Concentration of Selected Endocrine Disrupting Chemicals and Pharmaceutical and Personal Care Products Entering Wastewater Treatment Plants in South East Queensland

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The Urban Water Security Research Alliance (UWSRA) is a $50 million partnership over five years between the Queensland Government, CSIRO's Water for a Healthy Country Flagship, Griffith University and The University of Queensland. The Alliance has been formed to address South East Queensland's emerging urban water issues with a focus on water security and recycling. The program will bring new research capacity to South East Queensland tailored to tackling existing and anticipated future issues to inform the implementation of the Water Strategy.

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Description: Treated Effluent from Luggage Point Wastewater Treatment Plant
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FOREWORD

Water is fundamental to our quality of life, to economic growth and to the environment. With its booming economy and growing population, Australia's South East Queensland (SEQ) region faces increasing pressure on its water resources. These pressures are compounded by the impact of climate variability and accelerating climate change.

The Urban Water Security Research Alliance, through targeted, multidisciplinary research initiatives, has been formed to address the region's emerging urban water issues.

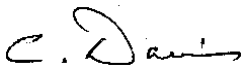
As the largest regionally focused urban water research program in Australia, the Alliance is focused on water security and recycling, but will align research where appropriate with other water research programs such as those of other SEQ water agencies, CSIRO's Water for a Healthy Country National Research Flagship, Water Quality Research Australia, eWater CRC and the Water Services Association of Australia (WSAA).

The Alliance is a partnership between the Queensland Government, CSIRO's Water for a Healthy Country National Research Flagship, The University of Queensland and Griffith University. It brings new research capacity to SEQ, tailored to tackling existing and anticipated future risks, assumptions and uncertainties facing water supply strategy. It is a $50 million partnership over five years.

Alliance research is examining fundamental issues necessary to deliver the region's water needs, including:

- ensuring the reliability and safety of recycled water systems.
- advising on infrastructure and technology for the recycling of wastewater and stormwater.
- building scientific knowledge into the management of health and safety risks in the water supply system.
- increasing community confidence in the future of water supply.

This report is part of a series summarising the output from the Urban Water Security Research Alliance. All reports and additional information about the Alliance can be found at http://www.urbanwateralliance.org.au/about.html.



Chris Davis
Chair, Urban Water Security Research Alliance

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LIST OF ABBREVIATIONS

BAC	Benzalkonium chloride
BPA	Bisphenol A
CAF	Caffeine
CBZ	Carbamazepine
COT	Cotinine
DNA	Deoxyribonucleic acid
E1	Estrone
E2	17β-estradiol
EE2	17β-ethynylestradiol
EDC	Endocrine disrupting chemical
GC-MS	Gas chromatography combined with mass spectrometry
GC-MS/MS	Gas chromatography combined with tandem mass spectrometry
IBU	Ibuprofen
LC-MS/MS	Liquid chromatography combined with tandem mass spectrometry
LLOQ	Lower limit of quantification
LOQ	Limit of quantification
MRM	Multiple reaction monitoring
NP	Nonylphenol
NPEO	Nonylphenol ethoxylate
OP	Octylphenol
PPPC	Pharmaceutical and personal care product
SEQ	South East Queensland
SIM	Selected ion monitoring
SNRI	Serotonin-norepinephrine re-uptake inhibitor
SPE	Solid phase extraction
STP	Sewage treatment plant
TCS	Triclosan
ULOQ	Upper limit of quantification
UV	Ultraviolet
WWTP	Wastewater treatment plant

EXECUTIVE SUMMARY

Background

- A routine sampling programme of wastewater treatment plants (WWTPs) was undertaken beginning in June 2008 to establish: (i) the removal efficiency of selected organic contaminants in two treatment plants; and (ii) to quantify the range of concentrations of different micropollutants discharged from the treatment plants in the treated effluents.
- A number of selected compounds, including selected number of endocrine disrupting chemicals (EDCs), pharmaceuticals and personal care products (PPCPs) were monitored in two different WWTPs.
- The WWTPs selected were based on their respective sources: Oxley Creek WWTP receives wastewater predominantly of a domestic nature, while Luggage Point receives a mixture of both domestic and industrial wastewater.
- In a parallel study, samples were taken from different stages of treatment at Luggage Point to understand the removal of micropollutants through the treatment train.

Key Findings

Influent and Effluent Concentrations of Endocrine Disrupting Chemicals

- The median concentrations of 17β-estradiol (E2) and Estrone (E1) in the influents and effluents showed about 90% removal of the two compounds. The concentration of the synthetic hormone EE2 was below the method limit of quantification (LOQ) (5 ng/L) in all the samples.
- The concentrations of E2 were higher than previously reported. The reasons are unclear, but there is growing awareness that gas chromatography combined with mass spectrometry (GC-MS) based methods can overestimate the E2 levels. Future studies should employ gas chromatography combined with tandem mass spectrometry (GC-MS/MS) methods.
- Despites its high and variable concentrations in the influent, nonylphenol (NP) was effectively removed (about 99%) during the treatment process.
- The concentrations of triclosan (TCS) and bisphenol A (BPA) in the influents and effluents demonstrated 93-95% removal of the two compounds during the treatment process.

Influent and Effluent Concentrations of Pharmaceuticals

- Ibuprofen (IBU) and caffeine (CAF) were present in relatively high concentrations in the influent (low µg/L range). However, both of these compounds were efficiently removed (> 94%) during the treatment.
- Conversely, carbamazepine (CBZ) concentrations (in low hundreds of ng/L) remained constant during the treatment process and no difference between the concentrations of CBZ in the influent and effluent streams were noted.
- The above findings are consistent with previous studies that have found high removal rates of CAF and IBU, whereas CBZ is highly resistant to removal during passage through WWTPs.

Overall Removal Efficiencies of the Treatment

- The intensive study on the assessment of the efficiency of different steps of treatment at Luggage Point in removal of 11 micropollutants showed that the aerobic steps were more effective in removal of these organic compounds than the anaerobic steps.
- We did not observe significant impact due to UV treatment on the concentrations of any of the compounds measured in the effluents at Oxley Creek.

- There was less than 5% variation in the removal efficiencies between the two treatment plants for most compounds.
- Most tested compounds were removed by > 95% during the treatment process. The removal efficiency of the two treatment plants for various compounds can be summarised as follows:
  - **> 95% removal**: caffeine, nonylphenol, nonylphenol diethoxylate, triclosan,
  - **> 90% removal**: bisphenol A, ibuprofen,
  - **> 80% removal**: 17β-estradiol (E2), estrone (E1), 4t-octylphenol (OP)
  - **< 80% removal:** nonylphenol monoethoxylate (includes production from higher chain compounds during breakdown).

Unexpected Contaminants Found

- During the monitoring study a few unexpected contaminants (or at least those that were not targeted) were picked up. Perhaps most significant among these is the benzalkonium chloride (BAC), which is a cationic surfactant compound used as a biocide in hospitals, laundries and possibly in other applications. The concentration of the compound in the influent stream was high (4000-17000 ng/L).
- Despite the high degree (> 95%) of removal of BAC during the treatment process, the concentrations in the effluents were mostly > 100 ng/L and occasionally even > 1000 ng/L. These levels are in line with other surveys of WWTPs and receiving waters in the USA and Europe.
- Other potential compounds of interest that were detected in both influent and effluent samples include the azole fungicide, propiconazole, and the serotonin-norepinephrine re-uptake inhibitor (SNRI) antidepressant, venlafaxine.
- Azole fungicides can be used as chemotherapy agents as one of their modes of action is to reduce estrogen levels by inhibiting aromatase (Zarn et al., 2003), indicating they have the potential to act as an endocrine disruptor.
- Venlafaxine has not been well studied but is reported to be poorly removed during wastewater treatment and has been detected in effluent and streams affected by effluents at concentrations > 2000 ng/L.
- Not all the above contaminants were targeted in the study, so the analytical results are subject to further optimisation of analytical methods using proper internal standards and recovery tests.

