

HUMAN HEALTH RISKS FROM ENDOCRINE DISRUPTORS IN RECLAIMED WATER

By

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To my late parents, may they rest in peace

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## LIST OF ABBREVIATIONS

ADI	Allowable Daily Intake
BPA	Bisphenol-A
BSTFA+TMCS	Bis-(trimethylsilyl)-trifluoroacetamide and trimethylchlorosilane
CSF	Cancer Slope Factor
CO <sub>2</sub>	Carbon-dioxide
DWEL	Drinking Water Equivalent Level
E1	Estrone
E2	17 $\beta$ -estradiol
EDC	Endocrine Disrupting Chemical
EE2	17 $\alpha$ -ethynylestradiol
EFED	Environmental Fate and Effects Division
EC <sub>50</sub>	Median Effective Concentration
GC	Gas Chromatograph
GC-MS	Gas Chromatograph - Mass Spectrometer
HEI	Highly Exposed Individuals
HPLC-MS	High Performance Liquid Chromatograph - Mass Spectrometer
Kow	Octanol – Water Partitioning Coefficient
LOAEL	Lowest Observable Adverse Effect Level
MDL	Method Detection Limit
NOAEL	No Observable Adverse Effect Level
NP	Nonylphenol

OPP	Office of Pesticide Programs
PCB	Polychlorinated Biphenyl
PPCP	Pharmaceuticals and Personal Care Product
PQL	Practical Quantitation Limit
RfD	Reference Dose
RQ	Risk Quotient
RSD	Risk Specific Dose
RW	Reclaimed Water
SD	Standard Deviation
SPE	Solid Phase Extraction
UF	Uncertainty Factor
USEPA	United States Environmental Protection Agency
WWTP	Wastewater Treatment Plant

Abstract of Thesis Presented to the Graduate School  
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Endocrine disrupting chemicals (EDCs) occur in reclaimed water (RW) and constitute unknown risks to humans. The presence of EDCs in reclaimed water used to irrigate turf and in nearby water retention ponds was determined, and used in the first step of an assessment of risk to a child playing on recently irrigated turf and subjected to dislodged EDC residues. Five EDCs (estrone,  $17\beta$ -estradiol,  $17\alpha$ - ethynylestradiol, bisphenol A and nonylphenol) were quantified in 28 samples of reclaimed waters (wastewater treatment plant effluents) and 88 samples from residential ponds. St. Augustine turf grass was irrigated with spiked RW to study dislodgement of the five EDCs using hand swipe and drag-sled methods. Grass clippings were analyzed to relate masses of EDC on grass with masses dislodged. EDCs were detected in all RW and pond water samples. Bisphenol A was detected most frequently and in greater amounts in both RW and pond samples. Maximum masses of EDCs were dislodged immediately after irrigation. Dislodged masses of estrone,  $17\beta$ -estradiol and  $17\alpha$ -ethynylestradiol decreased rapidly and were below detection limits 4 hours after application. Dislodged bisphenol-A and nonylphenol decreased more slowly and uniformly, but were not detected 6 hours after application. The human health risk

associated with EDCs in RW and retention ponds, and dislodged residues of EDCs from turf irrigated with RW was minimal.

## CHAPTER 1 INTRODUCTION

Increasing use of reclaimed water (RW) for crop irrigation, groundwater recharge, and landscape irrigation, increases the risk of human exposure to potentially harmful chemicals present in RW. Many trace organic chemicals, including so called “emerging chemicals of concern” like steroidal hormones, occur in surface and reclaimed water at concentrations ranging from below detection limits to several ng/ L (Duran-Alvarez et al., 2009, Liu et al., 2009). The human health risk associated with even small concentrations of EDCs is, however, uncertain. The development of more advanced analytical techniques, [e.g. gas chromatographs coupled with mass spectrometers (GC-MS) and high performance liquid chromatographs coupled with mass spectrometers (HPLC-MS)], allows detection of even lower concentrations. Better detection technology heightens public concern about harmful chemicals as the mere presence of a chemical in the environment may pose some risk to humans.

### **Reclaimed Water**

Exponential population growth and rapid development places enormous stress on potable water supplies, making more efficient water management crucial. Use of treated wastewater (instead of potable freshwater) for irrigation of landscapes, lawns, and agricultural fields is an increasingly popular approach and is expanding rapidly. Treated wastewater effluent reused for various purposes is called reclaimed or recycled water (RW). Secondary treated wastewater effluents are the source of reclaimed water in most cases (USEPA, 2012a). Irrigation of turf grass is one of the major water consuming activities in the United States and use of RW to irrigate turf is increasing.

Florida leads the U.S. in terms of reusable water production, as well as use. Out of 2740 million liters reclaimed water produced daily in Florida, 1590 million liters ( $\approx 58\%$ ) are used for irrigating golf courses, residential turf, and parks (FLDEP, 2012).

## **Benefits**

Wastewater reuse not only partially counters freshwater scarcity, but also helps to maintain ---

**Environmental sustainability:** by reducing pollutant loads and discharge into receiving water bodies,

**Economic efficiency:** through improved water conservation, promotion of water efficiency, waste reduction and harmonizing long term water demand and water supply,

**Food security:** (for some countries) through application of certain essential plant nutrients via treated wastewater reuse (Indian Institute of Technology Team, 2011).

## **Concerns**

Despite the many benefits of using RW, there are some potential disadvantages (Toze, 2006) including---

**Yuck Factor:** Many people are squeamish about using recycled water. Campaigns are sometimes necessary to educate people about the potential advantages and safety of RW. More and more people are, however, becoming more accepting of RW use.

**Presence of Pathogens:** Reclaimed water may contain greater numbers and species of certain enteric pathogens than potable freshwater.

**Presence of contaminants:** Chemicals like pharmaceuticals and personal care products (PPCPs), polychlorinated biphenyls (PCBs), flame retardants, steroids etc. can occur in wastewater effluents (Desbrow et al., 1998; Kuch and Ballschmiter, 2001;

Ternes et al., 1999a; Duran-Alvarez et al., 2009). Wastewater treatment plants are not fully efficient in removal of such organic chemicals from wastewater streams (Roberts and Thomas, 2006). As a result, trace organic chemicals are discharged into surface waters or are applied to agricultural fields, recreational areas, etc. with the reclaimed water. Many of the trace organic chemicals are endocrine disrupting chemicals (EDCs), and have been detected in reclaimed water up to several nanograms per liter (Liu et al., 2009). EDCs are natural and/or synthetic substances that when absorbed into body, from an outside source, can overstimulate/block and/or interfere with normal functioning of endocrine system of an organism producing adverse developmental, reproductive, neurological, and/or immune effects (Jobling et al., 1998; 2004). Many EDCs have estrogenic activity and either mimic or aid in excess production of body estrogens (Gore, 2007). Estrogenic activity of a chemical is a measure of potency of a chemical in terms of ability to stimulate normal endocrine system to produce estrogen (particularly, estradiol).

### **Sources of EDCs in Wastewater**

A wide variety of chemicals including pharmaceuticals and personal care products (PPCPs), polychlorinated biphenyls (PCBs), DDT and other pesticides, and plasticizers such as bisphenol A are considered endocrine disruptors, and are common in everyday products like plastic bottles, metal food cans, detergents, flame retardants, food, toys, cosmetics, and pesticides (NIEHS, 2011; Naz, 2005). Many pharmaceuticals and dietary supplements containing EDCs are only partially digested by the body and excreted intact through urine and feces (Redderson et al., 2002). Natural hormones produced by mammals (especially sex hormones like testosterone and estradiol) and their metabolites are also excreted in urine. Excreted hormones and pharmaceuticals

then enter the environment through effluents discharged by wastewater treatment plants (WWTPs) and wet-weather run-off (Shore et al., 1988; Shore and Shemesh, 2003). Partial removal or formation of an active form during the process of sewage treatment results in release of endocrine disruptors in surface waters and/or reusable water (Gomez et al., 2006). Other sources of EDCs in the environment include, but are not limited to, industrial solvents or lubricants and their byproducts.

### **Potential Impacts of EDCs**

EDCs not only contaminate environment but also affect organisms (especially aquatic) and possibly humans (Lewis, 1991; Jobling et al., 1998; Clayton, 2011).

#### **Ecological Impacts**

Some EDCs like steroidal hormones (e.g.  $17\alpha$ -ethynylestradiol) can cause biological effects on aquatic organisms at parts per trillion concentrations (Weber et al., 2005). Male fish held in cages exposed to sewage effluent undergo feminization effects, and rivers receiving significant amounts of sewage effluent contain significant numbers of “intersex” fish (Jobling et al., 1998, 2004; Sumpter, 2005; Rodgers-Gray et al., 2000). Floridian alligators suffer from under developed male gonads in juvenile male alligators due to the presence of estrogenic-like compounds in the Florida everglades (Guillette et al., 1994).

#### **Impacts on Humans**

Concentrations of EDCs in surface water bodies, reclaimed waters, and septic effluents are typically low (on the order of parts per billion or trillion). The risk of human exposure is, however, intensified as EDCs may have adverse effects on humans even at such low concentrations (Routledge et al., 1998). EDCs are thought to be linked with

the decline of sperm counts in adult males, increasing incidence of breast and testicular cancer, and the decrease in the age of puberty onset (Xiao et al., 2001; Hotchkiss et al., 2008). The endocrine disruptions produced can cause cancerous tumors, birth defects, and other developmental disorders (Lewis, 1991). Other supposed adverse effects of EDCs include decreased thyroid function and increased parathyroid hormone activity (NRC, 2006; Stewart et al., 2003; Zoeller, 2007), reproductive abnormalities (Guillette et al., 1994; Diamanti-Kandarakis et al., 2009), decreased sperm count in men (Carlsen et al., 1992), effects on brain of developing fetuses (McCally, 1997), and early puberty in females (Toppari and Juul, 2010).

The mere presence of EDCs in the environment, however, does not necessarily indicate significant risk. The extent of adverse effects of any chemical depends upon hazard and exposure-dose relationship. Further, some effects are also species dependent (Clayton, 2011). Thus, the risk from EDCs to humans is uncertain. The UK Environment Agency (2000) commented that “much of the published data relate to laboratory conditions and do not necessarily reflect the responses which would occur in the animal in the environment” (Clayton, 2011). Indeed, most of the published data suggests that the concentrations of EDCs to which human beings are subjected in the environment pose negligible to low risk of adverse health effects (Clayton, 2011). For example, the data suggesting a decline in male sperm concentration in adult males reported by Carlsen et al., (1992) were not confirmed by subsequent studies (Lukachko, 1999; Jørgensen et al., 2001; Clayton, 2011). However, with the increasing populations, increasing production and use of PPCPs, and new chemicals being developed on a daily basis, the risk from EDCs is likely to rise.

Risk assessment of EDCs is complicated because of many factors including the complex nature of effects produced, potential delayed on-set of effects, potential lack of a threshold for effect, suggested no relationship between dose and response, and effects at very low doses (NTP, 2001). Some EDCs can also be toxic and/or carcinogenic and some are suspected carcinogens, further increasing speculation about risk and thus increasing the importance of more research on EDCs.

### **EDCs in RW**

Current wastewater treatment plants (WWTPs) use primary and secondary treatments to produce effluents from which EDCs are incompletely removed (Deblonde et al., 2011). Secondarily treated wastewater effluents are the source of reclaimed water in most cases (USEPA, 2012a). Tertiary treatments like ozonation, active carbon adsorption and catalytic oxidation show high EDC removal efficiencies, but are rarely used in WWTPs (Yoon et al., 2006; Broseus et al., 2009). As a result, EDCs have been detected in effluents of wastewater treatment plants in many countries (Baronti et al., 2000; Belfroid et al., 1999; Desbrow et al., 1998; Kuch and Ballschmiter, 2001; Nasu et al., 2001; Snyder et al., 1999; Ternes et al., 1999a; Weber et al., 2005). Reuse of wastewater effluents containing EDCs can, thus, have potential adverse effects on human beings and/or the environment. Fortunately, there are restrictions on RW use depending upon the level of treatment (primary, secondary, and tertiary) undergone (e.g. secondary treated effluents are not potable). However, there are no federal regulations regarding the maximum load of EDCs allowable in effluents (Weber et al., 2005; USEPA, 2012a). Concentrations of various EDCs detected in effluents worldwide are given in Tables 1-1 and 1-2.

Table 1-1. Concentrations (ng/L)\* of hormones in effluents of WWTPs (Ying et al., 2002a).

Location	Number of Sample	Estrone (E1)	17 $\beta$ -Estradiol (E2)	17 $\alpha$ -Ethinylestradiol (EE2)
Italy	30	2.5-82.1 (9.3)	0.44-3.3 (1.0)	<0.9-1.7 (0.45)
Netherlands	6	<0.4-47 (4.5)	<0.1-5.0 (<0.1)	<0.2-7.5 (<0.2)
Germany	16	<1-70 (9)	<1-3 (<1)	<1-15 (1)
Canada	10	<1-48 (3)	<1-64 (6)	<1-42 (9)
UK	21	1.4-76 (9.9)	2.7-48 (6.9)	<0.2-7 (<0.2)
Japan	27x3	-	3.2-55 (14) <sup>b</sup> <1-43 (13) <sup>c</sup> 0.3-30 (14) <sup>d</sup>	-
USA	5	-	0.477-3.66 (0.9)	<0.05-0.759 (0.248)
Germany	16	<0.1-18 (1.5)	<0.15-5.2 (0.4)	<0.10-8.9 (0.7)

\* Concentration range; median in parentheses.

b = summer sampling. c = autumn sampling. d = winter sampling.

Table 1-2. Concentrations ( $\mu$ g/L) of nonylphenol (NP) and bisphenol-A (BPA) in effluents of WWTPs.

Location	NP (Ying et al., 2002b)		Number of Samples	BPA
	Number of Samples	Concentration range (median)		Concentration range (median) (reference)
Canada	8	0.8-15.1 (1.9)	36	0.07-1.68 (0.41) (Mohapatra et al., 2011).
UK	16	<0.2-5.4 (0.5)		
Switzerland	2	5-11		
Spain	3	6-343		
Japan	10	0.08-1.24		
USA	13	<0.1-37 (<.5)	> 50	0.0036-50 (0.01) (USEPA, 2010)
Germany	16	<0.05-0.77 (0.111)	18	0.018-0.702 (0.03) (Spengler et al., 2001).
Italy	12	0.7-4 (1.8)		

## EDCs in Surface Water Retention Ponds

Residential water retention ponds (present in many residential areas) may be contaminated with EDCs from sources like run-off and drainage of RW after irrigation and/or EDCs leaching from septic systems. RW used for irrigating lawns can collect in nearby water retention ponds along with run-off from other surfaces. Septic system effluents can also contain EDCs (Table 1-3) (Carrara et al., 2008) that leach through soil (both vertically and horizontally) and pollute nearby water retention ponds. Sandy soils of south Florida may increase leaching of EDCs from septic systems, while the underlying carbonates (less permeable) may facilitate lateral movement of septic effluents containing EDCs into the ponds. Unfortunately, no literature is available on concentrations of EDCs in residential water retention ponds.

Table 1-3. Concentrations ( $\mu\text{g/L}$ ) of EDCs reported in septic effluents

Chemical	Number of Samples	Concentration range (reference)
Estrone	42	> 2 (Sulleabhain et al., 2009)
17 $\beta$ -estradiol	42	> 2 (Sulleabhain et al., 2009)
17 $\alpha$ -ethynylestradiol	26	0.1-0.36 (Stanford and Weinberg, 2010).
Bisphenol-A	5	0.11-1.7 (Rudel et al., 1998)
Nonyl phenol	> 25	10-16 (Swartz et al., 2006).

