Behaviour and Health Risk Assessment of Endocrine Disrupting Chemicals from Wastewater

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Abstract

Water supply has become a social and economic issue in many countries as a result of global climate change, fast population growth, industrial and urban development. To address this issue, water recycling has been considered as a feasible technology to supplement the existing water supply. However, a major challenge with water recycling is the removal of harmful contaminants to meet drinking water guidelines and industrial requirements. Although various technologies can remove most contaminants efficiently, recent studies have shown that many endocrine disrupting chemicals (EDCs) can cause adverse health effects on wildlife species and humans at extremely low level.

EDCs from wastewater treatment effluent are the major point source entering the aquatic environment. Consequently, various adverse health effects have been observed in wildlife species, such as population changes, reproductive abnormalities, imbalanced sex ratios and behaviour changes. Many adverse human health effects such as prostate cancer, breast cancer and birth defects have also been implicated with the exposure to EDCs. Thus, it is important to study these environmental contaminants. The main aim of this work was to develop an understanding of the behaviour and health risks of EDCs from wastewater. This work focused on four estrogens, estrone (E1), 17β-estradiol (E2), estriol (E3), and 17α-ethinylestradiol (EE2) and three phenolic compounds, nonylphenol (NP), octylphenol (OP) and bisphenol A (BPA).

The behaviour of a chemical in the environment is largely dependent on its physicochemical properties such as aqueous solubility (S) and octanol-water partition coefficient (K_{ow}). Physicochemical properties, however, are related to chemical

structures. A quantitative structure-property relationship (QSPR) evaluation was conducted by using measured physicochemical properties and calculated molecular descriptors. With single and multiple linear regression methods, good linear relationships were found between the measured log K_{ow} values and three molecular descriptors: log FOSA (hydrophobic component of the total solvent accessible surface area), log FISA (hydrophilic component of the total solvent accessible surface area) and log PSA (Van de Waals surface area of polar nitrogen and oxygen atoms). Similar but weaker correlations were found between the measured log S values and each of the three molecular descriptors. The relationships can be used to obtain property values for various steroidal EDCs which may have potential environmental effects.

The behaviour of EDCs is also affected by some environmental parameters such as temperature, pH and equivalent biomass concentration (EBC). Several authors have noticed the effects of biomass concentration on degradation rate, but quantitative relationships have not been developed. So, this work conducted relationship studies between the measured degradation rate constants and EBC values. Simple linear regression indicated that the degradation rate constant generally increases with higher EBC values. Results showed that EE2 was most resistant to biodegradation, whilst E1 and E2 were relatively easily degraded at similar rates. The relationships obtained are useful for the prediction of the fate of steroidal EDCs in environmental media.

Often, the environmental fate of EDCs cannot be easily measured and mathematical simulation methods have to be used. A fugacity-based model was used to quantify the fate of E1, E2 and EE2 in a reservoir receiving recycled water in Queensland, Australia.

Under typical conditions, the simulated concentration in water after advanced water treatment were below 10^{-4} ng/L, implying negligible health risk when compared with *no-observed-adverse-effects-concentration* (NOAEC) for fish and Australia *Public Health Standards* (PHS) for humans. In addition, the simulated concentrations in water decreased when water temperature, reservoir water storage volume, EBC and reservoir water releasing rate increased. However the opposite trend was found with wastewater recycling rate and EDC concentration in the final recycled water.

To conduct health risk assessment for fish and humans, probabilistic techniques were used. A new risk characterisation method, the *overall risk probability* (ORP) was developed based on the cumulative probability distribution (CPD) of exposure and effect data. The ORP method obtained the same ranking of risk level for fish as the commonly used *hazard quotient* ($HQ_{95/5}$) method: EE2 ($HQ_{95/5}$, 250; ORP, 26.6%) > E1 ($HQ_{95/5}$, 63; ORP, 22.0%) > E2 ($HQ_{95/5}$, 16; ORP, 8.1%) > E3 ($HQ_{95/5}$, 1.2; ORP, 3.8%) > NP ($HQ_{95/5}$, 0.46; ORP, 0.6%) > BPA ($HQ_{95/5}$, 0.084; ORP, 0.4%) > OP ($HQ_{95/5}$, 0.057; ORP, 0.2%). All calculated $HQ_{95/5}$ and ORP values for estrogens were above their respective reference value of 1 in the $HQ_{95/5}$ method and 2.5% in the ORP method, implicating the contamination in surface water by estrogens is a global issue of concern. Due to the lack of human effect data, the ORP method was not used in human risk characterisation. Instead, the risk was quantified using *acceptable daily intake* (ADI) values developed by international and Australian agencies, which gave the ranked $HQ_{95/ADI}$ values in the order of E1 (3.16) > E2 (1.09) > BPA (0.200) > EE2 (0.0398) ≈ E3 (0.0398) > NP (0.0200) > OP (0.00252) for international ADI values and E1 (36.8) > EE2 (0.926) > E2 (0.632) > E3 (0.284) > BPA (0.200) > OP (0.00839) > NP (0.00667)

for Australian ADI values. Apparently, with both sets of ADI values, the $HQ_{95/ADI}$ values obtained for E1 were above the reference value of 1, showing significant level of risk to human health. Compared with the single-point $HQ_{95/5}$ method, the ORP method demonstrated the capability to reflect the information in the shape of cumulative distribution curves. Therefore, it is regarded as an improvement in risk characterisation.

Statement of originality

"This work has not been previously submitted for a degree or diploma in any university. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person except where due reference is made in the thesis itself".

Signature

August 2010

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List of abbreviations

ADI	Acceptable Daily Intake
APEO	Alkylphenol Ethoxylates
AWTP	Advanced Wastewater Treatment Plant
BCF	Bioconcentration Factor
BOD	Biological Oxygen Demand
BPA	Bisphenol A
cfu	Colony-forming Unit
CP	Cumulative Probability
CPD	Cumulative Probability Distribution
DDT	Dichlorodiphenyltrichloroethane
DES	Diethylstilbestrol
E1	Estrone
E2	17β-estradiol
E3	Estriol
EE2	17α-ethinylestradiol
EBC	Equivalent Biomass Concentration
BC	Biomass Concentration
EDCs	Endocrine Disrupting Chemicals
EE2	17α-ethinylestradiol
EEF	Estradiol Equivalent Factor
EEQ	Estradiol Equivalent Quantity
FISA	Hydrophilic Component of the Total Solvent Accessible Surface Area
FOSA	Hydrophobic Component of the Total Solvent Accessible Surface Area
H	Henry's Law Constant
HC	Hazard Concentration
HDD	Human Daily Dose
HED	Human Equivalent Dose
HQ	Hazard Quotient

HRT	Hydraulic Retention Time
k	Degradation Rate Constant
K_{oc}	Octanol-Carbon Partition Coefficient
K_{ow}	Octanol-Water Partition Coefficient
LOAEC	Lowest-Observed-Adverse-Effects-Concentration
MF	Microfiltration
MLSS	Mixed Liquor Suspended Solids
MLVSS	Mixed Liquor Volatile Suspended Solids
MOS	Margin of Safety
MW	Molecular Weight
NAS	Nitrifying Activated Sludge
NF	Nanofiltration
NOAEC	No-Observed-Adverse-Effects-Concentration
NOAEL	No-Observed-Adverse-Effects-Level
NP	Nonylphenol
OP	Octylphenol
ORP	Overall Risk Probability
P	Partition Coefficient
PAH	Polycyclic Aromatic Hydrocarbons
PCB	Polychlorinated Bisphenols
PHS	Public Health Standards
PSA	Van de Waals Surface Area of Polar Nitrogen and Oxygen Atoms
QSAR	Quantitative Structure-Activity Relationship
QSPR	Quantitative Structure-Property Relationship
RBA	Receptor Binding Affinity
RO	Reverse Osmosis
RPE	Relative Proliferative Effects
RPP	Relative Proliferative Potency
S	Aqueous Solubility
SMILES	Simplified Molecular Input Line Entry Specification

SRT	Solids Retention Time
T	Temperature
TBT	Tributyl Tin
T_m	Melting Point
UF	Ultrafiltration
V	Volume
VC	Viable Counts
VP	Vapour Pressure
VTG	Vitellogenin
WCRWP	Western Corridor Recycled Water Project
WWTP	Wastewater Treatment Plant

Publications arising from this work

Cao, Q., Yu, Q., and Connell, D. W. (2010) Health risk characterisation for environmental pollutants with a new concept of overall risk probability. (Accepted).

Cao, Q., Yu, Q., and Connell, D. W. (2010) Fate simulation and risk assessment of endocrine disrupting chemicals in a reservoir receiving recycled wastewater. Science of the Total Environment 408(24), 6243-6250.

Cao, Q., Garib, V., Yu, Q., Connell, D. W. and Campitelli, M. (2009) Quantitative structure-property relationships (QSPR) for steroidal compounds of environmental importance. Chemosphere 76(4), 453-459.

Cao, Q., Yu, Q. and Connell, D. W. (2008) Degradation rate constants of steroids in sewage treatment works and receiving water. Environmental Technology 29(12), 1321-1330.

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Chapter 1 Introduction

Rapid population growth, as well as industrial and urban development has placed great pressure on the water supply in many parts of the world. In some regions, environmental pollution has further reduced the water supply with acceptable quality. In response, many water authorities are now considering or building wastewater recycling facilities to supplement their current water supplies. For example, the largest water recycling project in the Southern Hemisphere, the Western Corridor Recycled Wastewater Project (WCRWP) was constructed in South East Queensland, Australia in 2006. For most recycling facilities, a technological challenge is to remove harmful substances, such as heavy metals, bacteria and viruses, and many organic compounds (e.g. dioxins, phenols, benzenes) to meet drinking water supply guidelines and industrial requirements. Although a variety of methods can remove these harmful substances to very low levels, recent research shows that some contaminants can impose adverse health effects on wildlife and humans at extremely low levels (e.g. ng/L) (Purdom et al., 1994; Hansen et al., 1998; Routledge et al., 1998; D'Ascenzo et al., 2003)

A particular group of wastewater contaminants, endocrine disrupting chemicals (EDCs), are able to cause disruption to the endocrine system in wildlife species and humans at very low levels (e.g. ng/L). For example, the sexual hormone 17α-ethinylestradiol (EE2) and 17β-estradiol (E2) were observed to cause an increase in vitellogenin (VTG) in male and juvenile female fish at the level of 0.1 and 1.0 ng/L respectively in wastewater effluents (Purdom et al., 1994; Bjerregaard et al., 2008). A wide range of substances can be classified as EDCs or potential EDCs. Some well known EDCs are pesticides (e.g.

DDT, organo phosphorpesticides), alkylphenols, bisphenols, polychlorinated bisphenols (PCBs), polycyclic aromatic hydrocarbons (PAHs), dioxins, furans, pharmaceuticals, hormones and some metals (e.g. mercury, lead, cadmium). They can be broadly divided into natural and man-made EDCs.

Endocrine disruption in organisms was studied as early as the 1930s (Dodds et al., 1938). Since the 1960s, it has become a major environmental and human health issue. For example, the use of diethylstilbestrol (DES) from the late 1940s to the early 1970s has been linked to increased cases in abortions, neonatal deaths, premature births and vaginal cancers in women (Birkett 2003). Another example is the extensive use of DDT during the 1950s and 1960s, which adversely affected the reproductive system in mammals and birds. This resulted in reproductive failure in raptors being reported from various countries, which caused a dramatic population decline (Hester and Harrison 1999).

From the 1990s, research on adverse effects of EDCs has been reported in numerous scientific publications (Birkett 2003; Khanal et al., 2006). Various adverse biological effects such as population decline, reproductive abnormalities, imbalanced sex ratios and behaviour changes have been observed in aquatic organisms, amphibians, reptiles, birds and mammals. Many human health effects such as prostate cancer, breast cancer and birth defects have also been linked to the exposure to EDCs (Sharp and Skakkebaek 1993; Whittemore 1994; Fernandez et al., 1998; Toppari and Skakkebaek 2000; Damstra 2002; Diamanti-Kandarakis et al., 2009)

Many of EDCs presented in the aquatic environment are from a variety of point and nonpoint sources (Birkett 2003; Rahman et al., 2009). Wastewater effluents from domestic and industrial wastewater treatment plants (WWTP) are the major point sources of EDCs entering the aquatic environment (Ternes et al., 1999b; Birkett 2003; Sharma et al., 2009). Hormonal EDCs are commonly detected in wastewater effluents from below detection limits to tens of ng/L. Other industrial chemicals such as nonylphenol (NP), octylphenol (OP) and bisphenol A (BPA) occur in concentrations up to several mg/L. In many cases, the level of EDCs in wastewater effluents is much higher than the NOAEC (no-observed-adverse-effects-concentration) values for fish and other aquatic organisms tested in laboratory studies. Consequently, adverse effects could be expected with aquatic organisms and other species, such as birds and even humans eating contaminated fish.

Evidence of endocrine disruption in wildlife species and humans is continuing to be reported in the scientific literature. Thus it is important to understand the occurrence, distribution, degradation and health risk of EDCs in the environment. This work focused on several EDCs, which are considered important based on their potency and quantity of usage. It will address specific aspects of their behaviour and health effects which will assist in management of these substances.

Chapter 2 Aims and objectives

This work aims to develop an understanding of the environmental behaviour and health risk of EDCs originating mainly from wastewater. Specifically, this work aims to achieve the following objectives:

Objective One

The first objective was to develop an understanding of the current knowledge on EDCs. This would be achieved by a critical review on physicochemical properties, occurrence, distribution, degradation, removal and health risk of EDCs in wastewater treatment processes and natural environments. In order to focus the research more specifically, it was decided to limit the work to seven EDCs, which can be divided into two groups: the group of four estrogens, estrone (E1), 17β-estradiol (E2) and estriol (E3), and 17α-ethinylestradiol (EE2), plus the group of three phenolic compounds, nonylphenol (NP), octylphenol (OP) and bisphenol A (BPA).

Objective Two

Generally, physicochemical properties and biological activity are related to chemical structures. The study of quantitative structure-activity relationship (QSAR) and quantitative structure-property relationship (QSPR) would provide information on properties useful for behaviour modelling. Therefore, the second objective was to investigate the relationships between important physicochemical properties (e.g. S and K_{ow}) and molecular descriptors describing chemical structures for 17 steroidal EDCs.

Objective Three

An objective outlined later (Objective Four) was to carry out fate modelling of EDCs but information on the degradation characteristics is lacking. Thus, the third objective was to develop quantitative relationships between the degradation rate constants and the equivalent biomass concentration (EBC) for three important steroidal EDCs.

Objective Four

Little information is available on the environmental fate of EDCs when they are discharged into a reservoir. When the fate can not be directly measured, simulation methods can be used. Thus, the fourth objective was to quantify the fate of EDCs in a reservoir receiving recycled water in Queensland, Australia using a fugacity based model. This model is a well established, well documented and widely used model (Mackay, 2001; Mackay and Macleod, 2002; Mackay, 2004). However, its application to simulate the fate of EDCs in a recycling scheme has not been reported.

Objective Five

Exposure to EDCs has caused various adverse health effects in wildlife species, particularly in aquatic organisms. Some human adverse health effects such as reproductive cancers are also linked to EDCs exposure. Thus, the fifth objective was to conduct a health risk assessment for fish and humans using probabilistic techniques with measured exposure and adverse effect data derived from scientific literature.

Chapter 3 Literature Review on behaviour and health risk of EDCs

3.1 Definition and categorization of EDCs

3.1.1 Definition of EDCs

In the scientific community and public media, *endocrine disrupting chemicals (EDCs)* has also been described as *endocrine disrupters*, *endocrine disrupting compounds*, and *endocrine disrupting contaminants*. This study adopts the term, *endocrine disrupting chemicals*, which has been most frequently used in the scientific community. Several definitions have been proposed for EDCs. In order to establish the scope, facilitate the identification and regulatory control of EDCs, the *European Workshop on the Impact of Endocrine Disrupters on Human Health and Wildlife* proposed a definition in 1997 (European Commission 1997):

An endocrine disrupter is an exogenous substance that causes adverse health effects in an intact organism, or its progeny, subsequent to changes in endocrine function.

Philips and Harrison (1999) pointed out that chemicals studied by *in vitro* methods should be distinguished from those studied by *in vivo* methods. They argued that chemicals with adverse health effects demonstrated by *in vitro* methods should be termed as *potential EDCs*. Thus, they have proposed the definition for potential EDCs:

A potential endocrine disrupter is a substance that possesses properties that might be expected to lead to endocrine disruption in an intact organism.

The difference between EDCs and potential EDCs were further clarified by the following definitions in a meeting of the *International Programme on Chemical Safety* (IPCS 1998):

An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations.

A potential endocrine disruptor is an exogenous substance or mixture that possesses properties that might be expected to lead to endocrine disruption in an intact organism, or its progeny, or (sub) populations.

The *Endocrine Disruptor Screening and Testing Advisory Committee* of US EPA also provided similar definitions but with emphasis on the scientific basis, weight-of-evidence and the precautionary principle (US EPA 1998; Phillips and Harrison 1999). Harrison and Philips (1999) pointed out two issues associated with the adverse effects in these definitions. The first issue is the adverse effects should be distinguished from the normal range of physiological variations. Secondly, the adverse effects should not be a secondary consequence of toxicity in other body systems.

3.1.2 Categorization of EDCs

According to the above definitions, a wide range of chemicals can be classified as EDCs. In a study conducted for the *European Commission* on gathering information on EDCs, 435 chemicals were selected as *potential EDCs*. Among them, 94 were considered as

EDCs by evidence of endocrine effects (Okkerman and van der Putte 2002). The list of EDCs is becoming more extensive with better detection methods and more scientific evidence on adverse heath effects. Many common EDCs include pesticides (e.g. DDT, organo phosphorpesticides), industrial chemicals (e.g. alkylphenols, bisphenols, polychlorinated bisphenols (PCBs), polycyclic aromatic hydrocarbons (PAHs), dioxins, and furans), pharmaceuticals (e.g. mestranol, EE2, DES), natural estrogens (e.g. estrone, estradiol, estriol, phytoestrogens) and some metals (e.g. mercury, lead and cadmium). The above mentioned EDCs can be broadly divided into two groups:

- Natural hormones such as estrone, estradiol, progesterone, testosterone from human and animal body, and some phytoestrogens from plants;
- Man-made substances such as pesticides, industrial chemicals, pharmaceuticals and metals.

Among the great variety of EDCs, a group of natural and synthetic hormones have attracted special scientific attention because of their feminising or masculinising effects. This group of hormones can be divided into estrogens and androgens, which have been defined separately (Birkett and Lester 2003):

Estrogens are any of a family of steroid hormones that regulate and sustain female sexual development and reproductive function.

Androgens are a class of male sex hormones related to the steroid androstane and produced in the adrenal cortex and the testes; includes testosterone, androsterone, and androstenolone responsible for the development of secondary male characteristics, such as a deep voice and facial hair.

Estrogens have attracted more scientific attention than androgens in current scientific research. Numerous laboratory studies have been conducted on E1, E2, E3 and EE2. Some non-steroidal EDCs, such as nonylphenol (NP), octylphenol (OP) and bisphenol A (BPA) can also cause estrogenic effects and they are termed as ‘xeno-estrogens’.

The majority of EDCs are man-made substances such as pesticides and industrial chemicals. Some chemicals such as DDT and tributyl tin (TBT) have shown estrogenic effects to wildlife and human health (Burlington and Kindeman 1950; Bitman et al., 1968; DeMora 1996). The use of such chemicals have been banned or restricted in many countries, but many other EDCs including some industrial chemicals and pesticides are still produced and used in vast quantities worldwide. Since the number of potential EDCs is so extensive, this review only focused on three important natural estrogens, E1, E2, E3, and one synthetic estrogen, EE2. In addition, three widely occurring phenolic compounds, NP, OP and BPA were also briefly reviewed, as they were studied in the health risk assessment of this thesis.

3.2 Chemical structures and physicochemical properties of EDCs

3.2.1 Chemical structures

Both estrogens and androgens are originated from the same parent compound, cholesterol. Inter-conversions can occur between these two groups as shown in Figure 1 (Brook and Nicholas 2001; Gracia et al., 2008). For example, the male hormone, testosterone can be converted into the female hormone, estradiol, by the aromatase enzyme. Thus, estrogens and androgens share many structural similarities as indicated by their structures in Figure 2. There are four hydrocarbon rings in all steroids: three hexagonal rings (rings A, B and C) and one pentagonal ring (ring D). A phenolic group in the A ring position occurs in estrogens, whilst androgens do not contain this group at the same position. The hydroxyl groups in estrogens at the positions C3 and C17 are susceptible to microbial attack, which may affect their degradation rate (Ying and Kookana 2003a).

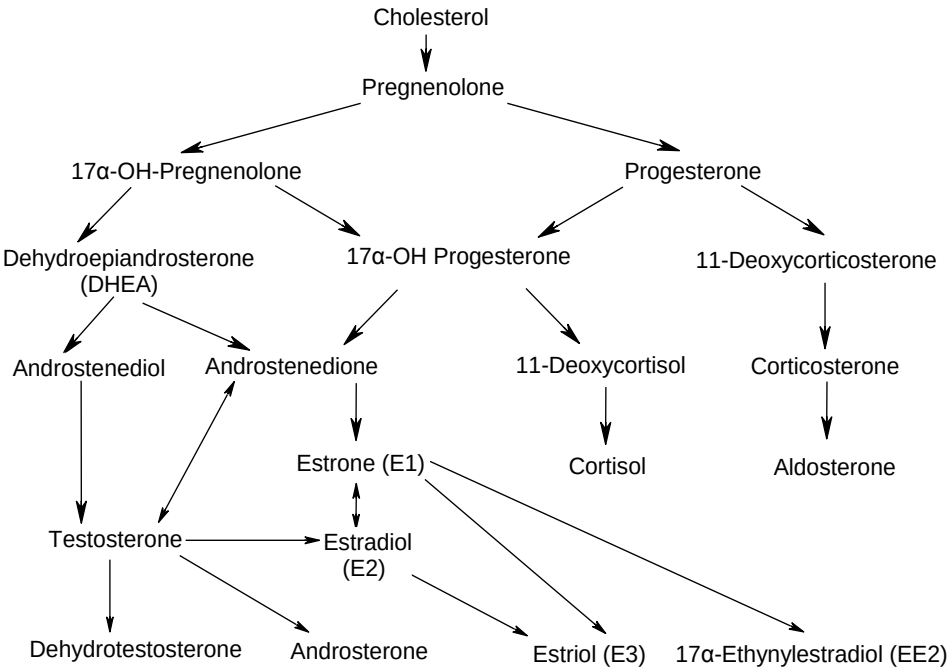

Figure 1. Inter-conversion and synthesis pathways of steroidal EDCs.

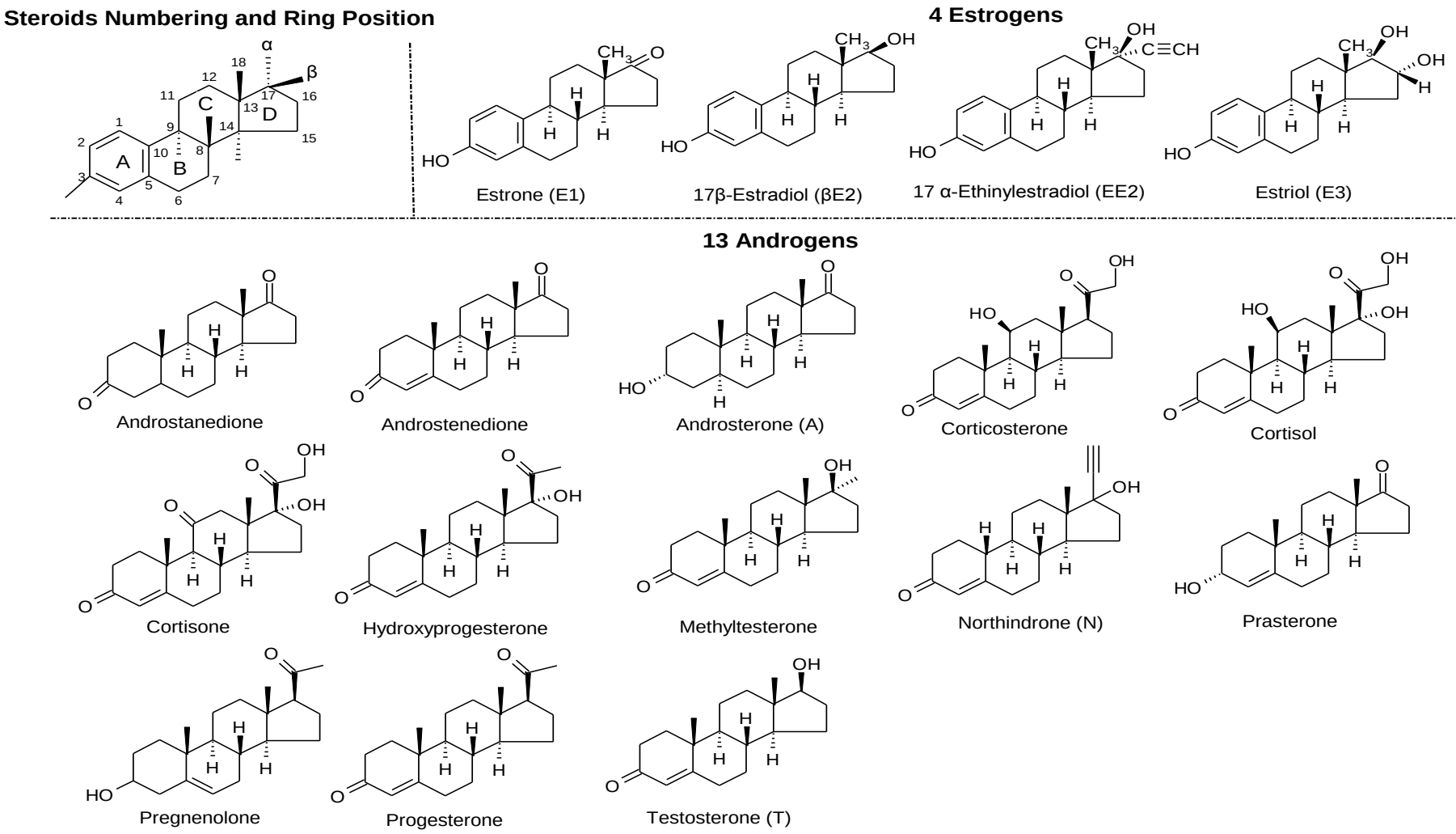

Figure 2. Chemical structures and nomenclature of estrogens and androgens.

With the phenolic compounds, NP and OP belong to the alkylphenol group with one alkyl group attached to their phenolic ring, as shown in Figure 3. This may lead to many isomer forms of NP and OP.

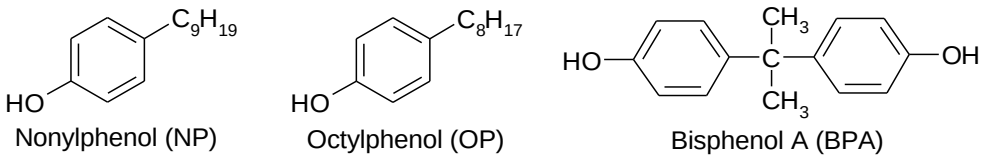

Figure 3. Chemical structures of NP, OP and BPA.

NP and OP can be synthesized from the alkylation of phenols. One of the manufacturing processes was illustrated in Figure 4 (Vazquez-Duhalt et al., 2005). Both NP and OP are important chemicals for the production of alkylphenol ethoxylates (APEOs), which are used in the formulation of a wide range of detergents, paints, lubricants, resins and pesticides (Renner 1997). By aerobic or anaerobic degradation, APEOs can be transformed back into NP or OP (Klecka et al., 2005; Sharma et al., 2009).

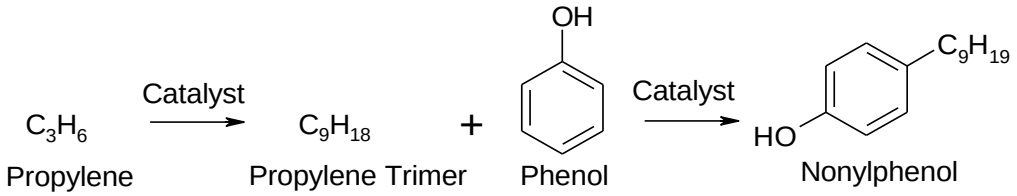

Figure 4. Synthesis process of NP.

The other phenolic compound, BPA, is mainly used for the production of polycarbonate plastics and epoxy resins such as food and beverage containers (Staples et al., 2002). Consequently, it has been detected in food released from coatings and packaging materials (Takino et al., 1999; Takahata et al., 2001; Muncke 2009). BPA can be manufactured through a condensation reaction with acetone and phenols as shown in Figure 5 (Uglea and Negulescu 1991).

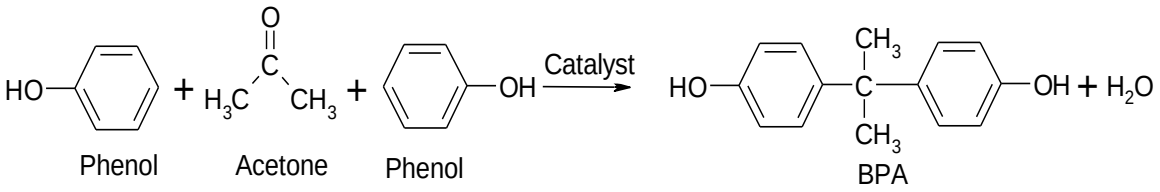

Figure 5. Synthesis process of BPA.

3.2.2 Physicochemical properties of EDCs

E1, E2, E3, EE2 and BPA are solids at normal conditions with specific weight slightly heavier than water. NP and OP however, are liquids and slightly lighter than water. Some of their important physicochemical properties were summarized in Table 1. As it is indicated by their octanol-water partition coefficient (K_{ow}) and aqueous solubility (S) values in Table 1, they are hydrophobic and almost insoluble in water, except for BPA with slightly higher solubility of 120 mg/L. For most organic compounds, log S is linearly correlated to log K_{ow}. Meylan et al. (1996) proposed a correlation based on a large set of organic compounds:

$$\log S = -1.020 \log K_{ow} - 0.312 \quad (R^2 = 0.786) \tag{1}$$

The organic carbon-water partition coefficient, K_{oc} can be calculated from K_{ow} by the following relationships:

$$K_{oc} = 0.411 K_{ow} \text{ (Karickhoff 1981)} \tag{2}$$

$$\log K_{oc} = \log K_{ow} - 0.317 \text{ (Means et al., 1982)} \tag{3}$$

$$\log K_{oc} = 0.904 \log K_{ow} - 0.543 \text{ (Chiou et al., 1983)} \tag{4}$$

Thus, log K_{oc} is related to log S by Equation (1) - (4), or by the following equations:

$$\log K_{oc} = -0.686 \log S + 4.273 \text{ (Means et al., 1982)} \tag{5}$$

With the above relationships, log K_{oc} values can be estimated from log K_{ow} or log S values, which were summarised in Table 1. These estimated values are consistent with a

wider range of measured values reported in the literature (Campbell et al., 2006). The log K_{oc} values of estrogens and phenolic compounds indicate these compounds have moderate to strong tendency of partitioning into organic matter in the environment and fat tissues in living organisms (de Mes et al., 2005). The bioconcentration factor (BCF) can be estimated from log K_{ow} values using Equation (6) or (7) (Schnoor et al., 1987):

$$\log BCF = 0.76 \log K_{ow} - 0.23 \quad (6)$$

$$\log BCF = 0.997 \log K_{ow} - 0.867 \quad (7)$$

Although the BCF values in Table 1 indicate low (estrogens and BPA) to moderate (NP and OP) bioconcentration potential, overdose could result in bioaccumulation if the detoxification pathways are saturated (Upmeier 2000).

The fate of EDCs in the aquatic environment is affected by factors such as temperature, pH, bacteria density and dissolved oxygen level (Aronson et al., 1999; Jurgens et al., 2002; Urase et al., 2005). Generally, EDCs have a half-life of several days in the environment, except for EE2 with half-life of several weeks or months. In aerobic activated sludge process, their half-lives are much shorter (see Table 1). Other important physicochemical properties such as the Henry's law constant (H) and vapour pressure (VP) are important for the study of their fate in the atmosphere. As their H and VP values in Table 1 indicate, estrogens and BPA can be regarded as non-volatile, and NP and OP slightly volatile from water. Therefore, exposure pathway through inhalation is negligible. When the estrogenic potency is compared, estrogens possess 1000 to 100, 000 times of higher potency than xeno-estrogens such as NP, OP and BPA, which were reflected by their *estradiol equivalent factor* (EEF) in Table 1 (Murk et al., 2002; de Voogt and van Hattum 2003; Vethaak et al., 2005; Vajda et al., 2008).

Table 1. Physicochemical properties of EDCs (estrogens and phenolic compounds)

Properties	E1	E2	E3	EE2	NP	OP	BPA
MW (g/mol)	270.37	272.39	288.39	296.40	220.35	206.33	228.29
S (mg/L)	0.8	3.9	3.2	9.7	5.43	12.6	120
log K_{ow}	3.13	3.57	2.45	3.67	4.48	4.12	3.32
log K_{oc}	2.29 – 2.81	2.68 – 3.25	1.67 – 2.13	2.77 – 3.35	3.51 – 4.16	3.18 – 3.80	2.46 – 3.00
pK_a	10.4	10.71	10.4	10.46 – 10.70	10.28	-	10.2 – 10.3
H (atm m^3/mol)	3.8×10^{-10}	3.6×10^{-10}	1.3×10^{-12}	7.9×10^{-12}	10^{-5} - 10^{-9}	10^{-5} - 10^{-6}	1.0×10^{-11}
VP (Pa)	3.0×10^{-8}	3.0×10^{-8}	2.7×10^{-8}	6.0×10^{-9}	10^{-2} - 10^{-3}	10^{-2} - 10^{-3}	3.9×10^{-5}
EEF	0.01	1	0.08	1.25	1.3×10^{-5}	6.5×10^{-5}	2.5×10^{-5}
BCF	141 - 179	304 - 492	38 - 43	362 - 619	1496 - 3977	797 - 1740	196 - 277
Aquatic $t_{1/2}$ (d)	2 - 4	2 - 4	5 - 7	15 - 80	0.3 - 10	1 - 5	2 - 4
AS $t_{1/2}$ (h)	min - 15	min - 5	20 - 40	25 - 70	min - 2	1 - 2	0.7 - 40

MW - molecular weight; S - aqueous solubility; log K_{ow} – octanol-water partition coefficient; log K_{oc} - organic carbon-water partition coefficient; pK_a - acid dissociation constant; H - Henry's law constant; VP - vapour pressure; EEF - E2 equivalent factor; BCF - bioconcentration factor; $t_{1/2}$ - half-life; AS - activated sludge (aerobic).

Physicochemical data was compiled from (Leszczynski and Schafer 1990; Hansch et al., 1995; Howard and Meylan 1997; Gomes et al., 2003; Ivashechkin et al., 2004; Campbell et al., 2006; Falconer et al., 2006; Cao et al., 2009; Ying et al., 2009).

log K_{oc} values calculated from Equation (2), (3) and (4); BCF values calculated from Equation (6) and (7); VP and H values for NP and OP vary with different isomers, only ranges were given. Half-life data estimated from published studies (Ike et al., 2000; Hesselsoe et al., 2001; Ying et al., 2002; Ying and Kookana 2003a; Ying et al., 2003b; Ankley and Johnson 2004; Shi et al., 2004; Shi et al., 2004a; Dubroca et al., 2005; Klecka et al., 2005; Urase and Kikuta 2005b; Shen et al., 2007; Cao et al., 2008).

3.2.3 Quantitative structure-activity/property relationships for EDCs

Due to a large amount of substances can be classified as EDCs or potential EDCs, it is impossible to conduct toxicity tests for all suspected substances (Liu et al., 2006). Fortunately, some structural features are linked to physicochemical properties and biological activities (Molnar and King 2001). Particularly, the estrogenic effects of EDCs are dependent on their binding affinity to target estrogen receptor (ER) in the living organisms (Bertosa et al., 2003; Asikainen et al., 2004). Therefore, the study on *quantitative structure-activity relationships* (QSAR) and *quantitative structure-property relationship* (QSPR) can be used to predict physicochemical properties and biological activities, which are important to prioritize target EDCs and consequently to reduce testing labour, testing animals and cost (Cunningham et al., 2004; Saliner et al., 2006).

Liu et al. (2006) developed QSAR models with 8 structural descriptors to predict estrogen receptor binding affinity (RBA) based on 132 estrogens. Their models were validated with satisfactory applicability. Another QSAR model based on k nearest principle (kNN) was developed by Asikainen et al. (2004) to predict RBA values. Based on interpretable mechanistic descriptors, Saliner et al. (2006) developed and validated a classification model with 117 aromatic compounds to predict estrogenicity. Cunningham et al. (2004) obtained two models to predict the relative proliferative effects (RPE) and relative proliferative potency (RPP) from 50 estrogenic and 72 non-estrogenic compounds. Their model achieved 88% and 72% of accuracy respectively. In a review conducted by Bradbury et al. (1998), they pointed out that the potency and agonistic or antagonistic effect of steroid hormones are dependent on both binding

affinity and the conformation of ligand-receptor complex. In order to develop the screening-level QSAR, they assessed the 3-dimentional flexibility of ligand.

In a QSAR study to predict biological activity based on similarities of molecular interaction fields, Bertosa et al. (2003) claimed that biological activity is also dependent on properties such as absorption, distribution, metabolism and excretion. Particularly, they pointed out that the lipophilicity parameter log K_{ow}, has influence on biological activity. For many EDCs, the measured properties such as S and K_{ow} are not readily available. In this case, the calculated values can be obtained from QSAR or QSPR. For example, Asikainen et al. (2004) used average consensus-QSAR model to predict the partition coefficient log P and obtained good results. Other authors have used molecular descriptors to calculate S and K_{ow} values for more general organic compounds (Warne et al., 1990; Jorgensen and Duffy 2002; Taskinen and Yliruusi 2003; Rytting et al., 2005; Balakin et al., 2006)

3.3 Sources of EDCs to the aquatic environment

The occurrence of EDCs in the aquatic environment is from a variety of point and nonpoint sources (Birkett 2003; Rahman et al., 2009). Discharges from domestic and industrial WWTP are the major point source entering the aquatic environment (Ternes et al., 1999b; Birkett 2003; Sharma et al., 2009). Estrogens are commonly found in WWTP effluents from several to 10's ng/L, and phenolic compounds from several 100's to 1000's ng/L (Belfroid et al., 1999; Baronti et al., 2000; Johnson and Williams 2004; Clara et al., 2005a; Lee et al., 2005a; Williams et al., 2007; Ying et al., 2009). As a result, the discharge of wastewater treatment effluent leads to the occurrence of EDCs

in surface water (Belfroid et al., 1999; Hohenblum et al., 2004; Lagana et al., 2004; Pojana et al., 2007; Zhao et al., 2009). For example, in a survey conducted in the USA between 1999 and 2000, EDCs were detected in about 80% of 139 investigated streams. It was reported that residential, industrial and agricultural sources contributed most of the occurrence (Kolpin et al., 2002).

Major nonpoint sources of EDCs include the runoff and underdrainage from agricultural land, animal farm, urban runoff and landfill leachate (Birkett 2003; Auriol et al., 2006; Benotti et al., 2009). For example, many pesticides found in surface water were originated from agricultural runoff (Chapman 2002). Most estrogens and androgens however, are naturally produced in human and animal body (e.g. E1, E2 and E3), plus the amount ingested for medical or growth promotion purposes (e.g. EE2, testosterone and progesterone) (Halling-Sorensen et al., 1998; Lintelmann et al., 2003). Sarmah et al. (2006) found significant amount of estrogens (up to thousands of ng/L) in the animal waste effluent in New Zealand farms. The highest concentration was found in the dairy farm, followed by the middle concentration in the goat farm and the lowest in the piggery farm. Hanselman et al. (2006) found similar level of estrogens in flushed dairy manure wastewater. In the raw wastewater of a swine farm in Japan, Furuichi et al. (2006) found even higher level of estrogens and phenolic compounds (e.g. μg/L). In addition, hospital wastewater is another important source of estrogens entering the aquatic environment (Ternes and Joss 2006; Kummerer 2008). The amount of hormones orally ingested and naturally produced in human and animal body is metabolised in the liver and excreted out mostly in conjugated forms (Young et al., 2004). Due to their incomplete removal in WWTP, estrogens have been frequently detected in the effluents.

In contrast, phenolic compounds NP, OP and BPA are manufactured in factories in large quantities worldwide. The estimated worldwide production of BPA was more than 2.5 million tonnes in 2001 (Staples et al., 2002). NP and OP have a wide range of industrial and household applications such as detergents, lubricants, defoamers, emulsifiers and paints (Renner 1997; Birkett and Lester 2003). The majority of BPA is used in the plastic industry to make food and drink packaging material or water supply pipes (Staples et al., 2002). Due to their wide applications, phenolic compounds eventually end up in domestic and industrial wastewater. They have been commonly detected with high levels in wastewater effluent and surface water (Halling-Sorensen et al., 1998; Ternes et al., 1999b; Ying et al., 2002).

Another important source of EDCs is from the fertilizers made from wastewater treatment sludge (Ternes et al., 1999b). A few studies showed the high adsorption affinity of EDCs to sludge (Clara et al., 2004; Andersen et al., 2005a; Urase and Kikuta 2005b), which makes sludge a major sink of these compounds (Xia et al., 2005). As discussed in Section 3.2.2, EDCs have moderate to strong tendency of partitioning into organic matter, as indicated by their log K_{oc} values. For example, Xia and Pillar (2003) measured NP in biosolids and composts with concentration up to 1380 mg/L. When sludge is applied to agricultural land, the adsorbed EDCs can be released into the environment at suitable conditions, such as the runoff by rainfall (Halling-Sorensen et al., 1998).

Stormwater canals, urban runoff and landfill leachate are also important sources entering the watercourse. Boyd et al. (2004) detected BPA with the concentration range

of 1.9 – 158 ng/L and 0.9 – 44 ng/L in stormwater canal and recreational urban waterway. In contrast, much higher level of EDCs was found in landfill leachate. Wintgens et al. (2003) detected high level of BPA with several μg/L in raw landfill leachate from two landfill leachate treatment plants in Germany. Yamamoto et al. (2001) detected BPA with a concentration range of 0.3 to 17 200 μg/L in Japan. In Sweden, 209 organic compounds including NP, OP and BPA were detected up to several hundred μg/L in the leachate (Paxeus 2000). Castillo and Barcelo (2001) found similar level of 7 organic pollutants in an industrial landfill leachate site in Italy (0.16 to 54.5 μg/L). These results showed that the concentration of EDCs in landfill leachate is comparable to, or even higher than the level found in WWTP influent. It was estimated that 50 000 out of the total 250 000 abandoned or closed landfills in Europe are contaminating or will contaminate groundwater and surface water in the near future (Castillo and Barcelo 2001). Thus, landfill leachate is a significant source of EDCs and it should be managed carefully.

3.4 EDCs fate in wastewater treatment processes

3.4.1 Excretion, conjugation and deconjugation before entering WWTP

Industrial discharges and residential detergents are the major sources of phenolic compounds entering WWTPs. In contrast, estrogens are mainly excreted from human and animal body after metabolism in the liver. Natural estrogens are produced by the ovaries and testis in the body, whilst synthetic estrogens are ingested as oral contraceptive pills, growth promoters or other medical purposes. The daily excretion of natural estrogens varies between different genders and age groups. The typical range of

daily excretion was listed in Table 2 for E1, E2 and E3. The highest daily excretions are from post-menopausal and pregnant women, whilst the smallest excretion is from children.

Table 2. Daily excretion of estrogens from human body

Gender		Daily excretion (μg/p/d)		
		E1	E2	E3
Female	Pre-menopausal	2.7 – 7.8	1.1 – 3.5	4.7 – 8.7
	Post-menopausal	1.4 - 23	0 - 14	0 - 72
	Pregnancy	209 - 2585	127 - 900	215 – 38 000
Male	Adult	3.0	1.6	3.4
Children	< 8 years	E1 + E2 + E3 total: 0.09 – 0.5		
	8 – 12 years	E1 + E2 + E3 total: 0.5 - 3		

Source: (Dao et al., 1973; D'Ascenzo et al., 2003; Young et al., 2004)

Before entering WWTPs, estrogens undergo two important processes, the conjugation and deconjugation processes. Two major types of conjugates are formed in the conjugation process: the glucuronide and sulphate conjugates, which were shown in Figure 6. Conjugation can occur at the C3, C16 and C17 position of the four-ring structure of estrogens. For E1 and EE2, conjugation occurs most often at the C3 and C17 position respectively, whilst E2 has conjugated groups both at the C3 and C17 position. E3, however, has glucuronide conjugates at the C16 position (Figure 6). Glucuronide conjugates are more susceptible to enzymatic hydrolysis, whilst sulphate conjugates are more stable. Deconjugation of glucuronide conjugates occur as early as they enter the sewer systems. A study conducted by Belfroid et al. (1999) showed no detection of glucuronide conjugates in the influent of a Dutch WWTP. Another study by D'Ascenzo et al. (2003) also found the complete removal of glucuronide conjugates in a WWTP. Therefore, it would be rare to find glucuronide conjugates in WWTP effluent (Panter et al., 1999; Johnson and Sumpter 2001). In contrast, the deconjugation process

of sulfate conjugates takes longer time and can continue in WWTP, or even in the natural environment (Baronti et al., 2000). For example, D'Ascenzo et al. (2003) found only 64% percent of conjugated E1-3S was removed in the WWTP. They discussed that E1 is mainly excreted in sulphate conjugates, and the desulfating arylsulfatase enzyme is less common as compared to the glucuronidase enzyme in WWTP (Andreolini et al., 1987; Baronti et al., 2000). Johnson and Williams (2004) argued that sulphate conjugates are mainly deconjugated by anaerobic desulfating strains, whilst much of the sewer systems and WWTP are aerobic. Therefore, it is more difficult to deconjugate sulphate conjugates in sewer system and treatment processes.