1. INTRODUCTION

Recently there has been an increasing number of new and emerging organic contaminants that have been detected in treated effluents from wastewater treatment plants (WWTPs). Similarly there has also been heightened public concern over the entry of such chemicals into aquatic environments including rivers, lakes and streams which supply input water for drinking water treatment plants. Endocrine disrupting chemicals (EDCs) and pharmaceutical and personal care products (PPCPs) have received particular attention in recent times due to some compelling evidence suggesting undesirable environmental or human health effects. Some of the potential contaminants include: (i) steroid hormones and pharmaceuticals and their metabolites (e.g. 17β-estradiol, estriol, estrone, 17α-ethynylestradiol and diethylstilbestrol); (ii) domestic and industrial detergent residues and degradates (e.g. linear alkyl benzene sulphonates (LAS) and nonylphenol ethoxylates (NPEOs); and (iii) widely used antibacterial agents such as triclosan (TCS).

The present study is a survey of selected WWTPs in Brisbane aimed at monitoring some of these EDCs and PPCPs. A routine sampling programme of two WWTPs was undertaken beginning in June 2008. Samples of water streams both entering the WWTP (influent) and leaving the WWTP (effluent) were taken. A number of selected compounds, including selected number of EDCs, and PPCPs were monitored in two different WWTPs. The WWTPs selected were based on their respective sources: Oxley Creek WWTP receives wastewater predominantly of a domestic nature, while Luggage Point receives a mixture of both domestic and industrial wastewater.

Briefly, triplicate samples were collected from both influent and effluent streams mid-morning at the respective WWTPs. Grab samples were collected in clean 1 L amber glass bottles, along with field blanks (1 L ultrapure water treated identically to samples to assess potential contamination). Samples were transported to the laboratory on ice and stored in the dark in a cold room maintained at +4°C, spiked with stable isotopic analogues of the target analytes and loaded onto Waters HLB solid phase extraction (SPE) cartridges. SPE cartridges were then eluted and split into samples to be prepared for analysis using high pressure liquid chromatography combined with tandem mass spectrometry (LC-MS/MS) and gas chromatography combined with mass spectrometry (GC-MS).

The target analytes listed in Appendix 2, Tables A2 and A3 include a number of EDCs and PPCPs. Stable isotopes used as surrogates for assessment of recovery in wastewater samples are also listed in Table 1. Analytical methods were also developed for a number of other pharmaceuticals, cytotoxic chemicals, pesticides and EDCs (*see* Appendix 2 Table A3) to expand the capability of understanding potential contaminants entering the wastewater stream. These methods were developed with both LC-MS/MS and a recently acquired GC-MS/MS at CSIRO's Adelaide Laboratory, although, due to surrogates not being identified for these additional compounds, only qualitative assessment of their presence in influent and effluent can be made with the available samples.

For the purpose of this report, only a snapshot of data collected from the initial sampling periods have been presented here. The detection of a non-target class of some new and emerging chemicals not previously reported in Australia has been briefly discussed in this report as a significant finding from this survey. The analytical procedures have been described in detailed in Appendix 1 at the end of this document.

2. RESULTS AND DISCUSSION

2.1. Concentrations of EDCs and Triclosan

Figure 1 shows the concentration of selected EDCs and PPCPs measured from the initial sampling at different treatment stages of Luggage Point in June 2008. The compounds included: estrogens (estradiol (E2) and estrone (E1) and ethynylestradiol (EE2)); 4t-octylphenol (OP), 4-nonylphenol (NP), and nonylphenol mono- and di-ethoxylates (NPE1-2); bisphenol A (BPA); and triclosan (TCS). The mean influent concentration of the surfactants ranged from 148±8 ng/L for OP up to 7323±2020 ng/L for NP. The concentrations of NPE1 and NPE2 were measured at 2996±249 and 2420±491 ng/L respectively. Gradual removal of all these compounds was observed down the treatment process. The overall effluent concentrations of the compounds indicated that greater than 99% of the most potent estrogenic surfactant by-product NP was removed.

The same trend was observed for the estrogens E2 and E1, TCS and BPA. The mean concentrations of E2 and E1 in the influents were 106.6±7.1 and 83.5±19.5 ng/L respectively, whereas the concentrations of TCS and BPA in the influent were measured at 603.4±130 and 261.2±4.5 ng/L respectively. Again, there was greater than 90% removal of all the compounds based on the effluent concentrations of the estrogens. The concentration of the synthetic hormone EE2 was below the method LOQ (5 ng/L) in all the samples.

The data from our long term monitoring survey of Luggage Point (see Figure 2) also indicated the same trends in concentrations and removal rates for all of the selected EDCs and PPCPs. For example, the concentrations of the estrogens were in the low ng/L concentrations although slightly higher levels of E2 were also observed in some cases. The concentrations of the xenoestrogens and TCS were measured at low µg/L range in influent, while the treatment process almost completely removed them from the effluent stream.

Similar trends were observed from the data for Oxley Creek WWTP (see Figure 3), where the effluent concentrations were also assessed pre- and post-UV treatment. These findings are consistent with the literature. We did not observe significant impact due to UV treatment on the concentrations of any of the compounds measured in the effluents at Oxley Creek. Some of the previous studies investigating the effect of UV treatment for removal of EDCs and PPCPs have also reported incomplete removal for the compounds (e.g. Snyder et al., 2003).

The results presented in Figures 2 and 3 are in accordance with the literature data for these compounds. For example, generally high removal rates for estrogens, alkylphenols and the antimicrobial TCS have been observed by many researchers, which maybe attributed, in part, to their physicochemical properties (Ying and Kookana, 2005; Ying and Kookana, 2007). The removal of TCS by activated sludge treatment process has been reported to be as high as 96%, where wastewater influent concentrations ranged from 3.8 to 16.6 ng/L and concentrations for final effluent ranged from 0.2 to 2.7 ng/L (McAvoy et al., 2002).

A recent study of four municipal sewage treatment plants (STPs) in South East Queensland (SEQ), (Tan et al., 2007) also showed similar results. Tan et al. (2007), who investigated the occurrence and fate of some steroid hormones, alkylphenols and BPA, reported low ng/L concentration ranges of the steroid hormones, while the concentrations of the xenoestrogens were higher (µg/L range). High removal efficiencies (80-99%) of the estrogens and xenoestrogenic compounds were observed with conventional treatment processes. Similarly, Clara et al. (2007) also recently reported high removal efficiencies (> 99%) of various surfactants based on data from nine municipal WWTPs from Austria.

