University of Strathclyde

Department of Civil Engineering

Fate and Transport of Oestrogenic Compounds from Sewage Effluent Irrigation Water

by

Edriyana A.Aziz

A thesis presented in fulfilment of the requirement for the degree of

Doctor of Philosophy

2010

Declaration of Author's Rights

Signed Date

Abstract

The presence of Environmental Endocrine Disruptors (EEDs) is increasingly significant due to their impact on human health and wildlife. Of the compounds implicated as EEDs, the most potent compounds to alter the normal functions of endocrine systems of organisms as well as humans are the natural and synthetic oestrogens. With the water problems faced by the entire world concomitant with global climatic change, the demand for clean water is increasing and this leads to reuse practices, particularly for purposes not requiring potable water quality such as the application of sewage treatment effluent as irrigation water. However, the extent to which the effluent is safe to be used as an irrigation water remains uncertain as most of the water reuse guidelines and standards presently stress the microbiological parameters while limiting the range chemical parameters. Natural and synthetic oestrogens are ubiquitous and present in the final effluents for the vast majority of sewage treatment plants (STPs). The need to further assess the extent to which effluent is safe to be used as an irrigation water and its potential to expose humans through vegetation uptake of contaminants is therefore the motivation of this thesis.

The major work in this study addresses the factors that governing the environmental fate and mobility of these oestrogens in soils. The work starts with the investigation of the sorption affinity of these oestrogens on soils and the relationship between sorption and the physico-chemical parameter, the octanol-water partition coefficient (K_{ow}). The results shows that the K_{ow} is a good predictor of sorption among contaminants. The observed and modelled data of soil column experiments further confirmed the mobility affinity of oestrogens in soils is strongly dependent to their K_{ow} values and retardation of oestrogens mobility in the soils is greatly dependent on the soil organic matter content. The work moves on to identify factors which influence the sorption behaviour of oestrogens in soils. Significant effects on oestrogens behaviour were observed in soils as salinity, pH and temperature were varied, which

is indicated by the changing sorption coefficient (K_d) obtained from batch sorption equilibrium experiments. The calculated sorption coefficient normalised to organic carbon (K_{oc}) indicates that all oestrogens increase in mobility in all soils as the pH increases whilst decrease in mobility as the temperature and salinity increase.

In this study, all oestrogens are predicted to have medium potential for uptake, either by plant roots or leaves following several irrigation techniques of sewage. From the calculated bioconcentration factors (BCFs), bioaccumulation in plant leaves is highly expected if the applied irrigation techniques have direct contact between sewage treatment effluent and plant leaves. Whereas, following direct contact between sewage treatment effluent and soils, calculated BCFs indicate lower occurrence of bioaccumulation in plants. Biomagnification in humans, especially infants from meat or milk consumption would be expected following irrigation with sewage treatment effluent. Thus, this study strongly recommends further investigation into the safety of reuse of sewage treatment effluent as irrigation water for crops and forage in view of human health effects and potential for exposure through vegetation and grazing animals.

The results in this study also indicate a considerable difference between experimental values and values generated by computational model, Estimation Program Interface (EPISUITE). EPISUITE uses a prediction model whereby the inherent physicochemical properties are used to determine the partition ratios for each environmental compartment. This study strongly shows that although the computational model is indicative it does not accurately assess the environmental impact of contaminants.

Acknowledgements

I would like to express my sincere gratitude to my primary supervisor Dr. Helen Keenan for her encouragement and support that she has always shown for my work.

I would also like to thanks Dr Nicholas Lazaridis who has been providing me HYDRUS-1D modeled data. I also wish to thank him for providing me with many valuable discussion and comments. A huge thanks also goes to Mrs Olga Cavoura for helping me in preparing this thesis and also to the technical staff at Strathclyde University and the rest of my lab mates who have created a pleasant and stimulating working conditions.

This work has been financially supported by the Malaysian Government which is gratefully acknowledged.

A special thank you goes to my husband, my parents, my children and also to the rest of my family for their love and support.

Nomenclature

APEO = alkylphenol ethoxylates

ASTM = American Society Testing Standard

BBP = butylbenzenephthalate

BCF = bioconcentration factor

BOD = biochemical oxygen demand

BS = Bitish Standard

BTC = breakthrough curve

C = carbon

$CaCl_2$ = calcium chloride

Cd = cadmium

Cl = chlorine

COD = chemical oxygen demand

CO_2 = carbon dioxide

DBP = dibutylphthalate

DDT = dichlorodiphenyltrichloroethane

DES = diethylstilbestrol

DMP = dimethylphthalate

DOP = dioctylphthalate

EDC = endocrine disrupting chemical

EFMs = environmental fate models

E1 = oestrone

E2 = 17β-oestradiol

EE2 = 17α-ethnyoestradiol

EPISUITE = Estimaton Program Interface

EU = European Union

FSH = follicle-stimulating hormone

GCMS = gas chromatography mass spectrometry

HCl = acid hydrochloric

Hg = mercury

HRT = hormone replacement therapy

KCl = pottasium chloride

k_{a1} = attachment rate coefficient

k_{d1} = detachment rate coefficient

K_d = sorption coefficient or soil-water partition coefficient

K_{oc} = sorption coefficient normalised to organic carbon

K_{ow} = octanol-water partition coefficient

LOD = limit of detection

LH = leutinizing hormone

MEHP = monoethlhexylphthalate

N = nitrogen

Ni = nickel

OECD = Organisation for Economic Co-operation and Development

P = phosphorus

PCB = polychlorinated biphenyl

PER = physical-chemical property estimation routine

pKa = acid dissociation constant

p,p'-DDE = dichlorodiphenyldichloroethylene

QSAR = Quantitative structure-activity relationship

R = retardation factor

R^2 = correlation coefficients

RP-HPLC = reverse phase high performance liquid chromatography

RSD = relative standard deviation

Sd = standard deviation

SHBG = steroid hormone-binding globulin

SPE = solid phase extraction

SS = suspended solid

STP = sewage treatment plant

$t_{1/2}$ = half-life

TDS = total dissolve solid

TOC = total organic carbon

UK = United Kingdom

USA = United State of America

USEPA = United State Environmental Protection Agency

UV = ultraviolet

WHO = World Health Organization

Z = fugacity capacity

Contents

List of Tables

List of Figures

Chapter 1

INTRODUCTION

1.1 Sewage Reuse

World population is regarded as a factor that rapidly increases clean water demand and at the same time decreases freshwater availability. In some regions, water scarcity has already detrimentally affected communities. As sewage is continually and abundantly produced, it is thought that sewage treatment effluent reuse has the potential to decrease the clean water demand from conventional sources and could become an alternative to clean water in various activities. Reservoirs, inter-basin transfer and abstraction from surface and groundwater are traditional approaches to secure a sufficient supply of water for agriculture; however, due to the unsustainable nature of the practices in the long term to the environment and eventually to world population, more sustainable means of re-using treated sewage effluent for agricultural water is thought to be the best approach. In fact, this application has long been practiced for agricultural irrigation. In Western Europe for instance, land application of sewage was started following the incidence of the "stink" smell from the River Thames as well as the cholera outbreaks approximately 150 years ago (CGER, 1996). An example of successful sewage effluent reuse can be found within Europe in Spain, for example, where 20 % of water used across all sectors is supplied from treated sewage including the irrigation of 5000 hectares of tomatoes and 2500 hectares of banana plantations (EEA, 2009).

The availability of new and upgraded treatment technologies of sewage treatment plants (STPs) has allowed much safer treated sewage effluent to be used in various

activities. In spite of its application for indirect purposes such as for agricultural, horticultural, industrial cooling towers or landscaping irrigation, it has now also been accepted as a potential source for potable purposes (drinking water) in countries such as Namibia and Singapore (WEM, 2007).

However, when considering sewage effluent reuse, a thorough evaluation of the advantages and disadvantages including possible risks has to be addressed. The case of fish feminisation in the UK rivers (Purdom et al., 1994) raised concerns about chemicals in discharged sewage treatment effluent that may induce endocrine disrupting activity. The Environmental Agency later revealed that natural and synthetic oestrogens particularly oestrone (E1), 17β-oestradiol (E1) and 17α-ethnyloestradiol (EE2) are the primary sources of endocrine disruption (EA, 1997) and these results were later confirmed using the *in vitro* oestrogen yeast assay toxicity identificaton and evaluation procedure (Desbrow et al., 1998). Thus, the extent to which effluent reuse is safe to be practised remains uncertain. In addition, most of the water reuse guidelines and standards presently focus on microbiological parameters, while the range of chemical parameters is limited.

Despite its potential as an alternative to freshwater which eventually could give an economical benefit, the potential exposure of humans to natural and synthetic oestrogens through land application of treated sewage effluent has to be understood in more detail. Thus, this thesis concentrates on the measurement of the adsorption of natural and synthetic oestrogens as well as their mobility through a series of soils in order to understand the correlation between the sorption coefficient (K_d) and their octanol-water partition coefficient (K_{ow}). The research also calculates the partition ratios for each of the environmental compartments based on experimental valuescompared to partition ratios obtained from a prediction model that uses inherent chemical and physical properties. Once establised, an evaluation of the efficiency of the environmental fate prediction tool, EPISUITE, a tool which is frequently used by regulators, is performed.

1.2 Objectives of the study

In summary, the objectives of this research are:

1. To understand the relationship between octanol-water partition coefficient (K_{ow}) and sorption partition coefficient (K_d) of natural and synthetic oestrogens.

2. To investigate the sorption behaviour of natural and synthetic oestrogens.

3. To identify factors which influence the sorption coefficient (K_d) of natural and synthetic oestrogens to the soil.

4. To determine the mobility of natural and synthetic oestrogens in soils.

5. To evaluate the efficiency of the EPISUITE program in predicting the fate of natural and synthetic oestrogens in the environment.

1.3 The endocrine system

The foundation of the endocrine system is the glands that secrete chemical messages known as hormones. Various glands are located throughout the body as shown in Figure 1.1 and these glands release hormones directly into the blood that signal or induce a physiological response in some target tissue which contains its own receptor. The major endocrine glands are the pituitary, thyroid, parathyroid, adrenal, pancreas and gonad glands.

A gland can be classified as either endocrine or exocrine (Keenan, 2000). Exocrine glands produce a variety of substances such as sweat, saliva and digestive enzymes and release them at the appropriate locations by means of ducts such as in the skin or inside the mouth. Endocrine glands function in a different manner to the exocrine glands. They are ductless and hormones produced by them are secreted directly into the bloodstream where they can be transported to the target tissues or organs.

In general the endocrine system is controlled by the hypothalamus which is connected to the anterior pituitary gland, also known as the master gland of the body. It is involved in regulating many bodily functions such as controlling blood sugar, growth, the function of reproductive system, regulation of metabolism, development of the brain and the rest of the nervous system and the development of an organism from conception through adulthood and old age, as well as maintaining homeostasis.

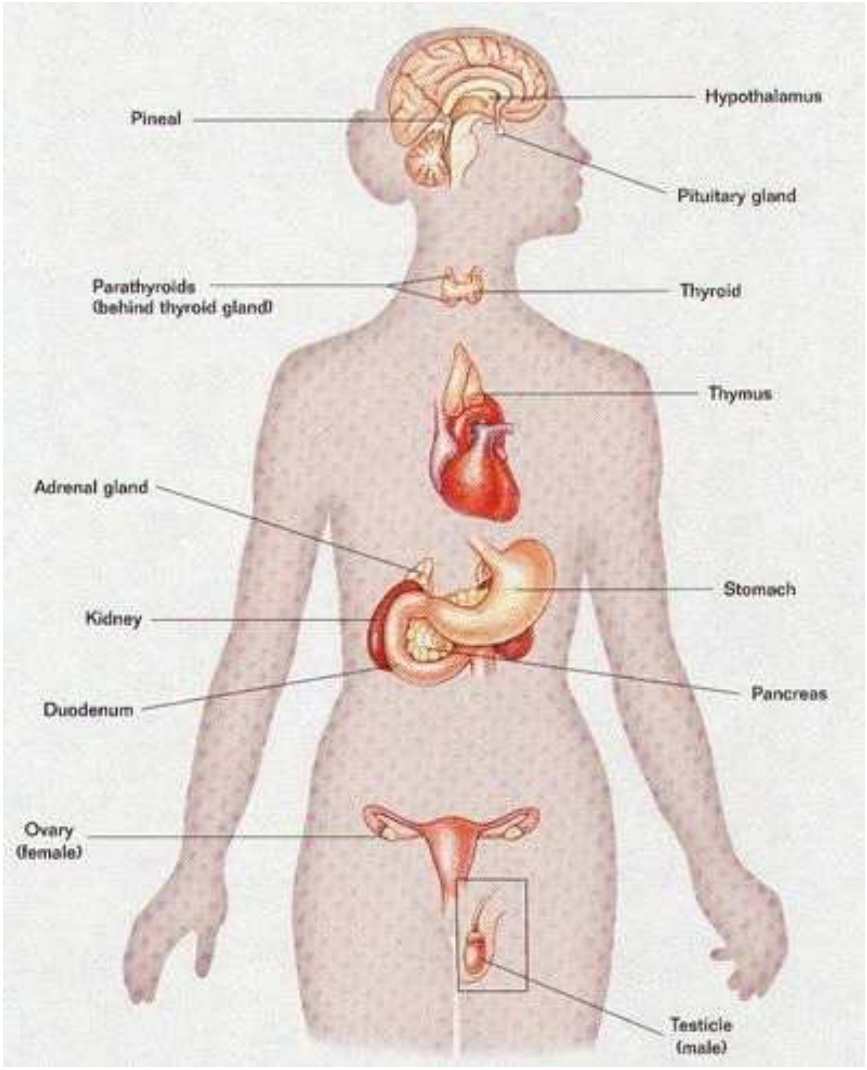


Figure 1.1: The endocrine system (adapted from USEPA website, http://www.epa.gov/).

1.3.1 Hormones

Hormones are natural secretory products of endocrine glands and travel via the bloodstream to act on target tissues or organs. The target tissues or organs under endocrine control include the mammary glands, bone, muscle as well as the male and female reproductive organs. Other endocrine tissues include the placenta, liver, kidneys and cells throughout the gastrointestinal tract. Along with the nervous system, the endocrine system coordinates the workings of the body. However, the systems work in different ways from each other. Unlike the endocrine system, the

messages in the nervous system are carried in the form of electrical impulses.

Figure 1.2 is a simplified diagram of hormone types which is based on their chemical structure. Hormones can be grouped into three classes; steroids, peptides and amines. Each hormone molecule has a specific shape that can be recognised by that hormone's target cells. The response to the hormonal signal is triggered by the combination of hormone-receptor. The action can be viewed as hormones travelling through the blood at low concentrations towards their target cell, where a special protein will bind to the hormone along the way and act as a carrier to the desired target cell and at the desired concentration. When the hormones reach their target cell, they will bind to specific cell surfaces or nuclear receptors and these hormone-receptor combinations trigger the responses to the hormonal signal and exert important regulatory, growth or homeostatic effects. There are hundreds of receptor types, each one designed for a particular chemical signal. The binding action of hormones and specific receptors is considered anologous with a lock and key situation whereby the key needs to fit the lock before a response is possible (adapted from Keenan, 2000).

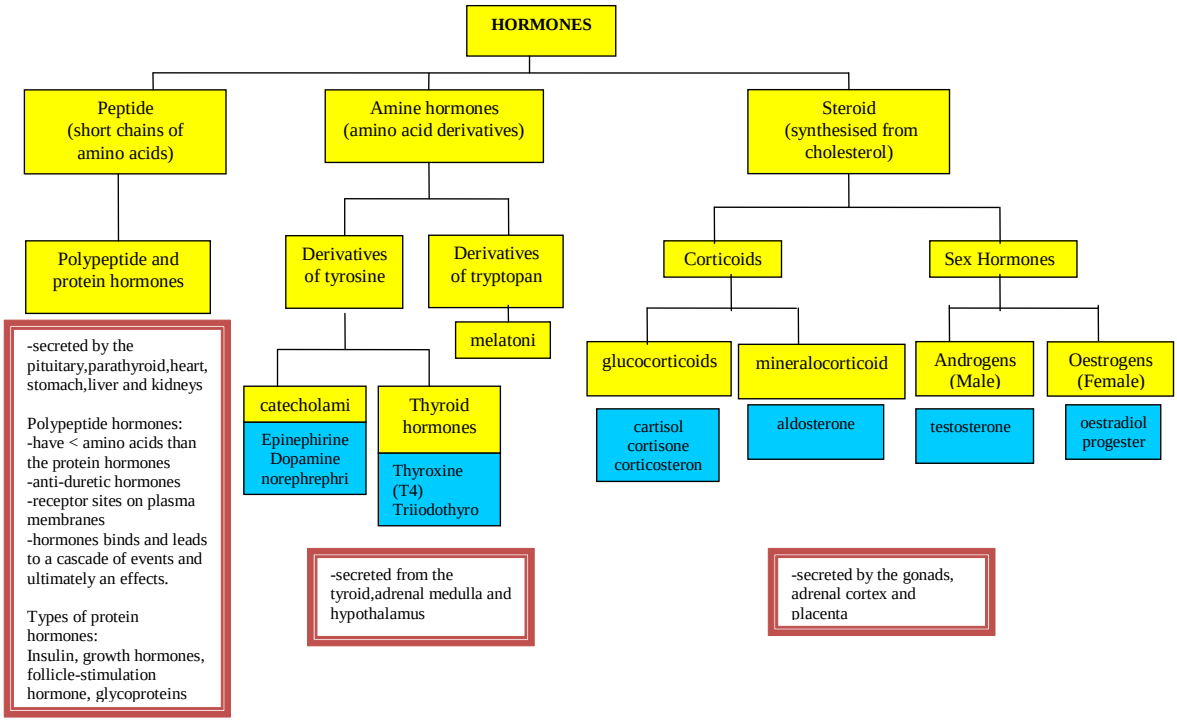


Figure 1.2: Simplified diagram of the types of hormone from previous literatures.

1.3.2 Steroid hormones and non-steroid hormones

Steroid hormones are all derived from cholesterol. Like their precusor, they all share similarities based on a 4-ring carbon skeleton (Figure 1.3 (a)) and they are classified by their substituents and functionalisation of the 4-ring carbon such as; oestrogens, A ring is aromatic (Figure1.3 (c)) ; androgens, hydroxyl group on D ring (Figure 1.3 (b)) ; gestagens, acetyl group on D ring (Figure 1.3 (d)) and corticoids, hydroxyl and carbonyl group on D ring (Figure 1.3 (e)).

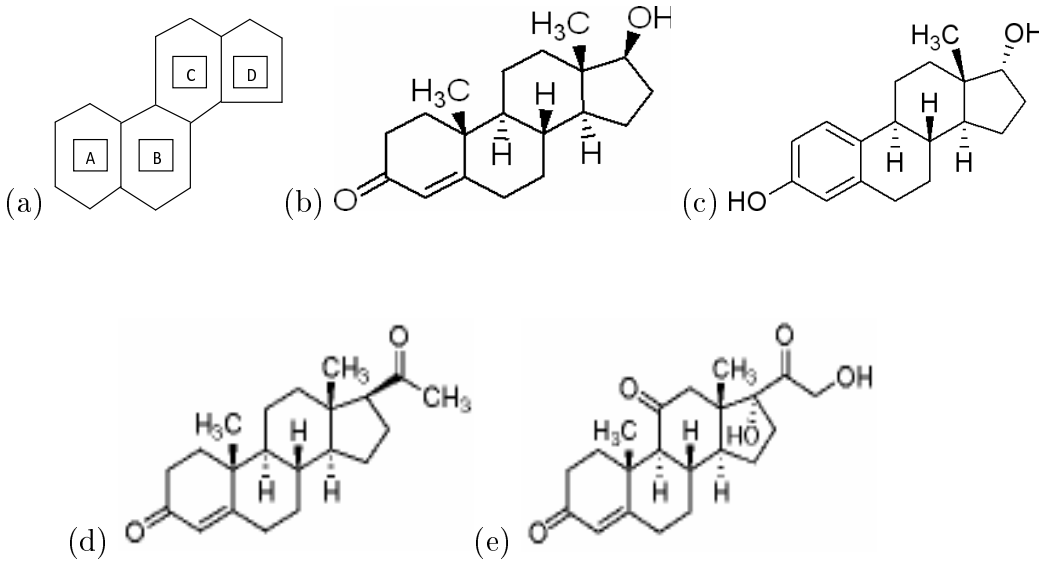


Figure 1.3: (a) Basic structure steroid (b) androgen (testosteron) (c) oestrogen (estradiol) (d) gestagen (progesteron) (e) corticoid (cortisone)

All the steroid hormones are lipid soluble and exert their action by passing through the plasma. Once inside the cell, they will bind to the nuclear membrane receptor and produce an activated hormone-receptor complex. On the other hand, non steroid hormones are peptide based and water soluble such as insulin. Therefore they do not enter the cell but will bind to the plasma membrane receptor and generate a chemical signal.

1.4 Endocrine disruption

In general disruption of the endocrine systems can occur directly or indirectly by endocrine disruptors. A direct disruption may be caused by one of two mechanisms: agonistic and antagonistic. Agonistic mechanisms occur when a particular chemical mimicks the action of the body's natural hormone, thus setting off the actual chemical reaction the natural hormone would produce in the body. On the other hand, an antagonistic mechanism occurs when the particular endocrine disrupting chemical blocks the receptors in the cells which normally receive the natural hormones, consequently preventing the actual action of the body's natural hormones.

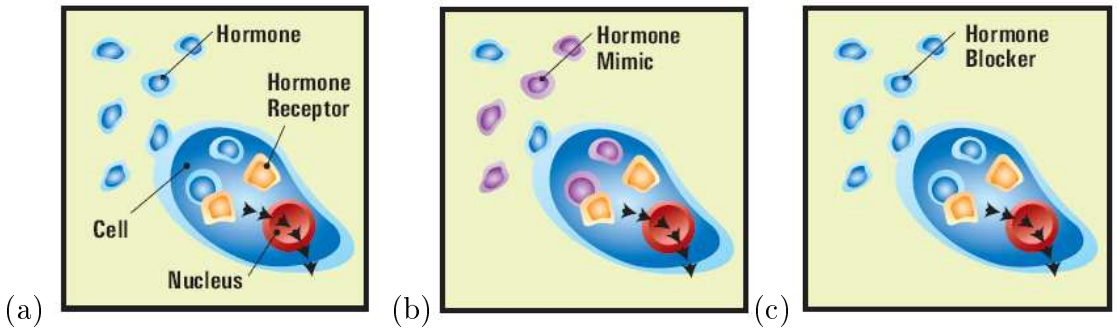


Figure 1.4: Mechanisms of endocrine disruptors (a) actual mechanism of the body's natural hormone (b) agonistic mechanism (c) antagonistic mechanism (adapted from http:// www.niehs.nh.gov/)

There are several ways in which endocrine disrupter chemicals could disrupt the endocrine system:

1. Altered hormone biosynthesis
2. Altered hormone storage and/or release
3. Altered hormone transport and clearance
4. Altered hormone receptor recognition/binding

Impaired hormonal control could occur as consequences of altered hormones resulting from the complexity of the cellular processes involved in hormonal communication.

1.4.1 Altered hormone biosynthesis

A number of compounds have the ability to alter glycosylation and biosynthesis of leutinizing hormones (LH) and follicle-stimulating hormones (FSH) by either mimicking or antagonizing the action of steroid hormones. For instance, oestrogen and testosterone have the ability to affect pituitary hormone synthesis either directly or by causing changes in the glycosylation of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Derelanko and Hollinger, 2001).

1.4.2 Altered hormone storage and/or release

The function for hormone storage is important in order to maintain normal concentrations of the hormones so that they can be released immediately when they are needed. However, steroid hormones are not stored intracellularly within membranous secretory granules. Testosterone for instance is produced and released by the activation of the luteinizing hormone (LH) receptor. Therefore, any compounds that inhibit the LH receptor can rapidly alter the secretion of testosterone hormones (Derelanko and Hollinger, 2001).

1.4.3 Altered hormone transport

The bioavailability of hormones at the target cells is dependent on the concentrations of the specialised carrier of the hormones in blood. Hormones are transported in blood in the free or bound state. Steroidal hormones are transported in the blood by a specialised carrier protein which is synthesised in the liver. For instance, oestrogen and testosterone hormones are transported by binding to globulin known as sex or steroid hormone-binding globulin (SHBG). Regulation of the concentration of those globulins in the blood is significant as there may be either increases or decreases which eventually could affect steroid hormones availability. For example, dichlorodiphenyltrichoroethane (DDT) is a pesticide that has been shown to possess an ability to modify the bioavailability levels by decreasing the concentrations of the carriers (Derelanko and Hollinger, 2001).

1.4.4 Altered hormone receptor recognition/binding

Hormones elicit responses from their respective target tissues or organs through direct interactions with either intracellular receptors or membrane bound receptors. Specific binding of the natural ligand to its receptor is a critical step in hormone function. A number of environmental compounds such as DDT may alter receptor recognition by mimicking the natural ligand and acting as an agonist or by binding and acting as antagonist to disrupt oestrogen receptor interaction. Many of the chemicals classified as environmental oestrogens, for instance the DDT metabolite p,p'-DDE, have been shown to possess an ability to inhibit binding to more than one type of intracellular receptor (Derelanko and Hollinger, 2001).

1.5 Classification of endocrine disrupting chemicals (EDCs)

Endocrine disrupting chemicals (EDCs) can be defined as "an exogeneous substance that causes adverse health effects in an intact organism, or its progeny subsequent to changes in endocrine function" (EC, 1997).

Endocrine disrupting chemicals (EDCs) with particular reference to oestrogenic activity can be classified as the following:

1. Steroidal oestrogens

- Natural oestrogens
- Synthetic oestrogens

2. Non-steroidal oestrogens

- Phyto-oestrogens
- Xeno-oestrogens

Oestrogen is a group of steroidal compounds produced by mammals. It is a collective term for the female hormones but it also acts as hormones in male-specific tissues

such as prostate, testis and epididymis. Oestrogens control female secondary sexual characteristics, maintain the lining of the uterus, and prepare the body for pregnancy. Oestrogens also affect the growth, differentiation and function of peripheral tissues of the reproductive system including the breasts, uterus, vagina and ovaries. In the brain, oestrogens may affect many factors important to regulating procreation including reproductive behaviour and mood. They are produced at significantly higher levels in women of reproductive age. Apart of their function to regulate the reproductory system, oestrogens also play an important role in bone development in both females and males.

Oestrogen can be divided into two types. One type, steroidal oestrogen, comprises of natural oestrogens produced by mammals as well as synthetic oestrogens. The other type, non-steroidal oestrogen, comprises of natural oestrogen produced by plants (also known as phyto-oestrogens) and any synthetic substances also known as xenoestrogens as well as substances produced by fungi that are known as mycoestrogens.

Steroidal oestrogens not only naturally regulate the development of female sex characteristic, mood and behaviour, reproductory process, but they have also been used widely as medicinal drugs. They are also used in a variety of veterinary treatments as well as in commercial products, for example in cosmetics.

1.6 Natural oestrogen

Natural oestrogen is mainly formed in the ovaries. The ovaries produce two types of active steroid hormones whose actions are interrelated in the regulation of the process of sexual development and together control almost all of the reproductive processes in women. The first group is referred to as the oestrogens and includes oestradiol, oestrone, and oestriol, while the second type of ovarian hormone are gestagens such as progesteron.

The most potent naturally occurring oestrogenic hormone is 17β-oestradiol (E2) and it is secreted along with its metabolite oestrone (E1) by the ovaries during the normal menstrual cycle and also by the placenta in pregnant women. These natural oestrogens have a very stable aromatic A ring and a phenolic hydroxyl group. They

are transported in blood bound to plasma globulins, metabolized in liver and mainly excreted in urine in an inactive form (conjugated with glucuronic or sulphuric acid) and only a very small amount is excreted via the faeces (unconjugated form) (Orme and Breckenbridge, 1983).

However, readily free oestrogens (active form) have been observed in sewage before entering STP treatment as well as during the treatment (Ternes et al., 1999a; Nasu et al., 2001; Andersen et al., 2003; D'Ascenzo et al., 2003). It has been reported that the presence of β-glucuronidase and arylsulfatase enzyme together are responsible for the deconjugation of the inactive form to free oestrogens (Ternes et al., 1999a; Johnson and Sumpter, 2001).

1.6.1 Production of oestrogens from humans

The amount of natural oestrogens excreted in the urine or faeces from any individual varies and depends on several factors such as sex, age, race, stage of menstruation, life style as well as stage of pregnancy. Approximately 0.3-5 μg per day of 17β-oestradiol (E2) are excreted in the urine of female and about 2-20 μg per day of oestrone (E1) while in males, the amounts per day of 17β-oestradiol (E2) and oestrone (E1) excreted in urine are 1.5 μg and 3 μg, respectively. Pregnant women have been reported to excrete by far the largest amount of natural oestrogens which is up to 1000 times higher (depending on the stage of pregnancy) followed by pre-menopausal women and oral contraceptive users while post-menopausal women excrete the least (Hoffmann and Evers, 1986 as cited by Shore and Shemesh, 2003). D'Ascenzo et al. (2003) investigated the urinary oestrogen excretion of one pregnant woman in the second trimester of pregnancy; the excretion values of oestrogens, oestrone (E1) and 17β-oestradiol (E2) were 940 and 258 μg per day. A healthy woman going through menopause was reported to excrete oestrogens in the range of 10-100 μg per day with the amount of oestrone (E1) excreted being two times higher than 17β-oestradiol (E2). After menopause, a woman only excretes between 5-10 μg of oestrogen per day (Aherne and Briggs, 1989). A woman with a normal menstrual cycle releases between 70-700 μg of oestrone (E1) and 17β-oestradiol (E2) per day (Hoffmann and Evers, 1986 as cited by Shore and Shemesh, 2003).

1.6.2 Production of oestrogens from livestocks

The amount of oestrogens and method of excretion varies among animal species as well as from the stage in their life cycle (Palme et al., 1996). For instance, sheep excrete most of the oestrone (E1) via faeces while horses and pigs excrete it through urine (Palme et al., 1996). Hoffmann and Evers (1986) reported excretion of 30 µg per kilogram of oestrogens in faeces and 15 µg per liter in urine per head of cow (as cited by Shore and Shemesh, 2003).

Other livestock which contribute to environmental oestrogen concentrations include poultry. Johnson and Van Tienhoven (1981) reported the excretion of oestrone (E1) and 17β-oestradiol (E2) via urine in laying hens was about 3 µg per day for both hormones while in non-laying hens, 0.5 µg per day and 2 µg per day were found respectively. Elevated excretion of oestrogens particularly 17β-oestradiol (E2) of up to six fold in urine (Callantine et al., 1961 as cited by Ullman, 2006) after administration of oestrogen supplements to enhance meat quality as well to increase weight gain of cattle has been documented (Popp et al., 1997).

1.6.3 Production of oestrogens from wildlife

Finlay Moore et al. (2000) reported the presence of wild turkey for several months was capable of contributing high levels of 17β-oestradiol (E2) and testosterone in the soil. Additionally, Shore and Shemesh (2003) through their personal observation over a four year period, reported 17β-oestradiol (E2) concentrations of 5 to 7 ngL^{-1} and comparable amounts of testosterone in a fish pond water. Oestrogens concentrations in elephant feaces has been reported at 1.0 μgg^{-1} (Fiess et al., 1999) and musk ox feaces at concentrations of 17 μgg^{-1}(Desaulniers et al., 1989). Further informaton about the occurrence of oestrogens from wildlife is limited. It is probably due to the difficulties in monitoring operations since there is no fixed sampling method or site compared to concentrated manure during animal farming, which is easier to sample.

1.7 Phyto-oestrogen

Phyto-oestrogen is a general definition that has been applied to any plant substance or metabolite that induces biological responses in vertebrates and can mimic the action of endogeneous oestrogen usually by binding to oestrogen receptor (FSA, 2002).

All phyto-oestrogens are non-steroidal with a structure similar to 17β-oestradiol and possess oestrogen agonist properties. However their oestrogenic effects are much less potent (Matsumura et al., 2005).

Phyto-oestrogens can be subdivided into three main classes (Murkies et al., 1998):

1. Isoflavones (e.g diadzein, genistein, equol, biochanin A and formononentin)
2. Lignans (e.g enterolactone and enterodiol)
3. Coumestans (e.g coumestrol and trifoliol)

Isoflavones and lignans are widely distributed within the plant kingdom; isoflavones are mainly present in legumes whereas lignans can be found in almost vegetables and cereals. Multiple phyto-oestrogens can be found in a single plant.

Consumption of phyto-oestrogen varies among individuals. Studies related to diet have found that the soy products contain isoflavones and lignans are able to help prevent several types of cancer, for example breast and prostatic cancer (Herman et al., 1995), and are capable to reduce risk of developing diabetes mellitus and heart disease (cardiovascular) of postmenopausal women (DeKleijn et al., 2002). However, a recent study of UK women showed that consumption of isoflavones contained in bakery products has increased the risk of breast cancer (Grace et al., 2004).

In the 1940's, phyto-oestrogens contain in grazing forage have been observed to cause reproductive difficulties in sheep (Adams, 1995). Since these observations, the risk of phyto-oestrogens to human fertility has been greatly discussed. Recent evidence shows that phyto-oestrogen treatment over time (6 months) can be used to increase sperm count and can eventually lead to successful conception for couples experiencing problems conceiving prior to treatment (Casini et al., 2006).