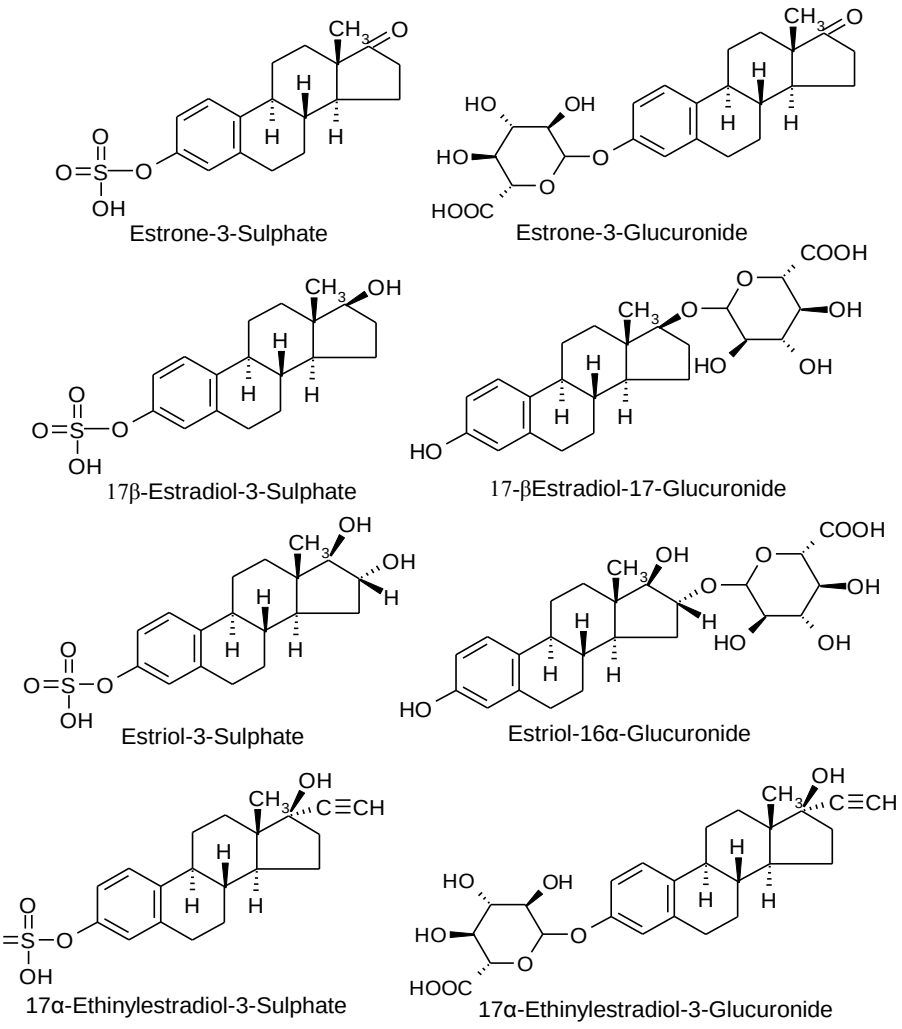

Figure 6. Chemical structures of commonly occurred estrogen conjugates.

Conjugation makes estrogens more water soluble and biologically inactive (Kozak et al., 2001; de Mes et al., 2005). For example, the estrogenic activity of conjugated estrogens is 10^5 to 10^7 times lower than E2 in a study using YES response (yeast estrogens screen) (Matsui et al., 2000). Deconjugation, however, can release free estrogens from the conjugates, which greatly increases the estrogenic activities (de Alda and Barcelo 2001; Svenson et al., 2003).

Most natural estrogens are excreted in the conjugated forms in the urine with small amount in unconjugated forms in faeces (Adlercreutz 1986; D'Ascenzo et al., 2003; Johnson and Williams 2004; Young et al., 2004; de Mes et al., 2005). Conjugates excreted from the bile are largely deconjugated by the natural intestinal flora prior to excretion from the bowel (Ternes et al., 1999a; Johnson and Williams 2004). After that, the presence of large amount of bacteria *Eschericia Coli* in faeces is able to deconjugate the conjugated estrogens into their free form by enzymatic hydrolysis (Andersen et al., 2004; Gomes et al., 2005; Khanal et al., 2006). Based on human daily excretion of E1, E2 and E3, the estimated percentage of conjugated and unconjugated forms of natural estrogens in urine and faeces was presented in Figure 7 (Larsson et al., 1999; Ternes et al., 1999a; D'Ascenzo et al., 2003; de Mes et al., 2005).

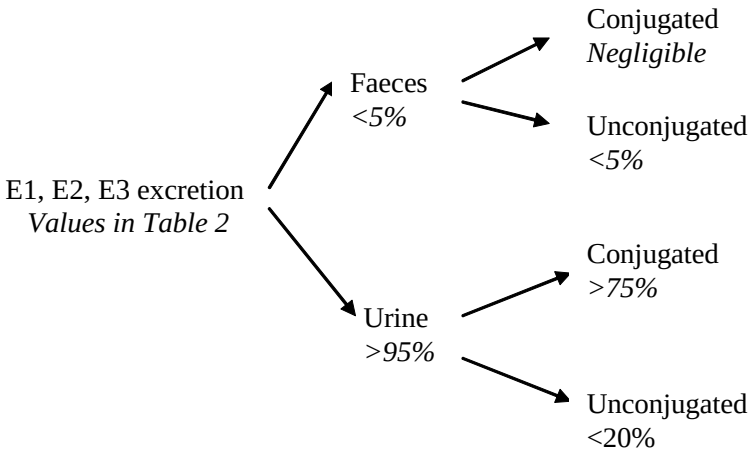

Figure 7. Conjugated and unconjugated natural estrogens in human urine and faeces.

For the synthetic estrogen EE2, the estimated daily dose is between 26 and 50 μg/p/d (Desbrow et al., 1998; Johnson et al., 2000; Johnson and Williams 2004; de Mes et al., 2005). About 20 to 43% of this ingested amount is metabolised in the body and the remaining is almost equally excreted in faeces and urine (Johnson and Williams 2004; de Mes et al., 2005). In faeces, free or unconjugated EE2 is dominant, whilst in urine the conjugated form is the majority. The estimated percentage of EE2 excreted in conjugated and unconjugated forms were presented in Figure 8. Compared with natural estrogens, there is a greater proportion of conjugated EE2 in faeces. Probably, the triple bond ethynyl group at the C17 position of EE2 structure increases deconjugation difficulties for the bacteria presented in faeces (Bolt 1979; Ying and Kookana 2005).

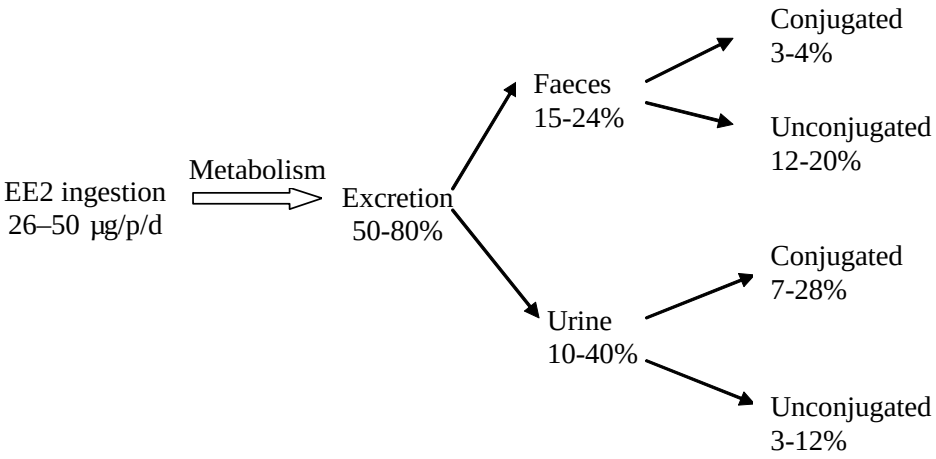

Figure 8. Conjugated and unconjugated EE2 in human urine and faeces.

3.4.2 Removal mechanisms of EDCs

EDCs can be removed in the WWTP and natural environment by a number of mechanisms such as hydrolysis, photolysis, volatilization, chemical oxidation, adsorption, biodegradation and membrane filtration (Johnson and Sumpter 2001; Ying et al., 2004a; Auriol et al., 2006; Zhang et al., 2007; Sharma et al., 2009; Liu et al., 2009b). In most WWTPs with biological treatment, sorption and biodegradation are the principal EDCs removal mechanisms. Particularly, aerobic biodegradation is the dominant removal mechanism (de Mes et al., 2005).

3.4.2.1 Hydrolysis

Hydrolysis is an important chemical transformation when chemical molecules have linkages separating highly polar groups (Rogers 1996). Because of the weak polarity and hydrophobic property of E1, E2, E3 and EE2, hydrolysis has very limited role in removing these compounds. However, it is the major reaction in the deconjugation of

glucuronide and sulphate conjugates (Andersen et al., 2004; Gomes et al., 2005; Khanal et al., 2006). Particularly, the rate of hydrolysis can be very fast when mediated by microorganisms (Connell 2005).

3.4.2.2 Photolysis

Photolysis is an effective removal mechanism mainly at the presence of catalysts such as titanium dioxide (TiO_2) (Jurgens et al., 2002; Nakashima et al., 2002; Ohko et al., 2002; Coleman et al., 2004; Coleman et al., 2005; Gray and Sedlak 2005). Without catalysts, photodegradation by direct sunlight occurs at very slow rate. For example, a half-life over 10 days was observed for E2 and EE2 by Jürgens et al. (2002). In contrast, ultra violet (UV) coupled with catalysts such as TiO_2 or H_2O_2 in laboratory studies has achieved satisfying removal results. For example, Ohko et al. (2002) found the complete removal of E2 within 50 minutes using UV radiation catalysed by TiO_2. Similar results were also obtained by Coleman et al. (2004). They observed the complete degradation of E1, E2 and EE2 within 1 hour. Without catalyst, the complete removal took about 400, 300 and 150 minutes for E1, E2 and EE2 respectively. In another photolysis study, Zhang et al. (2007) found half-life of 0.26 and 0.28 hour for E1 and E2 when the UV irradiation had a wavelength of 253 nm, which was close to the peak light absorption wavelength of 223 and 227 nm in the UV region of E1 and E2. In addition, the combination of UV and H_2O_2 was very effective to remove estrogens. Rosenfeldt et al. (2007) achieved 90% removal of E2 and EE2 using 5 mg/L of H_2O_2 and less than 350 mJ/cm^2 of UV irradiation.

Since UV is less often used in wastewater treatment, the removal of EDCs by photolysis plays insignificant role in WWTP. However, photolysis could be important for the fate of EDCs in surface water, particularly in tropical regions with sufficient solar irradiation. Due to the light scattering by water particles, seasonal changes and other factors, the removal through photodegradation is very limited (Ying et al., 2004).

3.4.2.3 Volatilization

To study the transfer of EDCs from water into air, the Henry's law constant (H) is a useful indicator of volatilization. Generally, a compound is regarded as slightly volatile from water with H value between 10^{-5} to 10^{-7} atm m^3/mole, and non-volatile with H value < 10^{-7} atm m^3/mole. As the H values in Table 1 indicate, NP and OP can be regarded as slightly volatile, and E1, E2, E3, EE2 and BPA as non-volatile. Thus, for the investigated EDCs, the amount removed by volatilization in WWTP is expected to be negligible (Namkung and Rittmann 1987; Rogers 1996).

3.4.2.4 Adsorption

Adsorption is an important removal mechanism for compounds with higher log K_{ow} values. Common coagulants such as aluminium sulfate and ferric chloride used in water and wastewater treatment are able to remove many EDCs. For compounds with log K_{ow} values larger than 3 are easily adsorbed into the organic coatings on the particles produced on coagulant addition. Compared with coagulants, activated carbon is more effective to remove non-polar organic compounds with log K_{ow} values larger than 2 (Ying et al., 2004a). Choi et al. (2005) found that *granular activated carbon* (GAC) can

effectively remove EDCs with high log K_{ow} values. Fukuhara et al. (2006) used commercial activated carbon to removal estrogens. With pure water, the amount adsorbed was 25.6 to 73.5 mg/g for E1 and 21.3 to 67.6 mg/g for E2 at equilibrium concentration of 1 μg/L. In contrast, with river water and sewage effluent, the E2 adsorption was only 0.1 to 0.2 μg/g and 0.3 to 1 μg/g respectively at a equilibrium concentration of 1 ng/L. Removal can be very limited if the background organic content is too high, because estrogens will not be able to compete with the organic content for sorption surface (Ying et al., 2004a). The hydrophobicity of target compound, specific surface area, and mean pore diameter of activated carbon are important factors affecting EDCs removal (Fukuhara et al., 2006).

The sorption equilibrium between the aqueous and solid phase is commonly described by the Freundlich isotherm (Schwarzenbach et al., 2003) in Equation (6):

$$K_f = \frac{C_s}{C_w^n} \tag{6}$$

where K_f is the sorption coefficient, C_s is the concentration in the solid phase, C_w is the concentration in the aqueous phase, and n is a sorption constant.

If the sorption constant, n, is close to unity, the sorption isotherm becomes a simple linear relationship. The ratio is termed as the distribution coefficient, K_d:

$$K_d = \frac{C_s}{C_w} \tag{7}$$

K_d can be also calculated from K_{oc} by using Equation (8) with the fraction of organic carbon, f_{oc}:

$$K_d = f_{oc} \times K_{oc} \tag{8}$$

Further, K_{oc} can be estimated from K_{ow} according to the relationship developed by Karickhoff (1981) for sorption of lipophilic chemicals in all environmental matrices:

$$K_{oc} = 0.411 K_{ow} \quad (9)$$

From Equation (8) and (9), K_d can be calculated from K_{ow} by Equation (10):

$$K_d = 0.411 f_{oc} \times K_{ow} \quad (10)$$

Specifically for activated sludge, K_d can be estimated directly from K_{ow} using the following relationship developed by Matter-Muller et al. (1980):

$$\log K_d = 0.39 + 0.67 \log K_{ow} \quad (11)$$

Both Clara et al. (2004) and Andersen et al. (2005a) discussed that the sorption isotherms of E1, E2 and EE2 in activated sludge fit better with a simple linear relationship (linear range of Langmuir isotherm) rather than a Freundlich isotherm. Holthaus et al. (2002) conducted a sorption study on river sediment, which supported the same conclusion. However, Lai (2000) obtained the sorption constant *n* of 1.37, 1.47, 1.20 and 1.75 for E1, E2, EE2 and E3 respectively, implicating that the sorption to solid phases in rivers and estuary systems cannot be simplified to linear isotherm. Holthaus et al. (2002) explained that Lai's results were possibly due to the higher loading of estrogens (10 to 1000 μ/L). Schäfer et al. (2002) and Urase and Kikuta (2005b) also claimed that a linear adsorption isotherm is adequate in the analysis with small concentration ranges.

For substances with log K_d values lower than 2, Clara et al. (2004) claimed that removal by sorption is negligible, but it can be a major removal pathway when log K_d values above 4. For most environmental compartments such as soil, sand and sediment, log K_d

values of estrogens are in the range of 0.5 to 2.5 (Holthaus et al., 2002; Loffredo and Senesi 2002; Lee et al., 2003; de Mes et al., 2005; Andersen et al., 2005a; Hildebrand et al., 2006). Therefore, adsorption in these natural systems is not considered as very significant. This conclusion was supported by the results from Holthaus et al. (2002) and Andersen et al. (2005a), which claimed less than 3% of estrogens would be absorbed by suspended sediments in river and suspended solids in WWTP effluent.

Compared with sand, soil and sediments in natural systems, there is much higher fraction of organic carbon in activated sludge. Both the measured and calculated log K_d values using Equation (11) are in a range of 2 to 4 for estrogens (de Mes et al., 2005; Andersen et al., 2005a). Therefore, higher adsorption potential was observed in activated sludge (Clara et al., 2004). However, due to the recycling of sludge with biological treatment, only a small amount of estrogens can actually be removed via the excess sludge disposal. Andersen et al. (2005a) calculated that only 1.5 to 1.8 % of estrogens can be removed at typical *mixed liquid suspended solids* (MLSS) of 4 g/L, despite adsorption rates of 61 ± 9% for E1, 66 ± 13% for E2 and 70 ± 6% for EE2. Another calculation carried out by de Mes et al. (2005) also concluded that less than 5% of E1, E2 and EE2 can be removed with excess sludge discharge.

3.4.2.5 Chemical oxidation

The removal by chemical oxidation was shown to be very effective. The basic mechanism is to generate hydroxyl free radicals (OH·) by adding strong oxidisers such as Cl_2, ClO_2, O_3, H_2O_2, FeO_4^{2-}, $S_2O_4^{2-}$ and MnO_2 (de Rudder et al., 2004; de Mes et al., 2005; Liu et al., 2009a). Chlorine is a widely used disinfectant in water and wastewater

industry. The oxidants, HOCl and OCl^- are formed by adding chlorine to water, which are capable of reacting with the phenolic ring in EDCs, leading to subsequent ring cleavage (Ying et al., 2002). However, due to the formation of many *disinfection by-products* (DBP), the estrogenic activities are not reduced significantly (Liu et al., 2009a).

In contrast, O_3 and H_2O_2 are more effective to reduce the overall estrogenic activities. Ozone can directly or indirectly oxidize EDCs by producing hydroxyl radicals. Direct oxidation with molecular O_3 dominates at acidic conditions whilst hydroxyl radicals dominate at higher pH conditions (Ying et al., 2002). The production of hydroxyl radicals can be facilitated by the exposure to UV light, the addition of H_2O_2 or other measures (Ying et al., 2004a). For example, Rosenfeldt and Linden (2004) showed removal efficiency of over 90% for BPA, E2 and EE2 using H_2O_2 coupled with UV. Chen et al. (2007) also experimented with the same combination (UV/H_2O_2) and achieved similar removal efficiencies. Interestingly, Snyder et al. (2006) found that the combination of O_3 and H_2O_2 increased the removal of dilantin, diazepam, DEET, iopromide, and meprobamate, but reduced the removal of pentoxifylline, caffeine, testosterone, progesterone, and androstenedione. Their results also showed a complete removal of estrogens within 24 minutes by either O_3 alone or combined with H_2O_2. Ermawati et al. (2007) achieved the complete removal of estrogens within 1 hour using ozone oxidation. However, in a review on ozone oxidation conducted by Ning et al. (2007), he concluded that phthalates are relatively stable to ozone oxidation. Probably the structures of phthalates are resistant to hydroxyl radicals attack.

Other oxidants were also investigated by many authors. For example, de Rudder et al. (2004) used manganese oxide (MnO_2) to remove EE2 and achieved a removal efficiency of 81.7 %. They found that EE2 was not only degraded, but also adsorbed to MnO_2 granules. If a self-regenerating cycle can be set up, this method would be cost-effective. Jiang et al. (2007) and Sharma (2007) reviewed the use of ferrate (VI) to remove water pollutants. They concluded that the ferrate (VI) not only possesses the highest redox potential, but also produces non-toxic by-products. In addition, the ferric oxide produced can act as an effective coagulant to remove metals, non-metals, radionuclides and humic acids (Sharma et al., 2009).

3.4.2.6 Membrane filtration

Membrane filtration includes microfiltration (MF, macropores > 50 nm), ultrafiltration (UF, mesopores between 2 to 50 nm), nanofiltration (NF, micropores < 2 nm), reverse osmosis (RO, dense < 2 nm), dialysis and electrodialysis (ED) (de Mes et al., 2005). Membrane filtration has been used to remove a wide range of micropollutants, such as pharmaceuticals, fragrances, hormones, disinfection by-products and pesticides (Kimura et al., 2003; Clara et al., 2005a; Bodzek and Dudziak 2006; Ozaki et al., 2008). Three major removal mechanisms in membrane filtration are size exclusion, charge repulsion and adsorption (Liu et al., 2009a). The removal efficiency varies from about 10 to over 99.9% (Bodzek and Dudziak 2006; Snyder et al., 2007; Yoon et al., 2007). Liu et al. (2009a) argued that the removal of EDCs is strongly dependent on their physicochemical properties (e.g. molecular weight, water solubility, K_{ow} and electrostatic property) and membrane types. Both Kimura et al. (2004) and Jung et al. (2007) observed a linear relationship between molecular weight and the removal

efficiency (or the rejection rate). Good removal was found with higher molecular weight. Jung et al. (2007) found that higher removal was associated with higher K_{ow} values. The removal efficiency is also affected by other factors such as membrane fouling and organic solutes. Agenson and Urase (2007) observed that organic fouling increased the rejection of BPA with NF, but decreased the rejection with RO. Chang et al. (2003) studied the effects of organic solutes, and observed smaller rejection with surface water and secondary effluent when compared with buffer solution. Schafer et al. (2003) investigated the effects of pH on estrone rejection. They found that the removal declined at higher pH because the adsorption was reduced.

3.4.3 EDCs removal in preliminary and primary treatment processes

Preliminary treatment mainly uses screens, comminutors and grit chamber to remove coarse materials (e.g. sand and gravel) and large floating objects (e.g. paper, rags and toys). The wastes are collected and then landfilled or incinerated. Preliminary treatment removes very little suspended or soluble organic matter (von Sperling 2007). Therefore, the removal of EDCs at this stage is negligible.

After the removal of coarse materials, wastewater enters the primary treatment units such as sedimentation tanks. Generally, primary treatment can remove 60 - 70% of suspended solids, which contain a large amount of organic matter. Accordingly, about 25 to 40% of *biological oxygen demand* (BOD) is removed at this stage (von Sperling 2007). The removal of EDCs is largely dependent on their adsorption to solids. As the log K_{oc} values summarised in Table 1 indicate, the 7 investigated EDCs have moderate to strong tendency of partitioning into organic matter. Therefore, a proportion of EDCs

is expected to be removed via the removal of suspended solids in primary treatment. For example, in a study conducted by Braga et al. (2005c), they observed that 14% of E1 and 5% of E2 were removed in a primary treatment plant. However, Muller et al. (2008a) observed similar concentration and estrogenicity in the influent and primary treated effluent, indicating weak removal by primary treatment. Therefore, it can be concluded that generally, the removal of EDCs by primary treatment would be less than 20%.

3.4.4 EDCs removal in secondary treatment processes

3.4.4.1 Aerobic, anaerobic and anoxic biodegradation

Degradation refers to '*the breakdown of the original molecule by the loss of the various component parts or by the fragmentation of the molecular into smaller substances*' (Connell 2005). Biodegradation is the degradation mediated by microorganisms. The biodegradation of estrogens can be regarded as first-order reactions described by Equation (12) (Johnson and Sumpter 2001; Jurgens et al., 2002; Andersen et al., 2004):

$$C_t = C_0 e^{-kt} \tag{12}$$

where C_t is the concentration at time t, C_0 is the initial concentration and k is the degradation rate constant. From Equation (12), the half-life can be calculated:

$$t_{1/2} = \frac{0.693}{k} \tag{13}$$

Biodegradation can be divided into aerobic, anoxic and anaerobic biodegradation. For most organic pollutants, aerobic biodegradation is the fastest, followed by slower anoxic and anaerobic biodegradation (de Mes et al., 2005). Generally, aerobic activated sludge process is able to remove over 80% of E2, EE2, NP, OP and BPA and over 60% of E1 and E3 (see Table 7 in Section 3.4.5).

The degradation rate was slightly slower for E2 at anaerobic conditions, but much slower for other estrogens (Andersen et al., 2004). The information on the anaerobic degradation of estrogens is limited in the literature. Czajka and Londry (2006) used lake water and sediment to study the anaerobic degradation of E2 and EE2 with initial concentrations both at 5 mg/L. They did not observe the degradation of EE2 over a three-year incubation period, but E2 was transformed into E1 at the rate of 99-176 μg/L/d. Jürgens et al. (2002) also found E2 was rapidly converted into E1 after an incubation of 2 days. Lee and Liu (2002a) used the supernatant of activated sludge to study anaerobic degradation of E2 and found about half of E2 was converted into E1 in 7 days. EE2 was also tested with river water under anaerobic conditions and no degradation was found over 46 days (Jurgens et al., 1999). The degradation at anoxic conditions is faster than anaerobic conditions. Joss et al. (2004) observed an half-life of 5.6 hours for EE2 under anoxic conditions compared to 11 hours under anaerobic conditions.

3.4.4.2 Factors affecting biodegradation

Biodegradation is affected by a number of factors, such as initial concentration (Ternes et al., 1999a), temperature (Li et al., 2005), pH (Kikuta and Urase 2003), retention time and the level of degrading bacteria (Andersen et al., 2004; Shi et al., 2004a; Li et al., 2005). High initial concentration sometimes can damage the proper functions of microorganisms. For example, it was found that initial concentration of EE2 over 10 mg/L exhibited toxic effects to microorganisms (Kozak et al., 2001). Urase and Kikuta (2005b) found acidic condition was preferable for the removal of estrogens, because acidic conditions increase the adsorption potential, which leads to faster transfer of

target compounds from the water phase to the sludge phase. Their results summarised in Table 3 showed that E1 and EE2 had highest degradation rates at pH 5.6, but E2 preferred lower pH of 4.4. However, pH variations in WWTP are generally small, which have minor impacts on biodegradation.

Table 3. Effects of pH on degradation rate constant (h^{-1})

pH	Degradation rate constant (h^{-1})		
	E1	E2	EE2
4.4	0.167	13.329	0.088
5.6	0.263	8.390	0.105
6.7	0.109	6.839	0.013
7.0	0.121	2.423	0.014

Source: (Urase and Kikuta 2005b).

In addition to pH, the *hydraulic retention time* (HRT) and *solids retention time* (SRT) are another two important parameters affecting biodegradation of EDCs. Longer HRT and SRT increase the removal efficiency of estrogens (Johnson et al., 2000; Johnson and Sumpter 2001; Andersen et al., 2004; Kreuzinger et al., 2004; Lee et al., 2004b). In WWTP with activated sludge process, HRT is normally between 10 - 14 hours and SRT between 6-25 days. Long SRT allows enough time for slow-growing bacteria to reproduce and consequently to establish a more diverse biocoenosis with broader physiological capabilities (Clara et al., 2005b). SRT does not only influence the biota, but also the characteristics and affinity of floc particles as sorbents (Johnson et al., 2000; Holbrook et al., 2002).

Compared with pH, HRT and SRT, temperature has a more profound impact on the biodegradation process. Layton et al. (2000) studied the biodegradation of E2 under two temperature ranges of 5 to 10 ℃ and 22 to 25 ℃. Their results showed faster

mineralization rates at the higher temperature range. In addition, the results summarised in Table 4 from studies conducted by Jürgens et al. (2002) and Li et al. (2005) also supported the same conclusion that EDCs degrades faster at higher temperature in both river water and activated sludge.

Table 4. Effects of temperature on degradation rate constant

Environment	T (℃)	k	
		E2	EE2
River water	5 - 10	4.2 d^{-1}	0.14 d^{-1}
	20 - 25	6.0 d^{-1}	0.29 d^{-1}
Activated sludge	5	2.12 h^{-1}	-
	20	3.26 h^{-1}	-
	35	4.79 h^{-1}	-

Source: (Jurgens et al., 2002; Li et al., 2005).

3.4.4.3 Microorganisms and degradation pathways

Microorganisms including bacteria, fungi, algae, protozoans and viruses play significant role in the biodegradation process (Connell 2005). To study the ability of specific species, Shi et al. (2002a) investigated the degrading ability of *Fusarium proliferatum strain HNS-1*. They found it was able to remove 97 % of EE2 at an initial concentration of 25 mg/L over 15 days with a first-order rate constant of 0.6 d^{-1}. Fujii et al. (2002) also isolated a gram-negative bacterium, a new *novosphingobium species* from a WWTP in Tokyo. They found that E2 can be degraded over 50 days at 1 mg/ml medium. Shi et al. (2004a) did a more comprehensive study on the degradation of estrogens using *nitrifying activated sludge* (NAS) and ammonia-oxidizing bacterium, the *nitrosomonas europaea*. Using NAS, they found the reaction obeyed first-order kinetics with degradation rate constants of 0.056 h^{-1} for E1, 1.3 h^{-1} for E2, 0.030 h^{-1} for E3 and 0.035 h^{-1} for EE2. Using *nitrosomonas europaea*, the degradation seems to obey zero-order

kinetics and the four degradation rates were similar. Also, they didn't find E1 during the degradation of E2, which implicated that E1 was not an intermedia degradation product of E2 by this bacterium. Vader et al. (2000) also used NAS capable of degrading EE2 with a maximum rate of 1 μg/g, dry weight/h at an initial EE2 concentration of 50 μg/L.

Ren et al. (2007) isolated a bacterium, *sphingobacterium sp. JCR5*, from the activated sludge of a WWTP in a factory producing oral contraceptives (EE2) in China. They reported that 87 % of the substrate (30 mg/L) added was metabolized within 10 days at 30 ℃. A degradation pathway of EE2 by strain JCR5 was proposed in Figure 9. They suggested that EE2 was firstly degraded into E1, and then a cleavage occurred in the B ring. Lee and Liu (2002a) argued that E2 and its metabolites were not persistent and could be easily degraded by sewage bacteria. The reaction was initiated at the D ring and E1 was produced. After that, they noticed new and unreported metabolites. Coombe at el. (1996) found that leakage can be initiated at the A ring of E1 by *Nocardia sp. (E 110)*, leading to the formation of dicarboxylic acid intermediate. From the above results, the degradation pathways of E1 and E2 were proposed and summarised in Figure 10. Ziegler et al. (1997) evaluated the validity and reproducibility of using immunoassay to quantify estrogen metabolism in women. He found that E2 was firstly transformed into E1, then into hydroxylestrones, hydroxyestradiol, E3 and other metabolites, which was shown in Figure 11. Sun et al. (2005) proposed three fragmentation pathways for E1 in Figure 12, suggesting that cleavage can also occur in the D ring.

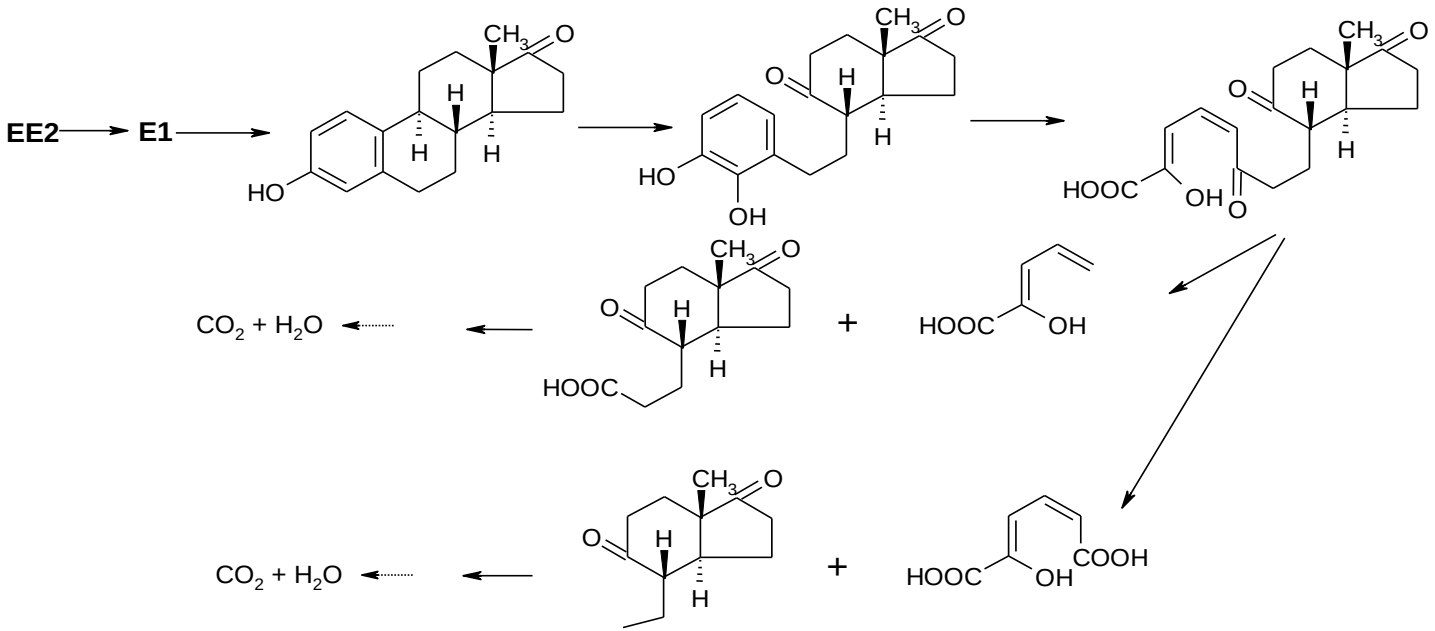

Figure 9. Degradation pathways of EE2 by activated sludge bacteria *JCR5* (Ren et al., 2007).

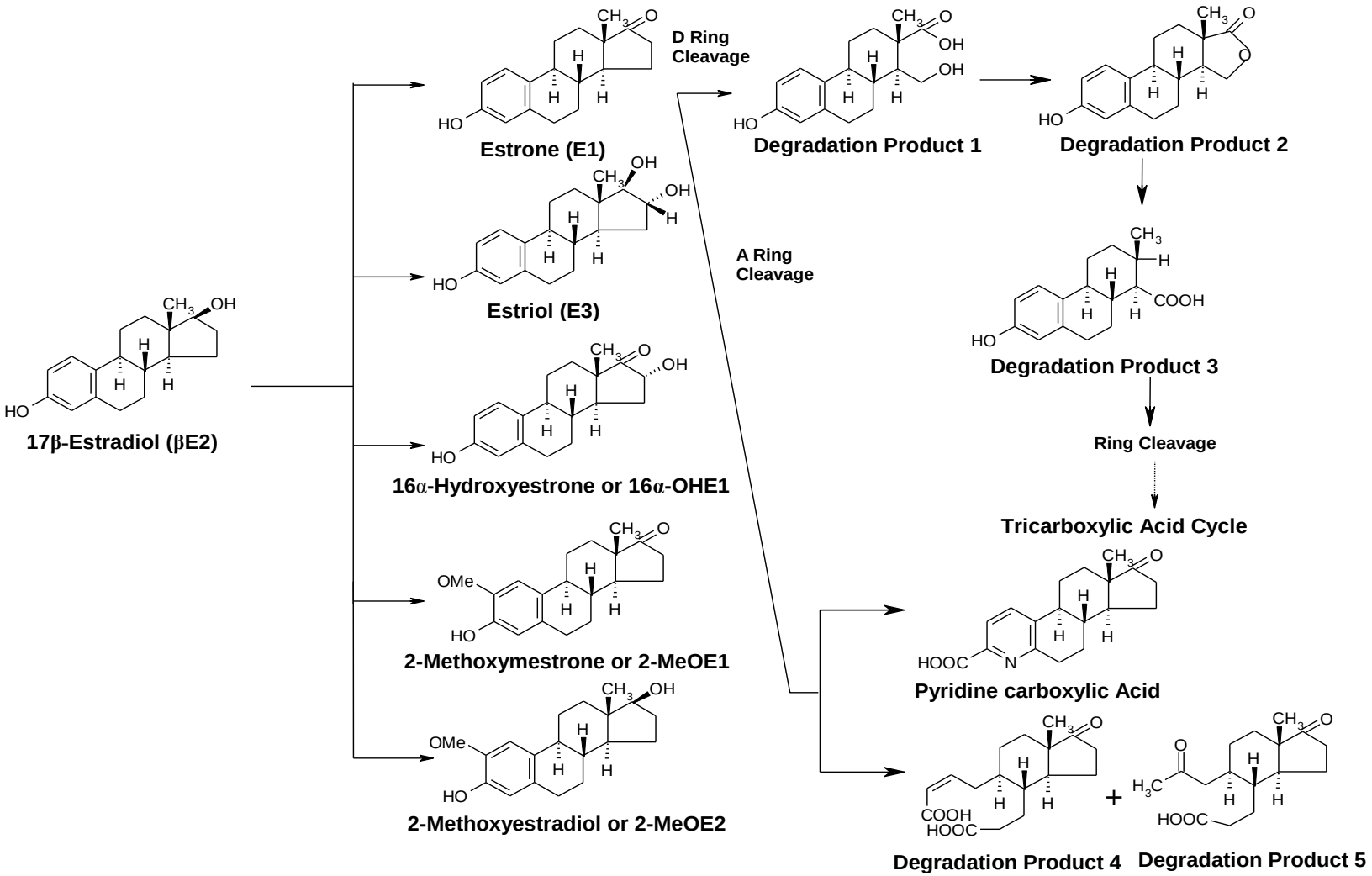

Figure 10. Degradation pathways of E2 and E1 by sewage bacteria (Coombe et al., 1996; Lee and Liu 2002a).

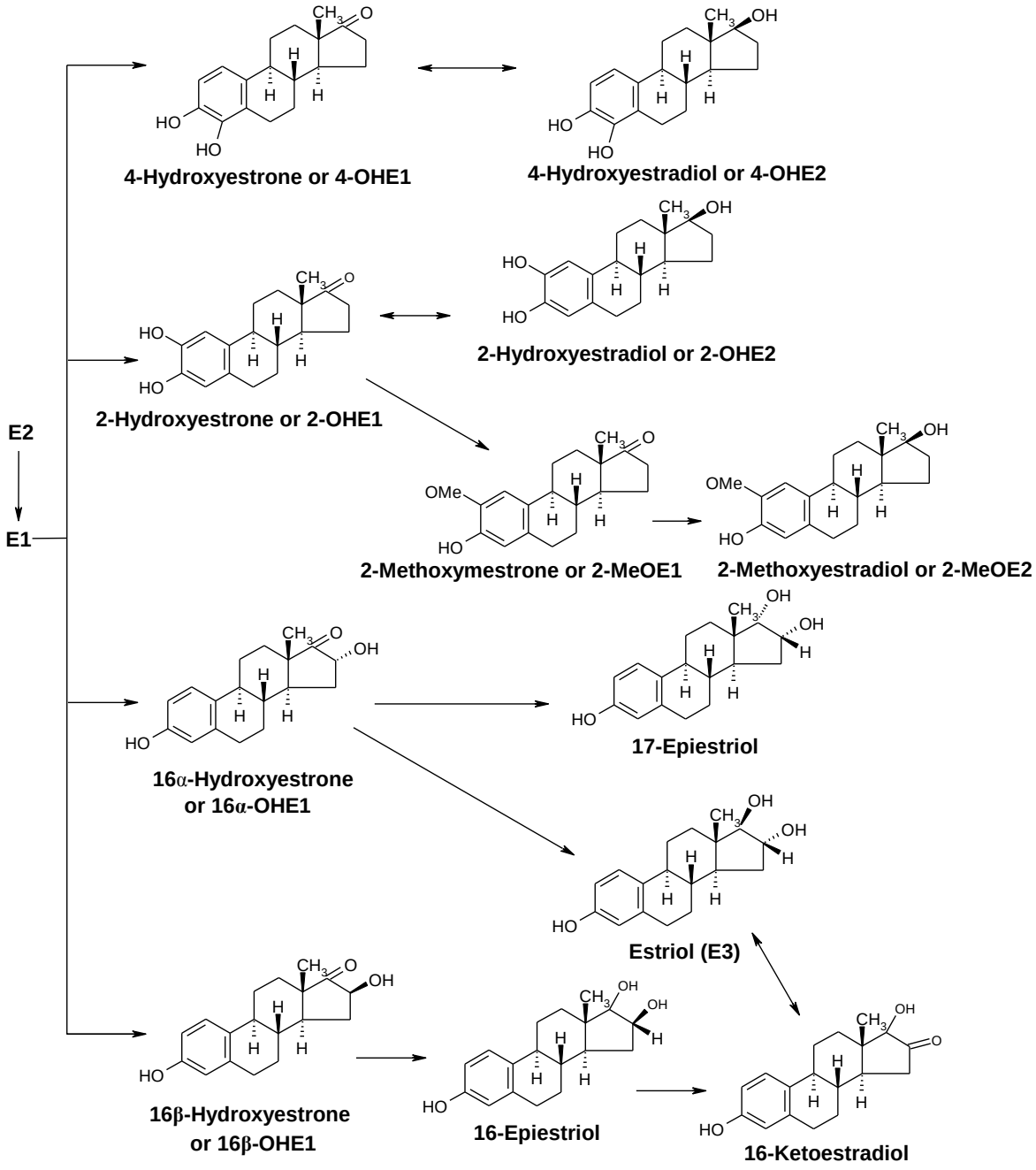

Figure11. Metabolic transformation of E2 and E1 in women (Ziegler et al., 1997).

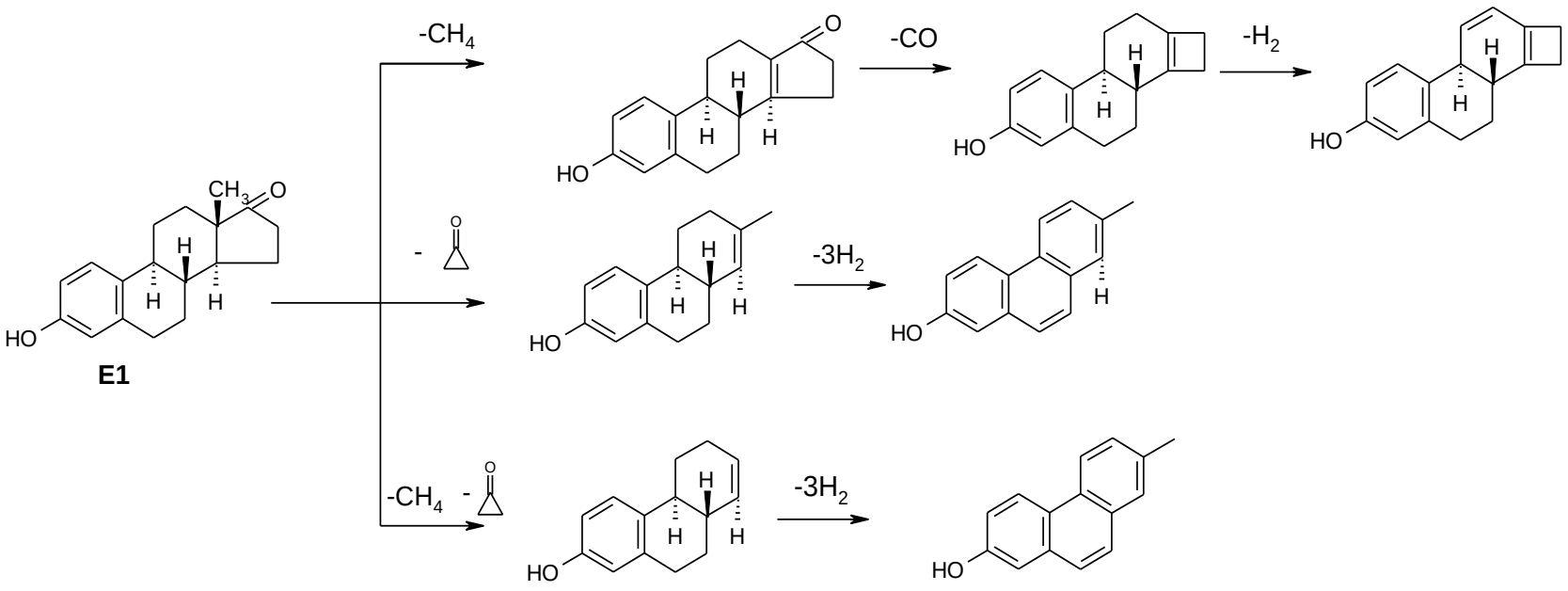

Figure12. Proposed fragmentation pathways of E1 (Sun et al., 2005).

3.4.5 Concentration of EDCs in WWTP influent and effluent

Since the 1990s, research on EDCs in wastewater has been increasing in scientific communities (Birkett and Lester 2003; Khanal et al., 2006). A substantial amount of data on the concentration measurement has been published in the literature. In order to know the current level of EDCs in WWTP, a worldwide survey of influent and effluent concentration were presented in Tables 5 and 6 for estrogens and phenolic compounds. The data was collected from 8 highly industrialized countries including Australia, Austria, Canada, France, Germany, Italy, The Netherlands, UK, and the largest developing country, China. The majority of surveyed WWTPs were equipped with activated sludge process. Generally, influent concentration of EDCs is normally higher from those WWTPs receiving large amount of industrial discharges, or located in highly urbanized regions.

From the summarised concentration values in Tables 5 and 6, something interesting was observed with the data from Lee et al. (2005a), Tan et al. (2007c), Nelson et al. (2007), Clara et al. (2005a) and Fernandez et al., (2007), that the effluent concentration of E1 was higher than the influent concentration. This can be explained by two reasons. The first one is the deconjugation of E1 in WWTP. As it was discussed in Section 3.4.1, sulphate conjugates are relatively more stable than glucuronide conjugates and they can partly survive the wastewater treatment (Johnson and Williams 2004). For example, D'Ascenzo et al. (2003) found E1-3S was still presented in the effluent. So, the release of free E1 from the deconjugation of E1-3S may partly contribute to concentration increase after treatment. The second reason is the oxidation of E2 into E1, which also increases the concentration of E1. The chemical structure of E2 has two hydroxyl

groups at the C3 and C17 position (see Figure 2), which are susceptible to microbial attack (Ying and Kookana 2003a). When the hydroxyl group at the C17 position is oxidised into a more hydrophobic ketone group by microbial action, E2 is transformed into E1 (Fukuhara et al., 2006), leading to the increase of E1 in the effluent.

A second observation from Tables 5 and 6 is that the concentration of phenolic compounds NP, OP and BPA was significantly higher than estrogens both in the influent and effluent. The concentration of estrogens was normally 10's ng/L in influent and several ng/L in effluent. In contrast, phenolic compounds were 100's to 1000's ng/L in influent and 10's to 100's ng/L in effluent. As discussed earlier in Section 3.3, phenolic compounds are industrial chemicals with annual production of millions of tonnes, whilst estrogens are mainly excreted by human and animal body. Therefore, their differences in sources also contributed to their concentration differences in influent and effluent.

The minimum, maximum, mean and median values from Tables 5 and 6 were collated in Table 7 for each compound. The median concentrations of estrogens were in the order of E1 (35 ng/L) > E3 (19 ng/L) > E2 (14 ng/L) > EE2 (3.2 ng/L) in influent, and E1 (10 ng/L) > E2 (2.7 ng/L) > E3 (1.5 ng/L) > EE2 (0.3 ng/L) in effluent. Apparently, the highest and lowest concentrations were found with E1 and EE2 respectively. For phenolic compounds, the median concentration were in the order of NP (2117 ng/L) > BPA (400 ng/L) > OP (248 ng/L) in influent and NP (1029 ng/L) > OP (40 ng/L) > BPA (31 ng/L) in effluent.

The individual removal efficiency for each set of influent and effluent concentration was calculated and listed in Tables 5 and 6. The median removal efficiency was obtained by ranking these individual removal efficiencies. For each compound, the median removal efficiency was ranked in the order of OP (90%) > E2 (87%) > NP (83%) > EE2 (81%) ≈ BPA (81%) > E3 (67%) > E1 (63%). It seems the removal efficiencies of these 7 EDCs can be divided into two groups: E1 and E3 as the group with lower removal efficiency and the remaining as the group with higher removal efficiency. Interestingly, the removal difference between these two groups related to their difference in log K_{ow} values. The log K_{ow} values of E1 and E3 in Table 1 are smaller than those of the other EDCs. As the transfer of EDCs from the water phase to the sludge phase is partly limited by the log K_{ow} value, which affects their removal.