### Human Exposure Scenario/ Research Problem

Use of RW to irrigate turf grass may increase risk of human exposure to EDCs. Dislodgment of EDCs from RW-irrigated turf poses a potential pathway of exposure to

humans present in the vicinity of the turf grass. Children playing on the contaminated turf could be at particularly high risk from the harmful effects of EDCs exposure. Knowledge of dislodgment rates of a particular chemical from this environmental niche is therefore important in assessing exposure of chemicals to humans.

The presence of EDCs in residential surface water retention ponds is another cause of concern. Water retention ponds may act as a potential source for exposure of EDCs, either directly via contact with water or indirectly through contamination of drinking water sources recharged by the ponds, or consumption of contaminated fish etc. grown in the ponds.

### **Overview of Human Health Risk Assessment**

The US Environmental Protection Agency (USEPA, 2004) defines risk as the chance of harmful effects to human health or to ecological systems resulting from exposure to an environmental stressor, a stressor being any physical, chemical or biological entity capable of inducing an adverse response. Risk is also defined as:

Risk= Exposure X Hazard.

The risk assessment process involves five principle steps:

**Hazard Identification:** Identification of hazard/contaminant, its properties (e.g., form, partitioning in the environment, concentrations in environmental niche of concern, mode of action, behavior in an organism, toxicity etc.) and other potential adverse environmental and/or human health impacts.

**Exposure Assessment:** Calculation of maximum amounts of the hazard to which an individual or a group of individuals may be exposed. Exposure assessment involves determination of concentrations of a chemical to which an individual can be exposed, identification of highly exposed individuals (HEIs) (the population which is

most susceptible to exposure either due to environment in which they live/work or by virtue of their habits, developmental stages etc.), pathways of exposure (e.g., air, water, soil, food etc.), routes of exposure (e.g., ingestion (food, water and hand to mouth ingestion), dermal absorption, and inhalation), and amount, duration and frequency of exposure.

**Dose-response assessment:** Reaction of the target organism, organ, tissue or cell after the hazard reaches inside the target. Dose-response assessments involve toxicological and epidemiological studies, plus the use of models to assess adverse response by/effect on the target. Widely used approaches for assessment of non-carcinogenic effects include:

No-observable adverse effect levels (NOAELs): Highest dose of a hazard administered without a statistically significant increase in adverse response.

Lowest observable adverse effect levels (LOAELs): Lowest dose of the hazard at which adverse response is significant/ unacceptable.

For carcinogens, acceptable risk (level or chance of damage/ adverse effect deemed tolerable by an individual or population. For instance, risk of death in an airplane crash is less than 1 in 50,000) is used, as there is no threshold exposure.

**Risk Characterization:** Quantification of risk and/or probability of risk in mathematical terms like additional risk to 1 individual per 100,000 individuals. Risk characterization involves determining risk factors, increased risk populations, etc. and answers questions like whether there is considerable risk, how severe the risk is, and the consequences of risk.

Many approaches to risk assessment of environmental chemicals have been proposed, but the fundamental concept of a risk quotient (RQ) is common to all:

$RQ = \text{Exposure (Concentration of contaminant that may reach inside a target organism)}/\text{Toxicity (NOAEL etc.)}$

The Risk Quotient Method is used by the Office of Pesticide Programs (OPP) and Environmental Fate and Effects Division (EFED) of the USEPA for screening and higher level probabilistic risk assessment. Risk Quotients (RQs) are calculated by dividing exposure estimates by the acute and chronic ecotoxicity values (USEPA, 2004).

RQ values are compared to levels of concern (LOCs) or concentrations of the chemical in question. LOCs are criteria used by OPP to indicate potential risk to organisms and the need to consider regulatory action. The LOC for acute toxicity to mammals is 0.5, and for chronic toxicity is 1 (USEPA, 2004). If  $RQ > LOC$ , then there is a risk of adverse effect from exposure to the chemical in question and a higher level probabilistic risk assessment is warranted. Calculations of risk quotients are essential to evaluate risk from chemical exposure.

Other important criteria used in risk assessment include:

$\text{Risk Ratio or (Relative Risk)} = \frac{\text{Incidence of disease in population with risk factor}}{\text{Incidence of disease in population without risk factor}}$ .

**Reference dose (RfD):** The RfD is a benchmark dose operationally derived from the NOAEL and used to set regulations for the concentration of a hazard to which an organism can be exposed daily for a long time without significant adverse effects.

As NOAELs and LOAELs are obtained from studies on animals, uncertainty factors (UF, usually 10-1000 times) are used to extrapolate data for humans. Thus,  $RfD = NOAEL / UF$ .

**Drinking Water Equivalent Levels (DWELs):** DWELs (mg/L) are the maximum safe concentrations of chemicals a person can drink in water, daily, without non-carcinogenic adverse effects, over a life time of exposure.

$DWEL = (RfD * \text{body weight}) / \text{daily water intake}$ .

The “Acceptable Risk” approach is used for carcinogens, as there is no dose-response relationship for adverse effects. Conceptually, even a negligible dose can result in increased risk of cancer. Cancer slope factors (CSFs or SFs) are used to calculate acceptable/maximum increased risk of cancer via exposure to a carcinogen. A Cancer Slope Factor is an age-averaged lifetime excess cancer incidence rate per unit intake of a carcinogen (or unit exposure for external exposure pathways) and is used to convert the intake to a cancer risk (USEPA, 1989). The unit of CSF is  $(\text{mg}/\text{kg}\cdot\text{day})^{-1}$ . From CSF one can obtain a risk specific dose (RSD) that defines the acceptable concentration of carcinogen an individual can be exposed to (per day), with a predetermined acceptable risk of developing cancer. RSD is expressed as  $\text{risk}/\text{CSF}$ . For instance, if the CSF of a chemical is  $20/\text{mg}/\text{kg}\cdot\text{day}$  and the acceptable risk of cancer is one in 1,000,000 then  $RSD = (0.000001/20) = 0.05 \text{ ng of carcinogen}/\text{kg}\cdot\text{day}$ . If the concentration of carcinogen an individual is exposed to is more than  $0.05 \text{ ng}/\text{kg}\cdot\text{day}$  for 70 years, the additional cancer risk exceeds the acceptable risk.

**Risk management and communication:** Review of risk among peers and development and implementation of necessary steps for risk mitigation. Risk

communication is the purposeful exchange of information about the existence, nature, form, severity, or acceptability of risk to alert the public and/or decision makers of a significant risk or to calm concerns about a small risk perceived as more serious and risk management refers to the actions taken to prevent or reduce risk through exposure control and/or monitoring etc.

### **Research Objectives**

- 1) Determine the occurrence of EDCs in reclaimed water and residential surface water retention ponds.

**Hypothesis:** EDCs will be detected in RW and residential surface water retention ponds, but at very low concentrations (ng/L).

- 2) Assess the risk associated with exposure of humans to EDCs from direct consumption of RW and pond water, and indirectly from EDC dislodgement from turf irrigated with reclaimed water.

**Hypothesis:** Risk associated with exposure to EDCs in RW is negligible owing to very low EDC concentrations and minimal exposure.

### **EDCs Selected for Study**

To address the issues related to EDCs in RW, five EDCs: estrone (E1), 17  $\beta$ -estradiol (E2), 17  $\alpha$ -ethynylestradiol (EE2), bisphenol-A (BPA) and nonylphenol (NP) were selected. E1 and E2 are estrogenic steroidal hormones naturally produced in bodies of humans and animals. Excreted in urine, the chemicals are introduced directly into the environment through municipal wastewater treatment plant (WWTP) effluents (Daughton and Ternes, 1999; Norris, 2007). EE2 is a synthetic estrogenic compound

used in oral contraceptives pills; some passes through the body and may contaminate the environment. BPA and NP are synthetic chemicals used to make plastics and surfactants, respectively. BPA belongs to the chemical class diphenylmethane derivatives and bisphenols whereas NP is an alkylphenol.

The three steroidal hormones, BPA, and NP were selected because they are amongst the most studied EDCs and the relative availability of data on their occurrence and fate in the environment. Various properties of the five chemicals are given in Table 1-4.

### **Estrone**

Estrone (E1) is a naturally occurring estrogen produced in the ovaries and the adrenal glands. The International Union of Pure and Applied Chemistry nomenclature (IUPAC name), of E1 is 3-hydroxy-13-methyl 6,7,8,9,11,12,13,14,15,16-decahydrocyclopenta[a]phenanthren- 17-one.

Estrone is also a primary estrogenic component in variety of pharmaceuticals (Petrovic et al., 2004) used in the treatment of estrogen deficiency. Incomplete metabolism by the users results in significant concentrations of E1 in wastewaters and surface waters at concentrations greater than 1 ng/L and as high as 80 ng/L with median concentration of less than 10 ng/L (Thomas and Colburn, 1992; Ying et al., 2002a). E1 may be linked to birth defects, infertility, immune system suppression, deformities of reproductive organs, and various other health problems (Thomas and Colborn, 1992).

Estrone is the least abundant of the three natural estrogenic hormones; estrone, estradiol and estriol. E1 is important to health because it can be converted to estrone

sulfate, a long-lived derivative, which can be converted to the more active estradiol.

Estrone sulfate is commonly found in wastewater, primarily from urine.

### **Estradiol**

Estradiol (E2) is a natural estrogen produced from estrone conversion metabolized by the liver and excreted from the body through urine (Gore, 2007). E2 is the predominant sex hormone found in females and the most abundant of the three natural estrogens. Estradiol is also present in males but at much lower levels.

The IUPAC name for estradiol is (17 $\alpha$  or 17 $\beta$ )-estra-1,3,5(10)-triene-3,17-diol. There are two isomers of estradiol;  $\alpha$  and  $\beta$ . 17 $\beta$ -estradiol is more prominent and was used in this study. Estradiol is the most biologically active naturally-occurring estrogen. It is also an active ingredient in many medications for birth control, hormone replacement therapy, infertility treatments and vaginal infections. Adverse effects of estradiol range from minor health problems such as nausea, migraines and dizziness to major health problems such as breast cancer, strokes and heart attacks. E2 has also been linked to birth defects and deformities of the reproductive organs. E1 and E2 are inter-convertible and have been reported in wastewater effluents (Ying et al., 2002a).

### **Ethinylestradiol**

Ethinylestradiol (EE2) is a synthetic, steroidal estrogen commonly used as the active ingredient in birth control medications. The IUPAC name of EE2 is 19-nor-17 $\alpha$ -pregna-1,3,5(10)-trien-20-yne-3,17-diol, and the chemical is synthesized from estrone. With an estrogenic activity of 246 (with respect to 100 for estradiol), EE2 is one of the most potent EDCs found in wastewaters (Balaguer et al. 1999; Pillon et al., 2005). EE2 concentrations as high as 42 ng/L with median of less than 10 ng/L have been reported in effluents (Ying et al., 2002a).

## **Bisphenol-A**

Bisphenol- A (BPA), 4,4'-(propane-2,2-diyl)diphenol, is used in the production of plastics and related materials. Globally, more than six billion kilograms of BPA are produced annually (Welshons et al., 2006). BPA has been detected at concentrations as high as 4090 ng/L in wastewater effluents (Deblonde et al., 2011). Median concentration, however, range between 350-400 ng/L (Mohapatra et al., 2011; Spengler et al., 2001). BPA is considered as an “everywhere chemical” and is found even in the ice caps of the arctic and antarctic circles (Fu and Kawamura, 2010). Concentrations of BPA up to 2550 ng/L have been reported in US groundwater (Barnes et al., 2008). Chapin et al. (2008) reported no data on human developmental exposure to BPA, but studies with rats suggest that doses of 0.01-0.2 mg/kg/day can cause neural and behavioral changes related to normal sex differences in male and female rats. NOAEL values with respect to reproductive toxicity of BPA on sub chronic or chronic (long term) exposures have been calculated to be 47.5 mg/kg/day in female and 4.75 mg/kg/day in male rats (Chapin et al., 2008).

## **Nonylphenol**

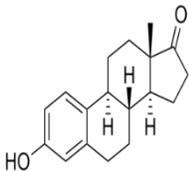
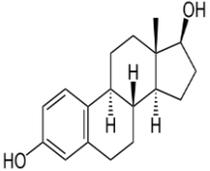
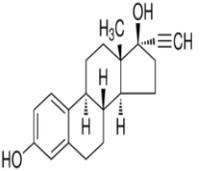
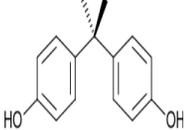
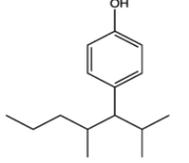
Nonylphenol (NP), 4-(2,4-dimethylheptan-3-yl)phenol, is an anaerobic breakdown product of the nonionic surfactant nonylphenol ethoxylate (NPEO). The surfactant is widely used in domestic liquid laundry detergents, industrial liquid soaps and cleaners, cosmetics, paints, pesticides and herbicides (Alkylphenols and Ethoxylates Research Council, 2001). Nonylphenol occurs in surface water and wastewater samples worldwide in concentrations up to 1 µg/L (Blackburn and Waldock, 1995; Ahel et al., 1996; Rudel et al., 1998; Snyder et al., 1999; Potter et al., 1999; and Kuch and Ballschmiter, 2001; Soares et al., 2008). Air concentrations of NP range from 2.2 to 70

ng/m<sup>3</sup> (Dachs et al., 1999; and Van Ry et al., 2000) suggesting that volatilization of NP should be considered when characterizing risk. However, the amount volatilized is very small (vapor pressure of NP is  $\sim 10^{-4}$  atm). Nonylphenol has been implicated as an endocrine disruptor in higher animals (Soto et al., 1991; and Gimeno et al., 1997) and mimics the natural hormone 17 $\beta$ -estradiol by competing for the binding site of the estrogen receptor (Lee and Lee, 1996; White et al., 1994; Ying et al., 2002b).

Table 1-4. Properties of the five EDCs studied.

Chemical→	Estrone	17 β-estradiol	17 α-ethynyl-estradiol	Bisphenol-A	Nonylphenol
Properties ↓					
Chemical Formula	C <sub>18</sub> H <sub>22</sub> O <sub>2</sub>	C <sub>18</sub> H <sub>24</sub> O <sub>2</sub>	C <sub>20</sub> H <sub>24</sub> O <sub>2</sub>	C <sub>15</sub> H <sub>16</sub> O <sub>2</sub>	C <sub>15</sub> H <sub>24</sub> O
CAS Number	53-16-7	50-28-2	77538-56-8	80-05-7	25154-52-3
Molecular Weight (g/mol)	270.37	272.38	296.41	228.29	220.35
Solubility (mg/L) in water (20°C)	30	13	4.8	120-270	5
log Kow	3.6-4.03	3.94	3.67-4.15	3.32	5.76
pKa	10.77	10.71	10.4	10.3	10.7
Half-life in aerobic water (days)	0.1-1.8	0.5-2	17	<2	2.5-10
Photolytic Half-life (clear water) (hours)	0.67	2-8	2-8	5	15

Table 1-4. Continued.

Chemical→ Properties ↓	Estrone	17 β estradiol	17α ethynyl- estradiol	Bisphenol-A	Nonylphenol
Estrogenic activity w.r.t E2	2.5	E2=100	246	0.66	0.32
Potential toxicity	Probable human carcinogen; possible teratogen.	Known human carcinogen (CSF <sub>0</sub> =39), human reproductive system disruptor.	Human carcinogen, human reproductive system disruptor.	May increase cancer risk, possible adverse effects on human reproductive system.	Possible human reproductive disruptor, possible human neuro-disruptor.
Major Routes of Exposure	Ingestion, dermal absorption.	Ingestion, dermal absorption	Ingestion, dermal absorption.	Ingestion, dermal absorption.	Ingestion, Inhalation, Dermal absorption.
Structure					

CSF<sub>0</sub>= Cancer Slope Factor for oral dose (mg/kg-day)<sup>-1</sup>.