Evaluating the detrimental effects of phyto-oestrogens on humans remain difficult as many factors are thought to have a role, including types of phyto-oestrogens consumed, cultural cooking practices, duration of studies, etc. However, it is thought that phyto-oestrogens are natural compounds that have evolved over time, can be flushed out from the body quickly (Darbre, 2006) and therefore may not be harmful to humans.

1.8 Synthetic oestrogen

Synthetic oestrogens refer to man-made hormones that are made from chemical substances to approximate the hormones the human body makes. They were produced as a result of major pharmaceutical research looking for any compound that was suitable for oral administration because natural oestrogens were ineffective when administered by mouth. Åstedt (1977) has reported that only 30-50 μg of synthetic oestrogen, 17α-ethynyloestradiol (EE2) administered per day was needed in order to exibit a comparable effect of 4 mg of the natural oestrogen oestradiol (E2) (as cited by Bolt, 1979).

Most synthetic oestrogens are used for therapeutic purposes such as for fertility treatment, hormone replacement therapy (HRT) and cancer treatment (Kuster et al., 2004). Some example cancer treatments include prostate cancer as well as to relieve certain symptoms of breast cancer in some women, as well as metastatic disease in men. Hormone replacement therapy (HRT) is given to menopausal as well as postmenopausal women as a treatment based on the assumption that it may prevent discomfort from for example hot flushes (sweating episodes); shrinking and irritation that occur in the vulva, vagina and urinary organs; loss of libido; oestoporosis; and ischemic heart disease, which is caused by the natural depletion of oestrogens that normally occurs when women reach an age of between 40 and 60 years.

The best known synthetic steroidal oestrogen is 17α-ethnyloestradiol (EE2), the main ingredient in the combined oral contraceptive pill that is the most popular method of preventing pregnancy. Diethylstilbestrol (DES) is also a non-steroidal synthetic oestrogen that was hailed as a wonder drug to prevent late pregnancy complication and at the same time to produce bigger and stronger babies; however, the drug was found to be mutagenic and teratogenic to the babies exposed as they reached sexual maturity (Stillman, 1982) and its usage has been withdrawn.

Unlike natural oestrogen, 17α-ethnyloestradiol (EE2) can not bind to steroid hormone-binding globulin (SHBG) but instead binds to albumin and favours faeces excretion (Bolt, 1979). As with natural oestrogen, the major urinary excretory products are glucuronide and sulphate and the excretion of 17α-ethnyloestradiol (EE2) is also in conjugated (inactive) form. The presence of hydrolysing bacteria *Escherichia coli* in sewage treatment plants (STPs), however is responsible for its deconjugation into

the active form (Johnson and Sumpter, 2001).

1.9 Xeno-oestrogens

Xeno-oestrogens are a group of industrial-made chemicals that differ chemically from naturally occurring oestrogens. They can be found in everyday household products such as plastic, which can contai Bisphenol-A and phthalates, for example, and also in cosmetics, which may contain parabens. They are also released into the environment from agricultural spraying of herbicides and pesticides; as by products of industrial processes; and from waste disposal (for example, polychlorinated biphenyls (PCBs) and dioxins); or as discharges from sewage treatment systems (for example, alkylphenol ethoxylates). They have also been shown to possess oestrogenic properties (Jobling et al., 1995).

1.9.1 Pesticides

Dicholorodiphenyltrichloroethane, DDT is a well known endocrine disruptor and has been implicated as a human carcinogen. Its use has been banned in the United States since 1972 however it is still used in developing countries, for example for mosquito control. Other organopesticides such as Endosulfan, Dieldrin etc are still used in many countries. Some of them however has been banned in UK and the rest of EU.

Endosulfan for instance is used on many crops including barley, broccoli, brussel sprouts, cabbage, lettuce, blueberries etc (USEPA, 2002). It has been identified as a pesticide of concern due to health and environmental problem associated with its use in Ecuador, Mauritus and Paraguay. Its use has been banned in Singapore, UK, USA, Sweden, and the Netherlands and restricted significantly in Australia, Bangladesh, Denmark, Findland, Japan, Indonesia and Canada (EJF, 2002).

1.9.2 Phthalates and Bisphenol A

Phthalates are used widely as platicisers. Commonly used phthalates include monoethylhexylphthalate (MEHP), dimethylphthalate (DMP), butylbenzenephthalate (BBP), dibutylphthalate (DBP) and dioctylphthalate (DOP). Phthalates can be found in cling film, ink printed on plastics, vinyl flooring, toys, emulsion paint as well as in adhesives used in packaging. They have also been reported in many cosmetic products (Darbre, 2006).

Bisphenol A is used primarily to make polycarbonate plastic which is a lightweight, high performance plastic in terms of its toughness, high heat resistence as well as electrical resistence. It is also used to make epoxy resin for applications such as electrical laminates for printed circuit boards, paints and adhesives.

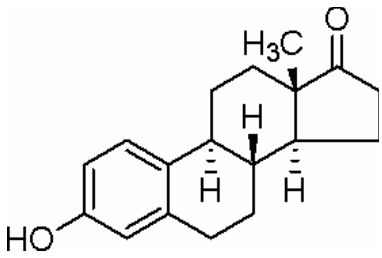
Both phthalates and Bisphenol A have been shown to have oestrogenic properties. Bisphenol A for instance has been shown to increase the risk of breast cancer (Brisken, 2008) while phthalates have been reported to reduce sperm counts. More recent research suggests that the exposure of a pregnant woman to Bisphenol A can result in a low birth weight in infants, which is a leading cause of death in children under five yeras of age, as well as increasing their risk of cardiovascular and metabolic disease in adulthood (Baker, 2009)

1.9.3 Alkylphenol ethoxylates (APEOs)

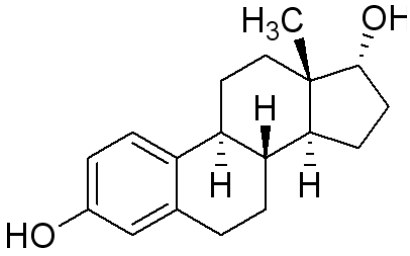
APEOs are surfactants that are used for industrial and agricultural purposes, for example in paper and pulp mills, textile processing resin paper manufacture and other chemical applications. APEOs are also used as ingredients in domestic household and industrial detergents that are mainly disposed of into the sewer system and biodegrade either in STP or in the environment, resulting in more recalcitrant metabolites such as nonylphenols and octylphenols.

1.10 Physicochemical properties

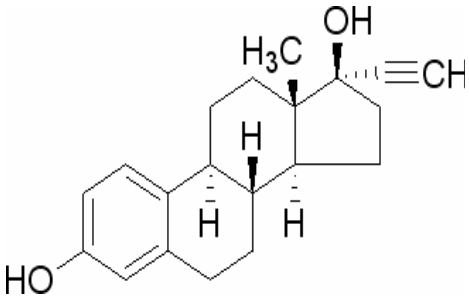
Figure 1.5 shows the molecular structure of oestrone (E1), 17β-oestradiol (E2) and 17α-ethnyloestradiol (EE2), the oestrogens in this study. Some relevant physicochemical properties of these oestrogens are given in Table 1.1. These physicochemical properties have an important impact on the compounds' behaviour in environmental matrices.



(a)



(b)



(c)

Figure 1.5: Molecular structure of (a) oestrone (E1) (b) 17β-oestradiol (E2) (c) 17α-ethnyloestradiol (EE2)

Table 1.1: Chemical and physical properties of oestrogens

	Oestrone (E1)	17β-oestradiol (E2)	17α-ethnyloestradiol (EE2)	Reference
CAS Number	53-16-7	50-28-2	57-63-6	
Molecular weight	270.4	272.4	296.4	(Lai et al., 2000)
Vapour pressure (Pa)	$3x10^{-8}$	$3x10^{-8}$	$6x10^{-9}$	(Lai et al., 2000)
Water solubility (mgL^{-1})	2.1 - 146.8	3.1 - 81.97	3.1 - 81.97	(Lai et al., 2000; Hanselman et al., 2003; Yu et al., 2004); EPISUITE
Henry's Law constant (atm m^3mol^{-1})	N.A	N.A	N.A	
Log K_{ow}	2.76 - 3.43	2.69 - 4.01	3.67 - 4.2	(Jurgens et al., 1999; Lai et al., 2000; Hanselman et al., 2003); EPISUITE

Chapter 2

POTENTIAL EXPOSURE TO STEROIDAL OESTROGENS VIA SEWAGE TREATMENT AND REUSE

2.1 Sewage treatment plants (STPs)

Conventional sewage treatment plants (STPs) contain a multitude of organic compounds such as natural and synthetic oestrogens derived not only from daily domestic applications but also naturally from human excretion. Evidence shows that natural and synthetic oestrogens are present in almost final effluents of conventional municipal sewage treatment plants (STPs) all over the world (Baronti et al., 2000; Johnson et al., 2000; Ternes et al., 1999b) at concentrations reported as low as nanogram per litre (ngL^{-1}) even though they have undergone a series of treatments.

From the removal rates reported by STPs all over the world, it can be deduced that conventional STPs are not able to deal completely with these contaminants. Oestrogens remain in effluent at concentrations known to induce vitellogenin in male fish. For example, final effluent samples from a series of UK STPs have been reported to contain 1-50 ngL^{-1}17β-oestradiol (E2), 0.2-220 ngL^{-1}oestrone (E1) and 0.2-7.1 ngL^{-1}17α-ethnyloestradiol (EE2) (Desbrow et al., 1998) and comparable concentrations ranges also have been reported in other countries such as Germany, (Kuch and

Ballschiter, 2001), the Netherlands, (Belfroid et al., 1999), Canada, (Ternes et al., 1999b) and Japan, (Nasu et al., 2001). It has been reported that the effective levels for these steroidal oestrogens to induce oestrogenic effects in sensitive species of aquatic organisms are in the range of 0.1-10 ngL^{-1}(Purdom et al., 1994).

Thus, removing a known chemical that has a capability to induce oestrogenic effects in sewage treatment effluent still remains a challenge. The removal rates published in previous literature vary greatly. Baronti et al. (2000) reported that the removal rates of oestrone (E1), 17β-oestradiol (E2) and 17α-ethnyloestradiol (EE2) by activated sludge treatment in Italian STPs were 61 %, 87 % and 85 %, respectively. Ternes et al. (1999b) reported the removal rates of oestrone (E1), 17β-oestradiol (E2) and 17α-ethnyloestradiol (EE2) by activated sludge treatment of Brazillian STPs were 67 %, 99.9 % and 78 %, respectively. In comparison, trickling filters were reported to remove only 8 % of oestrogenic compounds in Swedisch municipal STPs (Svenson et al., 2003) while Ternes et al. (1999b) reported the removal rates oestrone (E1), 17β-oestradiol (E2) and 17α-ethnyloestradiol (EE2) using trickling filters were 67 %, 92 % and 64 %, respectively, in Brazillian STPs. The variability in reported rates from one STP to another was attributed to several factors. Influent loading rates are important as are local conditions, in particular temperature. In addition, the capability of the sewage solids to adsorb contaminants and how quickly microorganisms present in the sewage degrade these contaminants are different from one STP to another. Moreover, most of the conventional STPs worldwide have been designed for waste stabilization, clarification, disinfection, nutrient (nitrogen and phosphorus) removal (Teske and Arnold, 2008; Bolong et al., 2009) and not the removal of endocrine disrupting chemicals.

Since conventional STPs are not proven to eliminate emerging disrupting chemical contaminants to a satisfactory level, advanced treatment processes that can remove these oestrogens have received extensive attention. Some preliminary research results indicate that various advanced oxidation processes such as photocatalysis have the potential to remove these oestrogens effectively (Malygina et al., 2005; Coleman et al., 2005). Coleman et al. (2000) for example first reported the potential of photocatalysis to be used as further treatment in STPs as this process was demonstrated to remove the natural oestrogen 17β-oestradiol (E2) by up to 98 % from a starting concentration of 0.82 mgL^{-1}in water. Due to this impressive result by Coleman et al. (2000), the same author further investigated the effects of silver and platinum metals on the

photocatalytic degradation of these natural and synthetic oestrogens so as to improve the removal rates. These additives produced lower concentrations of oestrogens in effluents than the levels that have been reported to induce oestrogenic effects in organisms in aquatic environment. However the presence of these metals does not enhance the photocatalytic oxidation of low concentrations of natural and synthetic oestrogens in raw sewage (Coleman et al., 2005). Malygina et al. (2005) demonstrated that alkaline conditioning of sewage would enhances the photocatalytic degradation process of oestrogens with highest efficiency.

Another advanced treatment that has recently received attention in sewage treatment management is the use of ultrasound. To date there is still limited information regarding the potential of this process in removing oestrogens from the water phase. However, Suri et al. (2006) reported that oestrogen removal by sonolysis was in the range from 40 % to 80 % from an initial concentration of 0.5 mgL^{-1} in raw sewage (as cited by Belgiorno et al., 2007).

Although these new processes offer some potential advanced treatment that could be employed in current STPs, more research for a cost effective operating process is essential as the treatment costs depend on various conditions. The volume and strength of sewage to be treated is important. Also the process design affects treatment operation and the water quality to be obtained must be considered. Adequate human resourses in operating the process are also necessary. In developed countries, where adequate financial, material and human resources are often available, implementation of various advanced treatments are appropriate; however, transferring these technologies to developing countries has many potential difficulties.

2.2 Conventional sewage treatment operation

A standard conventional sewage treatment process comprises the following stages: preliminary treatment, primary and secondary treatment, and finally disinfection and odour removal. Advanced treatment is increasingly suggested to be incorporated to produce higher quality treated effluents that can be reused for beneficial purposes.

2.2.1 Preliminary treatment

Preliminary treatment essentially is a physical operation which includes screening and grit removal. The screening process removes coarse solids such as twigs, rags, and branches that would interfere with the mechanical equipment. Grit removal separates sand, grit, cinders and small stones that would settle in channels and might result in clogging of aeration devices or take up capacity in tanks that consequently would interfere with the operation.

2.2.2 Primary treatment

With the screening completed and the grit removed, sewage still contains dissolved organic constituents, inorganic constituents and also suspended solids. Primary treatment removes the suspended solids from the sewage flow generally using primary clarifiers. This separation reduces total suspended solids as well as the biochemical oxygen demand (BOD) levels. It is estimated that 60 % of total suspended solids are removed while the BOD is reduced by 30 % through this process. The processes involved at this stage of treatment are gravity settling, sedimentation and chemical coagulation. The constituents that are removed sink to the bottom of the tank and are also referred to as 'primary sludge'. After primary treatment, the wastewater moves on to secondary treatment where a biological treatment process always takes place.

2.2.3 Secondary treatment

The secondary treatment employed by STPs involves the use of microorganisms to remove biodegradable organic material. The two most common methods used to achieve this are the 'suspended growth process' where the microorganisms are in suspension. Some example processes include activated sludge and the 'attached growth process', where microorganisms are attached to a media such as a trickling filter.

Half of the organic material is oxidized by the microorganisms to produce carbon dioxide and other end products and the remainder provides the energy and materials

needed to support the microorganism community. Sewage constituents become associated with secondary sludge following the microbial assimilation (e.g via sorption onto settleable solids or by incorporation into agglomerate particles) also referred to as bioflocculation. The excess biomass (as a result of the bioflocculation process) is separated in sedimentation tanks as a concentrated suspension called "secondary sludge".

2.2.4 Tertiary treatment

Tertiary treatment is the addition of any unit of treatment process in the flow schemes following conventional secondary treatment. Sometimes tertiary treatment can be found in conventional sewage treatment plants however it is only an option for the regulators. Tertiary treatment processes include filtration, lagooning, constructed wetlands and nutrient (nitrogen and phosphorus) removal.

2.2.5 Advanced treatment

Advanced treatment is considered when the discharge effluent is required to meet a higher water quality standards of the recieving water than the effluent produced by secondary treatment. The objectives of advanced treatment include further stabilization of the oxygen-demanding constituents in the sewage, the minimisation of nutrient enrichment of receiving water by nitrogen and phosphorus removal or the removal of toxic materials.

Advanced treatment can be divided into two major categories:

- Physical-chemical treatment
- Combined biological-physical treatment

Both categories of treatment have been developed in order to produce the highest quality of final effluent. Physical-chemical treatment is a treatment process which

may involve physical-chemical separation techniques such as adsorption, flocculation, membranes for advanced filtration, ion exchange and reverse osmosis (USEPA, 2004). In combination treatment, biological and physical-chemical treatment are both used.

2.3 Sewage reuse application

Sewage discharge into surface water has been a disposal means of STPs worldwide. Due to environmental circumstances disposal of sewage sludge into the sea has been banned in some countries such as in the UK since 1999 (Urban Wastewater Treatment Directive) (EA, 2010). It is generally accepted that land application of sewage sludge as fertiliser is a more environmental friendly method of disposing of treated sewage. However, treated sewage effluent discharge into surface water is still continuing. Although some regulators have imposed stringent water quality requirements before its release, the regulations are not comprehensively applied by other regulators in other parts of the world or even in the same countries. Many factors contribute to this lack of implementation such as poor management including bureaucratic, political and financial constraints. In addition, sewage itself contains a multitude of chemicals both identified and unidentified, and their treatment still remains a challenge especially for those compounds which have been classified as emerging endocrine disrupting chemicals.

As most of the surface waters in this world are the sources for potable water supply, deterioration of its quality has affected the entire water cycle. The increasing demand for freshwater from a multitude of water demanding activities such as agriculture in particular and industry in some regions worsen the condition as more clean water is needed to secure both sectors. Therefore, one of the options is to increase the reuse of treated sewage effluent produced by STPs. Moreover, reuse application is regarded as the best alternative approach of sewage disposal. In fact in developing countries, land application has always been the predominant method of disposing of sewage.

Sewage reuse can be seen largely in the agriculture sector either as treated effluent use for irrigation water or sludge use for fertilisers. For example about 41 % of treated sewage effluent is reused for irrigation in Japan, 60 % in California, USA, and 15 %

in Tunisia (Vigneswaran and Sundaravadivel, 2004). Other than irrigation, treated sewage effluent may be applied for industrial and domestic purposes including:

- non-potable purposes
- direct potable purposes

2.4 Sewage treatment effluent reuse for irrigation

Sewage treatment effluent reuse for land applications is a well established practice in many arid and semi-arid countries of the world as well as in developing countries. In arid and semi-arid countries, more than half of the effluent production (up to 70-90 percent) is used for land application purposes (Asano, 1987; Pettygrove, 2004). The application in agriculture and landscaping irrigation has been motivated by the fact that treated effluent is continuously produced, is rich in nutrients and is able to meet irrigation needs.

In developed countries, irrigation by treated sewage effluent for agricultural purposes was driven from the results of two pioneering studies that were conducted during the 1970s and 1980s in California called the *Pomona* and *Monterey* studies (Asano and Levine, 1996). The purpose of both studies was to determine the degree of treatment needed to minimise potential transmission of waterborne diseases through surface water and virus survival on crops and soils following effluent reuse as an irrigant. They concluded that 'microbiologically free' treated sewage reuse is possible only after virus removal is completed by tertiary treatment followed by disinfection of sewage. Even food crops that are consumed uncooked could be successfully irrigated by treated sewage effluent without adverse environmental and health effects. Meanwhile in Asia, Africa and Latin America, a recent survey conducted across 50 cities reports that irrigation by treated sewage effluent is a common practice in three-quarters of them (IWMI, 2006).

Although the use of treated sewage effluent has given some economical benefits, there are still a number of concerns related with its application to land:

- Significant concentrations of heavy metals may build up over time in the soil and can be taken up by food crops. This may affect their growth and produce adverse effects in both animals and humans if consumed.

- There is evidence that endocrine disrupting chemicals such as the synthetic oestrogens 17α-ethynloestradiol (EE2), oestrone (E1) and 17β-oestradiol (E2) are discharged via treated sewage effluents and occur in the environment in concentrations that detrimentally affect aquatic organisms. Thus, these compounds' behaviour in the terrestrial environment needs to be assessed for both the health of consumers as well as the protection of the environment.

- Potential occurrence of groundwater contamination as there are multitude of trace contaminants that still 'survive' in treated sewage effluents that may be highly mobile through the soil profile.
- Potential accumulation of contaminants in animal tissues and milk following vegetation uptake.

2.5 Sewage effluent reuse guidelines for irrigation

The first priority of all sewage effluent reuse guidelines and standards is to protect public health as well as the environment. Currently, most of the existing sewage effluent reuse guidelines and standards focus on the effluent quality produced by setting the concentration limits for parameters of concern as in Table 2.1.

Table 2.1: Summary of effluent quality parameters for effluent reuse for irrigation (Metcalf and Eddy, 2003; USEPA, 1992)

Parameter	Range in effluents aimed for effluent reuse	Significance for effluents reuse
Suspended solids	< 5 - 30 mg SS/L	Measurements of particles which can contribute to the clogging of the irrigation systems as well as lead to sludge deposits. Also can interfere with disinfection effectiveness.
Turbidity	< 0.1 - 30 NTU	
TOC	< 1 - 10 mg C/L	Measurement of organic substrate for microbial growth.
BOD_5	< 10 - 45 mg BOD/L	Their biological decomposition can deplete oxygen levels. Can favour bacterial re-growth in distribution systems and microbial fouling.
COD	< 20 - 90 mg COD/L	
Total coliforms	< 1 - 200 cfu/100 mL	Measurement risk of infection due to the presence of pathogens.
Faecal coliforms	< 1 - 103 cfu/100 mL	
Heavy metals	< 0.001 mg Hg/L, < 0.01 mg Cd/L, < 0.1 - 0.02 mg Ni/L	Measurements of toxic elements.
Inorganics	> 450 mg TDS/L	High salinity is harmful for irrigation. Can favour clogging to the distribution system and excessive salinity may damage crops. Extensive sodium for example may cause permeability problems in soil.
Chlorine residuals	0.5 - 1 mg Cl/L	To prevent bacterial re-growth. Excessive amounts of chlorine can damage some crops.
Nitrogen	< 1 - 30 mg N/L	Fertilisers for crops. However, excessive amounts can contribute to algal growth when surface runoff occurs.
Phosphorus	< 1 - 20 mg P/L	

However, the sewage effluent quality guidelines and standards implemented vary from country to country. In addition, the standards can sometimes vary in the

same countries. However, one criterion remains the same when considering the addition more of parameters to the the guidelines or standards and that is the cost of treatment and monitoring (Fatta and Kythreotou, 2005). Due to this economic reason, most developing countries have adopted a low cost approach in implementing sewage effluent reuse guidelines and standards, whereas developed countries have established a high cost approach as they have more advanced technology to produce a higher quality of effluents for reuse. However, even a high cost approach does not guarantee the prevention of adverse effects due to such factors such as insufficient operational experience, inadequate operational funds for maintenance and for the proper functioning of the treatment process. Limited information on the success of advanced technologies employed to treat a multitude of sewage contaminants such as the emerging endocrine disrupting chemicals (EDCs) is also a problem. Meanwhile, several developing countries have already adopted a low cost approach based on World Health Organisation (WHO) recommendations, which are thought too lenient for public health protection. Although it appears that the WHO guidelines are insufficient, there is no general agreement yet on the best approach to follow as there are several factors to consider. Local irrigation practices, local soil conditions, the desire to protect the public health, the existing local sewage treatment practices and the choices of the technologies available for irrigation systems as well as the financial costs must all be considered (Angelakis et al., 1999).

It is recognised that all the guidelines focus on the potential effects of the direct exposure of users to effluent. However, several exposure routes have been identified that may occur via irrigation for example surface runoff, leaching and plant uptake. As hundreds of organic compounds have been detected and identified in sewage and many may remain unidentified, an extension of the chemical parameters to be monitored for effluent quality is warranted in order to prevent adverse long terms effects to environmental compartments as well as to public health with a view to eventually obtain environmental sustainability. Once again how far regulators will be willing to implement more stringent quality parameters is uncertain as it involves a high cost both to regulate and to monitor the overall treatment process.

2.6 Occurrence in the environment

The occurrence of free oestrogens in the aquatic environment has been linked to the sewage effluents discharged by STPs into receiving waters. The observation of

hermaphrodite fish in caged male fish placed in streams nearby STPs by Purdom and co-workers (1994) confirmed the occurrences of endocrine disruption of freshwater fish which received the sewage effluents following disposal (Purdom et al., 1994). Further research done in the UK, Japan and Germany using a toxicity identification and evaluation approach based on the yeast estrogen screening assay (YES) concluded that the steroidal oestrogens oestrone (E1), 17β-oestradiol (E2) and 17α-ethnyloestradiol (EE2) appeared to be the most potent endocrine disrupters in sewage treatment plant effluent responsible for observed oestrogenic disruption (Matsui et al., 2000; Korner et al., 2000; Desbrow et al., 1998), and their potency was demonstrated to be over a thousand times greater than other xenobiotic oestrogen mimics (Thorpe et al., 2001; Routledge et al., 1998).

In general, concentrations of steroid oestrogens detected in surface water were as low as nanograms per litre (ngL^{-1}). Synder et al. (1999)analysed 13 surface water sites in the USA and reported the concentrations of 17β-oestradiol (E2) and 17α-ethnyloestradiol (EE2) were in the ranges between 0.1 to 2.67 ngL^{-1} and 0.05 to 0.52 ngL^{-1}, respectively due to the inevitable link to STP effluent discharges into the receiving waters. In seven out of eleven Dutch freshwater samples, oestrone (E1) was detected with a maximum concentration of 3.4 ngL^{-1}, while 17β-oestradiol (E2) and 17α-ethnyloestradiol (EE2) were detected with maximum concentrations of 5.5 $ngL^{-1}$and 4.3 ngL^{-1} respectively (Belfroid et al., 1999). In Germany, the maximum concentrations of oestrogenic steroids (E1, E2 and EE2) detected were 14 ngL^{-1}, 3.6 ngL^{-1} and 5.1 ngL^{-1}, respectively, in rivers receiving STPs effluents (Kuch and Ballschiter, 2001). Barel-Cohen et al. (2006) reported the occurrence of 17β-oestradiol (E2) at concentrations 2 to 4 ngL^{-1} over a 100 km stretch downstream of a sewage effluent discharge point during spring sampling meanwhile during autumn sampling, the concentrations of 17β-oestradiol (E2) were maintained within the first 30 km but the levels dropped below the detection limit only at the end of the stretch. Zuo et al. (2006) studied the possibility that oestrogenic hormones contained in effluents might have contributed to the observed decline in lobster abundance in Buzzards Bay, Massachusetts, and reported the occurrence of 17β-oestradiol (E2), oestrone (E1) and 17α-ethnyloestradiol (EE2) with the concentrations of 0.83 ngL^{-1}, 1.2 ngL^{-1} and 4.7 ngL^{-1}. From a two year survey on estuary water receiving a high discharge from domestic and industrial STPs, Noppe et al. (2007) reported the occurrence of oestrone (E1) at concentrations between 1 to 10 ngL^{-1}.

There is very limited data available on steroid oestrogens in drinking water. Recent data was reported by Kuch and Ballschiter (2001), who monitored drinking waters from three different treatment works and the concentrations detected were as follows; oestrone (E1) at 0.2-0.6 ngL^{-1}, 17β-oestradiol (E2) at 0.2-2.1 $ngL^{-1}$and 17α-ethnyloestradiol (EE2) at 0.15-0.5 ngL^{-1}.

A number of field studies have demonstrated that sediment could act as a sink for steroid oestrogens, not only to the river bed but also to the marine and estuarine bed. Kanda et al. (2001) reported the detection of oestrone (E1) at concentrations ranging between <0.1 to 0.386 μgkg^{-1}in sediment samples taken from two United Kingdom rivers (River Nene and Lea) while the concentrations of 17β-oestradiol (E2) and 17α-ethnyloestradiol (EE2) were below 0.1μgkg^{-1}(as cited byYoung et al., 2004). From the same site samplings of River Nene and Lea, Williams et al. (2003) also reported the concentrations of 17β-oestradiol (E2) and 17α-ethnyloestradiol were below 0.1 μgkg^{-1} and concentrations of oestrone (E1) ranged from < 0.04 to 0.36 μgkg^{-1}. In a recent study, Labadie et al. (2007) reported concentrations ranged from 3.0 ngg^{-1} at the surface sediment to 30 ngg^{-1} at the core sediment approximately 15 cm depth from the surface sediment for oestrone (E1) and concentration less than 1.1 ngg^{-1} for 17β-oestradiol (E2) in sediments samples taken from the freshwater and estuarine sites, both downstream of sewage discharges into the River Ouse (UK).

Due to the potential uptake and accumulation of oestrogens exceeding 300 ρgL^{-1} by the reef benthos (Atkinson et al., 2003), an attempt to study the abilility of steroidal oestrogens to accumulate in marine sediments in conjuction with deep ocean sewage outfall (Sydney, Australia) was carried out by Braga et al. (2005). The concentration of oestrogens reported ranged between 0.16 to 1.17 ngg^{-1} for oestrone (E1), 0.22 to 2.48 ngg^{-1} for 17β-oestradiol (E2) and < 0.05 to 0.5 ngg^{-1} for 17α-ethnyloestradiol (EE2). The concentrations reported were from samples taken 7 km from the outfall and they were comparably higher than the concentrations measured from the samples taken immediately adjacent the outfall. It was then suggested that oestrogens are attached to particles in the effluent, and as a result of contact with the high ionic strength of seawater, they are aggregating and being deposited on the seafloor. In Japan, Matsuoka et al. (2005) reported the concentrations of oestrogens in surface sediments of three selected bays in the ranges between 0.1 to 1.33 ngg^{-1} for oestrone (E1) and 0.17 to 2.33 ngg^{-1} for 17β-oestradiol (E2) at Osaka Bay; 0.21 to 0.87 ngg^{-1} for oestrone (E1) and 0.15 to 0.49 ngg^{-1} for 17β-oestradiol (E2) at the Otsuchi Bay; 0 to

2.16 ngg^{-1} for oestrone (E1) and 0.16 to 0.82 for 17β-oestradiol (E2) at Yamada Bay. Isobe et al. (2006) through his monitoring survey to investigate the distribution of steroid oestrogens in surface sediments of Tokyo Bay has reported the concentrations of oestrogens ranging between $<$ 0.07 to 0.59 ngg^{-1} and 0.05 to 3.60 ngg^{-1} for 17β-oestradiol (E2) and oestrone (E1) while no 17α-ethnyloestradiol (EE2) was detected.

A recent study shows that the significant concentrations available in sediments are able to infiltrate and contaminate shallow groundwater (Labadie et al., 2007). Labadie et al. (2007) confirms that oestrogen, particularly oestrone (E1), carried by sewage into rivers is able to seep through river sediments and is capable of polluting the groundwater. Previously, Matsuoka et al. (2005) reported a sudden increase in the level of oestrone (E1) in core sediment sampled at depth of 16 to 20 cm and suggested that the oestrogens in the sediment would be able to remain there over time. In their study, the oestrone (E1) was reported to have remained in the sediments for forty years (Matsuoka et al., 2005).

The exposure of soil to steroidal oestrogens is believed to occur from several sources such as land application of reused sewage treatment effluent, animal manure and leakage from on-site sewage treatment. However, soil samples have largely been overlooked and the information on this environmental compartment is limited in the literature. Most of the studies in the literature focus on the occurrence of these steroidal oestrogens in soils following applications of manure. Finlay Moore et al. (2000) reported the detection of 17β-oestradiol (E2) in a pasture topsoil (0-2.5 cm) after 4 days amendment with poultry litter with a concentration of 305-820 ngL^{-1} and after 88 days it was still detected in the range between 60-125 ngL^{-1}. Meanwhile, Beck et al. (2008) recently reported the concentration of oestrogens in the ranges between 3 $ngkg^{-1}$ and 25 $ngkg^{-1}$ for 17β-oestradiol (E2) and oestrone (E1) in a cropland soil that had been regularly amended with manure three times a year, and 2 $ngkg^{-1}$ and 12 $ngkg^{-1}$, respectively, in an intensively grazed pasture soil (0-15 cm) which was also amended with manure. A few studies reported that disposal of animal manure to agricultural land could lead to movement of these compounds into groundwater (Shore et al., 1995; Peterson et al., 2000). Shore et al. (1995) believed that a constant 17β-oestradiol (E2) concentration of about 5 ngL^{-1} in spring waters was caused by infiltration through the soil profile to the groundwater following manure application to land (as cited by Peterson et al., 2000). Peterson et al. (2000) studied the impact of disposal of poultry manure by the poultry industry had mea-

sured 17β-oestradiol (E2) concentrations ranging from 6 to 66 ngL^{-1} in mantled karst aquifers in northwest Arkansas, an area where a large segment of the agricultural economy of United States of America is located.

Recently, (Swartz et al., 2006) attempted a study to understand the fate of organic contaminants associated with sewage that are introduced to groundwater by installing a series of sampling wells immediately downgradient from a septic system that discharged sewage effluent to a shallow sandy aquifer of Cape Cod, Massachusetts, USA. The author reported the occurrence of 17β-oestradiol (E2) and oestrone (E1) with concentrations of approximately 10 ngL^{-1} and 80 ngL^{-1} at 5 m depth below the wells land surface and suggested that the concentrations detected indicate that potential groundwater contamination exists.

There is as yet no information regarding the occurrence of these hormones in the soil or groundwater following effluent reuse as irrigation.

2.7 Mobility of compounds in the environment

Like other organic compounds, oestrogens can undergo a variety of processes such as leaching, runoff and plant uptake after being released to the environment. Figure 2.1 illustrates the possible pathways of oestrogens that are governed by the above mentioned processes.

