Determination of Organic Contaminants of Emerging Concern in Domestic Wastewater from Urban Sources

Ali Shareef, Grace Tjandraatmadja, Rai Kookana and Mike Williams

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EXECUTIVE SUMMARY

This is the final report in the ‘Emerging contaminants in domestic wastewater’ series funded by the Smart Water Project on Household Sources of Priority Contaminants in Domestic Wastewater, jointly undertaken by CSIRO and the Smart Water Fund. The primary goal of this research was to evaluate priority and emerging contaminant sources from household waste streams and to understand their origins and potential contribution to municipal Wastewater Treatments Plants (WWTP).

WWTPs receive wastewater from domestic, trade and industrial sources. The composition and characteristics of each of these source streams can have a significant impact on the overall quality of the influent to the WWTP as well as on the effectiveness of treatment processes in capturing or removing contaminants. Because the focus of this project was domestic wastewater, only issues pertinent to domestic wastewater will be addressed in detail.

A number of factors may contribute to overall quality and composition of domestic waste streams. For example, (i) household water supplies and waste disposal infrastructure (e.g. water pipes and fittings), (ii) composition of black water and greywater which can depend on the nature of household usage of pharmaceuticals, cleaning and personal care products, as well as (iii) householder’s socio-economic background, lifestyle (e.g. variation in food preparation, grooming and cleaning) can affect the type of organic compounds entering WWTPs. However, very little is known on the nature and concentration of organic contaminants in domestic wastewater.

Increasing number of organic compounds of emerging concern have been detected in effluents of municipal WWTPs. These may be derived from human excretions, household chemicals, pharmaceuticals and personal care products. Many of these chemicals have been identified as endocrine disrupting chemicals (EDCs) which can interfere with the normal functioning of the endocrine systems of many aquatic and terrestrial organisms. Other potential chemicals of increasing concern are pharmaceuticals and personal care products (PPCPs). Typical EDCs include steroid estrogens (estradiol: E2, estrone: E1, estriol: E3) and xenoestrogens (alkylphenols: Octylphenol OP and Nonylphenol NP, and alkylphenol polyethoxylates: NPE1 and NPE2), while some of the common examples of PPCPs of concern, include pharmaceuticals (ethynylestradiol: EE2, carbamazepene: CBZ, ibuprofen: IBU), antimicrobials (triclosan), musk fragrances (tonalide and galaxolide), and insect repellents (DEET).

The identification and characterisation of organic contaminants of emerging concern in residential wastewater is the main objective of the work described in this report. This document should be read in conjunction with previous reports from this project (Shareef et al., 2008) and others in this project, e.g. (Tjandraatmadja and Diaper, 2007).

This report comprised three major components. These included (i) an abridged literature review through which potential contaminants and possible analytical methodologies have been identified, (ii) the development of analytical methods and (iii) Application of methods using a selected number of wastewater samples collected from an urban residential area of Melbourne (Shareef et al., 2008).
The literature review focused on identification of potential contaminants for analysis in domestic wastewater with emphasis on the following aspects:

(i) the potential impact on human and environmental health

(ii) prediction of expected levels of potential contaminants in domestic wastewater

(iii) the major pathways of their transport and fate in the environment

(iv) implications for municipal wastewater treatment facilities

(v) identification of impacts on wastewater recycling and reuse and land application of biosolids

(vi) availability of analytical methodologies for trace level analysis of the emerging contaminants of concern in domestic wastewater streams.

One of the conclusions from the literature review was that advanced analytical techniques were required for sensitive determination of typical domestic wastewater contaminants. The instrumentation and expertise available at CSIRO Adelaide Laboratories have been used in the development of analytical methods. Both gas chromatography mass spectrometry (GC-MS) and liquid chromatography tandem mass spectrometry (LC-MS/MS) analytical methods have been developed for determination of a range of major contaminants in domestic wastewater streams including greywater and black water samples. The initial list of potential contaminants identified through the literature review was diverse. Due to time and resource constraints, only the compounds given in the table below have been selected for analytical method validation using real domestic wastewater samples. The table also lists the analytical method used for detection of each compound.

The information from literature and the analytical methods developed for this project were used to identify the compounds analysed in samples of domestic wastewater (Shareef et al 2008, Shareef and Williams 2009).
Table 1. Selected EDCs and PPCPs method overview and analytical method parameters for selected compounds for in domestic wastewater samples.

<table>
<thead>
<tr>
<th>Analyte Class</th>
<th>Compound</th>
<th>Analytical method parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SPE Recovery</td>
</tr>
<tr>
<td>Steroid Hormones</td>
<td>Estradiol (E2) [50-28-2] MW: 272</td>
<td>120 ± 14</td>
</tr>
<tr>
<td></td>
<td>Estrone (E1) [53-16-7] MW: 270</td>
<td>125 ± 14</td>
</tr>
<tr>
<td></td>
<td>Ethynylestradiol (EE2) [57-63-6] MW: 296</td>
<td>127 ± 17</td>
</tr>
<tr>
<td>Plasticiser</td>
<td>Bisphenol A (BPA) [80-05-7], MW: 228</td>
<td>109 ± 5</td>
</tr>
<tr>
<td>Surfactants</td>
<td>Octylphenol (OP) [140-66-9], MW: 206</td>
<td>115 ± 25</td>
</tr>
<tr>
<td></td>
<td>Nonylphenol (NP) [25154-52-3], MW: 220</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NP-mono-ethoxylates (NPE1) [104-35-8], MW: NP1EO 264</td>
<td>163 ± 48</td>
</tr>
<tr>
<td></td>
<td>NP-di-ethoxylates (NPE2) NP2EO:308</td>
<td></td>
</tr>
<tr>
<td>Antimicrobials</td>
<td>Triclosan (TCS) [3380-34-5], MW: 289</td>
<td>113 ± 3</td>
</tr>
<tr>
<td>Pharmaceuticals</td>
<td>Carbamazepine [85756-57-6], MW: 236</td>
<td>103 ± 5</td>
</tr>
<tr>
<td></td>
<td>Ibuprofen [15687-27-1], MW: 206</td>
<td>93 ± 15</td>
</tr>
</tbody>
</table>

a Solid Phase Extraction recovery data obtained extraction of analytes spiked in worm casting leachate using Waters HLB cartridges (error is standard deviation of n=6 samples). LOQ= limit of quantitation, GC-MS= gas chromatography-mass spectrometry, LC-MS= liquid chromatography-tandem mass spectrometry.
The analytical methods developed in this study have been applied in a pilot study conducted to assess the composition of domestic wastewater. Analysing wastewater samples taken from two sites of 7 and 163 households in an urbanised region of Victoria (Frankston) indicated that most of the compounds were detectable at concentrations generally higher than WWTP influents reported in the literature. The impact of seasonal variations on concentration of the selected contaminants has also been evaluated. The findings from this preliminary study indicated that the concentration of the compounds were generally higher in winter than in summer samples for the sites selected, particularly for estrogens and nonylphenol. If the winter concentrations persist in water reaching the WWTP, treatment upgrade to advanced tertiary oxidation would be required to breakdown estrogens. Thus, further investigation is recommended.

Another interesting observation from this study was that concentrations of most of compounds showed an increasing trend towards the weekend. It was expected that the overall load of contaminants would be higher because people spend more time at home on weekends and have the opportunity to use more detergents for washing and cleaning etc.

Overall, the main conclusions from this study included:

1. A wide range of EDCs and PPCPs have been identified as potential contaminants likely to be present in wastewater. These included estrogens and xenoestrogens, which are often detected in ng L\(^{-1}\) to \(\mu\)g L\(^{-1}\) concentrations in WWTP effluents.
2. Analytical methods using GC-MS or LC-MS/MS developed in this project can be used for simultaneous determination of a wide range of the selected pollutants.
3. Although only a limited number of samples have been analysed, the results from the domestic wastewater case study indicated the presence of estrogens (E1, E2, EE2), alkylphenolic surfactants, bisphenol A, triclosan in composite wastewater samples with seasonal effects on concentrations apparent.
4. Surprisingly nonylphenol ethoxylates, which were thought to be derived mainly from industrial detergents, were measured in the residential wastewater at significant concentrations; hence further evaluation of its presence in household detergents is recommended. Sampling of greywater streams from within major household appliances indicates that the washing machine and dishwasher are likely to be significant contributor to NPE1 and NPE2 in households.
5. Carbamazepine and ibuprofen whilst reported in domestic wastewater in the literature were below the LOD in the current study.

Recommendations are:

1. Although the non-ionic surfactants nonylphenol polyethoxylates are thought to be an industrial detergent, the high levels of NPs and NPE1-2 measured in this study warrants a more comprehensive analysis of domestic wastewater to identify the source of these compounds. It may also be worthwhile conducting a study to determine the concentration of NPs in household detergents.
2. The effect of co-contaminants (e.g. estrogens and triclosan) deserves some research attention. For instance, while biodegradation of estrogens have widely been reported as a means of removal of the potential EDCs during wastewater treatment process, or in the environment, the occurrence of antibiotics and antimicrobials (e.g. triclosan) can have a significant impact on the effectiveness of treatments.