(Source: The National Library of Science; Hazardous Substance Database).

(Estrogenic activities: Balaguer et al. 1999; Pillon et al., 2005).

## Toxicological Data on the Five EDCs

Oral acute median effective concentrations ( $EC_{50 \text{ rats}}$ ) for the adverse endocrine effects of the five EDCs range from around 500  $\mu\text{g}/\text{kg}$  (Estrogens) to around 2  $\text{mg}/\text{kg}$  (BPA and NP) in rats (WHO, 2011; National Library of Science, Hazardous Substance Data Base; Spehar et al., 2010). Assuming a safety factor of 1000, the  $EC_{50}$  values for human toxicity, become 500  $\text{ng}/\text{kg}$  for estrogens and 2  $\mu\text{g}/\text{kg}$  for BPA and NP. The  $CSF_0$  for oral dose of E2 is 39/ $\text{mg}/\text{kg}\text{-day}$  (USEPA, 2009). Bisphenol-A does not exhibit significant carcinogenicity (USEPA, 2010).  $CSFs$  and other carcinogenicity data on the remaining three EDCs are not available.

Estimated NOAEL/LOAEL values, for humans, of the five EDCs, and uncertainty factors (UF) are given in Table 1-5.

Table 1-5. Human NOAEL/LOAEL (oral dose) values of EDCs

Chemical	Reference	NOAEL (Endocrine)* ( $\mu\text{g}/\text{kg}/\text{day}$ )	LOAEL (Endocrine)* ( $\mu\text{g}/\text{kg}/\text{day}$ )
Estrone	Mashchak et al., 1982	4	-----
17 $\beta$ -estradiol	JEFCA, 2000	5	-----
17 $\alpha$ - ethynylestradiol	Mashchak et al., 1982	-----	0.1
Bisphenol A	Tyl et al., 2002	50**	-----
Nonylphenol	Tyl et al., 2006	50**	-----

\* NOAELs/LOAELs for other toxicities are higher than the values given.

\*\* Extrapolated from rats by using UF of 100 for BPA and 30 for NP (Synder et al., 2008).

## Research Approach

Research was carried out in South Florida during the summer of 2012. Research consisted of:

Firstly, determining the occurrence and concentrations of the five EDCs in representative WWTP effluents in south Florida.

Secondly, determining the occurrence and concentrations of the five EDCs in several residential surface water retention ponds in south Florida via water sampling surveys. Comparisons between occurrence of EDCs in water retention ponds in neighborhoods using reclaimed water for lawn irrigation and in water retention ponds in the vicinity of neighborhoods having septic systems were also conducted.

Presence of  $5\beta$ -Coprostanol (used as an indicator of human contamination in RW and pond water samples) (Mudge and Lintern, 1999; Reeves and Patton, 2005) was also determined in the RW and pond samples to determine human contamination as the likely source of EDCs.

Thirdly, irrigating St. Augustine turf grass (*Stenotaphrum secundatum* (International Code of Nomenclature for algae, fungi and plants)) with RW spiked with EDCs and relating dislodgement of the five EDCs from the turf grass to total concentrations of EDCs present on the leaf surface.

Fourthly, Assessing Risk: The risk quotient method and comparison of exposure content to NOAELs/LOAELs/EC50 of the EDCs were used to determine the risk of human exposure to EDCs present in RW. NOAELs, LOAELs and other data available on toxicity of EDCs along with concentrations dislodged from turfgrass, routes of exposure, pathways of exposure (from RW to turfgrass to skin and/or from RW to

turfgrass to mouth via licking and/or from RW to turfgrass to air to nose) were used to calculate risk. NOAELs/LOAELs and RQchronic were used to calculate long term (chronic) risk, whereas EC50 (extrapolated from rats) and RQacute were used to calculate acute risk. Dermal EC50 data for the EDCs are not available, so EC50 values for oral dose were used assuming dermal EC50 values would be equal to or less than oral values. Risk to highly exposed individuals (HEI) was estimated. Children, because of their small body mass and nature of playing, rolling and/or even licking the irrigated grass, were considered HEIs for risk assessment. Total exposure was calculated using the USEPA Exposure Factors Handbook (USEPA, 2011) as a guide to calculate amount, duration and frequency of exposure. Risk from direct drinking of secondarily treated RW as well as water from residential ponds was evaluated as a worst case scenario of EDC exposure to humans.

## CHAPTER 2 MATERIALS AND METHODS

Estrone, 17 $\beta$ -estradiol, 17 $\alpha$ - ethynylestradiol, bisphenol A, nonylphenol, 5  $\beta$ -coprostan-3 $\beta$ -ol, 17 $\beta$ -estradiol-acetate, Sucralose, Bis-(trimethylsilyl)-trifluoroacetamide and trimethylchlorosilane (BSTFA+TMCS) and Pyridine were purchased from Sigma-Aldrich Inc. (St. Louis, MO). Preliminary experiments for compound extraction and analysis were conducted. Ten replicates were performed using a spiking mixture of estrone, 17 $\beta$ -estradiol, 17 $\alpha$ - ethynylestradiol, bisphenol A, nonylphenol and 5  $\beta$ -coprostan-3 $\beta$ -ol, in acetonitrile, at 1  $\mu$ g/mL concentration each. For each replicate, an aliquot (100  $\mu$ L) of the spiking mixture was added to 1 liter of double deionized water so that the final concentration of each chemical in water was 100 ng/L. An aliquot (100  $\mu$ L) of a standard 1  $\mu$ g/mL 17 $\beta$ -estradiol-acetate solution was also added to serve as an analytical surrogate. Percent recovery, method detection limit (MDL), and practical quantitation limit (PQL) were then calculated from the ten replicates after chemical extraction from the double deionized water using methylene chloride.

### **Sites and Site Conditions**

**WWTPs:** Effluents were collected from six WWTPs that produce and supply RW in South Florida. All WWTPs use primary and secondary treatment processes before releasing effluents to be used as RW. In these WWTPs, RW is produced either daily (4 WWTPs) or twice a week (2 WWTPs) and production ranges from 400,000 liters to 11 million liters per production day. Effluents are chlorinated with 5-15 mg of sodium or calcium hypochlorite per liter.

**Retention ponds:** Eight retention ponds were chosen in South Florida. Ponds 1, 4 and 5 are in communities that use reclaimed water to irrigate lawns. Irrigation usually occurs 2-3 times a week depending upon observed grass requirements and rainfall received. Grass mowing occurs weekly. Pond cleaning did not occur during the survey period, except once for pond 5. For operational and sampling purposes, ponds 1, 4 and 5 were categorized as relatively low (<5 houses around the pond), medium (5-15 houses), and high (>15 houses), respectively, in housing density. Ponds 2 and 3 are surface water retention ponds in the vicinity of public schools using reclaimed water for grass irrigation. The ponds are larger and accumulate greater run-off loads than ponds 1, 4 and 5 because of more irrigation and irrigation frequency. Ponds 6, 7 and 8 are in neighborhoods that do not use reclaimed water, but are serviced by septic systems, which are expected to affect retention pond water quality via leaching of EDCs. Ponds 6, 7 and 8 are relatively low, medium and high in housing density, respectively. Run-off from various surfaces like sidewalks etc. is also collected in these ponds along with run-off from nearby turf.

**Turf plots:** St. Augustine grass (*Stenotaphrum secundatum* (International Code of Nomenclature for algae, fungi and plants)) plots were located at the UF Indian River Research and Education Center at Fort Pierce, FL. The plots were mowed 2 days before treatments began (clippings removed) to an average height of 3 cm. The experimental area was an aesthetic lawn irrigated with groundwater. An average of less than one weed per 2.5 cm<sup>2</sup> and 20 weeds per 1 m<sup>2</sup> (from a total of fifty 2.5 cm<sup>2</sup> sections and ten 1 m<sup>2</sup> sections) were present in the experimental plot. Such low weed densities are indicative of typical St. Augustine grass lawns. Three sets of experiments were

performed to study dislodgeable residues of the EDCs from turf (described later). Heavy rain occurred the night before the first set of experiments. It was sunny throughout the time the experiment was conducted and the plot was directly exposed to sun. No stagnant water was detected, but the grass was moist prior to EDC application. For the second and third sets of experiment, weather was clear and sunny throughout, but the grass was moist prior to applications due to morning dew.

### **Water Samples**

One hundred and sixteen water samples (28 from WWTPs and 88 from ponds) were collected and analyzed for the five EDCs and coprostanol.

### **Sample Collection**

A total of 28 effluent samples (four from each of the six WWTPs and four additional samples from one randomly selected WWTP) were collected in two weeks between 10 am and 3 pm during December 2012. The four additional RW samples were used as matrix spike samples for quality assurance and quality control. One liter samples of RW were collected in one liter amber glass bottles from effluent discharge outlets with extreme caution to avoid contamination. Bottles were capped and put in ice immediately after sample collection. A total of 88 water samples from retention ponds (64 from the various ponds and 24 additional samples from one randomly selected pond) were collected in one liter amber glass bottles. Bottles were thoroughly cleaned with Nalgene L900 liquid detergent (obtained from Thermo Scientific Inc., Waltham, MA) and double deionized water, rinsed with acetone and dried prior to sample collection. The 24 additional samples were used as sample duplicate and matrix spike samples for quality assurance and quality control during each run. Samples were collected weekly

between 10 am and 3 pm for eight weeks during summer 2012. The sampler was a 3 meter long aluminum shaft, and was used to collect samples from up to 2.5 meter from the side of the ponds, 0.5 meter below the water surface. Each bottle was rinsed with the respective pond water prior to sampling, and the samples were capped and put in ice immediately after collection. Samples were extracted within 4-5 hours after collection.

### **Sample Extraction and Preparation**

Two extractions per water sample were done on automated shakers operated at 120 rpm. Sixty mL methylene chloride was used for each sample extraction. Each sample was shaken for 30 min and 20 min for 1st and 2nd extractions, respectively. Methylene chloride extracts were collected in glass flasks and the volume was reduced in a water bath adjusted at 68.8<sup>0</sup>C and then transferred to graduated glass tubes and completely dried in a Labconco rapidvap (Labconco Inc., Kansas City, Mo) vacuum evaporation system. Compounds in the dried samples were derivatized by addition of 50  $\mu$ L BSTFA+TMCS (99:1), plus 50  $\mu$ L pyridine as a stabilizer (Knapp, 1979). Following addition, extracts were covered with parafilm and derivatized for 40 min in the water bath at 68.8<sup>0</sup>C, after which samples were again dried using the nitrogen gas evaporation system. Hexane (200  $\mu$ L) was added to each sample, thoroughly mixed by shaking and then the extract was transferred to GC analysis vials. The same sample extraction and preparation method was applied for preliminary water experiments and the RW samples and retention pond water samples. However, the RW and retention pond water samples required an addition step of de-emulsification after extraction. De-emulsification involved passing the emulsified methylene chloride extract through screening filters (0.2 microns) using syringes, followed by addition of an additional 10

mL of methylene chloride to minimize or reduce chemical loss during filtering. Samples were analyzed for the five EDCs and 5 $\beta$ -Coprostanol.

### **Dislodged EDCs and EDCs on Grass Surface**

Preliminary experiments to study the amounts of the five EDCs dislodged from the turf grass were conducted by swiping turf grass from the residential lawns of water retention pond survey sites 1, 2, 4 and 5. Cotton facial puffs wetted with double deionized water were used to rigorously swipe the grass and the EDCs from puffs were extracted using 30 mL (two times) methylene chloride in an ultrasonic cleaner. Up to 20 ng of E1, 40 ng of EE2 and 100 ng of BPA were dislodged from turf grass.

### **Sample Collection**

Three sets of experiments were performed to study EDC dislodgement from turf. The experimental design for first set of experiments was a randomized block, consisting of 5 replicate plots of 2 meter by 1.2 meter each. Another 2 meter by 1.2 meter plot in the same area was used as a control. Two liters of double deionized water spiked with the five EDCs was used to simulate irrigation of the block. For ease of detection and to simulate a worst case scenario, the upper (higher) reported concentrations of estrone (80 ng/L), 17 $\beta$ -estradiol (64 ng/L) and 17 $\alpha$ - ethynylestradiol (42 ng/L) and environmentally relevant values (reported median concentrations) for bisphenol A (400 ng/L) and nonylphenol (400 ng/L) in reclaimed water were chosen as spiking concentrations for the spraying. Both hand swipe method and drag sled methods (USEPA, 1998) were used to collect samples for the first set of experiment. The hand swipe method involved use of circular polyvinyl pipes 12.7 cm in radius and 5 cm in height to mark the sampling area of 506 cm<sup>2</sup>. Kendall Excilon (Drain and I.V.) sponges (Covidien Inc., Mansfield, MA) were used as the swiping material. Prior to swiping, each

sponge was wetted with simulated sweat (70:30 v/v Phosphate Buffer: acetonitrile, phosphate buffer = 0.1M Sodium acid Phosphate). The hand swipe method provided a sampling area of  $\approx 506 \text{ cm}^2$ . Sampling was done in zig-zag pattern by exerting nearly equal pressure for 30 seconds per sampling area. In the drag sled method, sleds consisted of baking pans (19.05 cm\*19.05 cm each). Un-dyed cotton denim cloth was attached at the bottom of the pans, and was used for sampling. Sand bags (13.6 kilograms) were used as weight over each sled (approximating the weight of a 3-4 year child). Sleds were dragged in the irrigated replicates to cover an area of  $\sim 553 \text{ cm}^2$ . The sled was moved forward 10 cm, then back 10 cm and then forward 10 cm again. Reclaimed water was the sole source of irrigation and an irrigation depth of 2 cm was applied. The volume of water required to irrigate the plot area of 2 meter  $\times$  5 (replicates)  $\times$  1.2 meter per application was calculated to be 240 L. Since 1 L reclaimed water (RW) contained 80 ng of estrone, 240 L RW contained 19200 ng. Similarly, 240 L RW contained 15400 ng of 17 $\beta$ -estradiol, 10100 ng of 17 $\alpha$ -ethynylestradiol, and 96000 ng each of BPA and NP (Table 2-1).

Initial masses of EDCs (EDCs applied) present per sampling area of  $506 \text{ cm}^2$  for hand swipe and  $553 \text{ cm}^2$  for drag sled methods are given in Table 2-1.

Table 2-1. Masses of chemicals applied per sampling area.

Chemical	Mass per sampling area of $506 \text{ cm}^2$ (hand swipe)	Mass per sampling area of $553 \text{ cm}^2$ (drag sled)
E1	81 ng	89 ng
E2	65 ng	71 ng
EE2	43 ng	47 ng
BPA	405 ng	442 ng
NP	405 ng	442 ng

$$\text{Plot area} = 2 * 100 * 1.2 * 100 * 5 = 120000 \text{ cm}^2.$$

Two liters of double deionized water was spiked with 19200 ng estrone, 15400 ng 17 $\beta$ -estradiol, 10100 ng 17 $\alpha$ -ethynylestradiol, 96000 ng BPA and 96000 ng NP and sprayed uniformly over the entire plot with a compressed CO<sub>2</sub> backpack sprayer, adjusted to 207 kilopascal pressure and yielding an average sprayer flow rate of 25 mL/second (n=10). Application was done at a uniform height of 0.5 m above the ground. The control plot was sprayed with double deionized water. Spraying was done in the morning around 8 a.m.

Samplings were conducted before spraying, immediately after spraying, 4 hours after spraying, 8 hours after spraying and after rewetting the plot with double deionized water 8 hours after spraying. One sample per replicate per sampling was collected for each method in 250 mL glass bottles. Bottles were capped air tight immediately after sample collection and placed in ice before extraction.

