2007

Regulation issues and prediction of natural estrogen loads for wastewater treatment plants

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REGULATION ISSUES AND PREDICTION OF NATURAL ESTROGEN LOADS FOR WASTEWATER TREATMENT PLANTS

By

MICHELLE E JARVIE

A DISSERTATION

Submitted in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

(Environmental Engineering)

MICHIGAN TECHNOLOGICAL UNIVERSITY

2007

This dissertation "REGULATION ISSUES AND PREDICTION OF NATURAL ESTROGEN LOADS FOR WASTEWATER TREATMENT PLANTS" is hereby approved in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Engineering.

Department or Program:
(Non-departmental) Environmental Engineering

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AKNOWLEDGEMENTS

I would like to thank Dr. David W. Hand, without whose kind guidance and support, this work would never have been achieved. It is a rare occasion to meet an authority figure with such compassion. For your understanding and kind words in my darkest of times, I cannot express enough thanks.

I would also like to thank Dr. Marilyn Vogler, formerly of the graduate school, for warning me against the regret of quitting. Thank you for your wise words and assistance when my load was too heavy.

To Jill Arola, of the Writing Center, I give special thanks. Not only have you helped me to write, you have inspired me to live. Thank you for being a wise woman in my life, for saying the hard truths, and mothering me when I needed it most.

Special thanks to Dr. Bob Balliod and Dr. Mary Durfee, for taking a chance on letting me teach. The experiences I was given have forever altered my life path and future. I now know first hand, the joy of spreading passion for a subject, and watching minds blossom.

Thanks to Dr. Jim Mihelcic, a perpetual font of funding for our department. Without the GAANN and IGERT, none of this could have come to fruition. Your words of encouragement and straight forward advice about professional development are truly rare indeed. You make it all seem easier.

Thank you also to Dr. John Sutherland, and all who worked on the IGERT. The work you do may seem great and not worth the efforts, but IGERT and SFI gave this academic misfit a home. Without your work, I would have thought engineering was only for those who think inside the box.

I can hardly express how instrumental the support and love of my family has been, and continues to be, through all my life. It is a rare person who has such riches as I do. All of the knowledge I have gained in school means nothing compared to the wisdom of recognizing what is truly important in life. I feel great pride in this accomplishment. But at the end of my life, it is not the academic achievements I will look back on. It is the times with my family that have filled my heart with the greatest joy. To my father, Bob Jarvie, I would like to say thank you for supporting me, believing in me, and reminding me to keep going, even through grief. Thank you for being a father when I needed one most, even when I asked difficult things of you. To my sister, Ellie Jarvie, I can only say that I am lucky to have you as a friend. Your advice and unconditional love has meant more than I can ever express. To my sister, Kim Nelson, thank you for always being on my side, and supporting me no matter what I decide to do. To my niece, Angela Jarvie, thank you for all your fun visits. When life is hard, you make me laugh.

TABLE OF CONTENTS

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TABLE OF CONTENTS

INTRODUTCION

In the field of environmental engineering, concern is growing regarding trace chemical contaminants that disrupt the natural functions of hormonal systems, known as endocrine disruptors. Two potent endocrine disruptors, the natural estrogens, estrone and estradiol, are present in wastewater effluents. However, the concentration of estrogens in wastewater influents and effluents is not routinely measured.

Estrogens are known to cause endocrine responses in aquatic species. Wastewater effluent is known to contain estrogens, and has been shown to cause endocrine responses in aquatic species. Models developed for the removal of estrogens in wastewater treatment systems are dependant upon the accuracy of estimates of wastewater influent estrogen concentrations.

The primary goal of this research was determining the pseudo-first order kinetic constants for the biodegradation of both estrone (E1) and estradiol (E2) in sewers, and development of a model for the degradation/transformation of E1 and E2 within sewage collection systems.

The model predicts influent aqueous E1 and E2 concentrations at wastewater treatment plants based on the following inputs and mechanisms:

- Population distribution within age and gender
- Excretion data for each population group
- Sewer flows and residence times or collection system maps
- Deconjugation of estrogens
- Biodegradation of estrogens
- Transformation between estrogens
- Sorption to solids
- Temperature within sewer systems

As engineers, we should be concerned with creating a more sustainable society. The release of known endocrine disruptors into our surface waters through wastewater effluent directly affects the ability of aquatic species to reproduce. Thus, this work also includes an examination of the current framework for regulating chemical releases into surface waters within the US. It argues the case for the regulation of EEDCs within wastewater effluents through use of the current National Pollutant Discharge Elimination System (NPDES).

Engineering education has long been neglected in the studies and preparation of engineering Ph.D. students. The inclusion of engineering education articles in the appendices to this dissertation reflects a shift in the preparation of today's engineering educators, recognizing the importance of pedagogical knowledge. Active and collaborative learning techniques are at the fore front of strategies to improve the engineering classroom experience. Thus, Appendix C: Restructuring of CE4506 (Environmental Policy and Pollution Prevention Design) and Student Response Survey, was published in the peer-reviewed proceedings of the 2006 American Society of Engineering Education (ASEE) annual conference. This paper details the format change of a senior level environmental policy and pollution prevention class. The new format included class room strategies for active and collaborative learning. The paper includes qualitative results from a survey regarding student preferences for the new class structure in comparison to the previous structure.

Engineering has traditionally been dominated by white males within the United States. Certain disciplines, such as environmental engineering, are more successful than others at attracting women. However, recruitment of minority students remains a concern. Modern engineering educators must also recognize the importance of recruitment to under represented minorities within engineering disciplines. Appendix D: Minority Student Enrollment in Environmental Engineering, General Student Perceptions of the Discipline, and Strategies to Attract and Retain a More Diverse Student Body, was accepted for publication in the peer-reviewed proceedings of the 2007 American Society of Engineering Education (ASEE) annual conference. This paper takes a closer look at the data pertaining to the enrollment of minorities in environmental engineering programs, which indicates that just a few schools across the nation are enrolling minority students within environmental engineering. This paper presents studies regarding the perceptions of the discipline among k-12 and first year students, and highlights the need for research regarding the perceptions of the discipline among minorities and factors influencing career choice of minority students. Some suggestions are made for strategies which may increase the attraction and retention of minority students to the discipline.

CHAPTER 1:

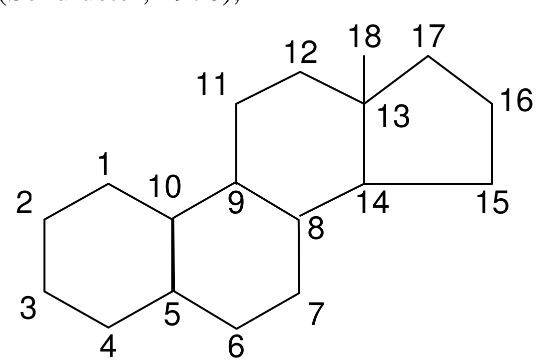
The Significance of Estrogens within Wastewater Systems

Concern for organic microcontaminants in drinking water supplies has been on the rise. Many of the organic chemicals of concern originate from our wastewater streams, are emitted into surface waters, and persist within the environment. As a result, these contaminants are present in the source waters for drinking water supplies. Among the emerging contaminants of concern, endocrine disruptors are fast becoming perceived as one of the greatest threats to human and ecological health.

Most simply stated, endocrine disruptors are chemicals which interfere with natural hormonal systems within the bodies of animals. Numerous chemicals abound that are considered endocrine disrupting compounds (EDCs). However, EDCs can be divided into the three main categories of estrogenic, androgenic, and thyroidal, based on the hormonal systems they disrupt (Snyder et al, 2003). Estrogenic and thyroidal EDCs affect the systems they are named after, while androgenic EDCs are those which interfere with the natural testosterone system. These classifications are not mutually exclusive, and one EDC may interfere with more than one hormonal system, or behave differently in males and females.
To develop an understanding of the significance of this research, the reader must first possess a basic knowledge of estrogens and their effects. The following section offers a chemical description of estrogens.

1. 1 What are Estrogens?

Estrogens are phenolic steroids that target the tissues of the uterus, vagina, oviduct, mammary gland, and parts of the brain (Paqualini, 1976). Estrogens have a common ring structure of three six-membered rings and one five-membered ring, with eighteen carbon atoms numbered as shown in the figure below (Schuluster, 1976),



All estrogens possess a hydroxyl group off the carbon 3 position. However, estrogens vary in which functional groups are attached to the 16 and 17 positions of the carbons.

The primary endogenous estrogen which binds to the human estrogen receptors is Estradiol (E2), which can be oxidized in metabolic processes to form Estrone (E1), and is further transformed into Estriol (E3) (Lai, 2003). When looking at the structures of the three natural estrogens, it is easy to see that the number (1, 2, or 3) following the "E" in their abbreviated names corresponds with the number of hydroxyl groups on each estrogen. These three estrogens encompass the natural estrogens found in wastewater treatment, and are shown in detail in Figure 1 from Hanselman (2003).

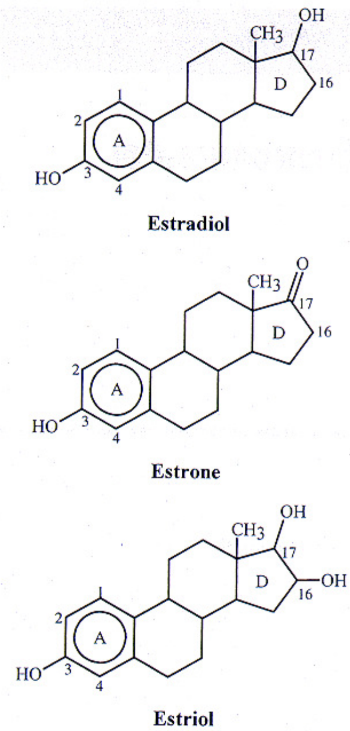


FIGURE 1. Molecular structures of estradiol, estrone, and estriol. The letters and numbers indicate the ring assignments and carbon numbers, respectively.

These estrogens can be excreted as conjugates of sulfuric and glucuronic acids, and are not biologically active as free steroids until environmental bacteria deconjugate them (Baronti et al, 2000). Estrogens are excreted in both urine and feces. Due to the presence of bacteria in feces, fecal estrogens are mostly excreted as free estrogens (Aldercreutz and Jarvenpaa, 1982).

Estrogen sulfates and estrogen glucuronates exist for all three forms of estrogens, and include: E1-3S, E1-3G, E2-3S, E2-17G, E2-3G, E3-3S, E3-16G, and E3-3G; where the "G" and "S" designations refer to glucuronate and sulfate groups, and the preceding number refers to the carbon chain position (Figure 2, from Johnson and Williams, 2004). A bacterium that synthesizes arylsulfatase is required to deconjugate the sulfated estrogens, while one that synthesizes the glucuronidase enzyme is necessary for deconjugation of the glucuronated estrogens.

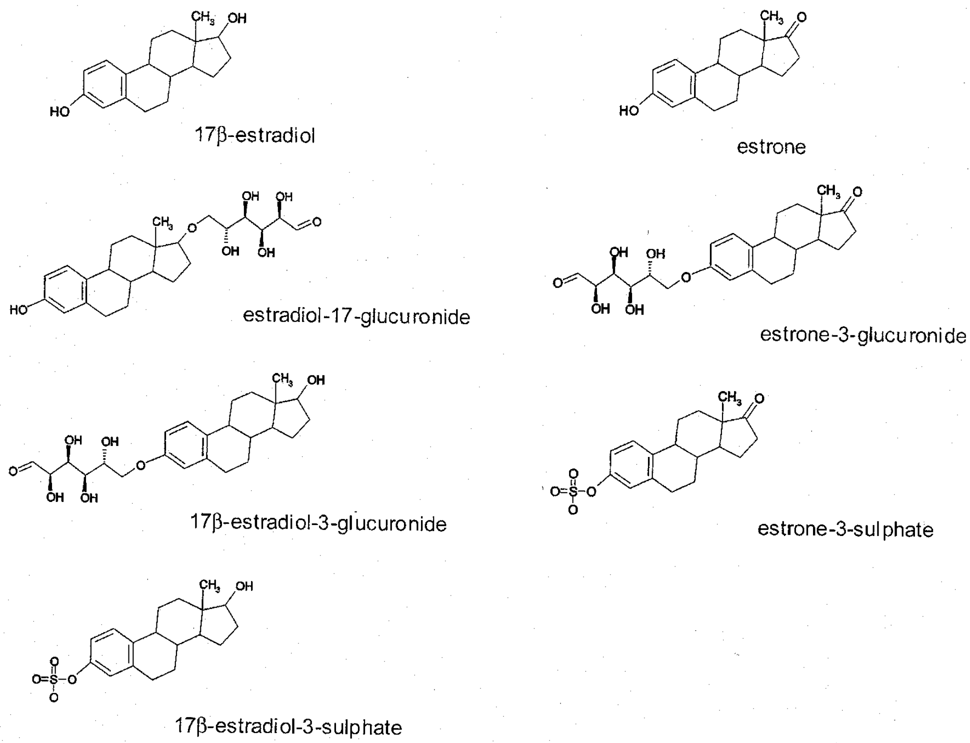


Figure 2. Conjugates of Estrone and Estradiol

Estrone (E1) is the most abundant estrogen excreted by cycling women. Due to its prevalence in urine, and the fact that E1 can form from the breakdown of E2; E1 is the most abundantly detected estrogen in activated sludge treatment plant effluents and in surface water (Belfroid, 1999).

1.2 What are the Effects of Estrogens?

Predicting the effects of human exposure to EDCs at varying concentrations is difficult because EDCs often do not follow the typical linear dose-response relationships used in classic toxicology, where greater exposure to a chemical has increased health effects (Vogel, 2004). Some EDCs are most potent at low exposure levels. Additionally, exposure to EDCs can have opposite effects when exposure occurs at differing developmental stages, making it very difficult to determine safe fetal exposure amounts. Although the results may be difficult to translate into human health effects for exposure, many studies have documented the effects on aquatics species of exposure to estrogens.

One result of exposure to estrogens is the presence of vitellogenin (VTG) in the male of a species. VTG is a precursor for egg yolk, and normally found in the serum of adult female oviparous vertebrates (Huang et al, 2003). In normal surface water environments, high levels of VTG are found only in adult females. High plasma VTG concentrations in juvenile fish and males are a definite result of exposure to estrogens (Hemming et al 2001). VTG concentrations vary in female fish by six orders of magnitude, depending on their reproductive stage. This wide range makes VTG a sensitive biomarker indicating the presence of estrogens in aquatic environments.

Another result of exposure to endocrine disruptors can be intersex, which is the growth of both oocytes and testicular tissue within male fish gonads, such as ovitestes. Perhaps the most disturbing effect is decreased fecundity, or the rate at which an individual produces viable offspring (Huang et al, 2003). At differing ambient hormone concentrations, the effects on aquatic life vary. Thus it is often useful to report the no-observed-effect concentration (NOEC), which is the concentration at which no adverse effects have been observed for a given species. Concentrations below the NOEC are assumed to be safe for the species in question.

Exposure to natural estrogens has been shown to affect fish life. Thorpe et al (2000) exposed juvenile female rainbow trout to 17β-E2 and found concentration dependant inductions of plasma VTG were optimal after 14 days, and the NOEC was <5 ng/L. Evidence also exists of 17β-E2 affecting the growth of oocytes in eel (Lokman et al, 2003). 17β-E2 has been proven to increase VTG synthesis in male rainbow trout and roach at concentrations within the ng/L range typically found downsteam of wastewater treatment plants (Routledge et al, 1998). E2 has also been shown to affect the connective tissue surrounding the sex accessory ducts of larval tiger salamanders (Norris et al, 1997). All of these effects result from a disruption in the normal activities of the estrogen system and can be referred to estrogenic responses. Thus, estrogens and chemicals that mimic them are known to cause estrogenic responses.

A review of estrogens and estrogenic effects has been presented. Obviously, estrogenic chemicals pose a severe threat to the ability of aquatic species to reproduce and survive. The release of these chemicals in the environment is not a sustainable practice, and identifiable sources of the chemicals should be minimized, if not eliminated altogether. One such source of estrogens in our surface waters is wastewater effluent.

1. 3 Estrogen is Present in Wastewater Effluent

Estrogens are excreted in human waste streams. Those that survive wastewater treatment enter surface water through wastewater treatment plant effluents. Estrogens have been well documented in the effluents of sewage treatment plants all over the world (Andersen et al, 2003; Baronti et al, 2000; Belfroid et al, 1999;

Joss et al, 2003; Johnson et al 2000; Lee & Pert, 1998; Matsui et al, 2000; Nasu et al, 2001; Servos et al, 2005; Ternes et al, 1999; Verstraeten et al, 2003). Highest concentrations were found in those plants that had only domestic influents, and among samples taken in colder months (Belfroid et al, 1999; Joss et al, 2003; Lee & Pert, 1998; Servos et al, 2005; Ternes et al, 1999; Verstraeten et al, 2003).

As sewage treatment plant effluents are documented to contain estrogens, surface waters downstream of wastewater treatment plants are likely places to find estrogens. Estrogen concentrations in water decline downstream from effluent entry points due to dilution, sorption, and degradation (Williams et al 2003). Most reports of estrogens in receiving waters are from colder climates, such as the northern US and northern Europe, or from wastewater plants that service large populations, resulting in large volume of discharge (Layton et al, 2000). This is due to the fact that biological activity decreases with temperature, and in colder climates, the bacteria present in wastewater treatment systems consume estrogens at a slower rate.

1.4 The Estrogenic Effects of Wastewater

In areas where wastewater effluents enter the environment, estrogenic effects have been observed on aquatic life. In Texas, a treated municipal wastewater effluent, flowing through a constructed wetland, was observed to cause a 3 to 4 order of magnitude increase in male fathead minnow plasma concentration of the egg yolk precursor, vitellogenin VTG (Hemming et al, 2001). In France, evidence has been documented of higher rates of intersex in fish downstream of wastewater plant effluents (Minier et al, 2000). In Iowa, wastewater from small municipal sources was shown to stimulate VTG production in male fathead minnows within treatment aeration lagoons (Bringolf et al, 2003). In Spain, male carp were documented to contain elevated VTG levels downstream of sewage treatment plants (Petrovic et al, 2002). In England, sewage effluent induced VTG production in trout at 15 different sewage treatment plants (Purdom et al, 1994).

Wastewater has also been shown to affect the sex ratios of populations of amphibian species exposed during larval development and metamorphosis (Bogi et al, 2003). Wastewater is known to contain components that cause estrogenic responses, as shown by the studies above where animals exposed to wastewater effluents exhibited estrogenic responses. Additionally, yeast estrogen screens (YES) were used to verify that two German wastewater plant effluents had estrogenic potential (Pawlowski et al, 2004).

There are a myriad of organic chemicals used as pharmaceuticals, personal care products, and household chemicals, which are found in wastewater streams. Non-estrogen chemicals present in wastewater, xenoestrogens, can also cause estrogenic responses in animals by acting as estrogen mimics. Of the chemicals which may act as xenoestrogens, perhaps the most attention has been given to nonylphenol (NP) and octylphenol (OP). NP and OP are formed from the breakdown of alkylphenol polyethoxylates (APE), which are domestic and industrial nonionic surfactants.

However, examination of domestic wastewater effluent to determine the chemicals responsible for estrogenic responses targeted only the natural estrogens E1, 17β-E2, and the artificial estrogen ethinylestradiol (EE2) as chemicals responsible for the estrogenic activity (or estrogenicity) of domestic wastewater (Desbrow et al, 1998). Of the natural estrogens present in wastewater, E3 is considered 300 times less active than E2, and did not emerge among the estrogens responsible for wastewater estrogenicity. Similarly, nonylphenol is considered 100 times less estrogenically active than E2 (Arnold, 2002).

In fact, phenolic xenoestrogens have been documented to only be responsible for 0.7-4.3% of the estrogenic activity of sewage treatment plant effluent (Korner et al, 2000). In Japan, estrogenicity in the effluent of the Shiga prefecture wastewater treatment plant was proven to be almost completely due to the presence of E2 (Matsui, et al, 2000). Similarly, a study of seven US wastewater facilities found that the majority of the estrogenic disrupting activity in both the primary and secondary effluents was due to the two natural hormones E2 and E3 (Drewes et al, 2005). Thus, based on the relative potencies and prevalence of the chemicals in wastewater effluent, the natural estrogens of E1 and E2 and artificial estrogen EE2 emerge as the primary estrogenic endocrine disruptors of concern in wastewater effluent.

EE2 is a major component of most birth control pills. However, the use of contraceptive pills varies greatly between cultures. The world average of pill use for women aged 15-49 is 15.9 % for developed nations and 6.2% among the less developed. But even among developed nations, pill use varies greatly. Western Europeans are among the highest users of the pill, with an average of 46.9% for the region, and a high of 58.6 % among Germans. Only 12.8% of Southern European women use the pill, with Italian use at 13.6%. While 24% of Australian women use the pill, and only 15.5 % in North America (UN, 2005).

Given the comparative rates of pill use between the countries, It should be of no surprise that higher average influent EE2 concentration (8.2 ng/L) was found at a German plant (Andersen et al, 2003), in comparison the average influent at five Italian plants was 3.1 ng/L (Johnson et al, 2000). Irregardless of influent concentration, EE2 in effluent was minimal, with the German plant and three of the five Italian plant effluents <LOQ for EE2. The remaining two Italian plants averaged an effluent EE2 concentration of 1.4 ng/L. Where EE2 is present in wastewater effluents, it is a cause for concern because of its comparatively strong potency as an endocrine disruptor. Within exposure studies on fathead minnows, EE2 was 25 times more powerful at stimulating VTG production than E2 (Brian et al, 2005; Sumpter et al, 2006).

The presence of artificial estrogens in wastewater systems varies with the use of chemical birth control between cultures. Additionally, it is possible that synthetic estrogens may be replaced by safer chemical alternatives. However, natural estrogens are normally excreted by healthy humans and their presence in sewage cannot be eliminated. Thus, this work is focused on the natural estrogens E1 and E2 within wastewater.