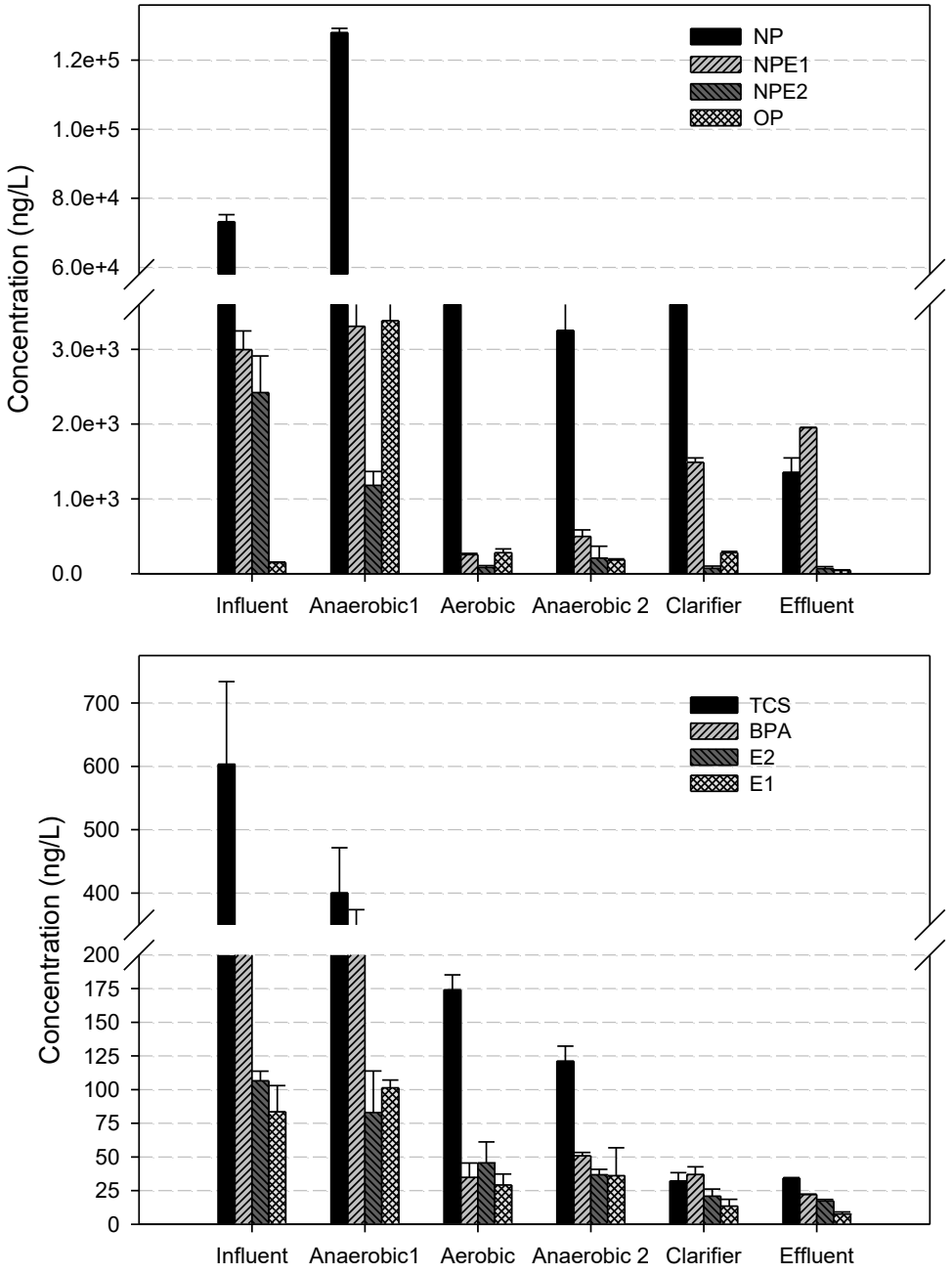

Figure 1 Concentrations of the selected estrogens (estradiol (E2), estrone (E1)); xenoestrogens, (4t-octylphenol (OP), 4-Nonylphenol (NP), nonylphenol mono and di- polyethoxylates (NPE1 and NPE2)); triclosan (TCS); and bisphenol A (BPA) in various stages of the treatment process at Luggage Point WWTP taken on one day (5th June 2008). Treatment stages within the WWTP are listed from left to right from influent to effluent. Error bars are standard deviation of triplicate samples.

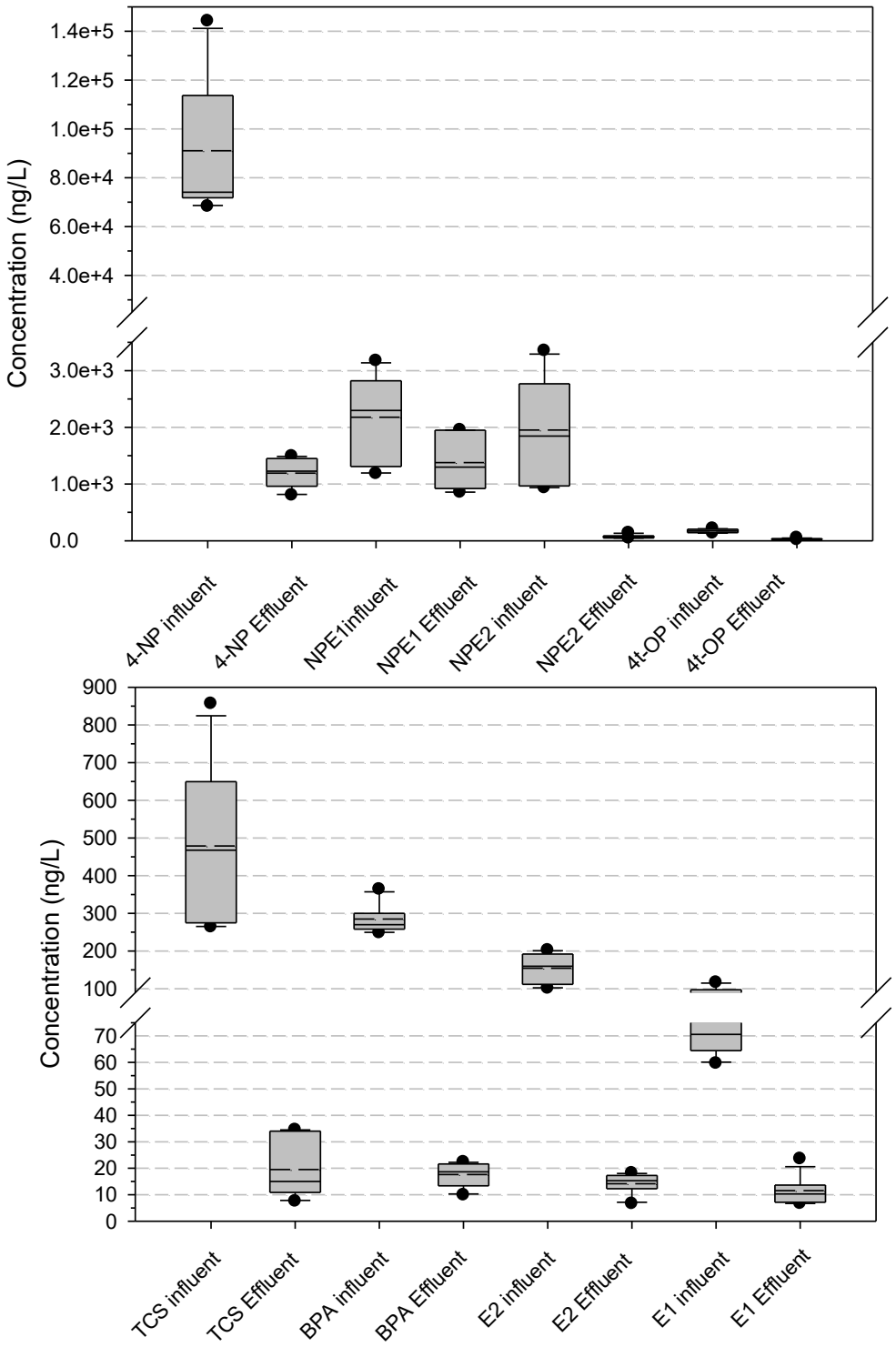

Figure 2 Overall concentrations of the selected estrogens (estradiol (E2), estrone (E1)); xenoestrogens (4t-octylphenol (OP), 4-Nonylphenol (NP), nonylphenol mono- and di-polyethoxylates (NPE1 and NPE2)): triclosan (TCS); and bisphenol A (BPA) from three months of sampling of Luggage Point WWTP influent and effluent streams in latter 2008. The dashed line indicates mean values, the solid line indicates median values, the bars represent 10th and 90th percentile values, the upper and lower boundaries of the box represent the 25th and 75th percentile values, respectively, while closed circles are maximum outlier values.