Results using the hand swipe method were inconsistent and highly variable, possibly due to the inconsistent pressure with which the grass was swiped. Due to wide differences in results and inability to set a standard pressure, the hand swipe method was determined to be unsuitable for measuring amount of EDCs dislodged from turf grass, and results will not be discussed.

The remaining two sets of experiment were conducted using only the drag sled method with slight modifications to obtain more reliable results. The same plot size (2 X 1.2 meters) and number of replicates (5 + Control) were used. The same drag sled method was followed. However, the area sampled per sampling was increased to 844 cm<sup>2</sup> to collect more dislodged mass and to improve detection reliability. Experiments were conducted on two consecutive days. Actual RW (instead of double deionized

water) was spiked with EDCs and applied to simulate real irrigation conditions. A portion of RW (1 L) was analyzed for EDC concentrations and the concentrations were found to be less than 20 ng/L for steroidal hormones and less than 60 ng for BPA and NP. The spiked EDC concentrations were adjusted accordingly. The backpack sprayer was adjusted to yield maximum possible droplet size to better simulate lawn sprinkler droplet size. Water droplets from sprinklers, by virtue of their large size and high release pressure may displace prior water droplets present on the turf grass. This may result in decreased potential for EDC dislodgement as the EDC in the displaced droplets may be sorbed to soil or grass thatch. The average sprayer flow rate was calculated to be 48 mL/sec (n=10). Initial masses of the EDCs for sampled area of 844 cm<sup>2</sup> were calculated to be 135 ng for estrone, 108 ng for 17β-estradiol, 71 ng for 17α- ethynylestradiol, 675 ng for BPA and 675 ng for NP. Sucralose (142200 ng) was also added to the spiking mixture to obtain a mass of 1000 ng for sucralose per sampled area. Sucralose was added as a conservative tracer to check the method and its reliability. Sucralose has a half-life on the order of months and is relatively resistant to degradation (Soh et. al., 2011) and thus can be used to check the method as sucralose detection by GC-MS if the EDCs are not detected suggest discrepancies in the method.

Samples were collected before spraying, immediately after spraying and 2 and 4 hours after spraying. Additional samplings occurred following rewetting of grass with double deionized water. Samples were collected 2 hours after rewetting (6 hours after initial spraying) and 4 hours after rewetting (8 hours after initial spray). Subsequently, the plot was again rewetted with double deionized water 8 hours after the initial spraying, and a last set of samples was collected.

Along with dislodged residue samples, grass clippings were collected from each replicate before the initial spraying, immediately after initial spraying, and before the first and second rewettings. For each grass sampling, grass clippings (0.5-0.7 cm above the soil) were collected from 506 cm<sup>2</sup> area, which corresponded to thirty grams (30±1 g) of grass (area to weight calculations determined from more than 200 samples). Sample bottles were capped and placed in ice immediately after sample collection. Initial masses of EDCs applied to 506 cm<sup>2</sup> of sampling area were calculated to be 81 ng for E1, 65 ng for E2, 43 ng for EE2, 405 ng for BPA, 405 ng for NP and 600 ng for sucralose. Samples were stored in a refrigerator for 8-10 hours before extraction.

### **Sample Extraction and Preparation**

For dislodged residue samples, two extractions per sample were performed using methylene chloride. 150 mL and 30 mL methylene chloride were used per extraction for drag sled and hand swipe methods, respectively. Extractions were done in an ultrasonic cleaner (15-335-222, Fisher Scientific, Waltham, MA), 20 min for first extraction and 15 min for second extraction per sample. After extraction, the methylene chloride extracts were collected in glass flasks and the volume was reduced in a water bath adjusted at 68.8<sup>0</sup>C. The rest of the extract preparation procedure was similar to that used for the water sample preparation.

A different method was used to extract EDCs on the surface of grass clippings since methylene chloride, or any other organic solvent to extract EDCs, would have resulted in extraction of interfering compounds from the leaves (e.g. pigments, lipids, etc.).

Modification of a method developed by Iwata et al. (1977) was used to determine total available EDC residues on foliage. Dioctyl-sulfosuccinate sodium salt (96%) was

purchased from Sigma-Aldrich Inc. (St. Louis, MO), and 1 gram was dissolved in 49 mL of double deionized water to get a 1:50 dilution of salt (Iwata et. al., 1977). One L of double deionized water was added to 30 grams of grass clippings collected in glass jars. Thirty drops (~ 6 mL) of diluted dioctyl-sulfosuccinate sodium salt were added to the bottle. Bottle ingredients were rigorously mixed for an hour using a vortex mixer. Solid phase extraction (SPE) was used (instead of liquid liquid extraction) to extract sucralose from water along with the EDCs. Because of the greater solubility of sucralose in water than in organic solvents, liquid-liquid extraction was not suitable for sucralose extraction. Samples were then subjected to solid phase extraction using 500 mg/ 6 mL Enviro Clean ECDVB 156 extraction cartridges (UCT Inc., Bristol, PA). The ECDVB 156 cartridge is capable of extracting both hydrophilic and hydrophobic chemicals. Each extraction cartridge was placed over each port of a 12 port vacuum manifold. The vacuum pressure was set at 25 bars, and the system was made air tight. Cartridges were cleaned twice with 6 mL (each time) of acetone. Water from each glass bottle containing EDC extracts was then passed through the respective extraction cartridges. The system was allowed to run for 5 hours to remove all water from the extraction cartridges. Extraction cartridges retained the EDCs, whereas water passed through the vacuum and collected in a waste bottle attached to the vacuum. When the system was stopped, graduated concentration tubes were placed inside the manifold such that each extraction cartridge corresponded to a single concentration tube. Vacuum was run again. The extraction cartridges were washed three times with 6 mL (each time) of methylene chloride. The methylene chloride (containing the EDCs) was collected in concentration tubes until the SPE cartridges appeared to be completely dry.

Methylene chloride was then completely dried in a Labconco rapidvap vacuum evaporation system (Labconco Inc., Kansas City, Mo), derivatized and prepared for GC-MS analysis following the same method used for dislodged residues. Percent recovery, method detection limit (MDL), and practical quantitation limit (PQL) were calculated prior to the analysis of actual samples.

### **Sample Analysis**

All samples were analyzed using GC-MS, consisting of an Varian 3800 gas chromatograph connected to Varian 4000 ion trap mass spectrometer with an electron ionization (EI) source (Varian, USA). Ionization energy was 70 eV, electron multiplier voltage was 1.19 kV, and ionization temperature was 200 °C. The target compounds were separated on a capillary column Rxi-5MS (30 m × 0.25 mm, 0.25 µm film thickness) (Restek Co., Bellefonte, PA, USA). Helium was the carrier gas, and was maintained at a constant flow rate of 1 mL min<sup>-1</sup>. The temperatures of the trap and manifold were set at 150°C and 50°C, respectively. A sample volume of 1 µL was injected in splitless mode at an inlet temperature of 280 °C. The column oven temperature program was as follows: the initial temperature of 130 °C was set and then increased at a rate of 10 °C min<sup>-1</sup> up to 280 °C. The final temperature was held for 5 min. The temperature of the transfer line was 270 °C. The solvent delay was set to 5 min. The characteristic ion fragments of the analytes were selected for quantification and confirmed in selected ion monitoring mode. The GC-MS was checked for air-water in the system, calibration gas levels, trap frequencies, and was used for analysis after it passed the auto tune check. Retention times and monitored ion fragments (m/z) ratios of the five EDCs analyzed using GC-MS are shown in Figure 2-1.

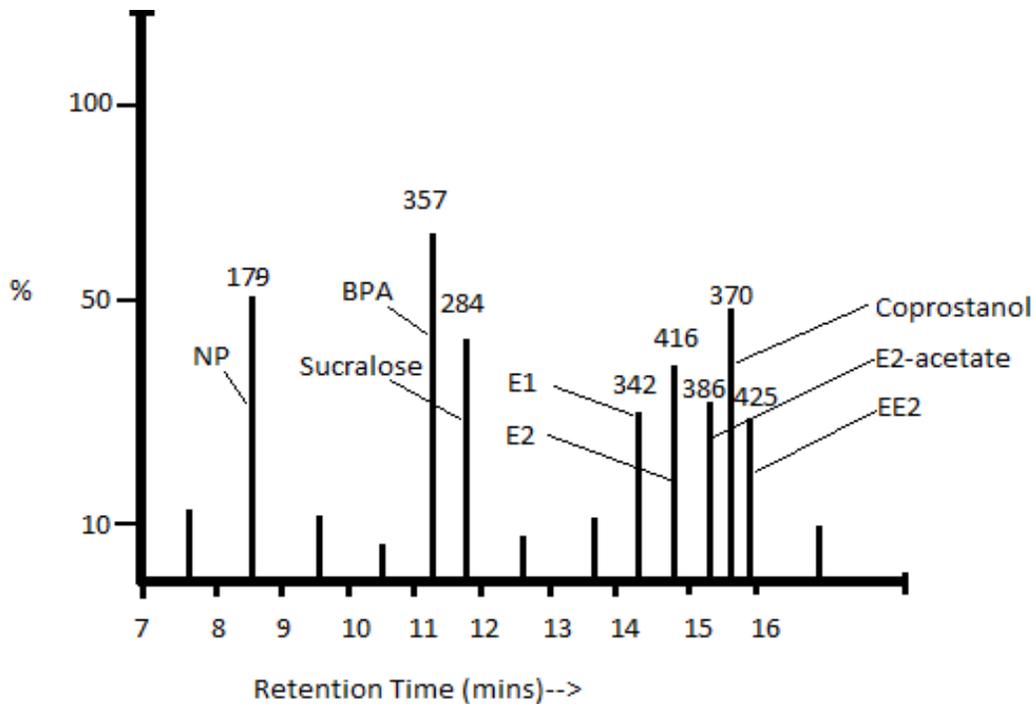


Figure 2-1. Retention times and m/z ratios of EDCs analyzed using GC-MS.

### Quality Assurance/Quality Control (QA/QC)

**Water samples:** For QA/QC purpose, 4 samples per sampling event were collected from one of the WWTPs and 4 from the retention pond site 2 (one sample, one sample duplicate, one matrix spike sample and one matrix spike sample duplicate). An aliquot (100  $\mu$ L) of the spiking mixture (1  $\mu$ g/ mL) was added to the matrix spike sample and the matrix spike sample duplicate. An aliquot (100  $\mu$ L) of stock solution of 17 $\beta$ -estradiol-acetate (1  $\mu$ g/mL) was added to each sample as an extraction surrogate to maintain extraction process quality as severe reduction or increase in surrogate recovery indicates something wrong with the extraction procedure. Method blanks were run along with the samples each time. Two additional 1 L double deionized water samples spiked with the spiking mixture (1  $\mu$ g/ mL) at concentrations of 45 ng/L and 100

ng/L were also run each time to check for contamination/problems in extraction or GC-MS analysis processes.

**Grass samples:** Samples were collected before application. A control plot was sampled along with each sampling of spiked plots. Method blanks and additional spiked samples (at masses of 45 ng and 100 ng) were run with each set of samples. An aliquot (100  $\mu$ L) of stock solution of 17 $\beta$ -estradiol-acetate (1  $\mu$ g/mL) was added to each sample as a surrogate to check the extraction process. Sucralose was added as a conservative tracer to check the method and its reliability.

#### **Method Detection Limit, Practical Quantitation Limit and Percent Recoveries**

Method detection limit (MDL), practical quantification limit (PQL) and percent recoveries of the five EDCs, E2-acetate, coprostanol and sucralose for water samples, dislodged residue samples and grass clippings are given in Table 2-2. MDLs and PQLs were calculated from the 10 replicates using EPA method (40 CFR 136, 2013).

Reduced recoveries of EDCs from spiked water samples, as compared to those from double deionized water, may have been due to matrix hindrances against extraction such as sorption of spikes onto colloidal organic matter, chemical reactions between spiking mixture and RW and the extra filtration of the samples to break emulsions when in RW.  $R^2$  values for the calibration curves, for all samples, were greater than 0.99.

Table 2-2. MDL, PQL and percent recoveries of the EDCs, E2-acetate, coprostanol and sucralose from different methods used.

Chemical ↓	Water Samples				Dislodged Residues			Grass Clippings		
	MDL (ng)	PQL (ng)	% recovery Double deionized water	% recovery Spiked sample	MDL (ng)	PQL (ng)	% recovery Denim (Methylene Chloride)	MDL (ng)	PQL (ng)	% recovery Surfactant
E1	5	20	85	63	5.6	21.8	73	6.7	28.7	81
E2	7.3	29	96	70	7.5	30.2	71	5.8	30.1	79
EE2	7.3	29	98	72	7.7	29.8	75	5.4	29.3	83.5
BPA	7.7	31	98	74	7.2	26.7	79	7.3	31.2	92
NP	4.9	19	95	69	6.3	22.5	80	7.9	31	88
E2-acetate	7.4	30	97	67	7.4	30.5	67	7.8	25.7	83
Coprostanol	5.7	23	87	66	-----	-----	-----	-----	-----	-----
Sucralose	-----	-----	-----	-----	5.8	23.8	82	5	21.9	94

## CHAPTER 3 RESULTS AND DISCUSSION

### EDCs in Water Samples

**Detection and concentrations in RW and ponds:** All five EDCs were detected in RW and ponds at various times during the survey. BPA was detected in greater amounts than the other EDCs (Table 3-1, Figure 3-1.) and with the greatest frequencies (> 96% for RW and 76% for all ponds, Figures 3-2 and 3-3.). Concentrations of all EDCs and coprostanol (not corrected for recovery percentages) were significantly lower (t-test,  $\alpha= 0.05$ ) in ponds near RW use than in reclaimed water samples indicating attenuation (degradation, dilution or adsorption) during drainage and/or runoff of RW (Figure 3-1).

Results suggest that the irrigation of turf grass with RW can play an important role in the transport and accumulation of EDCs in surface water ponds. The concentrations of EDCs were statistically similar (ANOVA,  $\alpha= 0.05$ ) in the water retention ponds 1, 4 and 5 suggesting housing density does not play a major role in the occurrence of EDCs in the retention ponds. Concentrations of EE2, BPA and coprostanol were significantly higher (t-test,  $\alpha= 0.05$ ) in retention ponds near homes with septic systems than near homes using RW for turf irrigation (Figure 3-1, Table 3-1) and were comparable to those in RW, consistent with less removal of the chemicals in septic systems than in WWTPs, or contributions from other (unidentified) sources.

BPA concentrations were more variable than other EDCs in all water types (i.e., RW, ponds near RW use and ponds near septic systems). Higher variability in BPA concentrations is in agreement with literature on the occurrence and fate of BPA (Belfroid et al., 2002), and may be attributed to variable initial concentrations in wastewater due to variable use in households.