Table 5. Measured concentration (ng/L) of estrogens in WWTP influent and effluent [a]

Country	E1			E2			E3			EE2			Reference
	Inf	Eff	η (%)	Inf	Eff	η (%)	Inf	Eff	η (%)	Inf	Eff	η (%)	
Italy	44	17	61	11	1.6	85	72	2.3	97	n.a.	n.a.		(D'Ascenzo et al., 2003)
Italy	35	16	54	25	6	76	31	1	97	n.d.	n.d.		(Lagana et al., 2004)
Italy	71	9.7	86	16	1.5	91	n.a.	n.a.		4	0.39	90	(Baronti et al., 2000)
Italy	67	4.1	94	9	0.9	90	n.a.	n.a.		3.4	0.55	84	(Baronti et al., 2000)
Italy	51	44.6	13	14	2.4	83	n.a.	n.a.		2.5	0.47	81	(Baronti et al., 2000)
Italy	35	30.3	13	9	1.9	79	n.a.	n.a.		2.9	0.53	82	(Baronti et al., 2000)
Italy	50	7.7	85	9	0.7	92	n.a.	n.a.		2.3	0.35	85	(Baronti et al., 2000)
Italy	37	13.8	63	11	1.0	91	n.a.	n.a.		2.9	0.38	87	(Baronti et al., 2000)
Germany	66	n.d.		63	n.d.		n.a.	n.a.		8	n.d.		(Andersen et al., 2003)
Netherlands	n.a.	2.7		n.a.	n.a.		n.a.	n.a.		n.a.	1.4		(Belfroid et al., 1999)
Netherlands	n.a.	15		n.a.	1.1		n.a.	n.a.		n.a.	0.2		(Belfroid et al., 1999)
Netherlands	n.a.	0.4		n.a.	n.a.		n.a.	n.a.		n.a.	1.8		(Belfroid et al., 1999)
Netherlands	n.a.	6.3		n.a.	0.7		n.a.	n.a.		n.a.	0.2		(Belfroid et al., 1999)
Netherlands	n.a.	2.1		n.a.	0.6		n.a.	n.a.		n.a.	0.3		(Belfroid et al., 1999)
Netherlands	n.a.	47		n.a.	12		n.a.	n.a.		n.a.	7.5		(Belfroid et al., 1999)
Netherlands	n.a.	11		n.a.	0.6		n.a.	n.a.		n.a.	1.8		(Belfroid et al., 1999)
Netherlands	n.a.	0.7		n.a.	1.8		n.a.	n.a.		n.a.	2.6		(Belfroid et al., 1999)
Netherlands	n.a.	0.4		n.a.	0.7		n.a.	n.a.		n.a.	0.3		(Belfroid et al., 1999)
Netherlands	n.a.	0.1		n.a.	0.4		n.a.	n.a.		n.a.	0.2		(Belfroid et al., 1999)

[a] - n.a. - not analysed; n.d. - not detected; Inf - influent; Eff - effluent; η - removal efficiency.

Table 5. Measured concentration (ng/L) of estrogens in WWTP influents and effluents (Cont'd)

Country	E1			E2			E3			EE2			Reference
	Inf	Eff	η (%)	Inf	Eff	η (%)	Inf	Eff	η (%)	Inf	Eff	η (%)	
Germany	n.a.	9		n.a.	n.d.		n.a.	n.a.		n.a.	1		(Ternes et al., 1999b)
Canada	n.a.	3		n.a.	6		n.a.	n.a.		n.a.	9		(Ternes et al., 1999b)
Germany	n.a.	1.5		n.a.	0.4		n.a.	n.a.		n.a.	0.7		(Kuch and Ballschmiter, 2001a)
France	17.6	7.2	59	11.1	4.5	59	14.9	7.3	51	5.4	3.1	43	(Cargouet et al., 2004)
France	15.2	6.5	57	17.4	7.2	59	15.2	5.0	67	7.1	4.4	38	(Cargouet et al., 2004)
France	9.6	4.3	55	11.6	6.6	43	12.3	5.7	54	4.9	2.7	45	(Cargouet et al., 2004)
France	11.2	6.2	45	17.1	8.6	50	11.4	6.8	40	6.8	4.5	34	(Cargouet et al., 2004)
Canada	52	11	79	3	2	33	n.a.	n.a.		n.a.	n.a.		(Lee et al., 2005a)
Canada	15	27	-80	13	2	85	n.a.	n.a.		n.a.	n.a.		(Lee et al., 2005a)
Canada	10	2	80	10	n.d.		n.a.	n.a.		n.a.	n.a.		(Lee et al., 2005a)
Canada	8	n.d.		14	n.d.		n.a.	n.a.		n.a.	n.a.		(Lee et al., 2005a)
Canada	18	5	72	5	2	60	n.a.	n.a.		n.a.	n.a.		(Lee et al., 2005a)
Canada	11	2	82	7	n.d.		n.a.	n.a.		n.a.	n.a.		(Lee et al., 2005a)
Canada	39	54	-38	22	2	91	n.a.	n.a.		n.a.	n.a.		(Lee et al., 2005a)
Canada	16	5	69	5	n.d.		n.a.	n.a.		n.a.	n.a.		(Lee et al., 2005a)
UK	n.a.	48.0		n.a.	48.0		n.a.	n.a.		n.a.	7.0		(Desbrow et al., 1998)
UK	n.a.	45.0		n.a.	42.0		n.a.	n.a.		n.a.	n.d.		(Desbrow et al., 1998)
UK	n.a.	32.0		n.a.	29.0		n.a.	n.a.		n.a.	n.d.		(Desbrow et al., 1998)
UK	n.a.	5.2		n.a.	3.7		n.a.	n.a.		n.a.	n.d.		(Desbrow et al., 1998)
UK	n.a.	8.5		n.a.	7.1		n.a.	n.a.		n.a.	n.d.		(Desbrow et al., 1998)

Table 5. Measured concentration (ng/L) of estrogens in WWTP influents and effluents (Cont'd)

Country	E1			E2			E3			EE2			Reference
	Inf	Eff	η (%)	Inf	Eff	η (%)	Inf	Eff	η (%)	Inf	Eff	η (%)	
UK	n.a.	8.9		n.a.	4.4		n.a.	n.a.		n.a.	n.d.		(Desbrow et al., 1998)
UK	n.a.	3.6		n.a.	2.7		n.a.	n.a.		n.a.	n.d.		(Desbrow et al., 1998)
UK	n.a.	1.8		n.a.	5.5		n.a.	n.a.		n.a.	n.d.		(Desbrow et al., 1998)
UK	n.a.	2.1		n.a.	6.3		n.a.	n.a.		n.a.	n.d.		(Desbrow et al., 1998)
UK	n.a.	13.0		n.a.	12.0		n.a.	n.a.		n.a.	n.d.		(Desbrow et al., 1998)
UK	n.a.	2.0		n.a.	4.9		n.a.	n.a.		n.a.	n.d.		(Desbrow et al., 1998)
UK	n.a.	9.4		n.a.	4.3		n.a.	n.a.		n.a.	n.d.		(Desbrow et al., 1998)
UK	n.a.	76.0		n.a.	10.0		n.a.	n.a.		n.a.	4.3		(Desbrow et al., 1998)
UK	n.a.	15.0		n.a.	6.5		n.a.	n.a.		n.a.	0.6		(Desbrow et al., 1998)
UK	n.a.	48.0		n.a.	9.8		n.a.	n.a.		n.a.	1.9		(Desbrow et al., 1998)
UK	n.a.	6.1		n.a.	4.9		n.a.	n.a.		n.a.	0.2		(Desbrow et al., 1998)
UK	n.a.	10.0		n.a.	5.7		n.a.	n.a.		n.a.	0.6		(Desbrow et al., 1998)
UK	n.a.	12.0		n.a.	4.0		n.a.	n.a.		n.a.	0.8		(Desbrow et al., 1998)
UK	n.a.	6.4		n.a.	6.1		n.a.	n.a.		n.a.	n.d.		(Desbrow et al., 1998)
UK	n.a.	1.4		n.a.	7.4		n.a.	n.a.		n.a.	n.d.		(Desbrow et al., 1998)
UK	n.a.	9.9		n.a.	6.9		n.a.	n.a.		n.a.	n.d.		(Desbrow et al., 1998)
UK	n.a.	6.4		n.a.	1.6		n.a.	3.0		n.a.	n.d.		(Xiao et al., 2001)
UK	n.a.	9.8		n.a.	2.6		n.a.	2.0		n.a.	n.d.		(Xiao et al., 2001)
UK	n.a.	29		n.a.	7.4		n.a.	4.0		n.a.	n.d.		(Xiao et al., 2001)
UK	59	n.a.		224	n.a.		n.a.	n.a.		n.a.	n.a.		(Jiang et al., 2005b)

Table 5. Measured concentration (ng/L) of estrogens in WWTP influents and effluents (Cont'd)

Country	E1			E2			E3			EE2			Reference
	Inf	Eff	η (%)	Inf	Eff	η (%)	Inf	Eff	η (%)	Inf	Eff	η (%)	
UK	57	n.a.		132	n.a.		n.a.	n.a.		n.a.	n.a.		(Jiang et al., 2005b)
UK	81.0	n.a.		188.7	n.a.		n.a.	n.a.		72.4	n.a.		(Jiang et al., 2005b)
UK	77.8	n.a.		182.6	n.a.		n.a.	n.a.		123.5	n.a.		(Jiang et al., 2005b)
Netherlands	60.5	n.a.		36.5	n.a.		n.a.	n.a.		3.2	n.a.		(Vethaak et al., 2005)
Netherlands	46	n.a.		31	n.a.		n.a.	n.a.		3.8	n.a.		(Vethaak et al., 2005)
Netherlands	n.a.	3.4		n.a.	n.d.		n.a.	n.a.		n.a.	2.6		(Vethaak et al., 2005)
Australia	54.8	n.d.		22.0	n.d.		n.a.	n.a.		n.d.	n.d.		(Braga et al., 2005a)
Australia	58.0	54.0		14.0	14.0		n.a.	n.a.		n.d.	n.d.		(Braga et al., 2005a)
France	n.a.	12.4		n.a.	14.8		n.a.	n.d.		n.a.	n.d.		(Vulliet et al., 2007)
France	n.a.	196.7		n.a.	n.d.		n.a.	n.d.		n.a.	n.d.		(Vulliet et al., 2007)
France	n.a.	20.8		n.a.	9.4		n.a.	n.d.		n.a.	n.d.		(Vulliet et al., 2007)
France	n.a.	18.7		n.a.	6.1		n.a.	n.d.		n.a.	n.d.		(Vulliet et al., 2007)
France	n.a.	28.1		n.a.	28.1		n.a.	n.d.		n.a.	5.6		(Vulliet et al., 2007)
France	n.a.	9.9		n.a.	n.d.		n.a.	n.d.		n.a.	n.d.		(Vulliet et al., 2007)
France	n.a.	66.4		n.a.	42.6		n.a.	n.d.		n.a.	n.d.		(Vulliet et al., 2007)
Australia	n.d.	n.a.		n.d.	n.a.		n.a.	n.a.		n.a.	n.a.		(Tan et al., 2007a)
Australia	14.5	n.a.		n.d.	n.a.		n.a.	n.a.		n.a.	n.a.		(Tan et al., 2007a)
Australia	37.5	n.a.		12.2	n.a.		n.a.	n.a.		n.a.	n.a.		(Tan et al., 2007a)
Australia	13.1	41.9	-220	16.6	1.6	90	110	n.d.		n.a.	n.a.		(Tan et al., 2007c)
Australia	1.7	n.d.		3.2	n.d.		n.d.	n.d.		n.a.	n.a.		(Tan et al., 2007c)

Table 5. Measured concentration (ng/L) of estrogens in WWTP influents and effluents (Cont'd)

Country	E1			E2			E3			EE2			Reference
	Inf	Eff	η (%)	Inf	Eff	η (%)	Inf	Eff	η (%)	Inf	Eff	η (%)	
Australia	8.3	n.d.		18.1	2.9	84	111	n.d.		n.a.	n.a.		(Tan et al., 2007c)
Australia	n.d.	1.3		226	n.d.		185	n.d.		n.a.	n.a.		(Tan et al., 2007c)
Australia	18.3	6.7	63	221	n.d.		155	n.d.		n.a.	n.a.		(Tan et al., 2007c)
Canada	3.3	27.2	-724	1.2	11.2	-833	9.1	4.9	46	n.a.	n.a.		(Nelson et al., 2007)
Canada	5.8	5.9	-2	1.9	2.0	-5	9.2	8.9	3	n.a.	n.a.		(Nelson et al., 2007)
Canada	3.4	24.1	-609	1.5	0.7	53	10.2	4.9	52	n.a.	n.a.		(Nelson et al., 2007)
Canada	5.7	1.3	77	0.2	0.1	50	12.4	4.9	60	n.a.	n.a.		(Nelson et al., 2007)
Canada	8.4	8.7	-4	1.9	1.7	11	11.7	8.2	30	n.a.	n.a.		(Nelson et al., 2007)
China	38.6	12.6	67	21.4	4.4	79	53.9	n.d.		n.a.	n.a.		(Jin et al., 2008)
Austria	34	72	-112	54	30	44	336	275	18	8	5	38	(Clara et al., 2005a)
Austria	51	8	84	35	n.d.		23	17	26	4	3	25	(Clara et al., 2005a)
Austria	670	n.d.		46	n.d.		143	n.d.		70	n.d.		(Clara et al., 2005a)
Austria	71	4	94	67	n.d.		326	n.d.		20	4	80	(Clara et al., 2005a)
Canada	9	41	-356	5	5	0	9	1	89	1	17	-1600	(Fernandez et al., 2007)
Canada	33	10	70	n.a.	n.a.		n.a.	n.a.		n.a.	n.a.		(Fernandez et al., 2007)
France	37.1	5.4	85	7.5	n.d.		n.a.	n.a.		n.d.	n.d.		(Stavrakakis et al., 2008)
Canada	30.2	13	57	8.1	n.d.		n.a.	n.a.		n.a.	n.a.		(Lishman et al., 2006)
Canada	49	17	65	15.6	1.8	88	n.a.	n.a.		n.a.	n.a.		(Servos et al., 2005)
Australia	n.a.	9.12		n.a.	1.37		n.a.	n.a.		n.a.	0.14		(Ying et al., 2009)
Australia	n.a.	9.30		n.a.	1.57		n.a.	n.a.		n.a.	0.11		(Ying et al., 2009)

Table 5. Measured concentration (ng/L) of estrogens in WWTP influents and effluents (Cont'd)

Country	E1			E2			E3			EE2			Reference
	Inf	Eff	η (%)	Inf	Eff	η (%)	Inf	Eff	η (%)	Inf	Eff	η (%)	
Australia	n.a.	25.59		n.a.	1.84		n.a.	n.a.		n.a.	0.36		(Ying et al., 2009)
Australia	n.a.	25.97		n.a.	1.64		n.a.	n.a.		n.a.	0.25		(Ying et al., 2009)
Australia	n.a.	32.22		n.a.	1.39		n.a.	n.a.		n.a.	0.40		(Ying et al., 2009)
Australia	n.a.	29.12		n.a.	5.69		n.a.	n.a.		n.a.	1.14		(Ying et al., 2009)
Australia	n.a.	21.33		n.a.	3.73		n.a.	n.a.		n.a.	0.57		(Ying et al., 2009)
Australia	n.a.	25.77		n.a.	6.35		n.a.	n.a.		n.a.	1.20		(Ying et al., 2009)
Australia	n.a.	17.64		n.a.	3.60		n.a.	n.a.		n.a.	0.75		(Ying et al., 2009)
Australia	n.a.	32.17		n.a.	4.71		n.a.	n.a.		n.a.	0.71		(Ying et al., 2009)
Australia	n.a.	20.6		n.a.	1.23		n.a.	n.a.		n.a.	0.24		(Williams et al., 2007)
Australia	n.a.	22.3		n.a.	5		n.a.	n.a.		n.a.	0.78		(Williams et al., 2007)
Australia	n.a.	5.76		n.a.	n.d.		n.a.	n.a.		n.a.	n.d.		(Williams et al., 2007)
Australia	n.a.	3.14		n.a.	1.42		n.a.	n.a.		n.a.	0.15		(Williams et al., 2007)
Australia	n.a.	39.3		n.a.	4.20		n.a.	n.a.		n.a.	0.40		(Williams et al., 2007)
Australia	n.a.	30.6		n.a.	6.22		n.a.	n.a.		n.a.	n.d.		(Williams et al., 2007)
Australia	n.a.	18.2		n.a.	3.60		n.a.	n.a.		n.a.	0.50		(Williams et al., 2007)
Australia	n.a.	20.9		n.a.	6.18		n.a.	n.a.		n.a.	0.28		(Williams et al., 2007)
Australia	n.a.	37.6		n.a.	2.80		n.a.	n.a.		n.a.	1.30		(Williams et al., 2007)
Australia	n.a.	12.7		n.a.	5.98		n.a.	n.a.		n.a.	0.19		(Williams et al., 2007)
Australia	n.a.	34.2		n.a.	3.90		n.a.	n.a.		n.a.	0.60		(Williams et al., 2007)
Australia	n.a.	30.9		n.a.	3.83		n.a.	n.a.		n.a.	0.41		(Williams et al., 2007)

Table 6. Measured concentration (ng/L) of phenolic compounds in WWTP influent and effluent

Country	NP			OP			BPA			Reference
	Inf	Eff	η (%)	Inf	Eff	η (%)	Inf	Eff	η (%)	
Italy	6573	1649	75	n.a.	n.a.		334	32	90	(Lagana et al., 2004)
Germany	n.a.	111		n.a.	14		n.a.	10	0	(Kuch and Ballschmiter 2001a)
Canada	18600	640	97	3240	40	99	1450	130	91	(Lee et al., 2005a)
Canada	24900	540	98	2840	20	99	1100	70	94	(Lee et al., 2005a)
Canada	11400	880	92	3560	40	99	2400	230	90	(Lee et al., 2005a)
Canada	25000	320	99	3350	10	100	690	20	97	(Lee et al., 2005a)
Canada	17900	3210	82	2980	320	89	2150	450	79	(Lee et al., 2005a)
Canada	9700	700	93	3180	170	95	580	250	57	(Lee et al., 2005a)
Canada	6240	2340	63	1590	470	70	2020	310	85	(Lee et al., 2005a)
Canada	2720	700	74	380	90	76	210	40	81	(Lee et al., 2005a)
UK	32	n.a.		85	n.a.		451	n.a.		(Jiang et al., 2005a)
UK	76	n.a.		112	n.a.		378	n.a.		(Jiang et al., 2005a)
UK	122.3	n.a.		545.7	n.a.		890	n.a.		(Jiang et al., 2005a)
UK	100.6	n.a.		611.1	n.a.		682.8	n.a.		(Jiang et al., 2005a)
Australia	120	n.a.		4.4	n.a.		n.d.	n.a.		(Tan et al., 2007c)
Australia	70.3	n.a.		2.6	n.a.		3.8	n.a.		(Tan et al., 2007c)
Australia	9610	n.a.		248	n.a.		704	n.a.		(Tan et al., 2007c)
Australia	3070	335	89	229	23.5	90	140	86.7	38	(Tan et al., 2007c)
Canada	968.3	1287.3	-33	n.a.	n.a.		44.6	61.1	-37	(Nelson et al., 2007)

[a] - n.a. - not analysed; n.d. - not detected; Inf - influent; Eff - effluent; η - removal efficiency.

Table 6. Measured concentration (ng/L) of phenolic compounds in WWTP influent and effluent (Cont'd)

Country	NP			OP			BPA			Reference
	Inf	Eff	η (%)	Inf	Eff	η (%)	Inf	Eff	η (%)	
Canada	680.2	591.7	13	n.a.	n.a.		41.9	45.4	-8	(Nelson et al., 2007)
Canada	647.3	313.7	52	n.a.	n.a.		51.2	17.3	66	(Nelson et al., 2007)
Canada	1513.2	207.5	86	n.a.	n.a.		71.8	2.9	96	(Nelson et al., 2007)
Canada	658.1	621.7	6	n.a.	n.a.		67.2	76.4	-14	(Nelson et al., 2007)
China	24791.6	4292.6	83	123.7	57.2	54	421.5	39.8	91	(Jin et al., 2008)
Austria	n.a.	n.a.		n.a.	n.a.		1710	1530	11	(Clara et al., 2005a)
Austria	n.a.	n.a.		n.a.	n.a.		1255	723	42	(Clara et al., 2005a)
Austria	n.a.	n.a.		n.a.	n.a.		720	125	83	(Clara et al., 2005a)
Austria	n.a.	n.a.		n.a.	n.a.		2025	26	99	(Clara et al., 2005a)
Canada	25912	10358	60	n.a.	n.a.		284	203	29	(Fernandez et al., 2007)
Canada	28207	4136	85	n.a.	n.a.		186	33	82	(Fernandez et al., 2007)
Canada	15427	1592	90	n.a.	n.a.		590	0	100	(Fernandez et al., 2007)
France	n.d.	3.4		16.3	24.6	-51	239.1	162.3	32	(Stavrakakis et al., 2008)
Australia	n.a.	1054		n.a.	26		n.a.	18		(Ying et al., 2009)
Australia	n.a.	1404		n.a.	30		n.a.	15		(Ying et al., 2009)
Australia	n.a.	1889		n.a.	46		n.a.	25		(Ying et al., 2009)
Australia	n.a.	1333		n.a.	45		n.a.	20		(Ying et al., 2009)
Australia	n.a.	2991		n.a.	56		n.a.	44		(Ying et al., 2009)
Australia	n.a.	614		n.a.	49		n.a.	13		(Ying et al., 2009)
Australia	n.a.	1029		n.a.	62		n.a.	31		(Ying et al., 2009)

Table 6. Measured concentration (ng/L) of phenolic compounds in WWTP influent and effluent (Cont'd)

Country	NP			OP			BPA			Reference
	Inf	Eff	η (%)	Inf	Eff	η (%)	Inf	Eff	η (%)	
Australia	n.a.	1679		n.a.	165		n.a.	20		(Ying et al., 2009)
Australia	n.a.	947		n.a.	55		n.a.	20		(Ying et al., 2009)
Australia	n.a.	1041		n.a.	17		n.a.	26		(Ying et al., 2009)
Australia	n.a.	1536		n.a.	49		n.a.	27		(Williams et al., 2007)
Australia	n.a.	893		n.a.	64		n.a.	20		(Williams et al., 2007)
Australia	n.a.	320		n.a.	60		n.a.	7		(Williams et al., 2007)
Australia	n.a.	514		n.a.	18		n.a.	16		(Williams et al., 2007)
Australia	n.a.	1525		n.a.	12		n.a.	50		(Williams et al., 2007)
Australia	n.a.	1412		n.a.	11		n.a.	4		(Williams et al., 2007)
Australia	n.a.	860		n.a.	13		n.a.	15		(Williams et al., 2007)
Australia	n.a.	599		n.a.	35		n.a.	n.d.		(Williams et al., 2007)
Australia	n.a.	2887		n.a.	41		n.a.	23		(Williams et al., 2007)
Australia	n.a.	635		n.a.	28		n.a.	n.d.		(Williams et al., 2007)
Australia	n.a.	1415		n.a.	37		n.a.	148		(Williams et al., 2007)
Australia	n.a.	1564		n.a.	13		n.a.	127		(Williams et al., 2007)

Table 7. Summary of influent and effluent concentration and removal efficiency[a] in WWTP

Concentration (ng/L)		E1	E2	E3	EE2	NP	OP	BPA
Influent	minimum	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	mean	45	37	76	12	7836	1101	645
	median	35	14	19	3.2	2117	248	400
	maximum	670	226	336	123.5	28207	3560	2400
Effluent	minimum	n.d.	n.d.	n.d.	n.d.	3.4	10	n.d.
	mean	18	5.3	12	1.3	1464	65	114
	median	10	2.7	1.5	0.3	1029	40	31
	maximum	196.7	48	275	17	10358	470	1530
Median removal (%)		63	87	67	81	83	90	81

[a] – Based on values in Table 5 and 6.

3.5 Fugacity-based fate modelling of environmental chemicals

To study the fate of chemicals in the environment, researchers have developed many models such as the QWASI (CEMC 2005), ECOS (Harris et al., 1993), DELWAQ (Boderie 1994) and EXAMS (US EPA 2005). These models can be generally categorised into concentration and fugacity based models. For example, ECOS, DELWAQ and EXAMS are concentration based models, whilst QWASI is a fugacity based model.

Among many fugacity based models, Mackay and his colleagues developed a multimedia fugacity based model, which is a well established, well documented and widely used model (Mackay 2001; Mackay and Macleod 2002; Mackay 2004). It has been applied to chemical fate studies in various environments by numerous researchers (Edwards et al., 1999; Baek and Park 2000; Hertwich 2001; Khan and Ongerth 2004a; Paraiba et al., 2007; Tan et al., 2007b; Contreras et al., 2008; Ao et al., 2009). Fugacity means the escaping or fleeing tendency of molecules. When equilibrium is reached between two phases, the fugacity of a compound in the two phases is the same. In modelling, fugacity (f, in Pa) can be expressed as proportional to concentration (C, in mol/m^3) by introducing a proportionality constant, Z:

$$C = Z \times f \tag{14}$$

where Z is defined as fugacity capacity with units of $mol/m^3/Pa$. The value of Z depends on temperature, the properties of chemicals and the nature of the environment into which the chemical is dispersed (Connell 2005). At equilibrium, the concentration ratio at the two phases is the same as the ratio of Z values:

$$\frac{C_1}{C_2} = \frac{Z_1}{Z_2} \tag{15}$$

The fugacity capacity can be calculated for each compartment using the following sets of equations (Connell 2005):

$$Z_{air} = \frac{1}{RT}, Z_{water} = \frac{1}{H}, Z_{soil} = \frac{K_D}{H}, Z_{biota} = \frac{K_B}{H} \tag{16}$$

where R is the ideal gas law constant in Pa m^3/mol/K, T is temperature (in K), H is the Henry's law constant (Pa m), K_D (L/mol) is the solid-water partition coefficient and K_B (L/mol) is the biota-water partition coefficient. In the fugacity model, the rate (R, in mol/h) of chemical transport and transformation is described as:

$$R = Df \tag{17}$$

where D (mol/Pa/h) is analogous to the first-order rate constant, representing processes like chemical reactions, advective transport, and diffusive exchange between phases. For chemical reactions:

$$D_{Ri} = k_i V_i Z_i \tag{18}$$

where i is phase i, k_i is the first-order rate constant (h^{-1}) and V_i (m^3) is the volume of phase i.

For advection:

$$D_{Ai} = G_i Z_i \tag{19}$$

where G_i (m^3/h) is the flow rate of the medium.

For diffusion:

$$D_D = 1/(1/k_1 A Z_1 + 1/k_2 A Z_2) \tag{20}$$

where k (h^{-1}) is the first-order rate constant, A (m^2) is the area between phase 1 and 2.

The fugacity based model includes three levels of steady-state mass balance calculations. Level I model describes the situation of a fixed quantity (M, in mol) of chemical is introduced into a closed environment under steady-state and equilibrium. It assumes that no degradation, no advective and intermedia transport processes:

$$f = \frac{M}{\sum V_i Z_i} \tag{21}$$

where M is the fixed quantity in mol, V_i is the volume of the environment system and Z_i is the fugacity capacity, which can be calculated from Equation (16).

Level II model describes the situation that a chemical is discharged into an environment at constant rate (E, in mol/h) and achieves steady-state and equilibrium at which input and output are equal:

$$f = \frac{E}{\sum D_i} \tag{22}$$

where E is the discharging rate in mol/h, and D_i is the individual removing process, which can be calculated from Equation (18), (19) and (20).

The Level III Model deals with the most complex conditions, which assume a chemical is discharged into a system at a steady state, but no equilibrium is achieved:

$$f_i \sum D_i = E_i + \sum D_{ji} f_i \tag{23}$$

The left part of Equation (23) is the rate of transport and transformation that removes chemical from the ith compartment, and the right are the emissions (E_i) into the ith compartment and transfers from jth compartments ($\sum D_{ji} f_i$). The Level III Model is built on the complexity of Level I and II. It better simulates the real situation when

compared with the simpler models. It is particularly useful in the study of how emission patterns affect environmental fate (Mackay and Macleod 2002).

3.6 Adverse health effects of EDCs

Since the introduction of EDCs into the environment, various adverse health effects, such as reproductive abnormalities, imbalanced sex ratios and behaviour changes have been observed in aquatic organisms, amphibians, reptiles, birds and mammals. Many human health effects such as prostate cancer, breast cancer and birth defects were also implicated with the exposure to EDCs (Sharp and Skakkebaek 1993; Whittemore 1994; Fernandez et al., 1998; Toppari and Skakkebaek 2000; Damstra et al., 2002; Diamanti-Kandarakis et al., 2009). For example, the use of diethylstilbestrol (DES) in the late 1940s to the early 1970s increased abortions, neonatal deaths, premature births and vaginal cancers in women. Another example is the worldwide use of DDT during the 1950s and 1960s, which adversely affected the reproductive system in mammals and birds. Breeding failure in raptors was reported in the USA, which resulted in the dramatic decline of population in the exposed region (Hester and Harrison 1999). In most cases, it is difficult to quantitatively determine the dose-response relationship, because the damages in wildlife are difficult to determine, and the exposure routes are always too complex (Lyons 2006). It is even more difficult to study the adverse health effects in humans, because of the complexity of human body system, and the sensitivity of ethical issues.

3.6.1 Endocrine system and endocrine disruption

The endocrine system in animals and plants plays an important role in regulating growth, reproduction, maintenance, homeostasis and metabolism. Natural hormones are produced by the glands in the endocrine system, and then transported to the target cells by the bloodstream (Ghijsen and Hoogenboezem 2000). The target cell has a receptor and an effector sites. Some hormone molecules attach to the receptor leading to changes in the effector site, which causes the desired response. The non-attached free hormone molecules will be inactivated by metabolic clearance processes in the liver and kidney before excretion (Birkett and Lester 2003). For xeno-estrogens (e.g. some industrial chemicals and pesticides), they can enter the bloodstream via various routes, such as food ingestion, inhalation or skin contact. A fraction of these chemicals is not fully metabolised and can enter the bloodstream and compete for the plasma protein binding sites. Ultimately they enter the cell nucleus and lead to changes in gene expression, by which the endocrine disruption occurs (Ghijsen and Hoogenboezem 2000).

The endocrine disruption is a very complex process including various mechanisms such as acting as mimics, stimulators, blockers, endocrine flushers, enzyme flushers and destructors. EDCs cause endocrine disruption mainly via two actions: *agonistic* and *antagonistic* effects. When an EDC mimic the native hormones by binding to the receptor and activate a response in the effector, it is called *agonistic effects*. When the binding causes no response in the receptor, then it is called *antagonistic effects* (Crain et al., 2000; Birkett and Lester 2003).

3.6.2 Adverse health effects observed in fish, rats and mice

The adverse health effects of EDCs observed in fish have been widely studied in the laboratory. The induction of female yolk protein, vitellogenin (VTG) in male and juvenile female fish has been commonly used as a biomarker. The early work carried out by Purdom's team in 1994 showed increased VTG level in male rainbow trout caged in WWTP effluent. Subsequent work by other researchers also used VTG to assess the adverse effects caused by different EDCs (Hansen et al., 1998; Gronen et al., 1999; Flammarion et al., 2000; Folmar et al., 2001; Rose et al., 2002; Sole et al., 2003; Van den Belt et al., 2003; Pawlowski et al., 2004; Scholz et al., 2004; Xie et al., 2005a; Bogers et al., 2006; Eguchi et al., 2007; Bjerregaard et al., 2008).

It was observed that the concentration of 3.3 ng/L of E1 (Thorpe et al., 2003), 1.0 ng/L of E2 (Hansen et al., 1998), 0.1 ng/L of EE2 (Purdom et al., 1994), 4 μg/L of NP (Kwak et al., 2001), 4.8 μg/L of OP (Jobling et al., 1996; Lintelmann et al., 2003) and 0.25 μg/L of BPA (Oehlmann et al., 2006) was able to induce elevated level of VTG in zebra fish, rainbow trout and fathead minnow fish. In addition to VTG induction, many other health effects have also been observed in fish, such as reproductive abnormalities (Panter et al., 1998; Young et al., 2004), altered sex ratio (Mills and Chichester 2005), mortality and behaviour changes (Van Miller and Staples 2005).

In addition to fish, rats and mice have been frequently studied in the laboratory to assess the health effects of EDCs. A wide range of adverse effects was observed in reproductive organs, brain development, body weight, sexual development, sperm production and mortality in offspring (Lai et al., 2002b; vom Saal and Hughes 2005).

The designed exposure routes were mainly through oral ingestion, implantation and injection. The dosages used were usually in the range of μg/kg, bw/day to several mg/kg, bw/day. The *No-observed-adverse-effect-level* (NOAEL) was between 50 μg/kg, bw/day to 2.5 mg/kg, bw/day for E2, 20 ng/kg, bw/day to 8 μg/kg, bw/day for EE2, 0.8 to 8 mg/kg, bw/day for NP, 7 to 600 mg/kg, bw/day for OP and 0.02 μg/kg, bw/day to 25 mg/kg, bw/day for BPA (Damstra et al., 2002; Okkerman and van der Putte 2002; Lai et al., 2002b; Van Miller and Staples 2005; vom Saal and Welshons 2006; Goodman et al., 2009). The NOAEL information of E1 and E3 has not been reported in the literature.

3.6.3 Adverse health effects observed in humans

Human epidemiological data showed a possible link between the exposure to EDCs and various human health effects. These health effects include effects on reproductive system, neural development, immune system, metabolism, insulin and glucose homeostasis, cancers and thyroid disruption. Some reported health effects were presented in the following list (Hester and Harrison 1999; Falconer et al., 2003; Diamanti-Kandarakis et al., 2009):

- Sexual development and behaviour
- Birth defects
- Changed sex ratio
- Decreased sperm counting and fertility
- Testicular cancer
- Prostate cancer
- Breast cancer

- Stillbirth
- Endometriosis
- Hypospadias and cryptorchidism
- Obesity

Despite the above various health effects, the current human epidemiological data is inadequate to make any definitive conclusions. In many cases, only casual link can be established (Falconer et al., 2006). Therefore, more research efforts are needed to establish the dose-response relationship of EDCs for human health.

3.6.4 Potency of EDCs

The potency of EDCs can be evaluated by their ability to cause estrogenic activities. Generally, the potency of endogenous steroid estrogens is 1000 to 100 000 times higher than xeno-estrogens such as pesticides, plasticizers, polychlorinated bisphenols (PCBs) and alkylphenols (Gomes et al., 2003; Hanselman 2003; Falconer et al., 2006). To compare the potency of different EDCs, an *estradiol equivalent factor* (EEF) has been defined (Sun et al., 2008):

$$EEF_i = \frac{EC50_{E2}}{EC50_i} \tag{24}$$

where $EC50_{E2}$ is the median effective concentration of E2, $EC50_i$ the median effective concentration of compound i.

Due to different bioassays have been used to determine the median effect concentration, Different EEF values were reported in the literature. The set of EEF values listed in Table 1 in Section 3.2.2 were based on the bioassay of MVLN cells. With EEF, the

estradiol equivalent quantity (EEQ) is calculated by multiplying with the concentration (Sun et al., 2008):

$$EEQ = EEF \times C \tag{25}$$

In the literature, EEQ sometimes also refers to EEF. For clarification, if the value carries a unit of concentration, it should be EEQ, otherwise it is EEF.

3.7 Health risk assessment of environmental pollutants

3.7.1 Framework of health risk assessment

The concept of 'risk' has a broad range of meanings across various disciplines. Generally it can be interpreted as the likelihood or probability of undesired events. In environmental sciences, it can be defined as the probability or likelihood that an adverse effect will occur in humans, wildlife or ecological systems exposed to a chemical, physical or biological agent under a specific set of conditions. This definition incorporates three important aspects of risk, which includes exposure, adverse effects and the likelihood. Without any of its three components, the risk is zero (Beer and Ziolkowski 1995; Paustenbach 2007).

Risk assessment is the process or procedures used to estimate the likelihood of concerned risk. Environmental health risk assessment consists of human health risk assessment and ecological risk assessment. The widely accepted framework of conducting human health and biological risk assessment has been developed by the National Academy of Sciences, USA (NAS 1983), which was shown in Figure 13:

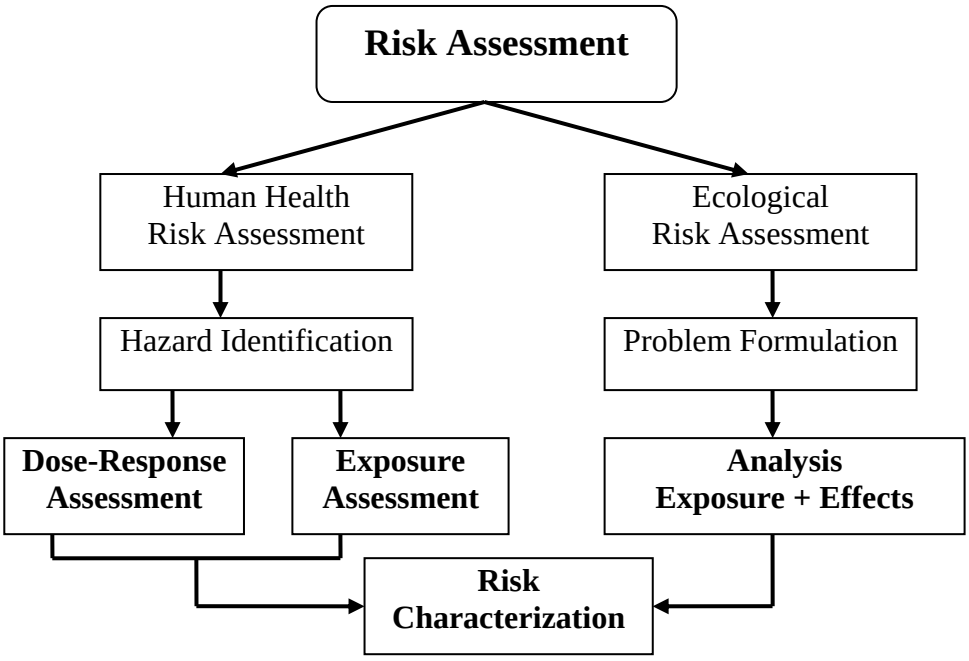

Figure 13. Framework of risk assessment.

3.7.2 Risk assessment using probabilistic techniques

Risk assessment using probabilistic techniques has been applied to engineering problems since the 1970s, such as the estimation of seismic risk and assessment of nuclear power plant safety (Hanauer 1975; Weichert and Milne 1979). Recently it has been applied to assess the risk of environmental pollutants (Solomon et al., 1996; Solomon et al., 2000; Djohan et al., 2007; Straub and Stewart 2007; Hamidin et al., 2008). In this method, the exposure and effect values are expressed in cumulative probability distributions (CPD). It provides probability distribution of risk, rather than single risk value as calculated in deterministic point estimate method (Solomon et al., 2000). The primary advantage of using this method is the quantitative analysis of uncertainty and variability, which enables a more comprehensive risk characterization than the point estimation approach. It also has the advantage of using multiple data sets in assessing both the exposure and dose-response effects. However, more resources,

time and expertise are required for the risk assessors, reviewers and risk managers (US EPA 2001).

The procedures of conducting a risk assessment begin with the collection of exposure and effects data. After these two sets of data are critically evaluated and converted into logarithm values, they are plotted in the same axis to produce CPD curves, which are illustrated in Figure 14. It should be pointed out here that all measurements below detection limits should also be counted for the calculation of the cumulative probabilities (CP) (Solomon et al., 2000).

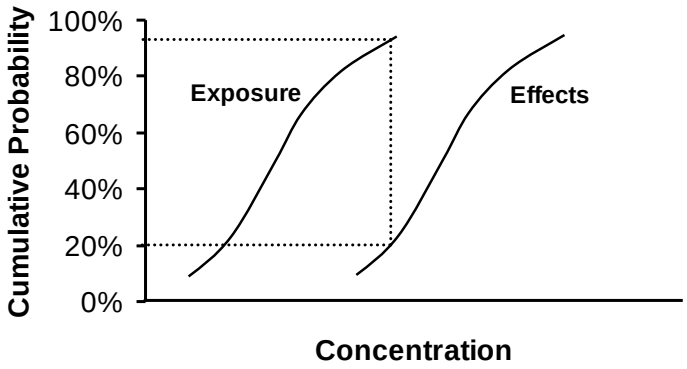

Figure 14. Cumulative probability distribution of exposure and effect concentration.

Within the overlapped range of CPD curves, each concentration value corresponds to two CP values: the $CP_{exposure}$ and $CP_{effects}$. The value of $1 - CP_{exposure}$ is calculated as the exposure exceedence, which is plotted against the $CP_{effects}$ values to obtain an exceedence curve as shown in Figure 15. The $CP_{effects}$ values can be regarded as the percent of affected samples. For example, point A refers to 20% of affected samples with about 8% of exposure concentration above effect concentration. This exceedence curve provides a tool to compare risks of different EDCs, because the risk increases when the exceedence curve is further away from the origin (Solomon et al., 2000).

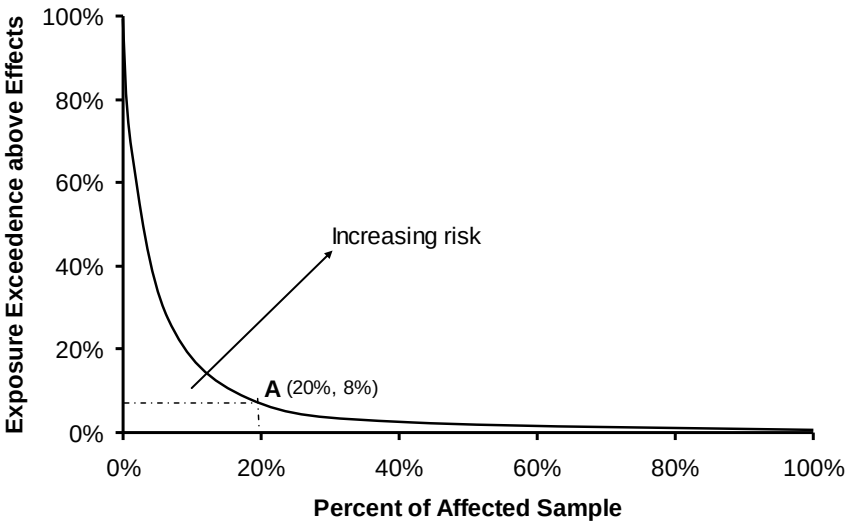

Figure 15. Exposure exceedence curve at different percent of affected samples.

In addition, hazard quotient (HQ) can be calculated as the exposure concentration divided by the effect concentration at each CP value (Solomon et al., 2000; Verdonck et al., 2002). All obtained HQ values can be plotted against all CP values to obtain a risk CPD curve as shown in Figure 16. More often, only a single $HQ_{95/5}$ value is calculated to show the risk level for the protection of the majority. It is a ratio of EC_{95} (exposure concentration at 95% of CP) to HC_5 (hazard concentration at 5% of CP). Sometimes, the reciprocal of $HQ_{95/5}$ is called *margin of safety* (MOS) (Solomon et al., 2000; Straub and Stewart 2007).

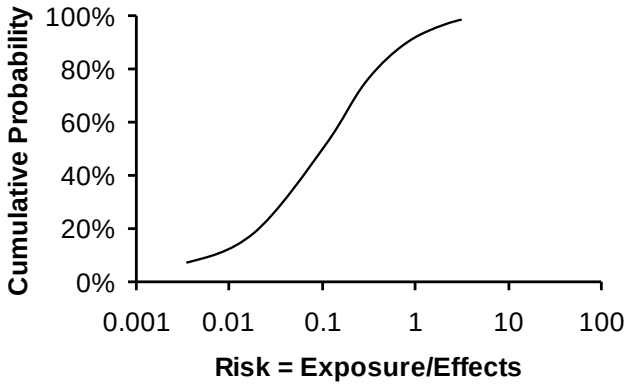

Figure 16. Cumulative probability distribution of hazard quotient values.

Chapter 4 Methodology

4.1 Quantitative structure-property relationships for steroidal EDCs

4.1.1 Subgroup classification

A group of 17 steroidal EDCs were divided into estrogens (E1, E2, E3 and EE2) and androgens (the remaining 13 compounds) based on their biological effects and structural differences (see Figure 2 in Section 3.2.1). There are four hydrocarbon rings in all steroidal compounds: three hexagonal rings (A, B, C) and one pentagonal ring (D). A phenolic group in the A-ring position occurs in estrogens, whilst the androgens do not contain this group at the same position. In order to investigate whether the relationships between molecular descriptors and physicochemical properties are different for each subgroup, these two subgroups were considered separately wherever applicable.

4.1.2 Measured and calculated properties

Measured aqueous solubility (S) values were drawn from Yalkowsky and He (2003). For the purpose of this study, the S values were selected from those measurements at a temperature between 20 to 25 ℃. If multiple values were available at this temperature range, the best value ranked by Yalkowsky and He (2003) was selected based on their five criteria. The S values for the 17 steroidal compounds have been listed in Table 16 with units of mg/L and μmol/L (logarithmic values). Table 16 also listed measured K_{ow} values between 20 to 25 ℃ for all 17 steroidal EDCs (Leszczynski and Schafer 1989; Hansch et al., 1995).

Calculated values of molecular descriptors and properties were obtained by using the QikProp program (Schrodinger 2007). The program calculates these values for organic compounds by the input of the compound structures in SMILES (simplified molecular input line entry specification) format. Results were generated for two properties (log S and log K_{ow}) and three independent molecular descriptors: log FOSA (hydrophobic component of the total solvent accessible surface area), log FISA (hydrophilic component of the total solvent accessible surface area) and log PSA (Van de Waals surface area of polar nitrogen and oxygen atoms). The calculated properties were then compared with the measured values to examine the reliability of the calculation methods.

4.1.3 Statistical analysis

QSPR were developed with relationships derived from single and multiple linear regressions. Microsoft Excel 2003 (Microsoft Office 2003) was used to determine the relationships between two variables whilst statistical program SPSS 13.0 (SPSS 2004) was used for multiple variable regression analysis. Multicollinearity was considered by examining the correlation matrix generated for the independent variables (MW, T_m, log FOSA, log FISA and log PSA) before the multiple variable regression analysis was conducted. If multicollinearity existed for these independent variables, the multiple regression analysis was not conducted. After the descriptor selection and the multicollinearity assessment, single and multiple variable linear regression analysis was carried out between these descriptors and properties. The same approach used by Liu et al. (2006) was used here, which started from the relationship between only one descriptor and one property. Then the number of descriptors was subsequently increased stepwise one at a time with log S and log K_{ow} separately.