3. This scoping study investigated a small dataset comprised of a limited number of samples (only over 7 days) to gain an *a priori* understanding of the concentrations of selected contaminants closer to residential sources of discharge in Melbourne. Hence more extensive monitoring of residential catchments would be recommended to confirm the concentration ranges in sewers throughout the year and reproducibility across various catchments.
# TABLE OF CONTENTS

Executive Summary ........................................................................................................ iv

Abbreviations .................................................................................................................. xi

1. Introduction ......................................................................................................................... 1
   1.1. Background .................................................................................................................. 1
   1.2. Objectives ................................................................................................................... 3
   1.3. Outline of This Report ............................................................................................. 3
       - Section 1: Introduction ............................................................................................. 3
       - Section 2: Overview Based on Literature ................................................................. 3
       - Section 3: Analytical Method Development ........................................................... 3
       - Section 4: Analysis of EDCs and PPCPs in Domestic Wastewater ..................... 4
       - Section 5: Conclusions ........................................................................................... 4
       - Section 6: Future Directions ................................................................................... 4

2. Literature Review .............................................................................................................. 5
   2.1. Characterisation of Organic Contaminants in Domestic Wastewater Streams ............. 5
   2.2. Identification of Potential Contaminants for Analysis ............................................. 5
   2.3. Environmental Occurrence, Fate and Effects of EDCs and PPCPs .......................... 6
       - EDCs and PPCPs ...................................................................................................... 6
       - Fate and Levels in the Environment ....................................................................... 6
       - Concentrations in Household Wastewater ............................................................... 7
       - Biological Effects ..................................................................................................... 7
       - Analytical Methods .................................................................................................. 8
       - Analytical Method Detection Limits ....................................................................... 8
       - Recommended List of Contaminants ...................................................................... 9

3. Analytical Method Development ..................................................................................... 11
   3.1. Selection of Analytical Methods ............................................................................... 11
   3.2. Sample Preparation ................................................................................................. 11
   3.3. Optimised Analytical Methods ............................................................................... 12
       - GC-MS ...................................................................................................................... 12
       - LC-MS/MS ............................................................................................................... 12
   3.4. Some QA/QC Considerations ............................................................................... 13
   3.5. Results and Discussions ....................................................................................... 13

4. Case Study: Analysis of EDCs and PPCPs in Domestic Wastewater ............................... 15
   4.1. Background ............................................................................................................. 15
   4.2. Potential Contaminants ......................................................................................... 15
   4.3. Sampling ............................................................................................................... 18
       - Site Description ....................................................................................................... 18
Sample Collection Protocols ........................................................................ 19
Sampling Regimes ....................................................................................... 20

4.4. Results and Discussions ....................................................................... 20
Daily Concentrations (site 2) ...................................................................... 21
Comparison of Same Day Concentrations (summer) .............................. 24
Seasonal Variations .................................................................................... 25
Source identification ................................................................................... 32

5. Conclusions .............................................................................................. 34

5.1. Literature Review .................................................................................. 34
5.2. Analytical Method Development ............................................................. 35
5.3. Preliminary Data on Domestic Wastewater Case Study ..................... 36

6. Future Directions ...................................................................................... 37

References ................................................................................................... 38
ABBREVIATIONS

AHTN  Tonalide
APEO  Alkylphenol ethoxylate
BPA   Bisphenol A
BSTFA N,O-bis-(trimethylsilyl)-trifluoroacetamide
CBZ   Carbamezapene
DEET  N,N-Dimethyl-m-toluamide
E1    Estrone
E2    17β-estradiol
E3    Estriol
EDC   Endocrine disrupting chemical
EE2   17α-ethynylestradiol
ELISA Enzyme linked immuno sorbent assay
GC-MS Gas chromatography-mass spectrometry
HHCB  Galaxolide
LAS   Linear alkylbenzene sulfonates
LC-MS/MS Liquid chromatography-tandem mass spectrometry
LOQ   Limits of quantification
NPE1  Nonylphenol monoethoxylate
NPE2  Nonylphenol diethoxylate
NPEs  Nonylphenol ethoxylates
OP    4-t-Octylphenol
OPEs  Octylphenol ethoxylates
PPCP  Pharmaceutical and personal care product
TCS   Triclosan
TMS   Trimethylsilyl
WWTP  Wastewater treatment plant
1. INTRODUCTION

1.1. Background

Water shortage has recently become one of the most significant challenges faced by many industrial as well as developing countries. There may be several reasons for this, including prolonged periods of low rainfall together with high evaporation rates (e.g. Australia), as well as increasing demands for fresh water resources due to exponential population growth (e.g. South East Queensland and Japan) (Eriksson et al., 2002).

Recycling and reuse of wastewater for both potable and non-potable purposes has been an important topic of debate on how to overcome the consequences of extensive drought conditions. Some of the potential options for conservation of potable water or freshwater resources include reuse of treated sewage effluents for non-potable purposes (e.g. irrigation of food crops and large fields and parklands). Reuse of greywater from households on gardens and for toilet flushing has also been suggested.

While greater use of recycled water is thought to be one of the solutions to Australia’s current water shortage and future water needs, there is mixed public attitude towards use of recycled water for potable purposes. There is considerable level of public concern about the use of treated effluents or greywater which can be attributed to the potential risks it poses on human or environmental health by pathogens or inorganic and organic contaminants. While some countries already sell purified recycled water (e.g. Singapore), there has been some strong opposition from some Australian community activist groups towards recycled water for potable purposes.

An increasing number of new and emerging organic contaminants has been detected in effluents from WWTPs, at concentrations shown to have adverse ecological impacts. Examples of potentially domestic wastewater based organic contaminants frequently detected in WWTP effluents include:

- steroid hormones and their metabolites (e.g. 17β-estradiol: E2, and estriol E3, estrone: E1)
- pharmaceuticals and their metabolites (e.g. 17α-ethynylestradiol: EE2, ibuprofen: IBU, and carbamazepine: CBZ)
- personal care products (e.g. insect repellent N,N-Dimethyl-m-toluamide: DEET, and musk fragrances galaxolide: HHCB and tonalide (AHTN)
- domestic and industrial detergent residues and degradates (e.g. linear alkyl benzene sulphonates (LAS), nonylphenol ethoxylates (NPEOs) and degradates (e.g. 4t-octylphenol: OP and 4-nonylphenol: NP)
- antimicrobial agents (e.g. triclosan, TCS)
- plasticiser (e.g. bisphenol A: BPA)
Reuse of treated effluents could minimize the ecological risk posed by exposure of organisms to such anthropogenic contaminants (Neal, 1996). On the other hand, direct discharge of treated effluents, land application of biosolids or the use of recycled water for non-potable purposes (e.g. irrigation) can introduce potential contaminants into the environment. Similarly, concern over potential human health risks associated with exposure to such xenobiotic chemicals is also increasing.

WWTPs generally receive wastewater from a number of different sources including residential, commercial and trade wastes. To date, very little is known about the composition of different input waste streams into WWTPs, which are not designed to remove all of the potential contaminants. The identification of sources of new and emerging contaminants may be an important step in effective treatment of wastewaters, or source control strategies for such contaminants of concern.

This report is part of the Smart Water Fund project Round 3 – Project 5 Household sources of priority contaminants in domestic wastewater aimed to understand the sources of priority and emerging contaminants in residential wastewater, and their contribution to the overall composition and characteristics of urban wastewater streams. The contaminants studied were selected because of their potential to influence the beneficial reuse of biosolids and the recycling of effluent.

Priority inorganic contaminants, i.e. heavy metals, cationic/anionic species, total dissolved solids and colour, for which standard analysis methodology is established, have been characterised by source and evaluated in detail in the other reports from the project (Tjandraatmadja et al 2009, 2010).

Emerging organic contaminants, which have in recent years have received increasing attention, were evaluated in domestic wastewater as an initial step to define the scope for future research.

Other reports from this project include:

- Sources of critical contaminants in domestic wastewater: a literature review (Tjandraatmadja and Diaper 2006);
- Sources of emerging organic contaminants in wastewater: an assessment based on the literature review (Shareef et al 2008);
- Sources of priority contaminants in domestic wastewater: contaminant loads from household products (Tjandraatmadja et al 2008);
- Sources of contaminants in domestic wastewater: contaminant loads from household appliances (Diaper et al 2008);
- Characterisation of priority contaminants in residential wastewater (Tjandraatmadja et al 2009);
- Investigation of seasonality effects on domestic wastewater quality (Tjandraatmadja et al 2009);
- Sources of priority contaminants in domestic wastewater II: nutrients and additional elements from household products (Tjandraatmadja et al 2010)
- Impact of source management strategies on quality and loads in residential wastewater: scenario analysis (Cook et al 2010).
1.2. Objectives

The key objectives of the work described in this report included:

(i) Identification of potential organic contaminants likely to be present in domestic wastewater derived from humans, household products and infrastructure (e.g. pipework) through a review of current literature.

(ii) To predict the expected threshold levels of potential contaminants in domestic wastewater derived from residential wastewater streams.

(iii) To develop robust and sensitive analytical methodologies for trace level analysis of the emerging contaminants of concern in domestic wastewater streams.

(iv) To determine the concentration of selected organic contaminants in domestic wastewaters.

(v) To evaluate the possible implications and challenges for municipal WWTPs based on domestic wastewater composition and concentrations of contaminants.

1.3. Outline of This Report

The report is comprised of the following sections.

Section 1: Introduction

A brief general introduction to the project and the issues pertinent to it are described here.

Section 2: Overview Based on Literature

In this section, an overview of available literature is presented (Shareef et al 2008). The literature review was undertaken to identify potential contaminants in domestic wastewater (including information of analytical methodologies).

Section 3: Analytical Method Development

The process of analytical method development has been described in this section. Based on chemicals identified as potential contaminants in residential wastewaters, suitable analytical methods have been identified and developed using a range of techniques including gas and liquid chromatographic-mass spectrometric techniques. Details of the process of methodology development are explained in detail in Shareef and Williams (2009).
Section 4: Analysis of EDCs and PPCPs in Domestic Wastewater

Application of the analytical methods developed in this project through a small survey of selected number of domestic wastewater samples during winter and summer periods of 2008, has been described here.

Section 5: Conclusions

General conclusions, drawn from the sections 1-4 are given here. These included the selection of potential contaminants, possible analytical methods, the outcomes of analytical method development process and the main findings from the case study on analysis of domestic wastewater.

Section 6: Future Directions

Some of the issues which arose from the key findings from this project and some of the possible future approaches and strategies to deal with them have been described here.
2. LITERATURE REVIEW

2.1. Characterisation of Organic Contaminants in Domestic Wastewater Streams

Recently there has been increasing number of new and emerging organic contaminants detected in treated effluents from WWTPs. These may include natural and anthropogenic chemicals including human derived chemicals (e.g. endogenous hormones or metabolic by-products) as well as various synthetic compounds used by humans such as pharmaceuticals and personal care and household cleaning products.

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 may be attributed to the potential adverse ecological impact of these contaminants. Similarly, there have been concerns about the potential human health risks following possible exposure though contaminated water (e.g. recycled water used for crop irrigation and other non-potable purposes). Certain chemicals classified as endocrine disrupting chemicals (EDCs), and pharmaceuticals and personal care products (PPCPs) have received significant attention recently due to the emergence of compelling evidence suggesting adverse environmental or human health effects.