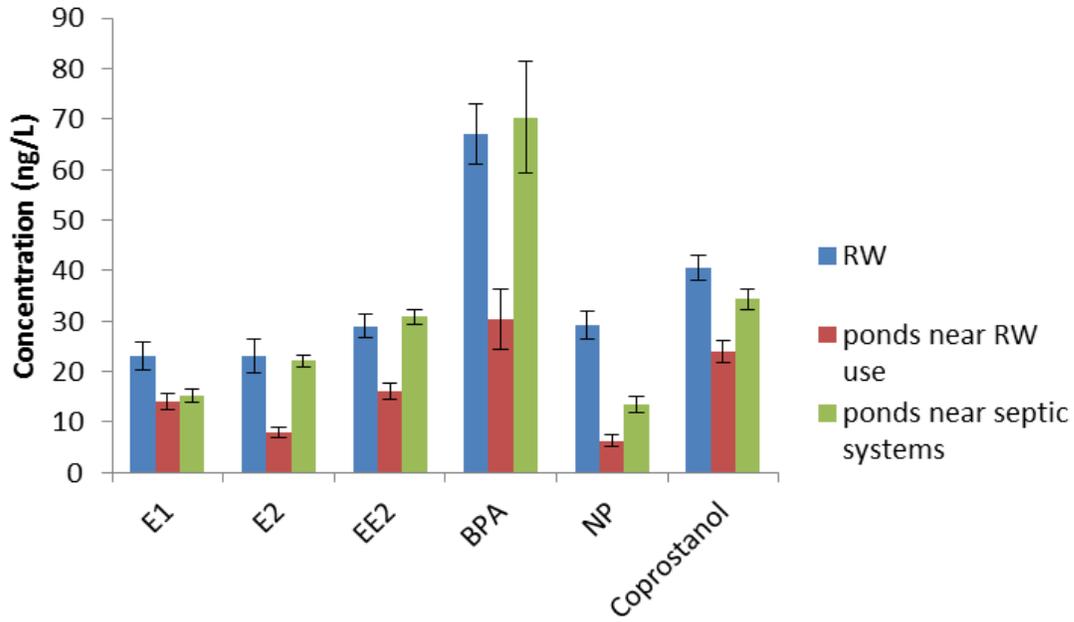


Figure 3-1. Concentrations of EDCs and coprostanol in RW (n=28), ponds near RW use (n=64) and ponds near septic systems (n=24).

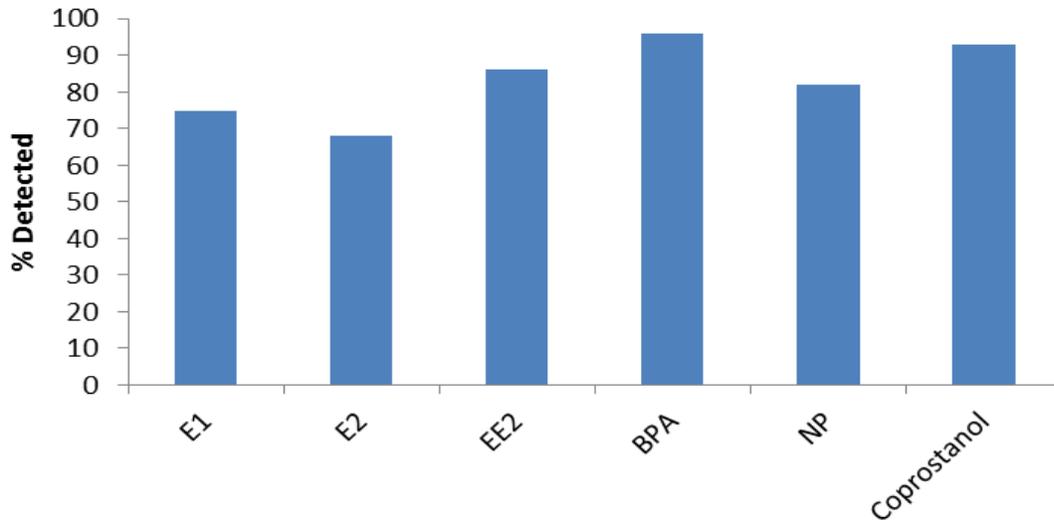


Figure 3-2. Frequency of detection of EDCs and coprostanol in RW (n=28).

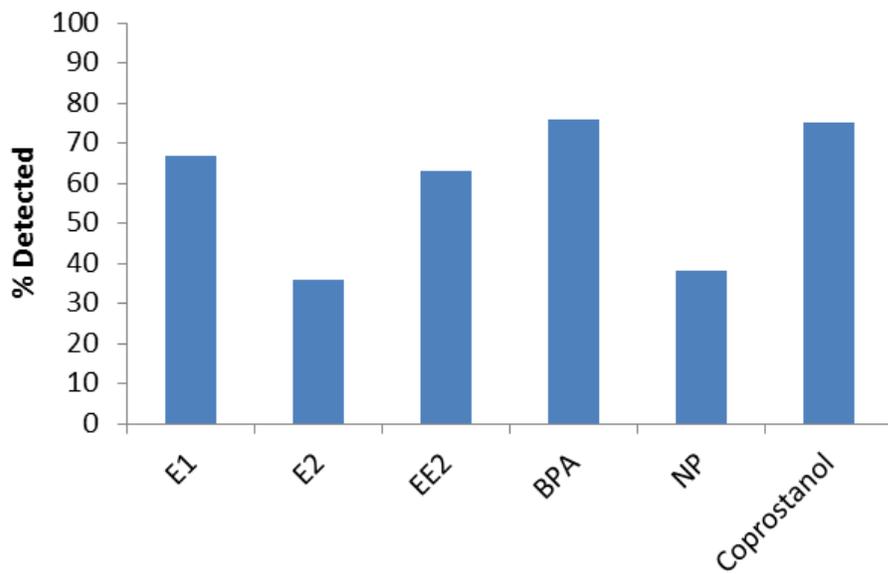


Figure 3-3. Frequency of Detection of EDCs and coprostanol in all ponds (n=88).

Table 3-1. Concentrations ( $\pm$ SD) of EDCs in RW (n=28), ponds near RW use (64), and ponds near septic systems (n=24).

Chemical ↓	Avg. conc. in RW (ng/L) $\pm$ SD	Highest conc. in RW (ng/L)	Avg. conc. in Ponds near RW use (ng/L) $\pm$ SD	Avg. conc. in Ponds near septic systems (ng/L) $\pm$ SD	Highest conc. in Ponds (all)
E1	23 $\pm$ 15	47	14* $\pm$ 10	15* $\pm$ 10	35
E2	23* $\pm$ 18	51	8* $\pm$ 7	22* $\pm$ 14	34
EE2	28* $\pm$ 14	49	16* $\pm$ 13	33 $\pm$ 15	58
BPA	67 $\pm$ 31	138	31 $\pm$ 93	73 $\pm$ 98	303
NP	29 $\pm$ 15	52	7* $\pm$ 14	14* $\pm$ 18	46

\*= concentration more than MDL but less than PQL. SD= Standard Deviation.

Table 3-2. Median concentrations of EDCs obtained from the study of RW and concentrations reported in literature.

Chemical	Median concentration found (ng/L)	Number of Samples	Median concentration reported (ng/L)
E1	26	104	<10 (Ying et al., 2002a)
E2	26*	185	<14 (Ying et al., 2002a)
EE2	31*	104	<10 (Ying et al., 2002a)
BPA	57	104	350-400 (Mohapatra et al., 2011; Spengler et al., 2001)
NP	31	50	400-500 (Ying et al., 2002b)

\*= Median more than MDL but less than PQL

## **Comparison to Published Data**

All EDC concentrations were in ng/L. The median concentration of E1 was higher than reported in literature. Median concentrations of E2 and EE2 were similar to literature values, whereas those of BPA and NP were less than the published data (Table 3-2). Also, the ranges (Tables 1-1, 1-2 and 3-1) were similar to peer reported concentration ranges. The concentrations of EDCs obtained in this study are, thus, in general agreement with, and are representative of, previously published data.

## **Likely Sources**

5 $\beta$ -Coprostanol was detected in more than 90% of the RW and 75% of all residential pond samples, suggesting human contamination in both RW and water retention ponds (Mudge and Lintern, 1999; Reeves and Patton, 2005). Human wastes in wastewater collected from houses and treated in WWTPs are the most likely source of EDCs in RW as WWTPs cannot fully remove such organic chemicals (Roberts and Thomas, 2006). Run-off and drainage of contaminated water from RW irrigated turf to nearby retention ponds likely contributes to detection of EDCs in retention ponds. Similarly, human wastes directed to septic systems are the likely sources of EDCs in retention ponds in the vicinity of neighborhoods using septic systems. Wastes from septic systems contain EDCs (Carrara et al., 2008), which could leach from the septic drainage fields consisting of high permeability sandy soils. Impermeable spodic and argilic horizons as well as bed rock materials below sandy surface layers could enhance lateral movement of the EDCs to retention ponds.

## **Possible Fate(s) of EDCs**

**Adsorption:** EDCs from RW strongly adsorb on organic components of soils (when sprayed on land), on grasses (when irrigated), and on sediments (when

discharged to surface water). EDCs in ponds may be bio-accumulated by fishes and adsorbed on sediments.

**Degradation:** Half-lives of the five EDCs, especially photolytic half-lives, are on the order of a few hours (Table 1-4), suggesting the possibility of rapid degradation. Frequent use of RW for turf irrigation and its run-off/ drainage, however, results in pseudo-persistence (maintenance of concentrations of otherwise quickly and/or easily degradable chemicals due to continuous release) of EDCs in surface water retention ponds. Similarly, the continuous leaching of EDCs from septic tanks could maintain chemical presence in nearby retention ponds.

**Contamination of groundwater:** Water containing EDCs, especially from retention ponds, may leach to the groundwater and/or contaminate the drinking water supply. The expected large dilution of leachate by uncontaminated groundwater, however, likely reduces EDC concentrations below detection limits.

**Human exposure:** Humans can be exposed to EDCs present in RW either directly (e.g., voluntary/involuntary consumption, swimming/bathing etc.) or indirectly (e.g., eating contaminated fish grown in ponds, etc.).

Results confirm that EDCs are present in RW as well as residential surface water retention ponds and that attention to their fates and human risk assessment is appropriate. By association, turf grass irrigated using RW is expected to come in contact with EDCs present in the water. Evaluation of the potential exposure to humans in contact with the grass following irrigation is warranted.

## EDCs in Dislodged Residue Samples and Grass Clippings

**Detection and masses dislodged via drag sled method:** Residues of all five EDCs were dislodged from the turf grass using the drag-sled method. Average masses (not corrected for percent recoveries) of EDCs dislodged from the three sets of experiments (set 1 extrapolated to 844 cm<sup>2</sup>) with time are shown in Table 3-3 and Figure 3-4 B. Dislodged masses of E1, E2 and EE2 were very small (few ng) immediately after application and were below detection limits 4 hours after irrigation. Dislodged masses of BPA and NP were significantly greater and disappeared more slowly and uniformly (Table 3-3.). Masses of all EDCs were, however, below MDLs 6 hours after irrigation. All EDC masses before spraying and from control plots were below MDLs.

The dislodged EDC masses decreased rapidly after spraying for E1, E2 and EE2, but changed more slowly for BPA, NP and sucralose with time (Figure 3-4 B). BPA masses (St. Dev. 43) showed greater variability among replicates than masses of the other EDCs. More uniform dislodgement of BPA, NP and sucralose with time may be attributed to their high initial masses, high water solubility of BPA (270 mg/L) and sucralose (283 g/L) and very low log Kow of -1.00 (USEPA, Estimation Program Interface Suite) of sucralose. Greater half-lives of sucralose (up to months) and NP (up to 10 days) likely also contributed to the more uniform dissipation of these EDCs as compared to E1, E2 and EE2.

The masses of EDCs dislodged via the drag-sled method from set 1 (extrapolated to 844 cm<sup>2</sup>), set 2, and set 3 were statistically similar (ANOVA,  $\alpha = 0.05$ ), suggesting reliability and consistency of the sampling method. No outliers were detected among the dislodged residue samples using Grubb's test ( $\alpha = 0.05$ ).

Rewetting of grass with double deionized water had no significant effect on the dislodgment of the EDCs (Table 3-3). Dislodged masses of sucralose increased after the first rewetting, but not after second rewetting. The increase in dislodged masses of sucralose after rewetting can be explained by its high water solubility (283 g/L) and suggests that sucralose dislodgement depends upon availability of water on the leaf surface. Sucralose was detected in relatively higher amounts than other EDCs in samples collected at various times confirming method reliability.

Table 3-3. EDC and sucralose masses (ng) dislodged, via drag-sled, with time.

Chemical →	E1	E2	EE2	BPA	NP	Sucralose
Before application	<5.6	<7.5	<7.7	<7.2	<6.3	<5.8
Immediately after application	21*±10	39±9	8*±12	230±43	303±13	638±21
2 hours after application	<5.6	12*±8	<7.7	79±7	192±17	391±21
4 hours after application	<5.6	<7.5	<7.7	53±5.5	91±12	130±27
After 1 <sup>st</sup> rewetting	<5.6	<7.5	<7.7	33±10	9*±15	149±20
6 hours after application	<5.6	<7.5	<7.7	<7.2	<6.3	45±5
8 hours after application	<5.6	<7.5	<7.7	<7.2	<6.3	<5.8
After 2 <sup>nd</sup> rewetting	<5.6	<7.5	<7.7	<7.2	<6.3	<5.8

\*= above MDL but below PQL.

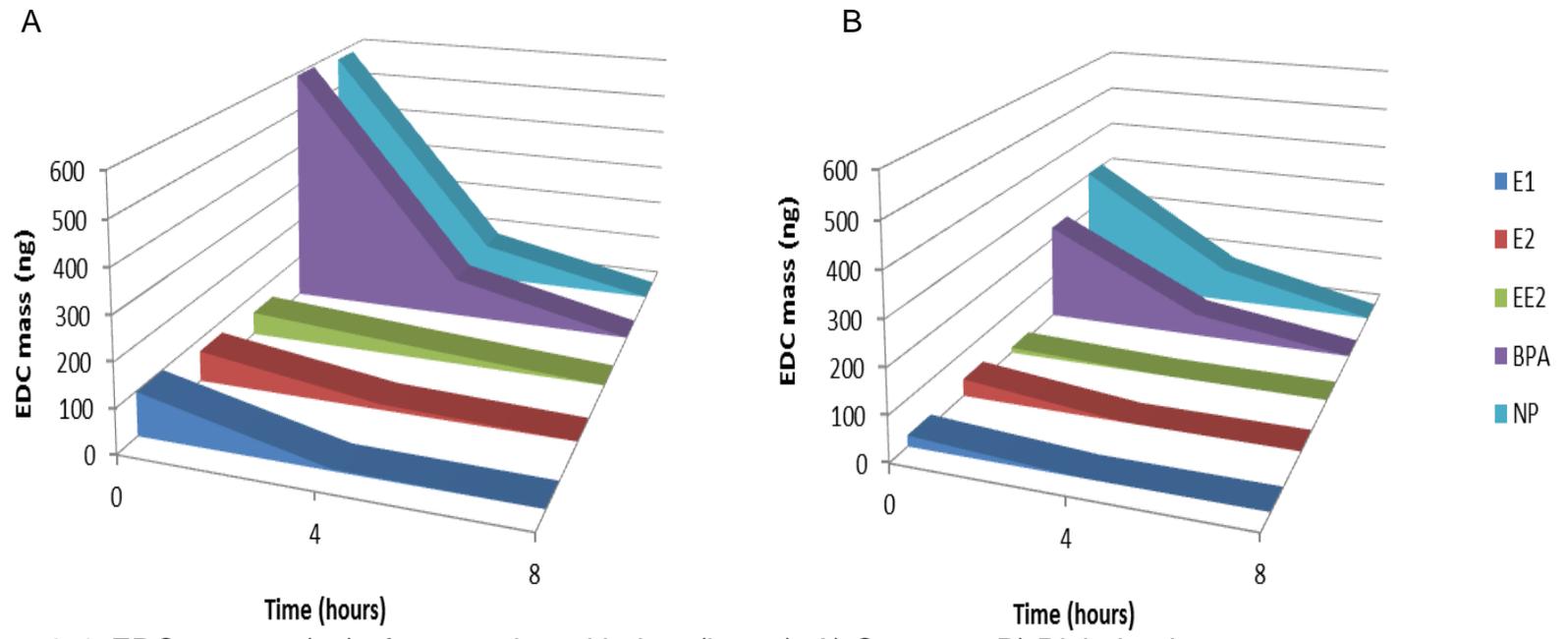


Figure 3-4. EDC masses (ng) after spraying with time (hours). A) On grass. B) Dislodged.