4.2 Relationship between degradation rate constant and equivalent biomass concentration for estrogens

4.2.1 Selection of compounds

This study principally relies on data published on the fate of estrogens in WWTP and receiving water. E1, E2 and EE2 are selected mainly because data on other estrogens are very limited. Furthermore, these three estrogens are the major contributors of estrogenic activities in sewage effluents and receiving water (Desbrow et al., 1998; Korner 2000; Matsui et al., 2000; Onda et al., 2003). Many *in vivo* studies showed that the potency of endogenous steroid estrogens is over 1 000 to 100 000 times greater than exogenous EDCs such as pesticides, plasticizers, polychlorinated bisphenols, and alkylphenols (Routledge et al., 1998; Lange et al., 2001; Hanselman 2003; Thorpe et al., 2003).

4.2.2 Data source and analysis

Rate constant data was collected from published studies for a variety of environmental media with different levels of biomass concentrations. Rate constants were either reported directly in units such as min^{-1}, h^{-1} and d^{-1}, or indirectly as half-lives. For the purpose of correlation with biomass concentrations, they were all converted into rate constants with the same unit in h^{-1}.

It is noted here that there is significant variability for the rate constants used in obtaining the correlations. The variability of the data is mainly from the differences between experimental and field conditions, and various sampling and analysis methods. Two assumptions were made in this study: the first is that all viable active

microorganisms play a role in the degradation of estrogens; and the second is that all microorganism species and substrates are similar in all WWTP investigated. Apparently, not all active microorganisms will have the same ability to degrade estrogens and some microorganism species may differ from locations to locations.

Rate constants from laboratory studies could possibly overestimate or underestimate the true degradation rates (Jurgens et al., 1999; Johnson and Sumpter 2001; Jurgens et al., 2002), because laboratory results were obtained under controlled conditions, such as the use of adapted microbial population, controlled temperature, enhanced aeration and much higher initial concentrations. Most experiments were conducted at about 20 ℃, which may represent a typical summer temperature in most places. Seasonal changes from summer to winter can reduce the degradation rate because the biomass is less active at lower temperature (de Mes et al., 2005). Initial concentrations used in many studies vary from several ng/L to hundreds of μg/L, even up to 1 mg/L (Tables 18, 19 and 20). High initial concentrations could possibly inhibit or even impose toxic effects on microorganisms involved in the degradation of estrogens (Kozak et al., 2001), resulting in lower rate constants in some cases (Ternes et al., 1999a; Jurgens et al., 2002). Various sampling and analysis methods can also lead to the variations in the determination of rate constants (de Mes et al., 2005).

Limited data on biomass concentration (BC) is available in the fate study of E1 and E2 in both surface water and activated sludge. Particularly, BC data for EE2 was only available for activated sludge. So, the average BC value in rivers (0.085 mg/L) of E1 and E2 (Tables 18 and 19) were used for EE2 (Table 20) in surface water. Three

different parameters with relation to BC have been used in literature: *mixed liquid suspended solids* (MLSS), *mixed liquid volatile suspended solids* (MLVSS), and *viable counts* (VC). The total amount of inorganic and organic matter in suspension in the mixed liquor (mixture of returned sludge and influent wastewater) is measured as MLSS. MLVSS is the organic component of MLSS. While *Viable count* (VC) is a direct counting method in which only viable cells are counted. It is usually measured as *colony-forming units per millilitre* (cfu/ml).

4.2.3 Relationship development

As it was reviewed in Section 3.4.4.1, the biodegradation of estrogens can be regarded as first-order reactions (Johnson and Sumpter 2001; Jurgens et al., 2002; Andersen et al., 2004). In the cases that results were reported as half-life, the rate constant can be obtained from half-life ($t_{1/2}$) by the rearrangement of Equation (13):

$$k = \frac{0.693}{t_{1/2}} \tag{26}$$

For convenience in describing the biomass concentration levels, a concept of '*equivalent biomass concentration*' (EBC) was used to describe the equivalent values as indicated by MLSS, MLVSS and VC. It is defined as all the active microorganisms which are involved in the biodegradation of E1, E2 and EE2. In activated sludge, microorganisms mainly consist of bacteria, fungi, algae, protozoa and metazoan (Henze et al., 2002). Bacteria are the dominant species and can make up about 95% of the activated sludge biomass. The major tasks of bacteria are the transformation and degradation of organic matter (Henze et al., 2002). Microorganisms consisting 70 to

80% of organic matter is often regarded as the same as MLVSS. A small fraction (less than 5%) of MLVSS is non-viable, inert organic matter (Seviour and Blackall 1999; Tchobanoglous et al., 2003). In this study, this small fraction is treated as negligible and the total content of MLVSS is regarded as EBC:

$$MLVSS \approx EBC \tag{27}$$

In activated sludge plants, MLSS is generally between 1500 to 5500 mg/L and MLVSS between 1500 to 4000 mg/L. The ratio of MLVSS to MLSS varies from 0.60 to 0.95 (Layton et al., 2000; Kumagai 2002; Coskuner et al., 2005). A typical ratio is about 0.80 and MLSS can thus be converted into EBC by Equation (28):

$$0.80MLSS \approx MLVSS \approx EBC \tag{28}$$

For VC measured in cfu/mL, the number of cfu is related to the viable number of microorganisms in the sample. Most commonly, a cfu consists of a single bacterium. The mass of a bacteria varies from 10^{-13} to 10^{-10} g (Tanner 1948; Davis et al., 1973) with a typical value close to 10^{-12} g (Lorian et al., 1985). Therefore, VC can be converted into EBC in unit of mg/L by Equation (29):

$$VC \times 10^{-6} \approx EBC \tag{29}$$

With each set of data for E1, E2 and EE2, it allows k to be plotted against EBC. By using the regression method, a trend line can be obtained to find out the relationship between k and EBC.

4.3 Fate modelling of estrogens in a reservoir receiving recycled wastewater

4.3.1 Selection of compounds

Three estrogens E1, E2 and EE2 were selected in this fate modelling study. In addition to the reasons discussed in Section 4.2.1, E1, E2 and EE2 were frequently detected in WWTP and surface water in South East Queensland and other regions of Australia (Leusch et al., 2005; Braga et al., 2005a; Williams et al., 2007; Tan et al., 2007b; Ying et al., 2009). More importantly, the investigated reservoir is a major source of drinking water supply to Australia's third largest city, Brisbane City. Many studies have shown that these estrogens can impose adverse effects on aquatic organisms even at low ng/L level. For example, as low as 0.1 ng/L of EE2 and 1.0 ng/L of E2 can increase the vitellogenin (VTG) level in fish (Purdom et al., 1994; Hansen et al., 1998; Routledge et al., 1998; D'Ascenzo et al., 2003). Therefore, these three estrogens were selected to study their fate in the reservoir.

4.3.2 Description of the recycling scheme and the reservoir

3.3.2.1 The recycling scheme

Effluent from 6 major wastewater treatment plants (WWTP) in South East Queensland, Australia is delivered to three advanced water treatment plants (AWTPs) located at Bundamba, Luggage Point and Gibson Island. In each of the AWTP, the incoming effluent is treated by screening, membrane filtration (MF/UF/RO), advanced oxidation (UV, H_2O_2), ion exchange and stabilisation. The project has a total capacity of 232

ML/day of recycled water. It was proposed that about 115 ML/day of this capacity would be discharged into the Wivenhoe Reservoir for drinking water supply and the remaining recycled wastewater would be pumped to three power plants in the region (SEQWater 2009).

4.3.2.2 The reservoir

The Wivenhoe Reservoir is situated on the Brisbane River in the Esk Shire, South East Queensland. It is one of three reservoirs managed by SEQWater for both drinking water supply and flooding water storage purposes. The reservoir has a full water storage volume of 2.62 million ML. Water is released from the reservoir into the Brisbane River and extracted downstream by the Mt Crosby water treatment plant (WTP). The major inflow into the reservoir is the water released from the Somerset Dam upstream (SEQWater 2009).

Stratification normally occurs in summer when maximum water temperature differences exist between the surface and bottom of the reservoir, resulting in poor mixing of water. It leads to two important biochemical consequences. The first one is the depletion of dissolved oxygen (DO) in the hypolimnion and the possible creation of anoxic conditions (Holdren et al., 2001). According to the measured DO level in the reservoir ((Burford and O'Donohue 2006a), aerobic condition is considered to be dominant in both winter and summer at the bottom of the reservoir.

The second consequence of stratification is the differences in vertical temperature profile. In summer, there is a difference of about 6 ℃ between the surface and the

bottom water of Wivenhoe Reservoir, whilst the difference is only 0.17 ℃ in winter when stratification would not be expected (Burford and O'Donohue 2006a). The effects of vertical temperature difference sometimes can be offset by the mixing caused by inflows, rainfall and wind (Holdren et al., 2002). Over half of the annual rainfall in the region of Wivenhoe reservoir falls in summer from December to March, which can enhance water mixing in the reservoir. Therefore, stratification was considered to occur in the reservoir to a very minor extent.

4.3.3 The fugacity approach based model

The theoretical basis of this model was introduced in Section 3.5. The Level III Model was selected for this modelling study, because it deals with the most complex conditions, assuming a chemical is discharged into a system at a steady state, but no equilibrium is achieved. It simulates the real situation better when compared with the simpler models of Level I and II. It is particularly useful in the study of how emission patterns affect environmental fate (Mackay and Macleod 2002). Computer programs for all three level models were developed by the Canadian Environmental Modelling Centre at Trent University in Canada, which was downloaded from www.trentu.ca/cemc. The detailed calculation steps in each model were illustrated in an Excel spreadsheet, which was also available from CEMC on request. In this study, the spreadsheet was used for the simulation. It was pointed out here that the quality of the results produced by the model is largely dependent on the quality of input data (e.g. physicochemical properties, half-life values, recycling parameters, reservoir characteristics and plant operations). Reasonable assumptions had to be made when measured values were unavailable, which would affect the accuracy of results to a minor extent.

4.3.4 Parameters used in the model

Six groups of parameters were used for mass balance calculations in the modelling, which include physicochemical properties, reservoir and recycling parameters, half-life values, plant operating parameters and mass transfer parameters. Important physicochemical parameters of E1, E2 and EE2 were listed in Table 1. The aqueous solubility and log K_{ow} values indicate that these compounds have low concentrations in water and are easily partitioned into organic matters in aquatic systems. Low vapour pressure values also suggest that the loss into air by vaporization is negligible.

Reservoir parameters in terms of their typical and range of values were summarized in Table 8 (Burford and O'Donohue 2006a; Burford et al., 2007). Based on the measured reservoir water temperature in summer and winter, a typical temperature of 20 ℃ was determined. The range of 15 to 25 ℃ was considered as appropriate for the climate condition in the reservoir region. For the reservoir water storage volume, a range of 20 to 100% of the maximum storage capacity was used, and 50% of the maximum was determined as the typical volume. Typical and range of reservoir area were determined similarly. The reservoir equivalent biomass concentration (EBC) was calculated from bacteria density in the reservoir. Burford and O'Donohue (2006a) and Burford et al. (2007) have reported that the bacteria density in the reservoir is in a range of about 10^2 to 10^6 cell/ml. A value of 10^4 cell/ml was used as the typical value, which was considered as reasonable for surface water (Jurgens et al., 2002). Using the method described by Cao et al. (2008) in Section 4.2.3, bacteria density was converted into EBC values (Table 8).

Table 8. Typical and range values of reservoir characteristics, recycling parameters and estrogen concentrations [a]

Parameters		Units	Typical Values	Range/Notes
Reservoir Water Temperature (T)		℃	20	15 – 25
Reservoir Storage Volume (V)		m^3	1.310×10^9	5.24×10^8 – 2.62×10^9
Reservoir Mean Depth (H)		m	10	mean depth 10.8
Reservoir Area (A)		m^2	1.310×10^8	5.24×10^7 – 2.62×10^8
Equivalent Biomass Concentration (EBC)		mg/L	0.01	0.0001 – 1
Reservoir Water Releasing Rate (F_d)		m^3/h	27778	13889 – 41667
WWTP Effluent Recycling Rate (F_r)		m^3/h	4792 [b]	0 – 10 000
Estrogen Concentrations in WWTP Effluent (C_r)	E1	ng/L	10	based on measured concentrations in Queensland
	E2		1	
	EE2		0.1	
Estrogen Concentrations in Recycled Water (C_e)	E1	ng/L	0.1	0.01 – 1
	E2		0.01	0.001 – 0.1
	EE2		0.001	0.0001 – 0.01

[a] – Data sources: (Schafer et al., 2003; Kimura et al., 2004; Rosenfeldt and Linden 2004; Nghiem et al., 2004a; Leusch et al., 2005; Burford and O'Donohue 2006a; Burford et al., 2006b; Chen et al., 2007; Williams et al., 2007; Tan et al., 2007c; Cao et al., 2008; SEQWater 2009; Liu et al., 2009a).
[b] – Designed capacity.

The recycling rate and operating parameters were determined using the information from WCRWP and SEQWater Company. The estrogen concentrations in recycled water in Table 8 were determined from measured effluent concentrations (Leusch et al., 2005; Leusch et al., 2006a; Williams et al., 2007; Tan et al., 2007c; Liu et al., 2009a) and estrogen removal efficiencies (30 – 95% for RO and 90 – 99% for advanced oxidation process) in WWTP from Southeast Queensland, Australia and other countries (Schafer et al., 2003; Kimura et al., 2004; Rosenfeldt and Linden 2004; Nghiem et al., 2004a; Chen et al., 2007).

The half-life values were obtained by several methods and listed in Table 9, which were necessitated by the difficulties of determining half life values for these compounds. In addition, most reported half life values were measured for the water compartment, whilst the half life values for other compartments were not readily available. The water compartment half life values were calculated by using the equivalent biomass concentrations, which were based on relationships between rate constants and biomass concentrations developed by (Cao et al., 2008) in Section 6.3.

Table 9. Typical half-life values for E1, E2 and EE2 at 20 ℃

Compartments	Half-life (h)		
	E1	E2	EE2
Water	96	78	2093
Sediment	1435	156	41857
Suspended solids	96	78	2093
Soil	232	228	8760
Biota	1	1	24
Air	1	1	1
Aerosol	1	1	1
Vegetation	1	1	24

Due to the lack of measured data for other compartments, the half life values in suspended solids were assumed to be the same as in the water compartment. This was based on their similar degradation mechanisms (e.g. aerobic condition, degrading bacteria). Longer half life was found with the degradation of estrogens in sediment, as anoxic or anaerobic conditions may exist. In addition, lower water temperature in the sediment might also prolong the half-life. Therefore, based on studies in sediment under anaerobic conditions (Jurgens et al., 2002; Andersen et al., 2004; Joss et al., 2004), the half life values in sediment were assumed to be approximately 15, 2 and 20 times longer than the half life values in water compartment for E1, E2 and EE2 respectively.

For soil and biota, reported half-life values were used (Das et al., 2004; Shi et al., 2004; Ying and Kookana 2005; Lucas and Jones 2006; Ternes and Joss 2006). Little information was available for degradation in vegetation compartment, so the half life values were assumed to be the same as in the biota. This assumption was also based on possibly similar degradation mechanisms involved. The half life in air were calculated by the program EPISuite 3.12 (EPISuite 2000). The half life values in aerosol were assumed to be the same as in air, as similar photochemical degradation mechanisms are involved in air and aerosol. The half-life assumptions made for vegetation and aerosol would not affect the simulation for other compartments, because the proportions of aerosol and vegetation in the modelled environment are very small. Due to low Henry's law constants (10^{-10} to 10^{-12}), the amount of estrogens lost by volatilization is negligible. For mass transfer values between different compartments, the default values proposed by (Mackay 2001) were used, which were considered as appropriate for such modelling applications when measured data were not readily available.

4.3.5 Simulated concentration under typical and random conditions

Under typical conditions as described in Table 8 in Section 4.3.4, the simulated concentration in different environmental compartments was calculated simply using physicochemical properties and half-life values of E1, E2 and EE2 in the model. At any random conditions, simulated concentrations for E1, E2 and EE2 were obtained using 500 sets of random values of T, V, F_d, EBC, F_r and C_e generated by Excel 2003 within ranges as shown in Table 8. These 500 sets of random values can be regarded as the results of 500 random sampling sessions in the reservoir. Although larger sample size (e.g. 10 000) provides better accuracy in Monte Carlo simulations, these 500 simulation points enable us to obtain reasonably accurate representation of the probability profile for risk assessment. The use of 500 as the smaller sample size in Monte Carlo simulation was considered as appropriate by other researchers, for example in Watanabe et al. (1992) and Straub (2008). In addition, we have tried increasing number of simulation points from 100 to 500. It was observed that after 500 points, further increase of sample size does not affect significantly the probability distribution profile. Therefore, we considered the sample size of 500 is appropriate in this case. To study the effects of temperature, a range of 15 to 25 ℃ was used. A small Visual Basic Macro Function was coded to calculate these 500 sets of concentrations. The obtained concentrations were arranged in ascending order with cumulative probability calculated for each concentration. The cumulative probabilities (CP) were then plotted against the simulated concentrations on a logarithmic scale.

4.3.6 Effects of reservoir and recycling parameters on simulated concentration

A number of factors can affect the concentration of estrogens in the reservoir, which include the dilution effects of rain and freshwater inflow, the reservoir water releasing rate into downstream Brisbane River, the biomass concentration in the reservoir, reservoir water temperature, the recycling flow rate and estrogen concentration in recycled water from the AWTP. These factors can be summarized as water temperature (T), reservoir water storage volume (V), the reservoir water releasing rate (F_d), the equivalent biomass concentration (EBC), the recycling rate (F_r) and estrogen concentration in final recycled water (C_e). Their effects were studied individually when the other parameters were set at typical values.

4.3.7 Risk characterisation for human health using simulated concentrations

With the simulated concentrations obtained for random condition, a health risk characterisation was carried out for fish and humans. This was achieved by calculating the *Hazard Quotient* (HQ) for each estrogen using the following equations:

$$HQ_{95/NOAEC} = EC_{95} / NOAEC \quad \text{(for fish)} \quad (30)$$

$$HQ_{95/PHS} = EC_{95} / PHS \quad \text{(for humans)} \quad (31)$$

where EC_{95} is the simulated concentration at 95% of CP, NOAEC is the effect concentration on fish vitellogenin (VTG), and PHS is human public health standards.

The level of plasma vitellogenin (VTG) in fish was used as a common biomarker for indicated adverse effects. Three lowest NOAEC values on VTG from published studies were used in Equation (30), which were 0.74 ng/L for E1 (Thorpe et al., 2003), 0.4 ng/L for E2 (Metcalfe et al., 2001) and 0.1 ng/L for EE2 (Young et al., 2004). For humans, a

set of PHS values was available in the *Australian Guidelines for Water Recycling* (2006)， which sets 30, 175 and 1.5 ng/L for E1, E2 and EE2 respectively. With NOAEC and PHS values available, the risk of each estrogen can be quantified using Equation (30) and (31) and compared among them. Apparently, higher HQ values mean higher level of health risk. Although HQ method is only a single-point risk estimation method, it provides important information for risk assessor. Particularly, it is useful for risk management by prioritizing target EDCs.

4.4 Health risk assessment of EDCs from water and food

4.4.1 Exposure assessment

Fish exposure to EDCs is mainly from surface water, whilst human exposure is mainly from drinking water and food (e.g. fish, milk, meat and fruits). Measured concentrations in the USA, Japan, China, Canada, UK and major European countries (e.g. Italy, Germany, the Netherlands and France) were derived from numerous published scientific literatures, which were summarized in Table 10 for surface water, Table 11 for drinking water and Table 12 for human food. All seven EDCs were detected in surface water, but E3 and EE2 were not reported in drinking water and food respectively. These concentration values were collated with other information such as detection limits, total number of samples, number of samples below the detection limits, methods of reporting these values (individual measurements, minimum, median or maximum values). After each set of concentration data for individual EDC was ranked and converted into logarithm values, they were cumulatively distributed. For humans, the exposure concentration in drinking water and food was converted into human daily dose (HDD)

by multiplying the concentration with daily water and food consumption (Food Standards Australia New Zealand 2008).

Table 10. Data sources for measured concentration values in surface water

EDCs	Surface water
E1	Belfroid et al., 1999; Adler et al., 2001; Xiao et al., 2001; Fawell et al., 2001a; Kuch and Ballschmiter 2001a; Isobe et al., 2003; Cargouet et al., 2004; Hohenblum et al., 2004; Lagana et al., 2004; Vethaak et al., 2005; Zuo et al., 2006; Kolodziej and Sedlak 2007; Noppe et al., 2007; Pojana et al., 2007; Williams et al., 2007; Benotti et al., 2009; Lei et al., 2009; Zhao et al., 2009
E2	Belfroid et al., 1999; Snyder et al., 1999; Adler et al., 2001; Shen et al., 2001; Xiao et al., 2001; Fawell et al., 2001a; Kuch and Ballschmiter 2001a; Isobe et al., 2003; Cargouet et al., 2004; Lagana et al., 2004; Vethaak et al., 2005; Morteani et al., 2006; Zuo et al., 2006; Kolodziej and Sedlak 2007; Noppe et al., 2007; Pojana et al., 2007; Williams et al., 2007; Benotti et al., 2009; Lei et al., 2009; Zhao et al., 2009.
E3	Adler et al., 2001; Xiao et al., 2001; Isobe et al., 2003; Cargouet et al., 2004; Hohenblum et al., 2004; Lagana et al., 2004; Morteani et al., 2006; Noppe et al., 2007; Lei et al., 2009
EE2	Belfroid et al., 1999; Snyder et al., 1999; Adler et al., 2001; Shen et al., 2001; Xiao et al., 2001; Fawell et al., 2001a; Kuch and Ballschmiter 2001a; Cargouet et al., 2004; Hohenblum et al., 2004; Lagana et al., 2004; Vethaak et al., 2005; Morteani et al., 2006; Zuo et al., 2006; Noppe et al., 2007; Pojana et al., 2007; Benotti et al., 2009; Lei et al., 2009
NP	Snyder et al., 1999; Shen et al., 2001; Kuch and Ballschmiter 2001a; Hohenblum et al., 2004; Lagana et al., 2004; Vethaak et al., 2005; Pojana et al., 2007; Benotti et al., 2009; Zhao et al., 2009
OP	Snyder et al., 1999; Kuch and Ballschmiter 2001a; Hohenblum et al., 2004; Vethaak et al., 2005; Zhao et al., 2009
BPA	Kuch and Ballschmiter 2001a; Hohenblum et al., 2004; Lagana et al., 2004; Vethaak et al., 2005; Pojana et al., 2007; Benotti et al., 2009; Zhao et al., 2009

Table 11. Data sources for measured concentration values in drinking water

EDCs	drinking water
E1	Adler et al., 2001; Kuch and Ballschmiter 2001b
E2	Adler et al., 2001; Kuch and Ballschmiter 2001b; Morteani et al., 2006
E3	not detected
EE2	Desbrow et al., 1998; Adler et al., 2001; Kuch and Ballschmiter 2001b; Morteani et al., 2006
NP	Kuch and Ballschmiter 2001b; Casajuana and Lacorte 2003; Benotti et al., 2009; Ghijsen and Hoogenboezem 2000
OP	Ghijsen and Hoogenboezem 2000; Kuch and Ballschmiter 2001b
BPA	Kuch and Ballschmiter 2001b; Casajuana and Lacorte 2003; Benotti et al., 2009

Table 12. Data sources for measured concentration values in human food

EDCs	food
E1	Henricks et al., 1983; Hartmann et al., 1998; Fritsche and Steinhart 1999
E2	Henricks et al., 1983; Hartmann et al., 1998; Fritsche and Steinhart 1999
E3	Caldwell et al., 2009
EE2	not detected
NP	Sasaki et al., 1999; Guenther et al., 2002; Tavazzi et al., 2002; Fernandes et al., 2003; Ademollo et al., 2008
OP	Sasaki et al., 1999; Tavazzi et al., 2002; Fernandes et al., 2003; Ademollo et al., 2008
BPA	Takino et al., 1999; Kawamura et al., 2001; Tavazzi et al., 2002; Kuo and Ding 2004; Zhang et al., 2009

For each EDC, the CP value was calculated as the assigned ranking number for concentration, i, divided by the total number of samples plus 1, which equals to i/(n+1). An issue has arisen regarding how to treat values below detection limits, and values above detection limits but were not given in the literature. For those values below detection limits, they should also be counted into the total sample number. For ranking purposes, they were assigned random values between 0 and the detection limit of that measurement using the computer program Excel (Microsoft Office 2003). For the values above detection limits but were not given by the author, they were also included

into the total sample number counting and assigned random concentration values between the minimum and maximum given values, or between the detection limit and the maximum depending on the type of value available. This solution was described in the following example with data from different sources as shown in Table 13:

Table 13. Sample data sets used for the calculation of cumulative probability

Concentration (ng/L)	Type of value	Total samples	Detection limit	Reference
2.3	individual	20	1.0 ng/L	source 1
5.4	individual			
6.7	individual			
12	individual			
1.4	individual			
7.5	individual			
2.8	minimum	15	0.5 ng/L	source 2
3.5	median			
5.4	maximum			
10.9	maximum	5	5 ng/L	source 3

In the left column of Table 13, although there were only 10 values available, the total number of samples counted in the probability calculation should be 40 as a sum of total samples in each data source (20 + 15 + 5). For source 1, only 6 values were above the detection limit of 1.0 ng/L, 14 values were below the detection limit. So, for ranking purposes only, these 14 values were assigned random values between 0 and the detection limit. For data source 2, all values were above the detection limit of 0.5 ng/L, but not all values were given by the author. There were 6 values missing between the minimum and the median, and also 6 values missing between the median and the maximum. So, during these two ranges, random concentration values were assigned. For data source 3, only the maximum was given. If all 5 samples were above the detection limit of 5 ng/L, then 4 missing values were assigned random values between the detection limit (5 ng/L) and the maximum (10.9 ng/L). If 2 values were below the

detection limit, then these two values were assigned random values between 0 and the detection limit. The other two missing values above detection limit were also assign random values between the detection limit and the maximum. Using this method, all 40 values were obtained including the values below detection limits, the values given and the non-given values. The CP value for the 10 given value was calculated as its ranking number in the total 40 values divided by 41 (total samples plus 1).

It is pointed out here that the 'true' cumulative distribution can never be obtained for the given values, because the true values of those samples below detection limits are unknown. By assigning random values generated by Excel (Microsoft Office 2003), the approximately true position of each point can be determined in the cumulative distribution curve. The generation of random values in Excel is based on the assumption of normal distribution. The real distributions of measured concentration for some individual cases, however, are not necessary a normal distribution. But for each small concentration range used to generate random values, their individual distribution has very limited impacts on the whole distribution curve. Particularly, the impact is negligible when the overall data set is large and from many sources. Therefore, the method described above was considered a satisfying approach for data treatment in the exposure assessment.

4.4.2 Effects assessment

In fish effects assessment, the induction of vitellogenin (VTG), a yolk protein, in fathead minnow, brown trout, rainbow trout, Japanese medaka, and zebrafish has been used as a biomarker for adverse health effects. The *no-observed-adverse-effects-*

concentration (NOAEC) and *lowest-observed-adverse-effects-concentration* (LOAEC) on VTG were collected for all EDCs from published scientific literature in Canada, the USA, UK, Japan, Belgium, France, Norway, Denmark and China, which were listed in Table 14. All NOAEC and LOAEC values were transferred into logarithm values and cumulatively distributed, except for E3, which has only one NOAEC and two LOAEC values available. These three values were not plotted in cumulative distributions for E3. Instead, they were indicated in Figure 51 of Section 8.2.3 for risk characterisation.

Table 14. Data sources of NOEAC and LOAEC values for fish

EDCs	fish
E1	Panter et al., 1998; Routledge et al., 1998; Metcalfe et al., 2001; Thorpe et al., 2003; Van den Belt et al., 2003; Holbech et al., 2006; Bjerregaard et al., 2008
E2	Hansen et al., 1998; Kramer et al., 1998; Panter et al., 1998; Routledge et al., 1998; Tyler et al., 1999; Metcalfe et al., 2001; Kang et al., 2002; Thomas-Jones et al., 2003; Thorpe et al., 2003; Van den Belt et al., 2003; Brion et al., 2004; Xie et al., 2005a; Holbech et al., 2006; Seki et al., 2006; Bjerregaard et al., 2008; Jin et al., 2009
E3	Metcalfe et al., 2001; Holbech et al., 2006
EE2	Purdom et al., 1994; Petersen et al., 2000; Lange et al., 2001; Metcalfe et al., 2001; Rose et al., 2002; Thorpe et al., 2003; Van den Belt et al., 2003; Balch et al., 2004; Jobling et al., 2004; Pawlowski et al., 2004; Young et al., 2004; Mills and Chichester 2005; Schafers et al., 2007; Scholze and Kortenkamp 2007; Bjerregaard et al., 2008
NP	Lech et al., 1996; Hansen et al., 1998; Korsgaard and Pedersen 1998; Kwak et al., 2001; Thorpe et al., 2001; Lintelmann et al., 2003; Van den Belt et al., 2003; Jobling et al., 2004; Jin et al., 2009
OP	Jobling et al., 1996; Routledge et al., 1998; Gronen et al., 1999; Lintelmann et al., 2003; Van den Belt et al., 2003; Van Miller and Staples 2005; Bjerregaard et al., 2008
BPA	Hansen et al., 1998; Groshart and Okkerman 2000; Lindholst et al., 2000; Kwak et al., 2001; Lintelmann et al., 2003; Van den Belt et al., 2003

With human biological effects evaluation, there is a lack of data in the literature. However, some surrogate animal studies are available, particularly reproductive effects in dose-response studies using rats and mice. These animal studies were reviewed in detailed by Lai et al. (2002b), Okkerman and van der Putte (2002), Van Miller et al. (2005), Vom Saal and Welshons (2006) and Goodman et al. (2009). With these data sources, the *no-observed-adverse-effects-level* (NOAEL) was used to extrapolate the *Human Equivalent Dose* (HED). Currently, there are three interspecies extrapolation methods: extrapolation based on caloric demand, body weight and body surface area (Vermeire et al., 1999). These methods have been reviewed and compared by several authors (Watanabe et al., 1992; Vermeire et al., 1999; Gad 2006; Reagan-Shaw et al., 2008). The body surface area method has been recommended by the US Food and Drug Administration (FDA 2005), which is described by Equation (32):

$$NOAEL_{HED} = NOAEL_{animal} \times \frac{K_{m,animal}}{K_{m,human}} \quad (32)$$

where $NOAEL_{HED}$ is human equivalent daily dose (ng/kg, bw/d), $NOAEL_{animal}$ is animal dose (ng/kg, bw/d), K_m is a factor calculated as the body weight (kg) divided by body surface area (m^2). Typical values listed in Table 15 were set by US Federal Drug Administration (FDA 2005). Instead of using 60 kg as adult human body weight, this study used a typical value of 70 kg for calculations. The K_m factor will not be affected by a slightly heavier body weight, as the body surface area will increase accordingly. Using Equation (32), the obtained human $NOAEL_{HED}$ values were ranked and transferred into logarithm values and plotted against CP values.

Table 15. Typical values used in the interspecies extrapolation of $NOAEL_{HED}$ values [a]

Species	Body Weight (kg)	Body Surface Area (m^2)	K_m
Human	60	1.6	37
Rat	0.15	0.025	6
Mouse	0.02	0.007	3

[a] – Values derived from (FDA 2005).

4.4.3 Risk characterisation

With the information obtained for exposure and effects assessment, the risk characterisation was carried out by plotting CPD curves for both exposure and effects data for each EDC, which was illustrated in Figure 17 for fish and Figure 18 for humans with important values of EC95, HC5 and ADI indicated. Risk can be observed from the overlapped part of these two curves. Generally, the more the two curves overlapped, the higher would be the risk level. Two methods are used to quantify the risk: the *hazard quotient* ($HQ_{95/5}$) and the *overall risk probability* (ORP).

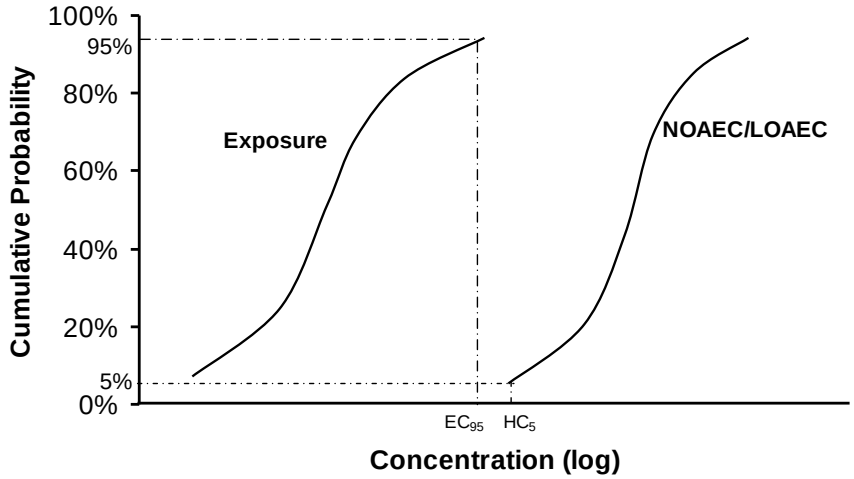

Figure 17. Cumulative probability distribution of exposure and effect values for fish.

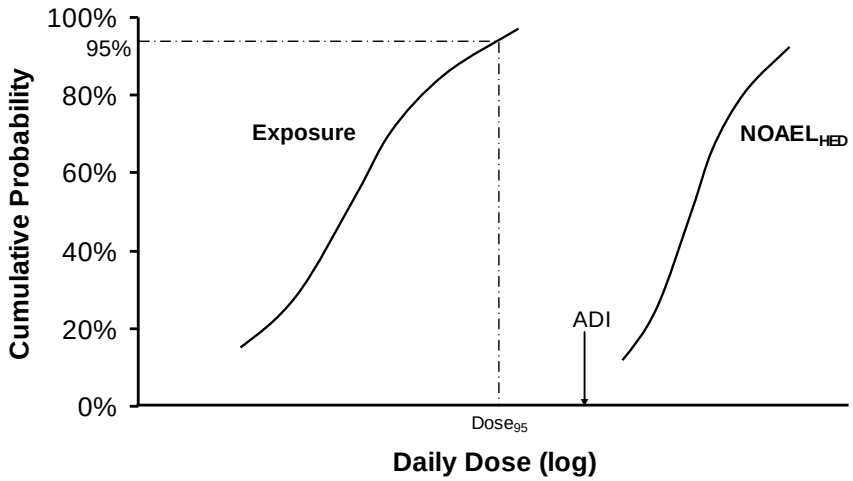

Figure 18. Cumulative probability distribution of exposure and effect values for humans.

4.4.3.1 Risk characterisation with $HQ_{95/5}$ method

The $HQ_{95/5}$ method is a single-point comparison between exposure and effect concentration, which is generally expressed as an exposure concentration divided by an effect concentration (US EPA 1998). For fish, the $HQ_{95/5}$ was calculated as the exposure concentration at 95% of CP (EC_{95}) divided by the hazard concentration at 5% of CP (HC_5) as described by Equation (33):

$$HQ_{95/5} = \frac{EC_{95}}{HC_5} \quad \text{(Fish)} \tag{33}$$

The EC_{95}, HC_5 values were obtained from the CPD curves and converted into non-logarithmic values. If $HQ_{95/5} < 1$, it means that less than 5% of fish will be affected by 95% of exposure concentrations. If HQ > 1, it means more than 5% of fish will be affected by 95% of exposure concentration. So, a $HQ_{95/5}$ value of 1 can be regarded as a reference value to assess whether a significant level of health risk occurs.

In this study, $HQ_{95/5}$ values can be directly calculated for all EDCs except for E3, because of the lack of effect data. Its HC_5 value can only be estimated from a linear relationship between the cumulative probability and the effect concentration:

$$CP = 0.4 \log C - 0.25 \quad (34)$$

Equation (34) was developed from the only available NOAEC value of 75 ng/L obtained by Metcalf et al. (2001) and an estimated slope of 0.4 based on the linear correlations for E1 and E2 as shown in Figures 43 and 44. Assuming this linear equation is CP = 0.4 log C + b, the intercept b was calculated as – 0.25 with the point of 75 ng/L at its 50% of CP. Although this linear relationship described by Equation (34) is not very accurate, it enables the risk assessor to compare the risk level among different EDCs when data is insufficient.

For humans, the hazard quotient ($HQ_{95/ADI}$) was calculated from the total daily dose at 95% of CP divided by the ADI values using Equation (35).

$$HQ_{95/ADI} = \frac{Dose_{95}}{ADI} \quad \text{(Humans)} \quad (35)$$

The total daily dose is the sum of doses from all food sources and drinking water. It can be approximated as the daily dose of food with the highest value among all food sources. This was because daily dose from drinking water and other minor food sources accounted for less than 5% of total daily dose when compared to the dominant source. Therefore, the highest daily dose of the food at its 95% CP was used as the $Dose_{95}$ in Equation (35) for calculations. In case of the lack of data points in the lower or higher tail of the CPD curves, the values at 95% and 5% of CP were extrapolated from the linear extensions of CPD curves.

The ADI values were drawn from standards set by Australia and international agencies. The first set of ADI values was collected from US EPA and UN Environmental Programme (UNEP 1995; Schlatter 1998; US EPA 2009), and the second set was drawn from the *Australian Guidelines for Water Recycling* (2006). Accordingly, $HQ_{95/ADI}$ values were calculated separately for these two sets of ADI values.

Human daily dose of EDCs from drinking water and food was also compared with $NOAEL_{HED}$ values extrapolated from rats and mice by body surface area method. Due to the more complex of human body system, $NOAEL_{HED}$ values may not reflect the true effective level of human dose. Therefore, only qualitative comparisons were made between the exposure and $NOAEL_{HED}$ values. Further, human daily dose of estrogens was also compared with female daily intake of the contraceptive pill, EE2, which is consumed at an average rate of about 500 μg/kg, bw/d (Ying et al., 2002). Similarly, $HQ_{95/EE2}$ values are also calculated for this comparison:

$$HQ_{95/EE2} = \frac{Dose_{95}}{Intake_{EE2}} \tag{36}$$

Smaller $HQ_{95/EE2}$ values implicate less health risk. Generally, if the $Dose_{95}$ is 1000 times smaller than the $Intake_{EE2}$, health risk can be regarded as negligible ($HQ_{95/EE2} < 10^{-3}$).

4.4.3.2 Risk characterisation with the ORP method

In addition to the $HQ_{95/5}$ method, health risk can also be quantified by the ORP method. The ORP method is based on the idea that the total risk can be indicated by the area under an exposure exceedence curve (Solomon and Takacs 2002). The exposure exceedence was described in Section 3.7.2. Briefly, for each CP of affected samples (or

percent of affected samples, e.g. 20% in Figure 19), it corresponds to an effect concentration (e.g. C_i in Figure 19). The CP of exposure at the same concentration C_i can be estimated from the distribution curve (e.g. 92% in Figure 19). So, exposure exceedence was calculated as $1 - CP_{Ci} = 8\%$. In other words, each CP of affected samples corresponds to an exceedence value in the exposure curve, which is also a probability. An exceedence curve can be obtained by plotting all CP values of the affected samples against these exceedence values, which was shown in Figure 20.

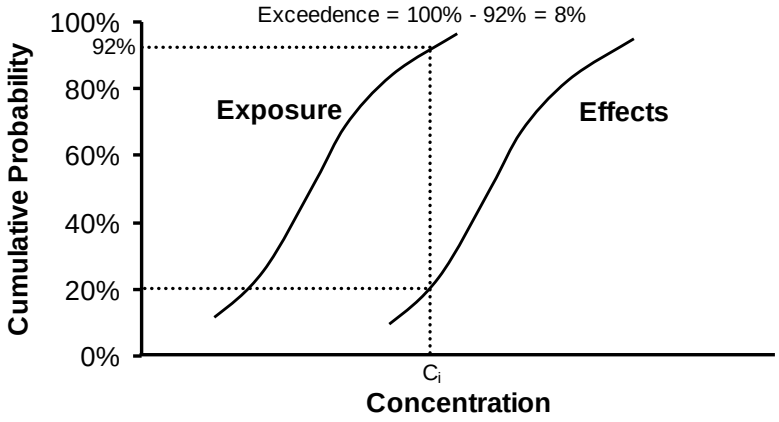

Figure 19. Exposure exceedence calculated from percent of affected samples.

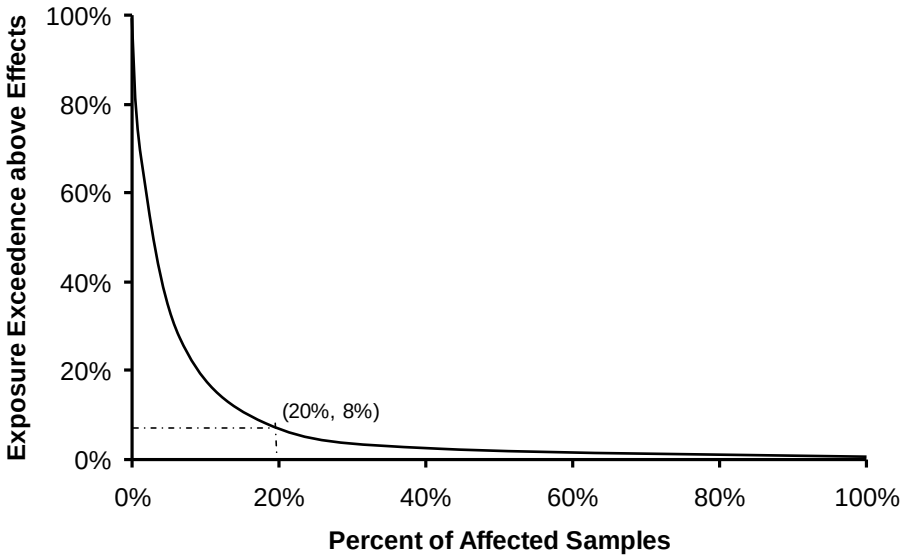

Figure 20. Exposure exceedence plotted against percent of affected samples.

The risk can be observed from the relative distance between the exceedence curve and the origin as shown in Figure 20. Larger distance means higher risk. Therefore, the exceedence curve provides a tool to compare the risk level among EDCs. Interestingly, the area under the exceedence curve increases when the exceedence curve moves away from the origin. Thus, the area can be used to quantify the risk, which is expressed as an ORP value without units by the multiplication of two probabilities.

4.4.3.3 The reference value in the ORP method

Corresponding to the reference value in the $HQ_{95/5}$ method, a reference value is also defined for the ORP method. This value is calculated as the area under a reference curve crossing the point of (5%, 5%) as shown in Figure 21. The point, (5%, 5%) corresponds to the reference value of 1 in the $HQ_{95/5}$ method. By taking half of the total area of the small rectangular and two triangulars in Figure 21, the reference value obtained for the ORP method is 2.5%.

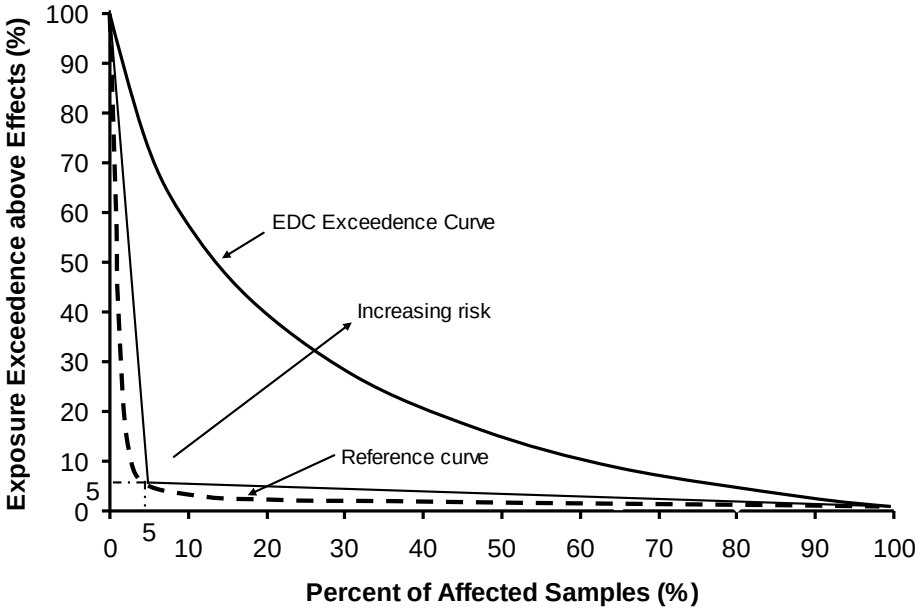

Figure 21. Overall risk probability represented by area under the exceedence curve.

With this reference value, it enables the risk assessor to judge whether a significant level of risk is imposed or not. For example, if the obtained exceedence curve for an EDC is above the reference curve, or if the ORP value calculated is larger than 2.5%, the risk is considered as significant.

4.4.3.4 Impacts of the relative position between exposure and effect CPD curves on the exceedence curve

The relative position between the exposure and effect CPD curves can affect the shape of exceedence curves. Accordingly, the ORP value calculated as the area under the exceedence curve will also be affected. For example, in Figure 22a, the exposure CPD curve is on the left of the effect CPD curve, resulting in exceedence curves below the diagonal line and curved towards the origin. On the contrary, if the exposure CPD curve is on the right of the effect CPD curve, the exceedence curve will be above the diagonal line and curved away from the origin, which was shown in Figure 22b. Apparently, the ORP value calculated from Figure 22b will be larger than the value calculated from Figure 22a, or the risk represented by exceedence curve in Figure 22b is larger than the one in Figure 22a.