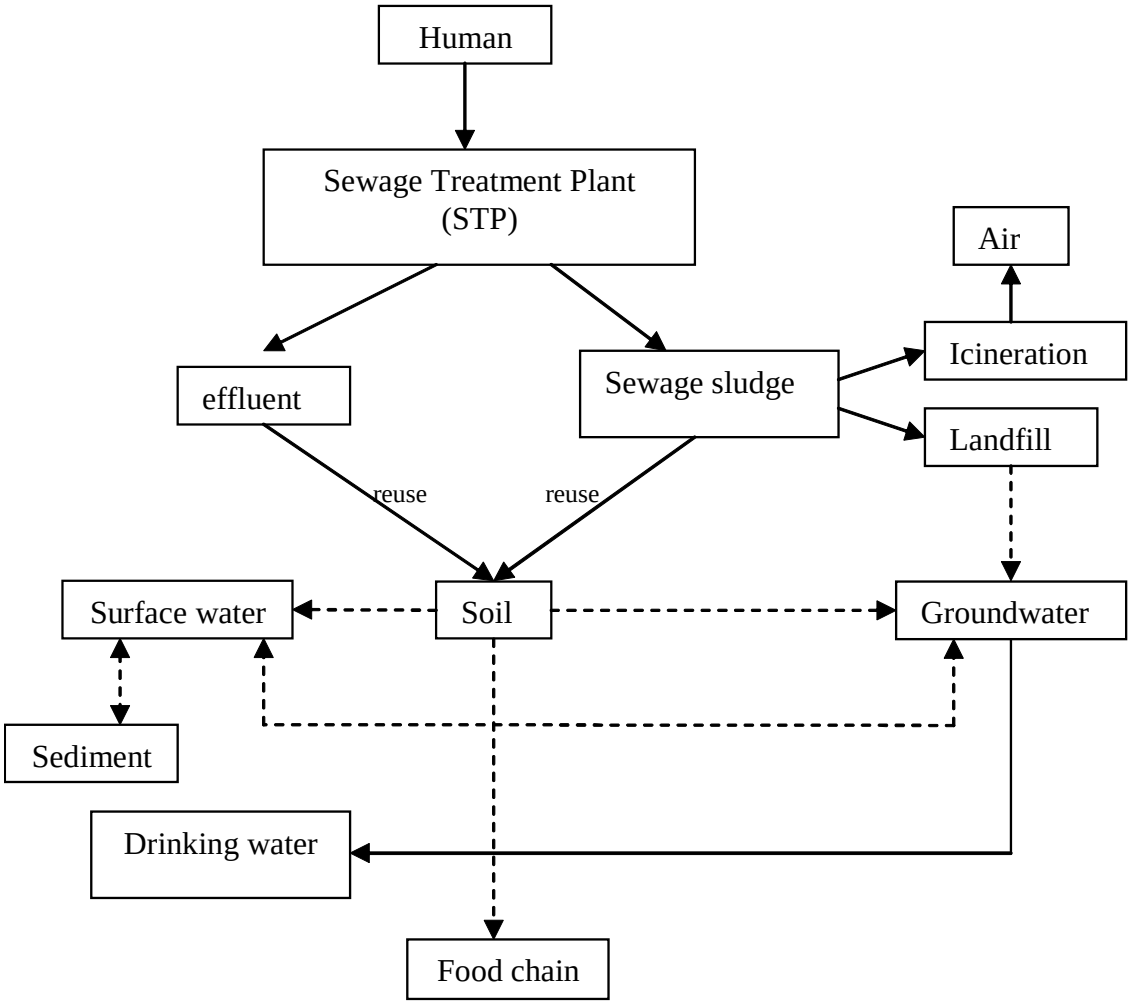


Figure 2.1: Pathways of environmental exposure following sewage reuse

2.7.1 Surface runoff

Most of the surface runoff of oestrogens has been observed in agricultural runoff following the application of livestock manure. Shore et al. (1995) reported the concentration of oestrogens in the streams draining farm field amended poultry manure increased from < 0.5 ngL^{-1} to 5 ngL^{-1}; however no application rates of poultry litter were specified (as cited by Hanselman et al., 2003). Nichols et al. (1997) in examining land application of broiler manure reported that the rate of runoff is significantly influenced by the application rates. The runoff concentrations increased reaching

a maximum of 1280 ngL^{-1} at an application rate of 1.76 to 7.05 Mg manure ha^{-1} (Nichols et al., 1997). Finlay Moore et al. (2000) showed that the concentrations in runoff was not only dependent on the application rate but also appeared to be time dependent. The author reported that the concentrations of 17β-oestradiol in runoff were high (305-820 ngL^{-1}) 4 days after manure application but after 88 days the concentrations decreased (60-125 ngL^{-1}). It was then suggested that the decrease in concentrations may have resulted from the movement of 17β-oestradiol into the soil profile or may be caused by surface runoff. However as no samples were collected from lower soil depth there can be no confirmation that the leaching process occurred.

2.7.2 Leaching

Only a few studies have reported the occurrence of oestrogens in groundwater (Peterson et al., 2000; Swartz et al., 2006). A recent field study conducted by Kjaer et al. (2007) reported that oestrogens are able to leach from manure treated soil and that oestrone (E1) in particular can leach in high concentrations within 3 months after application of slurry manure from the root zones into the tile drains. Labadie et al. (2007) through their field work suggested that the downward movement of oestrone (E1) into sediment core was possibly due to a change in sediment sorption properties or a change in the porewater velocity. Previous laboratory studies suggested that the risk of oestrogens to leach down the soil profile is low (Casey et al., 2005; Colucci et al., 2001; Colucci and Topp, 2001). From the soil column experiment, 17β-oestradiol (E2) was reported to be able to escape the 7 cm organic rich topsoil (Casey et al., 2005). The author however reported only 26 % of 8.5 mg of radiolabelled 17β-oestradiol (E2) was recovered in the leachate after 42 hours of continuous feeding of this radiolabelled compound in the soil column. Colucci et al. (2001); Colucci and Topp (2001) from their work establish in laboratory microcosm incubations to study oestrogens dissipation behaviour concluded that the radiolabelled oestrogens were readily biodegraded in soils under a range of temperatures from 4 to 30^{o} C within 3 days of contact, thus greatly reducing the risk of oestrogens mobility.

In conjungtion with limited study to studies on the leachability of these oestrogens in the soil profile (e.g soil column study), various models have been used to determine

the general leaching ability solely on the basis of their physico-chemical parameters. From the various models developed, the leaching ability of compounds could be determined by the properties K_{ow} and K_{oc} where K_{ow} is the octanol-water partition coefficient and K_{oc} is partitioning coefficient normalised to organic carbon content. The K_{oc} values provide a good indication of the likelihood of leaching through soil. Compounds with higher K_{oc} values tend to adsorb more strongly onto organic carbon. Compounds with lower K_{oc} are more readily leached as the organic carbon content lessens from the top to the bottom of a soil profile. Wilson et al. (1996) classified the mobility of compounds based upon their K_{ow} and K_{oc} values as shown in Table 2.2.

Table 2.2: Mobility classification (adapted from Wilson et al. (1996))

Classification	log K_{ow}	log K_{oc}	Mobility
1	> 3.78	> 3.7	immobile
1	-	3.3-3.7	slight
2	2.39-3.78	2.7-3.3	low
3	1.36-2.39	2.2-2.7	medium
4	0.08-1.36	1.7-2.2	high
5	< 0.08	<1.7	mobile

Based on limited reported K_{ow} and K_{oc} values from previous literatures, natural and synthetic oestrogens could be classified as low to immobile compounds. Thus, the leaching of these compounds down the soil profile is not expected. This is in contrast with the reported occurrence of oestrogens in groundwater (Peterson et al., 2000; Swartz et al., 2006).

Although the use of predictive models such as these can give an estimation of a compound's leachability, some limitations arise when applying these models to a real environment. In addition to the compound's physico-chemical parameters, the mobility of compounds in the environment is also influenced by other environmental characteristics such as soil type, the presence of colloidal materials and temperature. Thus, experimental assessment is vital to give absolute values to the ambient conditions.

2.7.3 Vegetation uptake

The uptake of chemicals by plants is important to assess as plants are the base of the food chain. It is necessary to incorporate plants in multimedia predictive models; however, it is difficult to model plant uptake of chemicals. A major obstacle in assessing chemical uptake is that plants grow very quickly and their circulatory system is not as efficient as animals (Mackay, 2001). However, there are still a number of field studies that examine the uptake of organic compounds by plants and limited study has specifically been carried out with respect to oestrogens. Hacker et al. (1967) demonstrated a presence of oestrogen at concentrations of less than 5 μgkg^{-1} in plant roots rather than other plant parts following application of diethylstilbestrol (DES) in manure as a fertiliser. Following mouse uterine weight assay of all affected plant parts, uterotrophic activity was negative except in radish leaves and in lettuce roots that were grown with soil at pH 7.5.

From the literature, there are several routes by which organic compounds present in soil may enter into plants (Topp et al., 1986). Duarte-Davidson and Jones (1996) through their work have developed a model to determine the potential uptake of organic compounds by the root system based on their physico-chemical parameter log K_{ow} as shown in Table 2.3.

Table 2.3: Root retention potential (adapted from Duarte-Davidson and Jones (1996))

Classification	Log K_{ow}	Potential for root retention
1	> 4.0	High
2	> 2.5 and < 4.0	Medium
3	< 2.5	Low

Based on this, the natural oestrogens, oestrone (E1) and 17β-oestradiol (E2) are classified as class 2 (medium root retention) and synthetic oestrogen, 17α-ethnyloestradiol (17αEE) is classified as class 1 (high root retention).

However, the potential of high K_{ow} compounds to adsorp to root surfaces might be retarded by the organic carbon content of the soil wherein soil with high organic carbon content strongly retains these compounds rather than releasing them to be taken up by plants (Chiou et al., 1986).

Due to several irrigation techniques, there are several ways organic contaminants could be transferred to vegetation. For example, in respect to spray irrigation, the compounds may be deposited on the leaf or stem of the plant whilst in the direct application of irrigant to the soil, volatilisation of the organic contaminants may occur. The compound deposited either on the leaf or stem may be adsorbed and remain in the waxy cuticles of the leaves if it has a high K_{ow} value, and if it has a low K_{ow} value, it may be transported into the plant via phloem (Duarte-Davidson and Jones, 1996). Thus, the K_{ow} value is essential to determine the fate of any compound deposited on the leaf of the plants.

Based solely on the K_{ow} and by referring to Table 2.4, the natural oestrogens, oestrone (E1) and 17β-oestradiol (E2) are classified as class 2 (medium/high potential) while the synthetic oestrogen, 17α-ethnyloestradiol (17αEE) is classified as class 1 (high potential). Thus, these classification indicate a high/medium/possible risk for foliar uptake of these natural and synthetic oestrogens. However, given low volatilisation potentials and strong retention by soils, it is unlikely that these compounds will be deposited onto foliage following the direct contact of sewage treatment effluent irrigation water onto soils.

Table 2.4: Potential for foliar uptake (adapted fromDuarte-Davidson and Jones (1996))

Classification	Volatility Potential		log K_{ow}	Potential for Foliar Uptake
1	High	and	> 4.0	High
2	High/Medium	and	> 2.5 and < 4.0	Medium/Possible
3	Medium	and	> 4.0	Medium/Possible
4	Low	or	< 2.5	Low

2.7.4 Animal uptake

The transfer of organic compounds to animals particularly livestock through treated sewage reuse has several potential routes:

1. ingestion of vegetation which has accumulated the chemical.

2. direct ingestion of soil amended with treated sewage or effluent.

3. ingestion of soil/sludge which has adhered to the vegetation.

The potential for livestock to accumulate the organic contaminants from the above routes mentioned is dependent on the chemical's persistence in the environment (which is normally expressed by its half-life, $t_{1/2}$), its potential to be uptaken by plants in the environment and other factors such as animal species and grazing habits. For example, sheep can ingest chemicals from soil by grazing on harvested forage as they are reported usually to ingest between 1-2% of soil in their diet and under special circumstances, this could be up to 26 % (Chaney, 1985).

Once again, Duarte-Davidson and Jones (1996) have estimated the likelihood of organic contaminants to be uptaken by livestocks through soil ingestion based on its physico-chemical properties. This is shown in Table 2.5. Based on limited half-life, $t_{1/2}$, and the physico-chemical property, log K_{ow}, all the oestrogens have medium potential to be uptaken by livestocks through soil ingestion.

Table 2.5: Potential for animal soil ingestion (adapted from Duarte-Davidson and Jones (1996)).

Classification	Half-lifes, $t_{1/2}$ (days)		log K_{ow}	Potential for Soil Ingestion
1	> 36	and	> 4.5	High
2	> 36	and	< 4.5 and > 3.0	Medium
3	< 36	and	< 3.0	Low

In regard to several irrigations techniques, based on the potential for animal intake via plant ingestion (Table 2.6) and by referring to the potential for foliar uptake (Table 2.4). If direct contact of compounds to the leaf or stem occurs following irrigation, these natural oestrogens would be classified as having medium to high potential to be uptaken by animal via plant ingestion. However, based on low volatility and strong retention by soils, low potential would be expected for animal intake by plant ingestion if these compounds were deposited on the soils following techniques other than spray irrigation.

Table 2.6: Potential for animal intake via plant ingestion (adapted from Duarte-Davidson and Jones (1996)).

Classification	Half lives $t_{1/2}$(days)		Translocation potential		Foliar uptake potential	Animal intake potential by plant ingestion
1	> 36	and	High	and/or	High	High
2	> 36	and	Medium	and	Medium	Medium
3	> 36	and	Medium	and	Low	Medium
4	> 36	and	Low	and	Medium	Medium
5	< 36	and/or	Low	and	Low	Low

Following animal ingestion, these organic compounds may be either metabolised and excreted in urine and faeces or accumulated in fat and transferred to milk (Duarte-Davidson and Jones, 1996). From the literature there are two chemical characteristics of the compounds that are important in determining the likelihood that it will be transported through terrestrial food-chain; persistence and lipophilicity. A compound with a low persistence in animal tissue tends to be less lipophilic and therefore is more easily metabolised. A compound with a high persistence and high lipophilicity tends to be stored and concentrated in fatty tissue of animals.

The most common and simplest approach to quantifying the accumulation of compounds in animals and their products is by using bioconcentration factors (BCFs). Various authors established a correlation between K_{ow} and BCFs in livestock (Kenaga, 1980; Travis and Arms, 1988). These relationship illustrated that the ability of compounds to accumulate in the animal tissues was based on lipophilicity; bioconcentration increases with increasing lipophilicity.

In the case of oestrogens, synthetic oestrogen 17α-ethnyloestradiol (17αEE) may cause adverse effects if it is inadvertently ingested. This oestrogen was designed to be highly orally active as compared to natural oestrogens, oestrone (E1) and 17β-oestradiol (E2), which are likely to elicit less physiological response.

Chapter 3

ENVIRONMENTAL FATE OF NATURAL AND SYNTHETIC OESTROGENS

3.1 Factors influencing fate and behaviour

Once a compound is released into the environment it may be transported to other locations by several processes as discussed in Chapter 2. These processes such as leaching and surface runoff are generally governed by a compound's fate and behaviour in the medium where it resides in the environment. A compound's fate and behaviour is dependent on its physico-chemical properties such as vapour pressure, aqueous solubility, octanol-water partition coefficient (K_{ow}) and Henry's Law constant. In addition, environmental factors such as temperature, organic matter content, pH and salinity also play a role for instance in governing a compound's half-life and bioavailability. The combination of these factors will reflect how specific compounds may be expected to distribute, persist and to bioconcentrate in different environmental compartments (Samiullah, 1990).

Of the physico-chemical properties, a compound's aqueous solubility will give an indication of its potential either to stay in solution or partition into the solid phase. A highly soluble compound has a tendency to stay in the polar phase while a compound with a lower solubility value will partition into a non-polar phase.

A compound's water solubility has also been linked to the octanol-water partition

coefficient (K_{ow}). A compound with high water solubility tends to have a lower K_{ow} value whilst a low solubility compound tends to have a high K_{ow} value. K_{ow} values have been used as a useful measurement of the partitioning of a compound between water and lipophilic phases such as lipids. Generally, a compound which has a low K_{ow} value dissolves more readily in water and is less likely to sorb onto solids or accumulate in lipids; thus, in human systems they would be excreted in the urine. In contrast, a compound with a high K_{ow} value will have a strong tendency to associate with lipids or in the environment to partition into organic matter of soils or sediments.

Considering the high octanol-water partition coefficients (K_{ow}) of these natural and synthetic oestrogens, they are expected to bind strongly to the organic matter in soils thus affecting the amount of oestrogens available to be transported and/or degraded either chemically or biologically (Shareef et al., 2006a).

From the literature, it can be deduced that there are many factors that contribute to the availability of a chemical in soils and sediments. It may be dependent on its behaviour such as how well it adsorbs to the soil or sediment matrix which is greatly influenced by its physico-chemical properties for example its aqueous solubility. Adsorption normally decreases bioavailability; strong sorption reduces the release rates of the compounds from soil and also the microbial degradation. The bioavailability of a compound will determine its tendency to bioaccumulate in the environment.

The following section discusses the literature available on the processes that governed the fate and behaviour of compounds in the environment as well as their potential to bioaccumulate.

3.2 Sorption

Sorption is regarded as the most important factor regulating a compound's fate and behaviour in a particular environmental compartment. It has a major influence on a compound's mobility in soils or sediments and on a compound's availability for other processess such as degradation. Sorption represents a process in which chemicals become associated with solid phases. It can be defined as " the net accumulation of a sorbate at an interface between a sorbent and the solution phase " (Samiullah, 1990).

Research first started in examining the sorptive capability of oestrogens as to remove them from aqueous solutions. Most of the studies have focused on sewage treatment specifically and removal techniques (Schafer et al., 2003; Nghiem et al., 2004; Yamamoto and Liljestrand, 2004). In addition, other studies have focused on the sorption capability of oestrogens on river bed sediments as a means of their removal from water columns(Lai et al., 2000; Holthaus et al., 2002; Yu et al., 2004). However, limited studies have been conducted on the sorption of oestrogens to soils following sewage effluent reuse from STPs.

From the literature sorption of oestrogens in any environmental compartment is largely influenced by the following factors:

- The octanol water partition coefficient (K_{ow}) of the compound.
- The organic carbon content of the sorbent.

Since the octanol-water partition coefficient (K_{ow}) is a useful tool for predicting the distribution of a compound in two immiscible phase, a K_{ow} value of oestrogens is then helpful in predicting the affinity of these compounds to partition onto solids. Considering the high K_{ow} values of these natural and synthetic oestrogens, they are expected to exhibit substantial sorption to organic matter. Organic carbon appears to provide a primary mechanism for the oestrogen sorption process to the soil (Lee et al., 2003). The hydrophobic nature of natural and synthetic oestrogens results in an affinity toward organic matter, thus yield high log K_{oc} values ranging from $<$ 3 to 4 (Lee et al., 2003) and in some cases higher than 5 (Yu et al., 2004). Alternative partitioning mechanisms other than to organic carbon have also been reported for example sorption to the binding sites provided by soil minerals. Some of the previous literature report alternative binding sites provided by clay surfaces (Casey et al., 2003; Hildebrand et al., 2006) and sorption of oestrogen is reported to be high in soil with high clay contents (Hildebrand et al., 2006). Of clay constituents, montmorillonite was found to adsorb 17β-oestradiol (E2) and oestrone (E1) much higher than other clay constituents such as goethite, illite, and kaolinite (Shareef et al., 2006b; Bonin and Simpson, 2007).

Most of the studies undertaken use site samples to study the sorption potential of oestrogens in environmental compartments. This has resulted in inconsistent sorption coefficient values, which differ by several orders of magnitude, primarily due to the differences in site characteristics which have yet to be resolved. In this study, a decision not to use sampling site soils but laboratory mixed soils was taken. It is believed that these laboratory mixed soils will represent benchmark samples in order to control variables and also allow intepretation of factors that governed the sorptive capabilities of these oestrogens in environmental compartments.

In addition to the physico-chemical properties (e.g aqueous solubility and K_{ow}) of compounds and the physical properties of environmental compartments where they reside (such as soil particle distribution), as well as other characteristics such as pH, salinity and temperature are considered as factors that may affect the sorption capability. Regarding the sorption of oestrogens in environmental compartments, a limited number of studies have been conducted which clearly indicate the potential of pH, salinity and temperature to affect sorption. Bowman et al. (2002) reported that there was no effect of salinity towards 17β-oestradiol (E2) sorption onto estuarine sediments however the salinity gradient present under estuarine condition did enhance the sorption coefficients of oestrone (E1), whereas Lai et al. (2000) reported a higher sorption of oestrogens onto sediments with higher water salinity. Meanwhile Yamamoto et al. (2003) reported no significant effect on the sorption of oestrone (E1) and 17β-oestradiol (E2) with pH values of 5, 7 and 9 onto organic matter surrogates (humic and fulvic acid). The pH range used by (Yamamoto et al., 2003) was limited and thus resulted in limited interpretation of the pH capability to influence oestrogens sorption potential in environmental compartments.

In this study, the characteristics of pH and salinity were considered as parameters to be varied in sorption experiments of oestrogens, oestrone (E1), 17β-oestradiol (E2) and 17α-ethnylestradiol (EE2) onto soils. The effect of salinity was reassesed in order to give a clear indication on the potential sorption capability. A wide range of pH values which also covered the acid dissociation constant (pKa) values of oestrogens was also studied in order to reassessed the pH effect on oestrogen sorption to the soils. As there are no studies reporting the effect of temperature on the sorption process although global temperature vary significantly, this parameter was also considered in order to study its effect on oestrogens sorption to soils.

3.3 Degradation

Degradation refers to any chemical alteration a compound undergoes in the environment. Degradation can be subdivided into abiotic degradation and biodegradation. Abiotic degradation is defined as the "transformation of organic compounds by chemical reactions such as oxidation, reduction, hydrolysis and photodegradation" (Cronin and Livingstone, 2004). Biodegradation is defined as the "transformation of organic compounds attributed to microbial activities via oxidation, reduction, and hydrolysis reactions"(Cronin and Livingstone, 2004). Abiotic degradation processes are usually incomplete and result in other organic products (Cronin and Livingstone, 2004).

The capability of microorganisms to degrade steroidal oestrogen compounds would be beneficial for sewage treatment processes, and previous studies report a degradation of oestrogens in sewage treatment systems (STPs). For example, the natural oestrogen, 17β-oestradiol (E2) was oxidized over time to oestrone (E1) while no oxidation occurred to synthetic oestrogen, 17α-ethnyoestradiol (EE2) in an experimental study conducted with activated sludge (Ternes et al., 1999b). During sewage treatment, it is common to describe the removal of oestrogens concentration due to both processes, sorption and degradation as a collective terms as it is difficult to distinguish the most responsible process involve.

As the degradation of oestrogens in STPs has been documented, it seems likely the same activities occur in the aquatic environment. This has been confirmed in various studies. Ying and Kookana (2003) for example reported a degradation of the oestrogens, oestrone (E1) and 17α-ethnyloestradiol (EE2) of more than 90 % within 56 days in seawater collected from coastal areas around Adelaide, Australia. Meanwhile Jurgens et al. (1999) reported a degradation of approximately 50 % of 17α-ethnyoestradiol (EE2) and oestrone (E1) within 17 days and between 2 to 8 days, respectively, in the aqueous phase (Jurgen et al., 2002). The determination of degradation rates of oestrogens in sediments has also been conducted and the rates reported also vary from one study to another. For example, one study reported 14 days for 17β-oestradiol (E2) to be fully degraded and longer for 17α-ethnyoestradiol (EE2) (Robinson and Hellou, 2009) as there was still a detectable level on day 14 and no further measurements were conducted. Ying and Kookana (2003) reported a degradation time of oestrogens in marine sediment samples of 2 days for 17β-oestradiol (E2) to degrade by approximately 50 % and 81 % for 17α-ethnyoestradiol

(EE2). (Kuster et al., 2004) reported a stable concentration of 17α-ethnyloestradiol (EE2) in sediments with a half-life of 46 days. The inconsistent degradation rates in aquatic environments reported may be due to site-specific conditions since different sites will have different adaptation of the native microbial communities. Chemical degradation of oestrogens may also occurr in aquatic environments. (Kang and Kondo, 2005) for example reported the chemical degradation of organic compounds in seawater and were also accelerated by the microbial activities present.

In the terrestrial environment, oestrogen degradation potential has received little attention. Only recently have researchers begun to investigate this matrix as a potential route through which the compounds can exert their potentially adverse effect on wildlife and on human health. Ying et al. (2003) reported a slow and incomplete degradation of 17β-oestradiol (E2). The presence of oestrone (E1) was reported following 17β-oestradiol (E2) degradation in soils (Colucci et al., 2001; Colucci and Topp, 2001). However, since oestrone (E1) was not analysed, there is no confirmation on the transformation of 17β-oestradiol (E2) to oestrone (E1) (Ying et al., 2003). For 17α-ethnyloestradiol (EE2) no transformation product has been reported so far. Xuan et al. (2008) in their study reported there was no significant degradation of 17β-oestradiol (E2) and 17α-ethnyloestradiol (EE2) was observed in sterilize silt loam soils and this in contrast with (Colucci et al., 2001) which reported the 17β-oestradiol (E2) degradation was under abiotic process, in which the autoclaved soils did not prevent the degradation of 17β-oestradiol (E2) and transformation to oestrone (E1) as its metabolite. Thus, further investigation is warranted to identify the responsible process to degrade oestrogens as the previous literature were conflicting.

The degradation rates of oestrogens in soils have been estimated to range from as short as a few hours to approximately 25 days (Colucci et al., 2001; Xuan et al., 2008). Despite this, however, they have been found to persist in soils as a recent study showed that these hormones persist in soil for time frames exceeding months (Stumpe and Marschner, 2007). Until today, there is still limited information regarding the capability of microorganism in soil to degrade oestrogens. An early investigation by Turfitt (1947) showed that no culturable bacteria found in loam and peat soils were able to degrade oestrogens, 17β-oestradiol (E2) and oestrone (E1); however, a strain of bacterial genus *Proactinomyces* isolated from an acid sand soil could degrade these oestrogens (as cited by Hanselman et al., 2003).

In this study, degradation measurement of oestrogens in soils is not given any con-

sideration; however, all soils used in this study were sterilised.

3.4 Partition coefficients

Partition ratios are essentially the distribution of a solute between two immiscible phase. One of the most useful parameters in predicting the environmental fate is the octanol-water partition coefficient (K_{ow}). It is described by Equation 3.1 and it is used as an input parameter for most exposure and risk assessment models (Cronin and Livingstone, 2004).

$$K_{ow} = \frac{concentration\ of\ the\ compound\ in\ octanol}{concentration\ of\ the\ compound\ in\ water} \qquad \text{(Equation 3.1)}$$

The K_{ow} values can be obtained by several approaches. Experimentally, K_{ow} can be measured directly via several classical separation methods, for example the slow stirring method and the shake flask method, or indirectly by the estimation from chromatographic methods such as via reverse phase high performance liquid chromatography (RP-HPLC) (OECD, 2004). A theoretical calculation of K_{ow} also exists and many studies report regression equations between K_{ow} and water solubility (Pinsuwan et al., 1995; Ran et al., 2002). However, the log K_{ow} values obtained rely on the regression equation and the quality of the solubility data. Finally, from the theoretical calculations an algorithm to calculate K_{ow} has been developed and has been computerised and is currently available as commercial software that may be assessed from the internet (Cronin and Livingstone, 2004).

Of the experimental approaches, the classical methods (e.g shake flask method) are more time consuming than the chromatographic method (RP-HPLC method), but it has the advantage of directly determining K_{ow} values. Via this method, an octanol-water mixture is spiked with the chemical under examination and shaken until equilibrium is achieved. The octanol and water phases are then separated and the concentrations either in one or both phases is determined (OECD, 2004). The RP-HPLC method is the most rapid and inexpensive method as it does not require any chemical quantification (Kah and Brown, 2008). The K_{ow} determination is based on a compound partitioning between a non-polar liquid organic phase and a polar

aqueous phase in a analytical column (OECD, 2004). A calibration graph has to be established by using known log K_{ow} values of several compounds and their respective log retention times on the RP-HPLC column as well as the void time of the HPLC system. This RP-HPLC method takes advantage of the fact that substances with high K_{ow} values elute later on reversed-phase columns than substances with lower K_{ow} values. A linear regression between the elution time of each compound and the log K_{ow} value can be established by using Equation 3.2.

$$\log K_{ow} = \text{a log t} + \text{b} \qquad \text{(Equation 3.2)}$$

where t is the retention time.

As K_{ow} has been reported to have a correlation with aqueous solubility, the retention of a compound also correlates with the solubility. In reversed-phase chromatography, the order of elution from a series of compounds is always that the most polar compound will elute first and the least polar will elute last. As the polarity is related to water solubility, the first compound eluted is the most water soluble. Therefore in relation to hydrophobicity, the first compound eluted is less hydrophobic in comparison to the other compounds in the elution series and has the lowest K_{ow} value of the series as it prefers the water phase.

In conclusion the K_{ow} value is an important parameter to predict a compound's partitioning in to soils and sediments and it is a very important molecular descriptor that often correlates well with the bioactivity of chemicals for estimating bioaccumulation in animals and plants. The value is also essential in most of quantitative structure-activity relationship (QSAR) studies in predicting the toxic effects of substances (Voutsas et al., 2002; Li et al., 2002).

In this study the determination of this parameter is considered. Both direct and indirect methods were conducted for several reasons. Firstly, various different K_{ow} values of natural and synthetic oestrogens have been reported thus it is essential to obtain an accurate value in ambient conditions. In addition no studies have been carried out to compare the oestrogens K_{ow} values obtained by direct and indirect methods. Finally it is necessary to determine if there is a difference between experimental and

default (generated by computational model) values.

3.5 Partition coefficient and bioconcentration

Bioconcentration, bioaccumulation and biomagnification are essential links in the sequential increase in concentration of a chemical when transferring from one trophic level to the next (Samiullah, 1990).

Bioconcentration is defined as a "process that causes an increase of chemical concentration inside an organism due to the uptake by absorption in the immediate environment". Whereas biomagnification occurs when a chemical enters the biota along different levels of the food chain through dietary uptake by predators in higher levels of the food chain of organisms which have a bioaccumulation of the substance in question.

Bioconcentration factors (BCFs) provide a useful measure of the bioconcentration potential of a chemical. They are often used to assess the uptake of existing and emerging chemicals by organisms. It can be simplified as the ratio of a chemical's concentration in an organism to that in the ambient environment at equilibrium.

$$\text{BCF} = \frac{chemical\ concentration\ in\ an\ organism}{total\ chemical\ concentration\ in\ immediate\ environment} \qquad \text{(equation 3.3)}$$

Numerous studies have been carried out (reviewed by Samiullah, 1990) illustrating a linear relationship between K_{ow} and BCFs with a general form:

$$\log\ (\text{BCF}) = \text{a} \log K_{ow} + \text{b} \qquad \text{(Equation 3.4)}$$

In humans, a relationship between the K_{ow} of a wide range of organic compounds and their BCFs in adipose tissue exists and can be expressed as the following (Geyer et al., 1987):

$$\log\ (\text{BCF}) = 0.756 \log K_{ow} - 1.415 \text{ (based on wet weight)} \qquad \text{(Equation 3.5) or}$$

$\log$ (BCF) = 0.745 $\log K_{ow}$ - 1.19 (based on lipid weight) (Equation 3.6)

To illustrate the relative value of BCF, Aherne and Briggs (1989) considered a potable water containing 17α-ethnyloestradiol (EE2) at a concentration of 4 ngL^{-1} . Approximately 250,000-12,500,000 litres of this water would be needed to achieve a therapeutic level of this oestrogen for contraception and hormone replacement therapy purposes (typically 1-50 mg accordance to British National Formulary (adapted from Keenan, 2000). However, with a BCF of approximately 80 (see Table 3.1) for humans (lipid weight), approximately 3,000-160,000 litres of water would be able to give a similar therapeutic dose.

Table 3.1: Bioconcentration factors (BCF) for oestrogens in human

Oestrogens	K_{ow}[a]	BCF in human (wet weight)	BCF in human (lipid weight)
Oestrone (E1)	3.43	15.07	23.19
17β-oestradiol (E2)	3.94	36.61	55.63
17α-ethnyloestradiol (EE2)	4.12	50.09	75.75

[a] default values

A large number of studies have concentrated on bioconcentrations in aquatic species; however, there have been relatively few studies investigating bioconcentration by plants. Kerler and Schonherr (1988) reported a good correlation of plant cuticle-water partition coefficient with $\log K_{ow}$ for a diverse set of lipophilic chemicals and the following linear regression obtained was:

$\log$ (BCF) = 0.97 $\log K_{ow}$ + 0.045 (Equation 3.7)

(Travis and Arms, 1988) modeled a BCF from soil to vegetation which they defined as:

$$\text{BCF} = \frac{concentration\ in\ above\ ground\ parts\ (mg\ compound\ /\ kg\ dry\ plant)}{concentration\ in\ soil\ (mg\ compound\ /\ kg\ dry\ soil)} \quad \text{(Equation 3.8)}$$

and a linear regression obtained was:

$$\log\ (\text{BCF}) = 1.588 - 0.578 \log K_{ow} \quad \text{(Equation 3.9)}$$

As indicated in Table 1.1, oestrogens have low vapour pressures thus they are likely to have very small Henry's law constants (Khanal et al., 2006). They are then not easily volatilised to the atmosphere. Therefore, their tendency to be taken up by plants from the atmosphere is negligable as their potential to volatalise from soil following sewage irrigation is low.