1. 5 Estrogens are Present in Surface Water

Investigating the presence of organic wastewater contaminants (OWCs) in surface water, United States Geological Survey (USGS) performed a reconnaissance survey of streams within mainland United States (Kolpin et al, 2002). Estrone (E1) was found in 7.1% of the samples taken, as a median concentration of 27 ng/L. 17α-estradiol (17α-E2) was found in 5.7% of the samples at a median concentration of 30 ng/L. 17β-estradiol (17β-E2) was found in 10% of the samples, at a mean concentration of 9 ng/L.

At first glance, the mean concentrations of natural estrogens detected in river water may seem low. However, these concentrations may have impacts on human and aquatic life. In fact, it has been shown that the no-observed effect concentration (NOEC) for 17β-E2 in female juvenile rainbow trout is <5 ng/L (Thorpe et al, 2000). Thus, at concentrations greater than 5ng/L, juvenile female rainbow trout will show responses to the presence of 17β-E2. Thus, where 17β-E2 is present in U.S. rivers, it exists at concentrations that are known to affect the sexual development of juvenile female rainbow trout. The presence of estrogens in surface water is not a strictly North American concern, and had been observed globally, including the Netherlands (Belfroid et al 1999), the United Kingdom (Lai et al, 2000), Germany (Verstraeten et al, 2003), and Italy (Baronti et al, 2000).

Currently, estrogens have been shown to be present in US surface waters at concentrations that are known to impact aquatic wildlife. Evidence is emerging of sexual abnormalities within aquatic populations in the wild. Ovotestes have been observed in male smallmouth bass in the Columbia River Basin (Hinck et al, 2004) and the Mississippi River Basin (McDonald et al, 2002); and in largemouth bass in the Rio Grande Basin (Schmitt et al, 2004). And a spring 2004, a USGS sampling of smallmouth bass on the Potomic River detected sexual abnormalities in 79% of the fish sampled (Cocke, 2004).

Observations of sexual abnormalities in aquatic life are not limited to North America. Intersex, or the presence of oocytes within testis tissue, among fish populations has been observed in other countries. In French rivers, intersex has been observed among roach, chubb, and gudgeon (Minier et al, 2000). In German rivers, intersex has been documented in three splined stickleback and perch, and among eelput in German coastal waters (Gercken and Sordyl, 2002). At one location in Italy, barbell captured in the Po River showed intersex gonads in 50%

of the fish sampled (Vigano et al, 2001). The endocrine response of fish is a good indicator of the health and safety of aquatic environments.

Natural estrogens and xenoestrogens may cause estrogenic endocrine responses. As many of these chemicals exist in surface water, it is likely that an aquatic organism is being simultaneously exposed to more than one estrogenic EDC. When this occurs, the effects of exposure to several estrogenic EDCs can be additive. In one study, the additive effects of VTG induction in juvenile rainbow trout could be predicted for a mixture of E2 and 17α-ethynylestradiol (EE2) by using the relative estrogenic potency of each chemical and concentration-response curves (Thorpe et al, 2003).

The estrogenic effects documented among fish in the wild may be due to the cumulative impact of several environmental micro contaminants, and it impossible to state that E1 and E2 are specifically to blame. However, E1 and E2 are present in relevant concentrations to be considered a contributing factor to endocrine responses in wildlife.

It should be noted that estrogens present in the environment may come from other sources than the waste of humans. Hormones are also present in livestock waste. In fact, though it is not the focus of this research, estrogens are also excreted by swine, cattle, and poultry, and can enter into surface water through farm or feed lot run off (Hansalman et al, 2003, Raman et al, 2004). Feedlot runoff from cattle operations has been documented to alter sexual hormone production in wild fathead minnows, resulting in smaller testis and lowered testosterone synthesis among males (Orlando et al, 2004).

Despite the presence of other estrogenic chemicals and other sources of natural estrogens in the environment, it is important to study E1 and E2 within wastewater due to the documented estrogenic responses in wildlife exposed to wastewater effluent. It is not an environmentally sustainable practice to knowingly release these chemicals into the environment. Research must be undertaken to understand the fate of natural estrogens within wastewater collection and treatment systems. Wastewater facilities should be operated in a way (or retrofitted with new technologies) that will minimize and ultimately eliminate their release into the environment.

CHAPTER 2:

Removal of Estrogens in Wastewater Treatment Unit Operations

It has been shown that estrogens exist within wastewater effluents. However, some estrogens are removed during wastewater treatment processes. This section reviews the literature investigating the effectiveness of various wastewater unit operations at removing estrogens.

2.1 Wastewater Treatment Unit Operations

Wastewater treatment processes are divided into the treatment levels of primary, advanced primary, secondary, secondary with nutrient removal, tertiary, and advanced. Each treatment level adds processes to the previous level that provide for greater removal of solids, organics, and other contaminants within wastewater.

- Primary treatment is a physical step that removes solids and some organics by settling within a primary clarifier. Most primary treatment plants in operation today are actually enhanced primary treatment, which utilizes the addition of a chemical or filtration to enhance physical removal.
- Secondary treatment includes the addition of a biological removal step after primary treatment, where the microorganisms naturally present in wastewater biodegrade organic matter within an aerated digestion tank. After aeration, the wastewater undergoes a second clarification step. Disinfection is often included in secondary treatment. Secondary treatment with nutrient removal includes the addition of process for the removal of nitrogen and/or phosphorus.
- Tertiary treatment includes additional suspended solids removal after secondary treatment, usually by microscreens or filtration. Nutrient removal and disinfection are often included in tertiary treatment.
- Advanced treatment includes the addition of other unit operations for the removal of contaminants that remain after biological treatment. Advanced treatment is most often required in water reuse scenarios.

Perhaps the most common type of secondary treatment is Activated Sludge Treatment (AST), as shown in Figure 3. In a plant using AST, wastewater enters the plant, passes through bar screens, a grit removal chamber, and primary clarification, after which it enters an aeration tank. From there, water enters the secondary clarifier, and is subsequently disinfected prior to release in a surface water body. Solids removed in primary clarification are sent to an anaerobic digester. Solids removed in secondary clarification are split into two streams, one of which goes to anaerobic digestion, the other of which is recycled to the aeration tank to seed the sludge with active microorganisms. Solids are reduced in the anaerobic digester, dewatered, and disposed of.

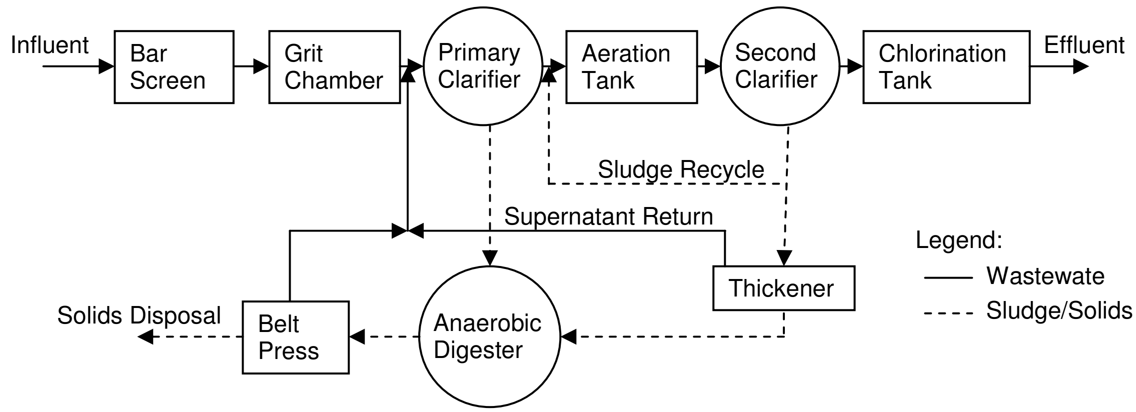


Figure 3. Activated Sludge Treatment

2.2 Estrogens in Wastewater Plant Influents & Effluent

Throughout the world, studies have documented typical inlet concentrations of estrogens in waste water treatment plants (Andersen et al, 2003; Baronti et al, 2000; Belfroid et al, 1999; Joss et al, 2004; Lee & Peart, 1998; Matsui et al, 2000; Nasu et al, 2001; Servos et al, 2005; Ternes et al, 1999; Verstraeten et al, 2003). Typical E1, and 17β-E2 concentrations found in the literature are presented in Table 1 below. When the average of the studies is taken, typical influent concentrations are found to be 50.9 ng/L of E1, 14.6 ng/L of E2, and average effluent concentrations are <12.7 ng/L of E1, <2.94 ng/L of E2. This results in average removals of >75.1 % of E1 and >79.9 % of E2.

Note that E1 has a smaller percent removal than E2. This may be due to the oxidation of E2 into E1. In some cases, E1 has even been found in greater effluent quantities than influent (Joss et al, 2004), documenting a net increase of E1 in wastewater treatment.

Table 1: Influent & Effluent Concentration of E1 & E2						
	Average Estrogen Influent Concentrations		Average Estrogen Effluent Concentrations		Average Percent removals	
Sewage Treatment Plant	E1 (ng/L)	E2 (ng/L)	E1 (ng/L)	E2 (ng/L)	E1	E2
Penha/Rio de Janeiro, Brasil[1]	40	21	6.8[A]	---	83	>99.9
Frankfurt/Main, Germany[1]	27	15	23	5.4	15	64
Wiesbaden, Germany[2]	65.7	15.8	<1	<1	>98.5	>93.7
Cobis, Rome, Italy[3]	71	16	9.6	1.5	86.5	90.6
Fregene, Rome, Italy[3]	67	9.2	4.1	0.92	93.9	90
Ostia, Rome, Italy[3]	51	15	45	2.4	11.8	84
Roma Sud, Rome, Italy[3]	35	8.6	30	1.9	14.3	77.9
Roma Est, Rome, Italy[3]	50	9.3	7.7	0.75	84.6	91.9
Roma Nord, Rome, Italy[3]	37	11	14	0.98	62.2	91.9
Average of 27 STPs, Japan[4]	---	45	---	14	---	68.9
Berlin-Ruhleben, Germany[5]	188	11.8	12.6	0.8	93.3	93.2
Guelph, Canada[6]	41	15	14	<5	65.9	>66.7
Burlington, Canada[6]	---	---	7	<5	---	---
Montreal, North Canada[6]	28	6	---	---	---	---
Montreal, South,Canada[6]	15	7	---	---	---	---
Dundas, Canada[6]	69	7	9	<5	87.0	>28.6
Netherlands, median of three ASTP[7]	---	---	4.5	.9	---	---
Japan[8]	---	36	---	4	---	---
Altenrhein, Germany[9]	7.3	4.9	8.6	1.0	+17.8	79.6
Kloten, Germany[9]	24	7.6	2.4	<0.5	90	93.4
Mean of 18 Canadian Plants[10]	49	15.6	17	1.8	65.3	88.5
Average of all studies	50.9	14.6	<12.7	<2.94	>75.1	>79.9

[1](Ternes et al, 1999), [A]Effluent of Activated Sludge Tank, nr: no reportable value
[2](Andersen et al, 2003)

[3](Baronti et al, 2000)
[4](Nasu et al, 2001), nm: not measured
[5](Verstraeten et al, 2003)
[6](Lee & Peart, 1998)
[7](Belfroid et al, 1999)
[8](Matsui et al, 2000)
[9](Joss et al, 2004)
[10](Servos et al, 2005)

Not all estrogens are excreted from humans as free estrogens available for breakdown by bacteria. The varying states at which estrogens are excreted directly affects their ability to be metabolized by bacteria.

Recall that both E1 and E2 can be excreted from humans as conjugates of either sulfuric or glucuronic acids, which are not biologically active as free steroids until bacteria deconjugate them (Baronti et al, 2000). *Escherichia Coli* present in human feces, sewers, and wastewater plants, can synthesize the β-glucuronidase enzyme, deconjugating those estrogens associated with them. Therefore, some estrogens are deconjugated by bacteria present within the distribution system, and arrive at the wastewater treatment plant as free active hormones. Estrogens excreted within feces exist mainly in their free form (Aldercreutz and Jarvenpaa, 1982). Thus, deconjugation becomes a concern for urine derived estrogens. Deconjugation is an important step in the breakdown of estrogens within a wastewater treatment plant, as estrogens can not be metabolized by bacteria until they exist in free forms.

Additionally, to be estrogenically active, the estrogens must exist in their free, non conjugated forms. It has been proven that male fathead minnows exposed to E2-3G exhibited no VTG alterations (Panter et al, 1999). However, when this same glucuronate associated hormone was spiked into a simulated biological wastewater treatment, the effluent did produce VTG responses in fish, indicating that biological wastewater treatment can deconjugate glucuronate associated hormones.

Although steroid de-sulfating bacteria have been isolated from human intestinal flora (Van Eldere et al, 1988), evidence exists that glucuronated estrogens are freed more easily on the way to the sewage treatment plant, when compared to sulfated estrogens (D'Ascenzo et al, 2003). In fact, in laboratory batch studies, the removal of glucuronate conjugated estrogens from wastewater occurred on a scale of a few hours, while significant removal of sulfate conjugated estrogens, required several days (D'Ascenzo et al, 2003). Considering sewer transit times of 3 to 5 hours, and plant hydraulic retention times of a few hours, sulfate conjugated estrogens may not spend enough nearly enough time in wastewater collection and treatment systems to be deconjugated. Thus, it is suspected that sulfate associated estrogens will be more likely to survive wastewater treatment.

The influent and effluent concentrations of estrogens in wastewater treatment indicate a definite removal of E1 and E2 by the wastewater treatment process. To identify which unit operations are responsible for the removal of estrogens, studies have been performed to profile the concentrations of estrogens throughout waste water treatment (Anderson et al, 2003; Bringolf et al, 2003; Esperanza et al, 2004; Matsui et al, 2000; Nasu et al, 2001;Ternes et al, 1999). Regardless of whether activated sludge treatment includes nitrogen removal, a trend emerged among these studies. The concentration of E2 was documented to increase from influent to the primary effluent, and subsequently decrease in the secondary effluent, with the majority of concentration decrease occurring between primary and secondary effluent. This reduction was shown to occur both in traditional activated sludge, and in systems including nitrogen removal (Esperanza et al, 2004; Matsui et al, 2000; Nasu et al, 2001; Ternes et al, 1999). A similar increase in concentration in primary effluent, and subsequent decrease in secondary effluent was observed for E1 (Anderson et al, 2003; Esperanza at al, 2004; Ternes et al, 2003).

The increase in free estrogen concentration of E2 from influent to primary effluent is suspected to be due to the deconjugation of estrogens, while the increase in free E1 concentration may be due to both the deconjugation of these estrogens and the oxidation of E2 to E1 in aerobic environments. The large decrease in estrogens from primary to secondary effluent indicates that the activated sludge process of aerobic digestion is the unit operation where estrogen degradation occurs.

Current wastewater treatment processes should be examined for their effectiveness at removing estrogenic EDCs. Some data exists within the literature about the ability of various wastewater plants to remove estrogenic EDCs.

2.3 Enhanced Primary Treatment

Enhanced primary treatment may remove some estrogenic EDCs, but it is not a very effective treatment.

Servos et al (2005) examined E1 and E2 in effluents of 18 wastewater treatment plants of various configurations within Canada. One plant utilized enhanced primary treatment, including alum addition for phosphorus removal. This plant exhibited no removal of E2, and an increase in E1, likely from the transformation of E2. This plant also had a relatively low hydraulic retention time (HRT) of 3 hours. Within this study, the primary treatment plant and another utilizing a trickling filter (attached growth process) were the two worst plants at removing estrogens.

Similar results were found by Johnson et al (2005), who examined the effluent of 17 sewage treatment works across Norway, Sweden, Finland, the Netherlands, Belgium, Germany France, and Switzerland. The highest estrogen values were detected in the plant effluent that only chemically enhanced primary treatment, including phosphorus removal (13 ng/L E2 and 35 ng/L E1). E1 influent concentrations were estimated, and this plant was also estimated to exhibit and increase in E1 concentration from influent to effluent. This plant also had a low HRT of 4 hours.

Braga et al (2005) examined the removal of E1, E2, and EE2 in two Australian wastewater plants. One plant was an enhanced primary plant, which utilized $FeCl_2$ addition for an HRT of 45 min. The enhanced primary plant had effluent concentrations (and percent removals) of 54.0 ng/L E1 (7% removal), 14.0 ng/L E2 (0% removal). EE2 was less than the limit of quantification (LOQ) in both influent and effluent. The immeasurably low influent concentrations of EE2 were attributed to a low percentage of birth control use by fertile-aged Australian women (26.7%) and that the majority of those women use low-EE2-dose contraceptives.

Svenson et al (2003) used yeast to quantify estrogenicity in untreated and treated effluents of 20 wastewater plants across Sweden. Estrogenic activity was expressed in terms of E2 equivalents. Enhanced Primary plants that utilized direct precipitation with either Al or Fe, averaged 18% removal of E2 equivalents.

From these studies it can be seen that enhanced primary treatment is not adequate for the removal of estrogenic EDCs within wastewater. As estrogens are known to be biodegradable, the absence of biological treatment is likely the reason for the poor performance of these systems. Additionally, they all have very low HRT values of four hours or less.

2.4 Secondary Treatment

If a treatment process utilizes biological treatment to reduce organic matter, it is considered secondary treatment. The natural microorganisms that exist within wastewater will degrade organics when provided with the correct environmental conditions and time. Biological treatment methods include lagoons (aerated or not), suspended growth reactors (such as activated sludge processes), and attached growth processes (such as trickling filters). Secondary treatment methods remove more estrogens overall than primary treatment. Of the secondary treatment methods, trickling filters do not perform as well as other processes for the removal of estrogens.

In the previously mentioned study of E1 and E2 in effluents of 18 Canadian wastewater treatment plants (Servos et al., 2005), the two plants that exhibited no removal of estrogens included one utilizing only primary treatment, and another utilizing a trickling filter (attached growth process). The trickling filter plant showed an increase in both E2 and E1. This plant operated with a system HRT of 6-8 hours, but a trickling filter HRT of only 1 hour and SRT of 1.9 days.

Other studies have also found trickling filters as inferior methods to activated sludge for the removal of estrogens. Ternes 1999a (1999) examined E1, E2, and EE2 in German, Canadian, and Brazilian wastewater plants and found that the activated sludge step removed estrogens with a greater efficiency than a biological trickling filter. While Svenson et al (2003) used yeast to quantify estrogenicity (expressed in terms of E2 equivalents) in untreated and treated effluents of 20 wastewater plants across Sweden. Plants with supported bacteria, such as trickling filters, averaged only 28% removal.

As the removal of estrogens is mainly a biological process, the poor performance of trickling filter among biological processes is likely due to their relatively low HRTs. In comparison to trickling filters, lagoon systems have very long HRTs and SRTs, and perform better at removing estrogens.

Lagoons are often used by small municipalities, as they are comparatively inexpensive to build and operate. It has been shown that when lagoons are employed in series, a reduction in the estrogenic potential (as measured by VTG induction in fathead minnows) of the lagoon water occurs with each subsequent lagoon (Bringolf et al., 2003).

Additionally, the reduction in VTG induction is correlated with increased retention time. Long retention time emerges as a common theme among plants with high removal of estrogens. In fact, once study of 18 Canadian plants (Servos et al, 2005) included four lagoon systems. All of these systems had >150 hours HRT and >150 days SRT. The lagoons performed much better than primary treatment, with an average of 93.2% E2 removal and 76.0% E1.

Activated sludge is a process which uses microorganisms to consume a portion of the organic material in wastewater. It characteristically includes primary sedimentation followed by an aeration step to encourage growth of aerobic organisms. Aeration is followed by the separation of solids by sedimentation, and a recycle of a portion of the solids to re-seed the aerobic digester with active microorganisms. Activated sludge plants tend to be more successful at removing E2 than E1. Additionally, higher removal percentages tend to occur in plants with higher SRT and HRT values.

In the previously mentioned study of E1 and E2 in effluents of 18 Canadian wastewater treatment plants (Servos et al., 2005), the authors claimed no statistical correlation between HRT or (solids retention time) SRT and estrogen removal. However, among the activated sludge plants, the two plants with the highest percent removals (98.9% & 98.2% of E2; 97.8% & 95.1% of E1; and 100% YES response) had very high HRTs (28 & 27 h) and SRTs (53 & 35.5

days). While two plants with comparatively low SRT (2.7 and 4.7 days) had elevated levels of estrone in effluent.

Svenson et al (2003) used yeast to quantify estrogenicity in untreated and treated effluents of 20 wastewater plants across Sweden. Estrogenic activity was expressed in terms of E2 equivalents. Those plants with activated sludge averaged 81% removal. The two plants that utilized Nitrogen removal with activated sludge had very high percent removals (>97% & >99%), but also had very high retention times for the biological step of 20 h and 7 days (wetland treatment).

Johnson et al (2005) examined the effluent of 17 sewage treatment works across Norway, Sweden, Finland, the Netherlands, Belgium, Germany France, and Switzerland. For the 16 plants using secondary treatment, E2 was only detected in the effluent of 6 plants (0.7-5.7 ng/L). EE2 was only detected in 2 effluents (<0.8-2.8 ng/L), one Finnish plant, and one Swiss plan tE1 removal rates were weakly correlated with SRT (r^2=0.28, p<5%) and HRT (r^2=0.39, p<5%), but not temperature (r^2=0.005). E1 was detected in the effluent of 13 plants (mean value of 3.0 ng/L). E1 influent concentrations were estimated, and a percent removal was calculated. Of the three plants with >99% removal E1, two utilized activated sludge; one with an HRT of 24 hours, and an SRT of 16 days, the other with 51 hours and 7 days. The third plant was an oxidative ditch with an HRT of 17.5 hours and an SRT of 30 days. In contrast, the worst performing plant, with an E1 removal of 51%, had an HRT of 11.8 hours, and an SRT of 5 days.

Baronti et al (2000) examined the influent and effluent of six Roman activated sludge wastewater treatment plants for estrogens, influent concentrations averaged 80 ng/L E3, 12 ng/L E2, 52 ng/L E1, and 3.0 ng/L EE2. Average percent removals from activated sludge treatment were 95% E3, 87% E2, 85% EE2, 61% E1. Hydraulic retention time for the plants was 12-14 hours.

Kreuzinger et al (2004) compared removal of various EDCs and pharmaceuticals, including the natural estrogens and EE2 in among wastewater plants of different configurations and operating at differing SRTs. Their work showed a correlation between SRT and removal of the EDCs and pharmaceuticals. Implementation of nitrification also resulted in an increase in removal efficiency.