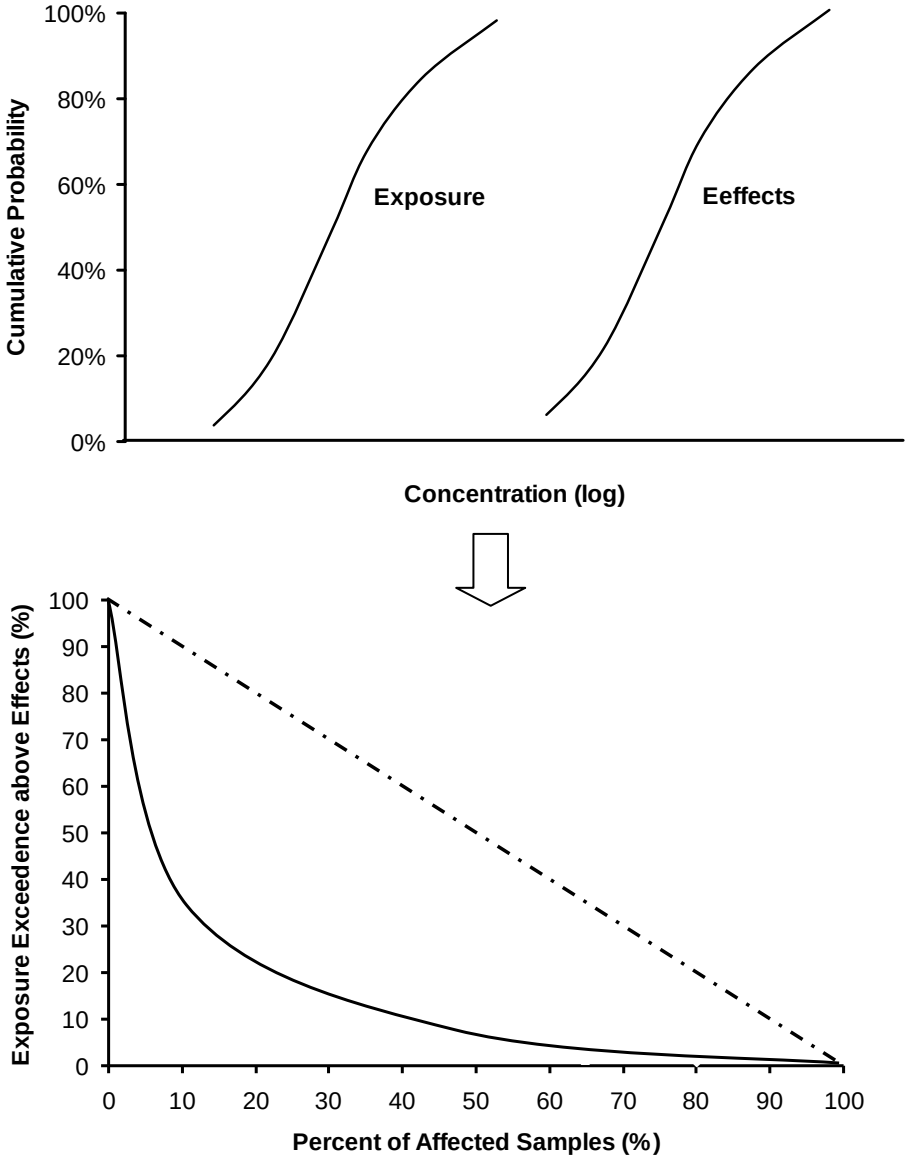

Figure 22a. Exposure CPD curve on the left of effect CPD curve, resulting in concave exceedence curve below the diagonal line.

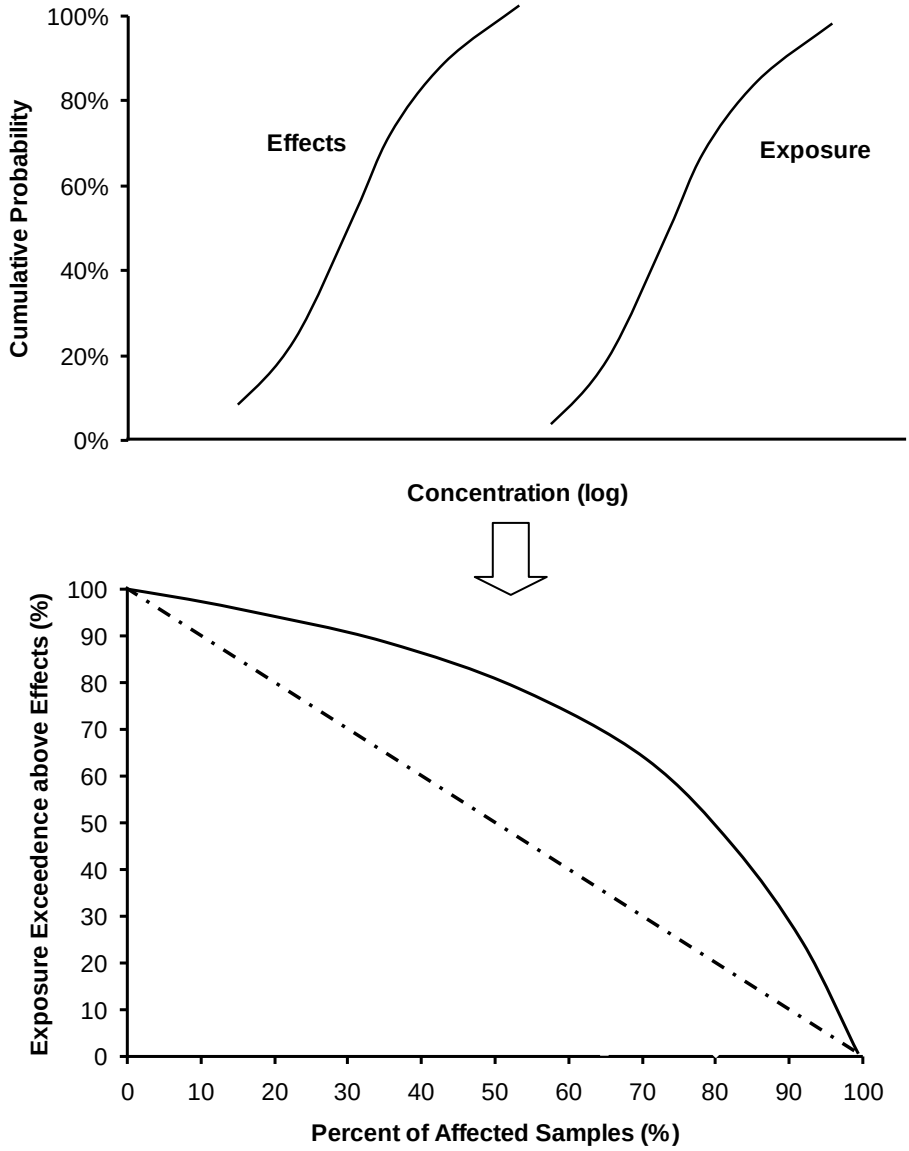

Figure 22b. Exposure CPD curve on the right of effect CPD curve, resulting in convex exceedence curve above the diagonal line.

In a rare situation, if the exposure curve is overlapped with effect curve as shown in Figure 22c, the exceedence curve will overlap with the straight diagonal line or become a curve separating the rectangular into half. Thus the ORP value can be simply calculated as the area of the triangular.

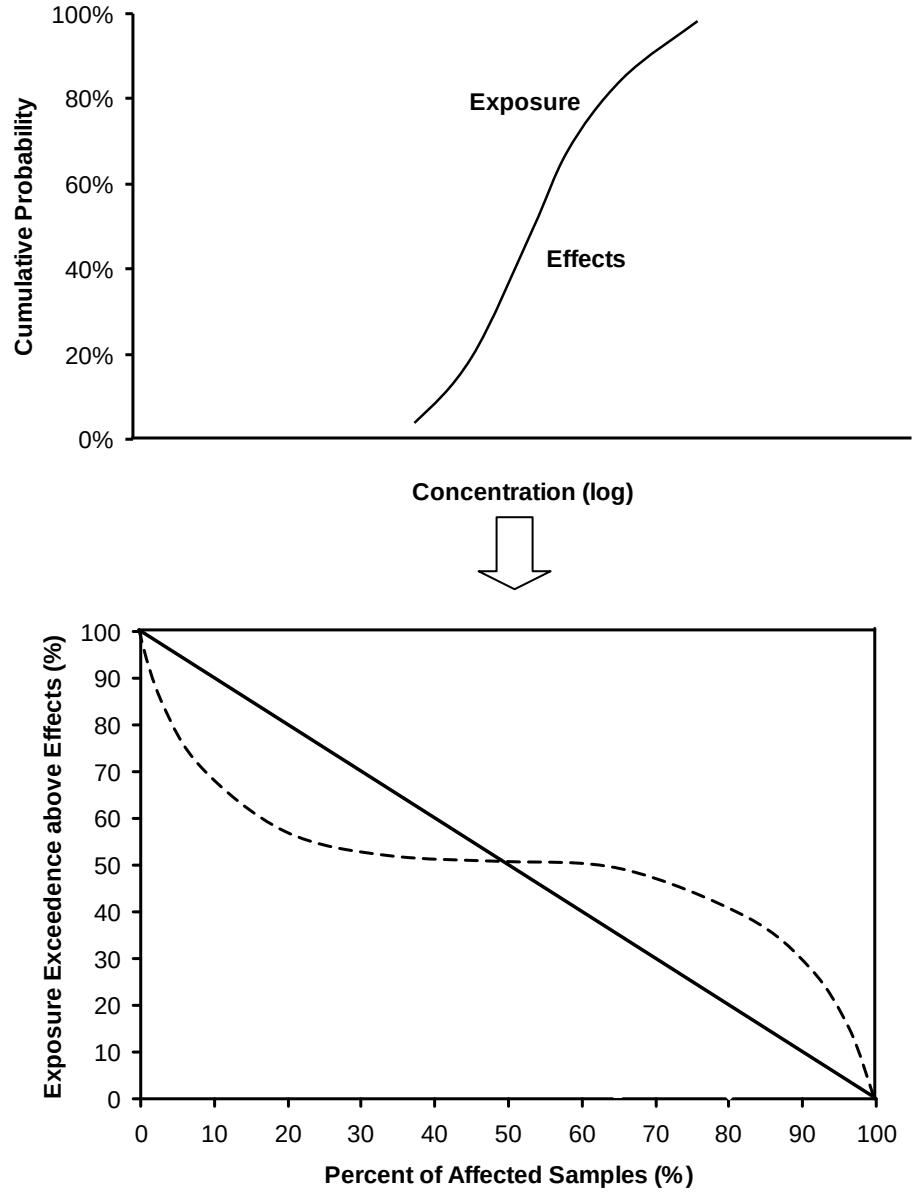

Figure 22c. Exposure and effect CPD curves overlapped, resulting in exceedence curves separating the rectangular into half.

4.4.3.5 Comparison between the $HQ_{95/5}$ and ORP method

The major difference between the $HQ_{95/5}$ method and the ORP method is that the former is only a single-point risk estimation method, whilst the latter takes into account of all points in exposure and effect CPD curves. In other words, the information of the shape of the CPD curves is included in the ORP method. However, the ORP method is more complicated and time-consuming. The calculation of exceedence values and the generation of exceedence curve require extra efforts to this methd.

Generally, larger $HQ_{95/5}$ values correspond to larger ORP values. For example, in Figure 23a, both $HQ_{95/5}$ and ORP values decrease when the effect CPD curve moves from B to C. The other two examples were illustrated in Figures 23b and 23c, both HQ and ORP values increase when the slope of exposure or effects curve decreases.

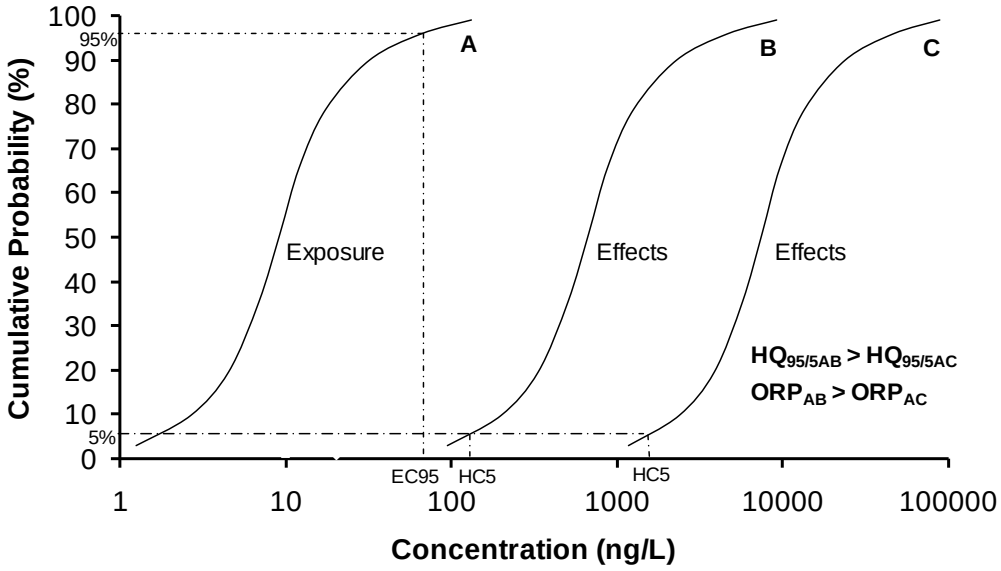

Figure 23a. Comparison of $HQ_{95/5}$ and ORP values when the relative distance between exposure and effect CPD curves changed.

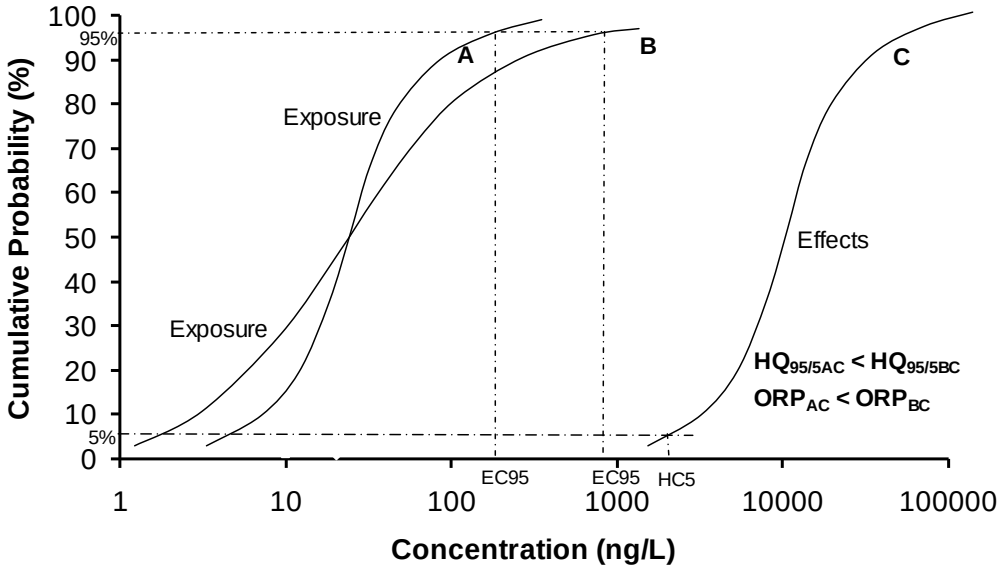

Figure 23b. Comparison of $HQ_{95/5}$ and ORP values when the slope of the exposure CPD curve changed.

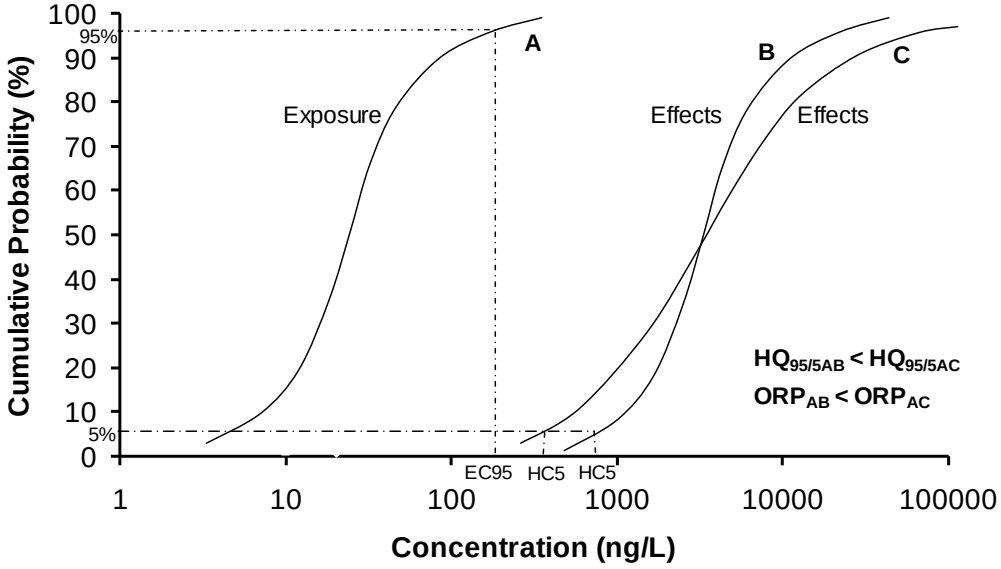

Figure 23c. Comparison of $HQ_{95/5}$ and ORP values when the slope of the effect CPD curve changed.

However, in other cases, the shape of exposure and effect distribution curves can cause disagreement between the ORP and $HQ_{95/5}$ values. For example, in Figures 24a and 24b, $HQ_{95/5}$ values calculated from the exposure and effect CPD curves are the same ($HQ_{95/5AC} = HQ_{95/5BC}$ in Figure 24a and $HQ_{95/5AB} = HQ_{95/5AC}$ in Figure 24b), because the exposure and the effect CPD curves are overlapped at the EC_{95} point in Figure 24a and at HC_5 point in Figure 24b respectively. However, the ORP values calculated from their exceedence curves are different, depending on the slope changes of the exposure curve (Figure 23a) or effects curve (Figure 23b). This was discussed in detail in the following example.

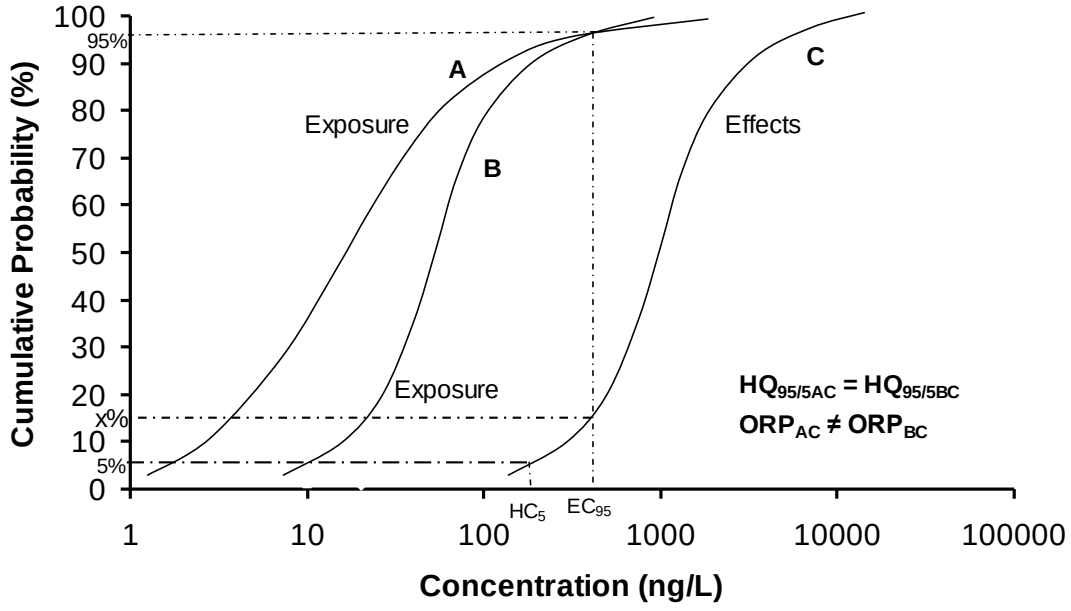

Figure 24a. Comparison of $HQ_{95/5}$ and ORP values when the exposure curves overlapped at the same C_{95} point.

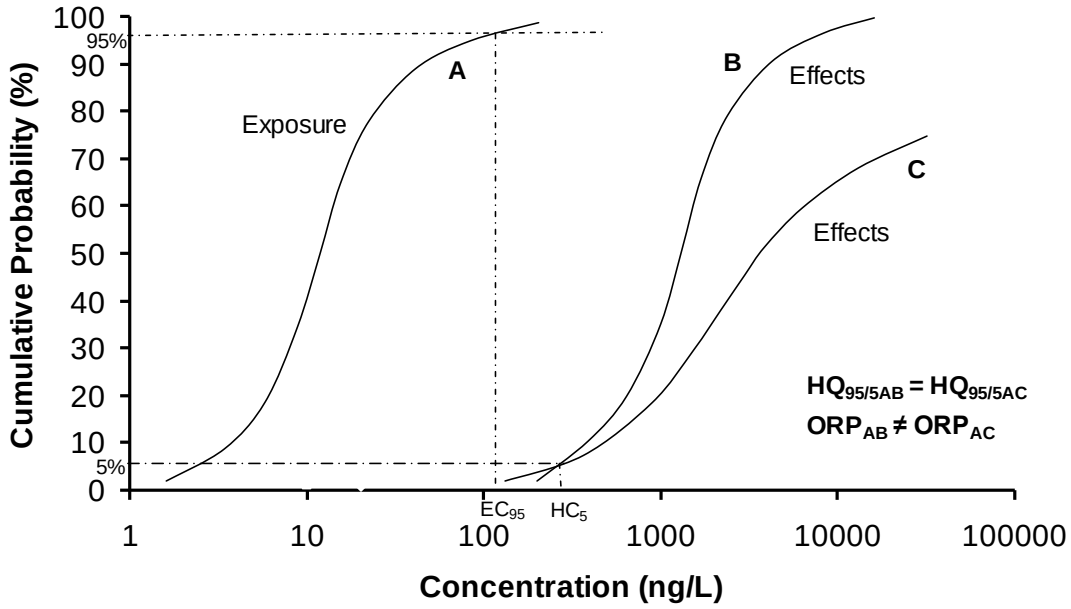

Figure 24b. Comparison of $HQ_{95/5}$ and ORP values when the effect curves overlapped at the same C_5 point.

Taking Figure 24a for example, the slope of exposure curve A is smaller than curve B, resulting in two different exceedence curves as shown in Figure 25. These two exceedence curves are overlapped at the point (x%, 5%), at which x% is the cumulative probability of the effects curve at the concentration of EC_{95} in Figure 24a. In Figure 25, the relative size of the area under these two exceedence curves depends on the position of the overlapped point of (x%, 5%). Only at a particular position when the size of these two shaded areas in Figure 25 are the same, ORP_{AC} equals ORP_{BC}. Similar analysis could be done with the two effect CPD curves in Figure 24b to show the impacts of the shape of CPD curves on the exceedence curve, which eventually affect their ORP values.

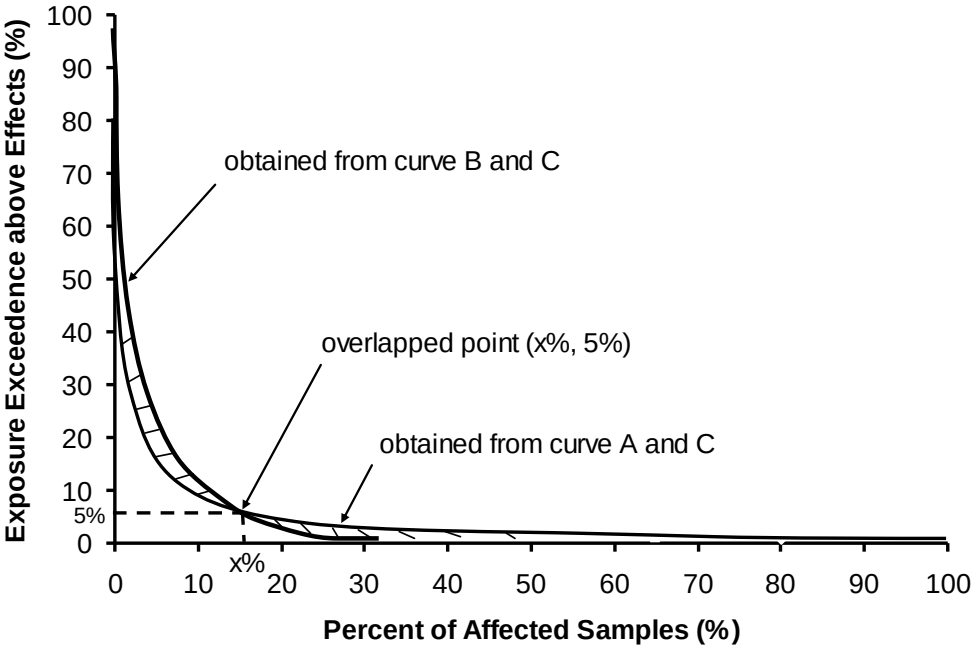

Figure 25. Comparison of $HQ_{95/5}$ and ORP values when the exposure CPD curves overlapped at the same EC_{95} point.

In some rare situations, due to extremely small standard deviation of exposure or effect data, their CPD curves form a vertical line compared with the one with larger standard deviation, which was shown in Figures 26a and 26b. If the exposure distribution form a vertical line, a 'Z' shaped exposure exceedence curve is obtained with a rectangular area of x % (ORP = x %). Similarly, if the effect distribution forms a vertical line (Figure 26b), a mirror 'Z' shaped exceedence curve is obtained. The area under the exceedence curve will be $(1 - x\ \%)^2$. This analysis again, indicates that the ORP method reflects more information regarding the distribution of exposure and effect data. It is therefore regarded as an improvement in risk characterisation.

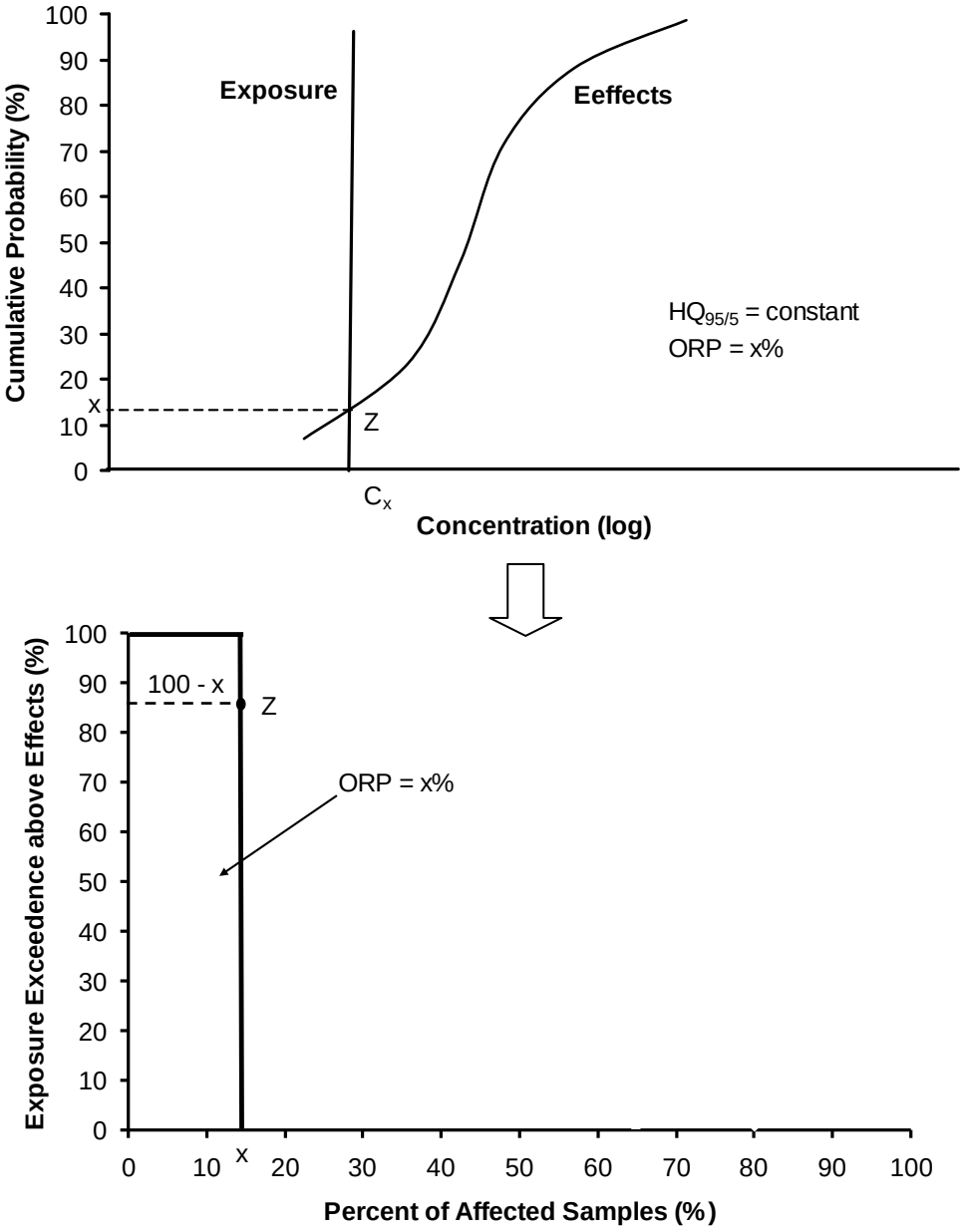

Figure 26a. A ‘Z’ shaped exposure exceedence curve resulting from a vertical exposure distribution.

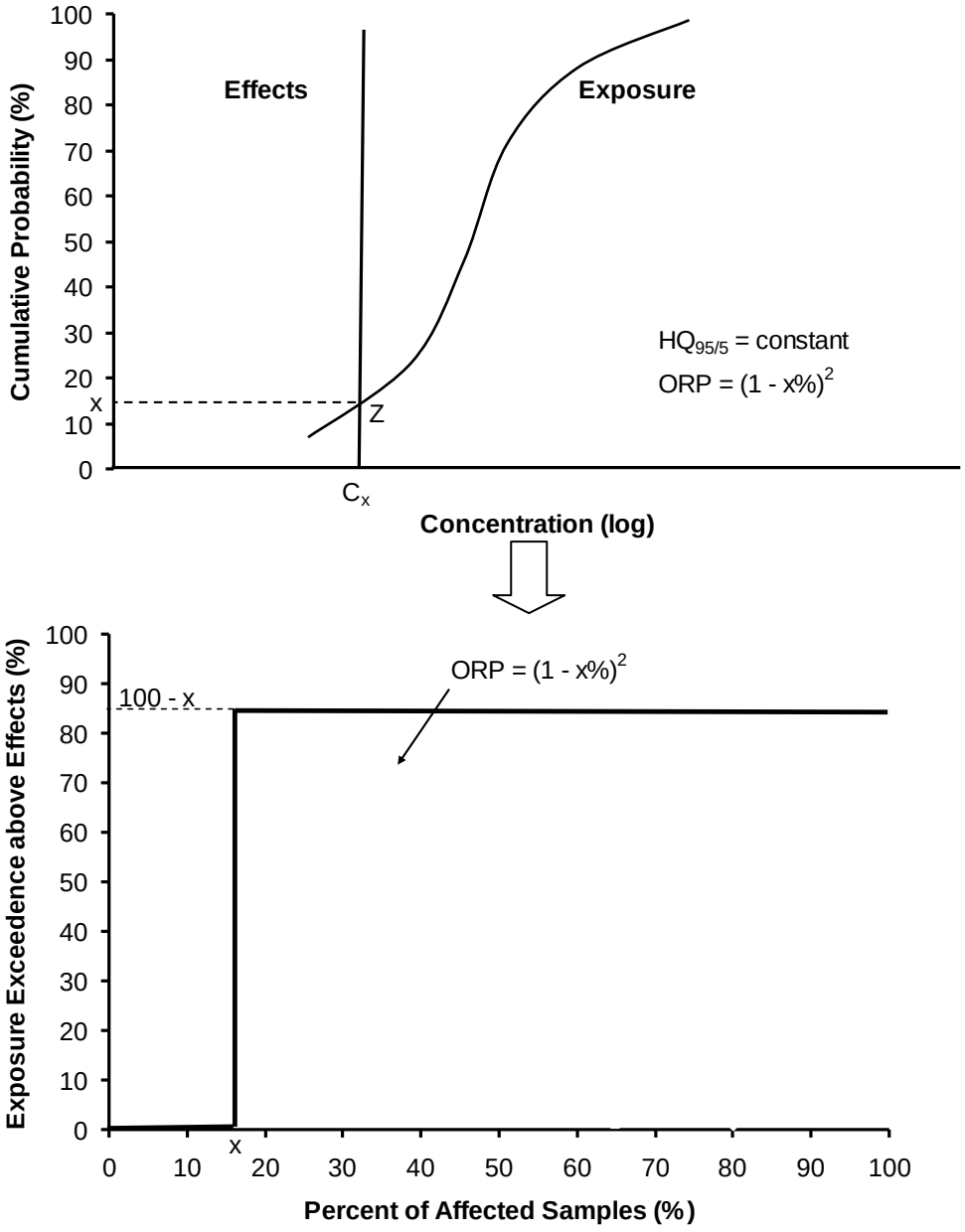

Figure 26b. A mirror 'Z' shaped exposure exceedence curve resulting from a vertical effect distribution.

Chapter 5 Quantitative structure-property relationships for steroidal EDCs

5.1 Background

A substantial amount of research has been carried out in order to understand the fate of EDCs in wastewater treatment processes (Ternes et al., 1999a; Ternes et al., 1999b; Johnson and Sumpter 2001; Ternes et al., 2002; Andersen et al., 2003; D'Ascenzo et al., 2003) and natural environments (Johnson et al., 1998; Jurgens et al., 1999; Lagana et al., 2004; Casey et al., 2005; Khanal et al., 2006). Previous studies mainly focused on adsorption and degradation mechanisms which depend not only on environmental parameters such as particulate size and surface roughness of adsorbent (Auriol et al., 2006), pH (Urase et al., 2005), temperature (Li et al., 2005), biomass concentration (Cao et al., 2008), but more importantly depend on molecular structure and properties (Clara et al., 2004; Cao et al., 2008). For example, S and K_{ow} are two important properties in the assessment and prediction of a chemical's behaviour in environmental systems.

Physicochemical and biological properties are principally determined by molecular structures, so molecular descriptors describing the structures can be used in QSPR or QSAR studies (Molnar and King 2001). For example Liu et al. (2006) developed QSAR models with 8 structural descriptors to predict the estrogen receptor binding affinity (RBA) of 132 estrogens. Also Pasha et al. (2005) also used quantum chemical descriptors and energy descriptors to predict RBA with multiple linear regression analysis. Other authors have used molecular descriptors to calculate S and K_{ow} values

for a general class of organic compounds (Warne et al., 1990; Jorgensen and Duffy 2002; Taskinen and Yliruusi 2003; Rytting et al., 2005; Balakin et al., 2006).

However, QSPR studies have not been developed for steroidal compounds which would give a deeper understanding of the manner in which structural factors influence environmental properties. For example, the fugacity modelling of steroidal compounds in natural environments requires an accurate knowledge of the K_{ow} values as well as the degradation rates. QSPR may provide new insights into both of these parameters. Importantly QSPR may provide information on the relationship between estrogenic activity and properties, molecular descriptors and other parameters. Thus this study aims to investigate the relationships between various measured and calculated physiochemical properties and molecular descriptors in order to develop QSPR for the environmentally important steroids.

5.2 Comparison of measured and calculated properties

Measured and calculated values of K_{ow} and S were summarized in Tables 16 and 17. It is noticeable in Table 16 that the measured S values for the group of 4 estrogens are generally lower (0.8 to 9.7 mg/L) as compared to the 13 androgens (7.0 to 297.0 mg/L) but this may reflect the relatively small sample size with the estrogens. In contrast, there is no apparent difference between estrogens and androgens with the calculated S values. On the other hand, there is no such difference existed for both measured and calculated log K_{ow} values between estrogens and androgens as shown in Tables 16 and 17.

Table 16. Measured property values for steroidal compounds

Compound	MW (g/mol)	T_m (℃)	log K_{ow} (-)	S (mg/L)	log S (μmol/L)
Estrogens					
Estrone (E1)	270.37[a]	260[a]	3.13[c]	0.8[e]	0.5[e]
Estradiol (E2)	272.39[a]	222[a]	3.57[c]	3.9[e]	1.2[e]
Estriol (E3)	288.39[a]	282[a]	2.45[c]	3.2[e]	1.0[e]
Ethinylestradiol (EE2)	296.41[a]	183[a]	3.67[d]	9.7[e]	1.5[e]
Androgens					
Androstanedione	288.43[a]	134[b]	3.60[c]	63.5[e]	2.1[e]
Androstenedione	286.42[a]	158[a]	2.68[c]	57.3[e]	2.3[e]
Androsterone (A)	290.45[a]	178[a]	3.69[c]	11.5[e]	1.6[e]
Corticosterone	346.47[a]	181[a]	1.85[c]	199.0[e]	2.8[e]
Cortisol	362.46[a]	220[a]	1.86[c]	297.0[e]	2.9[e]
Cortisone	360.45[a]	222[a]	1.47[d]	280.0[e]	2.8[e]
Hydroxyprogesterone	330.46[a]	220[a]	3.17[c]	6.5[e]	1.3[e]
Methyl testosterone	302.46[a]	164[a]	3.36[d]	33.9[e]	2.0[e]
Norethindrone	298.43[a]	204[a]	2.97[d]	7.0[e]	1.4[e]
Prasterone	288.43[a]	140[a]	3.23[c]	21.8[e]	1.9[e]
Pregnenolone	316.49[a]	192[a]	4.22[c]	7.1[e]	1.3[e]
Progesterone	314.47[a]	150[a]	3.87[c]	11.6[e]	1.6[e]
Testosterone (T)	288.43[a]	155[a]	3.17[c]	69.0[e]	2.4[e]

[a] - (EPISuite 2000).
[b] - (Wishart et al., 2007).
[c] - (Leszczynski and Schafer 1990).
[d] - (Hansch et al., 1995).
[e] - (Yalkowsky and He 2003).

Table 17. Calculated molecular descriptors and properties using QikProp program[a]

Compound	log FOSA ($Å^2$)	log FISA ($Å^2$)	log PSA ($Å^2$)	log K_{ow}	S (mg/L)	log S (μmol/L)
Estrogens						
Estrone (E1)	2.466	1.977	1.683	3.22	15.9	1.8
Estradiol (E2)	2.521	1.951	1.622	3.37	7.2	1.4
Estriol (E3)	2.425	2.149	1.806	2.14	80.2	2.4
Ethinylestradiol (EE2)	2.482	1.960	1.637	3.86	6.5	1.3
Androgens						
Androstanedione	2.624	1.942	1.712	2.89	50.5	2.2
Androstenedione	2.596	1.990	1.743	2.87	29.9	2.0
Androsterone (A)	2.635	1.948	1.682	3.17	15.3	1.7
Corticosterone	2.582	2.217	1.965	1.82	86.6	2.4
Cortisol	2.575	2.244	2.025	1.39	170.3	2.7
Cortisone	2.529	2.271	2.050	1.19	404.4	3.1
Hydroxyprogesterone	2.620	2.074	1.845	3.09	10.5	1.5
Methyl testosterone	2.622	1.951	1.689	3.75	4.1	1.1
Norethindrone	2.587	1.967	1.691	3.62	5.0	1.2
Prasterone	2.613	1.932	1.658	3.17	14.7	1.7
Pregnenolone	2.688	1.890	1.648	3.99	1.2	0.6
Progesterone	2.651	1.938	1.714	3.59	7.3	1.4
Testosterone (T)	2.600	1.963	1.688	3.10	17.2	1.8

[a] - (Schrodinger 2007).

When the measured log K_{ow} values were plotted against the calculated log K'_{ow} values in Figure27, a linear relationship was obtained:

$$\log K'_{ow} = 1.00 \log K_{ow} - 0.103 \quad (R^2 = 0.836) \qquad (37)$$

Equation (37) indicates that the calculated values are smaller than the measured ones. When estrogens and androgens were analysed separately, the coefficient of log K_{ow} and the coefficient of determination (R^2) did not improve. This suggests that there is sufficient structural similarity and function in this application, that the androgens and estrogens can be treated as one group.

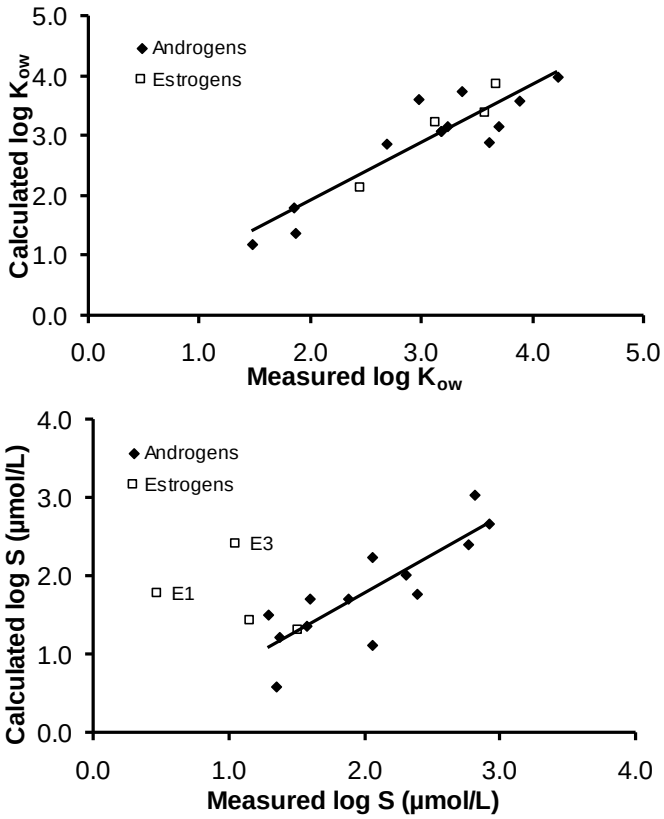

Figure 27. Plots of measured and calculated log K_{ow} and log S values

The calculated log S′ values were also plotted against measured log S values for all 17 compounds in Figure 27. The coefficient of 0.519 in Equation (38) indicates a substantial deviation between the calculated and measured values. There is a marked difference of the coefficient to the value of 1. As observed from Figure 27, two points (E1 and E3) can be regarded as outliers. In addition, the number of estrogens is limited in size. So, the regression based on all 17 compounds is not reliable for further analysis. With the exclusion of estrogens, the relationship for androgens was given in Equation (39) using linear regression analysis:

$$\log S' = 0.519 \log S - 0.851 \quad (R^2 = 0.323) \tag{38}$$

$$\log S' = 0.980 \log S - 0.184 \quad (R^2 = 0.700) \tag{39}$$

The R^2 value has been improved significantly from 0.323 to 0.700 and the coefficient increased from 0.519 to 0.980. Therefore the subgroup classification was effective for log S but not for log K_{ow} because the solubility data in Table 16 for estrogens is distinctly lower than androgens. In addition, Equation (39) also indicates that the calculated values were much smaller than the measured ones. This suggests that the solubility values for estrogens may be too low and perhaps a systematic error is involved in their measurement.

5.3 Relationship between measured properties

Measured log S and measured log K_{ow} were plotted for all 17 compounds in Figure 28, but the R^2 value is very low (0.354) and the coefficient of log K_{ow} is -0.529. By observation (see Figure 28), the subgroup of 4 estrogens does not follow the pattern of the androgens and two of these can even be regarded as outliers (E1 and E3). Therefore, for the reasons outlined above, small sample size and a possible systematic measurement error, this group of four estrogens was excluded and the correlation between log S and log K_{ow} was obtained for the group of androgens alone (Figure 28):

$$\log S = -0.561 \log K_{ow} + 3.72 \quad (R^2 = 0.665) \qquad (40)$$

By incorporating molecular weight (MW) and melting point (T_m) into the relationship in Equation (40), the R^2 value increased to 0.857. When estrogens were also included, the R^2 value was even higher at 0.905, while the coefficient of log K_{ow} changed slightly to -0.611:

$$\log S = -0.611 \log K_{ow} + 0.00680 MW - 0.0118(T_m - 25) + 3.56 \quad (R^2 = 0.905) \qquad (41)$$

Similar correlations were investigated by other researchers a using general group of organic compounds. For example, Meylan et al. (1996) obtained the following relationship:

$$\log S = -0.935 \log K_{ow} - 0.00468MW - 0.00820(T_m - 25) + 0.978 \quad (R^2 = 0.929) \qquad (42)$$

The R^2 value the authors (Meylan et al., 1996) obtained (0.929) was slightly higher than this study (0.905). Other differences observed between Equation (41) and (42) were probably due to the particular group of compounds used in this study. The coefficient of log K_{ow} in Equation (41) is close to a value of -0.650, which was used in the automated chemical property estimation program (PCCHEM) (US EPA 1987) for $-0.5 < \log K_{ow} < 3.2$.

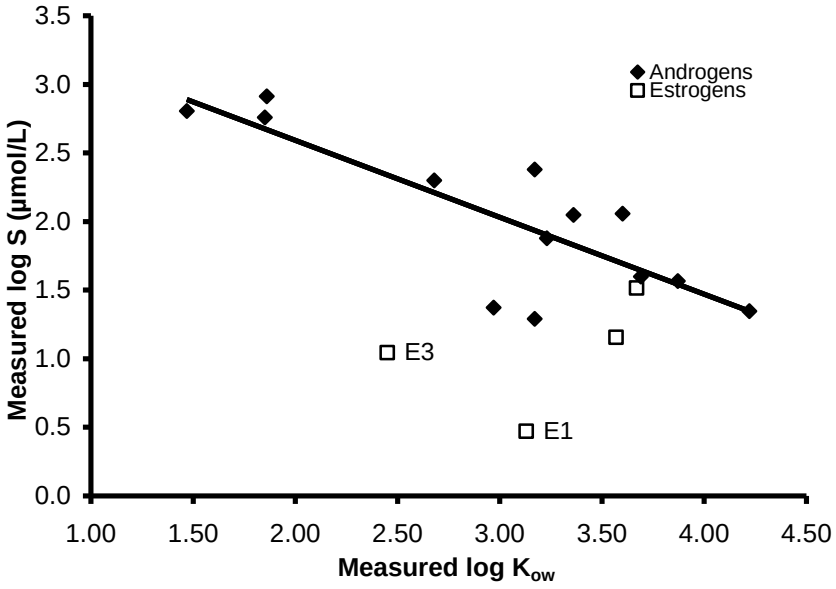

Figure 28. Plots of measured log S values against measured log K_{ow} values

5.4 Relationship between measured log S and molecular descriptors

It can be seen from Tables 16 and 17 that measured log S values and calculated log FOSA values for estrogens are distinctly lower than those of androgens. This could be attributed to the influence of the phenolic group in the estrogens lowering the FOSA values. Therefore, the subgroup classification was used for the relationships between log S and log FOSA. Estrogens were also excluded from the relationships between log S and the other two molecular descriptors log FISA and log PSA, because their inclusion in the analysis significantly lowered the R^2 values. When log S values were plotted against molecular descriptors in Figure 29 for androgens, weak correlations were found:

$$\log S = -11.0 \log FOSA + 30.6 \quad (R^2 = 0.546) \tag{43}$$

$$\log S = 3.23 \log FISA - 4.50 \quad (R^2 = 0.542) \tag{44}$$

$$\log S = 2.86 \log PSA - 3.05 \quad (R^2 = 0.505) \tag{45}$$

It is important to note the differences in the coefficients for log FOSA, log FISA and log PSA. The coefficient for log FOSA is negative in Equation (43), and positive for log FISA and log PSA in Equation (44) and (45) respectively. This reflects important structural influences on properties. FOSA is a measure of the hydrophobic property of a molecule and as it increases, the polarity of the molecule will decrease. As a result the aqueous solubility in polar water would be expected to decrease which would lead to a negative coefficient for log FOSA. On the other hand FISA, the hydrophilic component of total solvent accessible surface area, and PSA, Van de Waals surface area of polar nitrogen and oxygen atoms, both give measures of hydrophilic properties. As they increase, the polarity of the molecules will rise and aqueous solubility should rise in

accord with this leading to positive coefficients of log FISA and log PSA as shown in Equation (44) and (45).