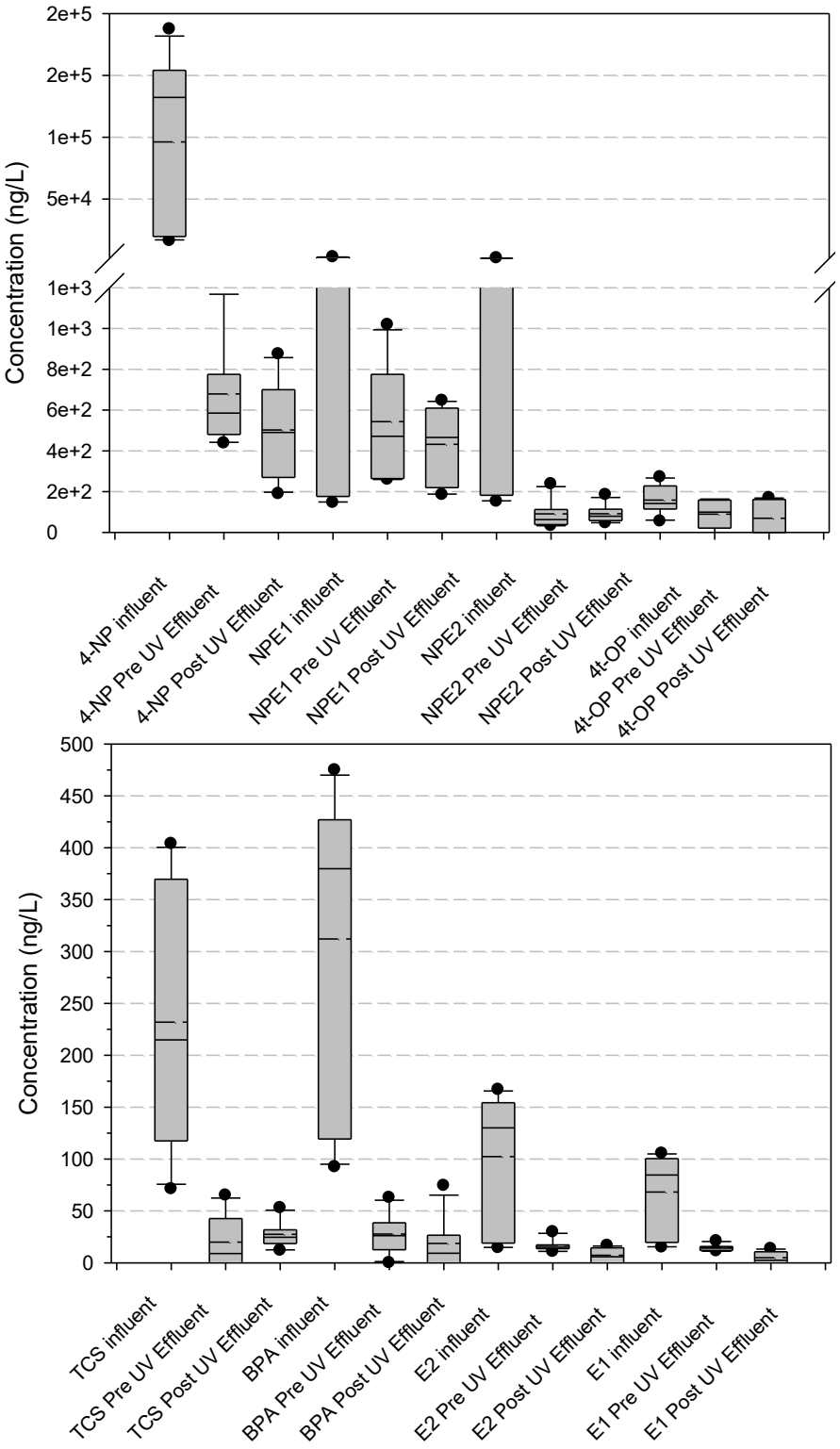

Figure 3. Overall concentrations of the selected estrogens (estradiol (E2), estrone (E1)); xenoestrogens (4t-octylphenol (OP), 4-Nonylphenol (NP, nonylphenol mono- and di-polyethoxylates (NPE1 and NPE2)); triclosan (TCS); and bisphenol A (BPA) from three months of sampling of Oxley Creek WWTP influent, pre-UV effluent and post-UV effluent streams in latter 2008. The dashed line indicates mean values, the solid line indicates median values, the bars represent 10th and 90th percentile values, the upper and lower boundaries of the box represent the 25th and 75th percentile values, respectively, while closed circles are maximum outlier values.

2.2. Concentrations of Pharmaceuticals

The initial assessment of Luggage Point WWTP involved sampling from all areas of the treatment process (Figure 4). Although concentrations of both caffeine (CAF) and ibuprofen (IBU) were in the low µg/L range during the initial stages of treatment, they were both substantially reduced to levels close to or below their respective LOQ (low ng/L; see Appendix 2, Tables A2 and A3). That is, removal due to the treatment process was > 99% for CAF and IBU. Conversely, carbamazepine (CBZ) concentrations remained constant during the treatment process, with there being no difference between the concentrations of CBZ in the influent and effluent streams.

This trend of removal rates was reflected in overall concentrations of the pharmaceuticals in the three monthly samples at Luggage Point WWTP. The concentrations of IBU and CAF were in the low µg/L range in influent, while it has been almost completely eliminated from the effluent stream. CBZ was present in relatively lower concentrations in the influent but was found at the same concentrations in the effluent, indicating it was highly resistant to the treatment process. A similar trend was noted for these compounds at Oxley Creek WWTP, where the concentrations were also assessed pre- and post-UV treatment. UV treatment did not have any apparent influence on the concentrations of CAF, IBU and CBZ in the effluent.

Furthermore, the concentrations of the pharmaceuticals were in a similar range between the Luggage Point (Figure 5) and Oxley Creek (Figure 6) WWTPs for both influent and effluent samples. This trend would be expected based on previous studies that have found CBZ is highly resistant to removal during passage through WWTPs, with high removal rates of CAF and IBU (Miao et al., 2005; Castiglioni et al., 2006; Gros et al., 2006; Vanderford and Snyder, 2006; Kasprzyk-Hordern et al., 2008; Reif et al., 2008; Spongberg and Witter, 2008). Based on the assessment of sorption of CAF and IBU, it is unlikely that partitioning to the solid phase (e.g. biosolids) would have been an important process for these two pharmaceuticals (Ternes et al., 2004; Thomas and Foster, 2005; Williams et al., 2006).

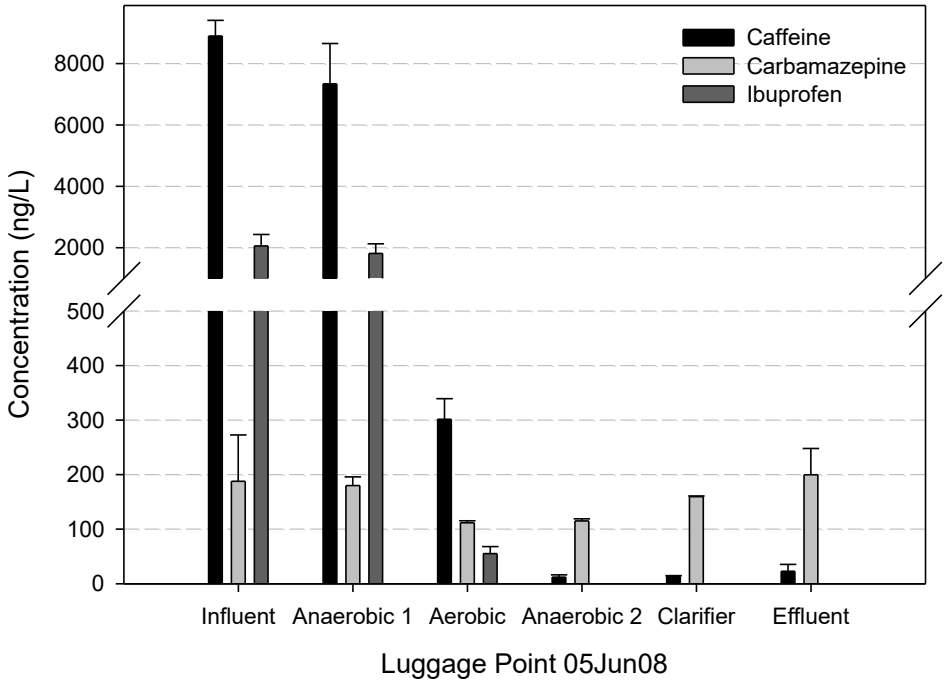

Figure 4. Concentrations of caffeine (CAF), carbamazepine (CBZ) and ibuprofen (IBU) in various stages of the treatment process at Luggage Point WWTP taken on one day (5th June 2008). Treatment stages within the WWTP are listed from left to right from influent to effluent. Error bars are standard deviation of triplicate samples.