3.6 Environmental fate prediction- partitioning models

Prediction of a chemical environmental fate based on evaluative mathematical models attempt to classify broadly the behaviour of a chemical in a hypothetical environment. By only using a minimum of information, a chemical environmental distribution patterns as well as the identification of affected environmental compartment could be predicted.

In its simplest form a partitioning model can be described as a distribution of a chemical between environmental compartments, based on the thermodynamics of the systems. Neely (1982) used a model called " Unit World " to represent the major environmental compartments, where a schematic representation of the " Unit World " used to model the major environmental compartments is given in (Samiullah, 1990). The volumes and physical properties of these compartments were selected to mimic a real environment as closely as possible. The model was operated by estimating the partition coefficients of the chemicals between the various media such as:

$C_{air}/\ C_{water} = H$ (Henry's constant)

$C_{soil}/\ C_{water} = K_d$ (soil sorption coefficient) and

$C_{fish}/\ C_{water} = BCF$ (bioconcentration factor)

By using the equations for estimating the partition coefficients summarised in Table 3.2 and data for molecular weight, vapour pressure and water solubility, the environmental distribution of any chemical can be calculated.

Table 3.2: Equations for estimating partition coefficients selected by Neely (1982) (reproduced from (Samiullah, 1990)

Equation	Reference
$H = PM16.04/TS$	(Dilling, 1977)
$\log BCF = 0.85 \log K_{ow}$ - 0.70	(Veith et al., 1979)
$K_d =$ % OC (0.6 K_{ow})	(Karickhoff et al., 1979)
$\log K_{ow} = 6.5$ - 0.89 (log S/M) - 0.015 ($M.P$)	(Banerjee et al., 1980)

where; P = vapour pressure (mm Hg)
M = molecular weight
T = absolute temperature
S = water solubility in g/m^3
K_{ow}= octanol/water partition coefficient
$M.P$ = melting point in ^{o}C
K_d = soil/water partition coefficient

Unfortunately, due to insufficient data for natural and synthetic oestrogens, this model could not be adequately operated. For example, an ambiguous water solubility value resulted in difficulty in calculating the Henry's Law constant values.

3.6.1 Fugacity approach

Environmental partitioning of a chemical can be explained by the concept of fugacity. Fugacity is regarded as the "escaping tendency" that a substance exerts from any given phase and has units of pressure, pascal (Pa). Any chemicals when

at equilibrium exists at different concentrations in air, water, sediment, and other environmental matrices.

Equilibrium is achieved between two phases when the "escaping tendency" from one phase exactly matches the other. If the five environmental compartments, atmosphere (A), soil (B), water (C), sediment (D) and aquatic biota (E) are considered as phases shown in Figure 3.1:

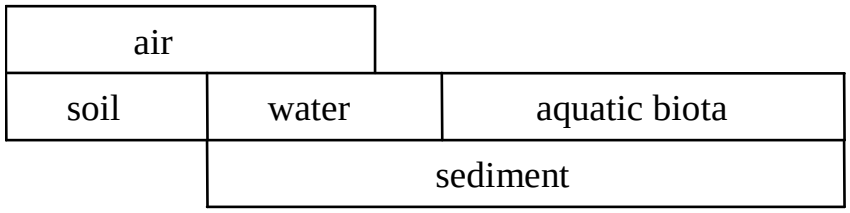


Figure 3.1: Five environmental compartments

then at equilibrium, fugacity (f) may be mathematically expressed as:

$$f_A = f_B = f_C = f_D = f_E$$

Fugacity can be related to concentrations (C) using fugacity capacity constant (Z) with units of molm^{-3}atm, as

$$C = Zf$$

Thus, if

$$f_A = f_B$$

it follows that:

$C_A/Z_A = C_B/Z_B$ or $C_A/C_B = Z_A/Z_B = K_{AB}$

where K_{AB} is the dimensionless partition coefficient controlling the distribution between two phases A and B.

This fugacity approach is simple to apply as it provides a means to process the available data by the use of equations that have inherent physical validity. Mackay and Paterson (1981) praised the elegence of this approach, which they illustrated by considering a 10-phase system, there were potentially 90 definable partition coefficients that were constrained in value with respect to each other and only 9 could be independently defined. However with fugacity approach, the 90 partition coefficients could all be determined just from 10 fugacity capacity (Z) values.

The fugacity capacity (Z) value is dependent on temperature, pressure, the nature of the substance and the medium in which it is present. The physical significance of fugacity capacity (Z), is that it quantifies the capacity of the phase for fugacity. At a given fugacity, if fugacity capacity (Z) is low, the concentration (C), is also low and only a small amount of substance is expected to exert an escaping tendency. Toxic substances for example tend to accumulate in phases where the fugacity capacity (Z) is high, therefore high concentrations (C) could escape without creating high fugacities (f) (Samiullah, 1990). Hydrophobic organics tend to partition into lipid phases as that is where their fugacity capacity (Z) values will be at a maximum (Mackay and Paterson, 1981).

The relationship between fugacity and partition coefficients can be illustrated as shown as in Figure 3.2.

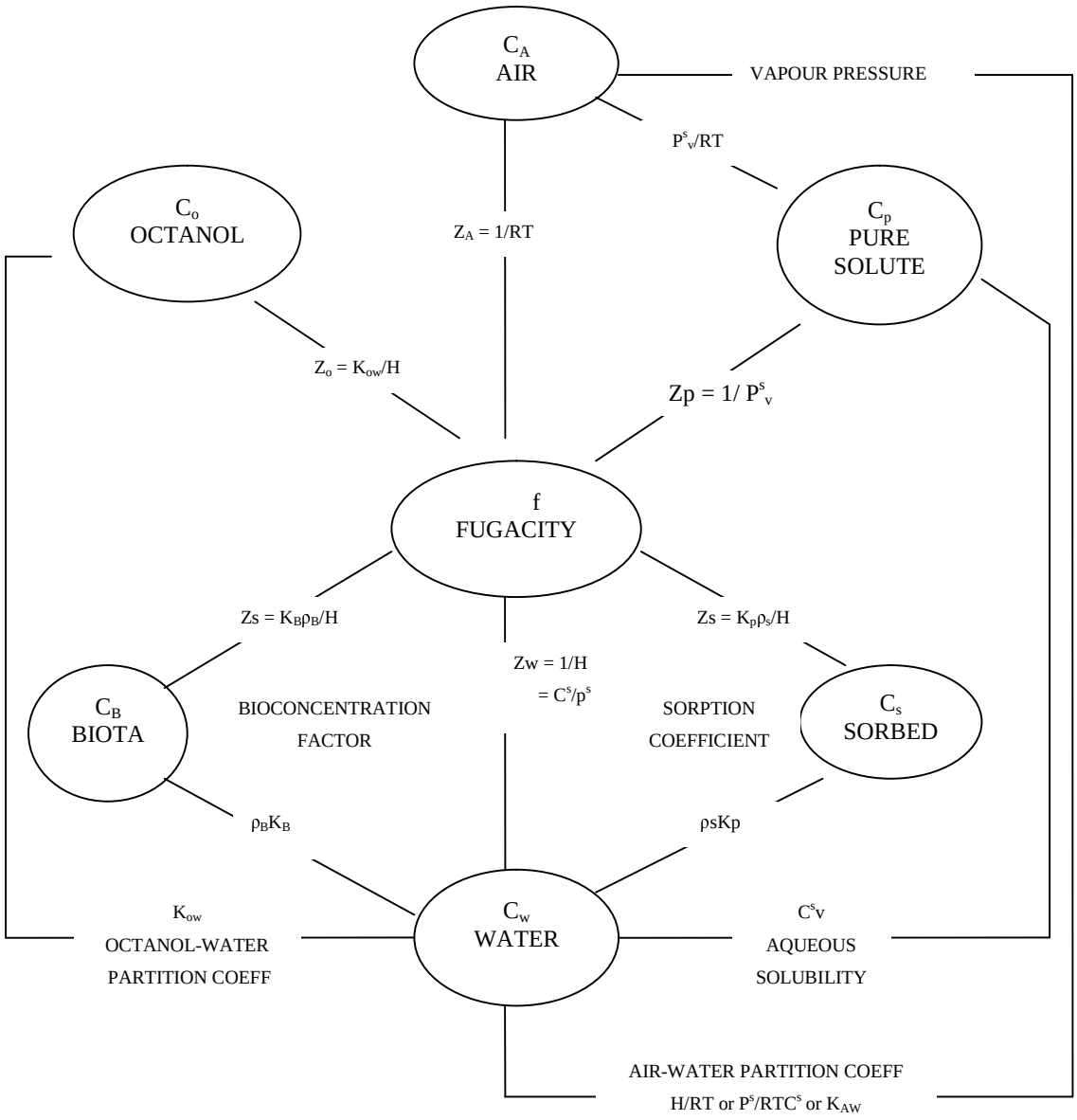


Figure 3.2: Relationship between fugacity capacities and partition coefficients (reproduced from Samiullah, 1990)

All symbols are defined in Table 3.3.

Table 3.3: Definition of fugacity capacities

Compartment	Definition of Z (mol m^{-3}Pa)
Air	$1/RT$ $R = 8.314$Pa m^3mol^{-1}K, T = temperature (K)
Water	$1/H$ or C^s/P^s C^s= aqueous solubility (mol m^{-3}) P^s =vapour pressure (Pa), H = Henry's Law constant (Pa m^3mol^{-1})
Solid sorbent (e.g soil, sediment, particles)	$K_d P_s/H$ K_d = partition coefficient (litre kg^{-1}), P_s= density (kg litre^{-1})
Biota	$K_B P_B/H$ K_B= Bioconcentration factor (litre kg^{-1}), P_s= density (kg litre^{-1})
Pure solute	$1/P^s v$ v = solute molar volume (m^3mol^{-1})

3.6.2 Fugacity capacity calculations

There are several levels of calculation involving fugacity:

Level I

The simplest application of fugacity is in the determination of the equilibrium distribution of a set quantity of a chemical. It is assumed that no transformation processes occur. Therefore the only requirement is the calculation of fugacity capacity (Z) values for each environmental compartments, see Samiullah (1990) for its illustration. It assumes that the chemical is conserved and is neither destroyed by reactions nor removed from the evaluative environment by flows in phases such as air and water. These assumptions can be quite misleading when determining the impact of a given discharge or emission of a chemical.

Level II

In this level of calculation, advection and reactions such as hydrolysis and biodegradation are added to the Level I calculation. Since the Level II calculations predict the proportions of a substance advected into the system and the proportion derived from local source, the relative effects of reducing local or external emissions can be calculated (Mackay and Paterson, 1981).

Level III

Both Level I and Level II calculations assume that the environmental media is in equilibrium; however, this is rarely the case in the real environment. Thus, Level III calculates the distribution of a chemical in non-equilibrium systems.

Level III calculations have been adopted by the multimedia fate computer programs, such as Estimation Program Interface (EPISUITE) used in this study. The user must provide physico-chemical properties of a substance such as its partioning values in order to deduce intermedia transport values (D). The intermedia transport values determination is important since in the real environment processes such as diffussion, advection and dispersion occur. Other parameters such as mass transfer coefficients are provided as default values (Mackay, 2001).

3.7 Estimation Program Interface (EPISUITE)

The application of prediction tools based on computational modeling to access the environmental fate of any environmental pollutant has been widely accepted and used in scientific communities and by regulators.

The most popular tool is the Estimation Program Interface (EPISUITE) which is freely available. This software was developed by the Environmental Protection Agency (EPA) (Office of Pollution Prevention and Toxic Substances) and Syracuse Research Corporation (SRC). The main purpose of this program is to support the EPA's decision making regarding the environmental fate, transport and potential toxicity of new chemicals in the environment.

The EPISUITE program consists of two modules where the first module is Physical-chemical Property Estimation Routine (PERs), which predicts important physico-chemical properties such as water solubility, vapour pressure, octanol-water partition coefficient (K_{ow}) and cerain types of reactivity (for example, biodegradation or atmospheric oxidation of a chemical). In conjuction with the second module, which is

the Environmental Fate Models (EFMs), this program is able to predict a chemical's fate and transport in environmental compartments.

Although this kind of program is helpful in assessing the environmental fate of any new emerging contaminant, experimental assessment still needs to be carried out. The model does not consider ambient conditions such as temperature, pH and soil type. Therefore experimental work and reporting is essential in order to ensure a realistic model of the environment is obtained for consideration as opposed to the default model given by the program.

The ambiguity problem between experimental and default values has led to the creation of EPISUITE program whereby inputting experimental values attempts to move to a more realistic model true to the environmental conditions under consideration.

3.8 Soil column study

Currently, there are limited studies investigating the mobility of oestrogens in soils via soil column experiments (Larsen et al., 2001; Casey et al., 2005; Sangsupan et al., 2006). Larsen et al. (2001) reported a loam soil was able to prevent a radiolabeled 17β-oestradiol (E2) from escaping the soil column and the radioactivity was mostly counted at the upper level of the column. They also reported that the escaping potential of radiolabeled 17β-oestradiol (E2) was high in a sand packed soil column where 85 % of radiolabelled 17β-oestradiol (E2) was recovered in collected leachate after 1.06 pore volumes were flushed into the column (Larsen et al., 2001). Casey et al. (2005) reported a capability of a radiolabeled 17β-oestradiol (E2) to escape from a soil consisting of 9.2 % organic matter where 26 % of the radiolabeled 17β-oestradiol (E2) was recovered in the soil column leachate at the end of a 42 hour breakthrough experiment.

It is noted that relatively high sorption coefficients indicate that oestrogens are strongly sorbed to soils. Thus, these oestrogens are unlikely to undergo soil transport. However, a few field studies report the occurrence of oestrogens in groundwater (Peterson et al., 2000; Swartz et al., 2006) and certain soil column experiments also demonstrate a leaching potential. Most of the oestrogen breakthrough studies in soil columns indicate involvement of chemical non-equilibrium processes (Casey

et al., 2003, 2005), which indicate the occurrence of sorption and other chemical reactions between oestrogens and soils such as mineralisation that would influence the transport of oestrogens. However, Sangsupan et al. (2006) suggested in addition to chemical non-equilibrium processes which may influence oestrogen mobility in soils, physical non-equilibrium processes (for example, the occurence of preferential flow) would also enhance their mobility and introduce them to groundwater.

Based on current information from limited studies, it is still difficult to assess the mobility potential of these hormones in soil and their potential to contaminate groundwater. In addition, it is difficult to draw comparisons between studies as different experimental approaches and soil samples are used. It is also noticed that all the compounds used for soil column experiments were radioisotopes/radiolabelled. The susceptibility of a chemical in a specific medium to reaction depends both on the inherent properties of the molecule and on the nature of the medium. In this respect, environmental chemicals are fundamentally different from the radioisotopes/radiolabelled chemicals used since conditions are not identical (Mackay, 2001). Thus, in order to improve understanding of oestrogens transport in the soils, a soil column experiment using compounds with > 95 % purity was used. In addition to this, it is also believed that the artificial soils used in this study will give a benchmark study to the transport behaviour of oestrogens in soils. As it is strongly believed that organic matter contained in soils retards oestrogen mobility, in this study, the soil column experiments were incorporated into the predictive model. Retardation factors which illustrate the degree of oestrogen retention in soil have not been reported prior to this study soil. Thus, by incorporating the measured data into the predictive model, this fundamental factor can be assessed and give more understanding to oestrogen mobility in soils.

Chapter 4

METHODOLOGY

This chapter illustrates the analytical methods that have been developed to analyse oestrogens in all samples involved in this study. Most of the presented methods have been utilised throughout the entire course of the project to investigate the behaviour of the oestrogens in soils by means of laboratory scale experiments.

4.1 Materials

4.1.1 Chemicals

Oestrone (E1, > 99% purity), 17β-oestradiol (E2, > 98% purity), 17α-ethnyloestradiol (EE2, > 98% purity), napthalene (99 % purity), anthracene (> 99 % purity), fluoranthene (> 99.5 % purity), chrysene (> 95 % purity), benzo (a) pyrene (> 97 % purity) and benzoic acid (> 99.5 % purity) were purchased from Sigma-Aldrich Ltd, United Kingdom. Acetonitrile (HPLC-grade) and methanol (HPLC-grade) were obtained from Fisher Scientific laboratory, United Kingdom. All other chemicals involved in this study were obtained from the Environmental Laboratory in the Civil Engineering Department of Strathclyde University, United Kingdom.

4.1.2 Soils

Three artificial soils were prepared in accordance with British Standards, BS 3882:1994 (BS, 1994). Soil material was prepared by mixing sand, silt, clay and humus. The

sand was commercial British sand. Silt was taken from a River Clyde bank, 15 km from Glasgow. The clay was excavated from Hallyard Quarry, 30 km from Glasgow and humus was purchased from a commercial shop.

All the individual material sizes were in accordance with British Standards, BS 1377-1:1990 (BS, 1990). A particle size micro-analyser (Malvern Master Sizer 2000) was used to analyse the size of each of the materials of the artificial soils, by laser scattering. Average particle size was expressed as the volume mean diameter. $D_{0.9}$, $D_{0.5}$ and $D_{0.1}$ are the particle diameters determined respectively at the 90^{th}, 50^{th}, and 10^{th}percentile of undersized particles. All soils were subjected to several tests in order to obtain their soils properties (e.g pH and organic matter content) which in accordance to British Standards and American Society Testing Standards (ASTM, 2000, 2001; BS, 1990). All soils used in this study were sterilized prior to use. Sterile soils were prepared by exposing the soils to a temperature of 121 $^{\circ}$C in an autoclave three times, 40 minutes for each time (Wolf et al., 1989).

4.2 Development of HPLC method of analysis

4.2.1 HPLC-UV system specification

Analysis of oestrogen analytes was performed on a Dionex Ultimate 3000 High Performance Liquid Chromatography (HPLC) system comprising a Dionex Thermostated Column Compartment TCC-300, a Dionex WPS-3000 SL and WPS-3000 RS Automated Sampler, a Dionex Ultimate 3000 Series-Pump, Dionex VWD-3100 and VWD-3400 RS detector and a Dionex Solvent Rack SR 3000. The injection loop size used was 10 µL. The analytical column used was an Allure Biphenyl 5µm, 60Å (150 x 4.6 mm).

4.2.2 HPLC-UV separation and detection

Initially the optimal detection wavelength (λ max) was determined by preparing a combined solution of oestrone (E1), 17β-oestradiol (E2) and 17α-ethnyloestradiol (EE2), each at 10 mgL^{-1} in the mobile phase. The UV absorbance of the oestrogens solution was measured on a Phillips PU8740 UV/VIS spectrophotometer from 190 to 300 nm in full scan mode.

Reversed phase liquid chromatography was used as the mode of separation with the UV spectrophotometric set at λ 220 nm. Several mobile phase compositions were employed and resultant chromatograms were assessed. The method was finally optimised by using isocratic elution with a mobile phase composition ratio of 60:40 (v/v) of acetonitrile to nanopurewater . The flow rate was constant at 1.0 $mLmin^{-1}$.

All oestrogens were intially identified by injecting single standards into the system and noting their respective retention times. Standard linearity was assessed in the concentration ranged of 1.0 to 6.0 mgL^{-1} and the detection limits of each oestrogen were calculated.

4.3 Development of solid phase extraction (SPE) method

The SPE tube used for this project was Supelclean Envi-18 (Supelco; 200mg). Although the solid phase extraction methodology for the conditioning of tubes and eluting the analytes is supplied by the manufacturer (Supelco), the tubes still required an evaluation for the extraction efficiency of natural and synthetic oestrogens. Thus, several conditioning methodologies from previous studies (Keenan, 2000; Lagana et al., 2004) were tested to obtain the best percentage of recovery.

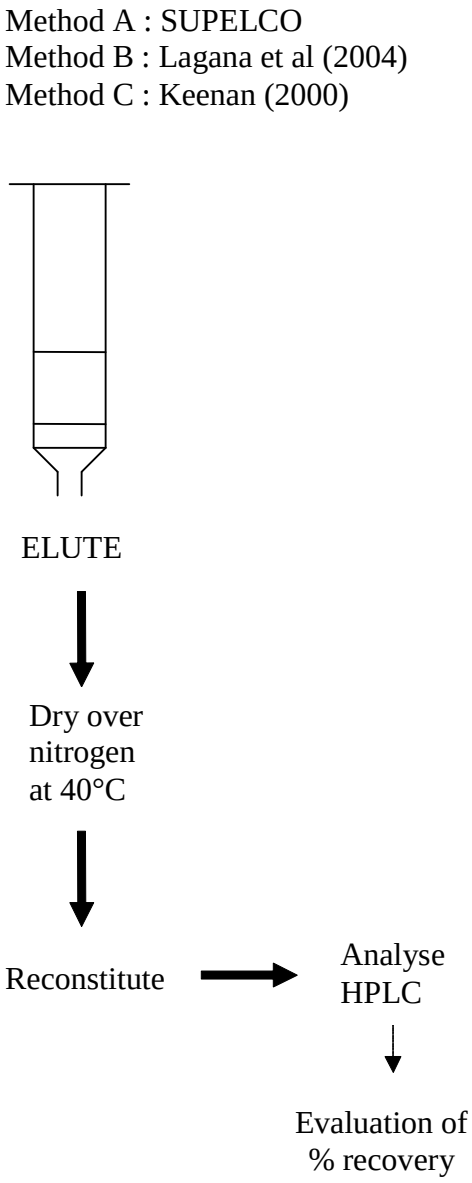


Figure 4.1: Schematic representation of SPE analysis

Table 4.1 displays conditioning methodologies from previous studies evaluated to develop SPE method for natural and synthetic oestrogens for the present study.

Table 4.1: SPE methods from previous literature

Method	A	B	C
Conditioning	12 mL toluene:MeOH (10:1)	10 mL of dicholomethane:MeOH (1:1)	5mL of 100 % MeOH
	6 mL of MeOH	5 mL of MeOH	5 mL of MeOH:nanopurewater (1:9)
	6 mL deionised water	10 mL of water	
Elution	2 mL toluene:MeOH (10:1)	7 mL of dicholomethane:MeOH (1:1)	2 mL of MeOH:isopropanol (1:1)

The SPE method development in this study was used to pre-concentrate all the concentrations which werebelow limit of the detection.

4.4 Determination of octanol-water partition coefficient (K_{ow})

The K_{ow} values for natural and synthetic oestrogens in this study were obtained by both direct and indirect methods. Both methods were in accordance with the Organisation for Economic Co-operation and Development (OECD) guidelines for the testing of chemicals (OECD, 2004). The OECD guidelines for the testing of chemicals are a collection of the internationally agreed testing methods used by government, industry and independant laboratories to assess the safety of chemicals.

4.4.1 Reverse Phase- HPLC method

Six reference substances (napthalene, 99 % Acros Organic; anthracene, 99 % Sigma Aldrich; fluoranthene, 99.5 % Sigma Aldrich; chrysene, 95 % Sigma Aldrich; benzo (a) pyrene, 97 % Sigma Aldrich and benzoic acid, 99.5 % Sigma Aldrich) were

selected. The selection of these reference substances was made as they are the recommended reference substances (OECD, 2004). The selection of reference substances should also be based on their log K_{ow} values which should encompass the range of reported log K_{ow} values of oestrogens. The log K_{ow} values for reference substances were in the range 1.90 to 6.11.

Two stock solutions were prepared in HPLC-grade methanol: A mixed standard containing 1 mgL^{-1} of all oestrogens under study and a mixed standard containing 1 $mgL^{-1}$of reference substances used in the study. The wavelength used for the reference substances was 254 nm while for oestrogens it was 220 nm. The dead time (t_0) was measured by using Thiourea (99 % Sigma Aldrich) (OECD, 2004).

The log K_{ow} of the reference substances were plotted against log capacity factor, k (log k). The capacity factor (k) of each substance was obtained from Equation 4.1.

$$k = \frac{t_R - t_0}{t_0} \qquad \text{(Equation 4.1)}$$

where

t_R =retention time of test substance

t_0 =dead time

The log K_{ow} of the oestrogens was calculated from the regression equation of the reference substance obtained following Equation 4.2:

$$\log K_{ow} = \text{a} + \text{b x} \log k \qquad \text{(Equation 4.2)}$$

where

a, b = linear regression coefficients

4.4.2 Shake-flask method

The determination of log K_{ow} for each oestrogen by the shake flask method was carried out at 20 $^{\circ}$C $\pm$ 0.1. In triplicate, each ratio of the individual oestrogens were shaken for 24 hours, as described below.

Initially, solutions of water saturated with n-octanol and n-octanol saturated with water were prepared. In a 25 mL test tube, three ratios of saturated n-octanol:water 1:1, 1:2, and 2:1 were equilibrated with oestrogen at a concentration of 1800 mgL^{-1}. Preliminary experiments of similar steps were conducted in order to obtain a suitable oestrogen concentration used.

After completion of shaking, all tubes were centrifuged for 15 minutes by laboratory centrifuge maintained at 20 $^{\circ}$C $\pm$ 0.1. The aqueous phase was withdrawn and analysed by HPLC.

4.5 Determination of solid-water partition coefficient (K_d)

Equilibrium batch sorption experiments were carried out accordance with ASTM standard E1195-01 (ASTM, 2001).

In order to determine a suitable contact time for the subsequent equilibrium batch sorption experiments, a Type IV nanopure water used as a background solution. It was spiked with an appropriate volume of stock solution of each oestrogens to yield aqueous concentrations of 1 mgL^{-1}. An aliquot of 25 mL of the Type IV water spiked with oestrogen was then added to 0.25 g of soil in a glass centrifuge tube equipped with a Teflon lined screw cap. Duplicate samples were prepared and samples were placed on a rotary shaker set at 300 min^{-1} in the dark at 20 $^{\circ}$C $\pm$ 0.1 temperature controlled room. At increasing time intervals (0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 24, 39, and 48 hours) the tubes were centrifuged at 4000 rpm for 20 minutes. After centrifugation, the supernatant was filtered through a 0.2 µm Whatman glass fiber filter (GF/C) and then analysed by HPLC. The samples that were below the detection limits were pre-concentrated by solid phase extraction (SPE).

The appropriate soil-water ratio for subsequent experiments was determined in accordance to the standard procedure (ASTM, 2001) in order to obtain the maximum adsorption of natural and synthetic oestrogens. Three soil to water ratios, 1:50, 1:100, and 1:250 were chosen to study this effect. Type IV water used as a background solution was spiked with an appropriate volume of stock solution of 17β-oestradiol (E2) to yield an aqueous concentrations in the range of 0 to 1.4 mgL^{-1}. This range was below its water solubility. An aliquot of 25 mL of the Type IV water spiked with 17β-oestradiol (E2) was added to 0.1g, 0.25g, and 0.5g of soils in a glass centrifuge tube equipped with a Teflon lined screw cap. Duplicate samples were prepared and placed on a rotary shaker set at 300 min^{-1} in the dark at 20 $^{\circ}$C $\pm$ 0.1 in a temperature controlled room untill equilibrium was achieved. After completion of shaking time, the tubes were centrifuged at 4000 rpm for 20 minutes. After centrifugation, the supernatants were further filtered through a 0.2 μm Whatman glass fiber filter (GF/C) and were subjected to HPLC for analysis. The samples which were below the detection limits were further pre-concentrated and the concentrated samples were then analysed by HPLC.

The experiments of equilibrium batch sorption were repeated with test mixtures in the range 0.1 to 0.5 $mgL^{-1}$of oestrogens with the appropriate soil-water ratio obtained. All centrifuge tubes were shaken on a rotary shaker set at 300 min^{-1} in the dark at 20 $^{\circ}$C $\pm$ 0.1 in a temperature controlled room. Each centrifuge tube was removed at apparent equilibrium time obtained and was centrifuged at 4000 rpm for 20 minutes. After centrifugation, the supernatant was filtered through a 0.2 μm Whatman glass fiber filter (GF/C) and was further pre-concentrated. The concentrated samples were then injected to HPLC for analysis.

In all experiments, in order to determine any losses to the tubes as well as to check for interfering peaks during analysis, blank controls were conducted with background solution containing oestrogen only without soil and background solution of without oestrogens in contact with the soils.

The sorption partition coefficient (K_d) indicating the partition between the solid and liquid phase was determined by dividing the adsorbed concentration (Q_e) by the solution concentration (C_e) and can be simplified as follows (Equation 4.3):

$$K_d = \frac{Q_e}{C_e} \qquad \text{(Equation 4.3)}$$

The soil partition coefficient normalised to organic carbon (K_{oc}) was then calculated from the obtained K_d values by using Equation 4.4:

$$K_{oc} = \frac{(K_d)}{OC\%} \text{ x } 100 \qquad \text{(Equation 4.4)}$$

The calculation of K_{oc} is essential as it is one of the parameters used in environmental fate predictive models. In addition, K_{oc} value is useful in predicting the mobility of soil organic contaminant and allows comparisons to be made between different contaminants and it is independent of soil type.

4.5.1 Effect of pH on sorption

In order to determine the effect of pH on oestrogen sorption, 0.25 g of soils were weighed into a series of 25 mL centrifuge tubes and 25 mL of oestrogen solutions with different pH and concentrations were then added.

Oestrogens solutions of varying pH were achieved by addition of either 0.1 M of acid (HCl) or base (NaOH) to yield pH 4, 6, 9 and 11. The concentration of the mixed oestrogens solution was in the range of 0 to 0.5 mgL^{-1}. After the addition of the oestrogen solution, the centrifuge tubes were capped and placed on a rotary shaker set at 300 min^{-1} at 20 $^{\circ}$C $\pm$ 0.1 in a temperature controlled room and in the dark. After completion of 24 hours, all the centrifuge tubes were centrifuged at 4000 rpm for 20 minutes.

After equilibrium and centrifugation, the pH of the supernatant was measured. The supernatant was then filtered through a 0.2 µm Whatman glass fiber filter (GF/C) and was pre-concentrated. The concentrated samples were then analysed by HPLC.

4.5.2 Effect of salinity on sorption

In order to determine the effect of salinity on oestrogen sorption, various salinities of oestrogen solutions were prepared. Various salinities were used to represent the salinity of effluent. The salinity range used was between 0 to 30 psu, by adjusting the addition of KCl to Type IV water. The concentration of oestrogens used ranged from 0 to 0.5 mgL^{-1}.

25 mL of oestrogen solution was added to 0.25 g of soils in a series of centrifuge tubes. They were then capped and placed on a rotary shaker set at 300 min^{-1} at 20 $^{\circ}$C $\pm$ 0.1 in a temperature controlled room and in the dark. After 24 hours the shaker was stopped and all the centrifuge tubes were then centrifuged at 4000 rpm for 20 minutes.

After centrifugation, the clear supernatant was filtered through a 0.2 μm Whatman glass fiber filter (GF/C) and pre-concentrated. The concentrated samples were then injected in the HPLC for analysis.

4.5.3 Effect of temperature on sorption

To study the effect of temperature on oestrogen sorption, equilibrium batch sorption experiments were conducted at various temperatures. The study temperatures were varied between 20 to 30 $^{\circ}$ C. The concentration of oestrogens used ranged from 0 to 0.5 mgL^{-1}.

25 mL of oestrogen solution was added to 0.25 g of soils in a series of centrifuge tubes. They were then capped and placed on a rotary shaker set at 300 min^{-1} at 20 $^{\circ}$C $\pm$ 0.1 temperature in the dark. To vary the temperature two other sets of experiments were prepared and placed in the shaker incubator and the temperatures were set at 25 and 30 $^{\circ}$C. After 24 hours, the shaker was stopped and all the centrifuge tubes were centrifuged at 4000 rpm for 20 minutes.

After centrifugation, the clear supernatant was filtered through a 0.2 μm Whatman glass fiber filter (GF/C) and pre-concentrated. The concentrated samples were then injected into the HPLC for analysis.

4.6 Prediction of environmental fate and persistence

The experimental values of log K_{ow} and log K_{oc} (calculated from experimental K_d values) obtained in this study were compared with the default values from the Kowwin-EPISUITE and PCKoc-EPISUITE program respectively. The experimental values of K_{oc} and K_{ow} were then input into the Fugacity Model Level III-EPISUITE program to obtain a more accurate prediction of oestrogens partitioning ratios in environmental compartment based on ambient conditions.

4.7 Soil column experiment

A glass column with a diameter of 2.7cm was packed with soil to a length of approximately 11.5cm. The column was saturated from the bottom up, over 24 hours, using a solution of 0.01 molL^{-1} $CaCl_2$. Following column saturation, two breakthrough curves (BTC) were run at a flow 108 cm^3/hr :

(i) Tracer-soil BTC: a solution pulse of 0.05 molL^{-1} $CaCl_2$ was introduced to characterize the transport through the column.

Following completion of non-reactive pulse, the column was flushed with 0.01 mol L^{-1} $CaCl_2$ to elute the tracer from the column;

(ii) Oestrogen-soil BTC: a solution pulse of approximately 1 mgL^{-1} of individual oestrogens were introduced to determine the retention behaviour of each oestrogen by the soil column.

Following completion of the oestrogen pulse, the column was flushed with 0.01 mol L^{-1} $CaCl_2$ to elute the oestrogen from the column.

In all cases, effluent samples were collected periodically and analysed. All the soil column experiments were conducted in a temperature controlled room where the temperature was set at 20 $^\circ$C $\pm$ 0.1. During the experiment, the entire length of the soil column was wrapped with aluminium foil to prevent in case the occurrence of photodegradation.