When MW and T_m were considered as independent variables for the relationships in Equation (43) to (45), the correlation matrix showed multicollinearity. This is because log FISA and log PSA, log FISA and MW, log FISA and T_m, log PSA and MW, log FOSA and T_m were highly correlated. Therefore, the multiple variable regressions using molecular descriptors, MW and T_m as independent variables were not conducted.

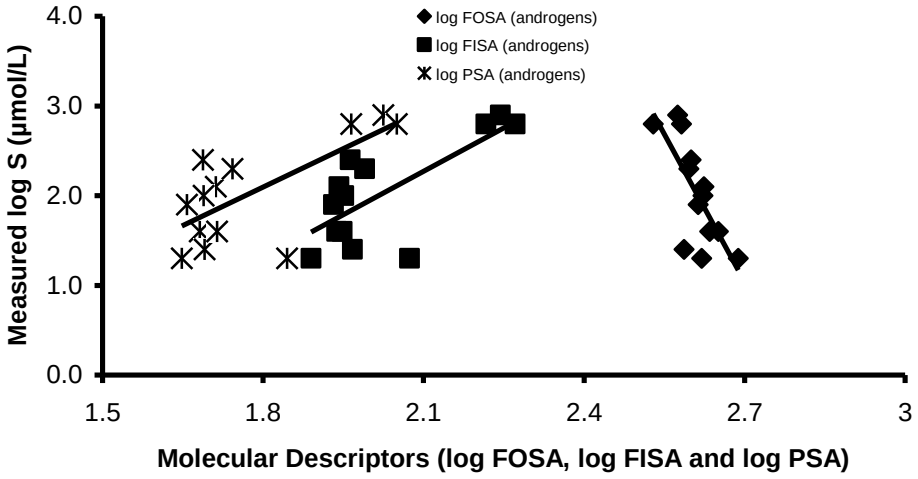

Figure 29. Correlations of measured log S values with molecular descriptors.

5.5 Relationship between measured log K_{ow} and molecular descriptors

It was found the measured K_{ow} was closely related to three molecular descriptors: FOSA, FISA and PSA ($0.777 < R^2 < 0.973$). When the measured log K_{ow} values were plotted against the log FOSA values in Figure 30, it clearly showed that the subgroup of 4 estrogens was different from the remaining 13 androgens. So, linear regression equation in Equation (46) was only obtained for androgens:

$$\log K_{ow} = 20.0 \log FOSA - 49.1 \quad (R^2 = 0.862) \qquad (46)$$

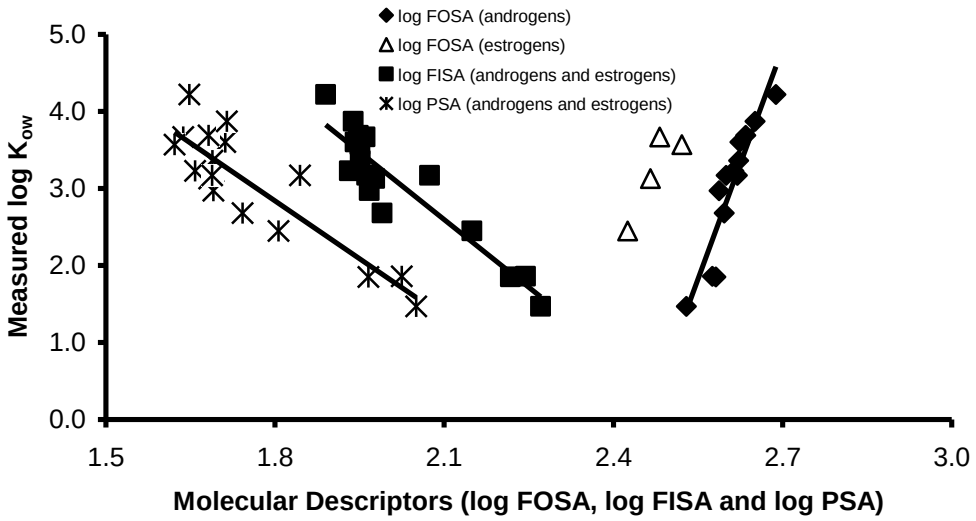

Figure 30. Correlations of measured log K_{ow} values with molecular descriptors.

The relationship between log K_{ow} and the other two descriptors were similarly processed. In contrast to the relationship with log FOSA, no significant variations were found for the R^2 value when estrogens and androgens were considered separately. This implies that the phenolic A-ring, in the subgroup of estrogens has a major impact on the hydrophobicity as reflected in the hydrophobic component of the total surface area (FOSA). Its increases size in the estrogens results in a lower log FOSA values for the estrogens than the androgens. On the other hand, it does not affect log FISA and log PSA values (Table 17). Therefore, the subgroup classification is not required for FISA and PSA. The relationships between measured log K_{ow} and log FISA and log PSA for all 17 compounds were also plotted in Figure 30. Two linear regression equations Equation (47) and (48) were obtained from these plots:

$$\log K_{ow} = -5.86 \log FISA - 14.9 \quad (R^2 = 0.861) \qquad (47)$$

$$\log K_{ow} = -5.01 \log PSA + 11.9 \quad (R^2 = 0.782) \qquad (48)$$

The multiple regression analysis was not considered because of multicollinearity existed for molecular descriptors, MW and T_m, as discussed in Section 5.4. Also, it is important to note that these relationships in Equation (46) to (48) are consistent with the relationships found with log S as noted in Equation (43) to (45). The value of K_{ow} is closely related to the ratio of the solubility in octanol to aqueous solubility allowing for the mutual solubility of octanol and water. Thus as aqueous solubility increases the K_{ow} value would be expected to decline. This would have the effect of reversing the negative and positive values of the coefficients before log FOSA, log FISA and log PSA in Equation (43) to (45) and Equation (46) to (48). Thus log S varies positively with log FISA and log PSA and negatively with log FOSA. On the other hand, log K_{ow} varies positively with log FOSA and negatively with log FISA and log PSA.

5.6 Chapter conclusions

Good simple linear relationships were found between the measured octanol-water partition coefficient (log K_{ow}) and three calculated molecular descriptors: log FOSA (hydrophobic component of the total solvent accessible surface area), log FISA (hydrophilic component of the total solvent accessible surface area) and log PSA (Van de Waals surface area of polar nitrogen and oxygen atoms). Similar correlations were conducted between the measured aqueous solubility (log S) and each of the three molecular descriptors, but only weak correlations were observed. With log S the coefficients of the hydrophobic parameter (log FOSA) was negative but with the hydrophilic parameters (log FISA and log PSA) was positive as would be expected from the influence of polarity on aqueous solubility. On the other hand, opposite values were observed for the coefficients of log FOSA, log FISA and log PSA in the relationship

with log K_{ow}, because increasing aqueous solubility has a negative effect of the value of log K_{ow}. The phenolic A-ring present in the structure of the estrogens has a major effect on their hydrophobicity. In addition, when the calculated log K_{ow} was plotted against the measured ones for all 17 compounds, it demonstrated that the calculation methods generally give reliable results. However, the calculated log S values agreed well with the measured values only for the subgroup of androgens. The relationships obtained in this study can be used to obtain property values for various steroidal compounds, particularly those with potential environmental effects.

Chapter 6 Relationship between degradation rate constant and equivalent biomass concentration for estrogens

6.1 Background

The biodegradation of EDCs in the environment is affected by a number of factors. A few studies revealed that higher temperature leads to faster degradation of estrogens (Layton et al., 2000; Jurgens et al., 2002; Li et al., 2005). Longer sludge retention time (SRT) and hydraulic retention time (HRT) can also slightly increase the degradation rate (Kreuzinger et al., 2004; Lee et al., 2004b). Additionally, two studies (Andersen et al., 2004; Li et al., 2005) indicated that biomass concentrations can impose impacts on degradation rate. Khan and Ongerth (2004a) assumed a direct linear relationship between the half-life (rate constant) and biomass concentration.

However, there have been no quantitative correlations describing the relationship between rate constants and biomass concentrations in the literature. Therefore, the aim of this study is to establish a quantitative correlation. The relationship obtained can provide an important tool to predict the fate of estrogens in wastewater treatment and receiving water under normal conditions. In particular, this is of significances in the context of water reuse and recycling with the evaluation of the relevant health risk.

6.2 Collation of rate constants and equivalent biomass concentration

Rate constants with corresponding EBC values for E1, E2 and EE2 are summarized in Tables 18, 19 and 20. As can be seen from these tables, equivalent biomass concentration (EBC) are much lower in surface water (0.003 to 0.35 mg/L) compared to activated sludge (208 to 3200 mg/L). Accordingly, the rate constants are also lower in surface water (0.00036 to 0.29 h^{-1}) compared to activated sludge (0.012 to 130 h^{-1}). This means both EBC and rate constants in activated sludge are higher compared to those in surface waters.

Temperatures, initial concentrations and environmental media were also listed in these tables. As pointed out earlier, factors such as temperature, pH, initial concentration, SRT and HRT can affect the rate constants. Because most experiments were conducted at temperatures close to 20 ℃ and pH values close to 7, the influences of temperature and pH were excluded from this study. In addition, the possibility of toxic effects caused by high initial concentrations is low in most studies. Although the SRT and HRT were unknown for most reports in our study, their influences were weak according to Johnson et al. (2005). So, EBC is regarded as the major factor which influences the degradation rate constants.

Table 18. Equivalent biomass concentrations (EBC) and rate constants (k) of E1 [a]

EBC (mg/L)	k (h^{-1})	T (℃)	C_i (μg/L)	Media
0.044	0.012	20	100	river water
0.047	0.29	20	100	river water
0.067	0.0066	20	100	river water
0.0096	0.058	20	100	river water
0.050	0.041	20	100	river water
0.017	0.016	20	100	river water
0.11	0.011	20	100	river water
0.14	0.041	20	100	river water
0.35	0.013	20	100	river water
0.24	0.019	20	100	river water
0.25	0.0070	20	100	river water
0.14	0.022	20	100	river water
0.17	0.014	20	100	river water
0.011	0.018	20	100	river water
0.17	0.011	20	100	river water
0.063	0.0067	20	100	river water
0.010	0.0090	20	100	river water
0.036	0.041	20	100	river water
0.014	0.0093	20	100	river water
0.0055	0.0040	20	100	river water
0.0039	0.010	20	100	river water
0.0030	0.0026	20	100	river water
208	0.042	20	1000	activated sludge
232	5.2	16	0.5	activated sludge
240	2.0	16	0.5	membrane bioreactor sludge
400	8.5	16-18	0.5	activated sludge
416	0.48	20	1	activated sludge
1600	4.2	20	16	activated sludge supernatant
3200	67[e]	16-18	0.5	activated sludge

[a] – data were drawn from (Jurgens et al., 2002; Onda et al., 2003; Andersen et al., 2004; Joss et al., 2004).

Table 19. Equivalent biomass concentrations (EBC) and rate constants (k) of E2 [a]

EBC (mg/L)	k (h^{-1})	T (℃)	C_i (μg/L)	Media
0.044	0.013	20	100	river water
0.047	0.032	20	100	river water
0.067	0.021	20	100	river water
0.0096	0.010	20	100	river water
0.050	0.017	20	100	river water
0.017	0.14	20	100	river water
0.11	0.032	20	100	river water
0.14	0.017	20	100	river water
0.35	0.011	20	100	river water
0.24	0.017	20	100	river water
0.25	0.012	20	100	river water
0.14	0.0069	20	100	river water
0.17	0.017	20	100	river water
0.011	0.096	20	100	river water
0.17	0.032	20	100	river water
0.063	0.012	20	100	river water
0.010	0.0070	20	100	river water
0.036	0.012	20	100	river water
0.014	0.0070	20	100	river water
0.0055	0.0033	20	100	river water
0.0039	0.014	20	100	river water
0.0030	0.0085	20	100	river water
0.0029	0.019	20	100	river water
208	1.1	20	1000	activated sludge
232	11	16	0.5	activated sludge
240	4.4	16	0.5	membrane bioreactor sludge
400	16	16-18	0.5	activated sludge
416	10	20	1	activated sludge
435	0.85	20	30	activated sludge
850	1.3	20	30	activated sludge
1600	5.9	20	16	activated sludge supernatant
1732	0.25	22-25	58	activated sludge
1750	3.3	20	30	activated sludge
3200	1.3E+02[e]	16-18	0.5	activated sludge

[a] – data were drawn from (Ternes et al., 1999a; Layton et al., 2000; Jurgens et al., 2002; Onda et al., 2003; Andersen et al., 2004; Joss et al., 2004; Li et al., 2005).

Table 20. Equivalent biomass concentrations (EBC) and rate constants (k) of EE2 [a]

EBC (mg/L)	k (h^{-1})	T (℃)	C_i (μg/L)	Media
0.085[b]	0.00063	20	280	river water
0.085 [b]	0.0017	20	100	river water
0.085 [b]	0.00036	20	1 μg/g	ground water
232	0.073	16	0.1	activated sludge
240	0.10	16	0.1	membrane bioreactor sludge
400	0.063	16-18	0.5	activated sludge
800	0.025	20	50	activated sludge
1732	0.012	22-25	58	activated sludge
2130	0.013[c]	20	100	artificial wastewater
3200	0.50[e]	16-18	0.5	activated sludge

[a] – data were drawn from (Jurgens et al., 1999; Layton et al., 2000; Vader et al., 2000; Jurgens et al., 2002; Kikuta and Urase 2003; Ying and Kookana 2003a; Andersen et al., 2004; Joss et al., 2004)
[b] – average value of EBC in river water calculated from Tables 18 and 19.
[c] – calculated.

6.3 Relationship between rate constants and equivalent biomass concentration

Using the data in Tables 18, 19 and 20, the relationship between EBC and degradation rate constants k was evaluated by plotting log EBC against log k values as illustrated in Figures 31, 32 and 33. Three logarithmic linear regressions in Equation (49), (50) and (51) were obtained for E1, E2 and EE2, respectively:

$$\log k = 0.52 \log EBC - 1.1,\ R^2 = 0.73 \quad (49)$$

$$\log k = 0.53 \log EBC - 0.99,\ R^2 = 0.79 \quad (50)$$

$$\log k = 0.44 \log EBC - 2.6,\ R^2 = 0.73 \quad (51)$$

The correlations are relatively good with correlation coefficients ranging from 0.73 to 0.79, which confirmed EBC is a major factor. The correlations are essentially based on two sets of data, one at very low EBC (surface water), and the other at high EBC

(activated sludge). When the data plotted in a linear form (not in logarithmic form), only E1 gave a good correlation coefficient (0.83). But for E2 and EE2, the correlation coefficients are too low only at 0.55 and 0.48 respectively. Therefore, the linear correlations were not used for further evaluation.

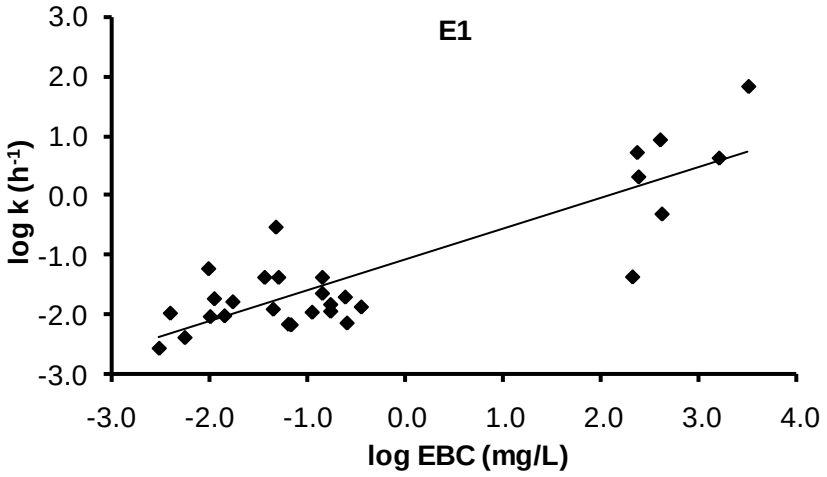

Figure 31. Correlations between log K and log EBC for E1.

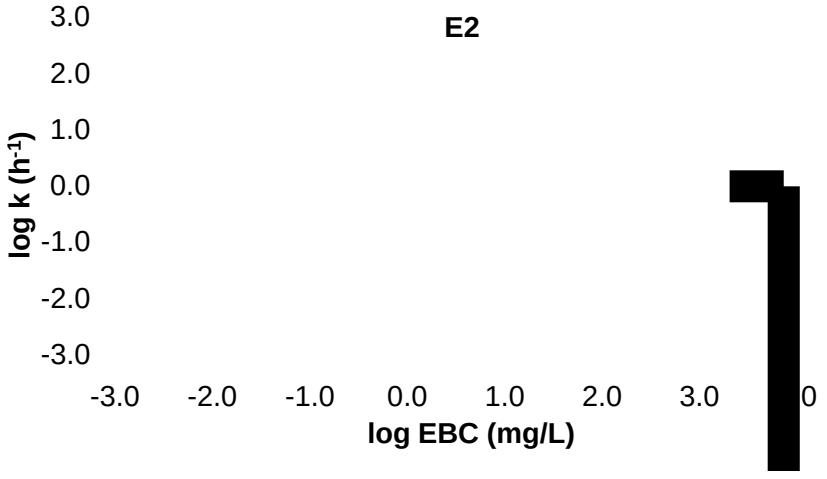

Figure 32. Correlations between log K and log EBC for E2.

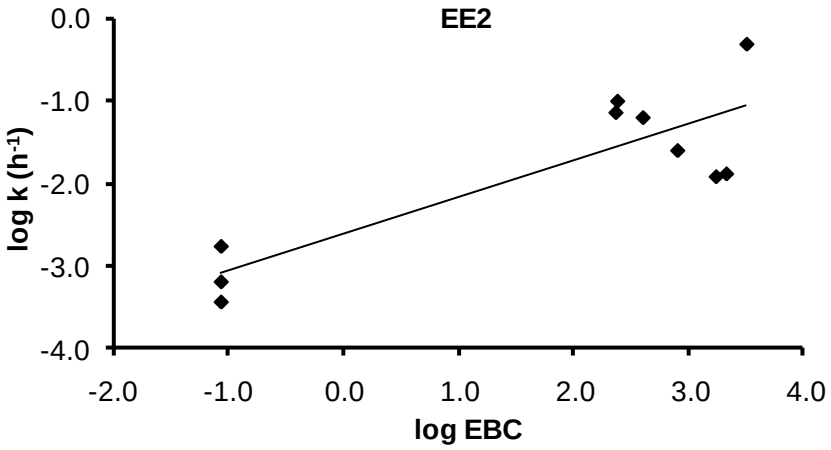

Figure 33. Correlations between log K and log EBC for EE2.

6.4 Limitations in the available data

There is an absence of biomass concentration data between the low concentration range in river water and high concentration range in activated sludge as shown in Figures 31, 32 and 33. Nevertheless, there are good correlations between the biomass concentration and rate constant when only the data of activated sludge at 20 ℃ was studied (e.g. R^2 = 0.82 for E2). Due to the limited source of data in river (only from one author), such correlations for river are not as good as activated sludge. For EE2, the data set is very limited and the average EBC values from other rivers can be used as substitute for the missing EBC values. The relationship obtained for EE2 is not as reliable as that for E1 and E2. However, it can be used for comparison purposes. These observations support the general positive relationships which have been found between EBC values and rate constant.

6.5 Degradation patterns

It is interesting to note that Equations (49), (50) and (51) have similar slopes despite the differences in chemical structures reflecting differences in physicochemical and chemical properties particularly the equations for E1 and E2. This is difficult to interpret but it implies the influences of EBC on degradation are somewhat similar in all cases.

The regression equations for E1 and E2 are very similar both in slope and intercept, reflecting the close similarity in molecular structure and properties of these compounds (see Equations (49) and (50)). In contrast to these compounds, EE2 has quite a different intercept (Equation 8). It can be seen that the equations for E1 and E2 have intercepts of -1.1 and -0.99, while the intercept for the equation representing EE2 is different at -2.6. This suggests that the compound EE2 degrades at a consistently slower rate than E1 and E2.

A comparison of the rate constants exhibited by compounds E1, E2 and EE2 was made by subtracting Equation (51) from Equations (49) and (50) as shown below:

$$\log k_{E1} - \log k_{EE2} = 0.08 \log EBC + 1.5 \quad (52)$$

$$\log k_{E2} - \log k_{EE2} = 0.09 \log EBC + 1.6 \quad (53)$$

Using these equations we can take an average river EBC of 0.085 mg/L from Tables 18 and 19, and a typical activated sludge EBC of 3200 mg/L and substitute these into Equations (52) and (53). These calculations indicate that the rate constants are 26 times (river) and 60 times (activated sludge) higher for E1, 33 times (river) and 84 times

(activated sludge) higher for E2 than those of EE2 respectively. Andersen et al. (2004) also calculated a similar ratio of 100 times between E2 and EE2 with an activated sludge EBC of 3200 mg/L (4 g/L MLSS).

6.6 Observations on degradation and structures

The relatively shorter half-lives (a few minutes to several hours) of E1 and E2 can be explained by reference to their molecular structures. The compound E2 has two hydroxyl groups, one at the C-3 and another at the C-17 position which is known to be susceptible to microbial attack (Ying and Kookana 2003a). When the hydroxyl group at C-17 is oxidized into a more hydrophobic ketone group by microbial action (Fukuhara et al., 2006), the compound E2 is transformed into compound E1. The compound E1 can then be further fragmented at slower rate from the polar ketone group at C-17 and the other hydroxyl group at C-3 (Sun et al., 2005).

In contrast, the compound EE2 is more resistant to biodegradation than compounds E1 and E2. Bolt (1979) and Ying and Kookana (2005) have explained that the triple bond ethynyl group in the chemical structure of EE2 (Figure 2) may block the formation of a ketone since it hinders access to the hydroxyl group in the C-17 position. This slows the formation of oxidation products resulting from this reaction. However the remaining segment of the EE2 molecule is the same as E1 and E2 and so once the reaction proceeds with this segment, the degradation of this molecule is similar to that of compounds E1 and E2. As a result the compound EE2 is more persistent in surface water (Ying and Kookana 2003a). For example, Jürgens et al. (1999, 2002) and Ying et al. (2003b) obtained half-lives of 17, 46 and 81 days respectively under aerobic

conditions using river and lake water. Under anaerobic conditions, Czajka et al. (2006) observed no degradation occurred during a three-year incubation period with lake water. This persistence of compound EE2 can allow the compound to bind to particulate and organic matter resulting in accumulation in sediments. In addition aquatic organisms may exhibit bioaccumulation since the compound has a relatively high K_{ow} value (1.41 x 10^4) and persistence (Flammarion et al., 2000; Lai et al., 2002).

6.7 Chapter conclusions

The degradation rate constants in aquatic systems for three estrogens (compounds E1, E2 and EE2) were normalised for biomass concentration and relationships to degradation rate evaluated. Acceptable linear relationships were established between the logarithm of EBC and logarithm of the rate constants for the three compounds. The three regression equations obtained indicate that EE2 is most resistant to biodegradation, while E1 and E2 are relatively easily degraded with similar rates. The regression equations all had similar slopes while the equations for E1 and E2 had similar intercepts but the one for EE2 was quite different. The degradation patterns and the regression equations can be explained by their molecular structures since E1, E2 and EE2 all have the same molecular structure except for the substitutions of the C-17 position. Although factors such as controlled temperature, pH, SRT, HRT and high initial concentrations in laboratory studies may affect the rate constants, the correlations obtained remain useful for the prediction of fate in various environmental media.

Chapter 7 Fate modelling of estrogens in a reservoir receiving recycled wastewater

7.1 Background

Water recycling is considered as an important solution to water supply issue in many countries. A major challenge with water recycling is the removal of harmful contaminants such as EDCs to meet drinking water supply standards.

In 2006, a major indirect water reuse project, the Western Corridor Recycled Wastewater Project (WCRWP) was launched in Southeast Queensland, Australia. It was proposed that about 115 ML/day of recycled water was to be discharged into the Wivenhoe reservoir for drinking water supply. Highly efficient processes including reverse osmosis and advanced oxidation were installed to treat the effluent from six major wastewater treatment plants (WWTP) before discharge into the reservoir (WCRWP 2009). It would be expected that these advanced processes would reduce EDCs. However, the effects of reservoir and recycling parameters on EDCs concentration in the reservoir are not known.

Therefore, this paper used a well established fugacity based model (Mackay 2001) to study the fate of E1, E2 and EE2 in the Wivenhoe reservoir. These three estrogens are generally considered to be the principle EDCs of concern in wastewater effluents (Gomes et al., 2003; Hanselman 2003; de Mes et al., 2005; Falconer et al., 2006). The effects of reservoir and recycling parameters on estrogen concentrations in the reservoir

were studied. Based on simulated results, a health risk characterisation using probabilistic techniques was carried out for both fish and humans.

7.2 Simulated concentrations under typical conditions

At typical conditions as described in Table 8 in Section 4.3.4, the simulated concentrations were calculated in Table 21 for water, sediment, soil, air, fish and suspended solids for E1, E2 and EE2 after secondary and advanced treatment. As noticed from the results, the highest concentrations were found in fish and suspended solids, whilst the lowest concentrations were observed in soil and air. Small Henry's law constant of E1, E2 and EE2 can explain the extreme low concentrations in air compartment (see Table 1). The distribution into soil was also low because the transfer through reservoir bank is very limited. Overall, the water reuse project significantly reduced estrogen concentrations in the reservoir by comparing the simulated concentrations under secondary and advanced treatment. The model also showed that 99.6%, 98.5% and 93.8% of E1, E2 and EE2 respectively were removed by degradation in the water compartment (Table 21), whilst the removal by other mechanisms in other compartments was very minor.

Table 21. Simulated concentrations in different compartments at 20 ℃

Compartments	without WCRWP (ng/L)			with WCRWP (ng/L)			Removal by degradation (%)		
	E1	E2	EE2	E1	E2	EE2	E1	E2	EE2
Water	5.0×10^{-3}	4.0×10^{-4}	1.0×10^{-3}	5.2×10^{-5}	6.0×10^{-6}	1.5×10^{-5}	99.6	98.5	93.8
Sediment	5.7×10^{-2}	1.1×10^{-2}	3.8×10^{-2}	6.0×10^{-4}	1.6×10^{-4}	5.7×10^{-4}	0.0749	1.29	0.173
Soil	1.4×10^{-8}	2.6×10^{-10}	2.2×10^{-9}	1.4×10^{-10}	3.8×10^{-12}	3.2×10^{-11}	1.09×10^{-6}	2.11×10^{-7}	4.62×10^{-7}
Air	2.1×10^{-14}	2.0×10^{-16}	4.7×10^{-17}	2.2×10^{-16}	3.0×10^{-18}	6.9×10^{-19}	4.46×10^{-6}	4.23×10^{-7}	9.75×10^{-7}
Fish (ng/kg)	3.4×10^{-1}	7.4×10^{-2}	2.4×10^{-1}	3.5×10^{-3}	1.1×10^{-3}	3.5×10^{-3}	0.638	1.40	1.87
Suspended Solids	8.3×10^{-1}	1.8×10^{-1}	5.9×10^{-1}	8.7×10^{-3}	2.7×10^{-3}	8.6×10^{-3}	0.0821	0.221	0.264

7.3 Simulated concentrations under random conditions

At any random conditions under advanced treatment, the cumulative distributions of simulated concentrations of E1, E2 and EE2 in the water compartment were presented in Figure 34. The concentrations for E1, E2 and EE2 at 50% of cumulative probability were corresponding to the values in the water compartment obtained at typical conditions in Table 21 (with WCRWP). At 95% of cumulative probability, the concentration for E1, E2 and EE2 were 2.7×10^{-3}, 3.7×10^{-4}, 5.2×10^{-4} ng/L respectively. Apparently, the advanced treatment demonstrated high efficiency for the removal of EDCs. This means for most of the time, the simulated concentrations of three estrogens in the reservoir were below current detection limits (generally over 0.01 ng/L).

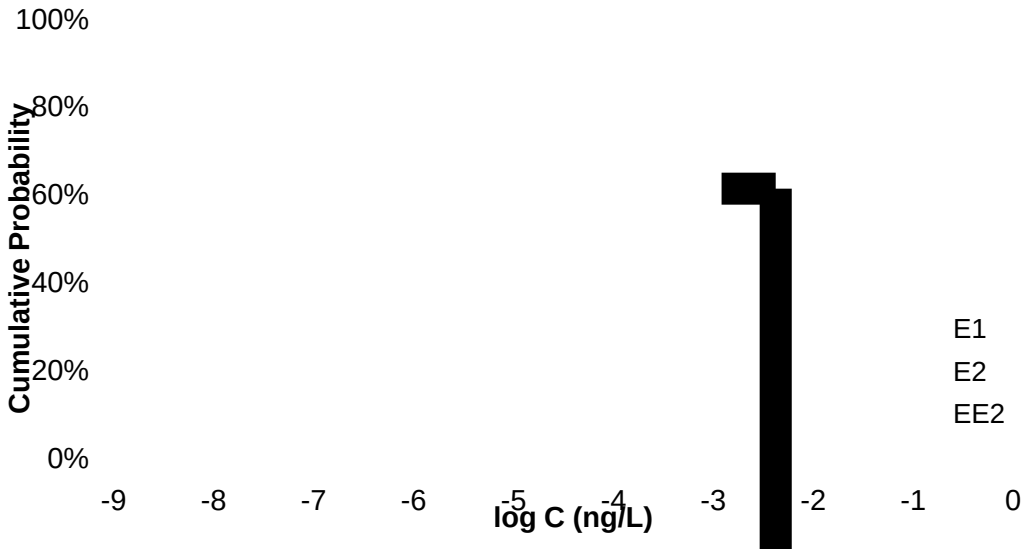

Figure 34. Simulated concentrations in the water compartment after advanced treatment under random conditions.

7.4 Effects of reservoir and recycling parameters on simulated concentration

The effects of reservoir parameters (T, V, EBC and F_d) and recycling parameters (C_e and F_r) on simulated concentration in the water compartment were illustrated in Figure 35. Similar trends were also found for other compartments but they were not the focus of this work. Figure 35A shows, when temperature increases, the estrogen concentrations in the reservoir will decrease, because estrogens degrade faster at higher temperatures. For the effects of reservoir water storage volume (Figure 35B), it is easy to understand that an increasing volume means more freshwater inflow (or rain) causing higher dilution. Therefore simulated concentration decreases with larger volume. The same trend was also found for the effects of EBC (Figure 35D). Higher EBC values mean more bacteria are available for estrogen degradation (Cao et al., 2008). On the contrary, the opposite trend was observed (Figure 35C) for reservoir water releasing rate (F_d). This can be explained by reduced dilution effects. However, this effect was less important as compared to other parameters. For the recycling parameters, higher F_r and C_e values both mean more estrogens were pumped into the reservoir leading to higher concentrations (Figure 35E and 35F). Understanding the effects of reservoir parameters (T, V, EBC and F_d) and recycling parameters (C_e and F_r) has practical implications. For example, during dry seasons, estrogens level in reservoirs or lakes will be higher than the wet seasons due to smaller storage volume (Figure 35B). Considerations should also be given to situations with higher emission (Figure 35E and 35F). In addition, microorganisms are less active in cold seasons, resulting in higher estrogens level (Figure 35A).

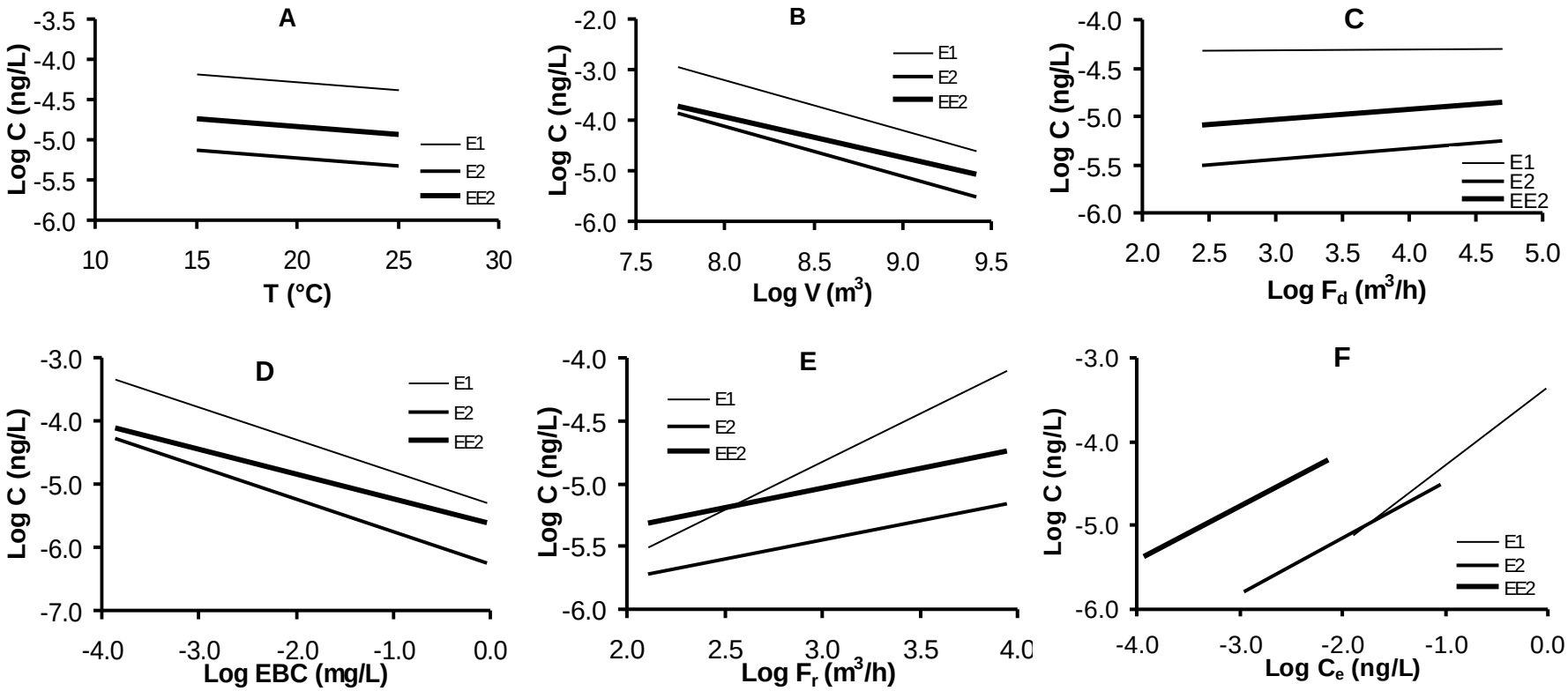

Figure 35. Effects of reservoir characteristics and recycling parameters on estrogen concentrations in reservoir water.

7.5 Risk characterisation for human health using simulated concentrations

Health risk was quantified as the simulated concentration at 95% of CP divided by NOAEC and PHS values, which was described by Equation (30) and (31). The simulated concentrations at 95% of CP were 2.7×10^{-3} ng/L for E1, 3.7×10^{-4} ng/L for E2 and 5.2×10^{-4} ng/L for EE2 respectively. With the available NOAEC and PHS values, the calculated HQ values for fish and humans were summarised in Table 22:

Table 22. HQ values calculated for fish and humans by simulated concentration

EDCs	EC_{95}	NOAEC (ng/L)	PHS (ng/L)	HQ	
		Fish	Human	Fish	Human
E1	2.7×10^{-3}	0.74	30	3.7×10^{-3}	9.0×10^{-5}
E2	3.7×10^{-4}	0.4	175	0.93×10^{-3}	2.1×10^{-6}
EE2	5.2×10^{-4}	0.1	1.5	5.2×10^{-3}	3.5×10^{-4}

The obtained small HQ values showed negligible health risk for fish and humans. When the risk level was compared among the estrogens, it was ranked in the descending order of EE2 > E1 > E2 for both fish and humans. Apparently, EE2 impose the highest level of risk among the three estrogens. EE2 does not only possess the highest potency (Murk et al., 2002; Gomes et al., 2003), but also has the ability to bioaccumulate in fish due to its high log K_{ow} value and long half-life in the environment (Lai et al., 2002). In comparison with synthetic EE2, natural estrogens E1 and E2 are less potent and have much shorter half-lives. Therefore, lower risks are generally associated with E1 and E2.

7.6 Chapter conclusions

The fate of E1, E2 and EE2 was quantified using a fugacity-based model in a reservoir receiving recycled water in Southeast Queensland (SEQ), Australia. At typical conditions, the simulated EDCs concentrations in the reservoir water compartments were very low (< 10^{-2} ng/L) when wastewater was treated by both secondary and advanced processes. The majority of estrogens were removed by degradation in the water compartment with removal rates of 99.6%, 98.5% and 93.8% for E1, E2 and EE2 respectively. Estrogen concentrations in the reservoir decreased when reservoir water temperature (T), reservoir water storage volume (V), biomass concentration (EBC) and reservoir water releasing rate (F_d) increased. However the opposite trend was found with higher EDCs concentrations in the final recycled water (C_e) and higher recycling rate (F_r). The hazard quotient (HQ) values obtained in the risk characterisation were less than 10^{-2}, showing negligible health risk for fish and humans. The ranked HQ values in the order of EE2 > E1 > E2 indicated that highest risk level was imposed by the synthetic estrogen EE2.

Chapter 8 Health risk assessment of EDCs from water and food using probabilistic techniques

8.1 Background

EDCs have been widely detected in WWTP effluents (see Table 5 in Section 3.4.5), surface waters (see Table 10 in Section 4.4.1) and even human food (see Table 12 in Section 4.4.1). Consequently, wildlife species and humans exposed to EDCs by food and water consumption are under health threat. Various adverse health effects have been observed in wildlife species and humans (see Section 3.6). Particularly, health risks to aquatic organisms (e.g. fish) are of great concern. Furthermore, other wildlife species and humans eating the contaminated fish are also at risk. Therefore, it is necessary to conduct a health risk assessment of EDCs for fish and humans.

The widely accepted risk assessment framework has been developed by the National Academy of Sciences, USA (NAS 1983), which was reviewed in Section 3.7.1. The recently developed risk assessment using probabilistic techniques has the advantage of express the exposure and effect data as a probability distribution rather than a single number (US EPA 2002). Probabilistic techniques have been applied to risk assessment for engineering problems several decades ago (Hanauer 1975; Weichert and Milne 1979). Until recently, it has gained popularity in risk assessment for environmental pollutants (Solomon et al., 1996; Djohan et al., 2007; Straub and Stewart 2007; Hamidin et al., 2008). Therefore, this work used probabilistic techniques to conduct health risk assessment for fish and humans exposed to EDCs from surface water, drinking water and human food.

8.2 Risk assessment of EDCs for fish with measured data

8.2.1 Exposure assessment of EDCs in surface water for fish

EDCs were frequently detected in rivers, lakes and other surface water in many countries around the world. Generally, high concentrations in surface water were found in highly industrialised regions, particularly for those EDCs originated from industrial sources. The data of measured EDCs concentration in surface water were collected from 15 countries worldwide such as Germany, France, Italy, the Nederland, UK, the USA, Canada, Japan and China. Totally, the collected concentration data included 747 samples for E1, 756 samples for E2, 348 samples for E3, 613 samples for EE2, 432 samples for NP, 403 samples for OP and 451 samples for BPA. The obtained concentration values were collated, ranked and transferred into logarithmic values.

The CPD curves of these measured concentrations were plotted in Figures 36 to 42 for each EDC using the methods described in Section 4.4.1. Only the measured values above the detection limit were plotted. It can be seen from these CPD curves that a large percentage of samples were below the lowest detection limit, which was 22% for E1, 38% for E2, 50% for E3, 71% for EE2, 16% for NP, 7% for OP and 15% for BPA. Particularly, EE2 and E3 were least frequently detected among all studied EDCs. Linear regressions were conducted within the CP range of 20 to 80% (otherwise slightly smaller range was used) for each EDC. With the regression equations obtained in Figures 36 to 42, the slopes were an indicator of the concentration range. Wider concentration ranges resulted in smaller slops. Thus, NP had the widest concentration range, whilst OP and E3 had the narrowest concentration range.

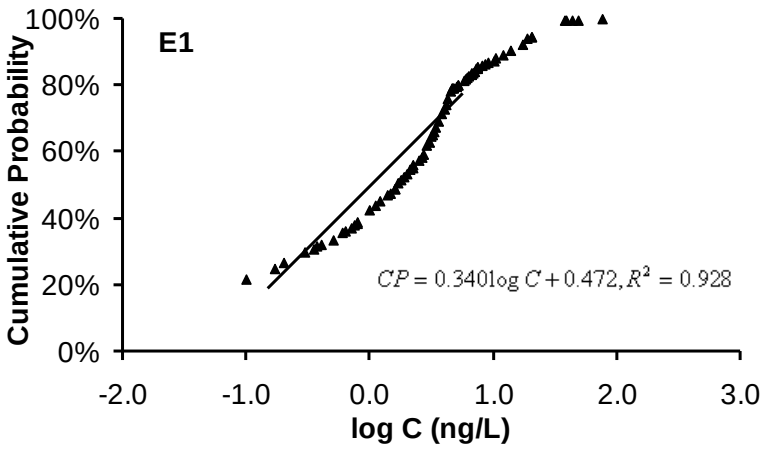

Figure 36. Cumulative probability distribution of measured E1 concentration in surface water from European countries, USA, Canada, Japan and China.

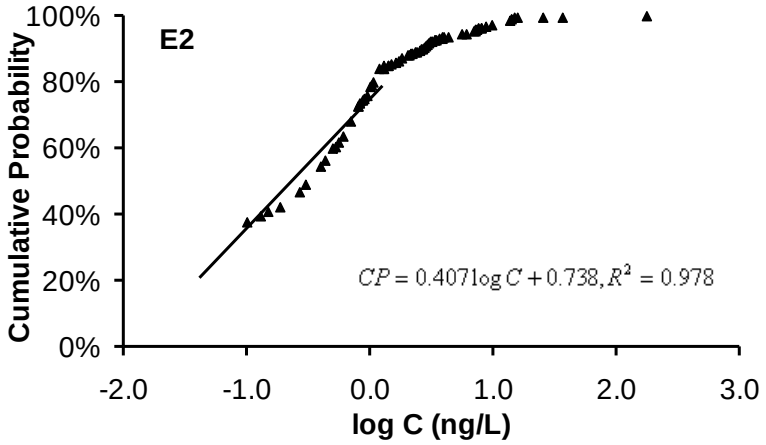

Figure 37. Cumulative probability distribution of measured E2 concentration in surface water from European countries, USA, Canada, Japan and China.

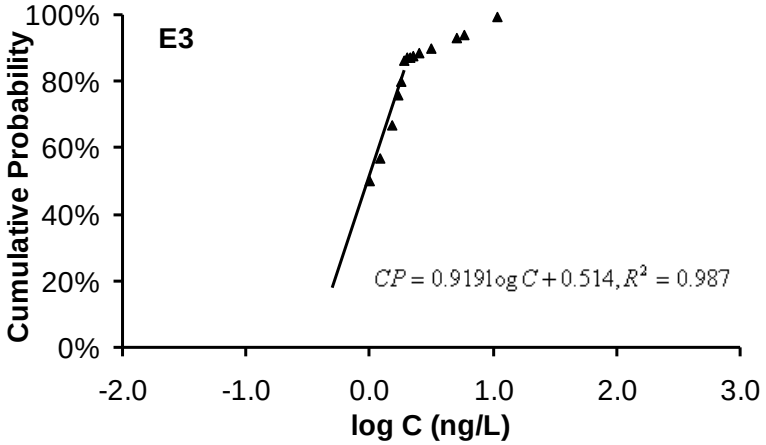

Figure 38. Cumulative probability distribution of measured E3 concentration in surface water from European countries, USA, Canada, Japan and China.

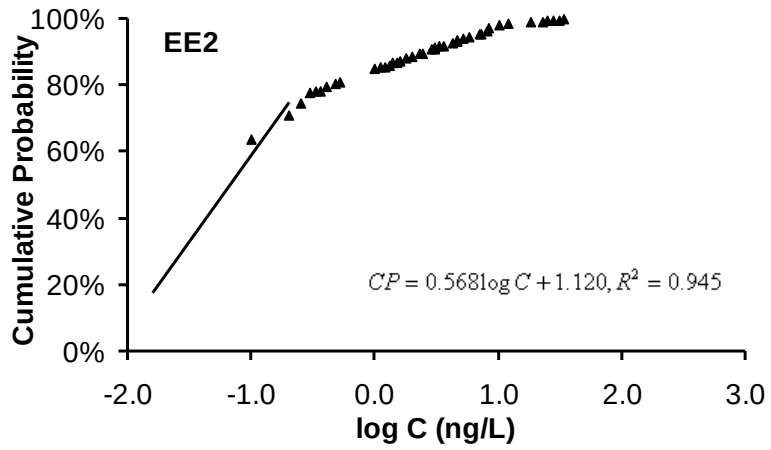

Figure 39. Cumulative probability distribution of measured EE2 concentration in surface water from European countries, USA, Canada, Japan and China.

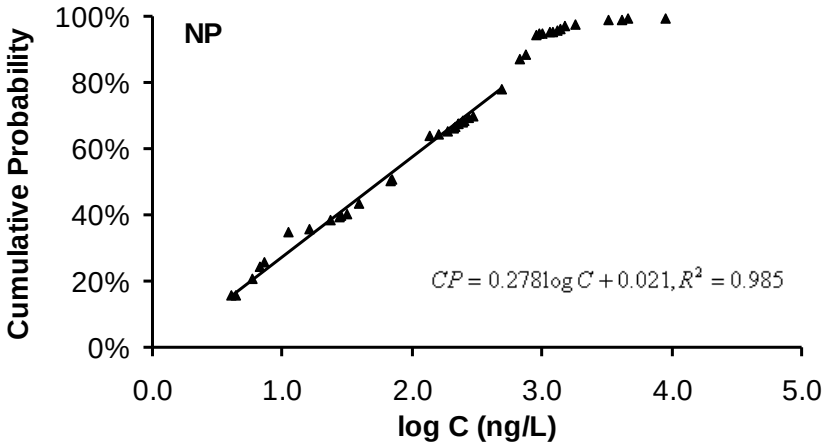

Figure 40. Cumulative probability distribution of measured NP concentration in surface water from European countries, USA, Canada, Japan and China.