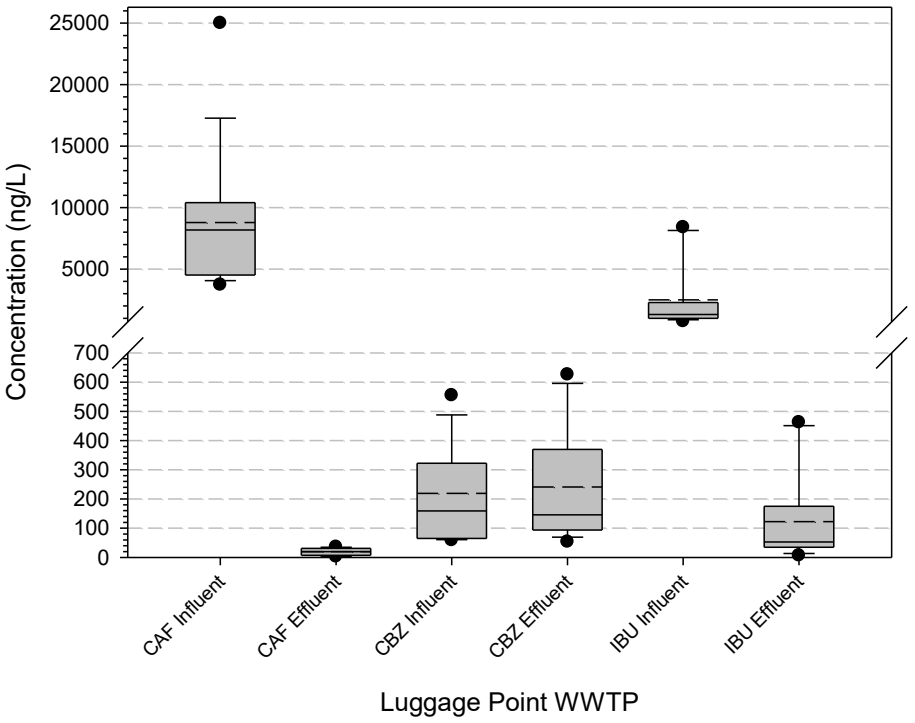

Figure 5. Overall concentrations of the pharmaceuticals caffeine (CAF), carbamazepine (CBZ) and ibuprofen (IBU) from three months of sampling of Luggage Point WWTP influent and effluent streams in latter 2008. The dashed line indicates mean values, the solid line indicates median values, the bars represent 10th and 90th percentile values, the upper and lower boundaries of the box represent the 25th and 75th percentile values, respectively, while closed circles are maximum outlier values.

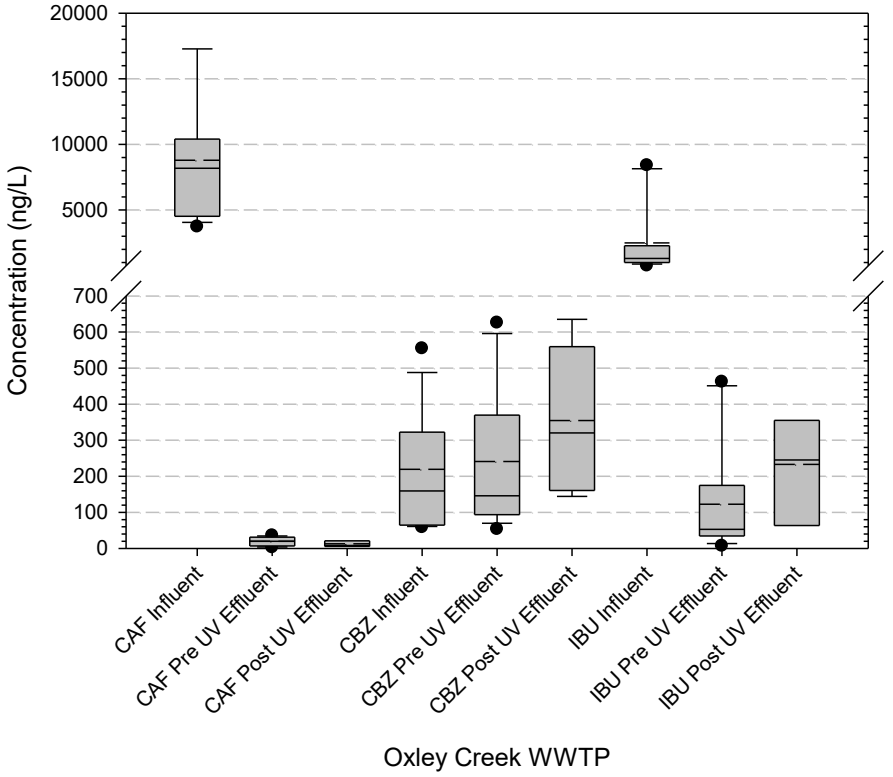

Figure 6. Overall concentrations of the pharmaceuticals caffeine (CAF), carbamazepine (CBZ) and ibuprofen (IBU) from three months of sampling of Oxley Creek WWTP influent, pre-UV effluent and post-UV effluent streams in latter 2008. The dashed line indicates mean values, the solid line indicates median values, the bars represent 10th and 90th percentile values, the upper and lower boundaries of the box represent the 25th and 75th percentile values, respectively, while closed circles are maximum outlier values.

2.3. Removal Efficiencies of the Two Wastewater Treatment Plants

A comparison of the two wastewater treatment plants (Luggage Point and Oxley Creek) was made on the basis of 11 different compounds ranging from hormones, alkylphenols pharmaceutical and personal care products. The data for percentage removal from influent to effluent concentrations are presented in Table 1. Key observations based on the data are as follows:

- There was little difference in the removal efficiencies of the two treatment plants for most compounds (Table 1). The variation in removal efficiency between the two plants was less than 5%.
- Most tested compounds were removed by > 95% during the treatment process. The removal efficiency of the two treatment plants for various compounds can be summarised as follows:
  - **> 95% removal: caffeine, nonylphenol, nonylphenol diethoxylate, triclosan,**
  - **> 90% removal: bisphenol A, ibuprofen,**
  - **> 80% removal: 17β-estradiol, estrone, octylphenol**
  - **< 80% removal: nonylphenol monoethoxylate (includes production from higher chain compounds during breakdown)**
  - **< 10% removal of carbamazepine**

Table 1. The removal efficiencies of the two wastewater treatment plants for a range of estrogenic and pharmaceutical compounds. The data is based on pre UV effluent concentrations.

Compound	Luggage Point		Oxley Creek	
	% removal	±SD	% removal	±SD
17β-estradiol (E2)	90.4	2.7	88.4	5.1
Estrone (E1)	85.4	7.9	83.2	4.1
Nonylphenol (NP)	98.3	0.4	99.6	0.2
Nonylphenol monoethoxylate	43.5	21.6	75.5	15.6
Nonylphenol diethoxylate	96.2	1.8	96.1	4.7
Bisphenol A	93.1	1.8	93.1	5.9
Octylphenol	84.9	6.5	72.0	52.8
Triclosan	96.8	2.6	95.8	12.7
Caffeine	99.8	0.1	99.8	0.3
Carbamazepine	No removal		No removal	
Ibuprofen	96.8	2.3	93.6	1.1

2.4. Unexpected Contaminants

A number of samples taken at Oxley Creek WWTP in October 2008 indicated the presence of some unexpected compounds in both influent and effluent. One compound of note was the surfactant / disinfectant benzalkonium chloride, or BAC (C_{12} alkyl chain), that was present in all influent and effluent samples. In order to apply a conservative concentration value to BAC (since quantitative analysis was not undertaken), its response was made relative to that of CBZ d10, which eluted closest to it (retention times of 15.7 and 12.5 minutes in 100% acetonitrile). This was a conservative approach since the recovery of BAC is considerably lower than CBZ (Appendix 2, Table A2) and any estimate of its concentration is likely to be an underestimation. Based on this approach, the concentration of BAC in seven influent and ten effluent samples is shown in Figure 7.