Some of these potential contaminants include (i) natural and synthetic steroid hormones and pharmaceuticals, and their metabolites (e.g. 17β-estradiol, estriol, estrone and 17α-ethynylestradiol), (ii) domestic and industrial detergent residues and degradates (e.g. linear alkyl benzene sulphonates (LAS) and nonylphenol ethoxylates (NPEOs), (iii) widely used antibacterial agents such as triclosan, TCS and plasticizers such as bisphenol A (BPA), as well as other personal care products (e.g. insect repellent N,N-Dimethyl-m-toluamide: DEET, musk fragrances galaxolide: HHCB, and tonalide:AHTN.

2.2. Identification of Potential Contaminants for Analysis

A recent literature review (Shareef et al., 2008) conducted as part of this project identified a range of organic contaminants of emerging environmental concern. The selection of potential contaminants for analysis in residential wastewater samples for this project were chosen in consultation with expert opinion from Australia and based on information from literature. The following broad criteria have been applied in the selection.

(i) the origins, occurrence, reported concentration and frequency of a given contaminant in WWTP effluents;

• degree of potential human and environmental health risks;

• fate and transport behaviour of a contaminant in the environment (including WWTP removal rates) and the implications on wastewater treatment processes;
• availability of suitable analytical methodologies for trace level analysis in wastewater or environmental samples.

2.3. Environmental Occurrence, Fate and Effects of EDCs and PPCPs

While the literature review conducted as part of this project provides extensive information on the environmental occurrence, fate and effects; an overview of literature is presented here for completeness of this report. For detailed information and of these aspects, reference to the full literature review is recommended (refer to Shareef et al, 2008).

EDCs and PPCPs

EDCs include a wide range of natural and synthetic chemicals that interfere with the normal function of the endocrine system in animals and humans (either mimicking or blocking endogenous hormones) (Hammond et al., 1979; Kelce et al., 1994; Patlak, 1996; Sonnenschein and Soto, 1998). The number of studies pertinent to environmental occurrence, fate and transport behaviour of EDCs and PPCPs are increasing (Snyder et al., 1999; Mol et al., 2000; Helaleh et al., 2001; Lopez de Alda and Barcelo, 2001; Xiao et al., 2001; Benijfs et al., 2002; Brossa et al., 2003; Ding and Chiang, 2003; Gomes et al., 2003; Lagana et al., 2004; Liu et al., 2004; Quintana et al., 2004; Rodriguez-Mozaz et al., 2004; Labadie and Budzinski, 2005).

Human excretion and improper disposal of pharmaceuticals (e.g. unused medicines from households), are among factors which can lead to variable levels of such drugs in domestic wastewaters. A range of pharmaceuticals including analgesics, antibiotics, anti-inflammatory drugs, blood lipid regulators, beta-blockers, antiepileptics, oral contraceptives and others have been frequently detected in the environment (Daughton and Ternes, 1999; Heberer et al., 2002; Khan and Ongerth, 2002; Ternes et al., 2003). Human and animal exposure to EDCs and PPCPs is suspected to come primarily from water contaminated by discharge from sewage treatment works. Therefore, the fate and transport behaviour of these compounds are important in better risk characterisation.

Fate and Levels in the Environment

Current technologies used in WWTPs are not designed to remove EDCs and PPCPs completely (Johnson and Sumpter, 2001; Braga et al., 2005). Snyder and co-workers (Snyder et al., 2003), who recently evaluated the removal efficiencies of some conventional wastewater treatment technologies for a range of common EDCs and PPCPs, reported that these chemicals are not completely eliminated by the WWTPs. However, biodegradation and Reverse Osmosis processes were generally found to result in greater than 90% removal of the most commonly investigated EDCs and PPCPs. Some studies have also reported relatively high removal rates (e.g. up to about 90%) for some of the EDCs (e.g. E2, EE2, NP) using conventional tertiary treatment processes (Andersen et al., 2003a; Lee et al., 2005). Also recent Australian studies (e.g. (Ying et al., 2008) (Tan et al., 2007)) reported good removal efficiencies of selected estrogens, some non-ionic surfactants and BPA in WWTPs
employing different treatment technologies. High removal rates for estrogens, alkylphenols and the antimicrobial TCS have been observed by many other researchers which maybe attributed, in part, to their physicochemical properties (Ying and Kookana, 2005; Ying and Kookana, 2007b).

The concentrations of some of the most potent chemicals from the class of EDCs (e.g. steroid estrogens) have been reported in rivers, waterways and ground water receiving treated effluents from wastewater treatment plants in the range of 0.1-17 ng L\(^{-1}\) for E1 and 0.1-5.1 ng L\(^{-1}\) for EE2 (Lee and Peart, 1998; Larsson et al., 1999; Ternes et al., 1999; Kuch and Ballschmiter, 2001; Promberger and Schmid, 2001; Gentili et al., 2002; Lagana et al., 2004; Quintana et al., 2004). This makes sewage and wastewater treatment plants major environmental sources of EDCs (Lai et al., 2000; de Rudder et al., 2003; Johnson Andrew and Williams Richard, 2004).

The wide range of PPCPs detected in the environment may originate from human usage and excretion, veterinary applications of a variety of products including prescription/non-prescription medications (Daughton and Ternes, 1999). Ternes and co-workers (Ternes et al., 2003) recently reported that effluents from a German STP contained concentrations of some five antibiotics ranging from 0.34 to 0.63 \(\mu g\) L\(^{-1}\), five betablockers ranging from 0.18 to 1.70 \(\mu g\) L\(^{-1}\), 4 antiphlogistics ranging from 0.10 to 1.3 \(\mu g\) L\(^{-1}\), two lipid regulator metabolites ranging from 0.12 to 0.13 \(\mu g\) L\(^{-1}\) and the antiepileptic drug carbamazepene at 2.1 \(\mu g\) L\(^{-1}\). PCPs are also detected in WWTP effluents. For example, concentrations of AHTN and HHCB from a German STP have recently been reported to be 0.12 \(\pm\) 0.03 and 0.75 \(\pm\) 0.14 \(\mu g\) L\(^{-1}\) respectively. It has also been estimated that the 50 most common drugs dispensed in Australia (including analgesics, anti-inflammatory, antibiotics, betablockers, antiepileptics and lipid regulators) are likely to be found in wastewater at concentrations ranging between 0.2 ng L\(^{-1}\) up to 8 \(\mu g\) L\(^{-1}\) (Khanh and Ongerth 2004).

**Concentrations in Household Wastewater**

The identification of sources of organic contaminants of emerging concern in WWTPs influents derived from residential wastewater streams is one of the aims of this project. There is very little information on composition of organic contaminants in domestic wastewater in the literature. However, an extensive survey of household greywater from Copenhagen, Denmark by Eriksson and co-workers (2003) reported concentrations of some common surfactants and plasticizers to be in the microgram per liter range (e.g. LAS: 25-450 \(\mu g\) L\(^{-1}\), NPEOs < 5 \(\mu g\) L\(^{-1}\) and Octylphenol ethoxylates: OPEOs < 3 \(\mu g\) L\(^{-1}\). Similarly, a recent Swedish study by Palmquist and Hanæus (2005) identified 46 organic compounds in greywater and 26 other compounds in black water; compounds included nonylphenol- and octylphenol ethoxylates, and triclosan, among others.

**Biological Effects**

The long-term effects of continuous, low-level exposure to EDCs and PPCPs and their metabolites are not well understood (Daughton and Ternes, 1999). This is a matter of concern since pharmaceuticals are designed to be biologically active and may affect non-target organisms. Steroid hormones (e.g. E1, E2 and EE2), xenoestrogens (e.g. NP, NPE1-2) are known to cause EDC effects in terrestrial and
aquatic organisms. For example, feminization of male fish indicated by the imposex condition or the production of the yolk protein vitellogenin is a classic example of the effect of low ng L⁻¹ exposure of EE2 to fish (Purdom et al., 1994; Folmar et al., 1996; Jobling et al., 1996; Harries et al., 1997; Jobling et al., 1998; Metcalfe et al., 2001; Wibe et al., 2002; Tyler et al., 2005). The ecological or human impact of exposure to EDCs is not fully understood.

Although a clear cause and effect relationship between exposure to EDCs in the environment and human health is yet to be established, some studies have shown decrease in sperm count and ejaculate volume, increase in testicular and breast cancer, increase in deformities of the genital organs in humans (Sharpe and Skakkebaek, 1993; Wolff et al., 1993; Sharpe, 2001), as well as increased sex reversal, reduced hatch/birth rate, and disrupted breeding behaviour in wildlife (Matlai and Beral, 1985; Henderson et al., 1988; Routledge et al., 1998; Iguchi and Tomomi, 2000).

Pharmaceuticals (e.g. antibiotics) can induce bacterial resistance resulting in the development of so called “super bugs” (Richardson, 2007), or the prevalence of antibiotic-resistant bacteria upon exposure to untreated hospital and domestic sewage effluents (Andersen et al., 2003b). Other PCPs such as musk fragrances (e.g. AHTN and HHCB) have also shown to cause reproductive and growth abnormalities in fish by affecting endocrine systems (Thorpe et al., 2001). Similarly, aquatic toxicities for algae, aquatic invertebrates, and fish following exposure to the antimicrobial TCS have been observed in a number of studies (Committee TSS, 2002; Consortium TT, 2002; SCCP SCoCP, 2005).

**Analytical Methods**

Both chemical and bio-analytical techniques have been commonly used in the identification of EDCs and PCPPs (e.g. Enzyme Linked Immuno-Sorbent Assay: ELISA, chromatographic-mass spectrometric methods). ELISA is a highly sensitive and rapid screening technique used in the determination of certain compounds for which antibodies have been developed. This technique is available for some steroidal hormones including E1, E2 and EE2 with a detection limit of 0.05 ng L⁻¹, but with some practical limitations (e.g. cannot be spiked with surrogates for recovery tests, operational pH, and temperature boundaries). Both gas and liquid chromatographic techniques (e.g. gas chromatograph-mass spectrometry: GC-MS, liquid chromatography: LC-MS and GC- or LC-MS-MS) have been employed more widely in the analysis of a wide range of emerging organic contaminants including endocrine disrupting chemicals, pharmaceuticals and personal care products (Kuch and Ballschmiter, 2000; Mol et al., 2000; Heemken et al., 2001; Lopez de Alda and Barcelo, 2001; Promberger and Schmid, 2001; Xiao et al., 2001; Brossa et al., 2003; Ding and Chiang, 2003; Liu et al., 2004; Quintana et al., 2004; Garcia et al., 2005; Labadie and Budzinski, 2005). Other techniques such as HPLC-UV, HPLC-Fluorescence detection methods have also been employed in the analysis EDCs and PPCPs.