H. Andersen, et al (2003) examined the concentrations of estrogens in the profile of a German activated sludge plant utilizing denitrification/nitrification with an SRT within the activated sludge system of 11-13 days. Influent estrogen concentrations were 65.7 ng/L E1, 15.8 ng/L E2, and 8.2 ng/L EE2. Primary effluent concentrations were 74.9 ng/L E1, 10.9 ng/L E2, and 5.2 ng/L EE2. Effluent concentrations from the first denitrification tank were 37.3 ng/L E1, 10.3 ng/L E2, and 1.5 ng/L EE2. Effluent concentrations from the second denitrification tank were 2.8 ng/L E1, < LOQ E2, and 1.2 ng/L EE2. Effluent concentrations from the nitrification tank were 1.8 ng/L E1, < LOQ E2, and < LOQ EE2. Secondary effluent concentrations were < LOQ for all three estrogens.

Secondary treatment is much more effective at removing estrogens than primary treatment alone. Secondary treatment processes with higher SRT and HRT values tend to exhibit greater removal of estrogens. Trickling filters do not remove a large amount of estrogens, but plants that include additional nutrient removal steps may increase estrogen removal.

2.5 Tertiary and Advanced Treatment

Increasingly, processes beyond secondary treatment are required to provide a higher quality of effluent by removing suspended, colloidal, and dissolved constituents. Higher effluent qualities are often required when wastewater will be used for another purpose, such as irrigation, recharge of ground water supplies, or potential drinking water sources. The removal of suspended and colloidal solids is a physical separation process. Typical advanced treatment techniques employed for this purpose include granular media filtration and membrane filtration. The removal of dissolved constituents generally requires additional membrane filtration or chemical processes. The removal of biological constitutions requires additional disinfection processes, such as UV light, Cl_2 (chlorine), or O_3 (ozone).

Membrane bioreactors (MBRs) are a biological treatment operation. They include an activated sludge process where physical separation of the finished water occurs by passage through a membrane. Membranes are classified by their pore size, which include Micro Filtration (MF), Ultra Filtration (UF). MF systems have the largest pore size (macropores of >50 nm) and typically remove TSS, turbidity, cysts, some bacteria, and viruses. UF systems have slightly smaller pores (mesopores of 2-50 nm), and remove macromolecules, colloids, most bacteria, some viruses, and proteins. MBRs can maintain very high solids concentrations and SRTs in comparison to traditional activated sludge.

Additional membrane processes include nanofiltration (NF) and reverse osmosis (RO). NF systems have small pore sizes (micropores of < 2 nm), and reject small molecules, and viruses. RO systems have a dense pore structure (< 2 nm) and can reject very small molecules and ions. NF and RO membranes typically require an upstream UF or MF membrane to prevent membrane clogging. Thus, NF and RO are often used an additional polishing step to remove more dissolved constituents.

One study compared the removal of micropollutants, including the artificial estrogen EE2, between a traditional activated sludge plant and an MBR pilot plant with a UF membrane operating at varying SRTs. The study found that biological degradation was dependant upon SRT. When the two systems were operated with comparable SRTs, no additional removal occurred through the use of the UF membrane (Clara et al, 2004). This study indicates that the actual removal is due to biological activity, and at the UF membrane pore size, physical separation provided no additional removal of estrogens.

Additionally, at a full scale plant in the UK, sand filtration post secondary treatment was found to provide no additional removal of E1 or E2 (Jiang et al, 2005). This study also indicated a lack of physical separation of the estrogens from wastewater.

However, a different study examined the removal of E1, E2, EE2, and other EDCs, from wastewater utilizing membranes of various pore sizes (Snyder et al, 2006). In some cases, UF and MF membranes allowed estrogens to pass through. However, systems utilizing reverse osmosis (RO) exhibited no detectable estrogens in their effluents. This is likely due to the extremely small pore size of RO membranes, small enough to reject the estrogen molecules.

Braga et al (2005) examined the removal of E1, E2, and EE2 in an Australian advanced secondary plant, which consisted of activated sludge treatment with two sequential batch reactors, an anoxic and aerobic, with a SRT of 16 days and a HRT of 4 hours within the batch reactors. Secondary treatment was followed by continuous micro filtration (CMF), reverse osmosis (RO), and chlorination/dechlorination. The influent and secondary effluent concentrations were 54.8 ng/L and 8.1 ng/L (85% removal) E1, 22.0 ng/L and 0.95 ng/L (96% removal), and less than the limit of quantification (LOQ) for EE2. The CMF influent and effluent concentrations were 4.1 ng/L and 1.2 ng/L (70% removal) E1, 0.75 ng/L and 0.1 ng/L (87% removal) E2, and <LOQ of EE2. The RO effluent and chlorination samples all had concentrations <LOQ for all three estrogens. The additional filtration steps beyond secondary treatment provided additional estrogen removal, with RO removing all estrogens below their LOQ values.

Estrogens may also be removed from water by adsorption. In fact adsorption onto granular activated carbon (GAC) has been shown to remove E1 and E2 from both water and wastewater in batch experiments (Zhang & Zhou, 2005). PAC has also been shown as effective at removing E1, E2, and EE2 from surface water in bench and pilot scales (Snyder et al, 2006). In this same study, it was observed that regular regeneration or replacement of carbon is required for good GAC bed performance.

Estrogens are also susceptible to chemical oxidation as a means of reducing their concentrations. Typical chemical oxidants utilized in wastewater treatment include chlorine and ozone. Oxidative processes are traditionally used in wastewater plants to provide a final pathogen disinfection step prior to the release of effluents into surface waters.

Leush et al (2005) examined the concentrations of E1 and E2 in an advanced biological nutrient removal plant (advanced processes included sand filtration, ozone contact) and UV disinfection, in Australia using GC/MS, and found influent concentrations of 19 ng/L E2 and 45 ng/L E1, and secondary and final effluents below the LOQ for both estrogens. This study also compared results with a breast cancer cell proliferation assay (E-screen), which indicates the full response of cells to a substance. E-screen results indicated no reduction in estrogenicity due to sand filtration. Additionally, the ozonation effluent was exhibited cytotoxicity in the E-screen, indicating that some toxic ozonation products may be formed.

The removal of E1 and E2 using Ferrate (VI) and electrochemical oxidation was examined in the laboratory. Starting concentrations were very high, ranging form 1 to 0.1 mg/L. However, both processes could reduce EDC concentrations to between 20 and 100 ng/L. Ferrate (VI) was more effective for removal (Jiang et al, 2005). Although testing should occur at lower concentrations, these results indicate that Ferrate (VI) may be effective in removing EDCs.

Chlorination was examined for its efficacy at removing E2 and other EDCs from aqueous solutions and decreasing the estrogenic activity of the solution. It was found that chlorine can remove both estrogenic activity and estrogenic chemicals, and may likely do so for other compounds with a phenolic ring. However, elimination of estrogenic activity was reaction time dependant. No significant decrease in E2 was found at a free chlorine dose of 1.5 mg/L for 10 minutes. At this same dose, complete elimination of the estrogenic activity of E2 required longer than 36 hours. A critical C x T (concentration of free chlorine x reaction time) for the removal of E2 was not determined (Lee, 2004).

Oxidation of various pharmaceuticals, including E1, E2, and EE2, using chlorine dioxide (ClO_2) was explored in laboratory studies. In ground water spiked with the estrogens at 1 µg/L each, and dosed with 0.1 mg/L of ClO_2, the estrogens reacted so quickly that their concentrations were below LOQ after just 5 minutes of contact time. Oxidation of estrogens by ClO_2 is an effective treatment method. Additionally, the authors compared the rate of reaction of EE2 with ClO_2 to that with published data from reaction with other oxidants. Ozone (O_3) was provided the fastest reaction rates, followed by ClO_2, and chlorine was the slowest acting oxidant (Huber et al, 2005). In fact, at concentrations normally used for drinking water disinfection, ozone has been shown to reduce the estrogenicity of water spiked with EE2 by a factor of over 200 (Huber et al, 2004). It has also been shown that ozone is effective for oxidizing EE2 (Huber et al, 2005) and E1 (Ternes et al, 2003) within wastewater effluents.

The literature indicates that certain advanced processes, such as carbon adsorption, and RO can completely remove estrogens to below the limit of quantification. However, not all plants utilize these advanced processes. Among the biological process, those with large HRT and SRT values show particular promise for the removal of estrogens. The next chapter will examine what is known about the kinetics of biological removal.

CHAPTER 3:

Kinetics of Estrogen Deconjugation, Degradation, and Adsorption

Within activated sludge plants, the primary pathways for the removal of estrogens are biodegradation (or biotransformation) and adsorption onto solids. Conjugated estrogens must be freed via a bacterial derived enzyme prior to bacterial consumption. This section reviews the literature regarding deconjugation, degradation, and adsorption of estrogens in batch studies and full scale plants.

3.1 Batch Studies of Estrogen Deconjugation & Degradation

It has been established that both estrone-3-sulfate (E1-3S) and estradiol-3-glucuronide (E2-3G) are readily deconjugated by bacteria available in human feces within an aerobic environment (Lombardi et al, 1977). However, not all sulfate associated estrogens can be freed by fecal bacteria. A study isolating steroid desulfating bacteria from feces found that estrone-3-sulfate (E1-3S), and estradiol-3-sulfate (E2-3S) were readily freed by several bacteria strains within feces, but β-estradiol-17-sulfate (E2-17S) was not freed by any bacteria present in feces (Van Eldere et al, 1988).

Evidence exists that wastewater treatment plants deconjugate glucuronide associated estrogens, such as E2-3G (Panter et al, 1999; Ternes et al,1999b) and E2-17G (Ternes et al, 1999b). However, sulfate associated estrogens are suspected to be more recalcitrant to deconjugation and subsequent degradation in waste water treatment. Batch studies were performed on septic tank wastewater spiked with the glucuronide associated estrogens E1-3G, E2-3G, E3-3G, E2-17G, E3-16G; and the sulfate associated estrogens E1-3S, E2-3S, E3-3S. These studies found that all glucuronide associated estrogens were readily deconjugated by the wastewater, with complete deconjugation occurring around 1 day; while sulfate associated estrogens displayed a day or more of lag time before they began to deconjugate (D'Ascenzo et al, 2003). Given typical range of 1.5-3 hours of HRT for high rate aeration processes (Metcalf and Eddy, 2003), it is likely that sulfate associated estrogens will survive wastewater treatment in their conjugated forms.

Once the estrogens are cleaved and in their free form, multiple strains of bacteria exist within wastewater that can degrade E1 and E2 (Yu et al, 2005). Many batch studies exist documenting the first-order degradation of estrogens in aerobic batch systems (D'Ascenzo et al, 2003; Li et al, 2005; Layton et al, 2000; Ternes et al, 1999b). In aerobic batch experiments of wastewater, spiked E2 readily oxidized to E1 within a matter of hours; E1 also readily degraded, but at a slightly slower rate, with about 50% reduction after 24 hours (Ternes &Mueller, 1999).

First-order rate expressions for the decrease in a chemical concentration with time are as follows:

$$\frac{dC}{dt} = -kC \qquad (1)$$

Where C is the concentration of the chemical (mass/volume), t is time, and *k* is the first-order rate constant (1/time). In its linear form, the equation is:

$$\ln\left[\frac{C}{C_0}\right] = -kt \qquad (2)$$

The dependence of a rate constant on temperature is well established and *k* values at varying temperatures can be calculated from a *k* at a known temperature using the following expression:

$$k = k_{20}\theta T^{-20} \qquad (3)$$

A study using biosolids from an American wastewater plant (Layton et al, 2000) spiked with ^{14}C-labeled estradiol performed aerobic batch studies for mineralization to ^{14}C-CO_2 and found first order coefficients that increased with temperature from 0.174 $hour^{-1}$ (5-10 ^oC) to 0.252 $hour^{-1}$ (22-25 ^oC). This study also examined operating conditions and estrogen removal between four plants and found no statistical correlation between hormone mineralization and either BOD removal or between the amount of ^{14}C in remaining in the aqueous phase and suspended solids removal.

A recent Japanese study included batch experiments performed with E2 spiked into activated sludge from a treatment plant at varying estrogen concentrations, temperatures, and microbial population densities (represented as mixed liquor volatile suspended solids, or MLVSS) found the first-order rate expression for the degradation of the estrogen (*k*), increased with MLVSS, and increased with temperature, and ranged from 0.23 $hour^{-1}$ to 4.79 $hour^{-1}$ (Li et al, 2005).

It has also been proven that the kinetics of estrogen biodegradation can vary with oxygen availability (Joss et al, 2004). Batch aerobic (molecular oxygen available in solution), anoxic (oxygen present as nitrate), and anaerobic (no oxygen available) studies were performed for E1 and E2 in samples from a conventional activated sludge wastewater treatment plant with 0.3 gSS/L. The degradation kinetics of E1 were found to be particularly sensitive to the absence of oxygen, with degradation rates of 2.03 $hour^{-1}$ for aerobic conditions, 0.375 $hour^{-1}$ for anoxic conditions, and 0.125 $hour^{-1}$ for anaerobic conditions. The degradation kinetics of E2 were found to be higher than those of E1, and less sensitive to the absence of oxygen with rates of 4.38 $hour^{-1}$ for aerobic conditions, 5.75 $hour^{-1}$ for anoxic conditions, and 2.19 $hour^{-1}$ for anaerobic conditions. Thus, E2 has high rates of biodegradation under all redox conditions, and will likely biodegrade faster than E1 in the limited oxygen environment of sewers.

3.2 Estrogen Partitioning to Solids

The most likely pathways for the removal of estrogens from aqueous mixes are sorption to solids and biodegradation/transformation (Johnson & Sumpter, 2001). As just discussed, the biodegradation of estrogens within aerobic batch studies has been established. This section will discuss previous studies on the partitioning of estrogens to solids.

One indicator of a chemical's tendency to sorb to solids is the octanol-water coefficient, K_{ow}, which is the ratio of the concentration of a chemical in octanol to the concentration in water at equilibrium and at a specified temperature. A larger number means that more of the chemical partitions into octanol. Such chemicals can be said to be more hydrophobic, and more likely to reside on suspended solids in aqueous solutions. Based on measured log K_{ow} of 3.1 for E2 and a modeled log K_{ow} of 4.3 for E1 (Johnson & Sumpter, 2001), both estrogens are considered weakly hydrophobic. Thus, it is unlikely that binding to sludge is the dominant estrogen removal mechanism within wastewater treatment.

A study using biosolids from an American wastewater plant (Layton et al, 2000) spiked with ^{14}C-labeled estradiol performed aerobic batch studies for mineralization to $^{14}C\text{-}CO_2$ and found first order coefficients that increased with temperature. The study also concluded that because mineralization rates of the labeled estrogen were similar to removal rates from the aqueous phase, sorption to solids is not a rate limiting step in the removal of E2.

One study measured the concentrations of estrogens in liquid and solid phases through a German wastewater plant utilizing denitrification-nitrification between two clarifiers (Andersen et al, 2003). This study found that the sum of E2 and E1 concentrations were reduced by >98% from the primary to the secondary clarifier, while the total estrogen concentration on solids remained relatively constant in the two reactors. This indicates that sorption kinetics are slow, and equilibrium is not reached between the dissolved estrogens and that on the solids. The final conclusion of this study was that only about 5% of the estrogens were sorbed onto solids.

Further evidence of the absence of equilibrium between sorbed and dissolved estrogens is provided by a pilot plant at the University of Cincinnati (Suidan et al, 2005). In this study, E2 exhibited 100% removal from primary to secondary effluents for both aqueous phase and in sludge. However, E1 exhibited 100% removal from primary to secondary solids, but only 88% removal from the aqueous phase. In short, 5 ng/L of E1 was found in the final effluent, but no E1 was found in the waste activated-sludge. This may be due to a greater SRT than HRT. Whatever the reason, it indicates a lack of equilibrium between aqueous and sorbed E1. This provides further evidence sorption is not a major mechanism for the removal of estrogens in wastewater treatment.

The Freundlich Isotherm gives the relationship between the amount of a chemical in aqueous solution and the amount adsorbed to solids as follows:

$$C_S = K_F g C_{aq}^{1/n} \quad (4)$$

Where CS is the concentration of estrogens adsorbed onto solids (ng/g SS), Caq is the aqueous estrogen concentration of estrogens (ng/L), and K_F is the capacity parameter ((ng/g)*(L/g)1/n), and 1/n is a dimensionless number accounting for adsorption site energy.

It has been shown that for E1 and E2, in the concentration range of concern in wastewater treatment, from low ng/L to high μg/L, the isotherm is linear and Freundlich 1/n = 1 (H. Andersen et al, 2005). Thus, the equation above reduces to:

$$K_D = \frac{C_{s,\ floc}}{C_{aq,\ floc}} = \frac{C_{s,\ reactor}}{(SS\ g C_{aq,\ floc})} \quad (5)$$

Where $C_{s,\ floc}$ is the mass of sorbed estrogens per mass of solids (g/gSS), and $C_{s,\ reactor}$ is the mass of sorbed estrogens per reactor volume (g/L).

Substituting eq 5 into eq 4 gives the following expression (Joss et al, 2004):

$$r = -k_{sor}\ (SS \bullet C_{aq,\ bulk} - (C_{s,\ reactor}/\ K_D\ )) \quad (6)$$

By using a first-order model for sorption, Schwarzenbach et al (2003) came up with the following expression:

$$C_s(t) = C_{s,\ eq} + (C_{S,\ 0} - C_{s,\ eq}) \bullet e^{-k_{sor}(SS-(1/K_D))t} \quad (7)$$

Where $C_s(t)$ is the amount of sorbed estrogen per reactor volume (ng/L) as a function of time, $C_{s,\ eq}$ is the mass of sorbed estrogen per reactor volume (ng/L) in equilibrium with the soluble estrogens, and $C_{S,\ 0}$ is the initial sorbed estrogen concentration per reactor volume (ng/L).

Recent batch experiments determined the sorption of estrogens onto activated sludge from a Dutch wastewater treatment plant (Andersen et al, 2005). K_D values were found to be constant with the range of low ng/L to high μg/L, and were 402± 126 L/kg for E1 and 476± 192 L/kg for E2. Thus, K_D values will likely not vary within estrogen concentration ranges typically found in wastewater. This study also calculated the estimated amount of estrogens removed with excess sludge as 1.5 -1.8% of the total estrogen loading, assuming equilibrium conditions.

However, as indicated in previous studies (Andersen et al, 2003; Layton et al, 2000; Suidan et al, 2005), equilibrium may not exist between sorbed and aqueous estrogens in sewage treatment. Whether or not equilibrium can be assumed, biodegradation, not sorption, remains the dominant mechanism for the removal of estrogens in wastewater systems.

CHAPTER 4:

Estrogen Excretion

The goal of this research was the development of a model for the prediction of influent estrogen concentrations at wastewater treatment plants. Within the model, initial estrogen concentrations, prior to sewer transit, are estimated based on a per capita excretion estimate. Thus, a literature search was performed to determine the amount of each estrogen excreted by males, non-gravid females, gravid females, and menopausal males. The excretion values found in the literature are presented in this section.

4.1 Estrogen Excretion Values

The table below reflects data sources found in the literature providing values of estrogen excretion within urine. Data on the amount of free estrogens in urine is difficult to find because it exists in such small amounts. In fact, leading researchers developing analytical methods of determining estrogen concentrations within urine focus on the conjugated forms of estrogens, and consider the free forms of minimal contribution (Aldercreutz et al, 2004).

Urinary Excretion Values in μg/24 hours									
	E1 total	E1 free	E1-3S	E1-3G	E2 total	E2 free	E2-3S	E2-3G	E2-17G
Males (literature)	3.89[d], 2.8[e], 5.41[f]	---	---	---	1.53[d], 1.6[e], 3.00[f]	---	---	---	---
Non-gravid Females (literature)	7.79[c1], 2.66[c2], 14.6[d], 15[e], 8.79[b]	--	5.4 [a]	16[a]	2.89[c1], 1.09[c2], 6.78[d], 5.4[e], 4.53[b]	--	3.5[a]	5.5[a]	2.5[a]
Gravid Females	1480[e]	---	450[a]	490[a]	360[e]	---	64[a]	104[a]	90[a]
Menopausal Females (literature)	3.95[d], 1.48[b]	---	3.2[a]	9.5[a]	2.32[d], 0.75[b]	---	1.3[a]	4.2[a]	1.5[a]

[a]D'Ascenzo et al, 2003.
[b]Key et al, 1996
[c]Aldercreutz et al, 1994 (c1 = Caucasians in Helsinki. c2 = Orientals in Hawaii)
[d]Fotsis & Aldercreutz, 1987
[f]Hämäläinen et al, 1987

The table below shows values for free fecal estrogens and total fecal estrogens, as provided by the literature. Values for the fecal excretion of estrogens in conjugated form were found as total conjugates, and it is difficult to find data on the distribution of conjugates between the sulfate and glucuronide associated forms. This is due to the fact that the majority of the estrogen in feces exists in the free form.

Feces Excretion Values in μg/24 hours									
	E1 total	E1 free	E1-3S	E1-3G	E2 total	E2 free	E2-3S	E2-3G	E2-17G
Men	0.247[a], 0.428[c]	0.201[a], 0.404[c]	---	---	0.175[a], 0.361[c]	0.170[a], 0.351[c]	---	---	---
Women	0.836[a]	0.210[a], 0.308[b1], 0.619[b2]	---	---	0.987[a]	0.956[a], 0.240[b1], 0.564[b2]	---	---	---
Preg W	98.2[d], 97.75[e]	96.5[d], 96.05[e]	---	---	203.4[d], 207.55[e]	203[d], 207.3[e]	---	---	---
Menopausal	---	0.127[a]	---	---	---	0.0926[a]	---	---	---

[a]Aldercreutz & Järvenpää, 1982
[b]Aldercreutz et al, 1994 (b1 = Caucasians in Helsinki. b2 = Orientals in Hawaii)
[c]Hämäläinen et al, 1987
[d]Aldercreutz et al, 1976
[e]Aldercreutz & Martin, 1976

CHAPTER 5:

Existing Models for Estrogens in Wastewater Collection and Treatment Systems

There have been two main attempts at modeling the fate of estrogens in wastewater treatment (Johnson & Williams, 2004; Joss et al, 2004). These models are limited by the accuracy of estimates for the concentrations of estrogens entering wastewater treatment plants. Thus, it is important to develop an accurate model for the fate of estrogens during sewer transit. This section reviews these models.