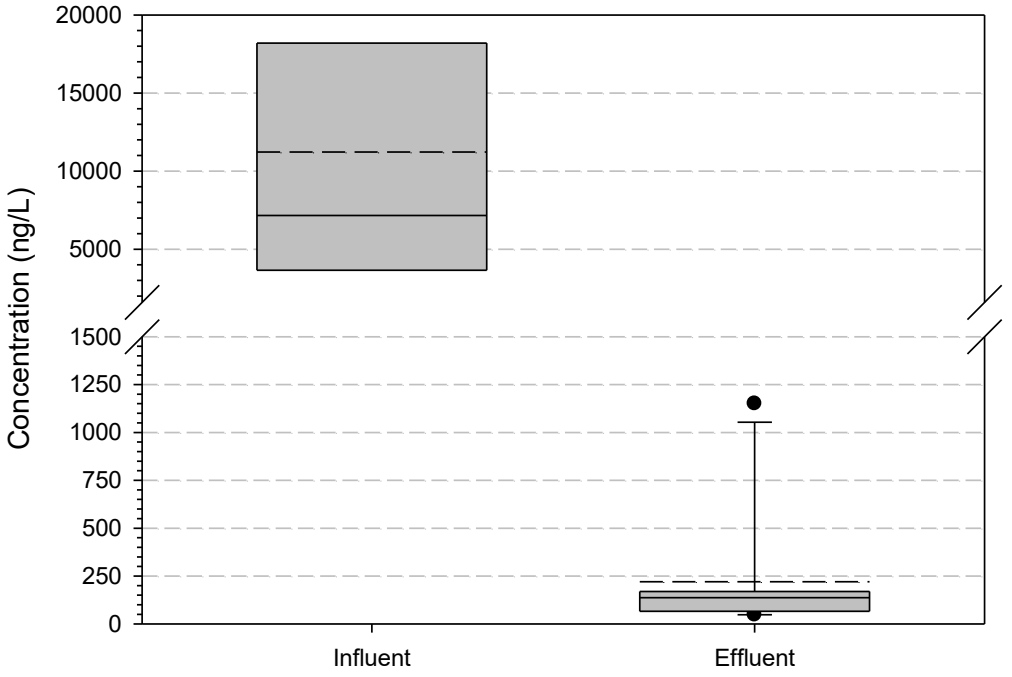

Figure 7. Overall concentrations of benzalkonium chloride C12 (BAC) in wastewater influent and effluent collected from Oxley Creek WWTP in October 2008 in 7 influent and 10 effluent samples taken on separate days. The dashed line indicates mean values, the solid line indicates median values, the bars represent 10th and 90th percentile values, the upper and lower boundaries of the box represent the 25th and 75th percentile values, respectively, while closed circles are maximum outlier values.

These data indicate that BAC was present in the influent at concentrations that are relatively higher compared with other organic contaminants (low µg/L). Also, there is a high degree of removal throughout the treatment process, resulting in only low to mid-ng/L concentrations in effluent. The concentration of BAC is similar to the findings by some of the surveys of WWTPs and receiving waters from the USA and Europe. For example, in an Austrian study, BAC concentrations ranged from 5-170 µg/L in influent, 140-2100 ng/L in effluent, and maximum surface water concentrations were 1900 ng/L (Martinez-Carballo et al., 2007). Similar ranges were found in another Austrian study (Clara et al., 2007). Concentrations in surface waters receiving WWTP effluent in the USA were in the low µg/L range (Ferrer and Furlong, 2001). However, typical effluents from German hospitals have been reported to have low mg/L concentrations (Kummerer, 2004). Despite the large extent of removal in WWTPs, the main concern relating to BAC include its potential to disrupt degradation of other organic contaminants, due to its cytotoxicity (Kummerer, 2004), and also due to its potential for genotoxicity (Ferk et al., 2007; Ferk et al., 2009).

Other potential compounds of interest that were detected in both influent and effluent samples collected for BAC analysis include the azole fungicide, propiconazole, and the serotonin-norepinephrine re-uptake inhibitor (SNRI) antidepressant, venlafaxine. Without adjusting the concentrations of propiconazole or venlafaxine to a surrogate, there was little apparent removal of both compounds during treatment with concentrations in both influent and effluent in the low to mid-ng/L range.

A previous study assessing removal of fungicides in Swiss WWTPs indicates there was also poor removal of propiconazole, where it was detected in effluents at similar concentration range as our study (Kahle et al., 2008). Azole fungicides can be used as chemotherapy agents as one of their modes of action is to reduce estrogen levels by inhibiting aromatase (Zarn et al., 2003), indicating they have the potential to act as an EDC.

While the environmental fate of the SNRI antidepressant venlafaxine has not been well studied, a Canadian group found that it was also poorly removed during treatment and detected in effluent in the mid-ng/L range (Lajeunesse et al., 2008). Also, a study of surface waters in the USA found levels of venlafaxine up to 2190 ng/L in a wastewater-dominated stream (Schultz and Furlong, 2008).

3. KEY FINDINGS

3.1. Influent and Effluent Concentrations of EDCs

- The median concentrations of 17β-estradiol (E2) and Estrone (E1) in the influents were 140±60 and 75±32 ng/L respectively. The concentration in treated effluents of E2 and E1 were 15±6 and 13±5, thus showing about 90% removal of the two compounds. The concentrations of E2 were higher than previously reported. The reasons are unclear but there is growing awareness that gas chromatography combined with mass spectrometry (GC-MS) based methods can overestimate the E2 levels. Future studies should employ gas chromatography combined with tandem mass spectrometry (GC-MS/MS) methods. The concentration of the synthetic hormone EE2 was below the method limit of quantification (LOQ) (5 ng/L) in all the samples.
- The median influent concentration of the surfactants ranged from 157±59 ng/L for octylphenol (OP) up to 74644±56430 ng/L for nonylphenol (NP). Despites its high and variable concentrations, NP was effectively removed during the treatment process; (about 99%). The final effluent concentration (median) was 880 ng/L.
- The concentrations of triclosan and bisphenol A in the influents were found to be 284±224 and 288±116 ng/L respectively. The corresponding effluent concentrations were only 12±21 and 19±16 ng/L respectively. This represented 93-95% removal of the two compounds during the treatment process.

3.2. Influent and Effluent Concentrations of Pharmaceuticals

- Ibuprofen (IBU) and caffeine (CAF) were present in relatively high concentrations in the influent (low µg/L range). However, both of these compounds were efficiently removed (> 94%) during the treatment.
- Conversely, carbamazepine (CBZ) concentrations (in low hundreds of ng/L) remained constant during the treatment process and no difference between the concentrations of CBZ in the influent and effluent streams were noted.
- The above findings are consistent with previous studies that have found high removal rates of CAF and IBU, whereas CBZ is highly resistant to removal during passage through WWTPs. Our laboratory partitioning experiments (see report on Experiment 1.4) also show that both CBZ and IBU are not removed substantially by sorption on sludge. Given that IBU was removed efficiently in the treatment shows that biodegradation was relatively rapid for this compound. The converse was true for CBZ.