**Analytical Method Detection Limits**

Although both GC-MS and LC-MS methods are suitable analytical methods, because of the low levels (ng L⁻¹) of typical contaminants in wastewaters and in the
environment, sample clean-up and pre-concentration using solid phase extraction (SPE) technique are necessary to achieve adequate sensitivities for quantitative determination of the target analytes. Pharmaceuticals and other polar compounds have commonly been analysed by LC-MS/MS in electrospray ionisation (ESI) mode. Detection limits of less than 5 ng L$^{-1}$ (e.g. EE2), are typically reported with good SPE recoveries from STP effluent samples (Miao et al., 2002; Pedrouzo et al., 2007). GC-MS has also been widely used for analyses of EDCs and PPCPs. For example, Zhang et al. (2007) recently used GC-MS and quantified many PPCPs including ibuprofen, TCS, BPA, carbamazepene, E1 and E2 in river water samples. GC-MS in electron impact (EI) ionisation mode has also been the most widely used technique in the analysis of the polycyclic musks for a number of reasons (Rimkus, 1999). Typically GC-MS detection limits ranged from 5-20 ng L$^{-1}$ for a wide range of compounds of interest in the present study.

**Recommended List of Contaminants**

Table 2.1 presents the list of potential organic contaminants of emerging concern derived from human and other household sources including personal care and cleaning products identified to be included in the case study analysis. The proceeding sections will describe the analytical method development undertaken in this project for these compounds.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Detection method (LOQ: ng L⁻¹)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>17β-estradiol (E2)</td>
<td>GCMS (5)</td>
<td>• Endocrine disrupting chemicals • Generally detected in low ng L⁻¹ in aquatic environments • Excreted by humans and animals as conjugates which are hydrolysed during transport in the sewerage systems • Undergoes aerobic biodegradation in the environment</td>
</tr>
<tr>
<td>Estrone (E1)</td>
<td>ELISA (0.05)</td>
<td></td>
</tr>
<tr>
<td>17α-Ethynylestradiol (EE2)</td>
<td>LCMSMS (0.1)</td>
<td></td>
</tr>
<tr>
<td>Estriol (E3)</td>
<td>GCMS (10)</td>
<td>• An EDC of industrial origin</td>
</tr>
<tr>
<td>Bisphenol A (BPA)</td>
<td>GCMS (10)</td>
<td>• Mainly industrial chemicals, with some limited domestic use • Ethoxylates degrades to the alkylphenols</td>
</tr>
<tr>
<td>4t-octylphenol (OP)</td>
<td>GCMS(20)</td>
<td>• Commonly found in personal care products • Low µg L⁻¹ in aquatic environments, higher levels in sludge and soils, sediments</td>
</tr>
<tr>
<td>4-Nonylphenol (NP)</td>
<td>GCMS (20)</td>
<td>• Metabolite of triclosan</td>
</tr>
<tr>
<td>NP-Ethoxylates (NP₁₂OE)</td>
<td>GCMS (20)</td>
<td>• Polycyclic musk fragrances • Weak EDC effects • µg L⁻¹ levels in the environment</td>
</tr>
<tr>
<td>Triclosan (TCS)</td>
<td>GCMS (10)</td>
<td>• Pharmaceutical compounds • Sertraline is highly prescribed antidepressant in Australia • Biological effects in aquatic species</td>
</tr>
<tr>
<td>Methyl- triclosan (MeTCS)*</td>
<td>GC-MS (-)</td>
<td></td>
</tr>
<tr>
<td>N,N-Dimethyl-m-toluamide (DEET)*</td>
<td>GC-MS (500)</td>
<td></td>
</tr>
<tr>
<td>Linear alkylbenzene sulphonates (LAS₉-1₃)</td>
<td>HPLC-FLD</td>
<td>• Common household detergent ingredient • Complex analysis</td>
</tr>
<tr>
<td>Galaxolide (HHCB )*</td>
<td>GC-MS (20)</td>
<td></td>
</tr>
<tr>
<td>Tonalide (AHTN)*</td>
<td>GC-MS (20)</td>
<td></td>
</tr>
<tr>
<td>Carbamazepine*</td>
<td>LC-MS/MS</td>
<td></td>
</tr>
<tr>
<td>Fluoxetine*</td>
<td>LC-MS/MS (3.3)</td>
<td></td>
</tr>
<tr>
<td>Sertraline*</td>
<td>LC-MS/MS (1.9)</td>
<td></td>
</tr>
<tr>
<td>Ibuprofen*</td>
<td>LC-MS/MS (low ng L⁻¹)</td>
<td></td>
</tr>
</tbody>
</table>

LOQ: Limit of quantitation based on analytical method validation using SPE clean-up and pre-concentration of 1 L of water sample spiked with appropriate chemical standards. * Literature LOQs.
3. ANALYTICAL METHOD DEVELOPMENT

Based on the information from literature, we set out to develop analytical methods for analysis of the selected contaminants in Table 2.1 in domestic wastewaters.

3.1. Selection of Analytical Methods

On the basis of the available literature and the analytical capabilities available at CSIRO, Adelaide laboratories, method development was conducted using (i) GC-MS, (ii) LC-MS/MS, and (iii) HPLC-Fluorescence (HPLC-FLD) techniques. Table 3.1 summarises the selection of analytical methods for the compounds of interest for this project.

<table>
<thead>
<tr>
<th>Compounds to be analysed</th>
<th>Analytical technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-4 Estrogens (Estrone: E1, Estradiol: E2, Estriol: E3, Ethynylestradiol: EE2)</td>
<td>GC-MS</td>
</tr>
<tr>
<td>5-8 Non-ionic surfactant (Octylphenol: OP, Nonylphenol: NP, NP- Ethoxylates: NPE1 and NPE2)</td>
<td>GC-MS</td>
</tr>
<tr>
<td>9-10 Antimicrobials (Triclosan: TCS and Methyl triclosan: Me-TCS)</td>
<td>GC-MS</td>
</tr>
<tr>
<td>11 Plasticiser (Bisphenol A:BPA)</td>
<td>LC-MS/MS</td>
</tr>
<tr>
<td>12 Insect repellent (N,N-Dimethyl-m-toluamide: DEET)</td>
<td>HPLC-FLD</td>
</tr>
<tr>
<td>13-14 Musks (Galaxolide: HHCB and Tonalide: AHTN)</td>
<td>HPLC-FLD</td>
</tr>
<tr>
<td>15 Carbamazepine</td>
<td>LC-MS/MS</td>
</tr>
<tr>
<td>16-17 Fluoxetine and Sertraline</td>
<td>HPLC-FLD</td>
</tr>
<tr>
<td>18 Ibuprofen</td>
<td>HPLC-FLD</td>
</tr>
<tr>
<td>19 Linear alkylbenzene sulphonates (LAS_{c_{10-13}})</td>
<td>HPLC-FLD</td>
</tr>
</tbody>
</table>

3.2. Sample Preparation

Waste samples were extracted and pre-concentrated by solid phase extraction. 1.0 L wastewater filtered through Whatman filters (GF/A and GF/C glass fibre filter) was returned to collection bottles, spiked with 100 μL of 1 mg L⁻¹ (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 4.0 mL milliQ water followed by 2 x 4.0 mL methanol and 2 x 4.0 mL of dichloromethane. After washing the cartridges with 2% methanol, and air drying (1-2h), the analytes of interest were eluted with 2 x 4.0 mL methanol, followed by 2 x 4.0 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 were stored at -18 °C, until they are ready for analysis, at which time the samples are eluted and prepared for analysis, as above.

3.3. Analytical Methods

**GC-MS**

For analysis by GC-MS analysis, 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 1h. 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 1mL min⁻¹ was used as the carrier gas. A splitless injection port liner was used for sample analysed in splitless injection mode. The oven temperature was programmed as follows: 75°C (1 min) to 150°C (10°C min⁻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 range between 25 and 1000 μg/L. Good linearity and reproducibility of analyses (R² > 0.99), were achieved.

**LC-MS/MS**

A Thermo-Finnigan TSQ Quantum Discovery Max (Thermo Electron Corporation) LC-MS/MS was used for analysis of the selected compounds. A BDS Hypersil C18 column (150 x 2.1 mm, 3 μm particle size) using a mobile phase flow rate of 0.2 mL min⁻¹ was used for separation of analytes. The mobile phase composition was 0.1 % acetic acid (A) and HPLC grade acetonitrile (B) using the following gradient parameters; 90 % -30 % A (0-5 min), 0 % A (6-15 min), 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 and the ratios between the transition ions.
3.4. Some QA/QC Considerations

Calibration curves for the analytes were obtained in the concentration ranges of typical environmental detection levels. For examples typical calibration curves with concentration ranges between 25 to 1000 \( \mu \)g L\(^{-1} \) were used for analyte quantification. Good linearity and repeatability \((r^2 > 0.99)\) were obtained.

Extraction efficiencies were optimised using different SPE cartridges (e.g. C18 and Oasis HLB). Using HLBS, recoveries exceeding 80% were achieved for most of the analytes from milliQ or tap water-spiked samples (low ng/L). However, due to the complex nature of wastewater samples, the relative recoveries of spiked wastewater samples are more variable, and can typically range from 60-120%. While initial tests with C18 cartridges also gave good recoveries, they were not used due to practical limitations (e.g. may not be allowed to dry during sample loading).

In order to avoid contamination, all sampling bottles were washed with laboratory grade detergent (e.g. Pyroneg and Extran MA03. Merck, Australia), rinsed with deionised, reverse osmosis, ultrapure water and acetone, before being baked at 300-400°C for at least 4 hours.