5.1 Models of Estrogens in Wastewater Collection & Treatment

Few attempts have been made at modeling the fate of estrogens within sewer systems. One early attempt at modeling the influent concentrations of estrogens to wastewater treatment plant consisted of the following simple equations for estrone (E1) and estradiol (E2) (Johnson et al, 2000):

$$\text{E1 ng/L} = P / 114.3\ F \qquad (8)$$

$$\text{E2 ng/L} = P / 23.64\ F \qquad (9)$$

Where P is the population served by the wastewater treatment plant and F is the flow ($m^3\ day^{-1}/1000$). These equations were arrived at by inputting known excretion data for various fractions of the population, and known sewer flows, and obviously did not account for the kinetics of degradation/transformation of the estrogens during sewer transit.

The final model by Johnson (Johnson & Williams, 2004) includes modeling both the influent and effluent concentrations at wastewater treatment plants. The influent estrogen concentrations are once again estimated using plant inflow and population. However, estrogens are accounted for in both urine and feces and in free and conjugated forms. The overall equation for the amount of estrogens arriving at a wastewater treatment plant is calculated by Johnson & William's (2004) using the following equation

$$S_T = (1-k_T)(U_T + F_T) + S_S \qquad (10)$$

Where S_T is the total of an estrogen (in free and conjugates forms), F_T is the total amount excreted in the feces (μg/d). U_T is the total amount excreted in the urine (μg/d). k_T is the overall fraction of the steroid "lost" during sewer transit, and is assumed to be zero for E1 and 0.5 for E2. Thus, the assumption is made that no degradation of E1 occurs within the sewers, while E2 is degraded by 50%. S_S is the generation within the sewers of an estrogen from another form (such as the oxidation of E2 to E1). This mass balance is performed for the following population groups: males, menstruating aged females, pregnant females, and menopausal females. A weighted sum is performed based on the fraction of the population that each group represents as follows for urine (Johnson & Williams, 2004):

$$U_T = \sum_{i=1}^{n} f_i (U_i^{'} + U_i^{g} + U_i^{s}) \tag{11}$$

Where f_i is the fraction of the population represented by each of the population groups, $U_i^{'}$ is amount of an estrogen in a particular form excreted in the urine by said population group (µg/d), and U_i^g is the amount of glucuronide associated estrogen excreted in the urine by the population group (µg/d), and U_i^s is the amount of sulfate associated estrogen excreted in the urine by the population group (µg/d).

A similar weighted sum is used to determine the fecal excretion (Johnson & Williams, 2004):

$$F_T = \sum_{i=1}^{n} f_i F_i \tag{12}$$

Where F_i is the amount of a particular estrogen excreted in the feces of a population group (µg/d). All fecally excreted estrogens are assumed to exist in their free and active form.

This model is lacking in any kinetic constants for degradation within sewage collection systems, and assumes the same 50% reduction of E2 regardless of initial concentration (which may vary widely based on the percentage of domestic contribution to the sewer flow) or mean sewer residence time. This model underestimated influent estrogen concentrations. When the mean predicted influent estrogen concentrations were divided by observed values, the model was found to predict approximately 78% of the actual E1 influent and 85% of the E2 influent.

Additionally, the model does not include any kinetic constants for the degradation of estrogens within wastewater plants, but simply predicts removal based on mean removal rates from wastewater treatment plants globally. Thus, the influent E1 is assumed to decrease by 64.7% and E2 by 81.7%, regardless of plant operating and flow conditions.

One other attempt exists at modeling of estrogens within wastewater plants. This study does not include an estimate of influent estrogen concentrations. Joss et al (2004) attempted to account for sorption of estrogens to sludge, as previously presented in equations 13-16.

$$r = -k_{sor} \bullet SS \bullet (C_{aq,\ bulk} - C_{aq,\ floc}) \quad (13)$$

$$K_D = \frac{C_{s,\ floc}}{C_{aq,\ floc}} = \frac{C_{s,\ reactor}}{(SS\ g C_{aq,\ floc})} \quad (14)$$

$$r = -k_{sor}\ (SS \bullet C_{aq,\ bulk} - (C_{s,\ reactor}/\ K_D\ )) \quad (15)$$

$$C_s(t) = C_{s,\ eq} + (C_{S,\ 0} - C_{s,\ eq}) \bullet e^{-k_{sor}(SS-(1/K_D))t} \quad (16)$$

However, sensitivity analysis of their model showed that model results were insensitive to the partitioning of estrogens to sludge. The lack of importance of sorption as a removal mechanism for estrogens was further verified by the fact that when this model was applied to a full scale wastewater plant, the amount of estrogens lost to sludge production was estimated at < 0.5 ng/L of the influent estrogen load.

In summary, an adequate model exists for the fate of estrogens within wastewater treatment plants (Joss et al, 2004). To be utilized as a predictive tool, this model must be paired with accurate predictions of influent estrogen concentrations. As previously stated, the existing model for the fate of estrogens in wastewater systems (Johnson & Williams, 2004), fails to account for the first order degradation of estrogens during sewer transit. Thus, a model was developed to predict the influent estrogen concentrations at wastewater treatment facilities. It is presented in Chapter 6, as an article under review for publication in *Water Environment Research.*

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The article presented in this chapter is currently under review for publication in *Water Environment Research.*

CHAPTER 6

Predicting Influent Estradiol and Estrone Concentrations for Wastewater Treatment Facilities

Abstract

A model was developed for the fate of the natural estrogens estrone (E1) and estradiol (E2) in municipal sewage collection systems. Six municipalities throughout the Northern Hemisphere provided data to aide in the calculation of the biotransformation/degradation kinetics of the natural estrogens estrone (E1) and estradiol (E2) in wastewater collection systems. The mean pseudo-first-order kinetic constant for the biotransformation of E2 into E1, kE2, was 0.030 hr-1 (ranging from -0.080 to 0.49 hr-1) and the mean value of the pseudo-first-order kinetic constant for the biodegradation of E1, kE1, was -0.18 hr-1(ranging from -0.44 to 0.38 hr-1) The mean total suspended solids concentration was 0.256 g/L (ranging from 0.103 to 0.450 g/L), and mean temperature was 16.8ºC (ranging from 12 to 24.5 ºC). Biodegradation k values were not found to be temperature sensitive within this range. Values for kE2 and kE1 displayed a trend of increasing with total suspended solids concentration.

Introduction

Estrogens are phenolic steroids that target the tissues of the uterus, vagina, oviduct, mammary gland, and parts of the brain (Paqualini, 1976). Estrogens have a common ring structure of three six-membered rings, one five-membered ring; with eighteen carbon atoms, and a hydroxyl group off the carbon 3 position (Schuluster, 1976). However, estrogens vary in which functional groups are attached to the 16 and 17 positions of the carbons. The primary endogenous estrogen which binds to the human estrogen receptors is estradiol (E2), which can be oxidized in metabolic processes to form estrone (E1)(Lai, 2003). They occur naturally in both men and women, and are excreted in the feces and urine of both sexes.

Although wastewater treatment does remove a significant amount of estrogens, some estrogens survive wastewater treatment and enter surface water through wastewater treatment plant effluents. Estrogen concentrations in the ng/L range have been well documented in the effluents of sewage treatment plants all over the world (Desbrow et al, 1998; Lee & Pert, 1998; Belfroid et al, 1999; Ternes et al, 1999a; Baronti et al, 2000; Johnson et al, 2000; Matsui et al, 2000; Nasu et al, 2001; H. Anderson, et al, 2003; Verstraeten et al, 2003; Joss et al, 2004; Johnson et al 2005; Servos et al, 2005).

Estrogens are known to cause estrogenic endocrine responses in aquatic species (Norris et al, 1997; Routledge et al, 1998; Thorpe et al, 2000; Länge et al, 2001; Metcalfe et al, 2001; L. Andersen et al, 2003; Lokman et al, 2003). Endocrine disrupting chemicals (EDCs) alter or interfere with the normal functions of one or more of the human body's three hormone systems: androgen, estrogen, and thyroid. Estrogenic effects include abnormalities that diminish the ability of aquatic species to reproduce. Wastewater effluent has been documented to cause estrogenic effects in aquatic species (Purdom et al, 1994; Hemming et al, 2001; Minier et al, 2000; Solé et al, 2000; Rodgers-Gray et al, 2001; Petrovic et al, 2002; Bőgi et al, 2003). EDCs are released into our surface waters, through various sources, such as wastewater effluents, and agricultural and confined feeding operations runoff. Additionally, within aqueous mixtures of estrogens, the total estrogenic activity is a result of the additive effects of the individual estrogenic chemicals (Thorpe et al, 2001; Thorpe et al, 2003). As a result of the presence of EDCs in surface water environments, sexual disruption of aquatic species has been observed in the wild (Jobling et al, 1998; Minier et al, 2000; Van Der Kraak et al, 2001; Vigano et al, 2001; Gercken & Sordyl, 2002; Bőgi et al, 2003).

Another natural estrogen exists, estriol (E3), which may also be present in wastewater plant effluents. A study of seven US wastewater facilities found that the majority of the estrogenic activity in both the primary and secondary effluents was due to the occurrence of two natural hormones E2 and E3 (Drewes et al, 2005). However, E3 is considered 300 times less active than E2 as an endocrine disruptor (Arnold, 2002). Additionally, one study examined the concentrations of the natural estrogens at six Roman wastewater treatment plants and found that although E3 had the highest average influent concentration of 80 ng/L (compared to 12 ng/L of E2 and 52 ng/L of E1), it also had the highest removal, 95%, in activated sludge plants (compared to 87% of E2 and 61% of E1) (Baronti et al, 2000). Due to the lack of potency of E3, and the low concentrations at which is found, E3 is most often neglected in studies examining the endocrine disrupting potential of sewage effluent.

Other chemicals present in wastewater, such as nonylphenols (NPs), can also cause estrogenic responses in animals by acting as estrogen mimics. However, NP is less of a concern as nonylphenol is considered 100 times less estrogenically active than E2 (Arnold, 2002). Additionally, in one German study, phenolic xenoestrogens were responsible for only 0.7-4.3% of the estrogenic activity of sewage treatment plant effluent (Korner et al, 2000). Thus, NPs do not emerge as major estrogenic EDCs in wastewater effluent.

In addition to the natural estrogens, wastewater effluent may contain artificial estrogens used as birth control or in hormone replacement therapy. The artificial estrogen ethinylestradiol (EE2), a major component of most birth control pills, is among the most commonly used of these hormones. EE2 has also been found in some wastewater effluents and is known to cause endocrine disruption. However, the use of contraceptive pills varies greatly between cultures. The world average of pill use for women aged 15-49 is 15.9 % for developed nations and 6.2% among the less developed. But even among developed nations, pill use varies greatly. Western Europeans are among the highest users of the pill, with an average of 46.9% for the region, and a high of 58.6 % among Germans. Only 12.8% of Southern European women use the pill, with Italian use at 13.6%. While 24% of Australian women use the pill, only 15.5 % of North American women use oral contraception (UN, 2005). Thus, the presence and relevance of EE2 in wastewater will vary with respect to the cultural context of the wastewater.

Given the comparative rates of pill use between the countries, It should be of no surprise that higher average influent EE2 concentration (8.2 ng/L) was found at a German plant (Andersen et al, 2003), in comparison the average influent at five Italian plants was 3.1 ng/L (Johnson et al, 2000). Irregardless of influent concentration, EE2 in the effluent was minimal, with the German plant and three of the five Italian plant effluent EE2 concentrations less than the limit of quantification. The examination of seven British domestic wastewater effluents found that, although EE2 did emerge as a component responsible for the estrogenicity of wastewater effluent, EE2 was below the limit of detection in 2/3 of the samples taken; and undetectable in four of the effluents (Desbrow et al, 1998). While the previously mentioned study of six Roman activated sludge treatment plants found a median effluent concentration of 0.45 ng/L of EE2 (Baronti et al, 2000). A study of two Australian wastewater plants did not detect EE2 in raw sewage (Braga et al, 2005). Five Dutch wastewater plant effluents were examined and among ten samples taken, EE2 was detected only twice (Belfroid et al, 1999).

One study found EE2 to be a contributing component to the estrogenic activity of two out of three Michigan wastewater treatment plant effluents (Snyder et al, 2001). However, when the concentrations of each estrogen were expressed in terms of 17β-estradiol equivalents (EEQs), E2 was contributed approximately three times more EEQs in the effluent than EE2. The third plant found no EE2 contributing to the estrogenic activity of the effluent. In Japan, estrogenicity in the effluent of the Shiga prefecture wastewater treatment plant was proven to be almost completely due to the presence of E2, despite the fact that E2 was responsible for only 34% of the estrogenicity of the raw sewage (Matsui, et al, 2000).

EE2 is produced by the chemical industry for use as a birth control. It is theoretically possible that a more benign alternative may be developed. Hormonal birth control methods may also be replaced with non-chemical, barrier, methods. However, E1 and E2 are naturally excreted from humans, and cannot be eliminated from our waste streams. Additionally, EE2 use varies greatly between cultures. In comparison, E1 and E2 are naturally occurring hormones, and will be EDCs of concern within wastewater across the globe. For these reasons, this effort has focused on the natural estrogens. Future modeling efforts could address EE2 and other synthetic pharmaceuticals.

The natural estrogens can be excreted either as free steroids or as conjugates. Estrogens may be associated with of sulfuric or glucuronic acids. The conjugates are not biologically active as free steroids until environmental bacteria deconjugate them. Both sulfate and glucuronide associated estrogens can be deconjugated by bacteria available in human feces (Lombardi et al, 1978), and as a result, conjugated estrogens can be freed during wastewater treatment (Panter et al, 1999). Glucuronide associated estrogens deconjugate quickly in wastewater environments, while sulfate associated estrogens are relatively recalcitrant to deconjugation (Van Eldere et al, 1988; Ternes et al, 1999b; D'Ascenzo et al, 2003). Deconjugation of the estrogens follows a pattern of pseudo-first-order degradation.

Within wastewater, multiple strains of bacteria exist that can biodegrade both E1 and E2 once the estrogens are cleaved and in their free form (Yu et al, 2005). Many batch studies exist documenting the first-order biodegradation of estrogens by the bacteria present in wastewater (Ternes et al, 1999b; Layton et al, 2000; D'Ascenzo et al, 2003; Li et al, 2005).

Although models exist for the fate of estrogens within wastewater treatment plants (Johnson & Williams, 2004; Joss et al, 2004), only one model attempts to estimate influent estrogen concentrations at wastewater treatment plants (Johnson & Williams, 2004). An earlier version of Johnson's model predicted influent estrogen concentrations based solely upon a per capita estrogen excretion estimate, the population served by a wastewater treatment plant, and the volumetric flow into the plant (Johnson et al, 2000). The model did not account for any changes in estrogen concentration during sewer transit.

A later version of Johnson's model made the assumption that 50% of E2 is converted to E1 during sewer transit, regardless of initial concentration (which may vary widely based on the percentage of domestic contribution to the sewer flow) or mean sewer residence time (Johnson & Williams, 2004). However, this model did provide a thorough literature review of the varying excretion amounts of E1 and E2 in the urine and feces of various population groups (males, menstruating aged females, gravid females, and menopausal females). Based on this data, and other assumptions that will be discussed later, the Johnson model included an estimate of per capita excretions of E1 and E2, which was utilized in this modeling effort. On average, the Johnson model tended to predict about 78% (ranging from 56 to 100%) of influent E1 concentrations and 85% (ranging from 67 – 110%) of E2. The objective of this work was to improve upon those efforts.

Due to time and cost constraints, influent estrogens are not normally measured as a part of the operations monitoring at wastewater treatment plants. Accurately predicting the removal of estrogens in wastewater systems depends upon developing a more accurate estimate of wastewater treatment plant influent concentrations. Thus, a comprehensive model for the fate of estrogens within wastewater systems will require the determination of the kinetics of estrogen biodegradation during sewer transit.

Model Development

The Figure 1 shows a graphical depiction of the estrogen sources and chemical pathways included in the model. Urinary and fecal sources for E1 and E2 are included, along with deconjugation, the oxidation of E2 to E1 (via biodegradation) and ultimate biodegradation of the free estrogens.

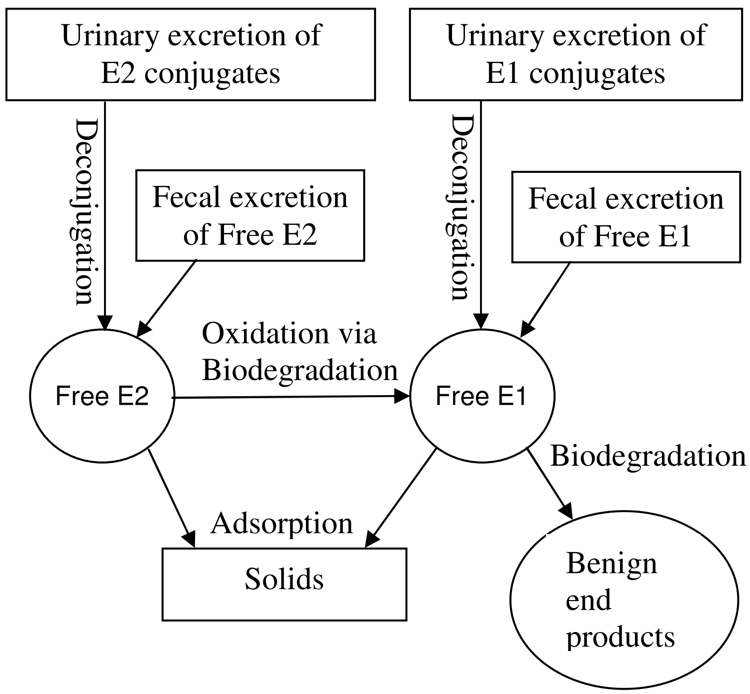

Figure 1: Schematic of the model including urinary and fecal sources, adsorption to solids, and the biodegradation of E1 and E2.

Eight municipalities from Europe and North America were selected on the basis of having published influent-estrogen concentration data (Lee & Peart, 1998; Joss et al, 2004; Verstraeten et al, 2003; Servos et al, 2005), and agreeing to participate in the study. The municipalities provided additional data regarding plant inflow, mean sewer residence time, influent temperature, influent total suspended solids concentrations, and population served. Six of these data sets (identified as plants 3-8) were used to determine the model kE1 and kE2 values, while two data sets (plants 1 and 2) were reserved to verify the model.

Estrogen Excretion per Capita

As fecal matter is abundant in bacteria that can deconjugate estrogens, the majority of the estrogen in feces exists in the free form. In comparison, healthy urine is relatively sterile, and urinary estrogens exist primarily as sulfate and glucuronate conjugates (Key et al, 1996). Among the estrogen conjugates, glucuronate associated estrogens deconjugate readily, while sulfate associated estrogens survive sewer transit relatively intact (D'Ascenzo et al, 2003).

Johnson et al (2004) performed an estimate of free estrogen excretion per capita, which assumes that all estrogens excreted within the feces are in the free form, and the glucuronate associated estrogens excreted within urine readily deconjugate. Their per capita excretion estimates are extremely well researched, accounting for the varying contributions of males, menstruating-aged females, menopausal females, and pregnant females. The mean (and range) values of 10.5 (7.2 – 13.4) µg/d of E1 excretion and 6.6 (5.3 – 8.4) µg/d of E2 excretion provided by Johnson et al (2004) are used within this model. Their model also assumed a that 50% of the E2 concentration was transformed into E1 during sewer transit. However, the per capita excretion values utilized in this effort do not make that assumption. It should be noted that the per capita estrogen excretion represents the total amount of free estrogens, including both aqueous and adsorbed estrogens.

Modeling Initial Sewer Concentrations

For the purpose of estimating the biotransformation kinetics of estrogens within sewage collection systems, an estimated initial concentration of estrogens is necessary, and was determined as follows:

$$C_{T,0,free} = \frac{1000\frac{ng}{\mu g} \cdot E_{free} \cdot P}{Q} \qquad (1)$$

Where CT,0,free is the total initial free estrogen concentration, representing the sum of the aqueous and sorbed phases of an estrogen in the sewer system (ng/L). Efree is the total amount of a free estrogen excreted (µg/person-d), as provided by Johnson et al (2004). P is the population served by a wastewater treatment plant. Q is the plant inflow (L/d). Eq. 1 was applied for both E1 and E2.

Modeling Solids Partitioning

The most likely pathways for the removal of estrogens from aqueous mixtures are sorption to solids and biodegradation (Johnson & Sumpter, 2001). However, sorption to solids does not pose itself as a dominant removal mechanism for estrogens in wastewater.

Batch studies have indicated that degradation of estrogens in the aqueous phase, and not adsorption to solids, is the dominant removal mechanism for estrogens in wastewater treatment (Layton et al, 2000). Full and pilot scale studies of wastewater treatment plants have indicated that equilibrium is not maintained between the dissolved estrogens and estrogens adsorbed onto the solids (H. Andersen et al, 2003; Esperanza et al, 2004). This lack of equilibrium is likely due to the extremely slow adsorption kinetics of estrogens.

For purposes of this model, it is assumed that the initial concentration of adsorbed estrogens is in equilibrium with the initial concentration of aqueous estrogens. However, as biodegradation occurs to estrogens in the aqueous phase, it is assumed that the concentration of adsorbed estrogens remains constant. Thus, adsorption equilibrium is not maintained as the estrogens biodegrade from the aqueous phase.

The Freundlich Isotherm gives the relationship between the amount of a chemical in aqueous solution and the amount adsorbed to solids as follows:

$$C_S = K_F \cdot C_{aq}^{1/n} \qquad (2)$$

Where CS is the concentration of estrogens adsorbed onto solids (ng/g SS), Caq is the aqueous estrogen concentration of estrogens (ng/L), and KF is the capacity parameter ((ng/g)*(L/g)1/n), and 1/n is a dimensionless number accounting for adsorption site energy.