3.3. Overall Removal Efficiencies of the Treatment

- The intensive study on the assessment of the efficiency of different steps of treatment at Luggage Point in removal of 11 micropollutants showed that the anaerobic steps were less effective in removal of these organic compounds than the aerobic steps. This is consistent with the laboratory degradation studies by CSIRO showing poor degradation of a range of micropollutants under anaerobic conditions.
- We did not observe significant impact due to UV treatment on the concentrations of any of the compounds measured in the effluents at Oxley Creek. Some other studies have previously reported incomplete removal of EDCs and PPCPs using UV treatment.
- There was little difference in the removal efficiencies between the two treatment plants for most compounds (Table 1). The variation in removal efficiency between the two plants was less than 5%.
- Most tested compounds were removed by > 95% during the treatment process. The removal efficiency of the two treatment plants for various compounds can be summarised as follows:
  - **> 95% removal**: caffeine, nonylphenol, nonylphenol diethoxylate, triclosan,
  - **> 90% removal**: bisphenol A, ibuprofen,
  - **> 80% removal**: 17β-estradiol (E2), estrone (E1), 4t-octylphenol (OP)
  - **< 80% removal:** nonylphenol monoethoxylate (includes production from higher chain compounds during breakdown).

3.4. Unexpected Contaminants Found

- During the monitoring study a few unexpected contaminants (or at least those that were not targeted) were picked up. Perhaps most significant among these is the benzalkonium chloride (BAC), which is a cationic surfactant compound used as a biocide in hospitals, laundries and possibly in other applications. The load of the compound in the waste stream was high (4000-17000 ng/L).
- Despite its high degree (> 95%) of removal of BAC during the treatment process, the concentrations in the effluents were mostly > 100 ng/L and occasionally even > 1000 ng/L. These levels are in line with other surveys of WWTPs and receiving waters in the USA and Europe. In particular, in German hospital effluents BAC has been detected at concentrations as high as mg/L.
- Recently, concerns have been expressed due to BAC's cytotoxicity (Kummerer, 2004) and also due to its potential for genotoxicity (Ferk et al., 2007; Ferk et al., 2009). Potential for DNA damage has been highlighted by Ferk et al. (2007).
- Other potential compounds of interest that were detected in both influent and effluent samples include the azole fungicide, propiconazole, and the serotonin-norepinephrine re-uptake inhibitor (SNRI) antidepressant, venlafaxine.
- Azole fungicides can be used as chemotherapy agents as one of their modes of action is to reduce estrogen levels by inhibiting aromatase (Zarn et al., 2003), indicating they have the potential to act as an endocrine disruptor.
- The antidepressant venlafaxine has not been well studied but is reported to be poorly removed during wastewater treatment and has been detected in effluent and streams affected by effluents at concentrations > 2000 ng/L.
- Not all the above compounds were targeted in the study, so the analytical results are subject to further optimisation of analytical methods using proper internal standards and recovery tests.

4. CONCLUSIONS AND FUTURE DIRECTIONS

- Data on steroid hormones and alkylphenols in effluents from treatment plants is now substantial from both Australia and overseas. This data allows a reasonable baseline of the levels of these compounds in treated effluents across a range of treatment technology. It is therefore recommended that no further work on monitoring of steroid hormones and alkylphenols (NP, OP and BPA) is needed. The only exception is the synthetic hormone ethinylestradiol (EE2) which needs to be analysed by more sensitive methods to establish its true baseline rather than the current finding of below the detection limit. E1 and E2 have been measured in some of the WWTP effluents at concentrations higher than expected. Artefacts in chemicals analyses by GC-MS for trace level determination of estrogens in complex environmental samples have been reported in the literature. The use of GC-MS methods for estrogen analyses has increasingly been recognised internationally as having potential for overestimation of estrogen concentrations in environmental samples. Therefore some of the estrogen data from this study are subject to re-evaluation, to be undertaken with more reliable analytical techniques such as GC-MS/MS.
- Considering the detection of some unexpected compounds, in particular bezylalkonium chloride (BAC), it is important that the presence of such compounds be properly assessed using improved analytical protocols. Considering the toxicological profile of the compounds of this class, it deserves further attention.
- Considering the recent literature and the direction of international efforts, greater emphasis should be placed on assessment of cytotoxic drugs, x-ray contrast media and pharmaceuticals.
- The drugs of abuse has also been highlighted in recent years as a group of compounds that warrants further investigations in terms of their removal in the treatment process.
- Future efforts should also be strategically guided by risk assessment needs and data deficiency. This may lead to greater emphasis on source control related monitoring closer to source, or to better understand the impact of various point sources that are more amenable to control.

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APPENDIX 1 - ANALYTICAL METHODS

LC-MS/MS Methods

For analysis by HPLC-MS/MS, a Finnigan TSQ Quantum Discovery Max (Thermo Electron Corporation) was used. HPLC separation was performed on a BDS Hypersil C_{18} 150 x 2.1 mm (3 □m particle size) column with a mobile phase flow rate of 0.2 mL/min. The mobile phase composition was 30 mM ammonium formate/0.1% formic acid (Method 1) or ultrapure water (Method 2) (A) and acetonitrile (B) using the following gradient parameters: 90%-30% A (0-5 min); 0% A (6-15 min); and 90% A (16-25 min). MS/MS analysis was undertaken using atmospheric pressure electrospray ionisation (ESI) in both positive and negative ionisation modes. Qualitative and quantitative analysis of compounds was based on retention time, multiple reaction monitoring (MRM) of two transition ions, the ratios between the transition ions and the relative response of stable isotopes in solution and stable isotopes in ultrapure water.

GC-MS Analysis

For analysis by GC-MS, a 0.5 mL aliquot from sample extracts was converted into trimethylsilyl esters by reacting the sample with BSTFA + 1% TMCS (100 µL) in pyridine solvent (400 µL) at 75°C for 1 hour. Following the derivatization, the mixture was then cooled to room temperature, concentrated to dryness and sample reconstituted in hexane containing the instrument internal standard (I.S), anthracene-d10 was added to the GC vial. A 1-2 µL aliquot analysed by an Agilent 6890 GC, coupled with a 5973 MS, equipped with a HP-5MS capillary column (30 m x 0.25 mm ID, film thickness 0.25 µm). Helium gas set at a linear flow rate of 1 mL/min was used as the carrier gas. A splitless injection port liber was used for sample analysed in splitless injection mode. The oven temperature was typically programmed as follows: 75°C (1 min) to 150°C (10°Cmin^{-1}) and then to 280°C (15°Cmin^{-1}) and held for 10 min. The injector and interface temperatures were set at 280°C, with the MS quad set at 150°C and the MS source at 230°C. The mass spectrometer was operated in the positive ion electron impact mode with an ionisation voltage of 70 eV using selected ion monitoring (SIM).Calibration curves for the analytes were obtained in the concentration ranges between 25 and 1000 µg/L. Good linearity and reproducibility of analyses ($R^2 > 0.99$) were achieved.

Recovery of the analytes varied considerably following SPE (see Appendix2, Tables A2 and A3) and due to matrix suppression during analysis. While this was usually overcome by comparing the response of the respective stable isotopes in the samples and in ultrapure water, it was sometimes difficult to overcome the issue of recovery. For example, while cotinine (COT) was often present at concentrations higher than its upper limit of quantification (ULOQ) its surrogate stable isotope COT labelled with three deuterium atoms (COT d3) was usually below its lower limit of quantification: (LLOQ, see Appendix2, Tables A2 and A3). Due to the extent of signal suppression of COT d3, it is likely that the actual concentrations of COT in influent were much greater than that measured. However, COT was never detected in any effluent samples, indicating the majority of it was removed during the treatment process.

Sample Collection Protocols

- Amber glass 1 L bottles were washed with detergent, rinsed with deionised, reverse osmosis, ultrapure water and acetone, before being baked at 300°C for four hours.
- Influent samples were collected at 9 a.m. on sampling days.
- 1 L of sample was transferred into amber glass bottle containing 0.5 mL conc. H_2SO_4, ensuring minimal headspace.
- Open field blank bottle containing MilliQ water and close.
- Place samples in icebox at 4°C and transport immediately to laboratory.