3.5. Results and Discussions

Table 3.2 presents information relating to analytical method development for the compounds for which analytical method development has been successful. Due to time and resources constraints, the compounds in Table 3.2 have been used in the case study analysis discussed in Section 4 of this report. The detailed method development is described in Shareef and Williams (2009).

Other compounds analysed were either below the LOD or could not be positively identified.
Table 3.2 Overview of methods and analytical method parameters for selected compounds for analysis in domestic wastewater samples

<table>
<thead>
<tr>
<th>Analytes</th>
<th>SPE Recovery (%) $^a$</th>
<th>Analytical method parameters</th>
<th>MS fragmentation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Method (LOQ: ngL$^{-1}$)</td>
<td></td>
</tr>
<tr>
<td>1. Estradiol (E2)</td>
<td>120 ± 14</td>
<td>GCMS (5)</td>
<td>231</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LC-MS/MS (1)</td>
<td>327,416$^*$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>271 [M+H]$^+$ $\rightarrow$ 183,145</td>
</tr>
<tr>
<td>2. Estrone (E1)</td>
<td>125 ± 14</td>
<td>GCMS (5)</td>
<td>342$^*$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LC-MS/MS (1)</td>
<td>218,244,257</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>269 [M+H]$^+$ $\rightarrow$ 145,143</td>
</tr>
<tr>
<td>3. Ethynylestradiol (EE2)</td>
<td>127 ± 17</td>
<td>GCMS (5)</td>
<td>425</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LC-MS/MS (1)</td>
<td>285, 300, 440$^*$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>295 [M+H]$^+$ $\rightarrow$ 145,159</td>
</tr>
<tr>
<td>4. Bisphenol A (BPA)</td>
<td>109 ± 5</td>
<td>GCMS (10)</td>
<td>357</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>191, 217, 372$^*$</td>
</tr>
<tr>
<td>5. Octylphenol (OP)</td>
<td>115 ± 25</td>
<td>GCMS (20)</td>
<td>278$^*$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>207, 263</td>
</tr>
<tr>
<td>6. Nonylphenol (NP)</td>
<td></td>
<td>GCMS (20)</td>
<td>179$^*$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>73, 277, 292$^*$</td>
</tr>
<tr>
<td>7. NP-mono-ethoxylates (NPE1)</td>
<td>163 ± 48</td>
<td>GCMS (20)</td>
<td>265$^*$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>251, 336, 307$^*$</td>
</tr>
<tr>
<td>8. NP-di-ethoxylates (NPE2)</td>
<td></td>
<td>GCMS (20)</td>
<td>295</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>281, 309, 351$^*$</td>
</tr>
<tr>
<td>9. Triclosan (TCS)</td>
<td>113 ± 3</td>
<td>LC-MS/MS (5)</td>
<td>200$^*$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>347$^*$, 362, 310</td>
</tr>
<tr>
<td>10. Carbamazepine</td>
<td>103 ± 5</td>
<td>LC-MS/MS (10)</td>
<td>237 [M+H]$^+$ $\rightarrow$ 193,194</td>
</tr>
<tr>
<td>11. Ibuprofen</td>
<td>93 ± 15</td>
<td>LC-MS/MS (30)</td>
<td>205[M-H]$^-$ $\rightarrow$ 161$^*$</td>
</tr>
</tbody>
</table>

$^a$ SPE recovery data obtained extraction of analytes spiked in worm casting leachate using Waters HLB cartridges (error is standard deviation of n=6 samples).

* Quantitation ions LCMSMS = mass/charge (m/z) based on transition of parent ion to daughters and GC-MS: base on EI fragmentation pattern of parent molecules.
4. CASE STUDY: ANALYSIS OF EDCS AND PPCPS IN DOMESTIC WASTEWATER

4.1. Background

Municipal WWTPs may receive heterogeneous wastewater from mixed sources including residential, trade and industrial sources. Therefore, the traditional end-of-pipe treatment of such wastewaters to meet regulatory guidelines is becoming increasingly difficult and expensive. This is especially true for new and emerging contaminants.

Recently there has been greater focus on options such as onsite treatment of wastewaters, source separation (e.g. black water and greywater separation in homes) to better manage and reduce the cost of wastewater treatment processes. The aim of this investigation was to evaluate the contribution of potential contaminants derived from (i) household products, (ii) appliance operation and (iii) infrastructure and human input.

Wastewater samples were collected from two selected sites within a residential catchment (Fig. 4.1). Samples from site 1 were composed of wastewater from 7 households, while site 2 samples were made up of wastewater contribution from 163 households, both receiving input from only residential sources (i.e. no trade waste or commercial input). The catchments were selected to ensure that wastewater monitored would be representative of residential discharge only, and would also be characteristic of the largest population segments in Melbourne. Detailed description of potential contaminants, site description, sampling protocols and sampling regimes are given in the following sections.

4.2. Potential Contaminants

The contaminants selected for analysis, based on the criteria described in Section 2.2 are given in Table 4.1. Information on physicochemical properties, WWTP removal efficiencies, possible effects and the availability of analytical methods have been included. A full description of such effects can be found in Shareef et al (2008).
### Table 4.1 Organic Contaminants of Emerging Concern: A Summary of Some of the Physico-Chemical Properties, Environmental Occurrence, Fate, Effects and Analytical Methods for Selected Compounds.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Compounds [CAS], MW</th>
<th>Log $K_{ow}$</th>
<th>WWTP Levels</th>
<th>WWTP Removal</th>
<th>Possible Effects</th>
<th>Detection Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroid Hormones</td>
<td>17β-estradiol (E2)</td>
<td>3.94 $^a$</td>
<td>Low ng L$^{-1}$</td>
<td>High, but incomplete</td>
<td>Potent EDCs</td>
<td>GC-MS</td>
</tr>
<tr>
<td></td>
<td>[50-28-2] MW: 272</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Estrone (E1)</td>
<td>3.43 $^a$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[53-16-7] MW: 270</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surfactants</td>
<td>4-Nonylphenol (NP)</td>
<td>5.76 $^b$</td>
<td></td>
<td>Generally good but incomplete</td>
<td>Weak EDCs</td>
<td>GC-MS</td>
</tr>
<tr>
<td></td>
<td>[25154-52-3], MW: 220</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nonylphenol ethoxylates (NP$_{1-2}$EO)</td>
<td>4.2 $^c$</td>
<td>µg L$^{-1}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[104-35-8], MW (NP1EO): 264, (NP1EO):308</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4-t-Octylphenol (OP)</td>
<td>4.12 $^b$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[140-66-9], MW: 206</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasticizers</td>
<td>Bisphenol A (BPA)</td>
<td>2.20 $^d$</td>
<td>low µg L$^{-1}$</td>
<td>Good</td>
<td>Weak EDC effects</td>
<td>GCMS</td>
</tr>
<tr>
<td></td>
<td>[80-05-7], MW: 228</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4.1 continued.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Compounds [CAS], MW</th>
<th>Log $K_{ow}$</th>
<th>WWTP Levels</th>
<th>WWTP Removal</th>
<th>Possible Effects</th>
<th>Detection Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antimicrobial Agents</strong></td>
<td><strong>Triclosan (TCS)</strong></td>
<td>4.7 $^f$</td>
<td>low $\mu$g L$^{-1}$</td>
<td>Good</td>
<td>Antibiotic resistance</td>
<td>GC-MS</td>
</tr>
<tr>
<td></td>
<td>[3380-34-5], MW: 289</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pharmaceuticals</strong></td>
<td><strong>17α-ethynylestradiol (EE2)</strong></td>
<td>4.15</td>
<td>low $\mu$g L$^{-1}$</td>
<td>Low to moderate</td>
<td>Possible effects in aquatic species</td>
<td>GC-MS or LCMS/MS</td>
</tr>
<tr>
<td></td>
<td>[57-63-6], MW: 296</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Carbamazepine</strong></td>
<td>2.45 $^g$</td>
<td>low $\mu$g L$^{-1}$</td>
<td>Low to moderate</td>
<td>Some EDC effects</td>
<td></td>
</tr>
<tr>
<td></td>
<td>[298-46-14], MW: 236</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Ibuprofen</strong></td>
<td>4.91 $^g$</td>
<td>low $\mu$g L$^{-1}$</td>
<td>Low to moderate</td>
<td>Possible effects in aquatic species</td>
<td>LCMS/MS</td>
</tr>
<tr>
<td></td>
<td>[15687-27-1], MW: 206</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$(Yu et al., 2004), $^b$(Ahel and Giger, 1993b), $^c$(Ahel and Giger, 1993a), $^d$(Dorn et al., 1987), $^e$(Vieno et al., 2007), $^f$(Halden and Paull, 2005), $^g$(Ahel and Giger, 1993a).
4.3. Sampling

4.3.1. Site Description

A residential catchment located north of Skye Rd in Frankston North along the Mornington coastline, Victoria, (Fig. 4.1) was selected for this study. The Frankston area is characterized by households with an average income range of $1200 to $1399 per week (ABS 2006). Households in the area are typically comprised of couples (39.3%), couples with children (36.5%), one parent families (22.4%) and other families (1.7%) (ABS 2006). The catchment was selected because of its residential wastewater, network characteristics and for being representative of one of Melbourne’s major population groups. The site selection process is described in Tjandraatmadja et al (2009).

![Fig. 4.1. Selected Catchment (SEWL 2006)](image)

The selected catchment was a residential zone 1 and 2 neighbourhood (see Fig. 4.2). Properties in the area were stand alone houses. The sewer network had been installed from 1970 to 1979 and from 1980 to 1989. It was comprised mainly of gravity sewers, but also had one section with a rising main after a pump station. Sewer pipes were made of mostly of vitrified clay, with short sections of cast iron, ductile iron, polyethylene and PVC. The catchment was in good condition (asset condition grading 1) and infiltration was assessed to be in the lowest classification band (0 - 5L m$^{-1}$ d$^{-1}$) (SEWL 2006).

Wastewater samples were collected from two manholes within the catchment. Site 1 was a manhole located on a nature strip over a vitrified clay pipe (DN 150mm) that received wastewater from 7 households. A weir was constructed in the manhole to allow sampling probes to be immersed in wastewater as flow on this site was intermittent throughout the day and the original wastewater level was too low for sample collection. Site 2 was a manhole located over a vitrified clay pipe (DN225mm) prior to entry into South East Water’s SPS 378 pump station at Lanena
Court, which received wastewater from 163 households and had 24h continuous flow.