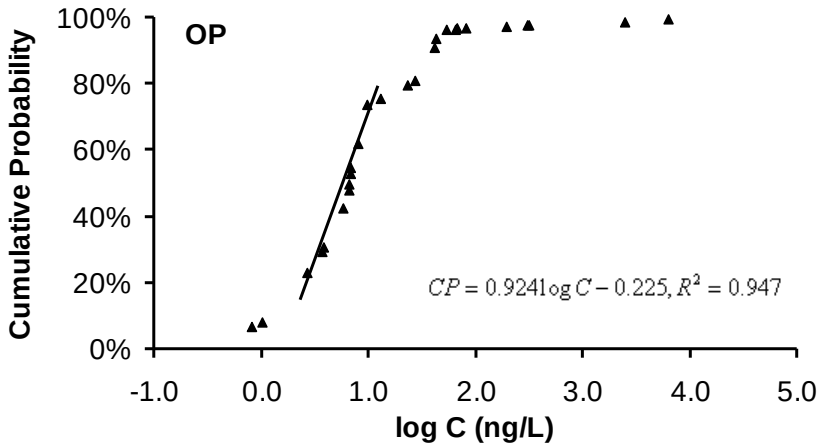

Figure 41. Cumulative probability distribution of measured OP concentration in surface water from European countries, USA, Canada, Japan and China.

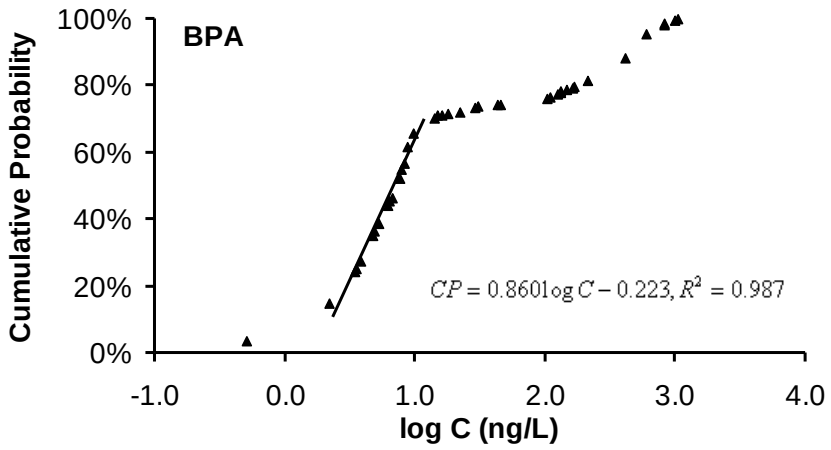

Figure 42. Cumulative probability distribution of measured BPA concentration in surface water from European countries, USA, Canada, Japan and China.

From the collected concentration data, the lowest detection limits, minimum, maximum and 50% median values were obtained and summarized in Table 23. The lowest detection limits for all EDCs were in a range of 0.04 to 0.1 ng/L. The minimum measured concentration above detection limits were between 0.1 and 4.0 ng/L. Apparently, there is no noticeable difference between estrogens and phenolic compounds for these two sets of values. However, the 50% median concentrations of phenolic compounds were 10 to 100 times higher than those of estrogens. The differences in the maximum concentrations were even bigger. For example, the maximum concentration of NP was about 880 times higher than that of E3.

For estrogens, the measured exposure concentrations in surface water span a wide range of 0.1 to 75 ng/L for E1, and 0.1 to 175 ng/L for E2, whilst smaller ranges were found for EE2 (0.1 to 34 ng/L) and E3 (1.0 to 10.8 ng/L). In contrast, even wider concentration ranges were observed for NP (4.0 to 8890 ng/L), OP (0.8 to 6300 ng/L) and BPA (0.5 to 1030 ng/L). The median concentration at 50% of cumulative probability was in the order of E1 > E3 > E2 > EE2 for estrogens. For phenolic

compounds, the 50% median concentrations were one to two orders of magnitude higher than estrogens, which were in the order of NP > BPA > OP.

Table 23. Summary of measured EDCs concentration (ng/L) in surface water [a]

EDC	Lowest detection limit	Minimum	50% Median	Maximum
E1	0.05	0.1	1.7	75
E2	0.05	0.1	0.3	175
E3	0.1	1.0	1.0	10.8
EE2	0.05	0.1	0.07[b]	34
NP	0.05	4.0	65.0	8890
OP	0.05	0.8	6.6	6300
BPA	0.04	0.5	7.3	1030

[a] – Values were drawn from references listed in Section 4.4.1. The minimum concentration refers to the minimum plotted values in each distribution curve.
[b] – Extrapolated values with extension of curve.

The large differences between the concentrations of estrogens and phenolic compounds can be attributed to two factors. The first is the difference in sources. As it was discussed in Section 3.3, phenolic compounds have been produced in large quantities worldwide by the chemical industry. For example, more than 2.5 million tonnes of BPA were produced in 2001 (Staples et al., 2002). In contrast, the sources of estrogens are mainly from human and animal body excretion, which are in relatively low volumes and concentrations. Consequently, the amount of estrogens entering WWTP would be expected to be much lower than phenolic compounds (see Table 5 and 6, Section 3.4.5). The second factor is that they have similar removal rate in WWTP. The removal rate of phenolic compounds in WWTP (see Table 7 in Section 3.4.5) is slightly higher than that of estrogens. Therefore, with similar removal rates, higher influent concentrations of phenolic compounds resulted in their higher level in surface water.

8.2.2 Effects assessment of EDCs for fish

As it was reviewed in Section 3.7.2, various health effects were imposed by EDCs on fish, such as reproductive abnormalities, altered sex ratio, mortality and changed behaviour. Among these adverse health effects, the induction of VTG in male and female juvenile fish was a more convenient biomarker to assess the adverse effects. From published results in the scientific literature, the NOAEC and LOAEC values on VTG induction were collated and CPD curve for each EDC was presented in Figures 43 to 48. Similar to the exposure distributions, linear regressions were conducted for NOAEC distributions with the CP range of 20 to 80% (otherwise smaller range was used in case of insufficient measured data). The slopes obtained for NOEAC were an indicator of the sensitivity of adverse effects imposed upon fish. Larger slope corresponds to higher sensitivity to that EDC. From these obtained regression equations as showed in Figures 43 to 48, it was found that fish was most sensitive to EE2 and NP.

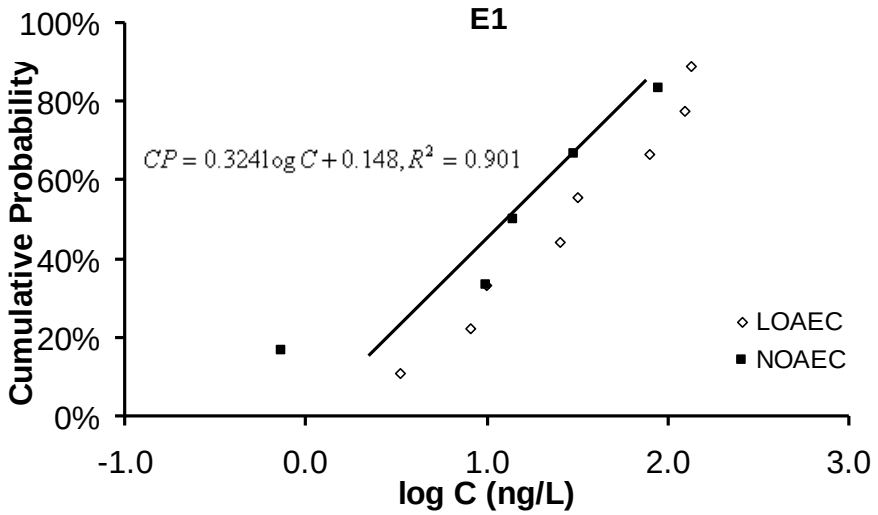

Figure 43. Cumulative probability distribution of NOAEC and LOAEC values of E1 for fish in surface water.

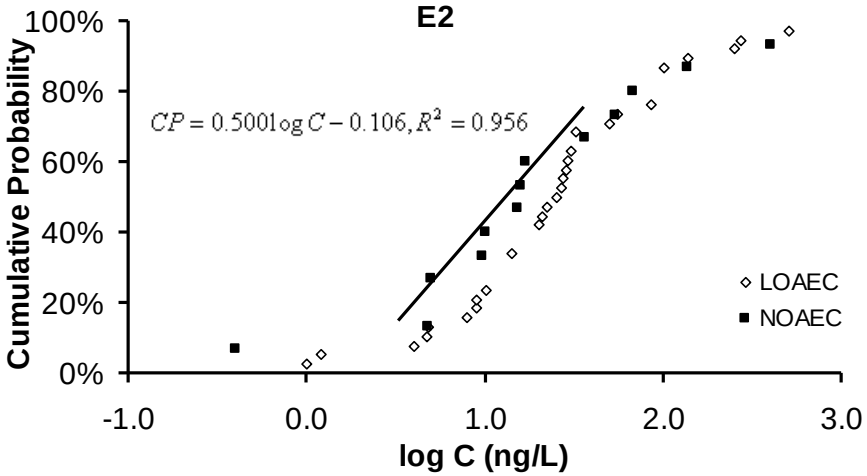

Figure 44. Cumulative probability distribution of NOAEC and LOAEC values of E2 for fish in surface water.

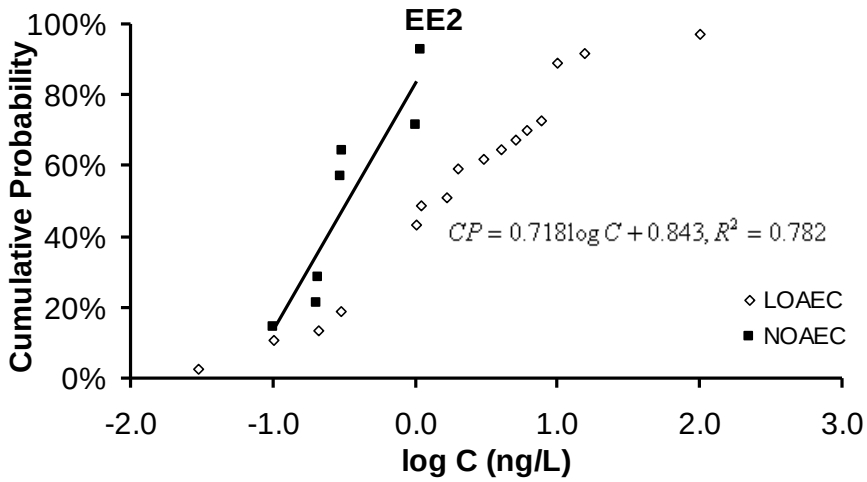

Figure 45. Cumulative probability distribution of NOAEC and LOAEC values of EE2 for fish in surface water.

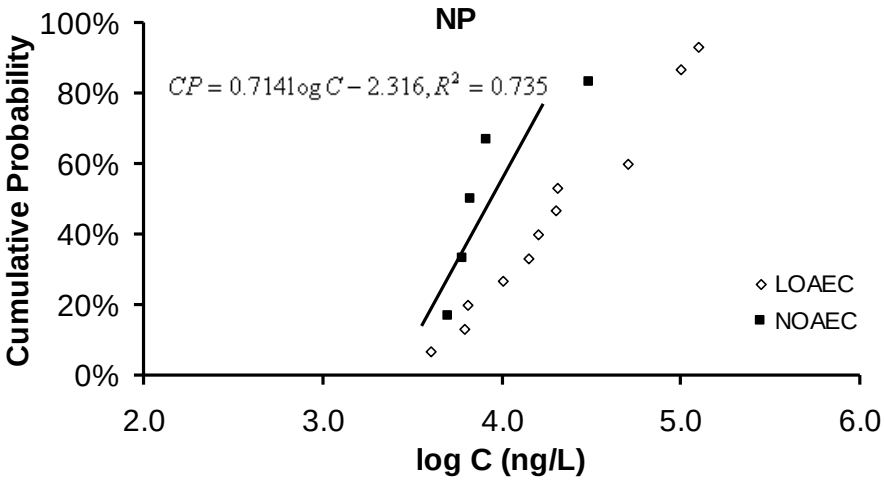

Figure 46. Cumulative probability distribution of NOAEC and LOAEC values of NP for fish in surface water.

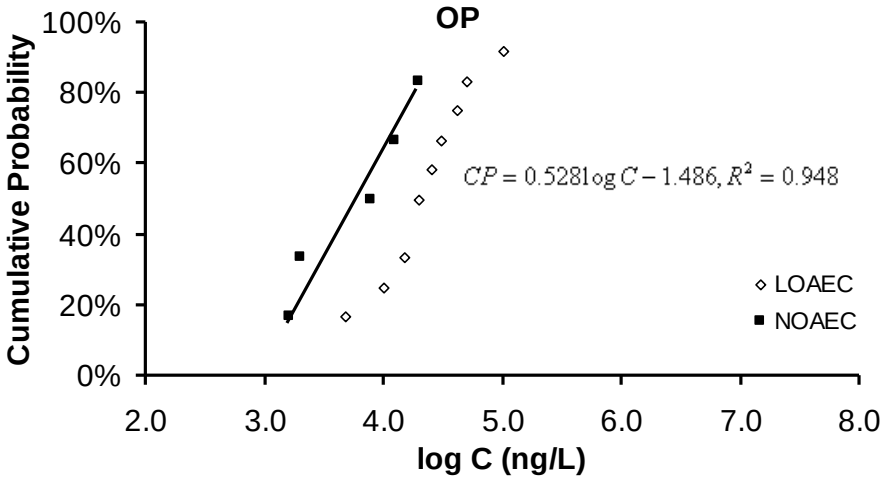

Figure 47. Cumulative probability distribution of NOAEC and LOAEC values of OP for fish in surface water.

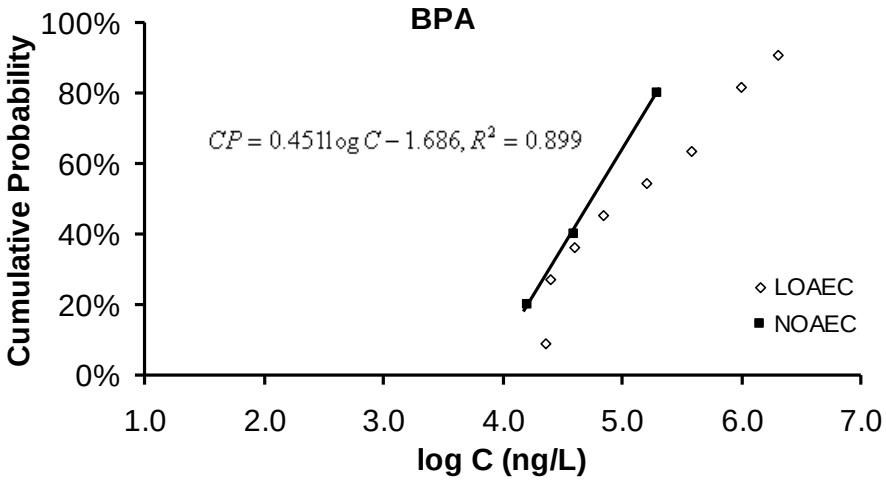

Figure 48. Cumulative probability distribution of NOAEC and LOAEC values of BPA for fish in surface water.

For each EDC, the minimum values in each set of NOAEC and LOAEC values are summarized in Table 24. Generally, estrogens are able to cause adverse effects at very low concentrations. For example, the minimum NOAEC and LOAEC values of EE2 were extremely low at 0.1 ng/L. Compared with estrogens, phenolic compounds were at several orders of magnitude less potent as indicated by their minimum NOAEC and LOAEC values shown in Table 24.

Table 24. Minimum NOAEC and LOAEC values (ng/L) for fish.

EDC	NOAEC	References	LOAEC	References
E1	0.74	(Thorpe et al., 2003)	3.3	(Bjerregaard et al., 2008)
E2	0.4	(Metcalfe et al., 2001)	1	(Bjerregaard et al., 2008)
E3	75	(Metcalfe et al., 2001)	600	(Holbech et al., 2006)
EE2	0.1	(Young et al., 2004)	0.1	(Purdom et al., 1994)
NP	5000	(Lintelmann et al., 2003)	4000	(Kwak et al., 2001)
OP	1600	(Lintelmann et al., 2003)	4800	(Jobling et al., 1996); (Lintelmann et al., 2003)
BPA	16000	(Lintelmann et al., 2003)	25000	(Van den Belt et al., 2003)

The median NOAEC and LOAEC values at 50% CP were estimated from the CPD curves in Figures 43 to 48 and summarized in Table 25. These values showed that, significant VTG increase occurred in half of the fish samples at concentrations of several ng/L for EE2, 10's ng/L for E1 and E2, 100's ng/L for E3, several µg/L for NP and OP, and 10's µg/L for BPA. Again, it clearly shows that EE2 is the most potent EDC, whilst phenolic compounds are much less potent.

Table 25. Median NOAEC and LOAEC values (ng/L) for fish

50% Median concentration	E1	E2	E3	EE2	NP	OP	BPA
NOAEC	14	16	75	0.3	6700	7800	79500
LOAEC	28	25	600	1.7	20140	20000	100000

8.2.3 Risk characterization of EDCs in surface water for fish

To conduct risk characterisation for fish, the CPD curves for exposure and effect were plotted in the same diagram for each individual EDC in Figures 49 to 55. For all EDCs, the exposure CPD curves were on the left of effect CPD curves. With E1, E2 and EE2, a large proportion of the exposure CPD curves were overlapped with effect CPD curves, showing significant level of health risk. Due to the lack of effect data for E3, its effect CPD curve can not be plotted. Instead, its one NOAEC and two LOAEC values were indicated in Figure 51. If more effect data was available for E3, probably there will also be some minor overlaps between its exposure and effect CPD curves because of chemical similarities with other estrogens. Among all estrogens, it was noticeable in Figure 52 that the measured EE2 exposure concentrations were completely overlapped with its effect concentrations. The lowest exposure and effect concentration observed with EE2 were both 0.1 ng/L (Belfroid et al., 1999; Kuch and Ballschmiter 2001a; Young et al., 2004), implicating high level of health risk. In contrast, the overlaps for phenolic compounds were less, showing much lower health risk for these compounds.

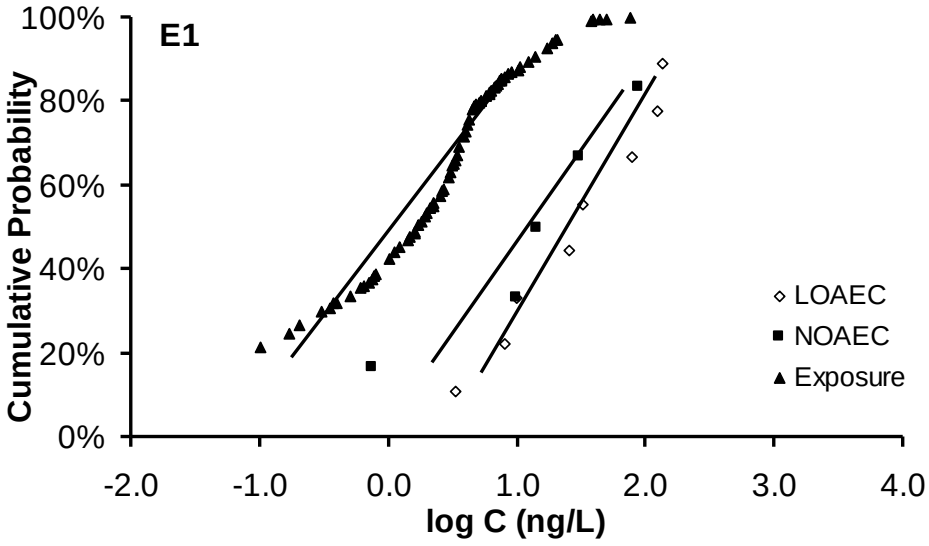

Figure 49. Comparison between the cumulative probability distributions of exposure and effect concentration of E1 for fish in surface water.

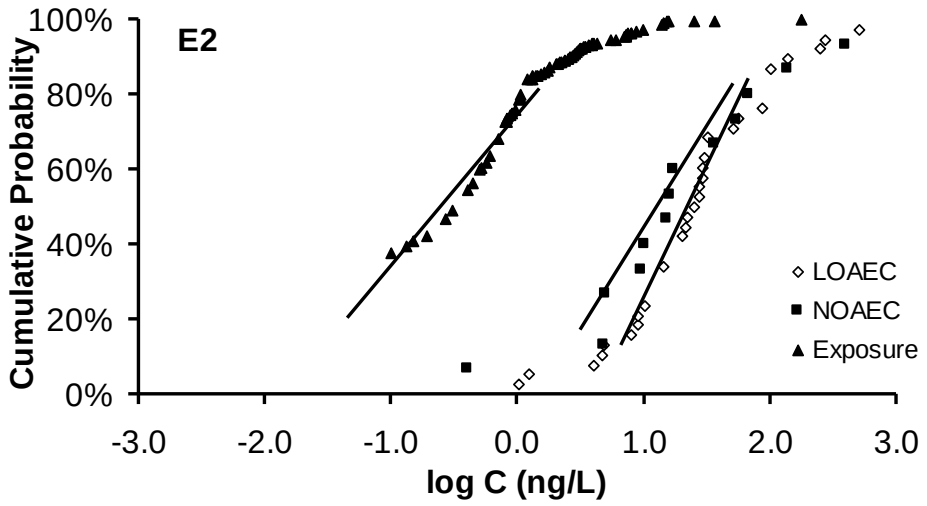

Figure 50. Comparison between the cumulative probability distributions of exposure and effect concentration of E2 for fish in surface water.

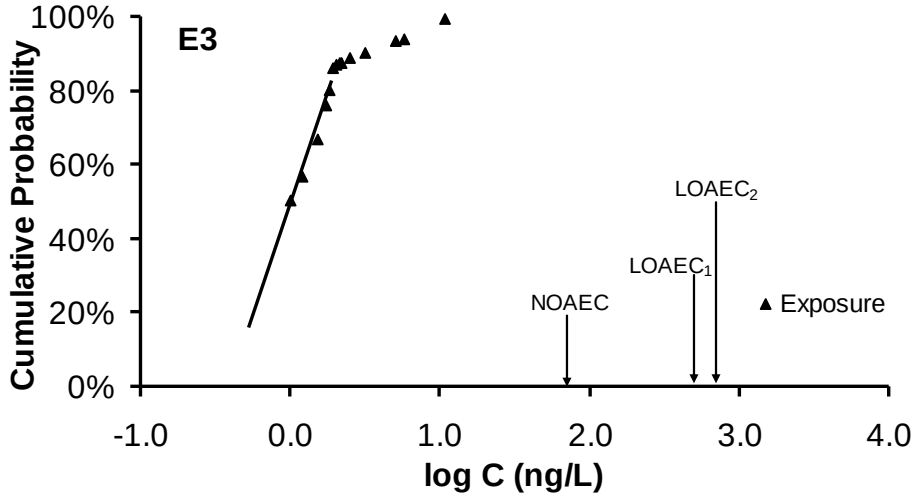

Figure 51. Comparison between the cumulative probability distributions of exposure and effect concentration of E3 for fish in surface water.

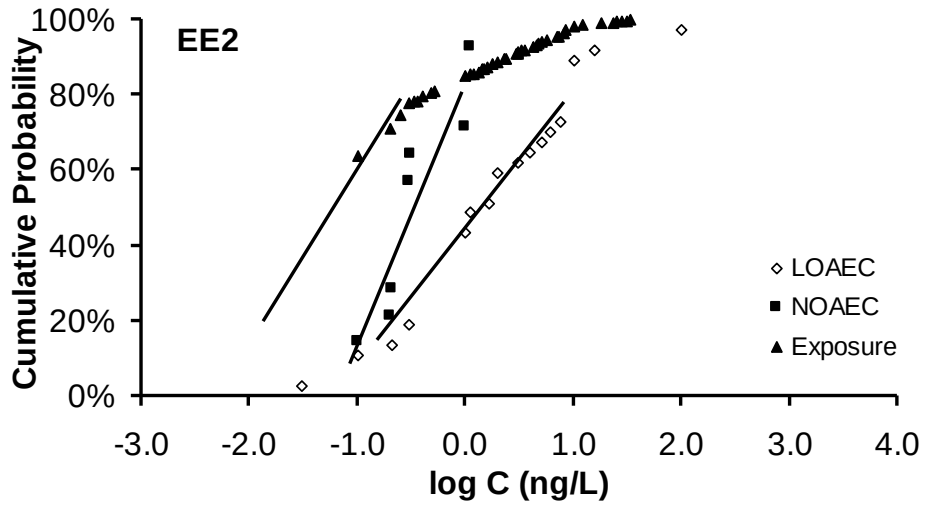

Figure 52. Comparison between the cumulative probability distributions of exposure and effect concentration of EE2 for fish in surface water.

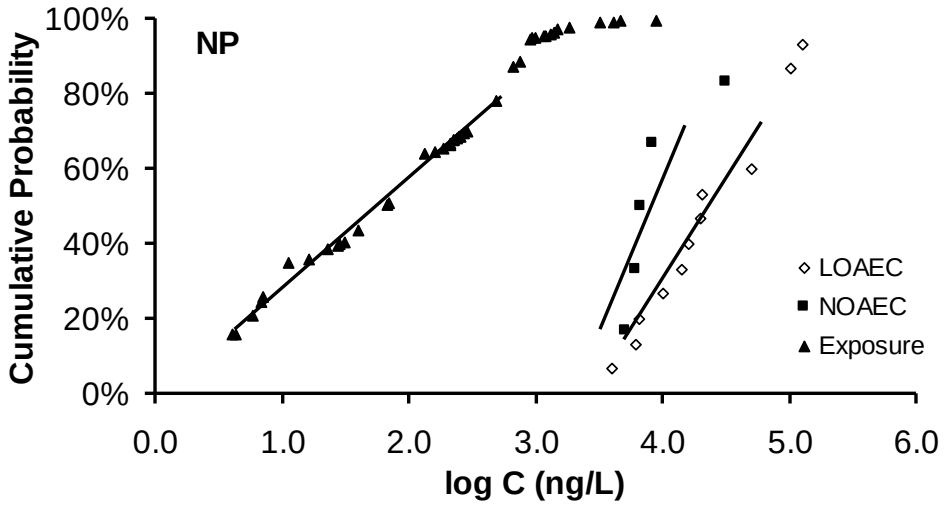

Figure 53. Comparison between the cumulative probability distributions of exposure and effect concentration of NP for fish in surface water.

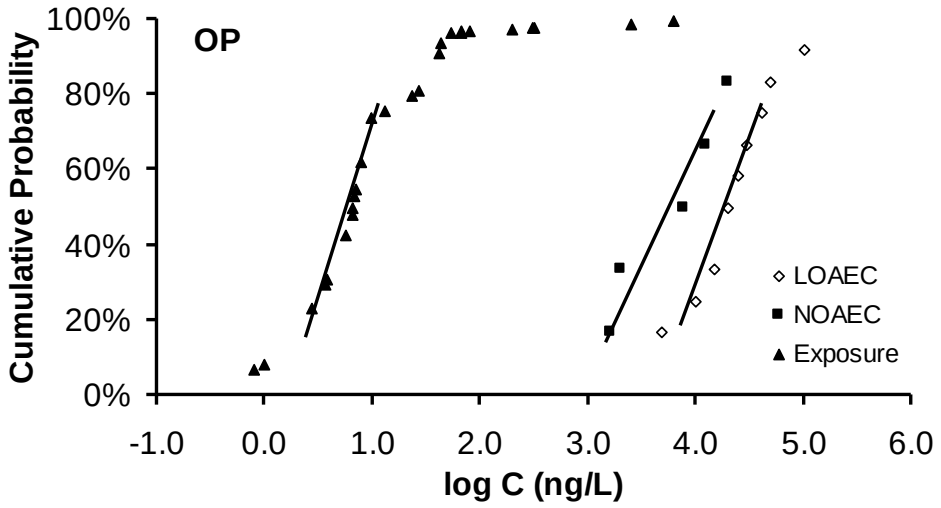

Figure 54. Comparison between the cumulative probability distributions of exposure and effect concentration of OP for fish in surface water.

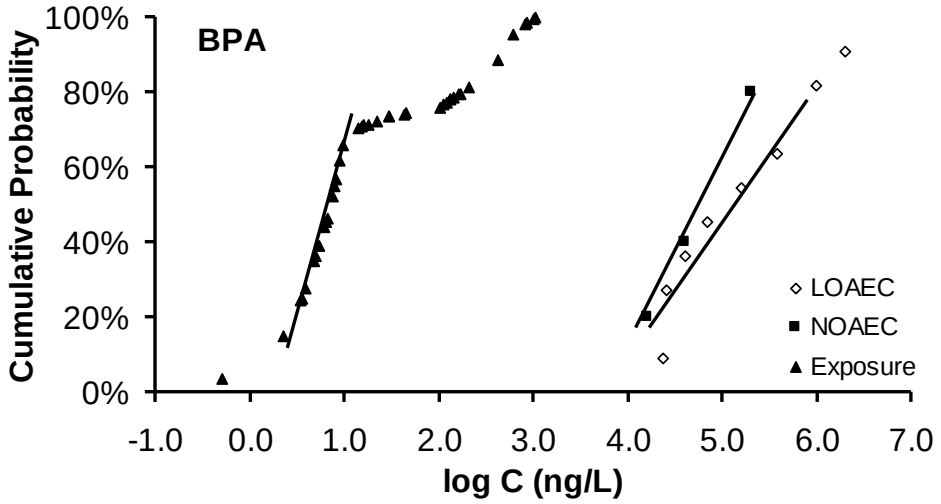

Figure 55. Comparison between the cumulative probability distributions of exposure and effect concentration of BPA for fish in surface water.

The health risk was quantified using two methods as described in Section 4.4.3. The first one was to calculate the $HQ_{95/5}$ values using Equation (33). With the EC_{95} and HC_5 (from NOAEC CPD curve) values estimated from Figures 49 to 55, the calculated $HQ_{95/5}$ values were summarised in Table 26. It was noted that $HQ_{95/5}$ values were larger than the threshold value of unity for estrogens and smaller than unity for phenolic compounds, indicating significant level of risk for estrogens. Particularly, the maximum

$HQ_{95/5}$ value of 250 was found for EE2, whilst the minimum $HQ_{95/5}$ value of 0.057 was found for OP. The ranked risk level among these 7 EDCs was in the order of EE2 > E1 > E2 > E3 > NP > BPA > OP.

Table 26. $HQ_{95/5}$ values calculated for fish

EDCs	E1	E2	E3	EE2	NP	OP	BPA
$HQ_{95/5}$	63	16	1.2	250	0.46	0.057	0.084

With the ORP method as described in Section 4.4.3.2, the exposure exceedence values were calculated and plotted with percent of affected fish samples in Figures 56 and 57. Due to the lack of measured effects data for E3, its exceedence curve was plotted with estimated effects values calculated by Equation (34). For the clarity of diagram presentation, estrogens and phenolic compounds were plotted separately in Figure 56 and 57. The obtained exceedence curve provides a convenient tool to observe the proportion of exposure concentration exceeding fish NOAEC value for any percent of affected fish samples. This could be interpreted by the following example. Taking point A in Figure 56 for example, at 10% of affected fish samples, there are about 67% of the measured EE2 exposure concentrations exceeding fish NOAEC value. Or we can say 67% of EE2 exposure concentrations will affect 10% of fish samples. Qualitatively, the level of risk can also be observed from the distance between the exceedence curves and the origin in the axis. The further away from the origin, the higher will be the risk level. Thus, the risk level for the investigated EDCs was ranked in the order of EE2 > E1 > E2 > E3 > NP > BPA > OP, which is consistent with the results obtained by using the $HQ_{95/5}$ method.

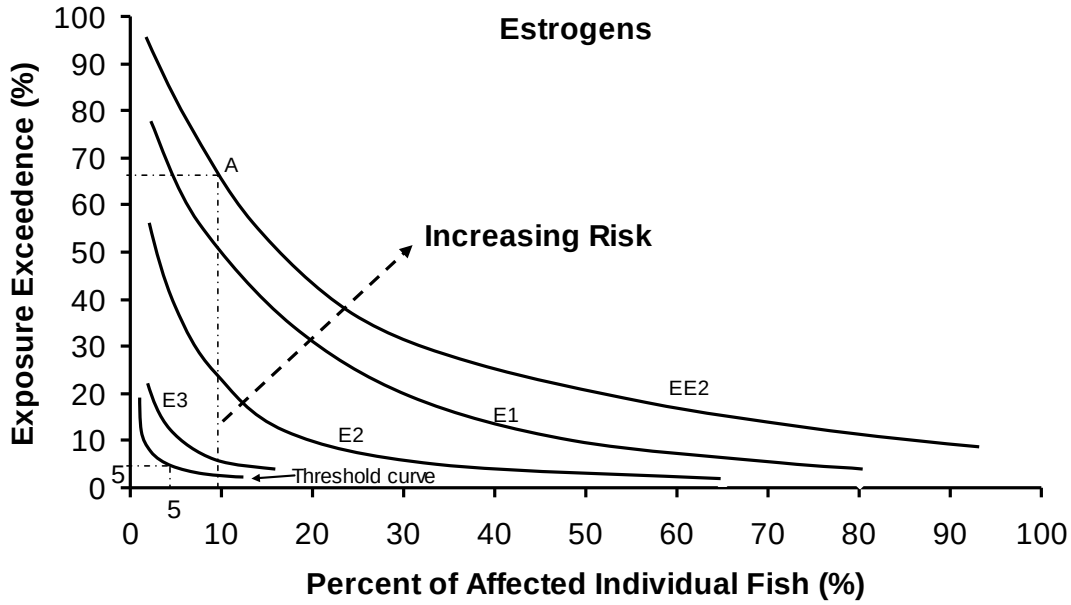

Figure 56. Exposure exceedence curves of estrogens for fish

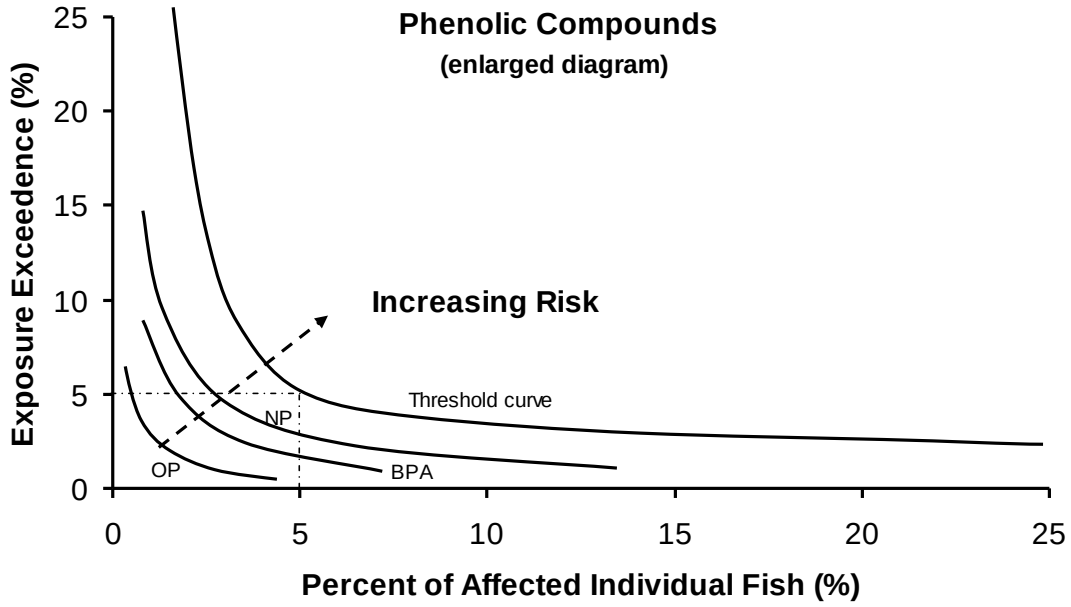

Figure 57. Exposure exceedence curves of phenolic compounds for fish

Furthermore, the overall risk was quantified as the area under each exceedence curve for individual EDCs. The ORP values were calculated and summarized in Table 27, which showed the same order of risk level as ranked by the $HQ_{95/5}$ method. Therefore, both

methods confirmed that estrogens impose much higher level of health risk than phenolic compounds. As it was noted from Figures 56 and 57, all exceedence curves for estrogens were above the threshold exceedence curve, which has an ORP value of 2.5%. In comparison, the exceedence curves of NP, OP and BPA were all below the threshold exceedence curve.

Table 27. Overall risk probability (ORP) values calculated for fish

EDCs	E1	E2	E3	EE2	NP	OP	BPA
ORP	22.0%	8.1%	3.8 %	26.6%	0.6%	0.2%	0.4%

8.3 Risk assessment of EDCs for human health with measured data

8.3.1 Exposure assessment of EDCs in drinking water and food

Human exposure to EDCs is mainly due to the consumption of drinking water and daily food such as fish, meat, dairy products, vegetables, eggs, rice, bread and pasta. Compared with EDC concentration in drinking water, significant level of EDCs was detected in food, particularly from food measured in developed countries such as Germany, Japan and USA. The measured concentration values were collated and cumulatively distributed in Figures 58 to 64. It was observed that in drinking water, most measurements of estrogen samples were below detection limits, whilst most measurements of phenolic compounds were above detection limits. In food, both estrogens and phenolic compounds were detected with high concentrations, particularly in meat and dairy products. Generally, EDCs found in food were one to two orders of magnitude higher than those found in drinking water.

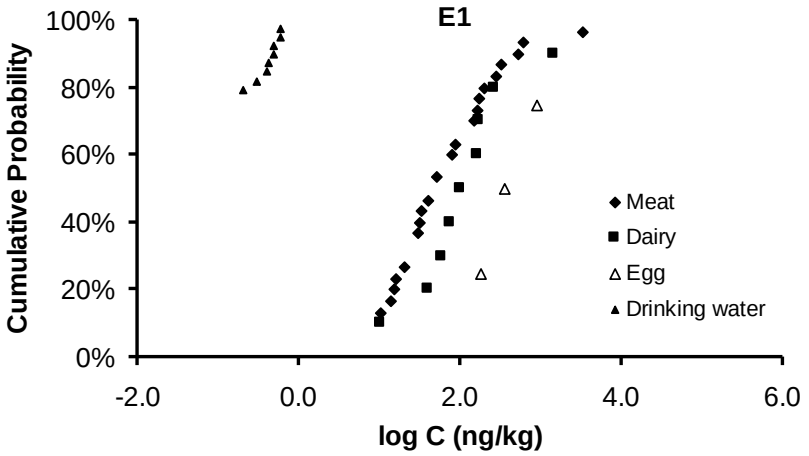

Figure 58. Cumulative probability distribution of E1 concentration in drinking water and human food.

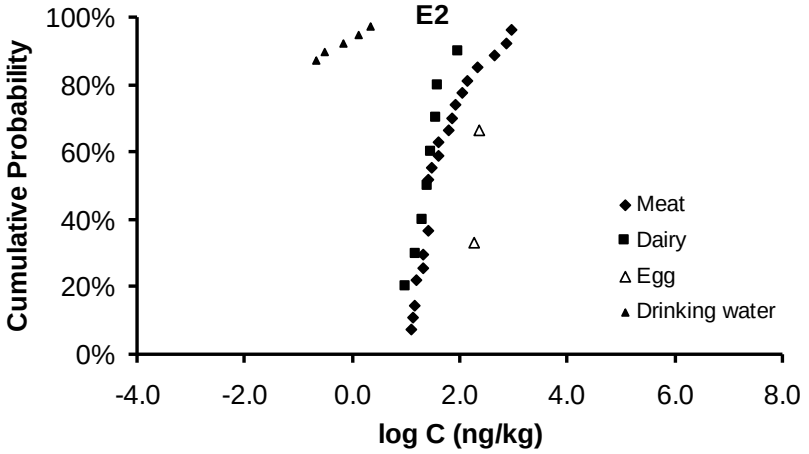

Figure 59. Cumulative probability distribution of E2 concentration in drinking water and human food.

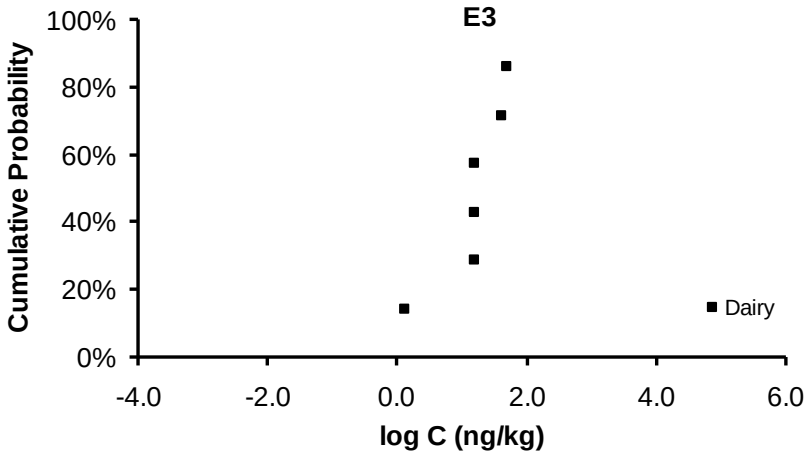

Figure 60. Cumulative probability distribution of E3 concentration in dairy products.

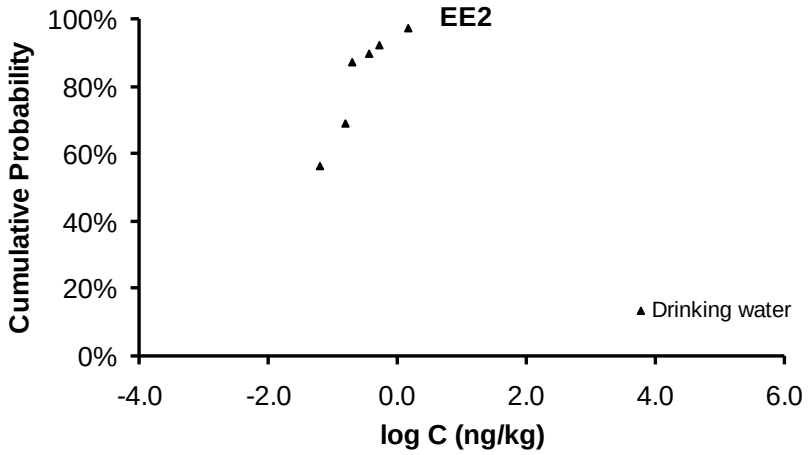

Figure 61. Cumulative probability distribution of EE2 concentration in drinking water.

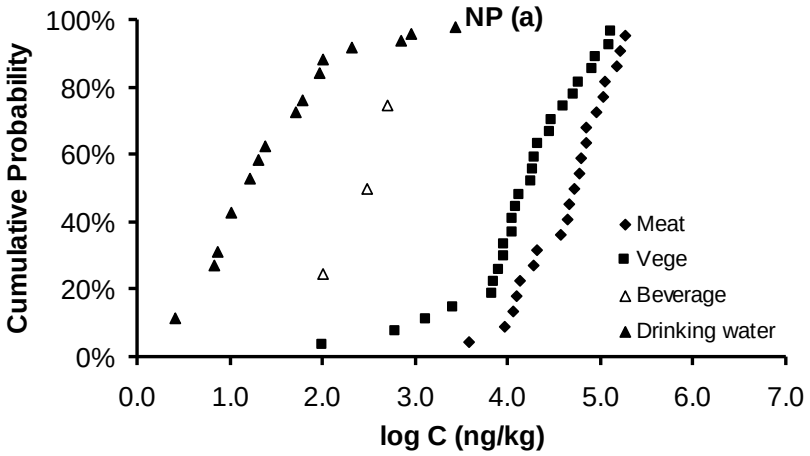

Figure 62a. Cumulative probability distribution of NP concentration in drinking water and human food.

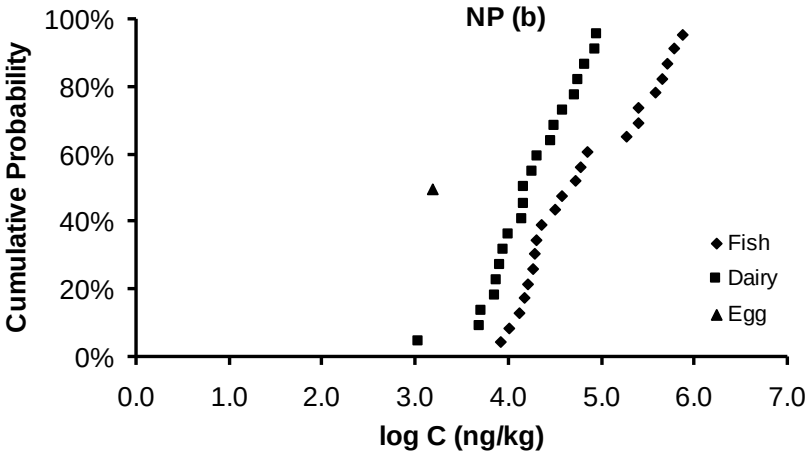

Figure 62b. Cumulative probability distribution of NP concentration in drinking water and human food.

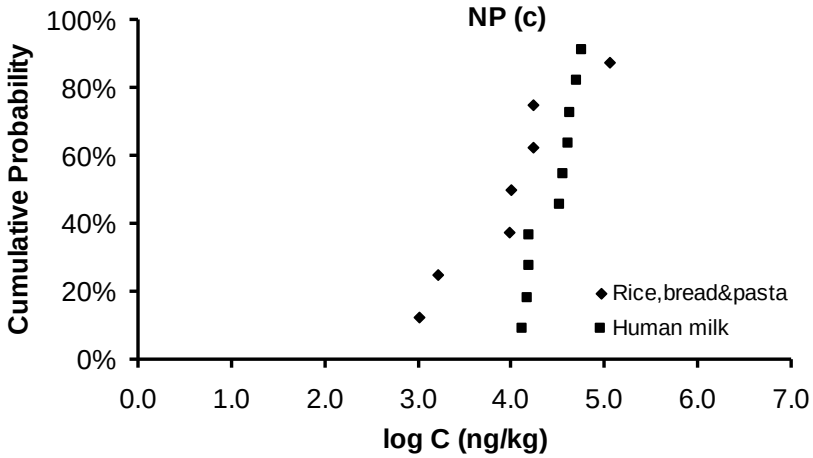

Figure 62c. Cumulative probability distribution of NP concentration in drinking water and human food.

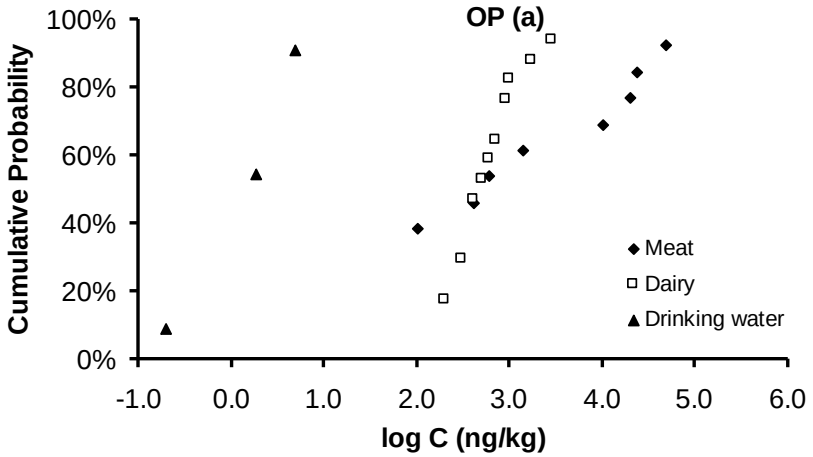

Figure 63a. Cumulative probability distribution of OP concentration in drinking water and human food.