It has been shown that for E1 and E2, in the concentration range of concern in wastewater treatment, from low ng/L to high µg/L, the isotherm is linear and Freundlich 1/n = 1 (H. Andersen et al, 2005). Thus, the equation above reduces to:

$$K_F = K_D = \frac{C_s}{C_{aq,0}} \qquad (3)$$

Where KD (L/g) is the distribution coefficient of the estrogens between the adsorbed and aqueous phases, CS is the concentration of estrogens adsorbed onto solids (ng/g SS), and Caq,0 is the initial aqueous estrogen concentration of estrogens (ng/L).

Batch experiments determined the sorption of estrogens onto activated sludge from a Dutch wastewater treatment plant (H. Andersen et al, 2005). KD values were found to be constant with the range of low ng/L to high µg/L, and were 0.402± 0.126 L/g for E1 and 0.476± 0.192 L/g for E2. Thus, KD values will likely not vary within estrogen concentration ranges typically found in wastewater.

A simple mass balance can be performed accounting for both aqueous and adsorbed estrogens:

$$C_{T,0} = C_{aq,0} + SS \cdot C_s \tag{4}$$

Where CT,0 represents the initial total amount of a free estrogen present (ng/L), Caq,0 is the initial aqueous estrogen concentration (ng/L), CS is the concentration of estrogens adsorbed onto solids (ng/g SS), and SS is the suspended solids concentration (g/L). Eq. 4 was applied for both E1 and E2.

Eq. 3 can be rearranged as an expression for Caq,0. When this is substituted into Eq. 4, and rearranged, the result is the following expression for the amount of estrogens adsorbed onto solids:

$$C_s = \frac{K_D C_{T,0}}{(1 + K_D \text{g} SS)} \tag{5}$$

When the total amount of estrogens (as estimated by Eq.1) and suspended solids concentrations (measured at wastewater treatment plant influents) are known, the amount of estrogens adsorbed onto solids can be calculated using published KD values (H. Andersen et al, 2005). Eq. 5 was applied for both E1 and E2. Once the initial total and adsorbed estrogen concentrations (CT,0 & Cs) are known, the initial aqueous estrogen concentration (Caq) prior to sewer transit was calculated as follows:

$$C_{aq,0} = C_{T,0} - C_s \text{g} SS \tag{6}$$

Estrogen Biotransformation/degradation

One of the model assumptions is that no transformation or degradation of sorbed estrogens will occur. Thus, biodegradation will only happen to free estrogens in the aqueous phase, Caq,0,free. In batch experiments and in full scale wastewater treatment pseudo-first-order degradation of aqueous estrogens occurs (Ternes et al, 1999b; Layton et al, 2000; D'Ascenzo et al, 2003; Li et al, 2005). This model assumes that E2 is transformed into E1 via biological activity. Thus, the rate of biotransformation of E2 can be written as:

$$r_{E2} = -k_{E2} C_{aq,freeE2} = \tfrac{d}{dt} C_{aq,freeE2} \tag{7}$$

Where kE2 is the pseudo-first-order rate constant for the biotransformation of E2, in units of 1/hours. Eq. 7 can be integrated and rearranged to yield the equation for the aqueous concentration of free and aqueous E2 arriving at the influent to a wastewater treatment plant after sewer travel time, t, as follows:

$$C_{aq,freeE2} = C_{aq,0,freeE2} e^{-k_{E2} t} \quad (8)$$

Caq,0,freeE2 is a an estimate of the representative initial aqueous concentration of free E2 in a sewer system prior to sewer transit, in ng/L. Caq,freeE2 is the concentration of free and aqueous E2 measured at the influent to the wastewater plant (ng/L) after sewer transit time t, in hours. The first-order rate expression for the biotransformation of E2 can be determined by the rearrangement of Eq. 8, as follows:

$$k_{E2} = -\frac{1}{t} \ln\left(\frac{C_{aq,t,freeE2}}{C_{aq,o,freeE2}} \right) \quad (9)$$

Eq. 8 was used to determine kE2 values for the biotransformation of E2 into E1 in six municipal sewer systems throughout Europe and North America. Values for the biotransformation of E2 into E1, kE2, ranged from -0.082 to 0.49 hr-1, with a mean value KE2 of 0.030 hr-1.

For E1, first order biodegradation is also assumed to occur. However it is also assumed that E2 is oxidized into E1 via biotransformation. Thus, the rate of disappearance of E2 is considered equal to the rate of appearance of E1. The overall rate expression for E1 then becomes the following first order non-homogeneous linear differential equation:

$$r_{E1} = k_{E2} C_{aq,freeE2} - k_{E1} C_{aq,freeE1} = \tfrac{d}{dt} C_{aq,freeE1} \quad (10)$$

The final concentration of E1, accounting for continuing biotransformation of E2 into E1, and biodegradation of E2 can be approximated using the following method:

At t = 0:

$$C_{aq,freeE2} = C_{aq,0,freeE2} \quad (11)$$

$$C_{aq,freeE1} = C_{aq,0,freeE1} \quad (12)$$

For ti = ti-1 + dt to tn = t

$$C_{aq,freeE2}(t) = C_{aq,freeE2}(t-1) e^{-k_{E2} dt} \quad (13)$$

$$dC_{aq,freeE2} = C_{aq,freeE2}(t-1) - C_{aq,freeE2}(t) \quad (14)$$

$$C_{aq,freeE1}(t) = C_{aq,freeE1}(t-1)e^{-k_{E1}dt} + dC_{aq,freeE2} \quad (15)$$

Iterative calculations were performed (utilizing a time step of 0.1 hours) to determine the kE1 value which would result in the least difference between the calculated and measured influent Caq,freeE1 concentration at a wastewater treatment plant, after sewer transit time, t in hours. Values for the biodegradation of E1 ranged from -0.44 to 0.38 h-1, with a mean value of -0.18 h-1.

Model Verification

Once the average kE1 and kE2 values were determined, these values were used to approximate the aqueous concentration of both E1 and E2 arriving at a wastewater treatment plant. Data from two municipalities (plants 1 & 2) was reserved for this verification. Eq.1 is used to approximate CT,0,free from the per capita estrogen estimates. Once the total, aqueous and adsorbed, estrogen concentration is known, Eq. 5 is used to determine concentration of the estrogen on solids, Cs. Then, Eq. 6 was used to approximate the initial aqueous concentration of an estrogen prior to transit within the sewer system. Eq. 8 was used to estimate the aqueous concentration of E2 arriving at a wastewater treatment plant influent. The numerical method described in Eqs. 11-15 were used to determine the aqueous concentration of E1 arriving at a wastewater treatment plant influent.

Results & Discussion

The current model accounts for the adsorption of estrogens onto solids, the kinetics of estrogen biodegradation, and sewer transit times. A comparison of the actual and model predicted influent estrogen values for plants 1 & 2 is shown in Figure 2. When mean predicted influent aqueous estrogen concentration was divided by the mean measured values for the plants in the study, the model predicted on average 92% (85-98%) of E1 and 96% (77-116%) of E2. In comparison, the Johnson model predicted a mean of 78% (56-100%) of E1 and 85% (67-110%) of E2.

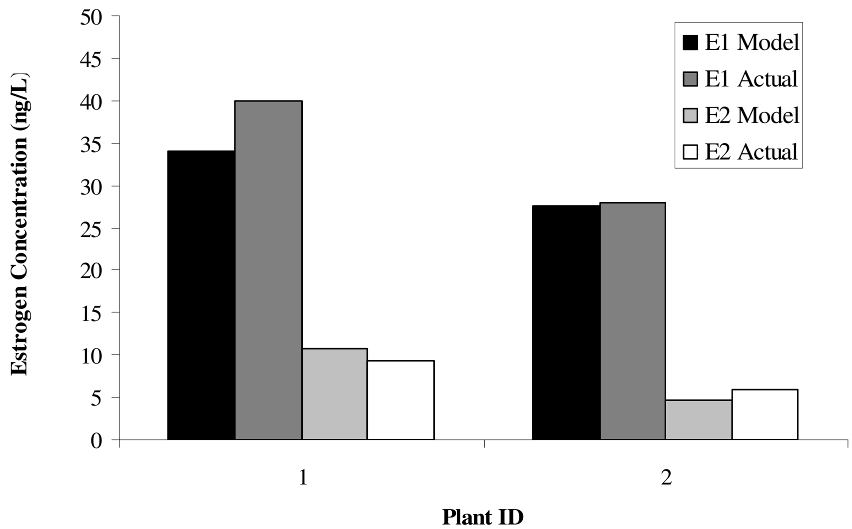

Figure 2: Model predicted and actual aqueous influent estrogen concentrations for Plants 1 & 2.

The kE1 and kE2 values for plants 3-8, used to determine the model mean value, are shown in Table 1. The range of kE1 and kE2 values is based on the excretion estimate range utilized. As stated previously, values for the biotransformation of E2 into E1, kE2, ranged from -0.082 to 0.49 hr-1, with a mean value KE2 of 0.030 hr-1. Although the overall mean kE2 value is positive, the majority of the kE2 values are negative, indicating a generation of E2 during sewer transit. In fact, for the mean kE2 value of 0.030 hr-1 the standard deviation is 0.23. Thus, the kE2 value varies greatly between systems.

Values for the biodegradation of E1 ranged from -0.44 to 0.38 h-1, with a mean value of -0.18 h-1. The negative kE1 value indicates a generation, rather than degradation, of E1 during sewer transit. This could indicate the generation of E1 from E2. However, among the data sets used to develop the model the amount of E2 transformed only accounts for a mean of 6.9% (ranging from 5.8% to 7.6%) of the amount of E1 generated. The transformation of E2 into E1 does not account for the majority of E1 generation.

Another source for the generation of E1 could be in the de-sorption of estrogens from the solids. To check the adsorption assumption, the model was reconfigured with no adsorption, meaning that all of the estrogens were aqueous and available for biodegradation/transformation. In this case, the mean kE2 value became 0.075 (h-1) and -0.0022 (h-1) for kE1. The E1 value still indicated the generation of E1. Additionally, the model became less accurate, predicting 89% of influent E2 and 82% of influent E1. The generation of E1 could not be accounted for by either transformation from E2 or lack of adsorption. It is likely that the E1 per capita excretion estimate utilized is low.

For the mean kE1 value of -0.18 hr-1, the standard deviation is 0.30. The variation of kE1 between systems is even greater than for kE2. However, temperature and suspended solids concentration also varied between systems. Thus, the relationship between the k values and each of these factors was explored.

Table 1: Values of kE2 and kE1 Utilized in Developing the Model Mean Value					
Plantw	Temp. (ºC)	TSS (mg/L)	Mean Sewer Residence Time (hours)	KE2 (hr-1) Mean (range)	KE1 (hr-1) Mean (range)
03	12	192	4.5	-0.061 (-0.11 to -0.0072)	-0.37 (-0.46 to -0.32)
04	14.3	356	2.65	-0.060 (-0.14 to 0.031)	-0.29 (-0.44 to -0.20)
05	21.4	231	2	-0.044 (-0.17 to 0.088)	-0.18 (-0.39 to -0.044)
06	24.5	203	3	-0.066 (-0.15 to 0.021)	-0.44 (-0.58 to -0.35)
07	16	450	2	0.49 (0.36 to 0.64)	0.38 (0.21 to 0.50)
08	12.5	103	6	-0.082 (-0.12 to -0.040)	-0.17 (-0.25 to -0.13)

Temperature

The rate of estrogen biodegradation has been shown to increase with temperature in batch studies (Layton et al, 2000; Li et al, 2005). Thus, the relationship between temperature and biodegradation k among the various municipalities was explored. A plot of the biotransformation/degradation k for E2 and E1 versus temperature is shown in Figure 3. The temperatures for each of the six municipal sewer systems used to develop the model ranged from 12 to 24.5 ºC, with an average of 16.8 ºC.

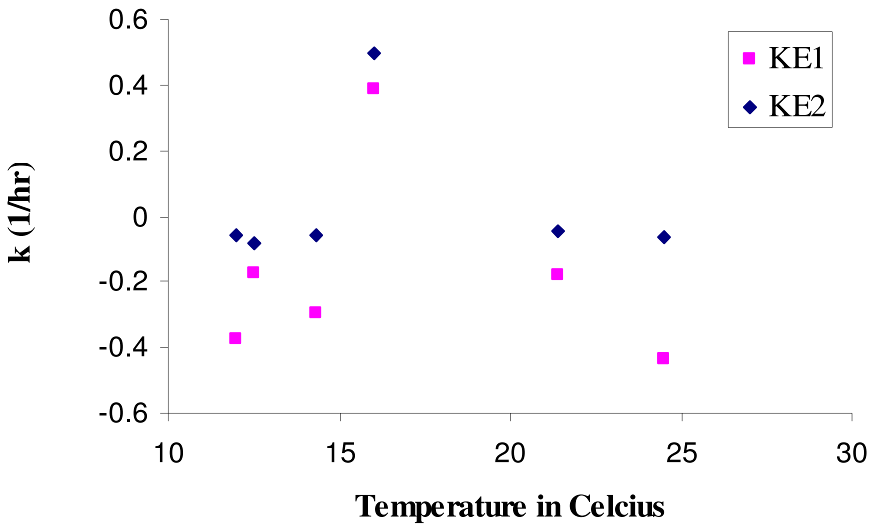

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Figure 3: Biodegradation k of E2 and E1 versus temperature. Biodegradation k values were not found to be dependant upon temperature for E1 or E2

Neither the biotransformation/degradation k of E2 nor E1 could be correlated with temperature. Linear regression yielded an r-squared value of 0.0276 for E1 and 0.0029 for E2. However, in batch studies, temperature differences of approximately15 ºC have exhibited a statistically significant effect on biodegradation kinetics (Layton et al, 2000; Li et al, 2005). Perhaps the model data does not span a wide enough temperature range to exhibit these effects. Thus, it is recommended that the model only be employed within the temperature ranges of 12 to 24.5 ºC.

It should be noted here that the municipalities in this study were all from North America and Europe. Most of the data for this study was taken from studies performed during the winter and fall months. Wastewater temperatures vary with season. Within the mainland US, wastewater temperatures have been observed to vary by almost 20 ºC between summer and winter months. The average temperature of the data sets used to determine the model biodegradation kinetics was 16.8 ºC, close to the representative mean annual wastewater temperature of 15.6 ºC for the United States (Tchobanoglous et al, 2003). Thus, the model will likely be of use to predict mean influent estrogen concentrations throughout the year within the mainland United States, but may not accurately predict seasonal variations in estrogen concentrations.

Wastewater temperatures also vary with location. In the United states alone, mean annual temperatures vary from 3 to 27 ºC (Tchobanoglous et al, 2003). In contrast, wastewater temperatures as high as 30 to 35 ºC have been found in Africa and the Middle East (Tchobanoglous et al, 2003). Future work could include data from more extreme temperatures to make the model more applicable to other climates, and more useful to predict seasonal variations.

Solids Concentration

Biodegradation k values for E2 have been found to increase with mixed liquor volatile suspended solids (MLVSS) concentrations (Li et al, 2005). The biotransformation/degradation k values of E2 and E1 for each of the six municipal sewer systems used to develop the model average data are shown in Figure 4. The biotransformation k values of E2 determined in this study displayed a trend of increasing linearly with total suspended solids (TSS) concentration ($R2 = 0.6048$). The biodegradation k values of E1 were less dependent on TSS ($R2=0.4452$).

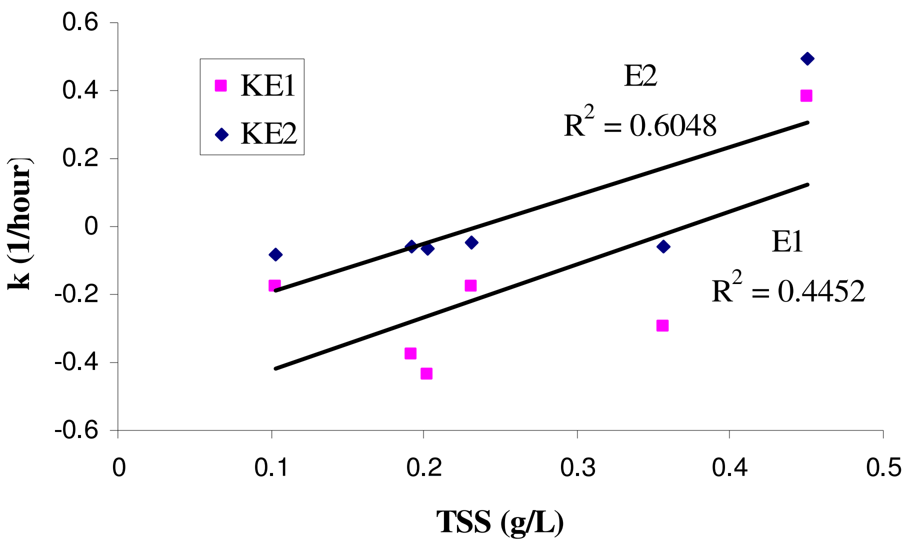

Figure 4: Biodegradation k for E1 and E2 versus total suspended solids concentration (TSS). The biodegradation k for E2 displays a trend of increasing linearly with TSS ($R2 = 0.6048$). The biodegradation k for E1 was less dependant on TSS ($R2=0.4452$).

Conclusion

The current model for estimating influent E1 and E2 concentrations at wastewater treatment plants includes adsorption to solids and biotransformation/degradation of estrogens in the aqueous phase. The mean pseudo-first order kinetic constant for the biotransformation of E2 into E1, kE2 was 0.030 hr-1 (ranging from -0.080 to 0.49 hr-1) and the biodegradation kE1 was -0.18 hr-1 (ranging from -0.44 to 0.38 hr-1).

Values for both kE2 and kE1 were not temperature sensitive for the range from 12 to 24.5 ºC. The model should only be applied within this rage, which is appropriate for mean annual wastewater temperatures within the United States. Data from temperatures outside this range could be used to increase the model's applicability. Values for both kE2 and kE2 increased linearly with TSS. However, KE1 values were less dependent on TSS.

Acknowledgements

The authors thank the municipal wastewater treatment plants which provided data for the study. Financial support was provided by the National Science Foundation (NSF) Integrative Graduate Education and research traineeship (IGERT) program and the Sustainable Futures Institute at Michigan Technological University. Special thanks to Bill Case and Dennis Lewandowski.

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CHAPTER 7:

History of the Clean Water Act

Estrogenic EDCs are present in wastewater effluent at concentrations known to impact aquatic wildlife. The release of these chemicals into surface water through wastewater effluent is not a sustainable practice. Currently, estrogenic EDCs are not regulated within wastewater effluents. This chapter explores the history of the Clean Water Act (CWA), and the EPA's responsibility to regulate chemicals, such as EDCs, within wastewater that can influence the health of our water resources.

7.1 History of the Clean Water Act

The original law pertaining to pollution in our nation's surface waters was the 1948 Federal Water Pollution Control Act (FWPCA) (P. L. 80-845), which has been amended many times to form the present day Clean Water Act (CWA) (33 U.S.C. 1251-1387). A history of the original law and its amendments is provided in this section.

Initially water pollution control was under the jurisdiction of the individual states. The 1948 Federal Water Pollution Control Act (FWPCA) focused on the protection of human health, and provided state and local governments with funding for water pollution control and the construction of wastewater treatment plants (P. L. 80-845). No federal standards were established for water quality, and states were encouraged to form mutual agreements to control interstate waters.

Through the 1950's and 1960's, Congress began to strengthen the federal role in water pollution prevention. The 1948 FWPCA was amended through the Water Pollution Control Act of 1956 (P. L. 84-660) and the Federal Water Pollution Control Act Amendments of 1961 (P. L. 87-88). Both laws provided additional funds for the construction of municipal wastewater treatment plants.

Water quality standards were introduced into the law in the Water Quality Act of 1965 (P. L. 89-234), which required states to develop interstate water quality standards by 1967, including permissible pollution levels and control measures. The Pollution Control Administration (PCA) was created under the Department of Health, Education, and Welfare. States were responsible for ensuring the quality of interstate waters, and determining waste load allocations so as not to exceed water quality standards they were to develop. In waters where they failed to do so, the federal government would assume jurisdiction, but must first prove that pollutant loads had either impacted human health or violated the agreed upon standards. Additional funds were also allocated for construction and research of wastewater treatment.

Recognizing the importance of proper wastewater treatment, congress allocated even more funds for the construction of wastewater plants in the Clean Water Restoration Act of 1966 (P. L. 89-753). States were required to share in the construction costs. More money was also given to research in pollution prevention and wastewater treatment.

Although the National Environmental Policy Act (NEPA) of 1969 (PL 91-190) is not considered to be part of the amendments to the CWA, it is included here for its significance in creating the EPA. This law stated that all federal agencies would protect the environment by considering environmental factors as part of their decision making processes. It established a Council on Environmental Quality (CEQ), in the Office of the President, responsible for coordinating environmental efforts at the federal level.

By 1970, the federal government was becoming concerned about the lack of state progress towards developing and enforcing water quality standards. It became apparent that a federal agency was required to handle environmental policies at the federal level.
In 1970, President Nixon consolidated the CEQ, the Pollution Control Administration, and environmental responsibilities of the Department of Interior and the Department of Agriculture into a single entity responsible for federal environmental policy, the Environmental Protection Agency. The EPA is ultimately responsible for enforcing and carrying out the provisions of the modern day CWA.

The Water Quality Improvement Act of 1970 (P. L. 91-224 Part I) strengthened the penalties for oil pollution. It also authorized the president to identify "hazardous polluting substances" for regulation, and required all federal agencies to comply with pollution standards.

The Federal Water Pollution Control Act Amendments of 1972 (P. L. 92-500) set the following goals, among others:

(1) It is the National Goal that the discharge of pollutants into the navigable waters be eliminated by 1985;
(2) It is the national goal that wherever attainable, an interim goal of water quality which provides for the protection and propagation of fish, shellfish, and wildlife and provides for recreation in and on the water be achieved by July 1, 1983;
(3) It is the national policy that the discharge of toxic pollutants in toxic amounts be prohibited;

It also expanded the federal financial support for the construction of wastewater treatment plants. By 1971, only about half the states had developed water quality standards, and it became apparent that the federal government would have to take responsibility for ensuring water quality standards were developed. Thus, in the

1972 amendments, congress authorized the EPA to set standards that would protect water quality by identifying toxic pollutants and placing appropriate limits on their release in water through the establishment of an effluent permitting system, the National Pollutant Discharge Elimination System (NPDES). This law also outlawed the discharge of chemical or biological warfare agents or radioactive waste. Additionally, penalties for violating the FWPCA were stiffened.