4.7.1 Soil column modeling

A number of hydraulic mechanisms dictate contaminant mobility in soils. Advection for example represents a primary factor that describes mass transport of a contaminant due to water flow. Dispersion that results from mixing due to variations in the flow velocity, further contributes to contaminant mobility. In order to further understand oestrogen mobility in soil, the measured data from soil column experiments was modeled using the computer program HYDRUS-1D (Simunek et al., 2008). HYDRUS-1D incorporates water flow and contaminant movement aspects in order to simulate the mobility of oestrogens in soils through the soil columns. This program calculates contaminant (solute) movement based on advective-dispersion principles.

The program uses an inverse modeling technique to fit the model solution to observed data in order to measure transport data. This inverse modeling approach uses a least-squares method that minimizes an objective function that provides a best-fit model solution to the measured transport data by finding the optimum combination of reaction and transport parameters (Lazaridis and Keenan, 2010).

Soil hydraulic characteristics used in the program were estimated by Rosetta, a built-in HYDRUS file, that implements pedotransfer functions (PTFs) using basic soil data such as soil texture and bulk density as predictors for water retention and saturated hydraulic conductivity (K_s) (Simunek et al., 2008). These estimated parameters were used for the remainder of the modeling.

Chapter 5

RESULTS AND DISCUSSION

5.1 Soil properties analysis

5.1.1 Soil particle distribution

Table 5.1 shows the individual sizes of sand, silt, clay, and humus used to prepare the three artificial soils in this study.

Table 5.1: Particle size analysis of individual materials.

Material	$D_{0.1}$µm	$D_{0.5}$µm	$D_{0.9}$µm	Size reference(BS, 1990)
Sand	530.983	811.98	1257.64	600 µm $<$ d.p $<$ 2000 µm
Silt	10.95	15.29	52.41	2 µm $<$ d.p $<$ 60 µm
Clay	0.46	0.63	1.37	d.p $<$ 2 µm
Humus	95.34	311.12	845.87	600 µm $<$ d.p $<$ 1000 µm

* d.p = particle diameter

The idea of using artificial soils rather than field-collected soils was to control the percentage of materials contained in soils and therefore present benchmark samples in order to control variables and to make interpretation clearly.

Three artificial soils (I, II and III) were prepared and their composition is shown in Table 5.2. Based on soil particle analysis, all soils were classified as sandy loam (BS, 1994).

Table 5.2: Soil particle analysis.

Soil	Sand (%)	Silt (%)	Clay (%)	Humus (%)
I	75	10	5	10
II	80	10	5	5
III	84	10	5	1

5.1.2 Soil total organic carbon (TOC) and pH determination

Total organic carbon for all soils was determined in accordance with the ASTM standard D2974-00 (ASTM, 2000). The carbonate content was assessed through the observation of any bubbles produced on addition of HCl from CO_2 liberated from breakdown of carbonates in the soils (Horvath et al., 2005). This simple experiment was negative for all soils. Thus, the total organic carbon is considered similar to the organic matter in all soils (ASTM, 2000). Soil pH was obtained accordance to the BS 1377-3:1990 (BS, 1990). The TOC and pH of all soils is shown in Table 5.3.

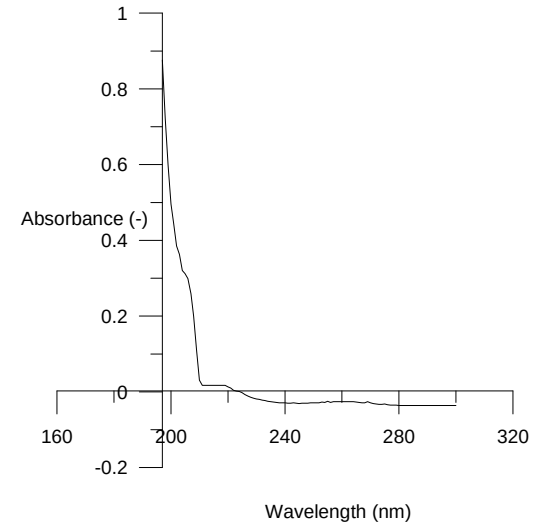
Table 5.3: Properties of the soils.

Soil	Sand (%)	Silt (%)	Clay (%)	Humus (%)	TOC (%)	pH
I	75	10	5	10	8.90	5.5
II	80	10	5	5	4.92	5.5
III	84	10	5	1	1.62	5.9

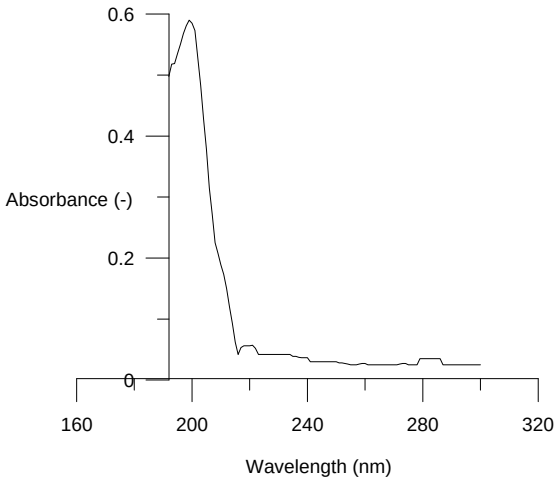
5.2 HPLC method of analysis

5.2.1 Optimum UV absorbance

Figure 5.1(b) displays the resulting UV absorbance spectrum of the mixed oestrogen standard for the wavelength range between 196 to 300 nm. The graph shows three absorbance peak at 200, 220 and 280 nm.



(a)



(b)

Figure 5.1: (a) Absorbance spectrum of mobile phase (b) Absorbance spectrum of mixed oestrogens in mobile phase

A wavelength of 220 nm was selected for the detection of the oestrogens in this study although at wavelength 200 nm the relative absorbance was six times higher than at 220 nm. The wavelength 200 nm was not chosen as it is believed that the absorbance of mobile phase occurred on that particular range (190-200 nm) as shown in Figure 5.1(a).

The predominant wavelength for oestrogen detection via UV reported in the literature varies between 201-280 nm (Keenan, 2000; Lee et al., 2003; Yu et al., 2004).

Thus, the UV absorbance spectrum found in the present study was in close agreement with previous studies.

5.2.2 Compound separation and detection

The oestrogens in this study were successfully separated on a biphenyl analytical column using a mixture of acetonitrile and nanopure water as a mobile phase. Previous studies showed that the run times of oestrogens are in the range of 6 to 20 minutes to elute oestrone (E1), 17β-oestradiol (E2) and 17α-ethnyloestradiol (EE2) at flow rates of 1.0-2.0 $mLmin^{-1}$ with varying acetonitrile to water ratios (Keenan, 2000; Lee et al., 2003). However, in the present study, the application of a biphenyl analytical column resulted in a significant reduction of the sample run time and elution of oestrone (E1), 17β-oestradiol (E2) and 17α-ethnyloestradiol (EE2) was achieved within 4 to 6.5 minutes with a mobile phase consisting of 60 % acetonitrile and 40 % nanopure water and a flow rate of 1.0 $mLmin^{-1}$. Figure 5.2 displays a standard chromatogram for oestrone (E1), 17β-oestradiol (E2) and 17α-ethnyloestradiol (EE2) and internal standard diethylstilbestrol (DES) with a concentration of 1 mgL^{-1} at a column temperature operated at 25 ° C.

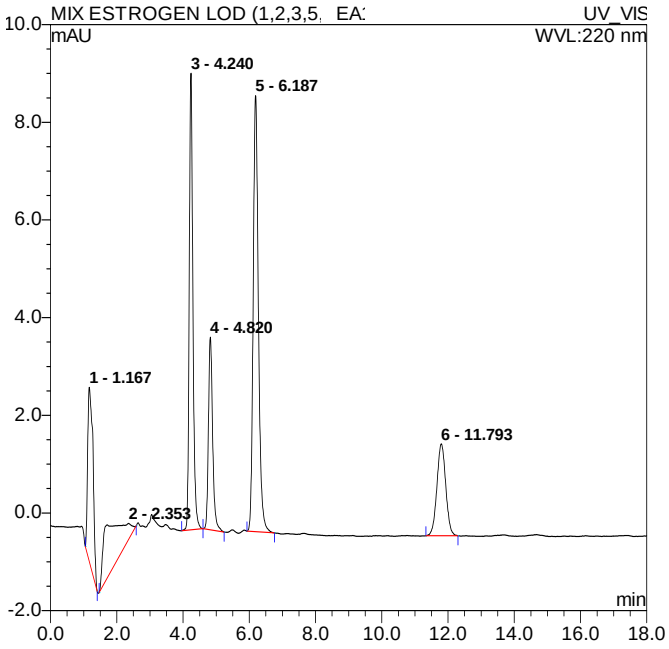


Figure 5.2: Chromatogram of 1 mgL^{-1} mixed oestrogens standard

5.2.3 Standard linearity and detection limits

The response for all compounds was linear in the range of 1.0 to 6.0 mgL^{-1} (n = 3). The correlations coefficients, R^2 for the standards were > 0.99 over the course of the entire experimental studies, and deviations in the correlation coefficient constituted a reliable indicator of faulty standard preparation. When variations occurred, a new standard series was prepared and rerun. Figure 5.3 displays the linear regression for each oestrogen (1.0-6.0 mgL^{-1}). The linear regression and equation for each oestrogen is shown in Table 5.4. The standard deviation (sd) and the relative standard deviation (RSD) of each oestrogens are shown in Table 5.5.

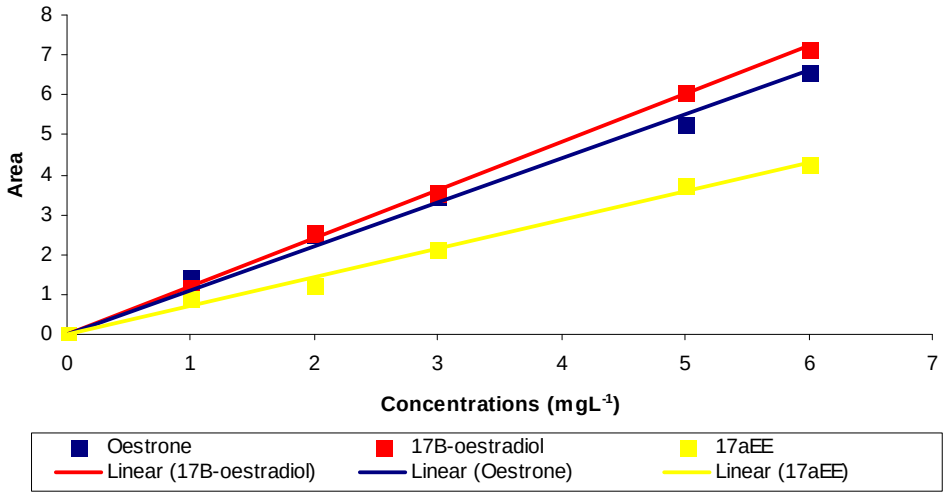


Figure 5.3: Calibration graphs for oestrogens (1.0 - 6.0 mgL^{-1})

Table 5.4: Linear regression equation.

Equation	$y = 1.2038x$	$y = 1.1036x$	$y = 0.7203x$
R^2	0.999	0.9908	0.9928

Table 5.5: Peak areas, standard deviation and relative standard deviation of oestrogens standard (1-6 mgL^{-1}).

Concentrations (mgL^{-1})	Peak Areas		
	Oestrone (E1)	17β-oestradiol (E2)	17α-ethnyloestradiol (EE2)
	1.424	1.196	0.877
1	1.398	1.199	0.893
	1.373	1.184	0.881
Mean	1.398	1.193	0.884
Sd	0.026	0.008	0.008
RSD %	1.824	0.665	0.942
	2.527	2.573	1.236
2	2.520	2.565	1.231
	2.523	2.570	1.231
Mean	2.523	2.569	1.233
Sd	0.004	0.004	0.003
RSD %	0.139	0.157	0.234
	3.539	3.628	2.167
3	3.404	3.515	2.137
	3.483	3.509	2.094
Mean	3.475	3.551	2.132
Sd	0.068	0.067	0.037
RSD %	1.952	1.888	1.720
	5.023	6.163	3.784
5	5.031	6.093	3.756
	5.772	5.985	3.729
Mean	5.275	6.080	3.756
Sd	0.430	0.089	0.028
RSD %	8.154	1.475	0.732
	6.290	7.319	4.300
6	6.711	7.059	4.223
	6.760	7.073	4.223
Mean	6.587	7.150	4.249
Sd	0.258	0.146	0.044
RSD %	3.922	2.045	1.046

The limits of detection (LOD) were calculated by 9 repetitions of 1 mgL^{-1} of each oestrogen standard by using the equation (3 x (σn-1) x concentrations). The calculation of LOD are shown in Table 5.6.

Table 5.6: Peak areas for a 1 mgL^{-1} of each oestrogens standards for LOD.

	Oestrone (E1)	17β-oestradiol (E2)	17α-ethnyloestradiol (EE2)
Peak areas of standard (mAU*min)	1.353, 1.324, 1.320. 1.319, 1.320, 1.320, 1.322, 1.320, 1.331	0.992, 0.994, 0.995, 0.998, 0.994, 0.998, 0.997, 0.997, 0.991	0.889, 0.893, 0.887, 0.887, 0.885, 0.887, 0.886, 0.899, 0885
Mean	1.325	0.995	0.889
Sd	0.011	0.003	0.011
RSD %	0.830	0.302	1.237
LOD (mgL^{-1}) (3 x Sd/Mean) x Conc	0.025	0.008	0.025

The results show that the calibration graphs of each oestrogen are linear over the tested range and the detection limits are in the range of 0.008 to 0.025 mgL^{-1}. The results are reproducible with a relative standard deviation (RSD) of < 2 % at 1 mgL^{-1} concentration used.

5.3 Evaluation of solid phase extraction (SPE)

The percentage recovery yield of all elution method tested on Supelclean Envi-18 tubes used in this project is indicated in Table 5.7. From this, method C was chosen as SPE method for the entire project because it showed the best recovery of the three methods. The recovery of the analyte was satisfactory where the percentage was in between 94 - 108% of all analytes.

Table 5.7: Percentage recovery of Supleclean Envi-18 tube using various conditioning and elution solvents, samples dried then reconstituted.

Compound	Peak areas after pre-concentration (mAU*min) A (SUPELCO)	Peak areas after pre-concentration (mAU*min) B (Lagana et al., 2004)	Peak areas after pre-concentration (mAU*min) C (Keenan, 2000)	Peak areas before pre-concentration (mAU*min)
Oestrone	1.300	1.523	2.000	0.342
(E1)	1.289	1.511	1.940	0.335
	1.446	1.535	1.950	0.319
Average	1.345	1.523	1.963	0.332
% Recovery	70.0	80.6	108.0	
17β-oestradiol	3.975	3.783	4.499	0.464
(E2)	3.959	3.961	4.501	0.486
	3.976	4.136	4.606	0.475
Average	3.970	3.960	4.535	0.475
% Recovery	85.0	84.8	97.0	
17α-ethnyloestradiol	4.574	5.111	6.089	0.633
	4.399	4.990	6.111	0.625
(EE2)	4.737	5.398	6.280	0.653
Average	4.570	5.166	6.160	0.637
% Recovery	70.0	79.0	94.2	

5.4 Octanol-water partition coefficient (K_{ow}) determination

5.4.1 Indirect K_{ow} measurement by RP-HPLC method

The reference materials showed a linear correlation ($y = 2.9133x + 1.5899$) as shown in Figure 5.4 with the coefficient correlation, $R^2 > 0.98$. Thus, it was suitable to measure the K_{ow} of all oestrogens by this method. The K_{ow} values obtained of all oestrogens are reported in Table 5.8. All the calculations to obtain log k values of reference substances are shown in the Appendix.

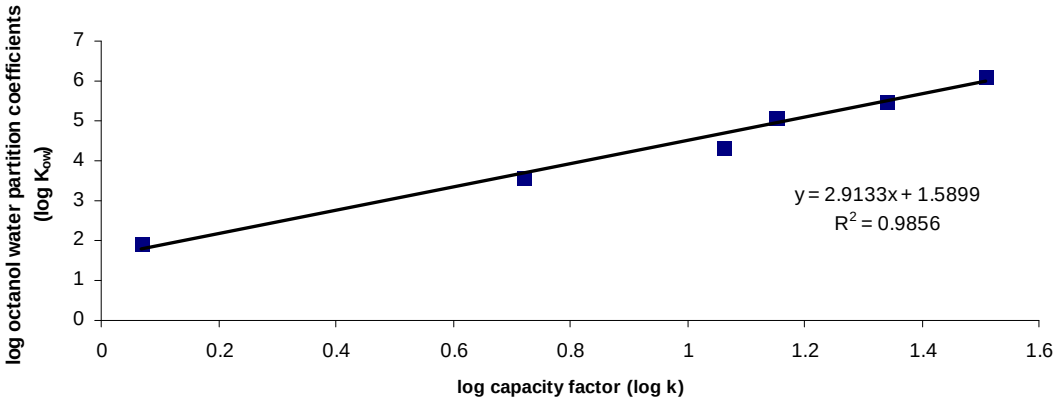


Figure 5.4: Correlation graph between log K_{ow} and log k of reference substances

Table 5.8: Calculated K_{ow} values of natural and synthetic oestrogens from RP-HPLC method.

Compound	Retention time (rt)	log rt	log K_{ow} ($y = 2.9133x + 1.5899$)
17β-oestradiol (E2)	4.24	0.63	3.42
17α-ethnyloestradiol (EE2)	4.92	0.69	3.61
Oestrone (E1)	5.90	0.77	3.83

From the retention time obtained as shown as in Table 5.8, 17β-oestradiol (E2) was eluted first followed by 17α-ethnyloestradiol (EE2) and oestrone (E1) was eluted last. From the equation obtained from reference materials correlation graph shown in Figure 5.4, log K_{ow} values derived for oestrone (E1) demonstrate the highest value. Therefore, from the fact that substances with high log K_{ow} values elute later on reversed-phase columns than substances with lower log K_{ow} values, oestrone (E1) demonstrate as the most hydrophobic compound followed by 17α-ethnyloestradiol (EE2) and 17β-oestradiol (E2). The hydrophobicity order obtained by using this method, (E1 $>$ EE2 $>$ E2) is not in agreement with the default hydrophobicity order obtained from the computational environmental fate model, Kowwin-EPISUITE. The default log K_{ow} values for oestrone (E1), 17β-oestradiol (E2) and 17α-ethnyloestradiol (EE2) are 3.43, 3.94 and 4.12 respectively and would give the hydrophobicity order as EE2 $>$ E2 $>$ E1.

As the hydropobicity is correlated with water solubility of compounds, a simple experiment to obtain water solubilities for these natural and synthetic oestrogens was conducted using the method from Shareef (Shareef et al., 2006a). Saturated solutions of each oestrogen were prepared by adding 20 mg of the solid into 25 mL glass centrifuge tubes, followed by 20 mL of Type IV water. The tubes were shaken and the supernatant was filtered through 0.2 μm glass microfiber filter. Initial tests showed that solutions were saturated and giving constant solubility data after 5 days. The water solubilities of all oestrogens measured at 20°C ± 0.1 and pH 6.7 is shown

in Table 5.9.

Table 5.9: Water solubilities of oestrogens obtained from experiment (n = 6).

Compound	Water solubility (mgL^{-1})
Oestrone (E1)	1.26 ± 0.07
17β-oestradiol (E2)	1.85 ± 0.02
17α-ethnyloestradiol (EE2)	1.88 ± 0.05

The results indicate oestrone (E1) is the least water soluble compound followed by 17β-oestradiol (E2) and 17α-ethnyloestradiol (EE2). The water solubilities order obtained in this study is in agreement with previous literature (Hurwitz and Liu, 1977; Yu et al., 2004; Shareef et al., 2006a) and the values obtained were in the range of previously reported values. The order of oestrogen water solubilities obtained is also consistent with the increasing polarities of the oestrogens from oestrone (E1) with one hydroxyl group, to 17β-oestradiol (E2) and 17α-ethnyloestradiol (EE2) with two hydroxyl groups.

From this experiment, it can be deduced that the most hydrophobic oestrogen, oestrone (E1) measured by this method is correlated with its least aqueous solubility. Between 17β-oestradiol (E2) and 17α-ethnyloestradiol (EE2), the elution order gained from the analytical column used by RP-HPLC method did not match with the water solubility order obtained from experiments. This study could not explain this discrepency; however, it is believed that the mechanisms involved in the analytical column used contributed to this situation.

5.4.2 Direct K_{ow} measurement by Shake Flask method

The K_{ow} by Shake Flask method was determined following the OECD guideline (section 4.4.2) at constant temperature of 20 °C ± 0.1. This method allows the user to select the compound concentration in octanol as well as the ratio of octanol-water used. The volume ratios between octanol and water studied were 1:1, 1:2, and 2:1. The log K_{ow} was obtained from the average results ($n = 3$) of each ratio, where the results varied by less than ± 0.3 units, which was achieved by maintaining

the concentration of each oestrogen dissolved in octanol at less than its maximum concentration allowed where the maximum concentration of test substance allows in each phase is 0.01 molL^{-1} (OECD, 2004). A concentration of 1800 mgL^{-1} dissolved in octanol for oestrogens was selected for the Shake Flask experiment. The pH recorded were in the range 6.1 to 6.3 for all solutions.

The log K_{ow} values for all oestrogens obtained is shown in Table 5.10. From this result, the hydrophobicity order obtained is E1 > EE2 > E2. This indicates that the least water solubility oestrogen, oestrone (E1) shows the highest log K_{ow} value. Regarding 17β-oestradiol (E2) and 17α-ethnyloestradiol (EE2), the log K_{ow} values obtained show only a slight different and indicate 17β-oestradiol is less hydrophobic than the 17α-ethnyloestradiol (EE2). The most water soluble oestrogen, 17α-ethnyloestradiol (EE2) does not indicate the lowest log K_{ow} values. As hydrophobicity has been reported to be well correlated with water solubility (Yalkowsky and Valvani, 1979 as cited by Pinsuwan et al., 1995), this result is contradictory. It is believed that this discrepency may due to the effect of octanol-water mutual saturation on the partition coefficient. In addition, the water solubility obtained for these two oestrogens are similar. This may have contributed to the difficulty in distinguishing their log K_{ow} as they posses a similar affinity to be distributed in the octanol-water phase.

Table 5.10: Measured log K_{ow} values of oestrogens obtained from Shake Flask method (n = 9).

Compound	Oestrone (E1)	17β-oestradiol (E2)	17α-ethnyloestradiol (EE2)
Log K_{ow}	3.95 ± 0.03	3.82 ± 0.02	3.85 ± 0.02

The log K_{ow} values obtained from this direct method for 17β-oestradiol (E2) and 17α-ethnyloestradiol (EE2) are lower than default values given by the computational model (Kowwin-EPISUITE); however, the experimental log K_{ow} values for oestrone (E1) was higher. This disparity between the experimental values and values given from the computational model is mainly because the default values given by the computational model (Kowwin-EPISUITE) were estimated from their structural

property without accounting for ambient conditions.

From both direct and indirect measurement of K_{ow} for oestrogens, one clear conclusion can be drawn. Water solubility indicates a strong correlation with hydrophobicity as both methods indicate the lowest water solubility oestrogen, oestrone (E1) is the most hydrophobic among them as shown by its highest log K_{ow} value.

5.5 Solid-water partition coefficient (K_d) determination

The rate at which oestrogens reach equilibrium in batch equilibrium studies varies from one study to another. Thus, an examination for different media prior to analysis is required. Figures 5.5 to 5.7 shows the equilibrium attainment of all oestrogens in all artificial soils used.

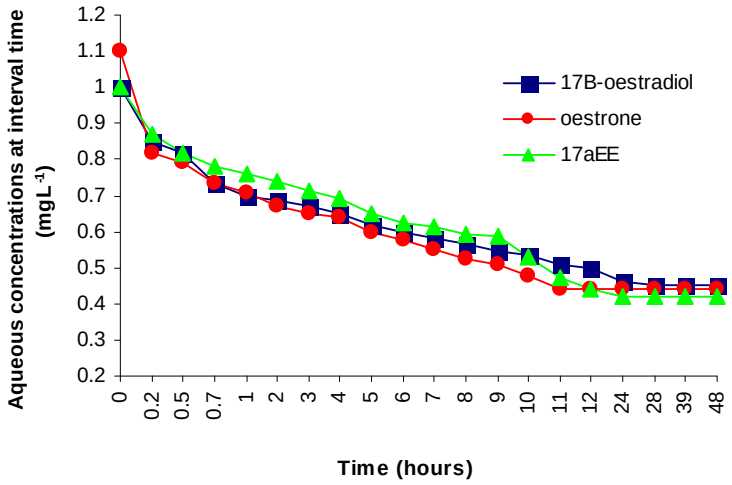


Figure 5.5: Equilibrium time attainment of all oestrogens in soil I

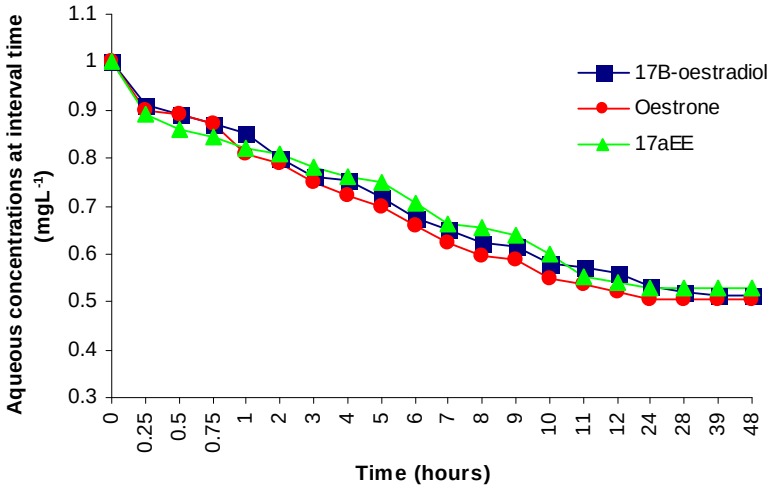


Figure 5.6: Equilibrium time attainment of all oestrogens in soil II

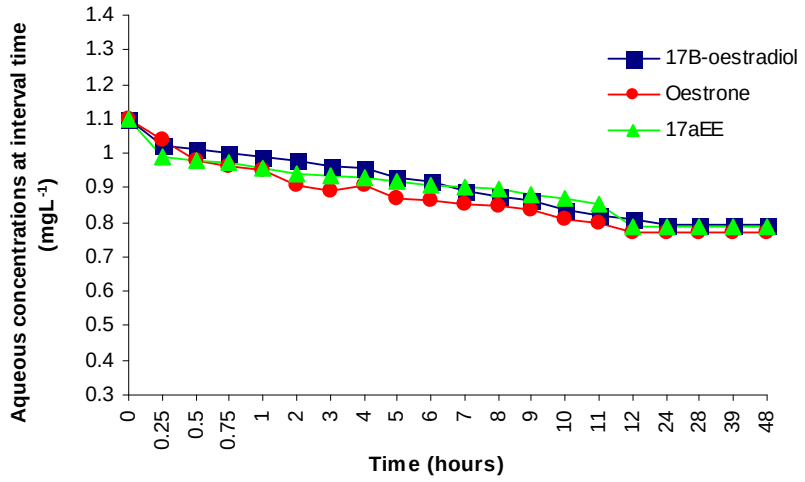
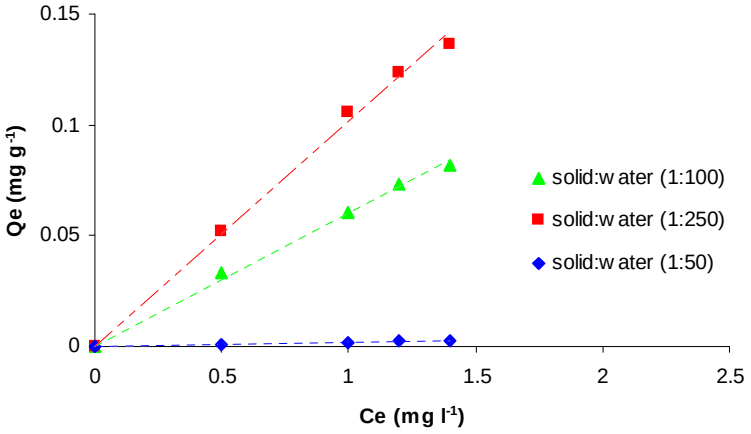


Figure 5.7: Equilibrium time attainment of all oestrogens in soil III

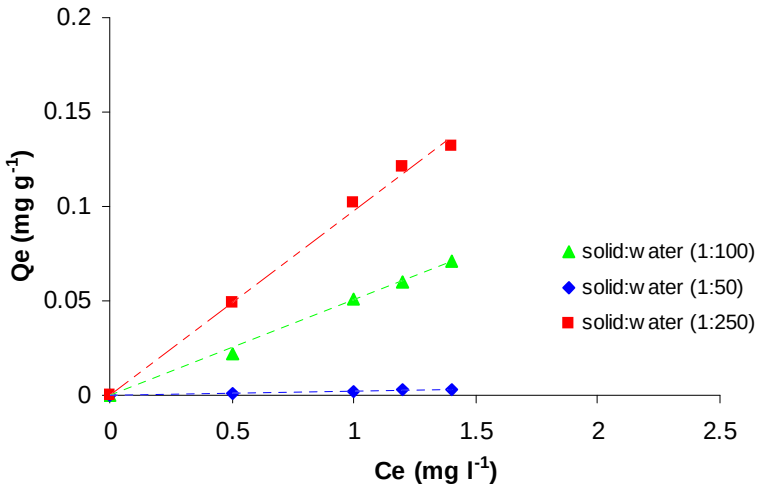
Soil material has a spectrum of binding site types, each exhibiting discrete equilibrium rates that contribute to chemical equilibration for a soil. This heterogeneity may create the disparity in equilibrium times observed between this and prior studies.

In this study, a 24 hour period was required to reach equilibrium of oestrogens in all artificial soils. Equilibrium periods of 24 hours or less have also been reported elsewhere (Ying et al., 2003; Yamamoto and Liljestrand, 2004; Keenan et al., 2008).

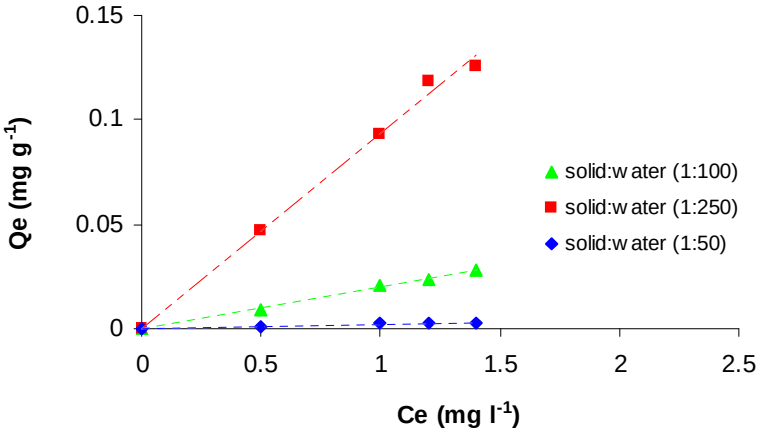
For the examination of maximum capacity of oestrogens, a soil-water ratio of 1:250 gave the highest adsorption among the tested ratios as shown in Figure 5.8. However, due to the technical problem in weighing the individual material for soil mixtures, a ratio 1 to 100 of soil-water was selected.



(a)



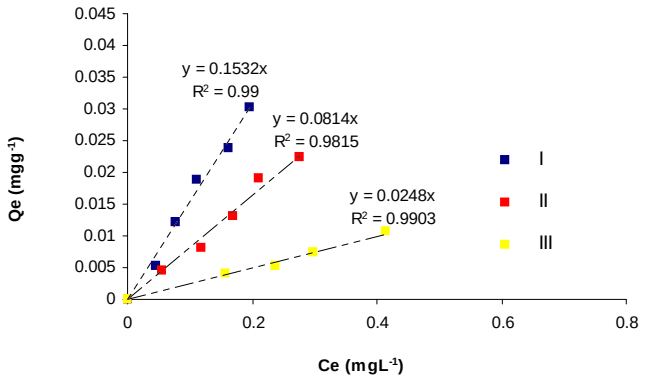
(b)



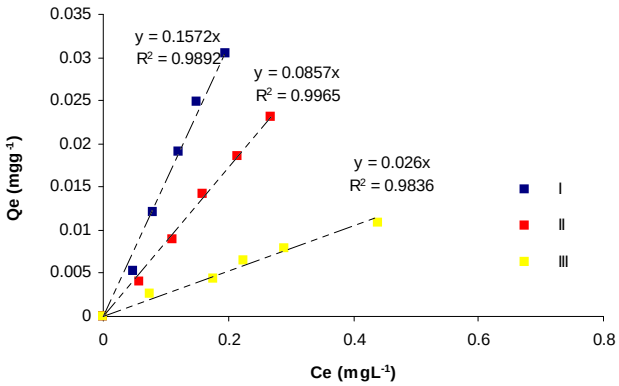
(c)

Figure 5.8: Adsorption isotherm of oestrogen, 17β-oestradiol (E2) at various soil-water ratios (a) soil I (b) soil II (c) soil III

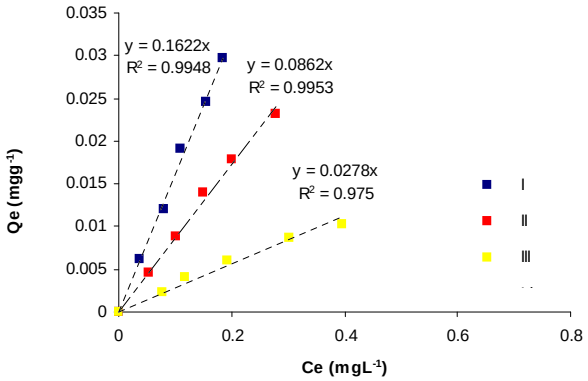
The batch equilibrium sorption experiments were then repeated with mixed oestrogen test mixtures ranging from 0.1 to 0.5 mgL^{-1} in 0.25g of soil in each 25 mL of glass centrifuge tubes which gave a soil to water ratio of 1:100. The tubes were shaken for 24 hours at a constant temperature, 20 $^{\circ}$C $\pm$ 0.1 in the dark room and were filtered and pre-concentrated as mentioned in section 4.3 before injected into HPLC for analysis.