Fig. 4.2 Schematic diagram showing the relative position of sampling sites (not drawn to scale). Each site was fitted with an automatic wastewater sampling device (Isco model 6712 autosampler) and a flow and conductivity meter supplied by ADS Environmental Services.

4.3.2. Sample Collection Protocols

Isco 6712 autosamplers (Fig. 4.3), fitted with twenty-four 300mL pyrex bottles were used for collection of wastewater. Each of the glass bottles was dosed with 0.5mL 5M H₂SO₄. A metal strainer was fitted to the end of the sampling hose, which was continuously submerged in wastewater. Each autosampler was housed in a metal enclosure for protection against tampering.
At each manhole, wastewater samples were collected on an hourly basis over a 24h period using an automated sampler filled with ice. Samples were collected and transported in an Esky with ice, to the CSIRO laboratories daily. Samples were collected as hourly composites. A 250mL volume of sample was collected every hour as an hourly composite. Hourly samples collected over 24h were combined to prepare a 24-hour daily composite sample based on the daily volumetric flow rate.

Tap water was sampled from garden taps located at the two sampling sites. Water was allowed to run continuously for 5 min to flush the line, before a grab sample was collected using pre-conditioned bottles.

Composite samples were acidified with H$_2$SO$_4$ (pH<2), and stored in 1L amber pyrex bottles with aluminium covered lids in the dark in a cool room (T< 4°C) until transport and analysis.

4.3.3. Sampling Regimes

- Site 1 (7 households): Triplicate one off sampling was conducted on 30 March 2008, at this point.

- Site 2 (163 households): Sampled from 12-17 March 2008 (summer samples, and then from 15-23 July 2008 (winter samples). Daily 24h composite samples made from hourly samples were prepared for analysis of organic contaminants.

- Greywater samples were also collected from a field-house where samples were collected from (i) a dishwasher, (ii) shower and (iii) a washing machine.

4.4. Results and Discussions

The concentration of selected organic compounds in samples from residential network sewer receiving only domestic wastewater including grey and black water are given in this section.
4.4.1. Daily Concentrations (site 2)

The daily variation in concentration of selected organic contaminants in domestic wastewater, from 24h composite samples taken from a catchment of 163 houses (Site 2, Fig. 4.2) in an urban region of Victoria is shown in Fig.4.4. A 7-day long sampling period was conducted during (i) winter (15-23 July) and (ii) summer (12-17 March) of 2008. The concentrations of the selected estrogens (estradiol: E2, estrone:E1) and the xenoestrogen 4t-octylphenol: OP, triclosan: TCS and bisphenol A: BPA are given in Fig. 4.4 (a) (winter), and Fig. 4.4(b) (summer), respectively.

These results indicate that except for a few compounds (e.g. BPA), the concentrations of the compounds tend to increase towards the weekend (from Thursday to Saturday). For example, the winter mean concentrations of the estrogens ranged from 61 ± 4 ng L⁻¹ on Tuesday to 123 ± 3 ng L⁻¹ on Saturday for E1 while that for E2 were between 105 ± 33 ng L⁻¹ on Tuesday and 265 ± 66 ng L⁻¹ on Friday. Although less significant, a very similar trend was generally seen for the summer samples. The observed trend is consistent with the general household activities and people’s lifestyles. People spend more time at home on weekends and therefore the contributions of EDCs and PPCPs in wastewater from homes are expected to be greater. However it should be noted that a more comprehensive sampling is necessary for establishment of such trends.

Similar trends in the concentrations were observed for 4-Nonylphenol (NP), and NP-ethoxylates (NPE1 and NPE2), shown in Fig. 4.5(a) (winter), and Fig. 4.5 (b) (summer), respectively. Detergents use may have increased during weekends as more people may do washing on weekends. However, the trend in summer for the surfactants (NP in particular) was not as prominent in winter. Summer concentrations was also lower, and this could be attributed to greywater diversion to gardens in summer, especially during this low rainfall period.
Fig. 4.4 Daily concentrations of the selected estrogens (estradiol: E2, estrone:E1), xenoestrogen (4t-octylphenol: OP), triclosan: TCS and bisphenol A: BPA in a Melbourne residential network sewer system receiving mainly domestic wastewater from 163 households. Sampling was carried out in two seasons: (a) winter (July 2008) and (b) summer (March 2008). Error bars are standard deviation of duplicate or triplicate samples.
Fig. 4.5 Daily concentrations of the selected xenoestrogens (4-Nonylphenol: NP, nonylphenol mono and di- polyethoxylates: NPE1 and NPE2) in a Melbourne residential network sewer system receiving mainly domestic wastewater from 163 households. Sampling was carried out in two seasons: (a) winter (July 2008) and (b) summer (March 2008) Error bars are standard deviation of duplicate or triplicate samples.
4.4.2. Comparison of Same Day Concentrations (summer)

The results from triplicate samples taken in summer (Saturday, 30 March 2008) at the site 1 (7 households), which was closer to the source compared to samples taken from Site 2 (163 households) in another Saturday in summer (15 March 2008) indicated that concentration of all compounds (except for OP) were higher in the Site 1 samples (see Table 4.1). These results may indicate decrease in concentration due to dilution of wastewater downstream in the catchment. Although it may be expected a higher input of contaminants from larger number of households, the lower concentration in site 2 samples may be due to dilution factor combined with possible losses due to degradation or sorption.

Site 1 results also confirm households as sources of BPA, NPE1 and NPE2. On the other hand E1 and EE2 was <LOD for wastewater at site 1 on that sampling day.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Site 1 (7 houses)</th>
<th>Site 2 (163 houses)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ng L⁻¹</td>
<td>s.d.</td>
</tr>
<tr>
<td>Octylphenol (OP)</td>
<td>15</td>
<td>1.4</td>
</tr>
<tr>
<td>Nonylphenol (NP)</td>
<td>106,387</td>
<td>8255</td>
</tr>
<tr>
<td>NP-mono-ethoxylates (NPE1)</td>
<td>4,596</td>
<td>1,295</td>
</tr>
<tr>
<td>NP-diethoxylates (NPE2)</td>
<td>4,061</td>
<td>407</td>
</tr>
<tr>
<td>Triclosan (TCS)</td>
<td>627</td>
<td>100</td>
</tr>
<tr>
<td>Bisphenol A (BPA)</td>
<td>491</td>
<td>24</td>
</tr>
<tr>
<td>Estrone (E1)</td>
<td>BDL</td>
<td>-</td>
</tr>
<tr>
<td>Estradiol (E2)</td>
<td>92</td>
<td>2</td>
</tr>
<tr>
<td>Ethynylestradiol (EE2)</td>
<td>BDL</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 4.1 Comparison of Site 1 and 2 samples* to assess the impact of catchment size (and distance from source) on the concentration of EDCs and PPCPs in wastewater samples.

*Sampling was conducted on two Saturdays (15 and 30 March) in summer of 2008. s.d. is the standard deviation from mean concentration for triplicate samples.
4.4.3. Seasonal Variations

Seasonal variations of concentration of the selected compounds are shown in Fig 4 and Fig. 5. The winter concentration of E1, E2, OP, TCS and BPA are given in Fig. 4A, while Fig. 4B shows the concentrations of the same compounds measured in summer samples.

![Box plot showing seasonal variations in concentrations of E1, E2, OP, TCS, and BPA](image)

**Fig. 4.6** Seasonal variations in concentrations of the selected estrogens (estradiol: E2, estrone:E1), xenoestrogen (4-t-octylphenol: OP), triclosan: TCS and bisphenol A: BPA in a Melbourne residential network sewer system receiving mainly domestic wastewater from 163 households. Sampling was carried out in two seasons: (a) winter (July 2008) and (b) summer (March 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.
Fig. 4.6 shows that the concentration of all the compounds detected (E1, E2, OP, TCS and BPA) were higher in winter (Fig. 4.6 (a)) compared to that in summer (Fig. 4.6 (b)). For example, the mean concentration of E2 was 190 ngL\(^{-1}\) for winter samples, while that in summer was 88 ngL\(^{-1}\). Similar trend was observed for all the analytes.

Similarly, the concentrations of the surfactants (NP, NPE1-2), given in Fig. 5A (winter) and Fig. 4.7B (summer) showed the same trends. Concentrations of sodium and sulphur which are also common constituents in detergent formulations, in wastewater, were also higher in winter (Tjandraatmadja et al. 2009).

Fig. 4.7 Seasonal variations in concentrations of the selected xenoestrogens (4-Nonylphenol: NP, nonylphenol mono and di-polyethoxylates: NPE1 and NPE2) in a Melbourne residential network sewer system receiving mainly domestic wastewater from 163 households. Sampling was carried out in two seasons: (a) winter (July 2008) and (b) summer (March 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.
The seasonal variations in concentration of contaminants (e.g. detergent ingredients) may be due to a number of factors: changes in inputs to sewage, dilution and/or degradation. For examples, families with active lifestyles (e.g. children who play sports), or increased outdoor activities in Summer, in general, may lead to increased use of laundry detergents due to more frequent laundry washing. Therefore, higher concentrations of the ingredients of laundry detergents would have been expected in summer. However, our results showed concentrations of almost half for the surfactants in summer compared to winter. This may be due to potential degradation of the compounds in summer (temperature min: 9 – max: 37 °C, (BOM, 2008), or as a result of a potentially less discharge to wastewater if greywater diversion for use in gardens occurs. The minimum temperature 5.5 and maximum of 17.7 °C were recorded for July 2008, with a rainfall of 83.7 mm. It is becoming a common practise in Australia to use grey water on gardens in warmer weather, especially during times of low rainfall (e.g. 9.6 mm in March 2008 (BOM, 2008)).

Table 4.2 presents the mean concentrations of the selected EDCs and PPCPs measured in samples from Site 2 (163 households) compared with some of the literature concentration for WWTP influent, effluent, grey water and blackwater.