Masses of EDCs present on the grass surface also decreased with time (Figure 3-4 A) and followed similar trends as the masses dislodged (Figures 3-4 B). E1, E2 and EE2 were detected on grass (not dislodged) four hours after application, but at masses near MDLs. None of the five EDCs were detected on grass eight hours after application. Figure 3-5 shows percentages of EDC masses present on grass that dislodged at different times after application. The percentages of EDCs present on grass that actually dislodged immediately after application were 50% for E2, 69% for BPA, 51% for NP, and 80% for sucralose. Percentages are consistent with the respective masses applied, log Kow values of the EDCs, and leaf area considerations. More initial mass/concentration of the chemical and smaller log Kow values resulted in greater percentages of the chemicals present on grass that was dislodged. Percentages of E1 (26%) and EE2 (19%) dislodged immediately after irrigation were much lower than other EDCs, which suggesting less dislodgement than other EDCs. The percent of sucralose dislodged decreased substantially to 26% four hours after application. This sharp decrease suggests that dislodgement of sucralose depends on availability of water. The amount dislodged decreases significantly once the grass dries as sucralose adheres more strongly to the leaf surface.

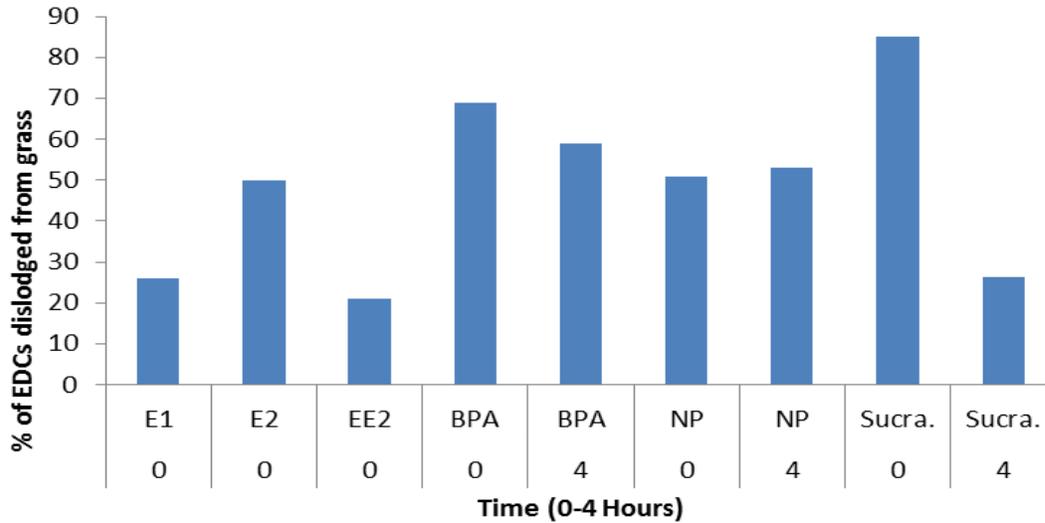


Figure 3-5. Percent of EDCs and sucralose dislodged from grass surface with time (hours).

### Human Health Risk Assessment

Risk was assessed using maximum EDC concentrations and masses (corrected for percent recoveries) obtained in the study.

#### Water Samples

**Worst case- contaminated water used for drinking:** To predict an extreme case of possible exposure, assume that the water containing highest concentrations of EDCs found (from RW and ponds combined) is used as drinking water. Drinking water equivalent levels (DWELs) for estrone, 17 $\beta$ - estradiol, 17 $\alpha$ -ethynylestradiol, bisphenol A and nonylphenol are given in Table 3-4 (USEPA, 2012b). Maximum chemical concentrations and the safety factors for non-carcinogenic adverse effects are also shown. Safety factors represent the ratio of mass of chemical consumed when chemical concentration is at DWEL to mass consumed when chemical concentration is

the maximum value found, and were calculated by dividing DWEL by maximum EDC concentration in water.

DWELs for carcinogens, assuming a 70 kg person consumes two liters of water daily for 30 years over a 70 year lifetime, can be estimated using the formula (Synder et al., 2008):

$$\text{DWEL } (\mu\text{g/L}) = (\text{acceptable risk} \times 70 \text{ kg} \times 70 \text{ yr} \times 1000 \mu\text{g/ mg}) / (\text{CSF } (/ \text{mg/kg-day}) \times 2 \text{ L/day} \times 30 \text{ yr}). \quad (3-1)$$

CSF for E2 is 39/mg/kg-day. Assuming an acceptable risk of one in 100,000 ( $10^{-5}$ ), DWEL for carcinogenic effects of E2 becomes=  $(10^{-5} \times 70 \times 70 \times 1000) / (39 \times 2 \times 30)$   
 $= 0.021 \mu\text{g/L}$ .

The highest concentration of E2 found in water samples was 0.073  $\mu\text{g/L}$ , approximately 3.5 times more than DWEL.

The data in Table 3-4 suggest that using water with such high EDCs concentrations for drinking purposes is not likely to cause non-carcinogenic adverse effects, except for 17 $\alpha$ -ethynylestradiol. The safety factors and carcinogenic dose (for E2) were generated using the highest obtained concentrations of EDCs. Further, DWELs are calculated for continuous consumption of a chemical in water, on daily basis, for 70 years (or 30 years for carcinogenic DWELs). Secondarily treated RW and/or retention pond water is not used for drinking and the incidence of even accidental drinking of the water is rare. Moreover, drinking water is more highly treated and concentrations of the EDCs are very low (e.g., <0.001  $\mu\text{g/L}$  for EE2) (Synder et al., 2008), suggesting minimal non-carcinogenic as well as carcinogenic (for E2) effects.

Table 3-4. DWELs and safety factor of EDCs when consumed in drinking water.

Chemical	DWEL (µg/L) (non-carcinogenic)	Max. conc. found (µg/L) (corrected for recovery)	Safety Factor
E1	0.46	0.075	6
E2	1.8 0.021 (carcinogenic)	0.073	25 0.3
EE2	0.0035	0.081	0.04
BPA	1800	0.409	4400
NP	1800	0.075	24000

### Dislodgeable Residues Exposure

The greatest masses of EDCs were dislodged immediately after application (Table 3-3). Maximum mass dislodged via drag-sled method was 33 ng for E1, 51 ng for E2, 25 ng for EE2, 296 ng for BPA and 323 ng for NP; from an area of 844 cm<sup>2</sup>. Masses corrected for percent recoveries become 45 ng for E1, 72 ng for E2, 33 ng for EE2, 370 ng for BPA and 403 ng for NP. Values extrapolated to a household turf grass area of 100 m<sup>2</sup>, become 53 µg for E1, 85 µg for E2, 39 µg for EE2, 438 µg for BPA and 478 µg for NP.

Assume a 3-year old child weighing 13.6 kg plays on contaminated turf (immediately after RW application) for an hour (USEPA, 2011) and drags on the contaminated grass an average of 20 times, covering an area of 2000 cm<sup>2</sup> each time. Further, assuming that the entire body of the child is exposed to the EDCs and all the EDCs are retained on the skin, the total dislodge able masses of EDCs the child contacts become 2133 ng of E1, 3412 ng of E2, 1564 ng of EE2, 17536 ng of BPA and 19100 ng of NP. Further, assume that only 5% of the adhering EDC masses are

absorbed into the child's body following 1 hour of exposure (Monteiro-Riviere et al., 2000; Demierre et al., 2012; Kao and Hall, 1987). Doses of the EDCs become 107 ng for E1, 171 ng for E2, 78 ng for EE2, 877 ng for BPA and 955 ng for NP. NP has an additional route of possible exposure- Inhalation. On an average, a 3 year old child inhales 10.1 m<sup>3</sup> of air/day (USEPA, 2011). Playing for an hour in the contaminated lawn, the child will inhale about 0.5 m<sup>3</sup> of air. Air concentrations of NP range from 2.2 to 70 ng/m<sup>3</sup> (Dachs et al., 1999; and Van Ry et al., 2000). Assuming a concentration of 70 ng/m<sup>3</sup> in the air surrounding the lawn, the child can inhale about 35 ng of NP through air. Thus, the total dose for NP becomes (955+35=) 990 ng.

The child may also ingest 100 mg/day (around 4 mg/ 1hour) of the soil and dust (USEPA, 2011). Child may ingest EDCs via licking contaminated grass and/or hands/ fingers. If 0.001% of the ingested material is EDC (as mass of EDCs is µg/ several hundred kg of turf, negligible EDC volatilization and there is no direct ingestion of grass or the soil below grass), 40 ng of each EDC will be ingested. The total dose of EDCs, thus, become 147 ng of E1, 211 ng of E2, 118 ng of EE2, 917 ng of BPA and 1030 ng of NP.

Assuming an EC<sub>50</sub> of 500 ng/kg (6800 ng/ 13.6 kg) for E1, E2 and EE2; and 2 µg/kg (27 µg/ 13.6 kg) for BPA and NP, RQs (Dose/ EC<sub>50</sub>) for acute endocrine related risks can be calculated (Table 3-5). RQs from chronic exposure (Table 3-5) were calculated using NOAEL/LOAEL (X 13.6 kg) values. Dose was multiplied by an additional factor of 10, where LOAEL data was used (Dose/NOAEL or Dose X10/LOAEL).

Table 3-5. RQs for the five EDCs: Acute and chronic toxicities.

Chemical →	E1	E2	EE2	BPA	NP
RQ <sub>acute</sub>	0.022	0.031	0.017	0.034	0.038
RQ <sub>chronic</sub>	0.003	0.003	0.87	0.0013	0.0015

RQ values for acute exposure are less than LOC of 0.5 and RQ values of chronic exposure are less than LOC of 1 for all five EDCs, suggesting minimal acute or chronic risk associated with the EDCs masses that dislodged in this study. NOAEL/LOAEL and EC<sub>50</sub> values (endocrine related toxicity) are based on oral dose; thus, the actual RQ values (from residues absorbed by skin) may be significantly less. Moreover the turf is irrigated two times (most cases) a week. For carcinogenic effect of E2, CSF<sub>0</sub> for E2 is 39/mg/kg-day and risk specific dose for a cancer risk of 1 in 100,000 is 0.26 ng/kg-day or 3.54 ng/ 13.6 kg-day. Considering that the lawn is irrigated twice every week, the per day E2 mass decreases to 36 ng and the dose becomes 2.7 ng/ 13.6 kg-day. Thus, the dose of E2 is less than risk specific dose for cancer development if the acceptable risk is 1 in 100,000. The risk associated with dislodgement of these EDCs is further reduced by the fact that it is not likely that the child plays in just irrigated (wet) turf. As the child ages, his/her body weight increases, soil/dust ingestion decreases and habits such as rolling /dragging on wet grass likely decrease; decreasing the masses of EDCs to which he/she is exposed.

The dislodged masses extrapolated to an area of 100 m<sup>2</sup> were used to calculate risk from an extreme case scenario (whole body of a 5 year old child is exposed to all the EDC mass available in 100 m<sup>2</sup> of contaminated turf just after irrigation). Based on very low concentrations of EDCs (few µg/100 m<sup>2</sup>), the child is likely to come in contact

with very small amounts of EDCs (assuming mass of 100m<sup>2</sup> grass+ upper 0.5 cm of soil (with bulk density of 1.33 g/cm<sup>3</sup>) to be 670 kg (mass of 0.5 cm of soil≈666 kg), percent of 100 µg of EDC, that a child can contact, would be less than 10<sup>-8</sup>% on mass basis).

If the child comes in contact with the entire 100 m<sup>2</sup> area and 1% of dislodged EDCs present in that area is retained on child's body, the total EDCs to which a 3 year old child could be dermally exposed become 530 ng of E1, 850 ng of E2, 390 ng of EE2, 4380 ng of BPA and 4780 ng of NP. If 5% of EDCs present on the child's body are actually absorbed by the skin, EDC doses become 27 ng for E1, 43 ng for E2, 20 ng for EE2, 219 ng for BPA and 239 ng for NP. If 40 ng of each EDC is ingested through licking grass or contaminated hands/fingers (assuming 4mg/hour of ingestion in contaminated area and 0.001% of the ingested material is EDC) and 35 ng of NP is inhaled, the total dose of EDCs would be 67 ng for E1, 83 ng for E2, 60 ng for EE2, 259 ng for BPA and 314 ng NP. These values are even lower than those calculated for exposure to dislodged residues and represent vanishingly small risk.

### **Assessment of Combined Risk (E2 Equivalent) from the five EDCs**

For calculations of human risk from combined exposure to the five EDCs, the concentrations and masses of EDCs were converted to E2 equivalents (Table 3-6) and summed. DWEL, NOAEL and EC<sub>50</sub> values for E2 were then used to calculate risk.

### **Water samples**

Maximum concentrations of EDCs in water (Table 3-4), when converted to E2 equivalents and summed, become 1724 ng. The DWEL for E2 is 1800 ng (Table 3-4). The concentration of E2 equivalents in water is less than DWEL, suggesting minimal

risk of combined exposure from the five EDCs from consumption of contaminated water. RW may contain numerous other EDCs, but their concentrations are likely to be similar to the ones studied. Moreover, EE2 is one of the most potent EDCs present in RW (Clayton 2011; Thorpe et al., 2003). The risk associated with the five EDCs was so small that it is unlikely that the risk from other EDCs would be considerable.

Table 3-6. Relative potencies of the five EDCs (Clayton, 2011; Thorpe et al., 2003).

EDCs	Potency
E1	0.4
E2	1
EE2	20
BPA	0.0002
NP	0.0000045

### Dislodged residues

Maximum masses of EDCs dislodged (Table 3-3) corrected for percent recoveries become 45 ng for E1, 72 ng for E2, 33 ng for EE2, 370 ng for BPA and 403 ng for NP. Converted to E2 equivalents and summed, the combined E2 equivalent mass become 750 ng. The total E2 equivalent exposure for a 3-year old child playing in recently sprayed grass (assuming the child drags in the contaminated turf 20 times, covering 2000 cm<sup>2</sup> each time) becomes 35.5 µg. Assuming that entire body of the child is directly exposed to the EDCs, the E2 equivalent exposure becomes is, thus, 35.5 µg. If 5% of the EDC, the child is exposed to, is absorbed by child's skin, the E2 equivalent dose becomes ~1800 ng. Adding 857 ng for EDCs ingested with soil and dust (40 ng for each EDC converted to E2 equivalent and summed), total dose would be ~2660 ng. The EC<sub>50</sub> for E2 is 500 ng/kg (6800 ng/13.6 kg) and NOAEL is 5000 µg/kg/day (68000 µg/13.6 kg/day). The RQ<sub>acute</sub> becomes (2660/6800=) 0.39 and RQ<sub>chronic</sub> becomes (2660/68000=) 0.039.

The RQ for acute toxicity is less than the LOC of 0.5 and RQ for chronic toxicity is less than LOC of 1, suggesting minimal risk ( on E2 equivalent basis) from combined masses of the five EDCs present in RW used for irrigating turf. Carcinogenic effects from long term exposure to these EDCs (when a person is exposed to several EDCs), however, may become a concern.

### **Risk Comparison to Published Data**

Concentrations used for risk assessment were towards the upper end of the concentration range for the EDCs reported in the literature. Results for risk assessment from human exposure to RW as well as dislodged residues of the EDCs, thus, represent a case where EDC concentrations are greater than reported median (Tables 1-1, 1-2 and 3-2) concentrations of the five EDCs. The risk associated with human exposure to EDCs at environmentally relevant concentrations should be minimal.