(a)



(b)



(c)

Figure 5.9: Sorption isotherm of oestrogens in soils (a) 17β-oestradiol (E2) (b) 17α-ethnyloestradiol (EE2) (c) Oestrone (E1)

The adsorption isotherm for natural and synthetic oestrogens is generally linear over the ranges of concentration in all soils used. The linear regression analysis gave R^2

> 0.97 for all compounds. From this experiment, the solid-water partition coefficient (K_d) of all oestrogens indicates good correlation to the organic carbon content as shown as in Figure 5.9. Soil with greater organic matter adsorbed more oestrogens than soil with less organic matter content.

From the calculated sorption coefficient normalised to organic carbon (K_{oc}), the values indicate a strong association of all oestrogens with organic carbon, as shown in Table 5.11. K_{oc} value of oestrone (E1) indicates as the least mobile oestrogen compared to 17α-ethnyloestradiol (EE2) and 17β-oestradiol (E2).

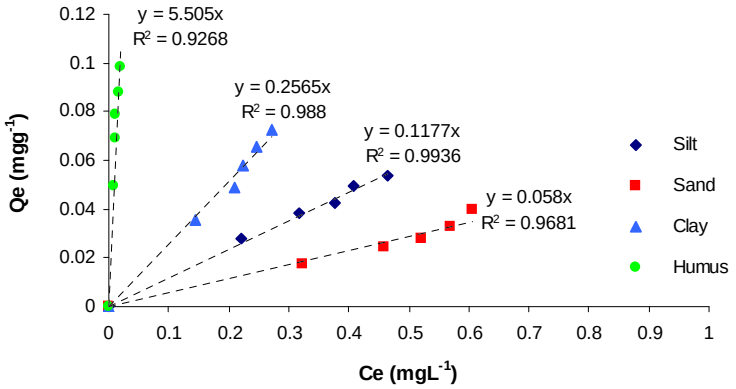
Table 5.11: The K_d and log K_{oc} for the linear isotherm fit for oestrogens in soils.

Soil	TOC %	K_d (Lkg^{-1})	K_{oc} (Lkg^{-1})	Log K_{oc}
			Oestrone (E1)	
I	8.90	162.2	1822.47	3.26
II	4.92	86.2	1752.03	3.24
III	1.62	27.8	1716.05	3.23
			17β-oestradiol (E2)	
I	8.90	153.2	1721.35	3.24
II	4.92	81.4	1654.47	3.22
III	1.62	24.8	1530.86	3.18
			17α-ethnyloestradiol (EE2)	
I	8.90	157.2	1766.29	3.25
II	4.92	85.7	1741.87	3.24
III	1.62	26.0	1604.94	3.21

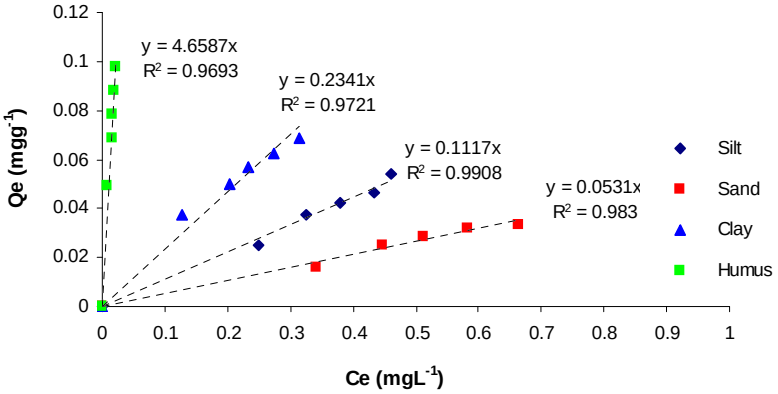
In order to confirm the correlation between sorption and organic carbon content, additional batch sorption experiments were conducted for the individual materials of the artificial soil. The adsorption of all oestrogens onto each material (sand, TOC 0.32% ; silt, TOC 1.44% ; clay, TOC 4.88% ; and humus, TOC 92.58%) was linear over the concentration range used as shown in Figure 5.10. The results shown in Table 5.12 demonstrate that the organic carbon is the dominant factor in explaining the sorption behaviour among oestrogens in soils of the present study. Humus, which has a high organic carbon content, demonstrates the highest sorption capacity for oestrogens followed by clay, silt and sand.

Table 5.12: The K_d and K_{oc} of individual material of soil.

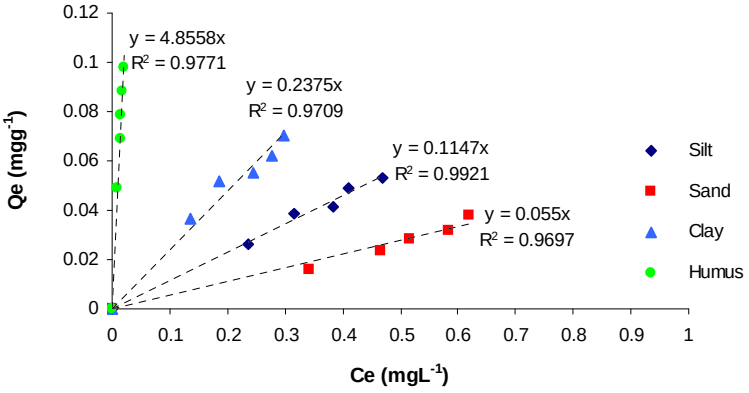
	Sand	Silt	Clay	Humus
TOC %	1.22 %	2.44 %	4.88 %	92.58 %
Oestrone (E1)				
$K_d(Lkg^{-1})$	58.0	117.7	256.5	5505.0
$K_{oc}(Lkg^{-1})$	4769.74	4817.85	5252.92	5946.40
17β-estradiol (E2)				
$K_d(Lkg^{-1})$	53.1	111.7	234.1	4658.7
$K_{oc}(Lkg^{-1})$	4366.77	4572.25	4794.18	5032.24
17α-ethnyloestradiol (EE2)				
$K_d(Lkg^{-1})$	55.0	114.7	237.5	4855.8
$K_{oc}(Lkg^{-1})$	4523.03	4695.05	4863.81	5245.15



(a)



(b)



(c)

Figure 5.10: Sorption of oestrogens onto soil materials (a) Oestrone (E1) (b) 17β-oestradiol (E2) (c) 17α-ethnyloestradiol (EE2)

Among the oestrogens, Oestrone (E1) shows the strongest adsorption onto all soils and it shows disparity from most of the previous studies which indicate 17α-ethnyloestradiol (EE2) as having the strongest adsorption onto soil. However, the stronger adsorption demonstrated by oestrone (E1) in this study is in agreement with a study reported by Hildebrand, (Hildebrand et al., 2006). Equal sorptive strength between 17α-ethnyloestradiol (EE2) and oestrone (E1) onto the same sorbent have also been reported (Yu et al., 2004).

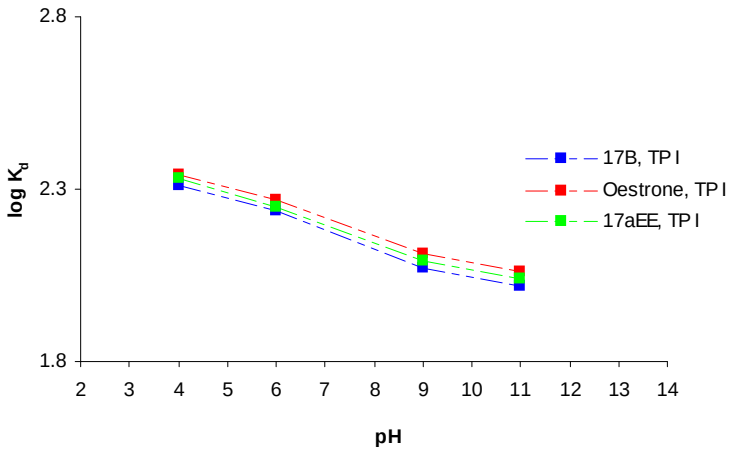
From this experiment, the sorption partition coefficient (K_d) of these oestrogens is in accordance with the hydrophobicity order obtained (based on log K_{ow} values). The least hydrophobic oestrogen, 17β-estradiol (E2) shows the weakest adsorption while Oestrone (E1), the most hydrophobic oestrogen, shows the greatest binding to all soils.

The results obtained in this study suggest that the octanol-water partition coefficient (K_{ow}) is an important parameter in predicting sorption in soils. The experimental of K_{ow} is vital in every study to determine the adsoprtion potential of any compounds in soil or sediment in order to allow clear interpretation. However, most of the previous studies either used default log K_{ow} given by computational models or values cited in other literatures for instance by Hildebrand (Hildebrand et al., 2006).

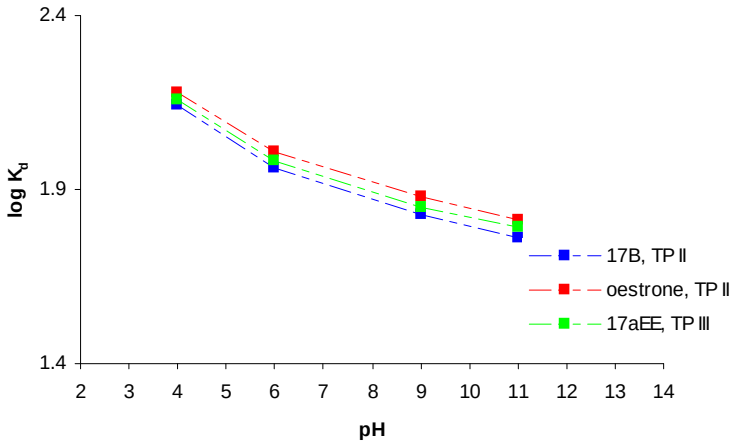
5.5.1 Effect of pH on sorption

The pH is an important factor in controlling the partitioning of oestrogen compounds, since this parameter not only affects the soil properties but also the property of the oestrogen itself. The effect of pH on K_d was studied with water at various pH. The pH of water was measured before and after the sorption experiment to confirm that it was constant.

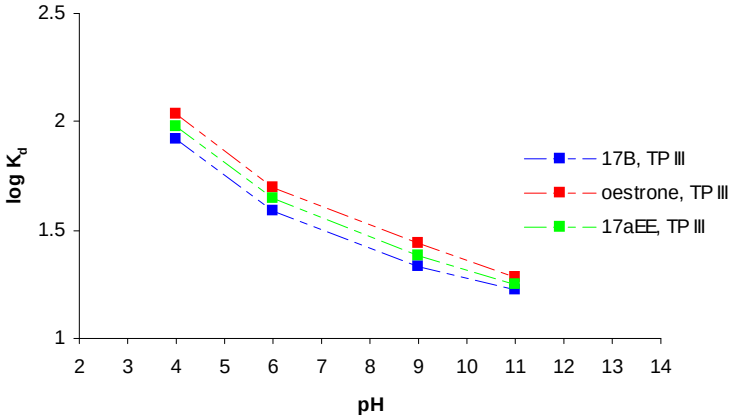
The variation of log K_d values are presented in Figure 5.11.



(a)



(b)



(c)

Figure 5.11: Relationship between log K_d of oestrogens and pH of water (a) soil I (b) soil II (c) soil III

The results indicate that for pH values between 4 to 11, sorption strength decreases as pH increases. It shows that at acidic pH, the sorption strength of all oestrogens is stronger than at alkaline pH. This behaviour can be explained by *LeChatalier's* principle. The acid dissociation constant (pKa) of all oestrogens reported is approximately in the range 10.2 to 10.4 (Hurwitz and Liu, 1977; Schafer et al., 2003; Yamamoto and Liljestrand, 2004). At pH $<$ pKa, the concentration of the hydronium ion increases and pushes the equilibrium point backward in accordance to *LeChatalier's* principle. Neutral species then become dominant, resulting in higher adsorption.

Of all soils, soil I shows the strongest sorption capacity for all oestrogens. The sorption affinity of all oestrogens for all soils decreased when the pH increased. A decrease of log K_d of oestrogens in all soils when pH increases could be explained by the occurrence of soil particle dispersion at higher pH (You et al., 1999). Soil particle dispersion increases the association of oestrogens onto colloid material being filtered into the solution phase.

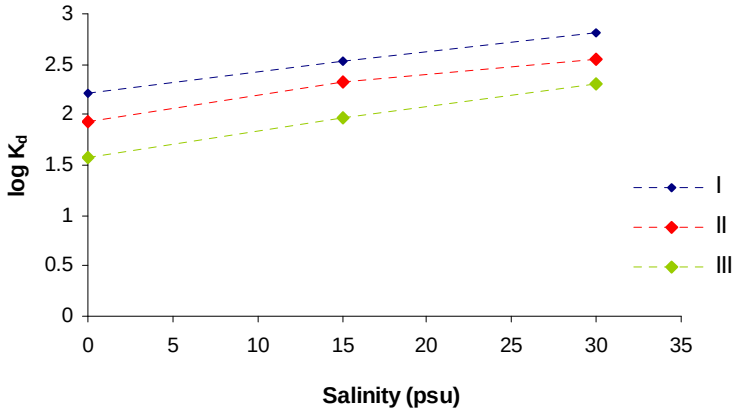
Table 5.13: Effect of pH on oestrogens mobility based on K_{oc} values in soils.

pH	I		II		III	
	K_{oc} (Lkg^{-1})	log K_{oc}	K_{oc} (Lkg^{-1})	log K_{oc}	K_{oc} (Lkg^{-1})	log K_{oc}
			(E2)			
4	2294.4	3.36	2804.9	3.45	5135.8	3.71
6	1952.8	3.29	1853.7	3.27	2401.2	3.38
9	1320.2	3.12	1373.9	3.14	1320.9	3.12
11	1176.4	3.07	1168.7	3.07	1024.7	3.01
			(E1)			
4	2458.4	3.39	3077.2	3.49	6765.4	3.83
6	2092.1	3.32	2079.3	3.32	3092.6	3.49
9	1447.2	3.16	1542.7	3.19	1697.5	3.23
11	1289.9	3.11	1313.0	3.12	1179.0	3.07
			(EE2)			
4	2402.2	3.38	2936.9	3.47	5895.1	3.77
6	1997.8	3.30	1941.1	3.29	2759.3	3.44
9	1382.0	3.14	1439.0	3.16	1475.3	3.17
11	1231.5	3.09	1254.1	3.10	1098.8	3.04

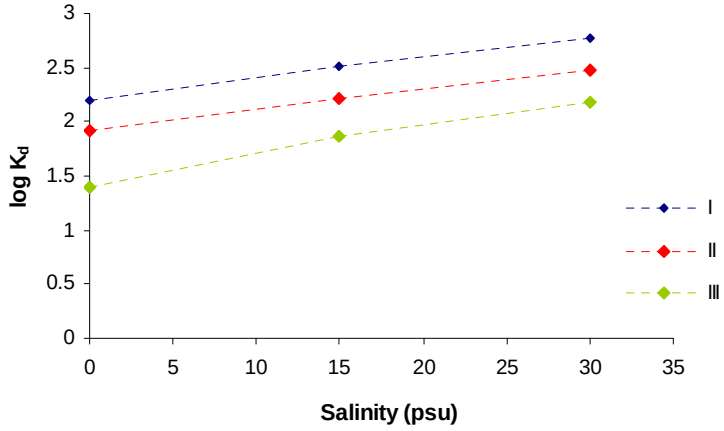
From the calculated sorption coefficient normalised to organic carbon (K_{oc}) (Table 5.13), the values indicate a strong association of all oestrogens with organic carbon. As the pH increases, all oestrogens become more mobile in all soils.

5.5.2 Effect of salinity on sorption

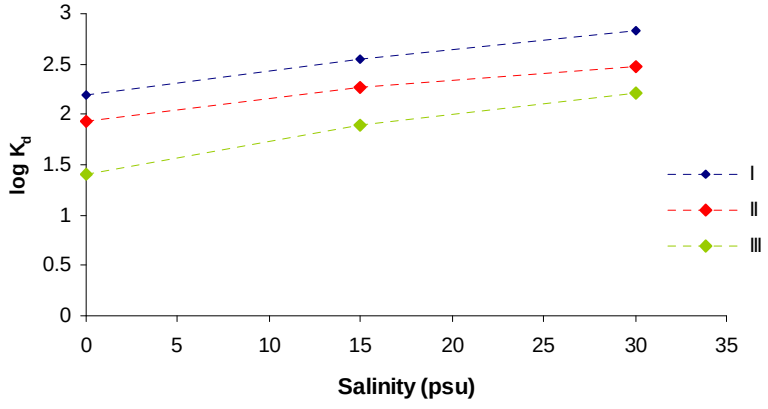
The K_d values at various salinities of water were investigated. The results are shown in Figure 5.12. Oestrogens adsorption is shown to increase with increasing salinity between 0-30 psu. The observed increase in sorption coefficient of all oestrogens with increasing salinity can be explained by the decreasing water solubility of the organic compounds (Schwarzenbach et al., 2003). Due to the presence of additional ions, it is more difficult for the organic compound to find a cavity to fit into which results in the compound being more attracted to the soil particles. This is usually referred to as the "salting out" effect of the organic compounds (Schwarzenbach et al., 2003). The results demonstrate that organic carbon is the dominant factor on oestrogen sorption with salinity. Higher organic carbon percentage in soil clearly shows a higher adsorption capacity of oestrogens with saline water compared to other soils with lower organic carbon content.



(a)



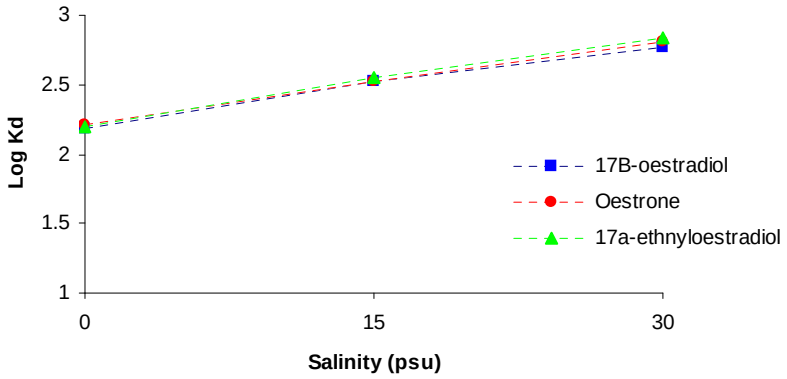
(b)



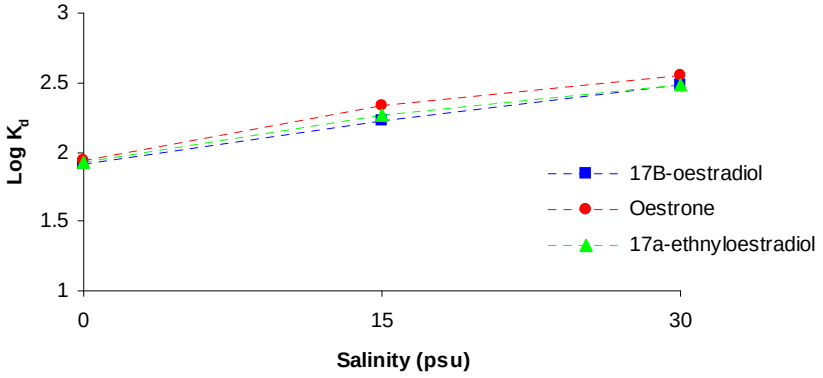
(c)

Figure 5.12: Relationship between log K_d and salinity of water (a) oestrone (E1) (b) 17β-estradiol (E2) (c) 17α-ethnyloestradiol (EE2)

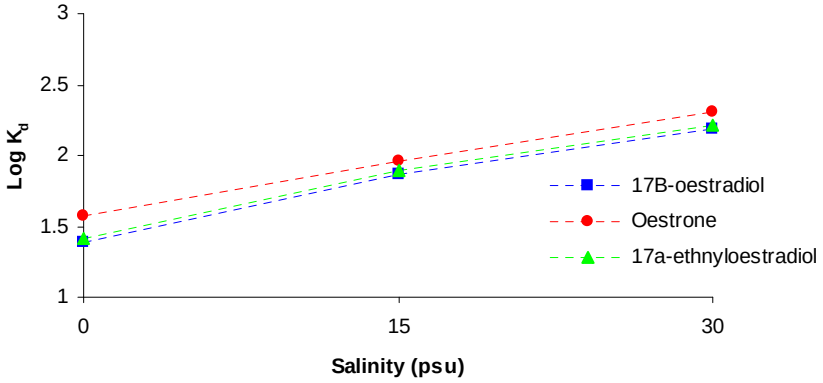
In Figure 5.13 (c), oestrone (E1) shows the highest adsorption at all concentrations of salinity used in this study, but no significant difference between 17α-ethnyloestradiol (EE2) and 17β-estradiol (E2). This behaviour may due to the water solubility properties where oestone (E1) is the least soluble compared to 17α-ethnyloestradiol (EE2) and 17β-estradiol (E2) which posses similar water solubility values. In soil II, (Figure 5.13 (b)), Oestrone (E1) also shows the highest adsorption at salinity 15 and 30 psu. However, at zero salinity, all oestrogens posses a similar adsorption. In soil I, for all salinities, there is no significant difference for the adsorption among these oestrogens, (Figure 5.13 (a)). This can be explained by the attainment of maximum adsorption capacity due to organic carbon, which may work as a limiting factor.



(a)



(b)



(c)

Figure 5.13: The effect of salinity on oestrogens adsorption (log K_d) in soils (a) soil I (b) soil II (c) soil III

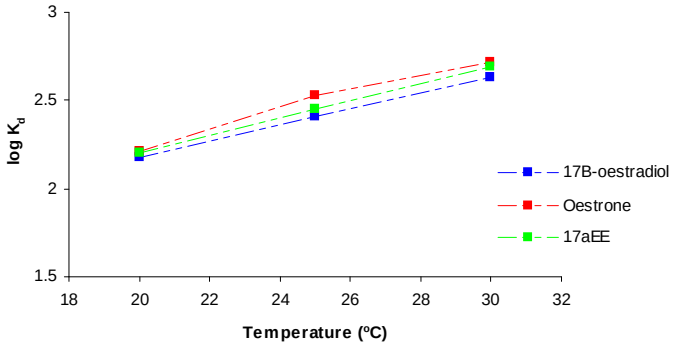
Table 5.14: Effect of salinity on oestrogens mobility based on K_{oc} values in soils.

Salinity (psu)	I K_{oc} (Lkg^{-1})	log K_{oc}	II K_{oc} (Lkg^{-1})	log K_{oc}	III K_{oc} (Lkg^{-1})	log K_{oc}
			(E2)			
0	1740.4	3.24	1652.4	3.21	3024.7	3.48
15	3720.2	3.57	3371.9	3.52	4574.1	3.66
30	6615.7	3.82	6138.2	3.79	9345.7	3.97
			(E1)			
0	1822.5	3.26	1770.3	3.25	3635.8	3.56
15	3986.5	3.60	4345.5	3.63	5629.6	3.75
30	7596.6	3.88	7211.4	3.86	12604.9	4.10
			(EE2)			
0	1780.9	3.25	1729.7	3.23	2512.3	3.40
15	3806.7	3.58	3784.6	3.58	4790.1	3.68
30	7255.1	3.86	6138.2	3.79	10012.3	4.00

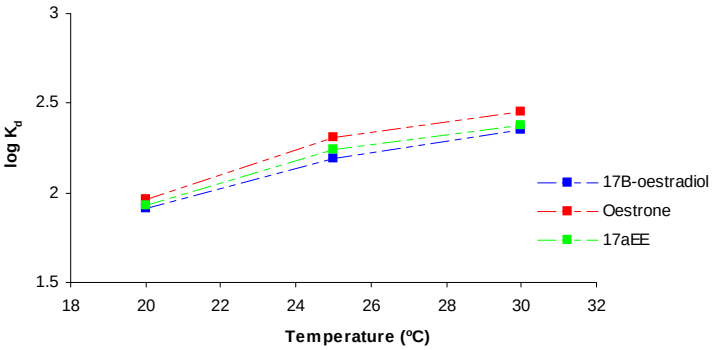
From the calculated sorption coefficient normalised to organic carbon (K_{oc}) (Table 5.14), the values indicate a strong association of all oestrogens with organic carbon. As salinity increases, all oestrogens become immobile in all soils. Among oestrogens, 17β-estradiol (E2) is the most mobile compound in all soils. The K_{oc} value of oestrone (E1) indicates that it is the most immobile compound in all soils followed by 17α-ethnyloestradiol (EE2).

5.5.3 Effect of temperature on sorption

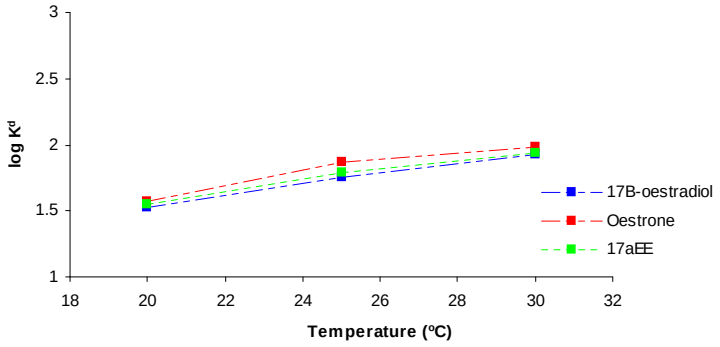
The effect of temperature was studied by varying the temperature from 20 to 30°C. The results are shown in Figure 5.14.



(a)



(b)



(c)

Figure 5.14: Relationship between log K_d and temperature (a) soil I (b) soil II (c) soil III

The results demonstrate that the sorption of oestrogens onto soils increases as the temperature increases. This behaviour can be explained by the concept of Gibb's surface free energy (Bajpai and Vishwakarma, 2003). Increasing temperature reduces the surface tension of water (Rinker et al., 1994) which then lowers the surface free energy. This leads to an increase in adsorption on the soil surface.

Table 5.15: Effect of temperature on oestrogens mobility based on K_{oc} values in soils.

Temperature ($^\circ$C)	I K_{oc} (Lkg^{-1})	log K_{oc}	II K_{oc} (Lkg^{-1})	log K_{oc}	III K_{oc} (Lkg^{-1})	log K_{oc}
			(E2)			
20	1701.1	3.23	1652.4	3.21	2092.6	3.32
25	2887.6	3.46	3148.4	3.50	3549.4	3.55
30	4793.3	3.68	4550.8	3.66	5253.1	3.72
			(E1)			
20	1822.5	3.26	1853.7	3.26	229.3	3.36
25	3806.7	3.58	4150.4	3.61	4574.1	3.66
30	5896.6	3.77	5727.6	3.7	5895.1	3.77
			(EE2)			
20	1780.9	3.25	1729.7	3.23	2191.4	3.34
25	3166.3	3.50	3532.5	3.54	3808.6	3.58
30	5503.4	3.74	4876.0	3.69	5376.5	3.73

From the calculated sorption coefficient normalised to organic carbon (K_{oc}) (Table 5.15), the values indicate a strong association of all oestrogens wih organic carbon. As temperature increases, all oestrogens become less mobile in all soils. Among oestrogens, oestrone (E1) K_{oc} value indicates the most immobile compound in all soils followed by 17α-ethnyloestradiol (EE2) and 17β-estradiol (E2) as the least.

From this experiment, it can be concluded that pH, salinity and temperature have a significant effect on the oestrogens sorption coefficient in soils and their mobility in soils.

5.6 Prediction of environmental fate and persistence

There is considerable difference between experimental values obtained for K_{ow} and K_{oc} in this study and the values generated by Kowwin-EPISUITE and PCKoc-EPISUITE computational model as indicated in Table 5.16.

Table 5.16: The partition coefficients and comparison of the prediction obtained using experiment and default values as the input for the EPISUITE program.

Parameter	Log K_d	K_{oc}	Log K_{oc}	Log K_{ow}	Partitioning[a] (%) / Air	Water	Half-life[a](hr) Soil
E1				3.83[b]/3.95[c]			
I	2.21	1822	3.26		$2.71x10^3/2$	11.60/900	87.20/1800
II	1.94	1752	3.24		$2.71x10^3/2$	11.60/900	87.20/1800
III	1.44	1654	3.22		$2.72x10^3/2$	11.60/900	87.30/1800
EPISUITE	N.A	30000	4.48	3.43	$2.33x10^3/2$	8.89/900	76.00/1800
E2				3.42[b]/3.87[c]			
I	2.19	1721	3.24		$2.68x10^4/2$	11.4/900	87.40/1800
II	1.91	1654	3.22		$2.68x10^4/2$	11.5/900	87.50/1800
III	1.39	1531	3.18		$2.68x10^4/2$	11.5/900	87.50/1800
EPISUITE	N.A	16000	4.20	3.94	$2.45x10^4/2$	9.76/900	81.20/1800
EE2				3.61[b]/3.82[c]			
I	2.20	1766	3.25		$6.58x10^4/2$	9.03/1440	89.90/2880
II	1.93	1742	3.24		$6.71x10^4/2$	9.52/1440	89.80/2880
III	1.41	1605	3.21		$7.20x10^4/2$	11.6/1440	89.20/2880
EPISUITE	N.A	47700	4.69	4.12	$5.23x10^4/2$	5.92/1440	73.10/2880

[a] The prediction data using K_{oc} and K_{ow} as the input values.
[b] Experimental values obtained from RP-HPLC method.
[c] Experimental values obtained from Shake Flask method.

These results show that the EPISUITE program underestimates the K_{ow} of oestrone (E1) while overestimates 17β-estradiol (E2) and 17α-ethnyloestradiol (EE2). The EPISUITE program also overestimates the K_{oc} values. The disparity between the experimental and the computational model results arise as the EPISUITE program calculation uses only the inherent chemical and physical properties of these compounds and does not account for ambient conditions.

Table 5.16 also shows a comparison of the prediction data on environmental distribution of oestrogens using both the input from experimental and default values. There is a significant difference in environmental distribution of these oestrogens depending on whether experimental or default input values were used. The results show that the EPISUITE program underestimates oestrogens partitioning in all environmental compartments. Thus, if this predictive model was used for decision making on sewage treatment effluent reuse, it would mislead the people making those decisions. However, there are no significant differences in percentage partitioning among the soils used in the experiment. It is believed that soils with a higher organic carbon content than the soils used in this study would give a better indication of the influence of organic carbon on soil partitioning. However, the organic carbon content in all soils used in this study is in the typical range of organic carbon found in soils (Mackay, 2001).

By inputting the experimental values of K_{ow} and K_{oc}, the persistence time (half-life) generated was no different with the default. It is because the experimental K_{ow} and K_{oc} input values only affect the partitioning behaviour calculation by the program. The half-life of oestrogens in ambient conditions is dependent on physical and biological properties of the environment, for example, the presence of microbial populations.

5.7 Prediction of vegetation uptake and bioconcentration

Until now, most environmental models have ignored plant uptake, including the EPISUITE program. Thus, the prediction of plant uptake and the bioconcentration factors of oestrogens was done by using a model developed by Duarte-Davidson and Jones (1996).

The potential uptake of oestrogens by the root system that is based on experimental log K_{ow} would be classified as 2 which indicates as a medium root retention for the oestrogen studied (Table 5.17).

Table 5.17: Root potential uptake of oestrogens, based on model developed by Duarte-Davidson and Jones (1996).

Compound	Log K_{ow}	Potential for root retention	Classification
Oestrone (E1)	3.83[a], 3.95[b]	Medium	2
17α-ethnyloestradiol (EE2)	3.61[a], 3.85[b]	Medium	2
17β-oestradiol (E1)	3.42[a], 3.82[b]	Medium	2

[a] RP-HPLC method
[b] Shake Flask method

Since during spray irrigation with sewage treatment plant effluent there is possible direct contact between effluent and plant leaves, it is also necessary to predict the foliar uptake of these oestrogens. Based on model developed by Duarte-Davidson and Jones (1996), see Table 2.6, the potential for oestrogens to be uptaken by the leaves can be classified based solely on log K_{ow} values. All oestrogens are classified as medium/possible for uptake following application of sewage treatment plant effluent as an irrigant.

Table 5.18: Potential for foliar uptake, based on model developed by Duarte-Davidson and Jones (1996).

Compound	Log K_{ow}	Potential for foliar uptake	Classification
Oestrone (E1)	3.83[a], 3.95[b]	Medium/possible	2
17α-ethnyloestradiol (EE2)	3.61[a], 3.85[b]	Medium/possible	2
17β-oestradiol (E1)	3.42[a], 3.82[b]	Medium/possible	2

As the oestrogens are classified as having medium potential for uptake by root and medium/possible potential for uptake by foliar uptake, it is essential to predict their bioconcentration following effluent reuse applications as irrigation water. Table 5.19 shows the bioconcentration factors (BCFs) that these oestrogens would give by using the Equation 3.7 and 3.9 mentioned in Chapter 3.