The concentrations of the selected compounds in domestic wastewater (black water and grey water) measured in this study are compared with literature values for influents, effluents. As was expected, the concentrations of the compounds measured in domestic wastewater were generally higher than the typical influent concentrations of municipal WWTPs (based on data available for WWTPs from South Australia and Queensland). This may be due to dilution factors as well as transformation of some of the compounds in the infrastructure and during transport to WWTPs.

Although unexpected, an interesting observation from this investigation was the detection of the non-ionic surfactants (OP, NP, NPE1 and NPE2) in almost all of the samples from this study. The concentrations were generally higher than expected. Given that appropriate QC samples have been used and that tap water blanks did not show significant background levels of these compounds, a question arose as to whether or not some of the household detergents actually contain some of these NPEs, which are generally attributed to commercial and industrial detergent formulations.

The higher estrogen concentrations in winter are partly caused by a reduction in wastewater flow at site 2, which was on average 33% lower in winter than in summer, whilst the mass load of estrogen which is attributed mainly to human excreta would have been expected to remain consistent throughout the year. This is confirmed for E1 in Figure 4.8. The mass loads of E1 was approximately 2978 µg d\(^{-1}\) during both seasons. But the load of E2 increased from 4765 µg d\(^{-1}\) by 43% in winter.

4.4.4. Comparison with literature

Table 4.2 presents the mean concentrations of the selected EDCs and PPCPs measured in samples from Site 2 (163 households) compared with some of the concentration for WWTP influent, effluent, greywater and blackwater from the literature. The typical influent concentrations of municipal WWTPs were estimated based on data collected from October 2004 to March 2005 at WWTPs in South Australia.
Australia and Queensland. Data for blackwater and greywater was derived from overseas studies.

As expected the concentration of hormones (E1 and E2) in wastewater in the network (this study) were significantly lower than in WWTP influents and blackwater (Table 4.2).

In summer, the concentrations in the network were:
- For estrone (E1): near the typical WWTP influent range of \(43.4 - 50.4 \text{ ng L}^{-1}\)
- For estradiol (E2): ten times higher than the influent range of \(1.37 - 9.36 \text{ ng L}^{-1}\), and
- For ethynylestradiol (EE2): below the limit of detection.

During winter, the concentrations of hormones measured in domestic wastewater were higher than the WWTP influent concentrations shown in Table 4.2.

As previously mentioned, this may be attributed to dilution as well as transformation of some of the compounds during transport to WWTPs in the warmer season.

Surfactants, antimicrobials and plasticisers in this study were within the concentration range measured at WWTPs in both seasons, except for nonyl phenol which was present at a significantly higher concentration (132 ng L\(^{-1}\)) in winter. The concentrations of these compounds in blackwater and greywater reported in the literature are also within the range observed at WWTPs.

4.4.5. Weekly patterns

Figure 4.8 shows the distribution of contaminant loads during a one week interval. The quantity of E1 was consistent through most of the week. Whilst E2 showed more variability from day to day. The quantity of NP fluctuated throughout the week. The smallest mass loads were detected on Monday and Wednesday and the peak load on the Tuesday, however for the remainder of the week the loads were consistent. There was no evidence of a higher load for NP in the weekend compared to weekdays.

The load of OP was constant from Sunday to Thursday and was the highest on the Saturday in the week of sampling. TCS discharged to sewer was constant throughout most of the week, with Friday displaying the peak load.

On the other hand the load of BPA increased markedly during the weekend. Determining the cause for such increase is not possible at this stage, as BPA has been reported to be present in a wide range of industrial and household sources, such as polycarbonate products, epoxy resins, plasticisers in plastics, lining of food cans, water supply pipes, selected dental compounds (Yamamoto and Yasuhara 1999, Fürhacker et al 2000), recycled toilet paper (in Germany) (Gehring et al 2002), food (Leusch et al 2009) and human excretion (Arakawa et al 2004).
The load of NPE1 and NPE2 increased towards the second half of the week, with Saturday registering the peak load for NPE1 and Sat-Sun the peak loads for NPE2.
Table 4.2. Comparison of median seasonal concentrations of contaminants in composite samples from catchment of 163 houses with literature

<table>
<thead>
<tr>
<th>Use/Class</th>
<th>Compound</th>
<th>Conc.</th>
<th>This Study (Mixed domestic)</th>
<th>Selected Data from Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Winter</td>
<td>WWTP Influent (Oct 04 - Mar 05)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Winter, Summer</td>
<td></td>
</tr>
<tr>
<td>Steroid Hormones†</td>
<td>Estrone (E1)</td>
<td>ng L⁻¹</td>
<td>79</td>
<td>43.4 - 50.4 †</td>
</tr>
<tr>
<td></td>
<td>Estradiol (E2)</td>
<td></td>
<td>190</td>
<td>1.37 – 9.36 †</td>
</tr>
<tr>
<td></td>
<td>Ethynylestradiol (EE2)</td>
<td></td>
<td>41</td>
<td>0.13 - 1.5 †</td>
</tr>
<tr>
<td>Surfactants‡</td>
<td>Octylphenol (OP)</td>
<td>µg L⁻¹</td>
<td>0.18</td>
<td>0.03 – 0.44 †</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.13</td>
<td>0.01 – 0.86 †</td>
</tr>
<tr>
<td></td>
<td>Nonylphenol (NP)</td>
<td></td>
<td>131.83</td>
<td>0.61 – 18.8 †</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9.16</td>
<td>1.15 – 8.6 †</td>
</tr>
<tr>
<td></td>
<td>NP-monoethoxylate (NPE1)</td>
<td></td>
<td>5.20</td>
<td>0.81 – 30.0 †</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.14</td>
<td>2.61 – 35.0 †</td>
</tr>
<tr>
<td></td>
<td>NP-diethoxylate (NPE2)</td>
<td></td>
<td>4.34</td>
<td>0.41 – 45.67 †</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.26</td>
<td>2.8 – 5.8 †</td>
</tr>
<tr>
<td>Antimicrobials³</td>
<td>Triclosan (TCS)</td>
<td></td>
<td>0.33</td>
<td>-</td>
</tr>
<tr>
<td>Plasticiser⁴</td>
<td>Bisphenol A (BPA)</td>
<td></td>
<td>0.61</td>
<td>0.013 – 2.73 †</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.40</td>
<td>0.04</td>
</tr>
</tbody>
</table>

* Australia, † Ying et al. (2008), ‡ Ying and Kookana (2007a), § UK (adapted from Ying et al. (2002)), †† Clara et al. (2007), †‡ Palmquist and Hanæus (2005), BDL: below detection limit. Methods of detection: ††, †‡ GC-MS. †EE2 was only picked up in two of the samples, †‡ Estimated using the model from Johnson and Williams (2004) and Carballa et al (2004), 2.24pe/household and 30.4L/cap/d blackwater (Roberts 2005), †§ adapted from Dekant and Völkel (2008) and 30.4L/cap/d blackwater (Roberts 2005)
Figure 4.8: EDC and PPCP mass load in wastewater at site 2 (Summer and Winter)
4.4.6. Source identification

From a limited number of samples, it was found that NP, NPE1 and NPE2 were coming from household detergents. For example both greywater from dishwasher and washing machine showed concentrations of the non-ionic surfactant ingredients at low μg L⁻¹ concentrations. Selected composite samples from Site 1 (7 houses) also indicated similar concentrations of the detergent ingredients (Table 4.3). None of the blank tap water samples taken at sites (1 and 2) had significant levels of any of the selected contaminants. No estrogens were detected in any of these samples, which was expected as estrogens are mostly of anthropogenic origin. It was not clear why we did not detect estrogens in the composite sample from Site 1 on this instance.

Among the greywater sources in the household, the washing machine discharge was the major source of NP, NPE1 and NPE2 at 21,868ngL⁻¹, 3,270 ngL⁻¹ and 2455ngL⁻¹, followed by the dishwasher. No TCS was detected in the household streams, which was expected as none of the products adopted in those appliances contained TCS in their formulation. BPA was below the LOD in the grey streams analysed, however BPA may be derived from human excretion and from recycled toilet paper according to the literature (Dekant and Vogel 2007, Gehring et al 2004).

Dietary intake of food and water consumption can also contribute to intake of NP, OP and BPA, with intake dependent on dietary habits and food/water provenience (Leusch et al 2009)

Table 4.3 Comparison of different sources of the selected compounds based on a snapshot of samples from different greywater sources and a composite sample from site close to source. Blank tap water from sampling sites also included as QC samples.

<table>
<thead>
<tr>
<th>Source</th>
<th>OP</th>
<th>NP</th>
<th>NPE1</th>
<th>TCS</th>
<th>BPA</th>
<th>NPE2</th>
<th>E1</th>
<th>E2</th>
<th>EE2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dish washer</td>
<td>11</td>
<td>1728</td>
<td>1953</td>
<td>BDL</td>
<td>BDL</td>
<td>599</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
</tr>
<tr>
<td>Shower low flow</td>
<td>BDL</td>
<td>602</td>
<td>692</td>
<td>BDL</td>
<td>BDL</td>
<td>451</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
</tr>
<tr>
<td>Washing machine</td>
<td>12</td>
<td>21868</td>
<td>3270</td>
<td>BDL</td>
<td>BDL</td>
<td>2455</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
</tr>
<tr>
<td>Site 2 Tap water (Blank)</td>
<td>BDL</td>
<td>118</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
</tr>
<tr>
<td>Tooloomba Sewer Average</td>
<td>170</td>
<td>56227</td>
<td>2102</td>
<td>281</td>
<td>305</td>
<td>2588</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
</tr>
<tr>
<td>Site 1 Tap water (Blank)</td>
<td>BDL</td>
<td>107</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
</tr>
</tbody>
</table>

BDL: Below detection limit (Estrogens 1-5 ng L⁻¹, xenoestrogens : 10-20 ng L⁻¹)
4.4.7. Implications

A limited range of organic contaminants were evaluated in this scoping study. The preliminary results here obtained suggest concentrations detected in the network were within the typical range detected for influent to WWTPs in summer for most compounds.

However, in winter significantly higher concentrations of selected compounds, such as steroid hormones EE2 and E2 and nonylphenol, could be detected in the wastewater of a residential network, which were also much larger than those verified in influents to WWTP.