### **Uncertainty**

A person can be exposed to numerous EDCs from numerous sources, both indoors and outdoors, which may increase the risk of adverse effects. Also, the cumulative effect of various EDCs on humans is largely unknown. The combined effect of numerous EDCs may be more drastic (synergistic) than the effect from a single EDC. Ingestion (via food and/or drinking water), indoor and outdoor inhalation, and dermal absorption of hundreds of chemicals (some of which are more toxic and/or carcinogenic than studied) used in everyday life could significantly increase the risk. Further, risk assessment is a dynamic field and involves many assumptions and uncertainties. For instance, this study lacked solid data on carcinogenicity of the EDCs. Further, many data points (for EDCs in water samples as well as dislodged residue samples) were below PQLs, so the true concentrations of the EDCs for these data points were not

known. Uncertainty also includes the many assumptions made throughout. Thus, further research is needed about risks associated with the combined intake as well as carcinogenicity of EDCs from various sources and about the combined effects of various EDCs on human bodies.

### **Risk Communication/Management**

To account for various uncertainties, the risk assessment was conducted to represent a conservative approach using worst case scenarios and maximum concentrations/masses of EDCs measured. The results suggest minimal risk associated with exposure to the five EDCs at current environmental concentrations. Also, the metabolites (degradation products) of the five EDCs are less estrogenic and less toxic than the parent compounds (Larcher et al., 2012; Kang et al., 2006; Hao et al., 2008). It is, however, prudent to wait for turf to dry before allowing children to play in the area irrigated with RW.

## CHAPTER 4 SUMMARY AND CONCLUSIONS

Risk of human exposure to EDCs from rapidly increasing use of RW for turf irrigation is a possible concern. To address the issue, the occurrence of five EDCs (estrone, 17  $\beta$ -estradiol, 17  $\alpha$ -ethynylestradiol, bisphenol-A and nonylphenol) was determined in RW and residential surface water retention ponds in South Florida. The information was then used to conduct a human health risk assessment for dislodged residues of the EDCs from St. Augustine turf grass irrigated with RW.

All five EDCs were detected in RW and water retention ponds at concentrations as high as 47 ng/L for E1, 51 ng/L for E2, 58 ng/L for EE2, 303 ng/L for BPA and 52 ng/L for NP. Concentrations of all compounds were similar to, or less than, concentrations reported by others. Concentrations of E2, EE2, BPA and NP in retention ponds in neighborhoods likely impacted from RW use were significantly less than concentrations in ponds from areas serviced by septic systems. EDCs were detected in both RW and retention ponds, but at low ng/L levels (similar to published values), supporting the first hypothesis.

Maximum masses of EDCs were dislodged from 844 cm<sup>2</sup> of RW-irrigated turf immediately after application and were up to 33 ng for estrone, 51 ng for 17 $\beta$ -estradiol, 25 ng 17 $\alpha$  ethynylestradiol, 296 ng for bisphenol-A, and 323 ng for nonylphenol. Dislodgement decreased rapidly with time after spraying, and was undetectable 6 hours after spraying. Re-wetting the turf failed to release significant amounts of additional chemicals.

Human health risk assessments for the EDCs suggested minimal risk associated with exposure to the EDCs, supporting the second hypothesis.

Environmentally relevant concentrations of E1, E2, EE2, BPA and NP in RW or retention ponds represent minimal risk of adverse health effects on humans. Even a 10 fold increase in EDC masses dislodged after turf irrigation with RW results in minimal non-carcinogenic risk. Such a 10 fold increase in EDC concentration in RW (long term) is highly unlikely.

Due to a lack of data on human toxicity, carcinogenicity and other adverse effects from the EDCs, the risk assessment conducted involved many assumptions. But, the assumptions were very stringent and conservative uncertainty factors were applied to the toxicological data. Toxicological data used to characterize risk were based on oral dose of EDCs. The entire risk assessment approach was highly conservative and represented worst case scenarios. Nonetheless, a person can be exposed to numerous EDCs from numerous other sources and a wide range of uncertainty exists regarding cumulative effects of various EDCs. More research regarding potential adverse effects of EDCs, especially on toxicity and carcinogenicity in humans, is needed to better assess the link between EDC concentrations and associated risk. But the risk associated with the five EDCs was so small that the risk from other EDCs should be minimal. Nonetheless, concerned parents may wish to restrict children's access to recently RW-irrigated turf until the grass is dry.

## LIST OF REFERENCES

- Ahel, M., Schaffner, C., Giger, W., 1996. Behaviour of alkylphenol polyethoxylate surfactants in the aquatic environment-- III. Occurrence and elimination of their persistent metabolites during infiltration of river water to groundwater. *Water Res.* 30, 37-46.
- Alkylphenols and Ethoxylates Research Council (2001). Safety of APEs, Retrieved on July 31, 2001 from <http://www.aperc.org/docs/safetyofapes.html>.
- Balaguer, P., Francois, F., Comunale, F., Fenet, H., Boussioux, A.M., Pons, M., Nicolas, J.C., Casellas, C., 1999. Reporter cell lines to study the estrogenic effects of xenoestrogens. *Sci. Total Environ.* 233, 47–56
- Barnes, K.K., Kolpin, D.W., Furlong, E.T., Zaugg, S.D., Meyer, M.T., Barber, L.B., 2008. A national reconnaissance of pharmaceuticals and other organic wastewater contaminants in the United States-I. Groundwater. *Sci. Total Environ.* 402, 192-200.
- Baronti, C., Curini, R., D'ascenzo, G., Di Corcia, A., Gentili, A., Samperi, R., 2000. Monitoring natural and synthetic estrogens at activated sludge sewage treatment plants and in a receiving river water. *Environ. Sci. Technol.* 34, 5059–5066.
- Belfroid, A.C., Van der Horst, B., Vethaak, D., Schäfer, A.J., Rijs, G.B.J., Wegener, J., Cofino, W.P., 1999. Analysis and occurrence of estrogenic hormones and their glucuronides in surface water and waste water in the Netherlands. *Sci. Total Environ.* 225, 101-108.
- Belfroid, A.C., Van Velzen, M., Van der Horst, B., Vethaak, D., 2002. Occurrence of bisphenol A in surface water and uptake in fish: evaluation of field measurements. *Chemosphere.* 49, 97–103.
- Blackburn, M.A., Waldock, M.J., 1995. Concentrations of alkylphenols in rivers and estuaries in England and Wales. *Water Res.* 29, 1623-1629.
- Broseus, R., Vincent, S., Aboulfadl, K., Daneshvar, A., Sauve, S., Barbeau, B., Prevost, M., 2009. Ozone oxidation of pharmaceuticals, endocrine disruptors and pesticides during drinking water treatment. *Water Res.* 43, 4707-4717.
- Carlsen, E.A., Giwercman, A., Keiding, N., Skakkebaek, N.E., 1992. Evidence for decreasing quality of semen during last 50 years. *Bri. Med. J.* 305, 609–613.
- Carrara, C., Ptacel, C.J., Robertson, W. D., Blowes, D. W., Moncur, M. C., Sverko, E., Backus, S., 2008. Fate of pharmaceutical and trace organic compounds in three septic system plumes, Ontario, Canada. *Environ. Sci. Technol.* 42, 2805–2811.
- Chapin, R.E., Adams, J., Boekelheide, K., Gray, L.E., Hayward, S.W., Lees, P.S., McIntyre, B.S., Portier, K.M., Schnorr, T.M., Selevan, S.G., Vandenberg, J.G.,

- Woskie, S.R., 2008. NTP-CERHR expert panel report on the reproductive and developmental toxicity of bisphenol A. *Birth Defects Res. B. Dev. Reprod. Toxicol.* 83, 157-395.
- Clayton, R., 2011. *Endocrine disruptors in environment*. 3<sup>rd</sup> (ed.). Foundation for Water Research, Marlow, Buckinghamshire, United Kingdom, pp. 3-22.
- CFR (Code of Federal Regulations) Environmental Protection Agency (2013). 40 cfr part 136 appendix b revision 1.11.
- Dachs, J., Van Ry, D., Eisenreich, S., 1999. Occurrence of estrogenic nonylphenols in the urban and costal atmosphere of the lower Hudson River estuary. *Environ. Sci. Technol.* 33, 2676-2679.
- Daughton, C.G., Ternes, T.A., 1999. Pharmaceuticals and personal care products in the environment: agents of subtle change? *Environ. Health Perspect.* 107, 907–937.
- Deblonde, T., Cossu-Leguille, C., Hartemann, P., 2011. Emerging pollutants in wastewater: A review of the literature. *Int. J. Hyg. Environ. Health.* 214, 443-448.
- Demierre, A.L., Peter, R., Oberli, A., Bourqui-Pittet, M., 2012. Dermal penetration of bisphenol A in human skin contributes marginally to total exposure. *Toxicol Lett.* 213, 305-308
- Desbrow, C., Routledge, E.J., Brighty, G.C., Sumpter, J.P., Waldock, M., 1998. Identification of estrogenic chemicals in STW effluent: 1. Chemical fractionation and in vitro biological screening. *Environ. Sci. Technol.* 32, 1549–1558.
- Diamanti-Kandarakis, E., Bourguignon, J., Giudice, L.C., Hauser, R., Prins, G.S., Soto, A.M., Zoeller, R.T., Gore, A.C., 2009. Endocrine-disrupting chemicals: an endocrine society scientific statement. *Endocr. Rev.* 30, 293–342.
- Duran-Alvarez, J.C., Bravo, E.B., Castro, V.S., Jimenez, B., Gibson, R., 2009. The analysis of a group of acidic pharmaceuticals, carbamazepine, and potential endocrine disrupting compounds in wastewater irrigated soils by gas chromatography–mass spectrometry. *Talanta.* 78, 1159–1166.
- FLDEP, 2012. 2011 Reuse Inventory. Water Reuse Program, Florida Department of Environmental Protection, Tallahassee, FL. Available at [www.dep.state.fl.us/water/reuse/inventory.htm](http://www.dep.state.fl.us/water/reuse/inventory.htm) accessed on 5/22/2013.
- Fu, P., Kawamura, K., 2010. Ubiquity of bisphenol A in the atmosphere. *Env. Poll.* 158, 3138-3143.
- Gimeno, S., Komen, H., Venderbosch, P.W.M., Bowmer, T., 1997. Disruption of sexual differentiation in genetic male common carp (*cyprinus carpio*) exposed to an alkylphenol during different life stages. *Environ. Sci. Technol.* 31, 2884-2890.

- Gomez, M.J., Mezcuca, M., Martinez, M.J., Fernandez-Alba, A.R., Aguera, A., 2006. A new method for monitoring oestrogens, N-octylphenol, and bisphenol A in wastewater treatment plants by solid-phase extraction-gas chromatography-tandem mass spectrometry. *Int. J. Environ. Anal. Chem.* 86, 3-13.
- Gore, A.C., 2007. Endocrine-disrupting chemicals: From basic research to clinical practice. *Contemporary Endocrinology*. Humana Press Inc., New Jersey, pp. 9-31. ISBN 978-1-58829-830-0.
- Guillette, L.J., Gross, T.S., Masson, G.R., Matter, J.M., Percival, H.F., Woodward, A.R., 1994. Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida. *Environ. Health Persp.* 102, 680–688.
- Hao, R.X., Liang, P., Zhao, M., Zhou, Y.W., 2008. Recognition and analysis on primary metabolites of nonylphenol. *Huan Jing Ke Xue.* 29, 1077-1081
- Hotchkiss, A.K., Rider, C.V., Blystone, C.R., Wilson, V.S., Hartig, P.C., Ankley, G.T., Foster, P.M., Gray, C.L., Gray, L.E., 2008. Fifteen years after “Wingspread”– environmental endocrine disrupters and human and wildlife health: where we are today and where we need to go. *Toxicol. Sci.* 105, 235–259.
- Indian Institute of Technology Team, 2011. Review of wastewater reuse projects worldwide. Collation of selected International case studies and experiences. Indian Institute of Technology, India. Available at [http://gangapedia.iitk.ac.in/sites/default/files/Second Set of Report/012\\_EQP\\_Reuse.pdf](http://gangapedia.iitk.ac.in/sites/default/files/Second Set of Report/012_EQP_Reuse.pdf)
- Iwata, Y., Knaak, J.B., Spear, R.C., Foster, R.J., 1977. Worker reentry into pesticide-treated crops. I. Procedure for the determination of dislodgable pesticide residues on foliage. *Bull. Environ. Contam. Toxicol.* 18, 649-655.
- JECFA, 2000. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additive Series: 43. Prepared by the fifty-second meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), Geneva, Switzerland. Available at <http://www.inchem.org/documents/jecfa/jecmono/v43jec05.htm> accessed on 6/16/2013.
- Jobling, S., Nolan, M., Tyler, C. R., Brighty, G., Sumpter, J. P., 1998. Widespread sexual disruption in wild fish. *Environ. Sci. Technol.* 32, 2498–2506.
- Jobling, S., Casey, D., Rogers-Gray, T., Oehlmann, J., Schulte-Oehlmann. U., Pawlowski, S., Baunbeck, T., Turner, A.P., Tyler, C.R., 2004. Comparative responses of molluscs and fish to environmental estrogens and an estrogenic effluent. *Aquat. Toxicol.* 66, 207–222.
- Jørgensen, N., Andersen, A. G., Eustache, F., Irvine, D. S., Suominen, J., Petersen, J.H., Andersen, A. N., Auger, J., Cawood, E. H. H., Horte, A., Kold-Jensen, T.,

- Jouannet, P., Keiding, N. B., Vierula, M., Toppari, J., Skakkebaek, N., 2001. Regional differences in semen quality in Europe. *Human Reprod.* 16, 1012-1019.
- Kang, J.H., Katayama, Y., Kondo, F., 2006. Biodegradation or metabolism of bisphenol A: from microorganisms to mammals. *Toxicology.* 217, 81-90
- Kao, J., Hall, J., 1987. Skin absorption and cutaneous first pass metabolism of topical steroids: in vitro studies with mouse skin in organ culture. *J. Pharmacol. Experi. Therap.* 241, 482.
- Knapp D., 1979. *Handbook of Analytical Derivatization Reactions*, Wiley-Interscience, New Jersey, pp. 449-453. ISBN 0-471-03469-X.
- Kuch, H.M., Ballschmiter, K., 2001. Determination of endocrine-disrupting phenolic compounds and estrogens in surface and drinking water by HRGC-(NCI)-MS in the pictogram per liter range. *Environ. Sci. Technol.* 35, 3201-3206.
- Larcher, S., Delbès, G., Robaire, B., Yargeau, V., 2012. Degradation of 17 $\alpha$ -ethinylestradiol by ozonation--identification of the by-products and assessment of their estrogenicity and toxicity. *Environ Int.* 39, 66-72.
- Lee, P.C., Lee, W., 1996. In vivo estrogenic action of nonylphenol in immature female rats. *Bull. Environ. Contam. Toxicol.* 57, 341-348.
- Lewis, R.J., 1991. *Carcinogenically active chemicals: A reference guide*. John Wiley & Sons, New York, pp. 133.
- Liu, Z.H., Ito, M., Kanjo, Y., Yamamoto, A., 2009. Profile and removal of endocrine disrupting chemicals by using an ER/AR competitive ligand binding assay and chemical analyses. *J. Environ. Sci. (China).* 21, 900-906.
- Lukachko, A., 1999. *Endocrine disrupters: A scientific perspective. A report prepared by the American Council on Science & Health, New York.* Available at <http://www.acsh.org/publications/booklets/enddis.html> accessed on 6/16/2013.
- Mashchak, C.A., Lobo, R.A., Dozono-Takano, R., Eggena, P., Nakamura, R.M., Brenner, P.F., Mishell, D.R. Jr., 1982. Comparison of pharmacodynamic properties of various estrogen formulations. *Am. J. Obstet. Gynecol.* 144, 511-518.
- McCally, M., 1997. Five years down the road from Rio. *Brit. Med. J.* 315, 3-5.
- Mohapatra, D.P., Brar, S.K., Tyagi, R.D., Surampalli, R.Y., 2011. Occurrence of bisphenol A in wastewater and wastewater sludge of CUQ treatment plant. *J. Xenobiotics.* 1, 9-16.
- Monteiro-Riviere, N.A., Van Miller, J.P., Simon, G., Joiner, R.L., Brooks, J.D., Riviere, J.E., 2000. Comparative in vitro percutaneous absorption of nonylphenol and