Table 5.19: Partitioning factors for natural and synthetic oestrogens based on model developed by Kerler and Schonherr (1988); Travis and Arms (1988).

Oestrogens	$K_{ow}{}^{a}$	$K_{ow}{}^{b,c}$	BCF vegetation[1] (from water to plant cuticle)	BCF vegetation[2] (from soil to plant)
Oestrone (E1)	3.43	3.83^{b} 3.95 ± 0.03^{c}	2355.59^{a} 5755.72^{b} 7524.89^{c}	0.40^{a} 0.24^{b} 0.20^{c}
17β-oestradiol (E2)	3.94	3.42^{b} 3.82 ± 0.02^{c}	7358.68^{a} 2303.56^{b} 5628.59^{c}	0.20^{a} 0.41^{b} 0.24^{c}
17α-ethnyloestradiol (EE2)	4.12	3.61^{b} 3.85 ± 0.02^{c}	11000.19^{a} 3521.28^{b} 6018.66^{c}	0.16^{a} 0.32^{b} 0.23^{c}

[a] default values
[b] experimental values in this study obtained by RP-HPLC method
[c] experimental values in this study obtained by Shake Flask method
[1] model developed by Kerler and Schonherr (1988)
[2] model developed by Travis and Arms (1988)

A compound with a higher K_{ow} value would give a higher bioconcentration factor (BCFs). In the case of effluent reuse for irrigation, the BCFs of these oestrogens from soil to plants would be lower than BCFs from water to plant cuticle. In regards to spray irrigation where direct contact occurs between sewage treatment plant effluent and plant leaves, high bioaccumulation would be expected as the BCF itself shows a several orders of magnitude higher than BCF of soil to plant.

Although the values of BCFs for vegetation from soil to plant is low, uptake by grazing animals may arise due to high sorptive affinity of these oestrogens in soils. It has been suggested that 6 % of the total dry matter intake of grazing stock is soil (Wild and Jones, 1992) and that a dairy cow typically consumes 15 kg of dry matter/day. Therefore if the 0.9 kg of soil ingested contains even 1 mg/kg of oestrogen, that would relate to 0.9 mg of oestrogen ingested per day. Given a BCF in a cow in the range 10 - 100 (Keenan, 2000), 0.9 mg of oestrogens ingested through

soil would give a theraupetic response equal to 9 - 90 mg/day of oestrogen ingested. Biomagnification in humans could occur from consumption of either dairy meat or dairy milk.

5.8 Soil column experiment

A mass balance revealed that all oestrogens have a strong sorption in all soils based on the volumes of solutions containing oestrogen used to feed the soil column until breakthrough is achieved.

Table 5.20 shows the percentage recovery for all oestrogens in all soils used in this study. Elution of a high percentage all oestrogens with high percentage following lowest displacement occurred in soil III indicating this soil as having the lowest sorption capacity. This may be attributed to the lowest percentage organic carbon content in soil III compared to soil I and II. In contrast, soil I displayed the highest retardation demonstrated by the highest pore volume application to achieve breakthrough. This behaviour can be explained by stronger oestrogen binding represented by their higher solid-water partition coefficient, K_d in soil I compared to soil II and III, see Figure 5.9, due to higher organic matter content.

Table 5.20: Percentage recovery of oestrogens in soils obtained from mass balance.

Soil	I	II	III
		Oestrone (E1)	
Effluent recovery (%) parent compound	87.2 (6402)	86.1 (4620)	84.4 (4010)
Effluent recovery (%) metabolite	n.d	n.d	n.d
		17β-oestradiol (E2)	
Effluent recovery (%) parent compound	85.7 (3410)	74.8 (2310)	85.9 (1540)
Effluent recovery (%) metabolite	n.d	n.d	n.d
		17α-ethnyloestradiol (EE2)	
Effluent recovery (%) parent compound	83.1 (3784)	81.9 (3520)	81.03 (2860)
Effluent recovery (%) metabolite	n.d	n.d	n.d

* value in paratheses represents pore volume (PV)

Among oestrogens in this study, oestrone (E1) has the strongest sorption affinity while 17β-oestradiol (E2) shows the weakeast sorption affinity in all soils. In soil I for example, 87.2% oestrone (E1) was recovered after approximately 6402 pore volumes of solution were introduced to the soil column while an 85.7 % recovery of 17β-oestradiol (E2) was collected in leachate at approximately 3410 pore volumes of solution were added as shown in Figure 5.15.

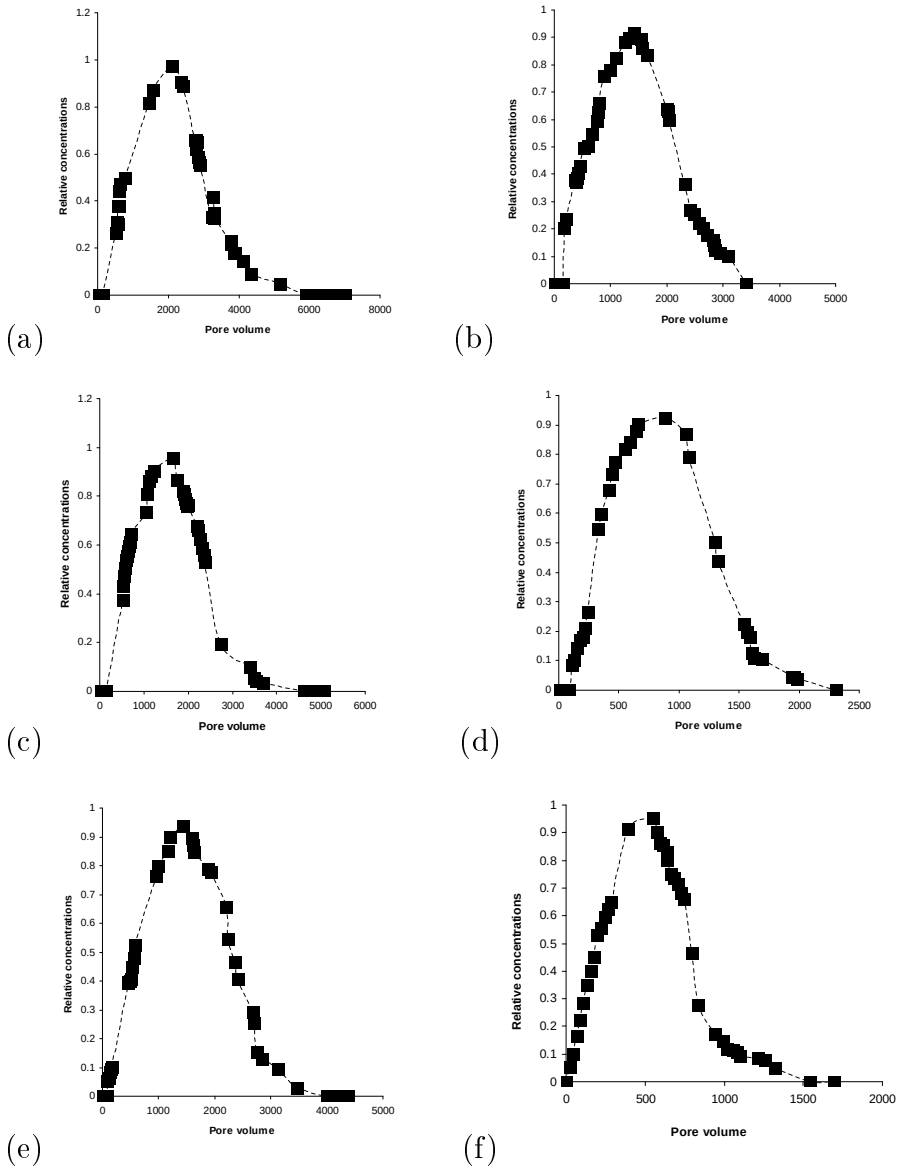


Figure 5.15: Breakthrough curve (BTC) in soil I (a) Oestrone (E1), soil I (b) 17β-oestradiol (E2), soil I (c) Oestrone (E1), soil II (d) 17β-oestradiol (E2), soil II (e) Oestrone (E1), soil III (f) 17β-oestradiol (E2), soil III

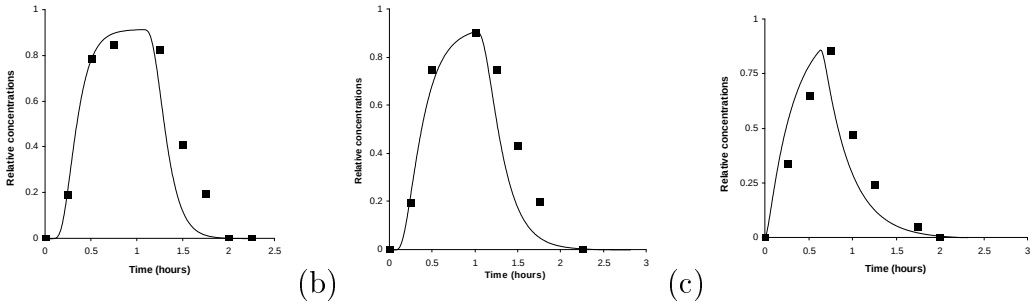
The percentage recovery collected for all oestrogens following high displacement obtained from soil column in this study indicates that under real fields study, their leaching potential would be low. Considering the oestrogens concentration used in this study (in mgL^{-1}) was much higher than the reported concentration in sewage effluent (e.g ngL^{-1} in UK's STPs, mentioned in Section 2.1). Therefore, their leach-

ing potential through soil profile would not be expected following sewage effluent irrigation.

Previous studies have shown that oestrogens undergo degradation in agricultural soils (Colucci et al., 2001; Colucci and Topp, 2001); however, in this study no metabolite was detected in the leachate during the experiments.

5.8.1 Soil column modeling

Figure 5.16 illustrates the measured and fitted breakthrough curves (BTC) for the tracer ($CaCl_2$) in the soil column. The recorded data for the tracer in all soils were almost symmetrical indicating an ideal transport behaviour.



(a) (b) (c)

Figure 5.16: Breakthrough curves of tracer, $CaCl_2$ in soils (a) soil I (b) soil II (c) soil III

A comparison between the breakthrough curve obtained for oestrogens, see Figure 5.17, and tracer indicates that the oestrogens travelled slower than a conservative tracer, $CaCl_2$. The later arrival of the oestrogens peak compared to tracer indicates their retardation in soils due to sorption. Among soils used, soil I, which has the highest organic carbon, is the slowest to reach a maximum outflow leachate concentration for all oestrogens, see Table 5.21.

Table 5.21: Maximum concentration and time until maximum for oestrogens breakthrough modeled by HYDRUS-1D from measured data.

Compound	Maximum relative concentration	Time until maximum (day)
17β-oestradiol (E2)		
I	0.90	2.92
II	0.92	1.67
III	0.95	0.92
17α-ethnyloestradiol (EE2)		
I	0.95	2.63
II	0.92	2.46
III	0.95	2.00
Oestrone (E1)		
I	0.95	4.00
II	0.95	3.13
III	0.93	2.71

The shapes of breakthrough curves for all oestrogens in all soils were basically the same sigmoidal shape. This similarity in shape shows that the K_d values input in this model do not introduce variations in the retreating slope of the breakthrough curves, but only affect the retardation factor.

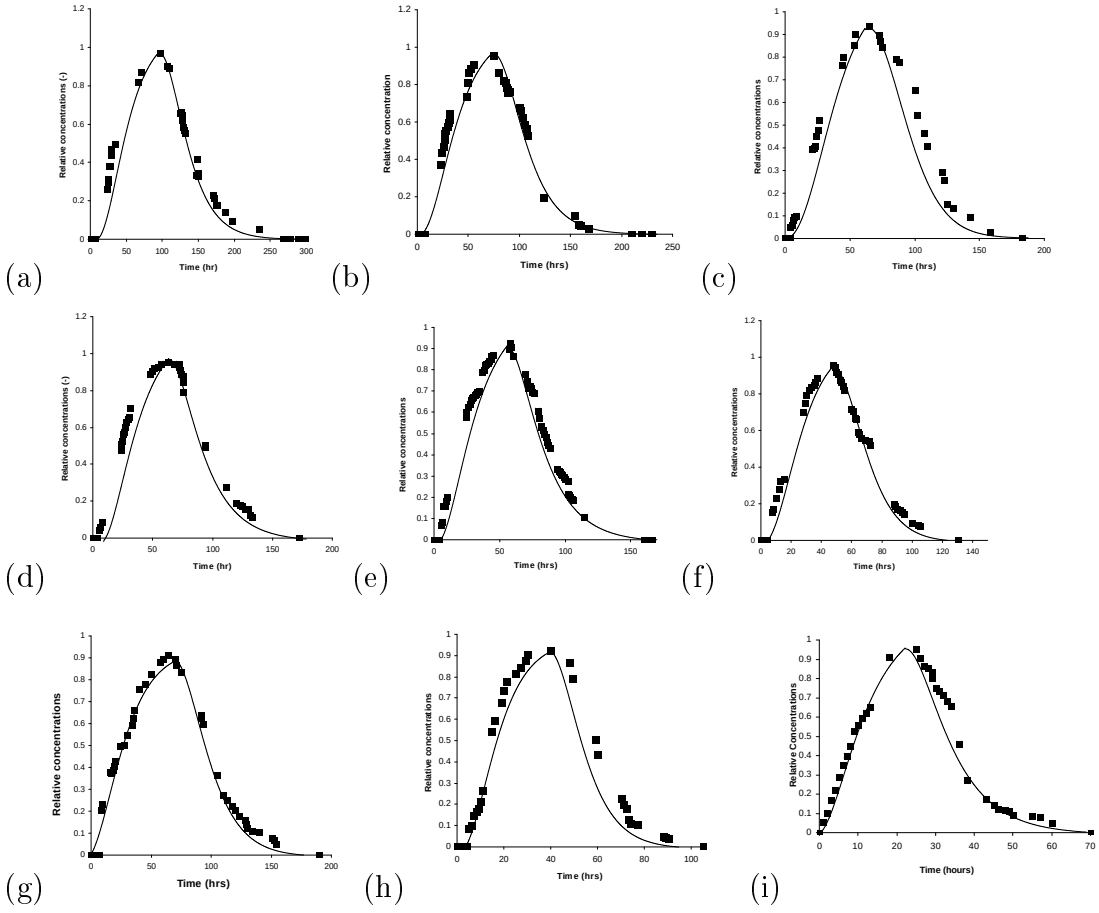


Figure 5.17: Measured and predicted breakthrough curves (BTCs) for oestrogens (a) oestrone (E1), soil I (b) oestrone (E1), soil II (c) oestrone (E1), soil III (d) 17α-ethnyloestradiol (EE2), soil I (e) 17α-ethnyloestradiol (EE2), soil II (f) 17α-ethnyloestradiol (EE2), soil III (g) 17β-oestradiol (E2), soil I (h) 17β-oestradiol (E2), soil II (i) 17β-oestradiol (E2), soil III.

The asymmetrical breakthrough curves of oestrogens from the measured data, which were illustrated by the curve tailing or late arrivals of oestrogens, see Figure 5.17, were further tested by fitting them into the non-equilibrium transport model HYDRUS-1D. Chemical non-equilibrium employing the attachment-detachment approach was used to describe the transport data.

The resulted transport parameters obtained are shown in Table 5.22. Soil I (TOC % 8.90) shows a greater retardation factor for all oestrogens compared to soil II (TOC % 4.92) and III (TOC % 1.62). These results indicate the impact of percentage organic carbon on retarding oestrogens mobility. The retardation factor given by

soil I for oestrone (E1) is approximately 1.5 times higher than that given by soil II and III. The strength of the retardation factor is also supported by the attachment and detachment rate coefficient given by HYDRUS-1D. From the values obtained for these coefficients, stronger attachment rate and weaker detachment rate result in a stronger retardation factor.

Table 5.22: Transport parameters for the soil column experiments.

Solute	Sorbent	Attachment rate coefficient (k_{a1}) (h^{-1})	Detachment rate coefficient (k_{d1}) (h^{-1})	Retardation factor (R) (-)
E1	Soil I	31.12 ± 0.051	0.141 ± 0.011	990
	Soil II	29.96 ± 0.550	0.174 ± 0.028	704
	Soil III	18.85 ± 0.090	0.304 ± 0.214	660
E2	Soil I	28.90 ± 0.026	0.155 ± 0.009	616
	Soil II	27.14 ± 0.344	0.180 ± 0.003	550
	Soil III	16.01 ± 0.012	0.311 ± 0.233	506
EE2	Soil I	30.11 ± 0.211	0.186 ± 0.001	572
	Soil II	28.89 ± 0.220	0.208 ± 0.072	407
	Soil III	26.95 ± 0.180	0.346 ± 0.125	219

The retardation of all oestrogens in all soils is well correlated with their hydrophobic characteristics. The most hydrophobic and the least water soluble oestrogen, oestrone (E1), shows high partitioning onto organic matter and as a result is the most retarded oestrogen in soils followed by 17α-ethnyloestradiol (EE2) and 17β-estradiol (E2).

This experiment further supports the finding that increased organic matter in soils increases retardation of oestrogens mobility. The order of oestrogen retardation by organic matter is well correlated with their hydrophobicity order obtained in octanol-water partition coefficient experiments.

Chapter 6

CONCLUSIONS AND FUTURE WORK

This chapter concludes the work in this thesis and gives some topics for future research study.

6.1 Conclusion

The aim of this study was to determine the environmental fate of oestrogens contained in sewage treatment effluent in soil environment following sewage reuse application. As a computational model is available to predict their environmental fate, experimentation and reporting is essential in order to ensure a realistic model of the environment under ambient conditions.

Of physical chemical properties, the octanol-water partitioning coefficient (K_{ow}) and sorption are the most important factors in regulating their environmental fate and mobility in soil. The results obtained indicate that the most hydrophobic oestrogen obtained from experiment, oestrone (E1) shows the strongest affinity to all soils followed by 17α-ethnyloestradiol (EE2) and 17β-oestradiol (E2). Thus, in this study it can be concluded that the K_{ow} is a good predictor and can be used as a tool in predicting oestrogens behaviour in the environment. The RP-HPLC method conducted in this study has shown to be suitable as a screening tool providing rapid results obtained that were similar with the hydrophobicity order obtained by the

Shake Flask method. However, as the K_{ow} values are derived from the elution time, thus the values are largely dependent to the packing material of analytical column used. Meanwhile, although Shake Flask method was time consuming and laborious, it is an appropriate method to determine the K_{ow} as the values were obtained directly from the concentrations determined in the aqueous phase of octanol-water system. In addition to that, the Shake Flask method also provide an accurate value as the ambient conditions for instance the temperature and pH can be varied. In this study, the hydrophobicity order of oestrogens obtained by both methods was in agreement with their water solubility order where the results obtained indicate that the least water soluble as the most hydrophobic compound. Thus, it can also be concluded that water solubility of a compound can be used to predict its hydrophobicity characteristic in the environment.

All oestrogens indicated a strong association with organic carbon content of the soils. The sorption of oestrogens onto soil increased when the organic carbon content increased. The sorption coefficients (K_d) obtained from the individual materials used to prepare the artificial soil also indicated a strong association with organic carbon. Thus, this study shows that organic carbon/matter plays an important role to bind oestrogens in soils. The results obtained in this study also showed a significant effect on oestrogens behaviour in soils as result of varying salinity, pH and temperature. All oestrogens showed stronger affinity to all soils as the temperature and salinity increased. Meanwhile, as the pH increased all oestrogens indicated a weaker binding to all soils. From the calculated sorption coefficient normalised to organic carbon (K_{oc}), all oestrogens become mobile in all soils with increasing pH whilst immobile as the temperature and salinity increased. These observtions are essential to predicting the consequences following sewage effluent reuse application as irrigation water. An application of saline and lower pH effluent for example would not increase oestrogens mobility in the soil profile. However, oestrogens may accumulate over time at the soil surface and may contribute to the surface runoff. Although increasing temperature resulted in the immobility of oestrogens in soils, in a real environment, the increasing temperature may affect the soil structure. Therefore, the mobilisation of oestrogens in the soil profile in countries with warmer climates needs to be further investigated. Field investigations at elevated temperatures will be essential to understand oestrogen mobility under realistic conditions in these environments.

From the observed and modelled data of soil column experiment, several conclusions can be drawn. The mobility affinity of oestrogens in soils is strongly dependent to their K_{ow} values; a higher K_{ow} value of an oestrogen indicates a stronger binding onto soils. Retardation of oestrogens mobility in the soils is greatly dependent to the soil organic carbon/matter content. From soil column experiments in this study, the leaching potential for all oestrogens in all soils were low as indicated by high displacement volume used to obtain breakthrough. Thus, this study suggested that contamination of groundwater following wastewater treatment effluent reuse as irrigation water may not occur. Moreover, the oestrogen concentration used in this study were higher than the concentration previously reported in wastewater treatment effluents. With lower concentration of oestrogens in effluent, leaching from a soil profile are not expected in to occur. However, the retardation of oestrogens in soil may not as strong as indicated by the sorption coefficient (K_d) values from batch experiments as several additional transport processes are involved in a real environment. For example, advection-dispersion of the oestrogen compounds becomes important in the column geometry; this process is not measured in batch experiment. Other processes present in real environment may enhance the oestrogens mobility in the real soil profile and in addition, three dimensional effects may be important. Thus, their potential to contaminate the groundwater is still need to be further clarified.

In this study, the results showed that there is considerable difference between experimental values of K_{ow} and the values generated by EPISUITE, the computational predictive model. The EPISUITE program under its Physical-chemical Property Estimation Routine (PERs) underestimated the K_{ow} of oestrone (E1) but overestimated K_{ow} for 17α-ethnyloestradiol (EE2) and 17β-oestradiol (E2). The result also shows that the EPISUITE program underestimated oestrogens distribution in environmental compartments. This ambiguity occurs as the computational model does not take into account their sorption coefficient (K_d) into calculation for estimating the sorption coefficient normalised to organic carbon (K_{oc}), whereby it can be only obtained through experimentation. Thus, by inputting experimental values into EPISUITE, a more realistic model close to the environment under consideration has been created. The consequences of using an inaccurate estimation K_{ow} by EPISUITE to model environmental distribution (i.e without incorporating the experimental values) risks affecting the decision making for the safe use of wastew-

ater treatment effluent as irrigation water. Thus, this study suggest strongly shows that although the computational model is indicative, it does not accurately assess environmental impact of contaminants.

Model developed by Duarte-Davidson and Jones (1996) which is based solely on the physico-chemical parameter, K_{ow} allows the prediction of oestrogens that would be uptaken by root and leaves. In this study, all oestrogens are predicted to have medium potential to be uptaken either by plant root or leaves following several irrigation techniques involving wastewater treatment effluent. From bioconcentration factor calculated from the model developed by Kerler and Schonherr (1988) and Travis and Arms (1988), bioaccumulation in plant leaves is expected if the irrigation techniques result in direct contact between effluent and plant leaves and thus may be transferred to animals or humans. Meanwhile, in the case of direct contact between effluent and soils (i.e no contact with plant leaves), the calculated bioconcentration factor of plants is much lower. More oestrogens are predicted to remain bound to soil rather than plant leaves and bioaccumulation is not expected to occur. Regarding effluent application for forage irrigation, the uptake of oestrogens by the grazing animals is possible. For instance, dairy cows also consume soils as part of their daily intake. As these compounds tend to bind strongly onto soils, grazing animals risk exposure. Thus, this study strongly recommends further investigation and possibly reconsidering re-use of effluent as irrigation water for crops and forage. Because these compounds can bioaccumulate, the potential for exposure through vegetation and grazing animals may result in biomagnification in humans from meat or milk consumption. Infants are particularly vulnerable.

Finally, this study demonstrates the effectiveness of a relatively affordable analytical technique involving SPE-HPLC. Techniques using far more sensitive analytical instrument equipment GC-MS may give a better indication of the whole results and allow the exploration of lower concentrations. However, in this study, the analytical techniques proved to be repeatable and reliable. One of the disadvantages of GC-MS instrumentation is that it is very expensive to buy and maintain and requires highly skilled and experienced operators. Many labs may not have the budget to afford this type of equipment or may lack the specialist knowledge required to use it effectively. Cost effective analytical methods such as those demonstrated in this study are important and would allow more researchers to become involved in this field. These methods are especially important in developing countries where budgets and skills

are the main constraints.

6.2 Future work

Several oppurtunities can be delivered from this research study for future works:

- The effect of octanol/water mutual saturation on the partition coefficient of oestrogen is important to characterise. It is essential to study the effect of octanol/water mutual saturation on the partition coefficient of oestrogens. If the effect is negligable then the octanol-water partition coefficient (K_{ow}) of oestrogens may be set equal to the solubility ratio of oestrogen in octanol and water. Therefore, it would give a better indication of their K_{ow} values.

- The effect of pH on oestrogens needs to be explored in more detail. The partition coefficient between octanol and water has been shown in this study as an important physicochemical parameter for characterising the hydrophobicity of oestrogens. As pH has effected oestrogens sorption affinity onto soils due to the affected water solubility, thus it is essential to obtain oestrogens K_{ow} values as a function of pH.

- The degradation rates and mechanisms of oestrogen in the environment need to be investigated further. Degradation is another factor that has been suggested to influence fate and behaviour of oestrogens in environmental compartments. However, the degradation mechanisms involved and their degradation rates reported in the literature, particularly in the soil environment are conflicting. Thus, further study is essential.

- A pilot study to assess plant uptake of oestrogens following several irrigation techniques and irrigation frequency is of particular importance. This follow-on study is essential since such information would be directly applicable to field conditions in agriculture. It would provide very useful information to guide decision on effluent reuse for crop irrigation in a manner that protects animals and humans from receiving these contaminants as a result of vegetation consumption.

Bibliography

Adams, N., 1995. Detection of the effects of phytoestrogens on sheep and cattle. J.Anim.Sci. Vol 73, pp 1509–1515.

Aherne, G., Briggs, R., 1989. The relevance of the presence of certain synthetic steroids in the aquatic environment. Jounal of Pharmaceutics and Pharmacology. Vol 41, pp 735–736.

Andersen, H., Siegrist, H., Sorensen, B., Ternes, T., 2003. Fate of estrogens in a municipal sewage treatment plant. Environmental Science and Technology. Vol 37(18), pp 4021–4026.

Angelakis, A., Marecos Do Monte, M., Bontoux, L., Asano, T., 1999. The status of wastewater reuse practice in the mediterranean basin: Need for guidelines. Water Research. Vol 33 (10), pp 2201–2217.

Asano, T., 1987. Irrigation with reclaimed municpal wastewater. Geo. Journal. Vol 15 (3), pp 273–282.

Asano, T., Levine, A., 1996. Wastewater reclamation, recycling and reuse: Past, present and future. Water Science Technology. Vol 33 (10-11), pp 1–14.

ASTM, 2000. Standard test methods for moisture, ash and organic matter of peat and other organic soils.

ASTM, 2001. Standard test method for determining a sorption constant (koc) for an organic chemical in soil and sediment.

Atkinson, S., Atkinso, M., Tarrant, A., 2003. Estrogens from sewage in coastal marine environments. Environmental Health Perspective. Vol 111, pp 531–535.

Bajpai, A., Vishwakarma, N., 2003. Adsorption of polyviylalcohol onto fuller's earth surfaces. Colloid Surface A. Vol 220, pp 117–130.

Baker, S., 2009. Phthalates danger: Chemicals in plastics put unborn babies at risk.Http://www.naturalnews.com.

Banerjee, S., Yalkowsky, S., Valvani, S., 1980. Water solublity and octanol/water partition coefficient of organics. limitation of the solubility partition coefficient correlation. Environmental Science and Technology. Vol 14, pp 1227–1229.

Barel-Cohen, K., Shore, L., Shemesh, M., Wenzel, A., Mueller, J., Kronfeld-Schor, N., 2006. Monitoring of natural and synthetic hormones in a polluted rivers. Journal of Environmenal Management. Vol 78 (1), pp 16–23.

Baronti, C., Curini, R., D'Ascenzo, G., Di Corcia, A., Gentili, A., Samperi, R., 2000. Monitoring natural and synthetic estrgens at activated sludge sewage treatment plants and in a receiving water. Environmental Science and Technology. Vol 34 (24), pp 5059–5066.

Beck, J., Totsche, K., Kogel-Knaber, I., 2008. A rapid and efficient determination of natural oestrogens in soils by pressurised liquid extraction and gas chromatography mass spectrometry. Chemosphere. Vol 71(5), pp 954–960.

Belfroid, A., Van der Horst, A., Vethaak, A., Schafer, A., Rijs, G., Wegener, J., Cofino, W., 1999. Analysis and occurrence of estrogenic hormones and their glucuronides in surface water and wastewater in the netherlands. Science Total Environment. Vol 225, pp 101–108.

Belgiorno, V., Rizzo, L., Fatta, D., Rocca, C., Lofrano, G., Nikolaou, A., Naddeo, V., Meric, S., 2007. Review on endocrine disrupting emerging compounds in urban wastewater:occurrence and removal by photocatalysis and ultrasonic irriradiation for wastewater reuse. Desalination. Vol 215, pp 166–176.

Bolong, N., Ismail, A., Salim, M., Matsuura, T., 2009. A review of the effects of emerging contaminants in wastewater and option of their removal. Desalination. Vol 239, pp 229–246.

Bolt, H., 1979. Metabolism of estrogens-natural and synthetic. Pharm. Ther. Vol 4, pp 155–181, editor Chadhury, R.R.

Bonin, J., Simpson, M., 2007. Sorption of steroid estrogens to soil and soil constituents in single and multi sorbate systems. Environmental Toxicology and Chemistry. Vol 26(12), pp 2604–2610.

Bowman, J., Zhou, J., Readman, J., 2002. Sediment-water interactions of natural oestrogens under estuarine conditions. Marine Chemistry. Vol 77, pp 263–276.

Braga, O., Smythe, G., Schafer, A., Feitz, A., 2005. Steroid estrogens in ocean sediments. Chemosphere. Vol 61, pp 827–833.

Brisken, C., 2008. Endocrine disruptors and breast cancer. CHIMIA International Journal for Chemistry. Vol 62, pp 406–409.

BS, 1990. Methods of test for soils for civil egineering purposes-part 1: General requirements and sample preparation.BS 1377-1990.

BS, 1994. Classification for topsoil.BS 3882:1994.

Callantine, M., Stob, M., Andrews, F., 1961. Fecal elimination of estrogens by cattle treated with diethylstilbestrol and hexestrol. Am.J.Vet.Res. Vol 22, pp 462–465.

Casey, F., Larsen, G., Hakk, H., Simunek, J., 2003. Fate and transport of 17 b-estradiol in soil-water system. Environmental Science and Technology. Vol 37, pp 2400–2409.

Casey, F., Simunek, J., Lee, J., Larsen, G., Hakk, H., 2005. Sorption, mobility, and transformation of estrogenic hormones in natural soil. Journal of Environmental Quality. Vol 34(4), pp 1372–1379.

Casini, M., Sandro, G., Vittorio, U., 2006. An infertile couple suffering from oligospermia by partial sperm maturation arrest:can phytoestrogens play a therapeutic role? a case report study. Gynecol Endocrinol. Vol 22 (7), pp 399–401.

CGER, 1996. Use of reclaimed water and sludge in food crop production. The National Academic Press.

Chaney, R., 1985. Potential effects of sludge-borne heavy metals and toxic organics in soils, plants and anmals, and related regulatory guidelines. in: Final report of the workshop on the international transportation, utilization or disposal of sewage

sludge including recommendations. Tech. rep., Pan American Health Organisation, Washington, DC. Annex 3, Workshop Paper 9, pp.1-56.

Chiou, C., Malcolm, R., Brinton, T., Klle, D., 1986. Water solubility enhancement of some organic pollutants and pesticides by dissolved humic and fulvic acids. Environmental Science and Technology. Vol 20, pp 502–508.

Coleman, H., Chiang, K., Amal, R., 2005. Effects of ag and pt on photocatalytic degradation on endocrine disrupting chemicals in water. Chemical Engineering Journal Chromatography. Vol 113, pp 65–72.

Coleman, H., Eggins, B., Anthony Byrne, J., Palmer, F., King, E., 2000. Photocatalytic degradation of 17b-oestradiol on immobilised titanium oxide. Applied Catalysis B : Environmental. Vol 24, pp 1–5.

Colucci, M., Bork, H., Topp, E., 2001. Persistence of estrogenic hormones in agricultural soils: I. 17b-oestradiol and estrone. J.Environ.Qual. Vol 30, pp 2070–2076.

Colucci, M., Topp, E., 2001. Persistence of estrogenic hormones in agricultural soils:ii.17a-ethnyloestradiol. J.Environ.Qual. Vol 30, pp 2077–2080.

Cronin, M., Livingstone, D., 2004. Predicting Chemical Toxicity and Fate. CRC Press, iSBN 0-415-27180-0.

Darbre, P., 2006. Environmental oestrogens, cosmetics and breast cancer. Best Practice & Research Clinical Endocrinology & Metabolism. Vol 20 (1), pp 121–143.

D'Ascenzo, G., Di Corcia, A., Gentili, A., Mancii, R., Mastropasqua, R., M., N., Samperi, R., 2003. Fate of natural estrogen conjugates in municipal sewage transport and treatment facilities. The Science of The Total Environment. Vol 302 (1-3), pp 199–209.

DeKleijn, M., Van Der Schouw, Y., Wilson, P., Grobbee, D., Jacques, P., 2002. Dietry intake of phytoestrogens is associated with a favorable metabolic cardiovascular risk profile in post menopausal u s women: The framingham study. Journal Nutrition. Vol 132 (2), pp 276–282.