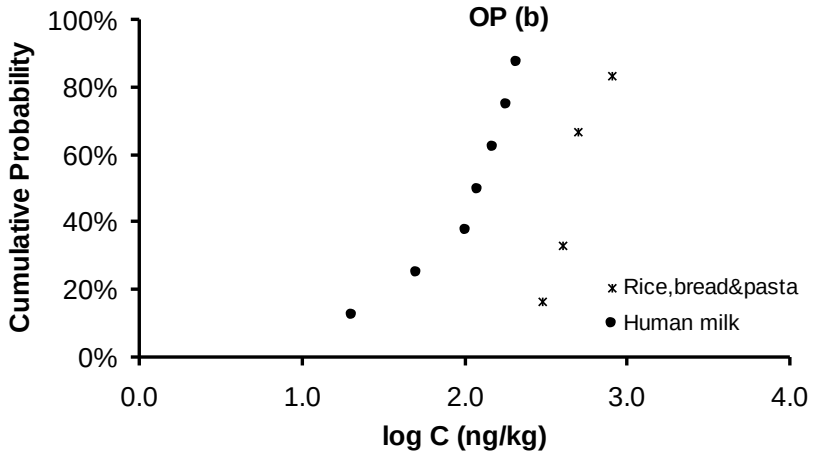

Figure 63b. Cumulative probability distribution of OP concentration in drinking water and human food.

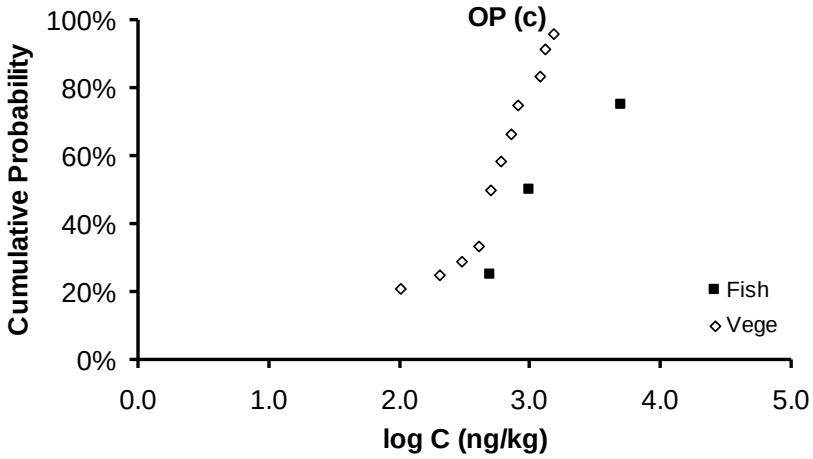

Figure 63c. Cumulative probability distribution of OP concentration in drinking water and human food.

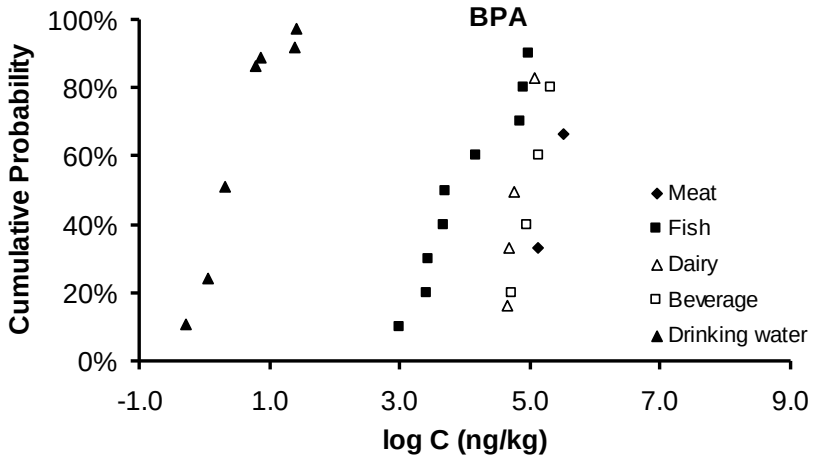

Figure 64. Cumulative probability distribution of BPA concentration in drinking water and human food.

In drinking water, the highest concentration detected for estrogens was 0.6 ng/L for E1 (Kuch and Ballschmiter 2001b), 2.1 ng/L for E2 (Kuch and Ballschmiter 2001a) and 2.4 ng/L for EE2 (Adler et al., 2001). There was no concentration values above detection limits reported for E3. In contrast, much higher concentrations of phenolic compounds were detected with up to 2700, 4.9 and 25 ng/L for NP, OP and BPA respectively. This difference between estrogens and phenolic compounds was consistent with their concentration found in surface water.

However, EDCs concentration in drinking water was minor when compared with concentration in food. Due to natural estrogens (E1, E2 and E3) are produced by metabolic processes in animal and human body, they were frequently detected with high concentrations in animal meat, internal organs, dairy products and eggs. The synthetic estrogen, EE2, was not reported among these sources, possibly implicated negligible food contamination by this EDC. However, the concentration of phenolic compounds was two to three orders of magnitude higher than those of estrogens. The highest levels of phenolic compounds were all reported in Japan with 723 ng/g in fish for NP (Sasaki

et al., 1999), 47 ng/g in chicken for OP (Sasaki et al., 1999) and 319 ng/g for BPA in cooked horse meat (Takino et al., 1999). In fact, these compounds were detected in a wide range of foods including meat, dairy products, seafood, fruits, eggs, rice, coffee, tea and human breast milk. Ademollo et al. (2008) detected 13.4 to 56.3 ng/ml of NP and 0.02 to 0.21 ng/ml of OP in human breast milk in Italy. BPA was also detected from 44 to 113 ng/g in powdered milk formulated for infants in Taiwan (Kuo and Ding 2004). This means infants and babies may be adversely affected by these compounds as milk is their major source of food during the early stage of development.

With the collected concentration data in drinking water and food, *human daily dose* (HDD) was calculated by multiplying the concentration in food with daily food consumption. In this work, Australian daily food consumption was used for calculations. The obtained HDD values, together with their E2 equivalent daily doses and percentage of total daily dose were summarised for each EDC in Table 28. It was found that meat, dairy products and fish in human diet accounted for the majority of total daily dose, whilst the proportion contributed by drinking water was negligible. Among all EDCs, E2 contributed to 92.47% of total daily dose, followed by BPA with 2.56%, E1 with 1.67%, EE2 with 1.28 and NP with 1.19%. The smallest daily doses were from E3 and OP, which were 0.56% and 0.27% respectively.

Table 28. Human daily dose of EDCs from food and drinking water [a]

EDCs / Food	Concentration in food (ng/g)	Australian Daily Food Consumption (g/day)	Daily dose (ng/kg, bw/day)
E1			
beef	0.05 (0.01 – 0.08)	96	0.07
pork	0.3 (0.03 – 0.59)	90	0.39
milk	0.07 (0.04 – 0.1)	310	0.31
cheese	0.17	29	0.07
chicken	0.16	113	0.26
egg	0.5 (0.18 – 0.89)	27	0.19
cream	0.26	38	0.14
yoghurt	0.16	144	0.33
butter	1.47	13	0.27
drinking water	0.42 ng/L	2 L	0.01
Total daily dose		2.04 ng/kg, bw/day	
Equivalent of daily E2 dose		0.0204 ng/kg, bw/day	
Percentage of total daily dose		1.67%	
E2			
beef	0.06 (0.012 – 0.11)	96	0.08
pork	0.47 (0.03 - 0.91)	90	0.60
milk	0.07 (0.04 – 0.09)	310	0.31
cheese	0.02 (0.01 – 0.03)	29	0.01
chicken	0.02	113	0.03
egg	0.2 (0.18 – 0.22)	27	0.08
drinking water	0.6 ng/L	2 L	0.02
Total daily dose		1.13 ng/kg, bw/day	
Percentage of total daily dose		92.47%	
E3			
butter	0.042	13	0.01
milk	0.016	310	0.07
cheese	0.016	29	0.01
drinking water	n.d.	2 L	0
Total daily dose		0.09 ng/kg, bw/day	
Equivalent of daily E2 dose		0.01 ng/kg, bw/day	
Percentage of total daily dose		0.56%	

[a] - Values in brackets are the ranges. Concentration in milk in ng/mL was approximated as ng/g. Concentrations in drinking water were median values from data in Figures 58 - 64. Percentage of potency was calculated using the EEF values listed in Table 1. References were listed in Section 4.4.1.

Table 28. Human daily dose of EDCs from food and drinking water (cont'd)

EDCs / Food	Concentration in food (ng/g)	Australian Daily Food Consumption (g/day)	Daily dose (ng/kg, bw/day)
EE2			
drinking water	0.44 ng/L	2 L	0.01
Total daily dose		0.01 ng/kg, bw/day	
Equivalent of daily E2 dose		0.0125 ng/kg, bw/day	
Percentage of total daily dose		1.28%	
NP			
fish	248 (10 – 723)	108	382.63
tuna	25 (8.1 – 37)	94	33.57
beef	28 (11 – 43)	96	38.40
pork	103 (20 – 180)	90	132.43
chicken	73 (3.8 – 163)	113	117.84
milk	9 (1.1 – 18)	310	39.86
cheese	33 (7.5 – 86)	29	13.67
sugar	6.8	37	3.59
butter	14.4	13	2.67
chocolate	14.1	35	7.05
egg	1.5	27	0.58
pasta	1	176	2.51
apple	35 (9 – 131)	158	79.00
rice	40 (10 – 114)	221	126.29
tomato	18.5	87	22.99
potato	0.6	167	1.43
beer	0.5	611	4.36
bread	1.6	90	2.06
broccoli	34 (29 – 39)	65	31.57
sweet corn	106 (89 – 123)	43	65.11
drinking water	289.6 ng/L	2 L	8.27
Total daily dose		1115.90 ng/kg, bw/day	
Equivalent of daily E2 dose		0.0145 ng/kg, bw/day	
Percentage of total daily dose		1.19%	

Table 28. Human daily dose of EDCs from food and drinking water (cont'd)

EDCs / Food	Concentration in food (ng/g)	Australian Daily Food Consumption (g/day)	Daily dose (ng/kg, bw/day)
OP			
tuna	0.8 (0.5 – 1)	94	1.07
beef	0.4 (0.1 – 1.4)	96	0.55
pork	5.1 (0.1 – 10)	90	6.56
chicken	22.6 (0.4 – 47)	113	36.48
cheese	4.2 (0.2 – 0.6)	29	1.74
butter	1.4 (0.7 – 2.8)	13	0.26
potato	0.1	87	0.12
rice	0.5 (0.3 – 0.8)	221	1.58
apple	0.8 (0.5 – 1.3)	158	1.81
broccoli	0.2 (0.1 – 0.2)	65	0.19
sweet corn	0.3 (0.1 – 0.6)	43	0.18
drinking water	2.3 ng/L	2 L	0.066
Total daily dose		50.61 ng/kg, bw/day	
Equivalent of daily E2 dose		0.0033 ng/kg, bw/day	
Percentage of total daily dose		0.27%	
BPA			
fish	16 (1 – 134.8)	108	24.69
tuna	71.7	94	96.28
coffee	174 (134 – 213)	200	497.14
tea	72 (53 – 90)	617	634.63
drinking water	11.9 ng/L	2 L	0.34
Total daily dose		1253.08 ng/kg, bw/day	
Equivalent of daily E2 dose		0.0313 ng/kg, bw/day	
Percentage of total daily dose		2.56%	

8.3.2 Dose-response assessment of EDCs for human health

There is a lack of information on does-response relationship for humans in the scientific literature, mainly because of experimental difficulties and sensitive ethical issues (Damstra et al., 2002; European Commission 2003; Diamanti-Kandarakis et al., 2009). So, human dose-response assessment can only be conducted indirectly with animal studies. Using the methods described in Section 4.4.2, $NOAEL_{animal}$ values based on reproductive effects in rats and mice were converted into $NOAEL_{HED}$ for *Human Equivalent Dose* (HED) using the body surface area extrapolation method (FDA 2005). The CPD curves of extrapolated $NOAEL_{HED}$ values were plotted in Figures 65 to 69 for all EDCs except for E1 and E3, which have no published animal studies available. Compared with estrogens, more dose-response studies were conducted with phenolic compounds in the literature, particularly with BPA.

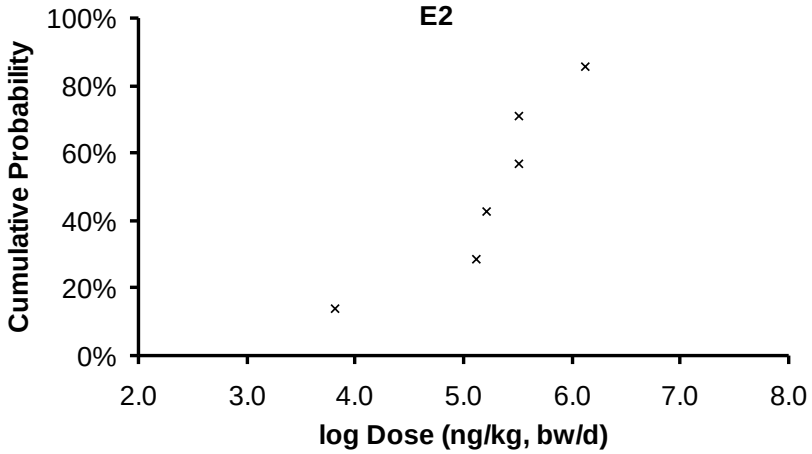

Figure 65. Cumulative probability distribution of $NOAEL_{HED}$ values for E2.

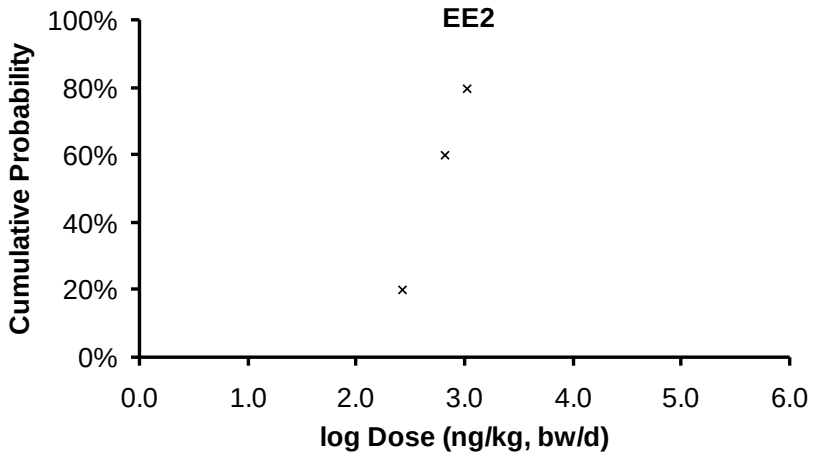

Figure 66. Cumulative probability distribution of $NOAEL_{HED}$ values for EE2.

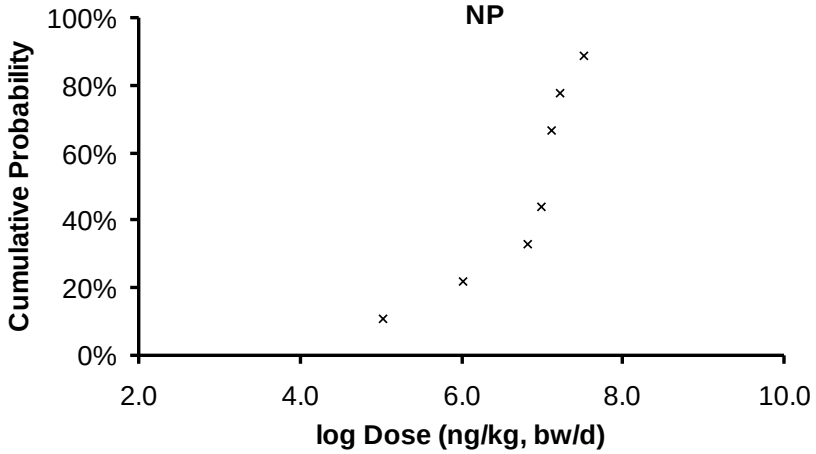

Figure 67. Cumulative probability distribution of $NOAEL_{HED}$ values for NP.

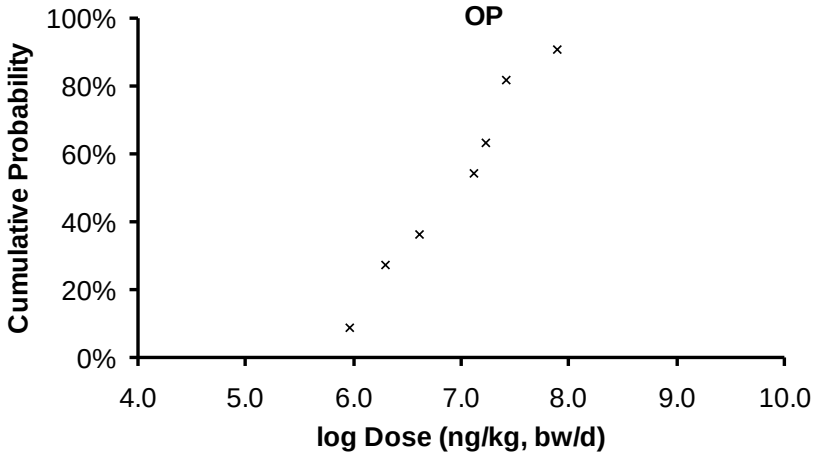

Figure 68. Cumulative probability distribution of $NOAEL_{HED}$ values for OP.

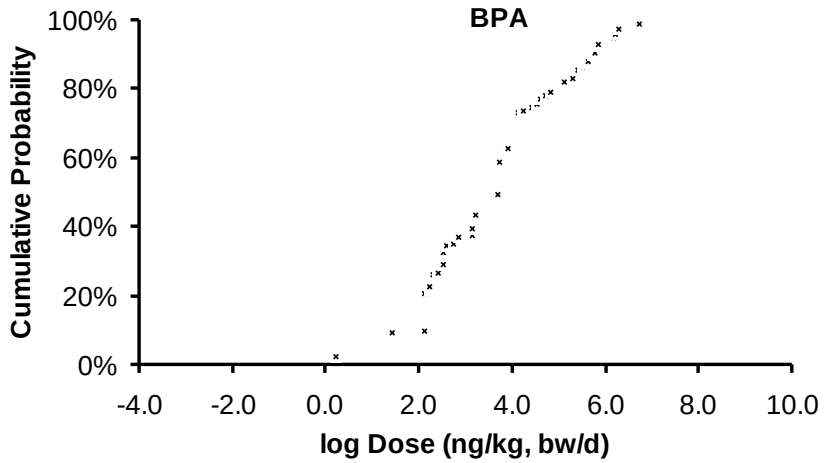

Figure 69. Cumulative probability distribution of $NOAEL_{HED}$ values for BPA.

It was observed in Figures 65 to 69 that estrogens generally exhibited higher potency than phenolic compounds by imposing adverse health effects at much lower $NOAEL_{HED}$ values. Particularly, EE2 was found to be the most potent EDC among all studied compounds, which is consistent with the NOAEC values obtained in fish studies. The median $NOAEL_{HED}$ value at 50% CP were estimated from the above CPD curves for E2, EE2, NP, OP and BPA, which were ranked in the order of EE2 < E2 < BPA < OP < NP. These median values were summarized in Table 29 together with the minimum and maximum $NOAEL_{HED}$ values.

Table 29. Summary of median and range of $NOAEL_{HED}$ values (μg/kg, bw/d)

EDC	Minimum	50% Median	Maximum
E1	n.a.	n.a.	n.a.
E2	6.4	200	1289
E3	n.a.	n.a.	n.a.
EE2	0.26	0.55	1.03
NP	103	11000	32230
OP	902	8000	77353
BPA	1.3	329	1647

8.3.3 Risk characterization of EDCs for human health

With the information obtained on human exposure (HDD), dose-response ($NOAEL_{HED}$ values) and public health standards (ADI), risk characterisation was carried out by plotting the CPD curves of HDD and $NOAEL_{HED}$ values together in Figures 70 to 76. The ADI values were also indicated in the log Dose axis, which were generally several orders of magnitude smaller than the $NOAEL_{HED}$ values. This is because ADI values were normally derived from $NOAEL_{HED}$ values and divided by a safety factor from 10 to 1000 for regulatory purposes (Vermeire et al., 1999).

When the HDD distributions were qualitatively compared with the $NOAEL_{HED}$ distributions, the former was generally several orders of magnitude lower than the latter for all EDCs except for BPA, which has an apparent overlap between HDD and $NOAEL_{HED}$ distributions. This overlap for BPA should be interpreted with caution, as $NOAEL_{HED}$ values were extrapolated from animal studies. The applicability of animal studies to humans is always questionable and the extrapolated values should be used with caution (Australia Guidelines for Water Recycling 2006). Nevertheless, this qualitative comparison remains some usefulness supported by other evidences in the decision-making process for risk assessors.

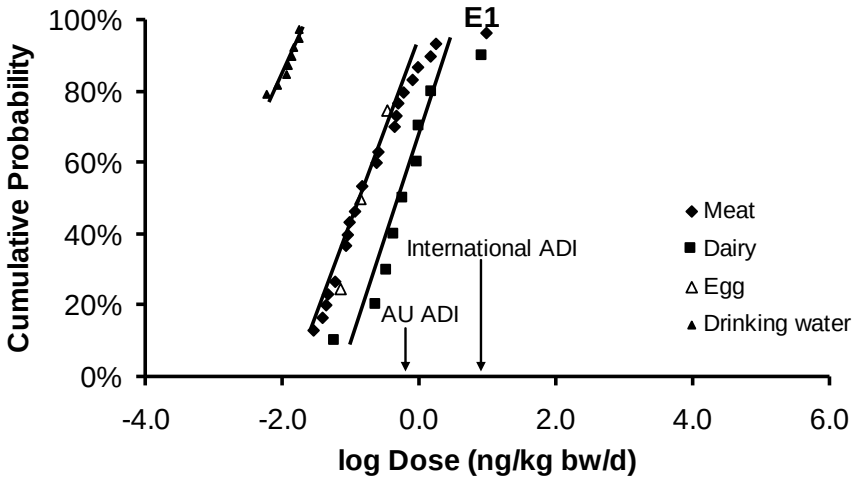

Figure 70. Comparison of human daily dose from drinking water and various foods with ADI values for E1.

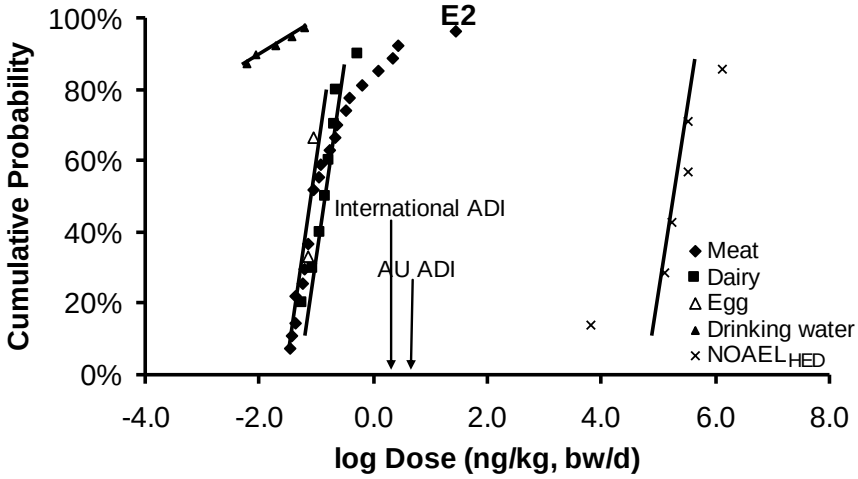

Figure 71. Comparison of human daily dose from drinking water and various foods with ADI and $NOAEL_{HED}$ values for E2.

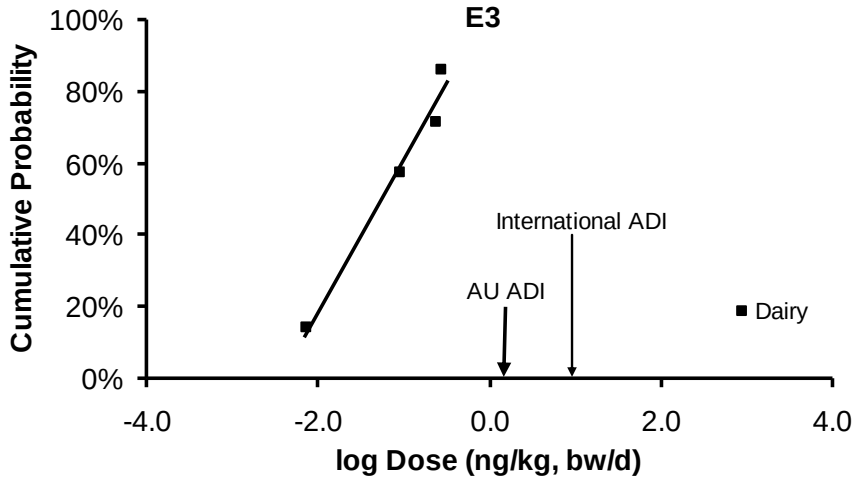

Figure 72. Comparison of human daily dose from dairy products with ADI values for E3.

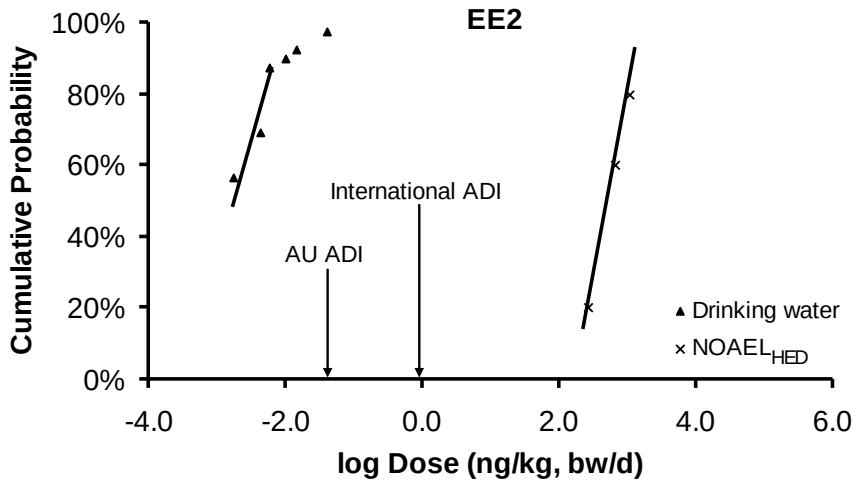

Figure 73. Comparison of human daily dose from drinking water with ADI and $NOAEL_{HED}$ values for EE2.

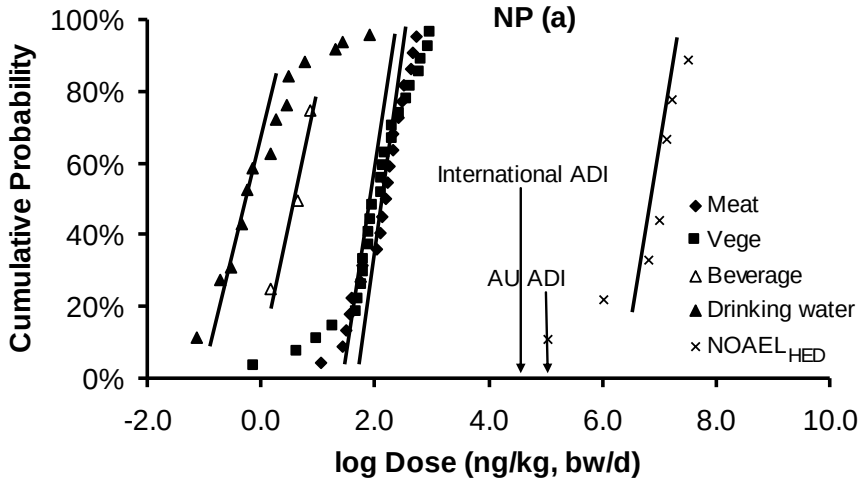

Figure 74a. Comparison of human daily dose from drinking water and various foods with ADI and $NOAEL_{HED}$ values for NP.

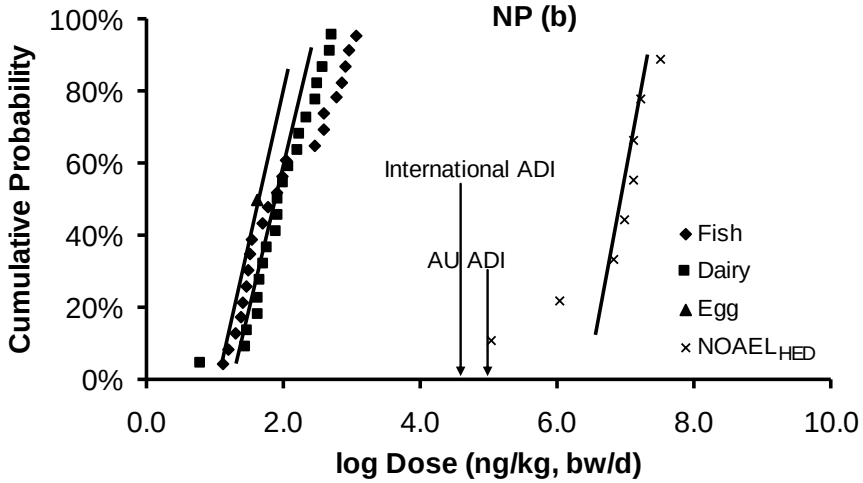

Figure 74b. Comparison of human daily dose from drinking water and various foods with ADI and $NOAEL_{HED}$ values for NP.

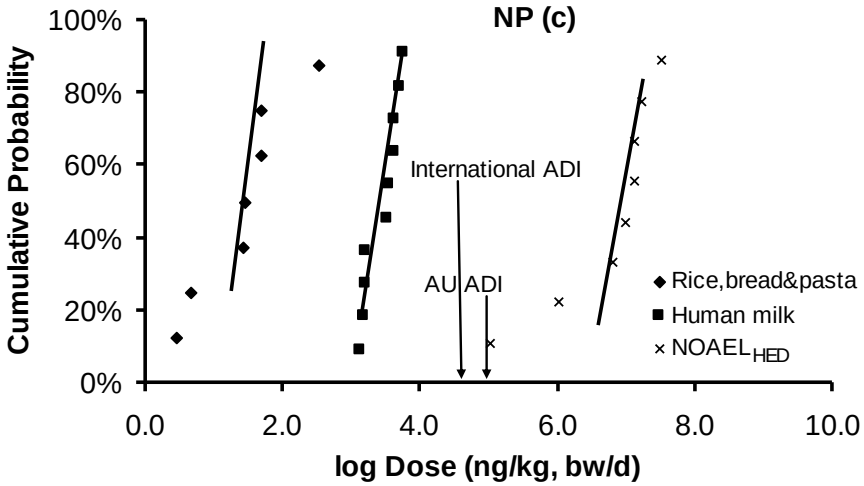

Figure 74c. Comparison of human daily dose from drinking water and various foods with ADI and $NOAEL_{HED}$ values for NP.

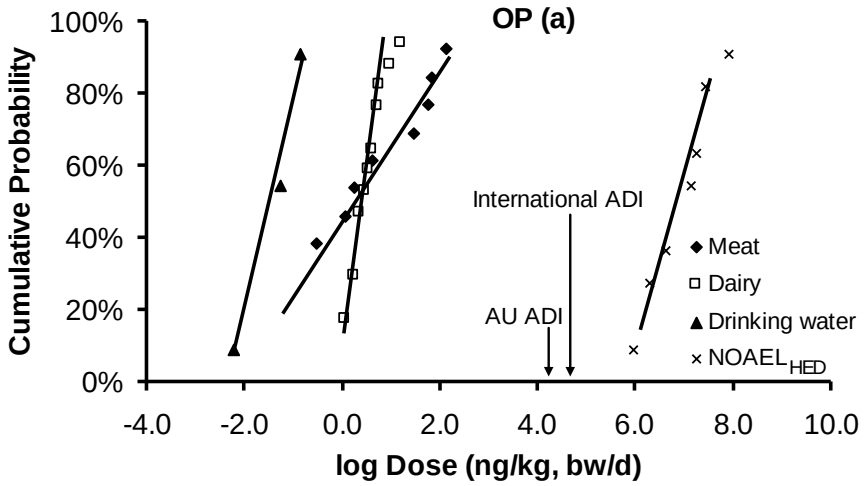

Figure 75a. Comparison of human daily dose from drinking water and various foods with ADI and $NOAEL_{HED}$ values for OP.

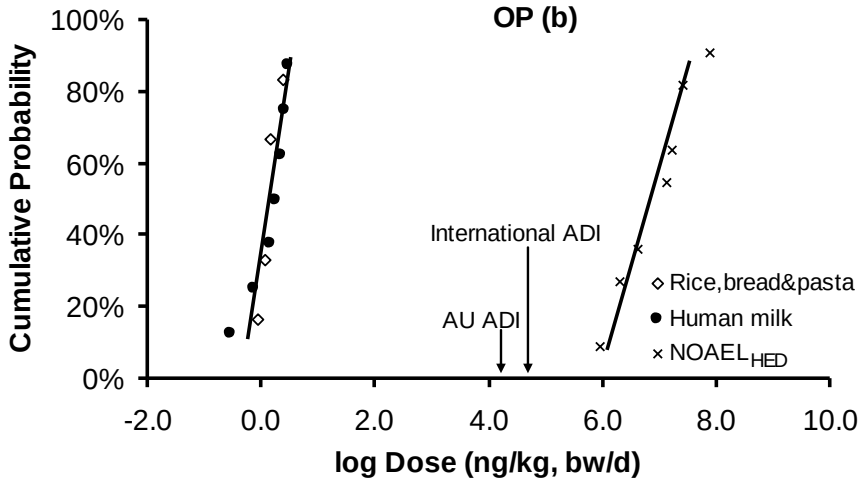

Figure 75b. Comparison of human daily dose from drinking water and various foods with ADI and $NOAEL_{HED}$ values for OP.

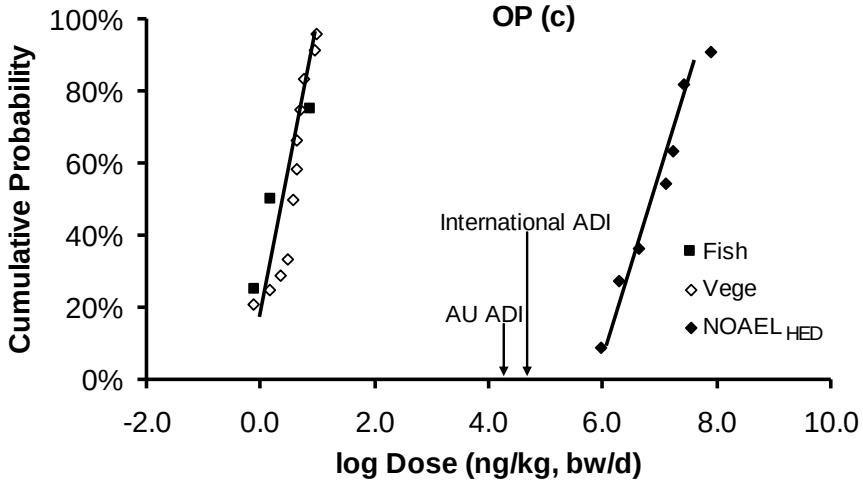

Figure 75c. Comparison of human daily dose from drinking water and various foods with ADI and $NOAEL_{HED}$ values for OP.

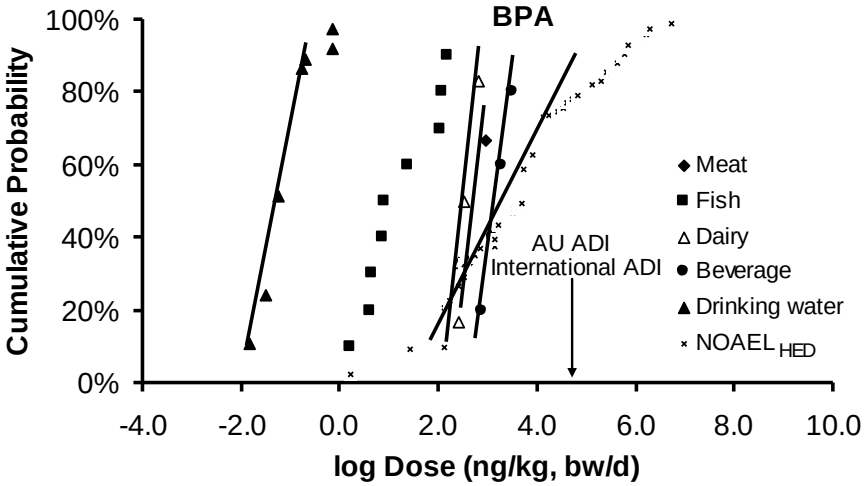

Figure 76. Comparison of human daily dose from drinking water and various foods with ADI and $NOAEL_{HED}$ values for BPA.

To quantitatively evaluate the level of risk for each EDC, $HQ_{95/ADI}$ values were calculated using Equation (35) as described in Section 4.4.3. The ADI values were drawn from International agencies and Australian guideline values, which were collated in Table 30. The ADI values for BPA were the same for these two sets of ADI values, but the Australian ADI values for E1, E3, EE2 and OP were slightly lower than those from International agencies. On the other hand, the ADI values of E2 and NP were higher in the Australian guideline values.

Table 30. International and Australian ADI values for EDCs

EDCs	International ADI (ng/kg, bw/d)	Australia Water Reuse ADI [e] (ng/kg, bw/d)
E1	10 [a]	0.86
E2	2.9 [a]	5.0
E3	10 [a]	1.4
EE2	1.0 [a]	0.043
NP	50 000 [b]	150 000
OP	50 000 [c]	15 000
BPA	50 000 [d]	50 000

[a] - from (Caldwell et al., 2009);
[b] - from (Schlatter 1998);
[c] - from (UNEP 1995);
[d] - from (US EPA 2009).
[e] - from (Australia Guidelines for Water Recycling 2006).

Using Equation (35), the calculated $HQ_{95/ADI}$ values were summarized in Table 31. The magnitude of $HQ_{95/ADI}$ values indicates the level of health risk. Higher $HQ_{95/ADI}$ value implicates higher level of health risk. The obtained $HQ_{95/ADI}$ values were ranked in the order of E1 > E2 > BPA > EE2 ≈ E3 > NP > OP for international ADI values and E1 > EE2 > E2 > E3 > BPA > OP > NP for Australia ADI values respectively. Apparently, E1 was found to be the one with the highest level of risk. Although its potency is not the highest, its risk level was contributed by its high exposure concentration. From Figure 70, it was estimated that there were about 40% and 7% of E1 HDD values exceeded Australia and international ADI values respectively. In contrast, NP and OP were found to have the lowest level of health risk, as the gaps between their HDD and ADI values were the largest among all EDCs.

Table 31. Hazard quotient calculated with international and Australia ADI values

EDCs	$HQ_{95/ADI}$ with International ADI	$HQ_{95/ADI}$ with Australian ADI
E1	3.16	3.68×10^{1}
E2	1.09	6.32×10^{-1}
E3	3.98×10^{-2}	2.84×10^{-1}
EE2	3.98×10^{-2}	9.26×10^{-1}
NP	2.00×10^{-2}	6.67×10^{-3}
OP	2.52×10^{-3}	8.39×10^{-3}
BPA	0.2	0.2

Among all estrogens, EE2 is a directly ingested contraceptive pill by women. Thus, the HDD of EE2 was also compared with female average daily intake of 500 μg/kg, bw/d (Ying et al., 2002), which was shown in Figure 77:

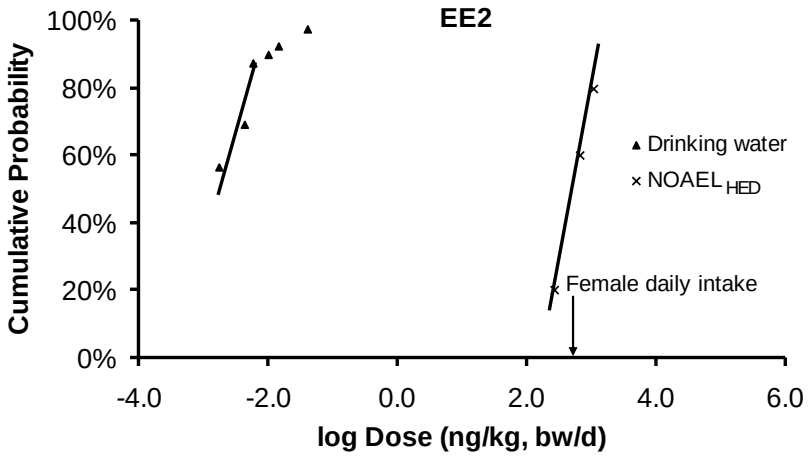

Figure 77. Comparison of human daily dose from drinking water with female daily intake of EE2 as oral contraceptive.

Similarly, a $HQ_{95/EE2}$ value of 7.96×10^{-5} was calculated using Equation (36) (see Section 3.4.3). This $HQ_{95/EE2}$ value indicated that human daily dose from drinking water was at least 10 000 times less than female daily intake of contraceptive pill. The human daily dose from food was unknown due to no concentration data in food was reported.

8.4 Chapter conclusions

The health risk assessment of EDCs was conducted for fish and humans by using probabilistic techniques. For fish, it was found that estrogens imposed much higher health risk than phenolic compounds. The obtained $HQ_{95/5}$ (1.2 - 250) and ORP (3.8% - 26.6%) values for estrogens were all above the reference value of 1 in the $HQ_{95/5}$ method and 2.5% in the ORP method, implicating estrogen contamination in surface water is a global issue of concern. In contrast, both the $HQ_{95/5}$ and ORP values obtained for phenolic compounds were below the reference values. With fish, the risk level of each EDC was ranked as EE2 > E1 > E2 > E3 > NP > BPA > OP with both the $HQ_{95/5}$ and ORP method. With humans, the majority of human daily dose (HDD) was due to the consumption of food. Generally, HDD from food was about 10 to 100 times higher than that from drinking water. Human risk was quantified as the $HQ_{95/ADI}$ value in the ranking of E1 (3.16) > E2 (1.09) > BPA (0.200) > EE2 (0.0398) ≈ E3 (0.0398) > NP (0.0200) > OP (0.00252) using international ADI values and E1 (36.8) > EE2 (0.926) > E2 (0.632) > E3 (0.284) > BPA (0.200) > OP (0.00839) > NP (0.00667) using Australia ADI values. The greatest human health concern was found to be associated with E1, which has $HQ_{95/ADI}$ values above the reference value of 1 with both sets of ADI values. Compared with the single-point hazard quotient method (e.g. $HQ_{95/5}$ and $HQ_{95/ADI}$), the ORP method takes into account the shape of individual cumulative probability distribution curve. Therefore, it is considered an improvement in risk characterisation.

Chapter 9 Overall conclusions

QSPR on Steroidal EDCs

Good linear relationships were found between log K_{ow} and each of the three molecular descriptors: log FOSA (hydrophobic component of the total solvent accessible surface area), log FISA (hydrophilic component of the total solvent accessible surface area) and log PSA (Van de Waals surface area of polar nitrogen and oxygen atoms). Similar relations were conducted between the measured log S and each of the three molecular descriptors, but only weak correlation coefficient (< 0.6) were observed. The phenolic A-ring present in the structure of estrogens has a major effect on lowering their hydrophobicity. The relationships obtained can be used to obtain property values for various steroidal compounds, particularly those with potential environmental effects.

Relationship between k and EBC for estrogens

Acceptable linear relationships were established between the equivalent biomass (log EBC) and the degradation rate constant (log k) for E1, E2 and EE2. Results indicate that EE2 is most resistant to biodegradation, while E1 and E2 are relatively easily degraded with similar rates. The triple bond ethynyl group in the chemical structure of EE2 may slow its biodegradation by blocking bacteria access to the hydroxyl group in the C-17 position. The correlations obtained are useful for the prediction of fate in various environmental media.

Fate simulation in reservoir for estrogens

Under typical conditions, the simulated estrogen concentrations in the reservoir water compartment were below 10^{-4} ng/L after receiving recycled wastewater treated by

advanced treatment. Most of the estrogens were removed by degradation in the water compartment with removal rates of 99.6%, 98.5% and 93.8% for E1, E2 and EE2 respectively. Simulated concentration of all estrogens decreased when reservoir water temperature (T), reservoir water storage volume (V), biomass concentration (EBC) and reservoir water releasing rate (F_d) increased. However the opposite trend was found with EDC concentration in the recycled water (C_e) and recycling rate (F_r). Under all conditions within the simulation ranges, the hazard quotient ($HQ_{95/NOAEC}$ and $HQ_{95/PHS}$) values obtained in the risk characterisation were smaller than 10^{-2}, implicating negligible health risk for fish and humans. The three estrogens were ranked in the risk level of EE2 > E1 > E2, showing EE2 is of highest health risk.

Health risk assessment for estrogens and phenolic compounds

Generally, estrogens impose much higher health risk than phenolic compounds for both fish and humans. The hazard quotient ($HQ_{95/5}$) and the overall risk probability (ORP) methods obtained the risk level in the same ranking of EE2 > E1 > E2 > E3 > NP > BPA > OP. The obtained $HQ_{95/5}$ (1.2 - 250) and ORP (3.8% - 26.6%) values for estrogens were all above the reference values (1 with $HQ_{95/5}$ method and 2.5% with ORP method), implicating estrogen contamination in surface water is a global issue of concern. In contrast, the $HQ_{95/5}$ and ORP values obtained for phenolic compounds were both below the reference values. For humans, most of human daily dose (HDD) was due to the consumption of food. Human risk was quantified as the $HQ_{95/ADI}$ value in the ranking of E1 (3.16) > E2 (1.09) > BPA (0.200) > EE2 (0.0398) ≈ E3 (0.0398) > NP (0.0200) > OP (0.00252) using international ADI values and E1 (36.8) > EE2 (0.926) > E2 (0.632) > E3 (0.284) > BPA (0.200) > OP (0.00839) > NP (0.00667) using Australia

ADI values. The greatest human health concern was found to be associated with E1, which has $HQ_{95/ADI}$ values above the reference value of 1 with both sets of ADI values. Compared with the hazard quotient method, the ORP method takes into account the shape of individual cumulative probability distribution curve. Therefore, it is considered an improvement in risk characterisation.

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