Sample Preparation in the Laboratory

Water samples were extracted and pre-concentrated by solid phase extraction. Typically, 1 L wastewater filtered through Whatman filters (GF/A and GF/F glass fibre filter) was returned to collection bottles, spiked with 100 µL of 1 mg/L (in methanol) solution of stable isotope standard mixture. Samples are loaded onto Waters Oasis HLB cartridges (500 mg, 6 cc) using Supelco large volume sampler siphon tubing. The cartridges were pre-conditioned with 2 x 4 mL water followed by 2 x 4 mL methanol and 2 x 4 mL of dichloromethane. After washing the cartridges with 2% methanol, and after a drying time of 1-2 hours, the analytes of interest were eluted with 2 x 4 mL methanol, followed by 2 x 4 mL dichloromethane. The combined methanol fractions were concentrated to dryness under a gentle stream of nitrogen before the elution step with dichloromethane into the same tubes. The dry extract was then reconstituted in 1.0 mL of methanol as appropriate for chemical analyses. SPE cartridges loaded with wastewater samples are usually stored at -18^{o}C until they are ready for analysis, at which time the samples are eluted and prepared for analysis, as above.

APPENDIX 2 - COMPOUNDS SCREENED IN WATER SAMPLES

Table A2. Overview of Compounds Selected for Screening in WWTP Effluent and Influent

Target Analytes	Surrogate[a]	Compound class/use	Analytical method parameters			
			Method	Recovery [b]	MS Target ions[c]	LOQ[d]
Bisphenol A (BPA)	BPA d16	Plasticizer	GC-MS	109±5	**357** 191,217,372	10 ng/L
Caffeine (CAF)	CAF $^{13}C_3$	Stimulant	LC-MS/MS	135±30	195$[M+H]^+ \rightarrow$110,138	6 – 100 ng/L
Carbamazepine (CBZ)	CBZ d10	Anti-epileptic	LC-MS/MS	103±5	237$[M+H]^+ \rightarrow$193,194	5 – 100 ng/L
Cotinine (COT)	COT d3	Nicotine metabolite	LC-MS/MS	55±29	177$[M+H]^+ \rightarrow$80,98	10 – 100 ng/L
Estrone (E1)	E1 d4	Endogenous estrogen	GC-MS	125±14	**342** 218,244,257	5 ng/L
17β-estradiol (E2)	E2 d4	Endogenous estrogen	GC-MS	120±14	**285** 231,327,416	5 ng/L
17β-ethynylestradiol (EE2)	EE2 $^{13}C_2$	Contraceptive	GC-MS	127±17	**425** 285,300,440	5 ng/L
Ibuprofen (IBU)	IBU $^{13}C_3$	NSAID[e]	LC-MS/MS	93±15	205$[M-H]^- \rightarrow$161	30 – 100 ng/L
4-nonylphenol (NP) and NPE1-2	NP d8	Surfactant degradation by-product	GC-MS	163±48	**179** 277,292	20 ng/L
4-*t*-octylphenol (OP)	NP d8	Surfactant degradation by-product	GC-MS	115 ±25	**207** 278,263	20 ng/L
Triclosan (TCS)	TCS methyl $^{13}C_{13}$	Antimicrobial	GC-MS	113±3	**200** 347,362,310	10 ng/L

[a] Stable isotopes added to samples to quantify analytes; isotopes were labelled with various numbers of atoms of deuterium (d) or ^{13}C.

[b] Recovery of compounds following extraction with Waters HLB cartridges from water spiked with worm casting leachate and analytes; error is standard deviation of n=6 samples.

[c] Target ions were mass/charge (m/z) and were based on transition of parent ion to daughter ions in LC-MS/MS and of fragment ions following electron impact (EI) ionisation in GC-MS.

[d] limit of quantification; samples higher than the upper range were diluted for analysis until responses were within quantifiable range.

[e] non-steroidal anti-inflammatory drug.

[f] methylenedioxyamphetamine.

[g] amphetamine-type stimulant.

[h] methylenedioxymethamphetamine.

[i] selective serotonin reuptake inhibitor.

[j] serotonin norepinephrine reuptake inhibitor.

Table A3. List of Compounds for which Analytical Methods have been Developed

Compound	Surrogate	Compound class/use	Analytical method parameters			
			Method	Recovery [b]	MS Target ions[c]	LOQ[d]
Amphetamine	AMP d8	Illicit stimulant	LC-MS/MS	71±16	136[M+H]$^+$→91,119	5 – 100 ng/L
Benzalkonium chloride	-	Surfactant/disinfectant	LC-MS/MS	44±6	304[M+H]$^+$→91,212	5 – 100 ng/L
Bicalutamide	-	Anti-androgen	LC-MS/MS	138±15	429[M-H]$^-$→185,255	5 – 100 ng/L
Chlorpheniramine	-	Antihistamine	LC-MS/MS	100±13	275[M+H]$^+$→230	5 – 100 ng/L
Cyclophosphamide	-	Chemotherapeutic	LC-MS/MS	94±9	261[M+H]$^+$→140,233	5 – 100 ng/L
Diclofenac	-	NSAID	LC-MS/MS	95±17	294[M-H]$^-$→214,250	10 – 100 ng/L
Diphenhydramine	-	Antihistamine	LC-MS/MS	118±8	256[M+H]$^+$→167	5 – 100 ng/L
Fenarimol	-	Fungicide	LC-MS/MS	118±12	331[M+H]$^+$→259,268	5 – 100 ng/L
Flutamide	-	Anti-androgen	LC-MS/MS	110±15	275[M-H]$^-$→202,229	5 – 100 ng/L
MDA[f]	MDA d5	ATS[g]	LC-MS/MS	81±15	180[M+H]$^+$→135,163	5 – 100 ng/L
MDMA[h]	MDMA d5	ATS	LC-MS/MS	87±7	194[M+H]$^+$→135,163	5 – 100 ng/L
Methamphetamine	MAP d11	ATS	LC-MS/MS	80±12	150[M+H]$^+$→91,119	5 – 100 ng/L
Methotrexate	-	Chemotherapeutic	LC-MS/MS	53±6	455[M+H]$^+$→134,308	5 – 100 ng/L
Metoprolol	-	Antihypertensive	LC-MS/MS	95±9	268[M+H]$^+$→159,191	5 – 100 ng/L
Myclobutanil	-	Fungicide	LC-MS/MS	118±7	289[M+H]$^+$→70,125	5 – 100 ng/L
Nicotine	-	Stimulant	LC-MS/MS	26±20	163[M+H]$^+$→106,132	5 – 100 ng/L
Pindolol	-	Antihypertensive	LC-MS/MS	73±50	249[M+H]$^+$→116,172	5 – 100 ng/L
Promethazine	-	Antihistamine/sedative	LC-MS/MS	88±13	285[M+H]$^+$→198,240	5 – 100 ng/L
Propiconazole	-	Fungicide	LC-MS/MS	102±12	342[M+H]$^+$→69,159	5 – 100 ng/L
Propranolol	-	Antihypertensive	LC-MS/MS	114±27	260[M+H]$^+$→116,183	5 – 100 ng/L
Sertraline	-	SSRI[i]antidepressant	LC-MS/MS	75±14	306[M+H]$^+$→159,275	5 – 100 ng/L
Simazine	-	Herbicide	LC-MS/MS	106±15	202[M+H]$^+$→124,132	10 – 100 ng/L
Triiodothyronine	-	Thyroid hormone	LC-MS/MS	197±52	652[M+H]$^+$→508,606	30 – 100 ng/L
Trimethoprim	-	Antibiotic	LC-MS/MS	95±6	291[M+H]$^+$→230,261	5 – 100 ng/L
Venlafaxine	-	SNRI[j]antidepressant	LC-MS/MS	107±4	278[M+H]$^+$→58,260	5 – 100 ng/L

[a] Stable isotopes added to samples to quantify analytes; isotopes were labelled with various numbers of atoms of deuterium (d) or ^{13}C.

[b] Recovery of compounds following extraction with Waters HLB cartridges from water spiked with worm casting leachate and analytes; error is standard deviation of n=6 samples.

[c] Target ions were mass/charge (m/z) and were based on transition of parent ion to daughter ions in LC-MS/MS and of fragment ions following electron impact (EI) ionisation in GC-MS.

[d] limit of quantification; samples higher than the upper range were diluted for analysis until responses were within quantifiable range.

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