Derelanko, M., Hollinger, M. (Eds.), 2001. Handbook of Toxicology-Mechanism of action of endocrine disrupters. CRC Press.

Desaulniers, D., Goff, A., Betteridge, K.J. ad Rowell, J., Flood, P., 1989. Can.J.Zool. Vol 67, pp 1148–1154, (As cited by Shore, L., and Shemesh, M., 2003).

Desbrow, C., Routledge, E., Brighty, G., Sumpter, J., Waldock, M., 1998. Identifiication of estrogenic chemicals in s t w effluent.1. chemical fractionatin and in vitro biological screening. Environmental Science and Technology. Vol 32 (11), pp 1549–1558.

Dilling, W., 1977. Interphase transfer processes ii evaporation rates of chloromethanes, ethanes, ethylenes, propanes, and propylenes from dilute aqueous solutions. comparisons with theoretical predictions. Environmental Science and Technology. Vol 11, pp 405–409.

Duarte-Davidson, R., Jones, K., 1996. Screening the environmental fate of organic contaminants in sewage sludge applied to agricultural soils. ii . the potential for transfers to plants and grazing animals. The Science of the Total Environment. Vol 185, pp 59–70.

EA, 1997. The identification and assessment of oestrogenic substances in sewage treatment works effluents. R&D Publication 7Environmental Agency.

EA, 2010. Urban wastewater treatment directive.Environmental Agency. http://www.epa.gov.

EC, 1997European Commission. http://www.epa.gov.

EEA, 2009. Water resources across europe-confronting water scarcity and drought. Tech. rep., European Environment Agency.

EJF, 2002. End of the road for endosulfan: A call for action against a dangerous pesticide. Tech. rep., Environmental Justice Foundation, London, UK.

Fatta, D., Kythreotou, N., 2005. Wastewater as valuable water resource concerns, constraints, and requirements related to reclamation, recycling and reuse. In: IWA International Conference on Water Economics, Statistics, and Finance, Greece.

Fiess, M., Heistermann, M., Hodges, J., 1999. Gen.Comp.Endo. Vo 115, pp 76–89., as cited in Shore, L.S. and Shemesh, M. (2003).

Finlay Moore, O., Hartel, P., Cabrera, M., 2000. 17b-oestradiol and testosterone in soils and runoff from grasslands amended with broiler litter. Journal Environmental Quality. Vol 29, pp 1604–1611.

FSA, 2002. Report of the working group on phyto-oestrogens. Tech. rep., http://www.food.gov.uk.

Geyer, H., Scheunert, I., Korte, F., 1987. Correlation between the bioconcentration potential of organic environmental chemicals in humans and their n-octanol/water coefficients. Chemosphere. Vol 16, pp 239–252.

Grace, P., Taylor, J., Low, Y., 2004. Phytoestrogen concentrations in serum and spot urine as biomakers for dietry phytoestrogens intake and their relation to breast cancer risk in european perspective investigation of cancer and nutrition-norfolk. Cancer Epidemiology Biomakers Prevention. Vol 13, pp 698–708.

Hacker, R., Crue, D., Shimoda, W., Hopwood, M., 1967. Uptake of diethylstilbestrol by edible plants. J. Anim.Sci. Vol 26, pp 1358–1362.

Hanselman, T., Graetz, D., Wilkie, A., 2003. Manure-borne estrogens as potential environmental contaminants: A review. Enviromental Science and Technology. Vol 37 (24), pp 5471–578.

Herman, C., Aldercreutz, T., Goldin, B., Gorbach, S.L.and Hockerstedt, K., Watanabe, S., Hamalainen, E., Markkanen, M., Makela, T., Wahala, K., Hase, T., Fotsis, T., 1995. Soybean phytoestrogen intake and cancer risk. Journal of Nutrition. Vol 125 (7), pp 1960.

Hildebrand, C., Londry, K. L., Farenhost, A., 2006. Soprtion and desorption of three endocrine disrupters in soils. Journal of Environmental Science and Health Part B. Vol 41, pp 907–921.

Hoffmann, B., Evers, P., 1986. Drug Residue in Animals. Academic Press, New York.

Holthaus, K., Johnson, A., Jurgens, M., Williams, R., Smith, J., Carter, J., 2002. The potential for estradiol and ethnylestradiol to sorb to suspended and bed sediments in some english rivers. Environmental Toxicology and Chemistry. Vol 21 (12), pp 2526–2535.

Horvath, B., Opara-Nadi, O., Beese, F., 2005. A simple methd for measuring the carbonate content of soils. Journal of Soil Sci. Soc. Am. Vol 69, pp 1066–1068.

Hurwitz, A. R., Liu, S., 1977. Determination of aqueous solubility and p k a values of estrogens. J.Pharm.Sci. Vol 66, pp 624–627.

Isobe, T., Serizawa, S., Horiguchi, T., Shibata, Y., Managaki, S., Takada, H, M. M., Shiraishi, H., 2006. Horizontal distribution of steroid estrogens in surface sediments in tokyo bay. Environmental Pollution. Vol 144 (2), pp 632–638.

IWMI, 2006. Recycling realities: Managing health risks to make wastewater an asset. Water Policy Briefing Series. Vol 17, pp 1–6.

Jobling, S., Reynolds, T., White, R., 1995. A variety of environmentally persistent chemicals, including some phtalate plasticisers, are weakly oestrogenic. Environmental Health Perspective. Vol 103, pp 582–587.

Johnson, A., Belfroid, A., Di Corcia, A., 2000. Estimating steroid estrogens input into activated sludge treatment works and observations on their removal from the effluent. Science Total Environment. Vol 256, pp 163.

Johnson, A., Sumpter, J., 2001. Removal of endocrine disrupting chemicals in activated sludge treatment works. Environmental Science and Technology. Vol 35 (24), pp 4697–4703.

Johnson, A., Van Tienhoven, A., 1981. Poultry Science. Vol 60, pp 2720–2723., as cited in Shore, L.S and Shemesh, M (2003).

Jurgens, M., Williams, R., Johnson, A., 1999. Fate and behaviour of steroid oestrogens in rivers. Tech. rep., Environmental Agency R&D Technical Report P161.

Kah, M., Brown, C., 2008. Log d : Lipophilicity for ionisable compounds. Chemosphere. Vol 72, pp 1401–1408.

Kang, J., Kondo, F., 2005. Bisphenol a degradation in seawater is different from that in river water. Chemosphere. Vol 60, pp 1288–1292.

Karickhoff, S., Brown, D., Scott, T., 1979. Sorption of hydrophobic pollutant of natural sediments. Water Research. Vol 13, pp 241–248.

Keenan, H., 2000. The development of a s p e- h p l c method to analysis to assess the environmental fate of natural and synthetic oestrogens. Ph.D. thesis, University of Stratchclyde.

Keenan, H., Sakultantimetha, A., Bangkedphol, S., 2008. Environmental fate and partition coefficient of oestrogenic compounds in sewage treatment process. Environmental Research. Vol 106, pp 313–318.

Kenaga, E., 1980. Correlations of bioconcentration factors of chemical in aquatic and terrestrial organisms with their physical and chemical properties. Environmental Science and Technology, Vol 14, pp 553–556.

Kerler, F., Schonherr, J., 1988. Accumulation of lipophilic chemicals in plant cuticles:prediction from 1-octanol/water partition coefficient. Arch.Environ.Contamination Toxicol. Vol 17, pp 1–6.

Khanal, S., Xie, B., Thompson, M., Sung, S., Ong, S., van Leeuwen, J., 2006. Fate, transport, and biodegradation of natural estrogens in the environment and engineered systems. Environmental Science and Technology. Vol 40 (21), pp 653–6546.

Kjaer, J., Olsen, P., Bach, K., Barlebo, H., Ingerslev, F., Hansen, M., Sorensen, B., 2007. Leaching of estrogenic hormones from manure-treated structured soils. Environmental Science and Technology. Vol 41, pp 3911–3917.

Korner, W., Bolz, U., Sussmuth, W., Hiller, G., Schuller, W., Hanf, V., Hagenmaier, H., 2000. Input/output balance of estrogenic active compounds in a major municipal sewage plant in germany. Chemosphere. Vol 40 (9-11), pp 1131–1142.

Kuch, H., Ballschiter, K., 2001. Determination of endocrine disrupting phenolic compounds and estrogens in surface and drinking water by hrgc- (nci) - ms in the picogram per liter range. Environmenal Science and Technology. Vol 35, pp 3201–3206.

Kuster, M., Lopez de Alda, M., Barcelo, D., 2004. Analysis and distribution of estrogens and progestogens in sewage sludge, sols, and sediments. TrAC Trends in Analytical Chemistry. Vol 23 (10-11), pp 790–798.

Labadie, P., Cundy, A., Stone, K., Andrews, M., Valbonesi, S., Hill, E., 2007. Evidence for the migration of steroidal estrogens through river bed sediments. Environmental Science and Technology. Vol 41 (12), pp 4299–4304.

Lagana, A., Bacaloni, A., DeLeva, I., Faberi, A., Fago, G., Marino, A., 2004. Analytical methodologies for determining the occurrence of endocrine disrupting chemicals in sewage treatment plants and natural waters. Analytica Chimica Acta. Vol 501, pp 79–88.

Lai, K., Johnson, K., Scrimshaw, M., Lester, J., 2000. Binding of waterborne steroid estrogens to solid phase river and estuarine systems. Environmental Science and Technology Vol 34, pp 3890–3894.

Larsen, G., Casey, B., Magelky, C., Pfaff, H. H., 2001. Sorption, mobiity and fate of 17b-oestradiol and testosterone in loam soil and sand.

Lazaridis, N., Keenan, H., 2010. Chitosan beads as barriers to the transport of azo dye in soil column. Journal of Hazardous Materials. Vol 173, pp 144–150.

Lee, L., Strock, T., Sarmah, A., Rao, P., 2003. Sorption and dissipation of testosterone, estrogens, and their primary transformation products in soils and sediment. Environmental Science and Technology. Vol 37 (18), pp 4098–4105.

Li, Z., Yu, H., Wei, D., Wang, G., Feng, J., Wang, L., 2002. Prediction of mixture toxicity with its total hydrophobicity. Chemosphere. Vol 36, pp 305–310.

Mackay, D., 2001. Multimedia Enviromental Models-The Fugacity Approach. CRC Press.

Mackay, D., Paterson, S., 1981. Calculating fugacity. Environmental Science and Technology. Vol 15, pp 1006–1014.

Malygina, T., Preis, S., Kallas, J., 2005. The role of p h in aqueous photocatalytic oxidation of b-oestradiol. International Journal of Photoenergy. Vol 07, pp 187–191.

Matsui, S., Takigami, H., Matsuda, T., Taniuchi, N., Adachi, J., Kawami, H., Shimizu, Y., 2000. Estrogen and estrogen mimics contamination in water and the role of sewage treatment. Water Science Technology. Vol 42 (12), pp 173–179.

Matsumura, A., Chosh, A., Pope, G., Darbre, P., 2005. Comparative study of oestrogenic properties of eight phytoestrogens in m c f 7 human breast cancer cells. The Journal of Steroid Biochemistry and Molecular Biology. Vol 94, pp 431–443.

Matsuoka, S., Sakakura, R., Takiishi, M., Kurokawa, Y., Kawai, A., Miyazaki, N., 2005. Determination of natural estrogens in the sediment of coastal area in japan. Coastal Marine Science 29(2), 141–146.

Metcalf, Eddy, 2003. Wastewater Engineering, Treatment and Reuse, 4th Edition. Mc Graw-Hill, New York.

Murkies, A., Wilcox, G., Davis, S., 1998. Phytoestrogens. Journal of Clinical Endocrinology and Metabolism. Vol 83 (2), pp 297–303.

Nasu, M., Goto, M., Kato, H., Oshima, Y., Tanaka, H., 2001. Study on endocrine disrupting chemiclas in wastewater treatment plants. Water Science and Technology. Vol 43, pp 101–108.

Neely, W., 1982. Review: Organising data for environmental studies. Environ.Toxicol.Chem. Vol 1, pp 259–266.

Nghiem, L., Schafer, A., Elimelech, M., 2004. Removal of natural hormones by nanofiltration membranes: Measurement, modeling, and mechanisms. Environmental Science and Technology. Vol 38, pp 1888–1896.

Nichols, D., Daniel, T., Moore, P., Edwards, D., Pote, D., 1997. Runoff of estrogen hormone 17b-oestradiol from poultry litter applied to pasture. Journal Environmental Quality. Vol 26, pp 1002–1006.

Noppe, H., Verslycke, T., DeWulf, E., Verheyden, K., Monteyne, E., Van Caeter, P., Janssen, C., DeBrabander, H., 2007. Occurrence of estrogens in the scheldt estuary: A 2 year survey. Ecotoxicology and Enviromental Safety. Vol 66 (1), pp 1–8.

OECD, 2004. Guidelines for the testing chemicals.

Orme, M.L.E.and Back, D., Breckenbridge, A., 1983. Transformation of a non-oestrogenic steroid metabolite to an oestrogenically active substance by minimal bacteria activity. Chemosphere. Vol 38, pp 3579–3596.

Palme, R., Fischer, P., Schildorfer, H., Ismail, M., 1996. Excretion of infused 14 c-steroid hormones via faeces and urine in domestic livestock. Anim.Reprod.Sci. Vol 43, pp 43–63.

Peterson, F., Davis, R., Orndorff, H., 2000. 17b-oestradiol as an indicator of animal waste contamination in mantled karst aquifers. Journal of Environmental Quality. Vol 29, pp 826–834.

Pettygrove, G., 2004. Irrigation forage with recycled water: problems and possibilities. In: National Alfalfa Symposium, 13-15 December 2004, San Diego, CA.

Pinsuwan, S., Li, A., Yalkowsky, S., 1995. Correlation of octanol/water solubility ratios and partition coefficients. J.Chem.Eng Data. Vol 40, pp 623–626.

Popp, J., McAllister, T., Burgevitz, W., Kemp, R., Kastelic, J., Cheng, K., 1997. Effect of trenbolene acetate estradiol implants and estrus supression on growth performance and carcass characteristics of beef heifers. Canadian J.Anim.Science. Vol 77, pp 325–328.

Purdom, C., Hardiman, P., Bye, V., Eno, N., Tyler, C., Sumpter, J., 1994. Estrogenic effects of effluents from sewage treatment works. Journal of Chemical Ecology. Vol 8, pp 275–285.

Ran, Y., He, Y., Yang, G., Johnson, J., Yalowsky, S., 2002. Estimation of aqueous solubility of organic compounds by general solubility equation. Chemosphere. Vol 48, pp 487–509.

Rinker, E., Oeschlager, D., Colussi, A., Henry, K., Sandall, O., 1994. Viscocity, density and surface tension of binary micxtures of water and n-methyldiethanolamine and water and diethanolamine and tertiaty mixtures of these amines with water over the temperature range 20-100 c. J. Cem.Eng.Data. Vol 39, pp 392–395.

Robinson, B., Hellou, J., 2009. Biodegradation of endocrine disrupting compounds in harbour seawater and sediments. Science of the Total Environment. Vol 407, pp 5713–5718.

Routledge, E., Sheahan, D., Desbrow, C., Brighty, G., Waldock, M. Sumpter, J., 1998. Identification of estrogenic chemicals in s t w effuents. 2. in vivo responses in trout and roach. Environ.Sci.Technol. Vol 32, pp 1559–1565.

Samiullah, Y., 1990. Prediction of the environmental of chemicals. Elsevier Applied Science, London and New York. ISBN 1851664505.

Sangsupan, H., Radcliffe, D., Hartel, P., Jenkins, M., Vencill, W., Cabrera, M., 2006. Sorption and transport of 17b-oestradiol and testosterone in undisturbed soil columns. Journal of Environmental Quality. Vol 35 (6), pp 2261–2272.

Schafer, A., Nghiem, L., Waite, T., 2003. Removal of natural hormone estrone from aqueous solutins using nanofiltration and reverse osmosis. Environmental Science and Technology. Vol 37, pp 182–188.

Schwarzenbach, R., Gschwend, P., Imboden, D., 2003. Environmental Organic Chemistry (Second Edition). John Wiley & Sons, Inc, Hoboken New Jerse.

Shareef, A., Angove, M., Wells, J., Johnson, B., 2006a. Aqueous solubilities of estrone, 17b-estradiol, 17a-ethnylestradiol and bisphenol a. Journal of Chemical and Engineering Data. Vol 51(3), pp 879–881.

Shareef, A., Angove, M., Wells, J., Johnson, B., 2006b. Sorption of bisphenol a, 17a-ethnyloestradiol and estrone to mineral surfaces. J.Colloid and Interface Science. Vol 297, pp 62–69.

Shore, L., Correll, D., Chakraborty, P., 1995. Relationship of fertlization with chick manure and concentrations of oestrogens in small streams -Animal Waste and the Land-Water Interface. Lewis Publisher, Boca Raton, FL., (As cited by Hanselman, T.A. et al., 2003).

Shore, L., Shemesh, M., 2003. Naturally produced steroid hormones and their release into environmental. Pure Applied Chemistry. Vol 75, pp 1859–1871.

Simunek, J., Sejna, M., Saito, H., Sakai, M., van Genuchten, M., 2008. The hydrus -1 d software package for simulating the one-dimensional movement of water, heat and multiple solutes in variably-saturated media, us salinity laboratory, u s d a - a r s. riverside, c a.

Stillman, R., 1982. In utero exposure to diethylstilbestrol: Adverse effects on the reproductive tract and reproductive performance in male and female offspring. America Journal of Obstetric and Gynecology. Vol 142, pp 905–921.

Stumpe, B., Marschner, B., 2007. Long term sewage sludge application and wastewater irrigation on the mineralization and sorption of 17b-oestradiol and testosterone in soils. Science of the Total Environment. Vol 374 (2-3), pp 282–291.

Suri, R., Fu, H., Chimcirian, R., 2006. Ultrasound indiced destruction of estrogen hormones in aqueous systems. In: 1st European Conference on Environmental Applications of Advanced Oxidation Processes. p. 243, (As cited by Belgiorno et al., 2007).

Svenson, A., Allard, A., Ek, M., 2003. Removal of estrogenicity in swedish municipal sewage treatment plants. Water Research. Vol 37, pp 4433–4443.

Swartz, C., Reddy, S., Benotti, M., Yin, H., Barber, L., Brownawell, B., Rudel, R., 2006. Steroid estrogens, nonylphenol ethoxylates metabolites, and other wastewater contaminants in groundwater affected by a residential septic on cape cod, m a. Environmental Science and Technology. Vol 40, pp 4894–4902.

Synder, S., Keith, T., Verbrugge, D., Synder, E., Gross, T., Kannan, K., Giesy, J., 1999. Analytical method for detection of selected estrogenic compounds in aqueous mixtures. Environmental Science and Technology. Vol 33, pp 2814–2820.

Ternes, T., Kreckel, P., Mueller, J., 1999a. Behaviour and occurrence of estrogens in municipals sewage treament plants. ii. aerobic batch experiment with activated sludge. The Science of the Total Environment 225, 91–99.

Ternes, T., Stumpf, M., Mueller, J., Haberer, K., Wilken, R., Servos, M., 1999b. Behaviour and occurrence of estrogens in municipal sewage treatment plants.i. investigation in german, canada, and brazil. The Science of the Total Environment. Vol 225, pp 80–90.

Teske, S., Arnold, R., 2008. Removal of natural and xeno-estrogens during conventional wastewater treatment. Rev. Environ. Sci. Biotechnol. Vol 7, pp 107–124.

Thorpe, K., Hutchinson, T., Hetheridge, M., Scholze, M., Sumpter, J., Tyler, C., 2001. Assessing the biological potency of binary mixtures of environmental estrogens using vitellogenin induction in juvenile rainbow trout (oncorhynchus mykiss). Environmental Science and Technology. Vol 35 (12), pp 2476–2481.

Topp, E., Scheunert, A., Forte, F., 1986. Factors affecting the uptake of 14- c labelled organic chemicals by plants from soil. Ecotoxicology and Environmental Safety. Vol 11, pp 219–228.

Travis, C., Arms, A., 1988. Bioconcentration of organics in beef, milk and vegetation. Environmenal Science and Technology. Vol 22, pp 271–274.

Turfitt, G., 1947. Microbiological agencies in the degradation of steroids.2. steroid utilisation by the microflora of soils. J.Bacteriol. Vol 54, pp 557–562, (As cited by Ullman, J.L., 2006).

Ullman, J., 2006. The chemical bahavior of estrone and 17 b-estradiol in the environment. Ph.D. thesis, Texas A &M University.

USEPA, 1992. Guidelines for water reuse: Manual. Tech. rep., U.S.EPA and U.S. Agency for Int Development.EPA/625/R-92/004.

USEPA, 2002. Reregistration eligibility decision for endosulfan. Tech. rep., United States Environmental Protection Agency.

USEPA, 2004. Primer for municipal wastewater treatment systems. Tech. Rep. EPA 832-R-04-001, United States Environmental Protection Agency (USEPA).

Veith, G., Austin, N., Morris, R., 1979. A rapid method for estimating log p for organic chemicals. Water Research. Vol 13, pp 43–47.

Vigneswaran, S., Sundaravadivel, M., 2004. Wastewater Recycle, Reuse, and Reclamation. UNESCO, Eolss , Oxford, UK.

Voutsas, E., Magoulas, K., Tassios, D., 2002. Prediction of the bioaccumulation of persistent organic pollutants in aquatic food webs. Chemosphere. Vol 48, pp 645–651.

WEM, 2007. Water reuse. World Environmental Magazine.

Wild, S., Jones, K., 1992. Organic chemicals entering agricultural soils in sewage sudges: Screening for their potential to transfer to crop plants and livestock. The Science of the Total Environment. Vol 119, pp 85–119.

Williams, R., Johnson, A., Smith, J., Kanda, R., 2003. Steroid estrogens profiles along river stretches arising from sewage treatment works discharges. Environmental Science and Technology. Vol 37, pp 1744–1750.

Wilson, S., Duarte-Davidson, R., Jones, K., 1996. Screening the environmental fate of organic contaminants in sewage sludges applied to agricultural soils: 1. the potential for downward movement to groundwaters. The Science of the Total Environment. Vol 185, pp 45–47.

Wolf, D., Dao, T., Scott, H., Lavy, T., 1989. Influenze of sterilization methods on selected oil micrbiological, physical, and chemical properties. J.Environ.Qual. Vol 18, pp 39–44.

Xuan, R., Blassengle, A., Wang, Q., 2008. Degradation of estrogenic hormones in a silt loam soil. Journal of Agricultural and Food Chemistry. Vol 56 (19), pp 9152–9158.

Yalkowsky, S., Valvani, S., 1979. J.Chem.Eng.Data. 24, 127–129, (As cited by Pinsuwan et al., 1995).

Yamamoto, H., Liljestrand, H., 2004. Partitioning of selected estrogenic compounds between synthetic membrane vesicles and water: Effects of lipid components. Enviromental Science and Technology. Vol 38, pp 1139–1147.

Yamamoto, H., Liljestrand, H., Shimizu, Y., Morita, M., 2003. Effects of ohysical chemical characteristics on the sorption of selected endocrine disruptors by dissolved organic matter surrogates. Environmenat Science and Technology. Vol 37, pp 2646–2657.

Ying, G., Kookana, R., 2003. Degradation of five selected endocrine disrupting chemicals in seawater and marine sediment. Environmental Science and Technology. Vol 37, pp 1256–1260.

Ying, G., Kookana, R., Dillon, P., 2003. Sorption and degradation of selected five endocrine disrupting chemicals in aquifer material. Water Research. Vol 37, pp 3785–3791.

You, S., Yin, Y., Allen, H., 1999. Partitioning of organic matter in soils: Effects of ph and water-soil ratio. The Science of the Total Environment. Vol 227, pp 155–160.

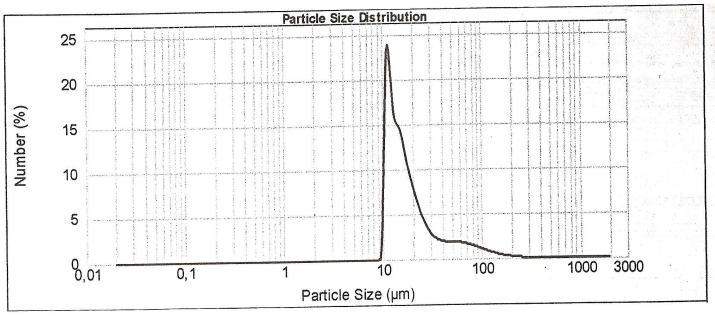
Young, W.F.and Whitehouse, P., Johnson, I., Sorokin, N., 2004. Proposed predicted no effect concentrations (pnecs) for natural and synthetic steroid oestrogens in surface waters. Tech. rep., Environment Agency.

Yu, Z., Xiao, B., Peng, P., 2004. Sorption of estrogens to soils and sediments. Environmental Toxicology and Chemistry. Vol 23 (3), pp 531–539.

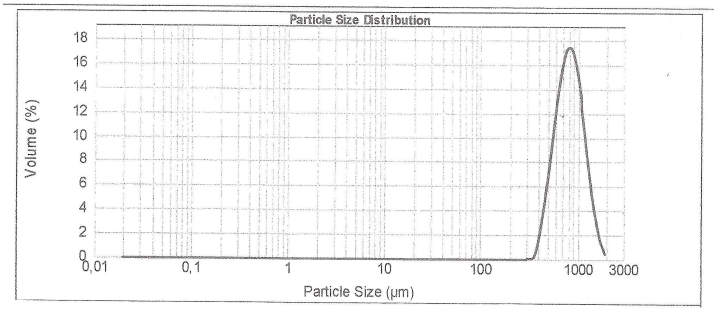
Zuo, Y., Zhang, K., Deng, Y., 2006. Occurrence and photochemical degradation of 17a-ethinylestradiol in acushnet river estuary. Chemosphere. Vol 63, pp 1583–1590.

Appendix

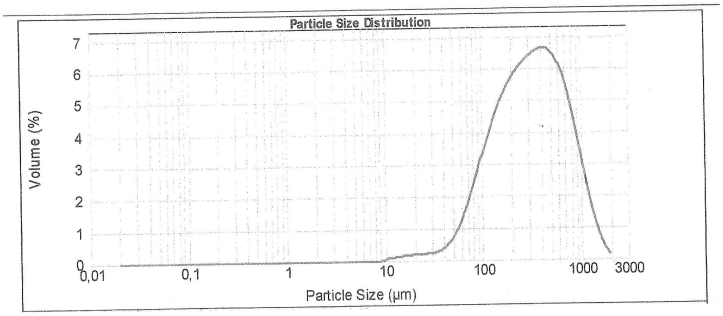
(a)



(b)



(c)



(d)

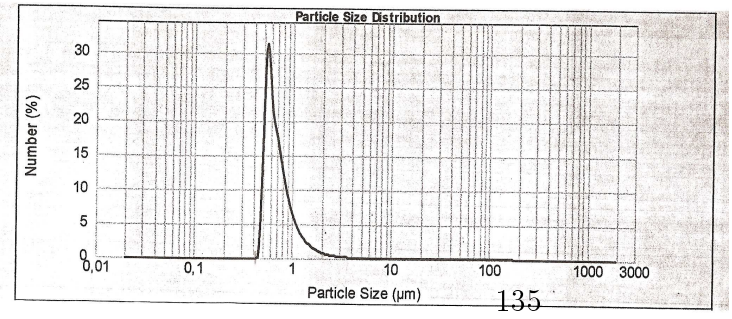


Figure 6.1: Particle size analysis diagram (a) silt (b) sand (c) Humus (d) clay

Table 6.1: Calculation of log $K_{ow}$by indirect method RP-HPLC

	rt				log Kow	log rt					log Kow	capacity factor, k				log k				
	1	2	3	mean		1	2	3	mean	sd		1	2	3 mean		1	2	3	mean	sd
Benzoic Acid	3.23	3.2	3.22	3.216667	1.9	0.50920252	0.50515	0.507856	0.507406	0.001685	1.9	1.182432	1.162162	1.175676	1.173423	0.072776	0.065267	0.070288	0.069455	0.003123
napthalene	9.27	9.3	9.18	9.25	3.6	0.96707973	0.968483	0.962843	0.966142	0.002398	3.6	5.263514	5.283784	5.202703	5.25	0.721276	0.722945	0.716229	0.720159	0.002855
Anthracene	18.5	18.5	18.37	18.45667	4.35	1.26717173	1.267172	1.264109	1.266153	0.001444	4.35	11.5	11.5	11.41216	11.47072	1.060698	1.060698	1.057368	1.059591	0.00157
fluoranthene	22.5	22.5	22.23	22.41	5.1	1.35218252	1.352183	1.346939	1.350442	0.002472	5.1	14.2027	14.2027	14.02027	14.14189	1.152371	1.152371	1.146756	1.150508	0.002647
crysene	33.97	33.97	33.57	33.83667	5.52	1.53109555	1.531096	1.525951	1.529388	0.002425	5.52	21.9527	21.9527	21.68243	21.86261	1.341488	1.341488	1.336108	1.339702	0.002536
benzo(a)pyrene	49.43	49.7	48.9	49.34333	6.11	1.69399061	1.696356	1.689309	1.693228	0.002928	6.11	32.39865	32.58108	32.04054	32.34009	1.510527	1.512965	1.5057	1.509741	0.003019

capacity factor, k = (tr - to)to
where;
tr = retentian time of substance
to = dead time

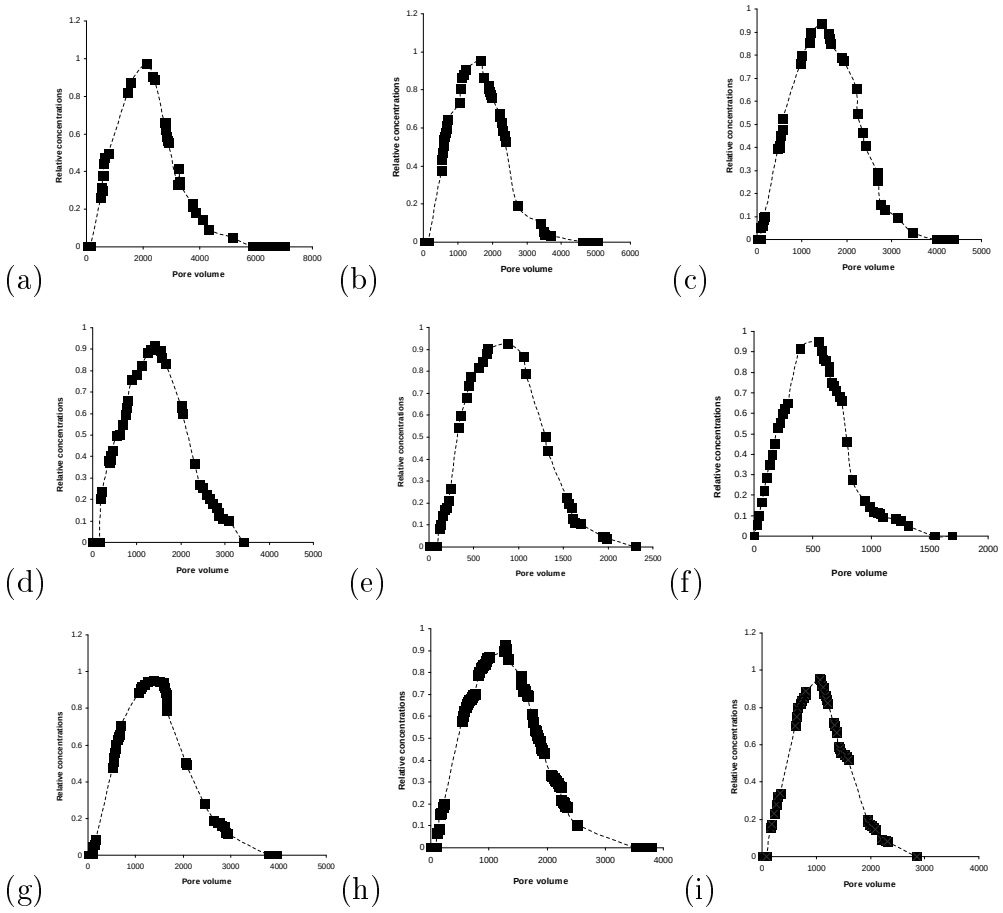
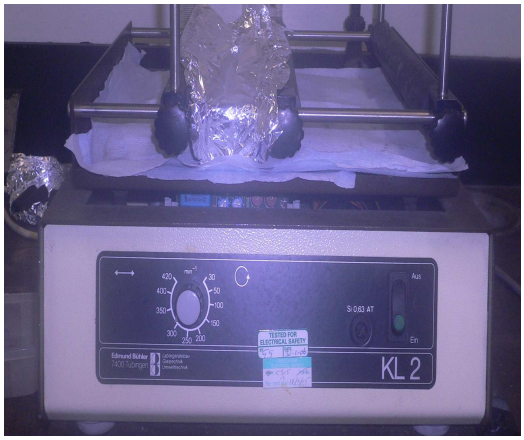


Figure 6.2: Breakthrough curve (BTC) of oestrogens in soils; (a) oestrone (E1), soil I (b) oestrone (E1), soil II (c) oestrone (E1), soil III (d) 17β-oestradiol (E2), soil I (e) 17β-oestradiol (E2), soil II (f) 17β-oestradiol (E2), soil III (g) 17α-ethnyloestradiol (EE2), soil I (h) 17α-ethnyloestradiol (EE2), soil II (i) 17α-ethnyloestradiol (EE2), soil III



(a)

(b)

(c)

Figure 6.3: The main equipment used in this study (a) mechanical shaker (b) centrifuge (c) HPLC