Chlorine Contact Chamber	Effluent	
			Plant 3	Plant 4		Station I	Station II
E1	12/16/08	3.25	3.18	3.67	*	1.43	1.82
	3/11/09	1.47	0.74	1.63	0.42	0.49	0.59
	6/3/09	ND	0.22	2.82	1.54	0.33	0.48
	9/9/09	1.29	ND	ND	ND	ND	ND
E2	12/16/08	4.62	1.30	1.38	*	ND	1.37
	3/11/09	2.29	ND	1.12	0.36	0.50	ND
	6/3/09	0.90	1.66	1.58	0.80	0.26	0.75
	9/9/09	1.58	0.73	0.50	ND	ND	0.67
E3	12/16/08	0.68	7.45	13.97	*	0.25	7.60
	3/11/09	110.71	126.53	29.19	86.71	83.43	59.23
	6/3/09	ND	37.51	7.29	7.66	13.08	2.76
	9/9/09	25.71	ND	ND	ND	ND	ND
EE2	12/16/08	ND	ND	ND	*	0.08	0.39
	3/11/09	7.89	ND	ND	0.16	ND	ND
	6/3/09	ND	ND	0.12	0.81	0.26	ND
	9/9/09	ND	ND	ND	ND	ND	ND
Triclosan	12/16/08	5.10	0.12	ND	*	0.26	0.13
	3/11/09	8.12	ND	0.77	ND	ND	ND
	6/3/09	1.90	0.44	ND	0.14	0.15	0.17
	9/9/09	0.70	ND	ND	1.41	0.18	0.35
Caffeine	12/16/08	23.60	ND	ND	*	N	ND
	3/11/09	41.04	0.35	ND	0.12	0.17	0.34
	6/3/09	45.48	5.35	ND	ND	ND	ND
	9/9/09	53.43	ND	ND	ND	ND	ND

*No sample
ND = Not detectable

Table 5 Concentrations of PPCPs in sludge

Compound	Date	Feed sludge chamber		Anaerobic digester	
		Solid phase (μg/g)	Liquid phase (μg/L)	Solid phase (μg/g)	Liquid phase (μg/L)
E1	12/16/08	3.27	39.87	1.60	28.17
	3/11/09	2.40	0.42	2.52	2.60
	6/3/09	0.70	3.22	ND	50.14
	9/9/09	6.59	14.12	1.16	11.20
E2	12/16/08	0.70	1.54	0.04	0.48
	3/11/09	2.23	2.84	1.50	3.47
	6/3/09	0.13	ND	0.22	ND
	9/9/09	ND	18.33	0.12	9.44
E3	12/16/08	ND	3.55	ND	3.49
	3/11/09	ND	46.50	ND	16.38
	6/3/09	0.01	2.76	0.01	0.61
	9/9/09	ND	ND	0.01	ND
EE2	12/16/08	0.34	ND	0.19	ND
	3/11/09	ND	ND	ND	ND
	6/3/09	ND	ND	ND	ND
	9/9/09	ND	ND	ND	ND
Triclosan	12/16/08	7.79	6.98	3.35	12.11
	3/11/09	3.52	2.84	3.39	3.73
	6/3/09	4.70	1.00	5.67	3.47
	9/9/09	18.62	11.59	2.48	4.22
Caffeine	12/16/08	ND	0.53	ND	ND
	3/11/09	0.02	ND	0.01	ND
	6/3/09	ND	ND	ND	ND
	9/9/09	ND	24.85	ND	ND

ND = Not detectable

Table 6 Concentrations (ng/g) of PPCPs in soil

Compound	Sampling Date	CL-11[a]			CL-29[b]			CL-43[a]			CL-48[b]		
		0-6"	12-18"	24-30"	0-6"	12-18"	24-30"	0-6"	12-18"	24-30"	0-6"	12-18"	24-30"
E1	3/9/09	*	*	*	7.55	10.04	8.52	7.82	34.52	20.61	5.30	8.68	6.73
	6/30/09	9.62	3.45	ND	9.53	7.33	4.96	3.28	9.59	20.83	4.60	7.63	6.71
	9/16/09	ND	ND	ND	ND	44.06	135.92	ND	ND	ND	2.03	ND	ND
E2	3/9/09	*	*	*	ND	ND	1.20	0.19	ND	3.33	0.17	0.58	0.65
	6/30/09	2.09	2.41	ND	ND	ND	ND	1.71	ND	ND	1.86	ND	ND
	9/16/09	2.84	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
E3	3/9/09	*	*	*	4.07	ND	ND	7.73	ND	ND	ND	ND	ND
	6/30/09	ND	1.01	ND	2.22	1.18	ND	1.63	3.14	1.20	2.98	1.00	1.08
	9/16/09	ND	ND	0.53	0.46	3.60	5.98	2.10	0.85	0.76	0.98	ND	ND
EE2	3/9/09	*	*	*	ND	ND	ND	ND	ND	ND	ND	ND	ND
	6/30/09	1.21	1.26	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	9/16/09	ND	2.62	ND	2.03	2.70	ND	ND	ND	ND	ND	ND	ND
Triclosan	3/9/09	*	*	*	5.24	3.20	ND	ND	ND	ND	ND	ND	ND
	6/30/09	ND	2.91	ND	8.16	1.24	1.09	1.94	1.33	7.81	ND	ND	ND
	9/16/09	ND	ND	ND	ND	ND	ND	19.15	ND	ND	ND	ND	ND
Caffeine	3/9/09	*	*	*	ND	ND	ND	ND	ND	ND	ND	ND	ND
	6/30/09	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	9/16/09	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

* No sample, ND = Not detectable, [a] inside pivot irrigation, [b] outside pivot irrigation

Table 7 Concentrations (ng/L) of PPCPs in groundwater

Compound	Sampling Date	CL-11[a]	CL-29[b]	CL-43[a]	CL-48[b]
E1	3/9/09	*	79.15	75.15	61.74
	6/30/09	ND	ND	ND	ND
	9/16/09	ND	ND	ND	ND
E2	3/9/09	*	12.16	146.54	34.30
	6/30/09	39.40	ND	ND	77.51
	9/16/09	ND	ND	ND	ND
E3	3/9/09	*	1744.62	874.16	538.32
	6/30/09	685.68	321.83	1660.75	675.96
	9/16/09	ND	ND	ND	ND
EE2	3/9/09	*	ND	230.32	101.66
	6/30/09	14.51	ND	ND	ND
	9/16/09	ND	ND	ND	10.87
Triclosan	3/9/09	*	16.69	15.74	11.57
	6/30/09	ND	44.73	53.27	ND
	9/16/09	ND	ND	ND	ND
Caffeine	3/9/09	*	118.57	166.17	163.52
	6/30/09	ND	ND	ND	ND
	9/16/09	16.03	ND	ND	ND
	9/16/09	ND	ND	ND	ND

* No sample
ND = Not detectable
[a] inside pivot irrigation
[b] outside pivot irrigation

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