Among the compounds analysed, steroid hormones E2 and EE2 have been identified as having the greatest potency to induce biological effect in the environment, with relative potency to E2 of 1.0 and 1.03 based on in vitro studies (Shareef et al 2008). By comparison nonyl phenol has a lesser relative potency (0.000009 E2). Yet, for those three compounds detrimental effects on aquatic systems have been reported at the respective concentrations of 20, 4 and 50 x 10\(^3\) ng/L (effect concentrations) (Shareef et al 2008).

The highest removal efficiency of existing secondary treatment processes adopted for WWTPs in South Australia for E2, EE2 and NP are 63, 72 and 92%, respectively (Ying et al 2008). However higher removal efficiencies up to 90% have been reported for WWTPs that use advanced tertiary treatment (Clara et al 2007).

Thus if the mean winter network concentrations are considered, the secondary and tertiary effluents derived would contain approximately 70ng/L, 11.5ng/L and 10.5 \(\mu\)g/L and 19ng/L, 4ng/L and 13 \(\mu\)g/L for E2, EE2 and NP, respectively.

Hence, secondary treatment processes alone would have reduced the concentration of NP below the effect concentration. But E2 and EE2 concentrations would have been larger than the effect concentrations without advanced tertiary treatment, potentially impacting aquatic biota. Thus further investigation is warranted.

As wastewater travels further to the WWTP mixing of various wastewater streams would be likely to occur, which could dilute the overall hormone concentrations. These were not evaluated in this study.

Analysis of a larger number of samples across the seasons distributed over a longer sampling period is recommended to confirm if the range of concentrations here detected are representative and the frequency at which high concentrations occur during a year. Mixing of wastewater streams could be likely to contribute to attenuation and could be considered for further research.
4.5. Literature Review

- Issue pertinent to wastewater recycling and reuse has recently become a topic of overwhelming debate on strategies for conservation of freshwater resources. While many believe the use of purified recycled water for potable purposes is a potential solution for alleviating the current water shortage in Australia, there is escalating concern over the potential harmful effects of contaminants. This can be the frequent detection of organic pollutants of emerging concern in treated effluents. Similarly, there have also been ongoing efforts on optimisation of treatment processes. In this regard, source characterisation of priority pollutants in wastewater input of WWTPs is also receiving increasing research attention.

- A review of recent literature on environmental occurrence, fate and effects of emerging organic contaminants has revealed substantial evidence suggesting the ubiquity of organic contaminants (including EDCs and PPCPs) in the environment with potential for ecological or human health implications. For example, steroid hormones are present in influents and effluents in the low ng L\(^{-1}\) range. The concentration of other potent xenoestrogens such as alkylphenolic detergent ingredients and degradates (OP, NP, NPE1-2), plasticiser BPA, antimicrobial TCS are detected in the \(\mu g\) L\(^{-1}\). Organic contaminants of pharmaceutical origin including analgesics (e.g. ibuprofen: IBU), anti-epileptic drug carbamazepine: CBZ, and antidepressants are typically reported to occur in \(\mu g\) L\(^{-1}\).

- Treated effluents from WWTPs are thought to be major pathway for these contaminants as treatment facilities are not designed to capture or remove such as diverse range of chemical pollutants. Degradation (biological or chemical) and sorption to filterable materials in the wastewaters, later extracted as biosolids are potential removal processes, and both treated effluents and biosolids are recycled and reused. Given the incomplete removal during treatment, a plethora of potential contaminants may enter the environment through these means.

- The identification of potential contaminants derived from residential wastewater is a key objective of this investigation. The composition of domestic wastewater is complex. Therefore, it is not possible to distinguish exactly which specific source contributes to certain chemicals because a large number of chemical substances are used as chemical ingredients in many household products including food and drinking containers (e.g. BPA), cleaning products (TCS, NP and NPEs), shampoos, cosmetics, and household and network infrastructure. However, source of certain endogenous chemicals (e.g. estrogens) and pharmaceuticals may be identified.
The most common analytical methods used for determination of estrogens and xenoestrogens as well as pharmaceuticals and personal care products are either gas or liquid chromatographic techniques coupled to mass spectrometric detection (GC or LC-MS, MS/MS).

Careful selection of the most appropriate analytical method is crucial for trace level determination of selected organic compounds in domestic wastewater streams. This can depend on a number of factors including the physicochemical properties of the analytes as well as the nature of samples, apart from the cost of analyses. GC-MS is generally used for volatile to semivolatile and thermally stable organic contaminants (e.g. triclosan, bisphenol A) while LC-MS or LC-MS/MS is more suitable for polar compounds (e.g. pharmaceuticals).

In some cases, both GC-MS or LC-MS can be used. For examples, while trimethylsilyl (TMS) derivatives of steroid hormones can be measured on GC-MS, enhanced sensitivities (e.g. 1-2 ng L\(^{-1}\) compared to 5 ng L\(^{-1}\) by GC-MS) are achievable on LC-MS/MS. One of the limitations of LC-MS often reported is signal suppression or enhancement, therefore, TMS-derivatives analysed on GC-MS is far more common.

4.6. Analytical Method Development

Analytical method have been developed using GC-MS and LC-MS/MS for trace level analysis of selected EDCs and PPCPs. Potential domestic wastewater contaminants from a range of chemical classes have been covered by the methods. For examples, our GC-MS method allowed quantitative determination of steroid hormones (E1, E2, EE2), alkylphenolic surfactants (OP, NP, NPE1 and NPE2), bisphenol A (BPA) and triclosan (TCS). Method limits of quantitation (LOQs) based on a preconstruction factor of 1000, using solid phase extraction technique, were 5 ng L\(^{-1}\) for the estrogenic compounds and 10-20 ng L\(^{-1}\) for xenoestrogens (OP, NP, NPE1-2, BPA, and TCS).

Given that the concentrations typical estrogens are in the sub ng L\(^{-1}\) levels, either LC-MS/MS or ELISA techniques were tested and validated, they were not used in the case study for this project due to time and resource constraints. Bioanalytical tools such as ELISA techniques were very expensive they also presented some analytical drawbacks, although LOQs below 1 ng L\(^{-1}\) can be achieved. Therefore analytical methods using LC-MS/MS had been developed for simultaneous determination of some pharmaceuticals (e.g. CBZ and IBU) and the estrogens in order to obtain improved detection limits. While we have validated both the GC-MS and LC-MS/MS methods using spiked wastewater samples, given the limited number of samples, we did not detect the two pharmaceuticals (CBZ and IBU) and the estrogens using LC-MS/MS. However, based on the data from method validation using spiked wastewater samples, excellent method linearity and reproducibility and high SPE recoveries have been obtained.
4.7. Preliminary Data on Domestic Wastewater Case Study

Based on the concentration data obtained from pilot study for steroid hormones and the xenoestrogens alkylphenols, bisphenol and triclosan in residential wastewater, the following conclusions have been made.

- All of the target compounds including the estrogens and xenoestrogens were detected in domestic wastewater samples. Concentrations of estrogens were in the low ng L\(^{-1}\) range while the xenoestrogens were detected at \(\mu g\) L\(^{-1}\) levels.

- The concentrations of both estrogens and xenoestrogens measured in residential effluents were generally higher than typical concentrations of these compounds reported in WWTP influent samples. This was not unexpected as the sampling for this case study was closer to the source and therefore was less dilution of wastewater compared to WWTP inputs. The samples were collected at close point on a residential sewer network receiving wastewater from only 163 houses.

- Based on the limited data available on domestic wastewater, the findings from this study were consistent with other studies. For example, the concentrations of surfactants in the present study reflected the data reported for greywater and blackwater concentrations of these compounds. NP was detected at a higher concentration in this study, but given the limited nature of these samples, we could not determine a particular trend.

- As expected, the data from this case study indicated clear dilution effects on the measured concentrations. Our data from same day samples collected from sites closer to source (7 house) compared to a site further (163 houses) indicated that concentrations generally decreased away from the source, as expected.

- Although our data were generally in the concentration ranges reported in the literature for typical residential effluents from WWTPs, for these compounds, a thorough investigation of domestic wastewaters is needed for establishment of the composition and concentration level of typical domestic wastewater contaminants.

- Although not extensive, the datasets from this study indicated clear seasonal trends in the concentrations of the selected contaminants. Summer and winter trends, as well as daily variations within each season were tested. Generally the concentrations of EDCs and PPCPs showed an increasing trend towards the weekend. The concentrations of the majority of the compounds were detected at a lower level in summer compared to winter. While it was anticipated that certain chemicals may be used more in summer (e.g. detergent ingredients) the concentrations did not show an increased trend in summer. Despite higher consumption of some products, contaminant ingredients from these sources may be reduced in summer due to increased diversion of domestic greywater on site (e.g. gardens and carwash, etc.). The winter concentrations for estrogens and particularly for nonylphenol were markedly higher than those in WWTP influents. Based on the concentrations detected, secondary treatment would be sufficient to reduce nonylphenol in WWTP effluents to low risk levels, but further tertiary treatment (advanced
oxidation) would be required to do the same for E2 and EE2 in the network water over winter.

- Limited datasets on greywater streams within the household indicate that the laundry and the dishwasher were two major sources of NP and derivatives.

5. FUTURE DIRECTIONS

- Given the reproducibility of the data presented here, we are confident the methods described here may be used in further comprehensive surveys of domestic wastewaters for determination of the potential contaminants.

- Although the non-ionic surfactants nonylphenol polyethoxylates are thought to be an industrial detergent, the high levels of NPs and NPE1-2 measured in this study warrants a more comprehensive analysis of domestic wastewater to identify the source of these compounds. It may also be worthwhile conducting a study to determine the concentration of NPs in household detergents.

- The effect of co-contaminants (e.g. estrogens and triclosan) deserves some research attention. For instance, while biodegradation of estrogens have widely been reported as a means of removal of the potential EDCs during wastewater treatment process, or in the environment, the occurrence of antibiotics and antimicrobials (e.g. triclosan) can have a significant impact on the effectiveness of treatments.

- This scoping study investigated a small dataset comprised of a limited number of samples (only over 7 days) to gain an a priori understanding of the concentrations of selected contaminants closer to residential sources of discharge in Melbourne. Further monitoring of residential catchments would be recommended to confirm the concentration ranges in sewers throughout the year and reproducibility across catchments.
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