Under the new law, limits for permissible pollutant concentrations were to be set by the EPA, and established based on either water quality or available technology. Water quality limits for a pollutant would be established to protect ecosystem and public health. Technology based limits would be established either using the best practicable technology (BPT) or best available technology (BAT). BPT standards would be set using the average of well operated plants, and consider the cost of implementing a technology with respect to the benefits of reduced pollution. BAT standards would be set based on the best achieved results of existing technologies. Cost would be considered in BAT standards, but cost would not have to be balanced against the benefits of reduced pollution. The act also set new source performance standards (NSPS), requiring that all new sources of pollution have state-of-the-art treatment technologies in place.

NPDES permits issued between 1973 and 1976 required industrial facilities to meet BPT standards by July 1, 1977 and BAT standards by July 1, 1983 respectively. Municipal facilities were required to meet biological secondary treatment standards by July 1, 1977. Biological Secondary treatment includes a physical settling process (primary treatment) for solids removal, followed by digestion of the organic materials via microbial activities (secondary treatment). Water quality limits were to be set on a chemical by chemical basis for toxic pollutants, according to the 1970 Amendments of the FWPCA. However, by 1977 the EPA had only issued standards for the following six chemicals: aldrin/dieldrin, endrin, DDT, toxaphene, benzidene, and polychlorinated biphenyls (PCBs) (Copeland, 1993).

EPA did not adequately address the regulation of toxics, and failed to develop all of the required effluent guidelines within the deadline set in the 1972 amendments. As a result, the Natural Resources Defense Council sued the EPA, resulting in a 1976 consent decree which identified the toxic priority pollutants to be controlled, the industries for which technology based controls would be developed, and the methods for regulating toxic discharges. These provisions were incorporated into a new law, the 1977 Clean Water Act.

The 1977 Clean Water Act (P. L. 95-217) is the origin of the modern day name of the amended FWPCA. The CWA classified three types of pollutants: conventional, toxic, and non-conventional, as shown in Figure 4. Five pollutants were designated as conventional pollutants: five day biological oxygen demand (BOD_5), total suspended solids (TSS), pH, fecal coliform, and oil and grease (O&G). It also mandated the establishment of a published list of priority pollutants, which are considered toxic alone or in combination. The original toxic pollutants list identified 65 pollutants or classes of pollutants, as listed in Appendix A. Non-conventional pollutants includes pollutants that are not identified as toxic or conventional, such as chlorine or ammonia. These changes marked a shift in water quality concerns away from conventional pollutants and emphasized emerging toxic pollutants.

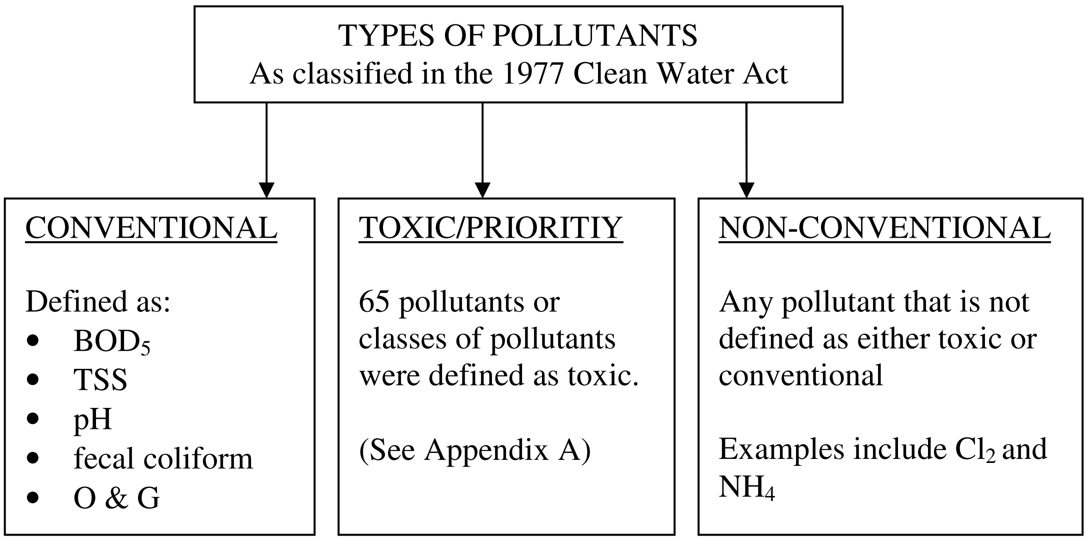

Figure 4: Classification of Pollutants

New technology based standards were included to address the pollutants. Under the 1972 law, the best practicable technology (BPT) was required for the control of the conventional pollutants. The 1977 law required more stringent controls for conventional pollutants by Best Conventional Pollutant Control Technology (replacing the BAT for conventional pollutants only), with a compliance deadline of July 1, 1984. The act stipulated the implementation of BAT controls with regards to toxic and non-conventional pollutants, with a compliance deadline of July 1, 1984.

The 1977 CWA also further extended the federal grant program for the construction of sewage treatment plants and strengthened oil pollution control measures. Recognizing the importance of sewage treatment, congress continued to fund the construction of new plants through the Municipal Wastewater Treatment Construction Grant Amendments of 1981 (P. L. 97-117).

The Water Quality Act of 1987 (P. L. 100-4) continued to offer financial support, establishing a state revolving loan fund for wastewater treatment and pollution control. It established the National Estuary Program and expanded programs regarding diffuse pollution, such as urban runoff. Deadlines for compliance with water quality standards were extended, and maximum penalties for violations were increased. Projects pertaining to water quality in specific water bodies were legislated, such as Chesapeake Bay, the Great Lakes, and Boston Harbor.

No major amendments to the Clean Water Act were passed from 1988-2006 (Hull, 2006). However, in 2007, a new amendment of the CWA was proposed. HR 720: Water Quality Financing Act of 2007 will authorize appropriations for state water pollution control revolving funds, and other purposes. The bill passed the house on March 9, 2007, but as of this writing, has yet to be voted on by the President or signed by the Senate (GovTrack.us, 3/29/07).

CHAPTER 8:

Implementation of the Current Clean Water Act

As stipulated in the CWA, the EPA is responsible for the oversight of the national pollutant discharge eliminations system (NPDES) program. Any point source which discharges a pollutant into US waters is required to have an NPDES permit. Each point source has a unique permit stipulating the chemical and biological quality limits, and permissible quantity of liquid effluent, for the effluent stream.

When changes are made to the NPDES permit requirements, the EPA first issues proposed rule. The proposed rules are published in the federal rule and a period of time is given for public comments to be submitted about the rule. The federal register is a government wide daily record of publications. After reviewing the comments, the EPA will issue a final rule to the federal register. Every year, all final rules from the federal register are compiled into the code of federal regulations (CFR).

8.1 Pollutants Regulated in Wastewater Effluents

Wastewater treatment plants are considered a point source, and as such, must possess a NPDES permit. Some industrial sources also directly discharge into water and must posses a NPDES permit. Other industrial dischargers send their effluent through sewers to a wastewater treatment plant, and are called indirect sources of pollution. Indirect sources are not required to posses a NPDES permit, but are subject to the National Pretreatment Program. Regardless of whether a wastewater treatment plant receives indirect discharges, each plant is responsible for meeting the conditions of their specific NPDES permit.

As previously stated, pollutants may be regulated in one of three possible categories: conventional, toxic/priority, and non-conventional. Currently, the discharge of all publicly owned treatment works (POTWs) must monitor the conventional pollutants of BOD-5 or CBOD-5, fecal coliform, pH, TSS. Additionally, all POTWs must also monitor the design flow rate and temperature (40 CFR 122.21 (j) as of July, 2005).

POTWs with a flow of 0.1 MGD or greater must also monitor the conventional pollutant of O & G, as well as the non-conventional pollutants of ammonia (as N), total residual chlorine (TRC), dissolved oxygen (DO), nitrate/nitrite, kjeldahl nitrogen, phosphorus, and total dissolved solids (TDS). An additional 105 pollutants may be measured in selected POTWs, as listed in Appendix B (40 CFR 122.21 (j) as of July, 2005). It should be noted that none of these pollutants include the natural or artificial estrogens.

8.2. Goals of the Current Clean Water Act

The CWA has gone through many amendments over time. The CWA as it exists today establishes national goals to "maintain the chemical, physical, and biological integrity of the Nation's waters". The goal of net zero discharge into navigable waters by 1985 was not met. However, this goal establishes an emphasis on reducing the impact of aquatic pollution by continually working towards the elimination of discharges.

The national goal "*that wherever attainable, an interim goal of water quality which provides for the protection and propagation of fish, shellfish, and wildlife and provides for recreation in and on the water be achieved by July 1, 1983"* also did not meet its deadline. Today, evidence exists of endocrine disruption in aquatic species within surface waters. It is the responsibility of the EPA to determine adequate water quality goals, with respect to EDCs, that will allow for the "protection and propagation" of aquatic wildlife.

The CWA establishes the EPA's responsibility for determining the effects of pollutants, identifying the best practicable technologies for the removal of pollutants, and setting appropriate maximum contaminant levels that will ensure the propagation of aquatic species.

The CWA also states *"It is the national policy that a major research and demonstration effort be made to develop technology necessary to eliminate the discharge of pollutants into the navigable waters, waters of the contiguous zone, and the oceans"* indicating a responsibility on the part of the EPA for the research and development of technology that will eliminate pollution.

Another goal of the CWA, as stated in its current text, is as follows:

> *"It is the national policy that areawide waste treatment management planning processes be developed and implemented to assure adequate control of sources of pollutants in each State"*

The EPA is implementing an "areawide waste treatment management planning" approach by regulating contaminants within a watershed. As releases within the same watershed ultimately affect the same water bodies, the EPA has adopted a watershed based permitting approach.

In summary, the goals of the CWA charge the EPA with the responsibility of determining which pollutants must be regulated; researching the effects of the pollutants; establishing acceptable limits for the pollutants; researching, developing, and identifying the best practicable technologies for removing these pollutants form wastewater streams; and establishing a program for permitting these pollutants within wastewater streams for the protection of a watershed.

8.3 EPA's NPDES Program Initiatives

As previously stated, wastewater treatment plants are considered point sources discharging into water, and each is required to have an NPDES permit. The EPA oversees the NPDES program, by developing its rules. However, most states (excluding Alaska, Idaho, Massachusetts, New Hampshire, New Mexico, and the District of Colombia) have EPA approved programs that issue the permits within their states. The permits must still meet the provisions of the CWA. The EPA issues rules pertaining to the implementation of NPDES permits, which are complied in the code of federal regulations each year, and followed by the states. In addition, the EPA develops the approaches to permitting to be used by the states, including technology based permitting, water quality based permitting, and the permitting for environmental results (PER) strategy.

Within wastewater treatment, technology based standards have been developed for secondary treatment. Secondary treatment consists of biological treatment, which is proceeded by physical settling (primary treatment). The secondary treatment standards established by the EPA determine the required effluent quality for secondary treatment with regards to BOD-5 and TSS removal. The secondary treatment standards also provide standards on a case-by-case basis for technologies equivalent to secondary treatment, such as trickling filters and waste stabilization ponds. The standards also consider the nature of the sewer systems (combined or separated).

These technology based standards are meant to protect our aquatic resources, and represent the minimum requirements for NPDES permits. Permit writers must be aware of the quality of receiving water, and ensure that water quality standards are met. States develop the water quality goals, also called standards, for each water body. Standards identify the designated uses of the water body and the scientific criteria to protect each use, such as recreational fishing or a drinking water source.

When technology based limits are insufficient to protect the quality of the receiving water for its indicated uses, the permit writer is required by the CWA (section 303(b)(1)(c)) and NPDES regulations (40 CFR 122.44(d)) to develop more stringent effluent limits based on water-quality. If a water body does not meet its water quality standards, it is considered impaired. It is placed on a list of impaired waters, as required by section 303(d) of the CWA, and total maximum daily loads (TMDLs) must be developed for the water body. A TMDL is the maximum amount of a specific pollutant that can be released per day into a water body, while still meeting water quality standards.

Water quality based permitting must take into consideration the impacts of all pollutant loads within a watershed. The EPA has issued a guide for watershed based permitting, offering advice on the selection of a watershed and determination of its boundaries, identification of stakeholders and facilitation of their participation, the collection and analysis of data for permit development, the development and issuing of watershed based permits, and the measuring and reporting of progress (EPA 833-B-03-004, 2003).

Watershed based permitting addresses water quality problems by considering the impacts from multiple point sources within a watershed. Rather than permit each source independently of the others, permits for each source are issued taking the impacts of other sources into consideration. When a pollutant has multiple sources, portions of the TMDL are allocated to each point source contributing to the total load on the water body. Thus, each NPDES permit is issued considering the impacts of other pollution sources. Non-point source reduction efforts may also be encouraged within a watershed to reduce the pollutant load. The control of both point and non-point sources to meet water quality objectives is also a goal stated in the CWA.

The EPA's Water Quality Trading Policy allows the trading of effluent emissions for a specific pollutant between sources within a watershed. The permits within a watershed are reviewed every five years to reassess whether the water quality standards are met and renew permits. Non-point sources within the watershed are not subject to permitting. They are addressed by the Nonpoint Source Management Program, which regulates them mostly through voluntary programs.

Chemical specific water quality criteria are addressed by the allocation of portions of a TMDL to different point sources through NPDES permits. However, the combined impact of these chemicals on the health of aquatic life should also be considered. When issuing NPDES permits with the goal of attaining water quality objectives, the EPA recommends that permit writers utilize whole effluent toxicity (WET) tests.

WET represents the cumulative toxic effect of an effluent on aquatic organisms, and do not require identification of the contributing pollutants to the total effect. WET tests include and acute test for mortality within 96 hours, and chronic test for impacts on an organism's growth, reproduction, and mortality within a 7 day life cycle test. EPA has developed WET tests for freshwater and marine species. The EPA also recommends testing for invertebrate, vertebrate, and plant effects to determine the most sensitive indicator species. This helps the permit writer to determine which WET tests to include in permit limits and monitoring requirements. Inclusion of WET in permits is yet another measure that can protect water quality standards as determined for each water body.

The use of technology based permits is often insufficient to protect water quality. Water quality based permits include the consideration of multiple pollutant sources within a watershed, development of TMDLs when necessary, the allocation and trading of portions of the allowable pollutant load within a water shed, and the consideration of a the cumulative effects of exposure to multiple pollutants. These efforts combine to create permits that aim to protect the designated uses of each water body, and focus on the environmental results desired for each watershed.

CHAPTER 9:

The Regulation of Estrogens in the Surface Waters of United Kingdom

As previously mentioned, the US has yet to develop TMDLs for estrogens within surface waters. In contrast, the United Kingdom Environment Agency (UKEA) has been more proactive on this front. The UKEA has been researching the effects of steroid estrogens on aquatic life. In response to the growing amount of scientific data pertaining to the effects of estrogens, the UKEA proposed predicted-no-effect-concentrations (PNECs) for estrogens in surface waters (UKEA, 2004). The suggested PNECs were established for the protection of fish in freshwater and saltwater environments. The suggested PNECs were 0.1 ng/l for 17α-EE2 and ng/L for 17β-E2. The EA acknowledged that current analytical limits of detection for 17α-EE2 are 0.1-0.5 ng/L, making the compliance with the suggested PNEC difficult to monitor. The UKEA also suggested that a "provisional target range of 3-5 ng/L may be appropriate" for E1.

These proposed PNECs were incorporated into threshold exposure limits for estrogens of 0.1 ng/L for EE2, 1 ng/L for 17β-E2, and 3 ng/L for E1(Burke, 2004). The threshold limits for exposure were set at a level meant to protect fish life within surface waters. Prior to establishing the exposure thresholds, the UKEA reviewed scientific data on the aquatic toxicity and bioaccumulation potential of steroid estrogens (EA, 2004). Enough evidence existed to warrant the establishment of the threshold exposure limits by the UKEA. Although the establishment of the threshold limits is a step in the right direction, the UKEA does not regulate estrogens within wastewater or industrial effluents (Brown, EA Policy Advisor, 2006). Regardless, the threshold exposure limits could be used as a starting point for the development of TMDLs within the US.

CHAPTER 10:

History of EDC Regulation in US

Although estrogenic EDCs are not regulated within the wastewater industry, there have been efforts to regulate EDCs in food and water sources within the US. Attempts at regulation of EDCs by the federal government began in 1996. In that year, congress passed amendments to both the Food Quality Protection Act (FQPA) and the Safe Drinking Water Act (SDWA). The FQPA amendments required the EPA to develop a screening program to determine the endocrine disrupting effects of pesticide chemical residues in food and regulate them, giving special consideration to ensure that "no harm will result to infants and children from aggregate exposure to the pesticide chemical residue" (FQPA Amendments of 1996, Sec 405). The amendments to the SDWA authorized the EPA to screen "substances that may be found in sources of drinking water in which a substantial population may be exposed" for endocrine disrupting potential (SDW Amendments of 1996, Sec 136). These amendments began the federal examination of EDCs within food and water sources. It should be noted that neither of them addressed EDCs within the wastewater effluents, despite the fact that many chemicals found in surface water, which may be used as drinking water sources, originate from waste waters.

In 1996, the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) was formed. The purpose of EDSTAC was to make recommendations to the EPA about how to develop the EDC screening and testing program. EDSTAC included representatives from industry, government, environmental and public health groups, worker safety groups, and academia. In 1998, EDSTAC issued a final report, based on which the EPA developed a tiered approach for the Endocrine Disruptor Screening Program (EDSP), including the development of four categories for chemicals, (EPA, 1998, FRL-6021-3), as shown in figure 5. Chemicals are placed into the categories during an initial sorting based on existing, scientifically relevant information. Category 1 is considered a "hold" category. These chemicals are not likely to interact with the estrogen, androgen, and thyroid systems and have the lowest priority for additional analysis. A Chemical may be moved down to Category 1 from another category if it is determined that the chemical is not likely to interact with the endocrine system. Category 2 is considered the "priority setting/tier 1 analysis" category. Chemicals in Category 2 lack sufficient information to determine whether they are likely to interact with the estrogen, androgen, and thyroid systems. These chemicals will undergo priority setting and Tier 1 analysis to determine which chemicals are not likely to interact with the estrogen, androgen, and thyroid hormonal systems. After Tier 1 analysis, a chemical will be either moved to Category 1 (hold) or move on to Category 3, Tier 2 Analysis. Within Category 3 (Tier 2 Analysis), it will be determined wither a chemical "may have an effect on humans similar to that of naturally occurring hormones and to identify, characterize, and quantify those effects for estrogen, androgen, and thyroid hormones (EPA, 1998, FRL-

6021-3). Chemicals for which sufficient information is known regarding their interaction with the three previously mentioned hormonal systems, may go straight to Category 4, Hazard Assessment. The exact content of Tier 1 and Tier 2 analysis must be determined, but it would consist of various assays, which are tests used to determine the endocrine disrupting potential of a substance.

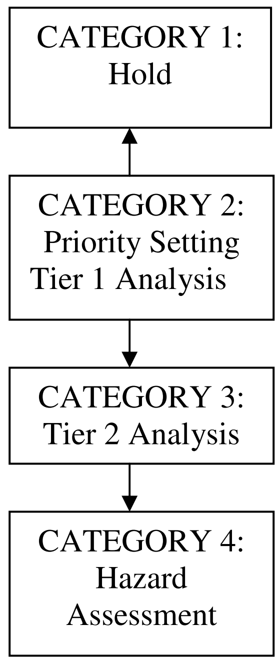

Figure 5: Endocrine Disruptor Screening Program

In August of 1999 the National Resources Defense Council (NRDC) filed suit with other public interest groups against the EPA for failure to meet the deadline for implementation of the EDSTAC recommended EDSP. In 1999, the EPA entered into a settlement agreement with the plaintiffs. The settlement included agreements that the EPA would use best effort to (US District Court, 2001):

- Complete the architecture of the endocrine disruptor priority setting (EDPSD) database by July 31, 2001.
- Complete and validate the quantitative structure-activity relationship (QSAR) portion of the EDPSD by Dec 31, 2001.
- Ensure that the EDPSD will be operational by May 31, 2002. If it is not operational by then, EPA will notify the plaintiffs and make semi-annual reports of efforts to make EDPSD operational.
- Determine within one year of receipt of any results or data from Japan related to its high throughput prescreening (HTP) effort whether to incorporate such results into the EDPSD.

- Publish and solicit comment on an initial list of chemicals for screening no later than Dec 31, 2002. If EPA fails to meet the deadline, it will notify the plaintiffs and provide semi-annual reports of efforts to complete the list.
- Complete validation of all Tier 1 screens no later than Dec 31, 2003; except the frog thyroid assay, which the EPA shall determine whether to include in Tier 1 by Dec 31, 2002.
- Start requiring testing for certain Tier 1 screens by Dec 31, 2003.
- Complete the validation of Tier 2 mammalian two-generation assays by Dec 31, 2004, and other Tier 2 tests by Dec 31, 2005.
- Start requiring testing for certain Tier 2 screens by Dec 31, 2004.
- The EPA also agreed to keep the public informed of all results of priority setting, screening, and testing by publishing these results in a centralized place readily accessible to the general public.

In 2001, The Endocrine Disruptor Methods Validation Subcommittee (EDMVS) was established to provide technical advice to the EPA regarding selection, design and validation of Tier 1 and Tier 2 assays. Assay development involves identifying scientific methods of use within the literature, comparing the cost and robustness of each, and recommending an assay procedure. After an assay is developed, it must be pre-validated, which often requires laboratory studies to ensure a procedure has reproducible results and to optimize and standardize the assay conditions. Validity testing proves the reliability of the protocol and transferability to other laboratories by performing the assay at multiple laboratories. Document regarding essay development, pre-validation, and validation, along with assay results, are then compiled for peer review. All EDSP methods must pass peer review prior to approval for regulatory use.