- nonylphenol ethoxylates (NPE-4 and NPE-9) through human, porcine and rat skin. *Toxicol. Ind. Health.* 6, 49-57.
- Mudge, S.M., Lintern, D.G., 1999. Comparison of sterol biomarkers for sewage with other measures in Victoria Harbour, B.C. Canada. *Estuarine, Coastal and Shelf Sciences.* 48, 27-38.
- Naz, R.K., 2005. Endocrine disruptors: effects on male and female reproductive systems. CRC Press, Boca Raton, Florida, pp. 21-35. ISBN 0-8493-2281-2.
- NTP, 2001. The National Toxicology Program's Report of the Endocrine Disruptors Low Dose Peer Review. Available at <http://ntp.niehs.nih.gov/>.
- Nasu, M., Goto, M., Kato, H., Oshima, Y., Tanaka, H., 2001. Study on endocrine disrupting chemicals in wastewater treatment plants. *Water Sci. Technol.* 43, 101-108.
- NIEHS, 2011. National Institute of Environmental Health Sciences, Child Development and Environmental Toxins. Available at [http://www.niehs.nih.gov/health/assets/docs\\_a\\_e/child\\_development\\_and\\_environmental\\_toxins\\_508.pdf](http://www.niehs.nih.gov/health/assets/docs_a_e/child_development_and_environmental_toxins_508.pdf). Accessed on 6/16/2013
- National Library of Science, Hazardous Substance Database. <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB> accessed on 6/16/2013.
- NRC, 2006. Fluoride in Drinking Water: A Scientific Review of EPA's Standards. National Research Council -Committee on Fluoride in Drinking Water. The National Academies Press, Washington, D.C. Available at <http://www.actionpa.org/fluoride/nrc/NRC-2006.pdf> accesses 6/16/2013.
- Norris, D.O., 2007. Xenoestrogen actions on reproduction: Implications for health of wildlife and humans. American Water Resources Association 2007 summer specialty conference on Emerging Contaminants of Concern in the Environment: Issues, Investigations and Solutions. Vail, Colorado.
- Petrovic, M., Eljarrat, E., De Alda, M. J. L., Barcelo, D., 2004. Endocrine disrupting compounds and other emerging contaminants in the environment: A survey on new monitoring strategies and occurrence data. *Anal. and Bioanal. Chem.* 378, 549-562.
- Pillon, A., Servant, N., Vignon, F., Balaguer, P., Nicolas, J.C., 2005. In vivo bioluminescence imaging to evaluate estrogenic activities of endocrine disruptors. *Anal. Biochem.* 340, 295-302
- Potter, T.L., Simmons, K., Wu, J., Sanchez-Olvera, M., KostECKI, P., Calabrese, E., 1999. Static die-away of a nonylphenol ethoxylate surfactant in estuarine water samples. *Environ. Sci. Technol.* 33, 113-118.

- Redderson, K., Heberer, T.H., Dunnbier, U., 2002. Occurrence and identification of phenazone drugs and their metabolites in ground- and drinking water. *Chemosphere*. 49, 539-545.
- Reeves, A.D., Patton, D., 2005. Faecal sterols as indicators of sewage contamination in estuarine sediments of the Tay Estuary, Scotland: an extended baseline survey. *Hydrology and Earth System Sciences*. 9, 81–94.
- Roberts, P.H., Thomas, K.V., 2006. The occurrence of selected pharmaceuticals in wastewater effluent and surface waters of the lower Tyne catchment. *Sci. Tot. Environ.* 356, 143-153.
- Rodgers-Gray, T.P., Jobling, S., Morris, S., Kelly, C., Kirby, S., Janbakhsh, A., Harries, J.E., Waldock, M.J., Sumpter, J.P., Tyler, C.R., 2000. Long term temporal changes in the estrogenic composition of treated sewage effluent and its biological effects on fish. *Environ. Sci. Technol.* 34, 1521.
- Routledge, E.J., Sheahan, D., Desbrow, C., Brighty, G.C., Waldock, M., Sumpter, J.P., 1998. Identification of estrogenic chemicals in STW effluent. 2. In vivo responses in trout and roach. *Environ. Sci. Technol.* 32, 1559–1565.
- Rudel, R.A., Melly, S.J., Geno, P.W., Sun, G., Brody, J.G., 1998. Identification of alkylphenols and other estrogenic phenolic compounds in wastewater, septage, and groundwater on Cape Cod, Massachusetts. *Environ. Sci. Technol.* 32, 861-869.
- Soares, A., Guieysse, B., Jefferson, B., Cartmell, E., Lester, J.N., 2008. Nonylphenol in the environment: A critical review on occurrence, fate, toxicity and treatment in wastewater. *Environ. Int.*, 34, 1033-1049.
- Shore, L.S., Shemesh, M., Cohen, R., 1988. The role of estradiol and estrone in chicken manure silage in hyperestrogenism in cattle. *Aust. Vet. J.* 65, 68.
- Shore, L. S., Shemesh, M., 2003. Naturally produced steroid hormones and their release into the environment. *Pure and Applied Chem.* 75, 1859–1871.
- Snyder, S.A., Keith, T.L., Verbrugge, D.A., Snyder, E.M., Gross, T.S., Kannan, K., Giesy, J.P., 1999. Analytical methods for detection of selected estrogenic compounds in aqueous mixtures. *Environ. Sci. Technol.* 33, 2814-2820.
- Soh, L., Connors, K.A., Brooks, B.W., Zimmerman, J., 2011. Fate of sucralose through environmental and water treatment processes and impact on plant indicator species. *Environ. Sci. Technol.* 45, 1363–1369
- Soto, A.M., Justicia, H., Wray, J.W., Sonnenschein, C., 1991. p-Nonyl-Phenol: An estrogenic xenobiotic released from “modified” polystyrene. *Environ. Health Persp.* 92, 167-173.

- Spehar, R.L., Brooke, L.T., Markee, T.P., Kahl, M.D., 2010. Comparative toxicity and bioconcentration of nonylphenol in freshwater organisms. *Environ. Toxicol. Chem.* 29, 2104–2111.
- Spengler, P., Korner, W., Metzger, J.W., 2001. Substances with estrogenic activity in effluents of sewage treatment plants in southwestern Germany. 1. Chemical analysis. *Environ. Toxicol. Chem.* 20, 2133-2141.
- Stanford, B.D., Weinberg, H.S., 2010. Evaluation of on-site wastewater treatment technology to remove estrogens, nonylphenols, and estrogenic activity from wastewater. *Environ. Sci. Technol.* 44, 2994-3001.
- Stewart, P.W., Reihman, J., Lonky, E.I., Darvill, T.J., Pagano, J., 2003. Cognitive development in preschool children prenatally exposed to PCBs and MeHg. *Neurotoxicol. Teratol.* 25, 11–22.
- Sulleabhain, C.O., Gill, L.W., Misstear, B.D.R., Johnston, P.M., 2009. Fate of endocrine-disrupting chemicals in percolating domestic wastewater effluent. *Water Environ. J.* 23, 110-118.
- Sumpter, J.P., 2005. Endocrine disrupters in the aquatic environment: an overview. *Acta. Hydrochim. Hydrobiol.* 33, 9–16.
- Swartz, C.H., Reddy, S., Benotti, M.J., Yin, H., Barber, L.B., Brownawell, B.J., Rudel, R.A., 2006. Steroid estrogens, nonylphenol ethoxylate metabolites, and other wastewater contaminants in groundwater affected by a residential septic system on Cape Cod, MA. *Environ. Sci. Technol.* 40, 4894-4902.
- Synder, S.A., Trenholm, R.A., Synder, E.M., Bruce, G.M., Pleus, R.C., Hemming, J., 2008. Toxicological relevance of EDCs and pharmaceuticals in drinking water. Awwa Research Foundation, Denver, Colorado. ISBN 978-1-60573-040-0.
- Ternes, T., Kreckel, P., Mueller, J., 1999a. Behaviour and occurrence of estrogens in municipal sewage treatment plants—II. Aerobic batch experiments with activated sludge. *Sci. Total Environ.* 225, 91– 99.
- Thomas, K.B., Colborn, T., 1992. Organochlorine endocrine disrupters in human tissue. Chemically induced alterations in sexual and functional development: The Wildlife/Human Connection, Princeton Science Publishing Company Inc., Princeton, New Jersey, 365-394.
- Thorpe, K.L., Cummings, R.I., Hutchinson, T.H., Scholze, M., Brighty, G., Sumpter, J.P., Tyler, C.R., 2003. Relative potencies and combination effects of steroidal estrogens in fish. *Environ. Sci. Technol.* 37, 1142-1149.
- Toppari, J., Juul, A., 2010. Trends in puberty timing in humans and environmental modifiers. *Mol. Cell Endocrinol.* 324, 39–44.

- Toze, S., 2006. Water reuse and health risks-real vs. perceived. *Desalination*. 187, 41-51.
- Tyl, R.W., Myers, C.B., Marr, M.C., Thomas, B.F., Keimowitz, A.R., Brine, D.R., Veselica, M.M., Fail, P.A., Chang, T.Y., Seely, J.C., Joiner, R.L., Butala, J.H., Dimond, S.S., Cagen, S.Z., Shiotsuka, R.N., Stropp, G.D., Waechte, J.M., 2002. Three-Generation Reproductive Toxicity Study of Dietary Bisphenol A in CD Sprague-Dawley Rats. *Toxicol. Sci.* 68, 121-146.
- Tyl, R.W., Myers, C.B., Marr, M.C., Castillo, N.P., Seely, J.C., Sloan, C.S., Veselica, M.M., Joiner, R.L., Van Miller, J.P., Simon, G.S., 2006. Three-generation evaluation of dietary para-nonylphenol in CD (Sprague-Dawley) rats. *Toxicol. Sci.* 92, 295-310.
- UK Environment Agency, 2000. Endocrine disrupting substances in the environment: The environment agency's strategy. Environmental Issues Series. The Stationery Office, London, United Kingdom. ISBN 1 857 05261 7.
- USEPA, Estimation Program Interface Suite. Available at [http://www.epa.gov/sab/panels/epi\\_suite\\_review\\_panel.htm](http://www.epa.gov/sab/panels/epi_suite_review_panel.htm). Accessed on 6/16/2013.
- USEPA, 1989. Risk assessment guidance for superfund volume I. Human health evaluation manual (Part A). Office of Emergency and Remedial Response, EPA/540/1-89/002, U.S. Environmental Protection Agency, Washington, D.C.
- USEPA, 1998. Guideline 875.2100. Transferable residue dissipation: Lawn and turf. U.S. Environmental Protection Agency, Washington, D.C. Available at <http://www.epa.gov/scipoly/sap/meetings/1998/march/chapb-4.pdf> accessed on 5/22/2013.
- USEPA, 2004. Overview of the ecological risk assessment process in the office of pesticide programs, U.S. Environmental Protection Agency: Endangered and threatened species effects determinations. Office of prevention, pesticide, and toxic substances. Washington, D.C. Available at <http://www.epa.gov/espp/consultation/ecorisk-overview.pdf> accessed on 5/22/2013.
- USEPA, 2009. Contaminant information sheets for the final CCL 3 chemicals. EPA 815-R-09-012. U.S. Environmental Protection Agency, Office of Water, Washington, D.C.
- USEPA, 2010. Bisphenol A action plan. U.S. Environmental Protection Agency, Washington, D.C. Available at [http://www.epa.gov/oppt/existingchemicals/pubs/actionplans/bpa\\_action\\_plan.pdf](http://www.epa.gov/oppt/existingchemicals/pubs/actionplans/bpa_action_plan.pdf) accessed on 5/22/2013.

- USEPA, 2011. Exposure Factors Handbook. EPA/600/R-090/052F. U.S. Environment Protection Agency, United States. Office of Research and Development, Washington, D.C.
- USEPA, 2012a. Guidelines for water reuse. EPA/600/R-12/618. U.S. Environmental Protection Agency, Office of Wastewater Management, Washington, D.C.
- USEPA, 2012b. 2012 Edition of the drinking water standards and health advisories. EPA 822-S-12-001. U.S. Environmental Protection Agency, Office of Water, Washington, D.C.
- Van Ry, D., Dachs, J., Gigliotti, C., Brunciak, P., Nelson, E., Eisenreich, S., 2000. Atmospheric seasonal trends and environmental fate of alkylphenols in the lower Hudson River estuary. *Environ. Sci. Technol.* 34, 2410-2417.
- Weber, S., Leuschner, P., Kampfer, P., Dott, W., Hollender, J., 2005. Degradation of estradiol and ethinylestradiol by activated sludge and by a defined mixed culture. *Appl. Microbiol Biotechnol.* 67, 106–112
- Welshons, W.V., Nagel, S.C., Vom Saal, F.S., 2006. Large effects from small exposure. III. Endocrine mechanisms mediating effects of bisphenol A at levels of human exposure. *Endocrinology.* 147, S56-S69.
- White, R., Jobling, S., Hoare, S.A., Sumpter, J.P., Parker, M.G., 1994. Environmentally persistent alkylphenolic compounds are estrogenic. *Endocrinology.* 135, 175-182.
- WHO, 2011. World Health Organization Guidelines for Drinking-Water Quality. Fourth Edition. Geneva, Switzerland.
- Xiao, X-Y., McCalley, D.V., McEvoy, J., 2001. Analysis of estrogens in river water and effluents using solid-phase extraction and gas chromatography–negative chemical ionization mass spectrometry of the pentafluorobenzoyl derivatives. *J. Chromatography A.* 923, 195–204
- Ying, G.G., Kookana, R.S., Rub, Y.J., 2002a. Occurrence and fate of hormone steroids in the environment. *Environ. International.* 28, 545– 551.
- Ying, G.G., Williams, B., Kookana, R.S., 2002b. Environmental fate of alkylphenols and alkylphenol ethoxylates – a review. *Environ. International.* 28, 215-226.
- Yoon, Y., Westerhoff, P., Snyder, S.A., Wert, E.C., 2006. Nanofiltration and ultrafiltration of endocrine disrupting compounds, pharmaceuticals and personal care products. *J. Membrane Sci.* 270, 88-100.
- Zoeller, R.T., 2007. Environmental chemicals impacting the thyroid: targets and consequences. *Thyroid.* 17, 811–817.

## BIOGRAPHICAL SKETCH

Harmanpreet Sidhu was born in 1987, in Northwest India. Coming from a magnificent region situated in the foot hills of Himalayas, Harman learned to admire, protect and preserve the known Universe's great spectacle- Earth. After his sophomore year of high school, Harman's natural interest towards scientific mysteries of the planet, his zeal and aim propelled him to take biology, physics and chemistry as the main subjects for his senior secondary examination.

After high school, Harman was admitted to Punjab Agricultural University, Ludhiana, India, for a 4 year bachelor's degree in agriculture (hons.). It was in that university, that his interest in environmental sciences encouraged him to pursue a career in related field. He then decided to major in soils. Harman graduated from Punjab Agricultural University in 2011.

Harman began his graduate career at the University of Florida in August of 2011, pursuing master's in Soil and Water Science Department under the advisement of Dr. George A. O'Connor. His master's research focused on human risk assessment of endocrine disrupting chemicals in reclaimed water. Harman enjoys the work he does and hopes to continue a similar scientific research in his Ph.D. at University of Florida.