EPA is developing and validating the assays which will make up Tier 1 and Tier 2. Tier 1 screening assays currently under consideration include: the amphibian metamorphosis assay, receptor binding *in vitro* assays, aromatase *in vitro* essay, fish screen assay, Hershberger assay, pubertal female assay, pubertal male assay, steroidogenesis *in vitro* assay, uterotrophic assay, and the 15-day adult intact male assay. Tier 2 assays under consideration include: the amphibian development, and reproduction assay, the avian 2-generation assay, the fish lifecycle assay, the invertebrate lifecycle assay, and the mammalian 2-generation assay. Also under consideration is the *in utero* through lactation assay.

It is obvious that developing the methods to test and screen EDCs is time consuming. However, even if testing methods existed, it is estimated by the EPA that as many as 87,000 chemicals may be potential endocrine disruptors. In fact, if testing protocols did exist, it could take 59,000 years to test all existing potential EDCs (Vogel, 2004). Thus, developing methods to test and screen every suspected EDC is not only time consuming and expensive, it is not a practical regulatory solution to the problem of EDCs within wastewater.

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CHAPTER 11:

An Examination of the Significance and Regulation of Estrogenic Endocrine Disrupting Chemicals (EEDCs) within Municipal Wastewater Effluents

1. Introduction

The Clean Water Act establishes the Environmental Protection Agency's responsibility to regulate the release of pollutants into surface waters. For this purpose, the National Pollutant Discharge Elimination System (NPDES) requires a permit for all industrial and municipal effluents discharged into U. S. waters, which limit the biological and chemical characteristics of each effluent.

Estrogenic endocrine disrupting chemicals (EEDCs), such as the natural and artificial estrogens, are known to cause sexual abnormalities that affect the ability of aquatic species to reproduce. They exist in human waste streams, and can be present in wastewater effluents at concentrations that may affect aquatic species. Evidence of estrogenic endocrine disruption is emerging in U. S. surface waters, and has been documented downstream of wastewater outfalls. Currently, EEDCs are not regulated within municipal wastewater effluents and should be addressed within the current NPDES permitting system. Technology and water quality based standards should be developed for EEDCs, and the estrogenic effects of a whole effluent should be considered when issuing National Pollutant Discharge Elimination System (NPDES) permits for wastewater effluents.

2. Regulation of Pollution within Municipal Wastewater Effluents

The release of point source pollution into U. S. surface waters is regulated under the Clean Water Act (CWA). The Environmental Protection Agency (EPA) is ultimately responsible for enforcing and carrying out the provisions of the modern day CWA.

The 1972 amendments of The Federal Water Pollution Control Act (P. L. 92-500) set several national goals, among them is the goal "*that wherever attainable, an interim goal of water quality which provides for the protection and propagation of fish, shellfish, and wildlife and provides for recreation in and on the water be achieved by July 1, 1983"* did not meet its deadline. As will be discussed later, evidence exists of endocrine disruption in aquatic species within surface waters, which directly effects the propagation of aquatic species. It is the responsibility of the EPA to determine adequate water quality goals, with respect to endocrine disrupting chemicals (EDCs), that will allow for the "protection and propagation" of aquatic wildlife.

Additionally in the 1972 amendments, congress authorized the EPA to set standards that would protect water quality by identifying toxic pollutants and placing appropriate limits on their release in water through the establishment of an effluent permitting system, the National Pollutant Discharge Elimination System (NPDES). Any point source which discharges a pollutant into US waters is required to have an NPDES permit stipulating the chemical and biological quality limits, and permissible quantity of liquid effluent.

Wastewater treatment plants are considered point sources discharging into water, and each is required to have an NPDES permit.

Currently, the discharge of all publicly owned treatment works (POTWs) must monitor the effluent oxygen demand, fecal coliform, pH, TSS, design flow rate, and temperature. POTWs with a flow of 0.1 MGD or greater must also monitor the conventional pollutant of oil and gas, as well as the non-conventional pollutants of ammonia (as N), total residual chlorine (TRC), dissolved oxygen (DO), nitrate/nitrite, kjeldahl nitrogen, phosphorus, and total dissolved solids (TDS). An additional 105 pollutants may be measured in selected POTWs. It should be noted that none of these pollutants include the natural or artificial estrogens, which are currently not monitored or regulated in wastewater effluent.

Effluent limitations are established based on either available technology or water quality. Technology based limits consider the performance abilities of current and available technologies for treating municipal and industrial effluents, and represent the minimum requirements for NPDES permits. Permit writers must be aware of the quality of receiving water, and ensure that water quality standards are met. States develop the water quality goals, also called standards, for each water body. Standards identify the designated uses of each water body and the scientific criteria to protect each use, such as recreational fishing or a drinking water source.

When technology based limits are insufficient to protect the quality of the receiving water for its indicated uses, the permit writer is required by the CWA to develop more stringent effluent limits based on water quality. If a water body does not meet its water quality standards, it is considered impaired. It is placed on a list of impaired waters and total maximum daily loads (TMDLs) must be developed for pollutants of concern in the water body. A TMDL is the maximum amount of a specific pollutant that can be released per day into a water body, while still meeting water quality standards. Portions of the TMDL are allocated to different point sources through NPDES permits.

In cases where multiple chemicals exist that may be toxic to aquatic wildlife, the combined impact of these chemicals on the health of aquatic life should also be considered. For this purpose, the EPA recommends that permit writers utilize whole effluent toxicity (WET) tests within NPDES permits. WET represents the cumulative toxic effect of an effluent on aquatic organisms, and does not require identification of the individual pollutants. Inclusion of WET in permits is yet another measure that can protect water quality standards as determined for each water body.

In summary, the goals of the CWA charge the EPA with the responsibility of determining which pollutants must be regulated, and establishing a program for permitting these pollutants within wastewater streams. As will be discussed, estrogenic endocrine disruptors are emerging as contaminants of concern that should be regulated within NPDES permits.

3. Estrogenic Endocrine Disrupting Compounds (EEDCs) within Wastewater

Estrogenic endocrine disrupting chemicals (EEDCs) are chemicals which interfere with the estrogen system. EEDCs that exist in wastewater effluents include the natural estrogens, artificial estrogens and chemicals which mimic the activity of estrogens, called xenoestrogens. There are three natural estrogens: $C_{18}H_{24}O_2$ or Estradiol (E2), $C_{18}H_{22}O_2$ or Estrone (E1), and $C_{18}H_{24}O_3$ or Estriol (E3). Natural estrogens have been well documented in the effluents of sewage treatment plants all over the world at concentrations ranging from <1 ng/L to 45 ng/L of E1 and <1 ng/L to 14 ng/L of E2 (Andersen et al, 2003; Baronti et al, 2000; Belfroid et al, 1999; Johnson et al 2000; Johnson et al 2005; Lee & Pert, 1998; Servos et al, 2005; Ternes et al, 1999).

Other EEDCs found in wastewater effluent include pharmaceutically produced artificial estrogens. 17α-ethinylestradiol (EE2) is the most commonly used birth control. The use of contraceptive pills varies greatly between cultures. Western Europeans are among the highest users of the pill, with a high of 58.6 % among Germans. In comparison, only 13.6 % of Italian women use the pill (UN, 2005). Not surprisingly, one study found a higher average influent EE2 concentration (8.2 ng/L) at a German plant (Andersen et al, 2003), in comparison to the average influent at five Italian plants, of 3.1 ng/L (Johnson et al, 2000). Irregardless of influent concentration, EE2 in the effluent was often less than the limit of quantification (LOQ).

In addition to natural and synthetic hormones, there are a myriad of organic chemicals used as pharmaceuticals, personal care products, and household chemicals, which are found in wastewater streams that may act as xenoestrogens. Perhaps the most attention has been given to nonylphenol (NP) and octylphenol (OP), which are formed from the breakdown of nonionic surfactants.

As EEDCs are present in wastewater effluents, it should come as no surprise that in areas where wastewater effluents enter the environment, estrogenic effects have been observed on aquatic life. High plasma vitellogenin (VTG), a precursor for egg yolk, concentrations in juvenile fish and males are abnormal, and a definite result of exposure to estrogenically active substances. Increased VTG levels have been documented among fish in constructed wetlands used for wastewater treatment (Hemming et al, 2001), in aerated sewage lagoons (Bringolf et al, 2003), downstream of wastewater plants (Petrovic et al, 2002), and from effluent exposure (Purdom et al, 1994). Another result of exposure to estrogens is intersex, or ovotestes, the growth of both oocytes and testicular tissue within male fish gonads. Evidence has been documented of higher rates of intersex in fish downstream of wastewater plant effluents (Minier et al, 2000).

Evidence is also emerging of sexual abnormalities within aquatic populations in the wild. Ovotestes have been observed in male smallmouth bass in the Columbia River Basin (Hinck et al, 2004) and the Mississippi River Basin (McDonald et al, 2002); and in largemouth bass in the Rio Grande Basin (Schmitt et al, 2004). And a spring 2004, a USGS sampling of smallmouth bass on the Potomac River detected sexual abnormalities in 79% of the fish sampled (Cocke, 2004).

Due to the documented estrogenic responses in wildlife exposed to wastewater effluent, and the growing evidence of endocrine disruption among aquatic species in the wild, it is important to regulate estrogens within wastewater effluents. The CWA provides a framework for the regulation of aquatic pollutants in the United States.

4. Possible regulatory scenarios

As stipulated in the CWA, technology based standards are the first measure utilized for the control of conventional pollutants in wastewater. When technology based standards are insufficient for protecting aquatic resources, water quality based standards are developed.

4.1 Technology Based Standards

The effectiveness of EEDC removal varies among wastewater plants due to differing treatment techniques and operating conditions. Wastewater treatment processes are divided into the treatment levels of primary (gravity settling), advanced primary (chemical addition or filtration to aid in solids removal), secondary (biological breakdown of organics), tertiary (additional suspended solids removal and disinfection), and advanced (additional unit operations for the removal of contaminant).

Enhanced primary treatment is not very effective at removing EEDCs. Studies of full scale enhanced primary treatment plants, including phosphorous removal, have found no removal of E2 (Braga et al, 2005; Servos et al, 2005). E1 removal rates were either low (Braga et al, 2005) or increased from plant influent to effluent (Johnson et al, 2005; Servos et al, 2005). As estrogens are known to be biodegradable, the absence of biological treatment is likely the reason for the poor performance of these systems.

Biological treatment methods include lagoons (aerated or not), suspended growth reactors (such as activated sludge processes), and attached growth processes (such as trickling filters). Trickling filters do not perform as well as other processes for the removal of estrogens (Ternes, 1999). One full scale Canadian wastewater treatment plant utilizing a trickling filter, with an HRT of only 1 hour, displayed an increase in both E2 and E1 from plant influent to effluent (Servos et al., 2005). The poor performance of trickling filters among biological processes is likely due to their relatively low HRTs.

Comparatively, lagoon systems have very long HRTs and solids retention times (SRTs), and perform better at removing estrogens. One study of 18 Canadian plants (Servos et al, 2005) included four lagoon systems. All of these systems had >150 hours HRT and >150 days SRT. The lagoons performed much better than primary treatment, with an average of 93.2% E2 removal and 76.0% E1 removal.

Activated sludge is a biological process, characterized by aeration to encourage the growth of aerobic organisms, followed by the separation of solids by sedimentation, and a recycle of a portion of the solids to re-seed the aerobic digester with "activated" microorganisms. Most studies of activated sludge systems claim either no or weak statistical correlation between HRT or SRT and estrogen removal (Servos et al, 2005; Johnson et al, 2005). Despite this fact, plants that perform better at removing EEDCs tend to have higher SRT and HRT values. One study of E1 and E2 in the effluents of 18 Canadian wastewater treatment plants found that the two plants with the highest percent removals (≥ 98.2% of E2; ≥ 95.1% of E1) had very high HRTs (≥ 27 h) and SRTs (≥ 35.5 days) (Servos et al., 2005). Similarly, Johnson et al (2005) examined the effluent of 16 plants using secondary treatment. Three of the plants had >99% removal E1, two of which utilized activated sludge; one with an HRT of 24 hours, and an SRT of 16 days, the other with 51 hours and 7 days. The third plant was an oxidative ditch with an HRT of 17.5 hours and an SRT of 30 days. Thus, despite the weak statistical evidence, plants with higher HRT and SRT values perform better at removing estrogens.

Increasingly, processes beyond secondary treatment are required to provide a higher quality of effluent by removing colloidal, dissolved, and biological constituents. The removal of suspended and colloidal solids is a physical separation process. Typical advanced treatment techniques employed for this purpose include granular media filtration and membrane filtration. The removal of dissolved constituents generally requires additional membrane filtration or chemical processes. The removal of biological constituents requires additional disinfection processes.

Among the physical removal processes, sand filtration is not effective at removing estrogens. At a full scale plant in the UK, sand filtration post secondary treatment was found to provide no additional removal of E1 or E2 (Jiang et al, 2005). A better physical process for the removal of estrogens is adsorption. Adsorption onto granular activated carbon (GAC) has been shown to remove E1 and E2 from both water and wastewater in batch experiments (Zhang & Zhou, 2005). PAC has also been shown as effective at removing E1, E2, and EE2 from surface water in bench and pilot scales (Snyder et al, 2006).

Membranes bioreactors (MBRs) are an activated sludge process where physical separation of the finished water occurs by passage through a membrane. Membranes are classified by their pore size. MF systems have the largest pore size (macropores of >50 nm), while UF systems have slightly smaller pores (mesopores of 2-50 nm). However, MBR plants operating with a UF membrane provide no additional removal of estrogens when compared to activated sludge processes with similar SRT values (Clara et al, 2004).

Reverse osmosis (RO) is an additional membrane process with a dense pore structure (< 2 nm), which can reject very small molecules and ions. Systems utilizing reverse osmosis (RO) exhibited no detectable estrogens in their effluents (Braga et al, 2005; Snyder et al, 2006). This is likely due to the extremely small pore size of RO membranes, which reject the estrogen molecules.

Estrogens are also susceptible to chemical oxidation as a means of reducing their concentrations. Typical chemical oxidants utilized in wastewater treatment include chlorine and ozone. Oxidative processes are traditionally used in wastewater plants to provide a final pathogen disinfection step prior to the release of effluents into surface waters. One study compared the rate of reaction of EE2 with various oxidants. Ozone (O_3) provided the fastest reaction rates, followed by ClO_2, and chlorine was the slowest acting oxidant (Huber et al, 2005). Evidence also exists that Ferrate (VI) may be effective for removal of EDCs from aqueous solutions (Jiang et al, 2005).

In summary, primary treatment offers little removal of estrogens. Technology based standards should be developed for activated sludge systems and advanced treatment processes. This would require further research to establish the removal efficiencies of various unit operations based on their operating parameters, such as SRT and HRT in activated sludge.

4.2 Water Quality Based Standards: TMDLS

Water quality standards include the identification of which pollutants are items of concern for a specific water body and the establishment of total maximum daily loads (TMDLs) for each pollutant. In the U.S., steroid estrogens are not currently regulated within surface waters. Other countries, however, are making progress towards regulating estrogens in surface waters.

To protect fish life within surface waters, the United Kingdom Environment Agency (UKEA) has established threshold exposure limits for estrogens of 0.1 ng/L for EE2, 1 ng/L for 17β-E2, and 3 ng/L for E1(Burke, 2004). Prior to establishing the exposure thresholds, the UKEA reviewed scientific data on the aquatic toxicity and bioaccumulation potential of steroid estrogens (EA, 2004). Although the establishment of the threshold limits is a step in the right direction, the UKEA does not regulate estrogens within wastewater or industrial effluents (Brown, EA Policy Advisor, 2006).

Within the US, there are no established TMDLs for E1, E2, or EE2. However, the UKEA's threshold exposure limits for steroid estrogens within surface waters could be used as a starting point for the regulation of EEDCs.

4.3 Whole Effluent Estrogenicity

It is known that waste water effluents contain multiple chemicals that can cause estrogenic endocrine responses. The establishment of effluent limitations for each EEDC would require knowledge of the exact effects of each chemical at varying concentrations. As the effects of the natural and artificial estrogens have been proven to be additive (Thorpe et al, 2003), determining the allowable effluent concentration for each EEDC would require consideration of the comparative concentrations of every other EEDC within an effluent. Rather than addressing the permissible effluent concentration of each chemical individually, EEDCs could be regulated as a class of chemicals within effluents.

Bioassays can be used to determine an organism's response to exposure to a chemical. One of the most commonly used bioassays for the evaluation of the estrogenic activity of a sample is the yeast estrogen screen (YES), which is used for the detection of possible estrogenic substances (Routledge & Sumpter, 1996). The response of cells to a mixture of EEDCs within wastewater effluents can be measured utilizing YES, and expressed as estradiol equivalents (E2-EQs) for comparison purposes.

It is possible that YES would be utilized within the NPDES permit system in a similar manner as whole effluent toxicity (WET) tests. YES represents the total estrogenic endocrine disrupting potential of a mixture of chemicals, which can be translated into an equivalent concentration of estradiol. Limits for E2-EQs, as measured by YES, could be included in NPDES permits.

5. Conclusion

EEDCs are present in wastewater effluent at concentrations that can affect aquatic organisms. The CWA establishes the EPA's responsibility for the protection of the ability of aquatic species to reproduce. Technology based standards should be developed for the removal of EEDCs in wastewater treatment. Biological processes with long SRT and HRT values show promise for the degradation of estrogens, as do advanced treatment processes, such as RO and oxidative processes. Water quality based standards for EEDCs should also be developed. The UKEA has established threshold exposure limits for estrogens within their surface waters, which could be used as a starting point for the establishment of TMDLs. However, each EEDC cannot be addressed independently of the others, as multiple EEDCs exist within wastewater, which may have cumulative effects. It is suggested that YES results be additionally utilized for the regulation of EEDCs within the NPDES permit system.

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APPENDIX A:

40CFR401.15

[Revised as of July 1, 2002]

The following comprise the list of toxic pollutants designated pursuant to section 307(a)(1) of the Act:

1. Acenaphthene
2. Acrolein
3. Acrylonitrile
4. Aldrin/Dieldrin (Effluent standard promulgated 40 CFR part 129)
5. Antimony and compounds (including organic and inorganic compounds)
6. Arsenic and compounds (including organic and inorganic compounds)
7. Asbestos
8. Benzene
9. Benzidine (Effluent standard promulgated 40 CFR part 129)
10. Beryllium and compounds (including organic and inorganic compounds)
11. Cadmium and compounds (including organic and inorganic compounds)
12. Carbon tetrachloride
13. Chlordane (technical mixture and metabolites)
14. Chlorinated benzenes (other than di-chlorobenzenes)
15. Chlorinated ethanes (including 1,2-di-chloroethane, 1,1,1- trichloroethane, and hexachloroethane)
16. Chloroalkyl ethers (chloroethyl and mixed ethers)
17. Chlorinated naphthalene
18. Chlorinated phenols (other than those listed elsewhere; includes trichlorophenols and chlorinated cresols)
19. Chloroform
20. 2-chlorophenol
21. Chromium and compounds (including organic and inorganic compounds)
22. Copper and compounds (including organic and inorganic compounds)
23. Cyanides
24. DDT and metabolites (Effluent standard promulgated 40 CFR part 129)
25. Dichlorobenzenes (1,2-, 1,3-, and 1,4-di-chlorobenzenes)
26. Dichlorobenzidine
27. Dichloroethylenes (1,1-, and 1,2-dichloroethylene)
28. 2,4-dichlorophenol
29. Dichloropropane and dichloropropene
30. 2,4-dimethylphenol
31. Dinitrotoluene
32. Diphenylhydrazine
33. Endosulfan and metabolites
34. Endrin and metabolites (Effluent standard promulgated 40 CFR part 129)
35. Ethylbenzene

36. Fluoranthene
37. Haloethers (other than those listed elsewhere; includes: chlorophenylphenyl ethers, bromophenylphenyl ether, bis(dichloroisopropyl) ether, bis-(chloroethoxy) methane and polychlorinated diphenyl ethers)
38. Halomethanes (other than those listed elsewhere; includes methylene chloride, methylchloride, methylbromide, bromoform, dichlorobromomethane
39. Heptachlor and metabolites
40. Hexachlorobutadiene
41. Hexachlorocyclohexane
42. Hexachlorocyclopentadiene
43. Isophorone
44. Lead and compounds (including organic and inorganic compounds)
45. Mercury and compounds (including organic and inorganic compounds)
46. Naphthalene
47. Nickel and compounds (including organic and inorganic compounds)
48. Nitrobenzene
49. Nitrophenols (including 2,4-dinitrophenol, dinitrocresol)
50. Nitrosamines
51. Pentachlorophenol
52. Phenol
53. Phthalate esters
54. Polychlorinated biphenyls (PCBs) (Effluent standard promulgated 40 CFR part 129)
55. Polynuclear aromatic hydrocarbons (including benzanthracenes, benzopyrenes, benzofluoranthene, chrysenes, dibenz-anthracenes, and indenopyrenes)
56. Selenium and compounds (including organic and inorganic compounds)
57. Silver and compounds (including organic and inorganic compounds)
58. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)
59. Tetrachloroethylene
60. Thallium and compounds
61. Toluene
62. Toxaphene (Effluent standard promulgated 40 CFR part 129)
63. Trichloroethylene
64. Vinyl chloride
65. Zinc and compounds (including organic and inorganic compounds)

APPENDIX B:

40 CFR 122.21 (j)

(Revised as of July 2005)

NPDES Permit Testing Requirements for Publicly Owned Treatment Works

Table 1A--Effluent Parameters for All POTWS

Biochemical oxygen demand (BOD-5 or CBOD-5)
Fecal coliform
Design Flow Rate
pH
Temperature
Total suspended solids

Table 1--Effluent Parameters for All POTWS With a Flow Equal to or Greater Than 0.1 MGD

Ammonia (as N)
Chlorine (total residual, TRC)
Dissolved oxygen
Nitrate/Nitrite
Kjeldahl nitrogen
Oil and grease
Phosphorus
Total dissolved solids

Table 2--Effluent Parameters for Selected POTWS

1. Hardness
2. Metals (total recoverable), cyanide and total phenols
3. Antimony
4. Arsenic
5. Beryllium
6. Cadmium
7. Chromium
8. Copper
9. Lead
10. Mercury
11. Nickel
12. Selenium
13. Silver
14. Thallium
15. Zinc

16. Cyanide
17. Total phenolic compounds
18. Volatile organic compounds
19. Acrolein
20. Acrylonitrile
21. Benzene
22. Bromoform
23. Carbon tetrachloride
24. Chlorobenzene
25. Chlorodibromomethane
26. Chloroethane
27. 2-chloroethylvinyl ether
28. Chloroform
29. Dichlorobromomethane
30. 1,1-dichloroethane
31. 1,2-dichloroethane
32. Trans-1,2-dichloroethylene
33. 1,1-dichloroethylene
34. 1,2-dichloropropane
35. 1,3-dichloropropylene
36. Ethylbenzene
37. Methyl bromide
38. Methyl chloride
39. Methylene chloride
40. 1,1,2,2-tetrachloroethane
41. Tetrachloroethylene
42. Toluene
43. 1,1,1-trichloroethane
44. 1,1,2-trichloroethane
45. Trichloroethylene
46. Vinyl chloride
47. Acid-extractable compounds
48. P-chloro-m-creso
49. 2-chlorophenol
50. 2,4-dichlorophenol
51. 2,4-dimethylphenol
52. 4,6-dinitro-o-cresol
53. 2,4-dinitrophenol
54. 2-nitrophenol
55. 4-nitrophenol
56. Pentachlorophenol
57. Phenol
58. 2,4,6-trichlorophenol
59. Base-neutral compounds
60. Acenaphthene
61. Acenaphthylene

62. Anthracene
63. Benzidine
64. Benzo(a)anthracene
65. Benzo(a)pyrene
66. 3,4 benzofluoranthene
67. Benzo(ghi)perylene
68. Benzo(k)fluoranthene
69. Bis (2-chloroethoxy) methane
70. Bis (2-chloroethyl) ether
71. Bis (2-chloroisopropyl) ether
72. Bis (2-ethylhexyl) phthalate
73. 4-bromophenyl phenyl ether
74. Butyl benzyl phthalate
75. 2-chloronaphthalene
76. 4-chlorophenyl phenyl ether
77. Chrysene
78. Di-n-butyl phthalate
79. Di-n-octyl phthalate
80. Dibenzo(a,h)anthracene
81. 1,2-dichlorobenzene
82. 1,3-dichlorobenzene
83. 1,4-dichlorobenzene
84. 3,3-dichlorobenzidine
85. Diethyl phthalate
86. Dimethyl phthalate
87. 2,4-dinitrotoluene
88. 2,6-dinitrotoluene
89. 1,2-diphenylhydrazine
90. Fluoranthene
91. Fluorene
92. Hexachlorobenzene
93. Hexachlorobutadiene
94. Hexachlorocyclo-pentadiene
95. Hexachloroethane
96. Indeno(1,2,3-cd)pyrene
97. Isophorone
98. Naphthalene
99. Nitrobenzene
100. N-nitrosodi-n-propylamine
101. N-nitrosodimethylamine
102. N-nitrosodiphenylamine
103. Phenanthrene
104. Pyrene
105. 1,2,4,-trichlorobenzene

APPENDIX C:

Restructuring of CE4506 (Environmental Policy and Pollution Prevention Design) and Student Response Survey

Abstract

This paper details the format change of a senior level environmental policy and pollution prevention class offered to 51 civil and environmental engineering students in Spring of 2005. The old format entailed 3 lecture hours per week, and traditional hour exams. The new format included class room strategies for active and collaborative learning, had no exams, and reduced the lecture to one class hour each week. The paper includes qualitative results from a survey regarding student preferences for the new class structure in comparison to the previous structure.

1. Background

Historically, engineering education has been dominated by a lecture only format, in which students are expected to retain and memorize lecture material, reproducing it on demand for exams[1]. This one-way transfer of information from teacher to student has been termed the "banking concept of education" by Paulo Freire[2].

In the 1970's Feire criticized "banking education" for its inability to actively involve the students as critical thinkers. Freire viewed banking education as a form of domination, in which the teacher maintained total control over the lecture material, and the students became intellectually unengaged, passive recipients of facts.

As an alternative to banking education, Freire suggested that modern educators should utilize dialogue within the class, creating an environment that develops critical thinking and focuses on education as a process of inquiry. In this new pedagogy, teachers and students would become co-investigators about the subject at hand. The roles would become more fluid, with teachers learning from students and students learning from teachers. Freire emphasized the fundamental importance of dialogue in education, writing "Without dialogue there is no communication. And without communication there can be no true education"[2]. Freire believed that banking education was a means of dominance, which required the passive acceptance of facts by students. He proposed that education should be a practice of freedom, engaging students to become involved in class and critically examine the material at hand.

Currently, many engineering educators are unknowingly practicing Freire's liberation pedagogy through the inclusion of active learning techniques. Active learning has been "generally defined as any instructional method that engages students in the learning process"[3]. As Freire called for, active learning includes students as engaged participants in learning, rather than passive recipients of facts.

One type of active learning is collaborative learning, which may include "any instructional method in which students work together in small groups toward a common goal"[3]. A key element of collaborative learning is student interactions. The goal of collaborative learning is for students to grow beyond rote memorization, by questioning the material and its application, and develop a deeper understanding of the meaning of class material. In fact, it has been proposed that the goal of education itself is to "induce students to develop a deep approach to subjects"[1]. This questioning and deeper understanding are the building blocks of Freire's pedagogy of freedom.

As paraphrased from Felder & Brent, classroom strategies that can foster "deep approach" to learning possess the following characteristics, among others:

- Interest in and background knowledge of the subject
- Assessment that emphasizes conceptual understanding
- Long-term engagement with learning tasks
- Opportunities to exercise reasonable choice in the content and method of study[1]

The terms cooperative and collaborative learning are often used interchangeably. Smith et. al., make a distinction between cooperative and collaborative learning in that "cooperative learning requires carefully structured individual accountability, while collaborative does not"[4]. Regardless of whether it is cooperative or collaborative, working together requires students to develop the interpersonal communication and team building skills required for success in the engineering work place[5]. In the professional engineering world, communication skills are required in the three forms of written, oral, and graphic communication[6].

This article focuses on the restructuring of a class from the traditional "banking education" approach, to include elements of a more progressive active learning approach. Collaborative formal discussion groups and research projects provided active learning experiences, which included written, oral, and graphic communication.

2. History of Class format:

CE4506 Environmental Regulation and Pollution Prevention design has been taught for several years at Michigan Technological University. From 2002-2004, CE4506 was taught each spring by different departmental faculty or staff. The class was taught mostly in the traditional “banking education” format of a lecture class with the course grade based on a few home work assignments and exams.

In Spring 2005, the course was restructured and offered to 51 civil and environmental engineering juniors and seniors. Students were surveyed half way through the semester to determine student preference between the new structure and the old. Although these students had not taken this specific course under the old structure, the majority of the classes in their engineering curriculum would have exposed them to the “banking education” format. Thus, it is valuable to gather information regarding students’ preference with regard to the new course structure.

3. New Structure:

In Spring of 2005, CE4506 was team taught by two new instructors. Instructor A was a professional engineer at a local firm. Instructor B was a Ph. D. candidate in Environmental Engineering at Michigan Tech. The two instructors rotated on a weekly basis. Thus, each taught every other week. The class was taught on Monday, Wednesday, and Friday, in one hour sessions. Course format changes included alterations to the components of the student grade and the classroom teaching methods as described in the following sections.

3.1 Student Grade Components

Student grades for the class were based on the following components:

- Weekly quizzes (40%)
- Research project on the application of environmental regulations for a specific contamination site or persistent environmental problem:
  - Paper (15%)
  - In-class presentation (10%)
- Research project on a specific pollution prevention technology:
  - Paper (15%)
  - In-class presentation (10%)
- Class participation (10%)

Quizzes were administered each Friday. The weekly quizzes were based on material covered in Monday’s lecture and case studies discussed in class on Wednesday. Quiz results were promptly returned to students to provide timely feedback, as faculty feedback has been related to student’s self-reported gains from classes[7]. The intention of quizzes was to provide incentive for attendance and routine feedback to the students (and the instructors) regarding the students’ grasp of the course material.

The first research project was due at midterm. The students were expected to work in pairs to research an environmental problem (or specific contamination site), the regulations that pertained to the problem, and the application of the regulations for this problem. The second research project was due at the end of the semester. Again in pairs, the students were expected to research a sustainable pollution prevention technology, and explain what environmental problem it relieved, how it was developed, and how it worked. Presentations accompanying each of these projects were performed in class on Fridays throughout the semester. The students had approximately 7 weeks to complete each project.

The research projects were included in the place of formal exams, as a tool to assess the students' understanding of the course material and improve the students' written communication skills. Students were asked to propose their own research subjects for instructor approval, and encouraged to select topics they found interesting. The research assignments met the previously mentioned aspects of tools that foster a "deep approach" to learning. By choosing the subjects themselves, students tailored assignments to their own interests. If students did not choose subjects for which they already had some background knowledge, this knowledge was surely gained through their research. The research paper was intended as a venue where students could convey their conceptual understanding of the course material. Additionally, the research papers required a relatively long term engagement of 7 weeks. By selecting their own topic and research methods, the students exercised choice in the content and method of study. Thus, the research projects were likely candidates for fostering a deep understanding of the course material.

Attendance was taken each Wednesday, to ensure students were attending discussion days. Participation points were assigned for attendance and for each time a student took a leadership role in the group, as described in section 3.2.

3.2 Classroom Format

The first alteration to classroom format was the reduction of lecture hours from three-per-week to one-per-week. The weekly lecture was always presented on Monday. This served as an introduction to the week's topic and provided background information for that week's case study and group discussions. Background knowledge of the subject has been listed as one of the elements that can lead to a deep learning approach among students[1]. As the traditional lecture format will only capture the interest of students with a reproducing orientation to learning (i.e. memorization)[1], lecture hours were reduced to make time for other, active learning techniques, while still providing a foundation of background knowledge for each week's subject.

The second alteration to the classroom format was the addition of one group discussion hour each Wednesday. It was anticipated that group discussion with meaningful dialogue would be difficult with 51 students. Thus, the first Wednesday of the class, students were asked to form discussion groups of 6-7 students each. The groups were capped at 7 because when groups become much larger than 6 students, it is more likely that some students will become passive observers in the group, and students tend to have less sense of responsibility to larger groups[8]. Eight groups were formed. The first discussion day, group bonding was facilitated by introductions and ice-breaker activities. To encourage group ownership, the students were each asked to name their discussion groups. The following group names were chosen:

Crazy 8's
The Planeteers
Civility, Hostility, and the Notorious Yankee Swap
I Got Worms
C-Blerb Run
The A-Team
Booze-Hounds
JJ-Talk

Each Monday, students were assigned out-of-classroom research and reading to be done as preparation for Wednesday's discussion groups. For example, when the Superfund cleanup process was lectured about on Monday, students were required to locate and read the Record of Decision (ROD) for a site near their home communities. The ROD is a public document that explains which alternatives will be used to clean up a Superfund site. The ROD is based on scientific and engineering information gathered during two other processes: Remedial Investigation and Feasibility Study. Students were also required to locate and read the Remedial Investigation and Feasibility Study for their chosen site. This material would be brought to class for group discussion as to whether the students' agreed with the ROD for each case based on the information gathered.

Student involvement and leadership of the discussion groups was considered key to the success of the discussion groups. Thus, each group was to have a rotating group leader, with each student required to lead their group twice during the semester. The role of the leader was to attend class with prepared discussion topics and questions (in writing) for the subject at hand, facilitate group discussion, take notes on group discussion, take attendance of all the group members, and submit all of this information to the instructor. Participation points were based on this information. Discussion days were included as a technique to promote dialogue about the course material, improve student verbal communication skills, encourage student leadership, allow for reasonable student control over the subjects discussed, and promote a deeper understanding of the course material.

Fridays were reserved for student presentations of their research projects, and weekly quizzes. Student presentations, with a required Power Point slides, were included as a method to develop the verbal and graphic communication skills of the students. Additionally, as the course subject had a wide range of application to unique circumstances, the presentations were intended to give the students a sense of the myriad of ways in which both environmental policies and pollution control technologies can be applied in varying situations. These presentations were intended to enrich the class material with numerous “real world” examples of the application of the course concepts that would be difficult for the instructors to research in the normal time allowed for class room preparation.

4. Findings & Discussion:

Qualitative methods are used to gather data about human perspectives[9]. Surveys are an established qualitative method for capturing data about students’ perceptions of engineering classroom experiences[9-10]. An open ended survey was administered half way through the semester to gather information regarding preference about the new class structure in comparison to the old structure. The survey was administered in class. Although there were 51 students in the course, only 41 students responded to the survey. The remaining ten included students that were absent that day and students that did not respond to the survey. The surveys were anonymous, but letter identification was assigned to each survey (A through NN) for purposes of identifying each respondent uniquely during qualitative data analysis. The survey is presented in Appendix A.

The results presented in this section are based upon qualitative analysis of the responses to each survey question. Upon examination of the surveys, student preferences for the following were revealed: reduced lecture hours, the inclusion of one discussion hour per week, preference for research papers and presentations over exams, and student acceptance of a grade based on quizzes papers and presentations.

4.1 Student Preference for the Reduced Lecture Hours:

The first survey question inquired as to whether students believed one lecture hour per week was adequate, or if the students preferred the traditional format of three hours per week. Of the 41 students surveyed, only two expressed criticism of the decreased number of lecture hours. However, even those students criticizing the Monday lecture did not express a preference for three lecture hours per week. Thus, none of the students surveyed expressed a preference for the traditional format.

Displeasure with the reduced lecture format was expressed by Student A in the following words:

> *Having just (one) lecture hour a week cuts down on the topic detail and number of topics discussed.*

Student A expressed a concern for lost lecture topics and depth. However, several students indicated a deeper level of intellectual engagement during discussion periods, as opposed to traditional lectures. In this format, the lectures were intended as an introduction to each week’s topic. The preparatory assignments and discussion days were intended as a venue where a deeper conceptual understanding of the week's topic could be gained. In fact, cutting the lecture hours by 2/3 must result in less material conveyed in this format. But it is arguable that these topics were covered in much greater detail through group discussions and research projects, which, as previously discussed, possess characteristics which promote a deep approach to learning.

Three students (T, V, W) commented that they preferred the reduced lecture format specifically because of the inclusion of the group discussions. Student T explained that s/he did not prefer three lecture hours per week because "I enjoy being able to discuss things and explore on my own", indicating that this student was more engaged in the new class format. While Student V stated:

> *I feel that I get a pretty good idea of the topic by one lecture, and then discussing it on Wednesday helps to give a practical application of the regulations.*

This statement confirms that, for some students, the class format did reinforce and add depth to the lecture material. Student E also expressed that Monday lectures served as a good introduction to the topic of the week:

> *I think the Monday lectures are good because it prepares you for the rest of the week*

As a new topic was covered each week, lecture and discussion days were paired, with each lecture introducing a subject for out of class room research/reading and in class discussion. In effect, Monday lectures functioned as an introduction to concepts to be explored more in depth on Wednesdays. A cohesive subject for the lecture and discussion days is an essential element in the success of this format.

The only other criticism of the reduced lecture format was given by Student DD as follows:

> *Monday lectures are sufficient, although sometimes rushed.*

With the reduction in lecture hours, it is likely that the instructors did rush lectures in an effort to provide a comprehensive introduction to each new topic. However, students may have been aided in their retention of faster lectures by reviewing lecture slides electronically accessible to all (the departmental R-drive), as expressed by student U:

> *I feel like I'm learning enough in the one lecture period especially since I can review the lectures on the R-drive.*

One final reason, expressed by three students (N, S, X), for preferring the reduced lecture format, was that it was more interesting than the traditional format.

Student N explained that the new format was “more interesting than three lecture hours per week”. While Student S explained, “This way is something different and makes it more interesting. When asked specifically about three lecture hours per week, Student S replied “every other class is like that” and student X replied “Three lecture hours becomes monotonous”.

Overall, students indicated a preference for the reduced lecture format. Despite the two criticisms of this format, none of the 41 students surveyed indicated a preference for the three-lecture-per week format. Students indicated an increased understanding of the application of the course material was gained through the added discussion sessions. Additionally, the new structure, with fewer lectures, was more interesting to the students than the traditional format. The inclusion of collaborative learning through student-lead discussion groups made the class more appealing than lectures alone.

4.2 Student Preference for the Inclusion of a Group Discussion Hour:

The second survey question inquired as to whether Wednesday discussions improved student understanding. As discussion days usually required the students to perform some research and reading on their own and bring the materials into class, students were asked if they preferred this work to traditional graded homework assignments,

Only one student in the class expressed a desire not to have in class discussions. In the words of student A:

> *I feel that I get very little from the discussions and would prefer to write a memo on my findings.*

It should be noted that Student A was the only student that indicated a preference for not including group discussion days in the class format. Thus, 40 of the 41 students surveyed expressed a preference for including weekly discussions. Recall that Student A also expressed a concern, discussed earlier, for a loss of lecture topics and detail with the reduced format. These statements indicate that Student a may perceive the traditional “banking education” lecture format as providing more facts to the students, or that this student has a reproduction orientation to learning. However, the aim of this course was not rote memorization of environmental law, but for the students to gain a conceptual understanding of the investigation and application of environmental regulations and sustainable pollution control technology. In fact, several students indicated that the preparatory assignments and discussions provided a greater understanding of the application of the course material.

Four students (V, T, U, and KK) specifically mentioned the value of the research assignments performed in preparation for discussion days. In the words of Student V:

I think looking up stuff relating to the topic and then discussing it really shows how those reg(ulation)s apply to the real world.

While student U explained:

I love the discussions. They not only allow me to see practical applications of the reg(ulation)s but also familiarize me with how to find the info…

Thus, it is not only the discussions themselves, but the preparatory out of class room reading and research that enriched the students' learning. Despite the fact that the assignments accompanying discussions were found useful to those who did them, two students, O and F, specifically mentioned that it was difficult to be motivated to prepare for discussion as there was no classroom check on student preparedness. Future attempts at teaching this course could easily be adapted so that preparatory materials brought to discussion days were handed in to the instructor and included in the course grade component. This may provide increased student motivation to complete preparatory assignments for discussion days.

In total, 13 students (EE, U, O, NN, CC, C, E, AA, X, Z, DD, W, N) specifically mentioned that the discussions aided in understanding of the application of the course material or increased learning. Student E described discussions as:

gets you involved and talking about the topics which helps me to learn better.

While student NN explained about discussions:

It makes the information sink in well through the use of real-life application

These statements both reflect increased student learning through the discussions themselves. Thus, the students found both the preparatory assignments and the discussions as tools that increased their understanding of the course material.

Student leadership of the discussion groups resulted in no complaints. In fact, only one student commented negatively on the discussion group format, in the words of Student H:

Discussions should be better formatted. Sometimes it is hard to talk about certain topics. We need to have some other thought provoking questions as well.

This student was the only one who requested additional discussion aides beyond what the student group leader provided. However, Student H did not express direct criticism of the student leadership, merely a request for additional assistance. Additional discussion points and questions could easily be prepared by an instructor for each discussion day. However, these materials should be used sparingly. One of the elements that make the discussion groups likely candidates to foster deep learning is the ability of the students to exercise control over the discussions themselves. By providing students with scripted discussion topics, the students would be lead through the exploration process, rather than develop a true investigative approach to learning on their own.

Three students (P, N, II) explained that they preferred discussion days because they found discussions interesting when compared to traditional lectures. In the words of student II:

> *It is both interesting and fairly fun.*

Overall, the students expressed a preference for the inclusion of discussion days in the class format. They indicated that the *research*/reading assignments followed by discussions created a deeper conceptual understanding of the application of the course material. The students also found the discussion days more interesting than traditional lectures.

4.3 Student Preference for Research Papers and Presentations:

The third survey question inquired about the usefulness of research papers and presentations for enriching the students' understanding of the course material, and whether the students would prefer traditional exams in place of these assignments.

Only two students (Q and HH) stated that they would prefer exams. One, Student Q, explained, "I would prefer exams over presentations, but I like the research papers".

Student Q expressed displeasure with the knowledge gained from the research assignment:

> *These papers/presentations, as far as individual research is concerned, do not give us a full scope of the reg(ulation)s. I would prefer traditional exams if they help to show me the real work situations and how I would apply the reg(ulation)s.*

It should be noted that only student Q expressed this displeasure with the research paper and presentations. This student expressed a desire to be tested on the way the regulations would be applied in a work setting. However, the application of environmental regulations is site-specific, with no one answer that would be applicable to all circumstances. The student presentations of research assignments were intended to enrich to the class with many examples of the application of environmental regulations. In fact, several students stated that the reason they preferred the papers were that they were more in depth than exams and that watching each other's presentations exposed them to more real world examples. Overall, 39 of the 41 students surveyed expressed a preference for research papers and presentations over exams.

In total, 19 students (K, H, Y, A, CC, E, I, O, R, S, U, V, AA, BB, DD, FF, II, JJ, NN) specifically mentioned learning more detail, or gaining a greater understanding of the regulations and their applications through the research paper assignment. In the words of student R:

> *The research has us learn and understand the law, as opposed to just memorizing them.*

While Student AA explained:

> *I think this method is better than exams because application of the material is required more than just repeating the info.*

And Student E stated:

> *By doing research you get a better understanding of the material. It is not just memorizing something.*

The statements above indicate a deeper understanding of the course material was provided by the research papers in comparison to the memorization required for traditional exams. It exemplifies the student preference for deeper learning over "banking education" methods.

Additionally, several students stated that listening to other's presentations in class gave them a sense of how environmental regulations could be applied in various settings. In the words if Student U:

> *The research projects are better than exams. I get in depth knowledge of my topic plus all the other groups topics.*

While Student JJ explained:

> *Not only do we still learn the course material, but we get to hear 15 or so different applications of them, exams = bad!*

These statements reflect that students valued the presentation for reasons beyond the experience in presenting itself. In fact, none of the students mentioned increased presentation skills as part of the value of this assignment. The students valued the presentations for their ability to learn from each other the wide variety of ways that environmental regulations are applied.

Of those who mentioned learning gains from the research papers, four students (K, H, Y, CC) cited a preference for research papers because of the “real world” examples brought to the class. As Student CC explains:

> *The papers and presentations are good because you see the real world applications to topics as opposed to just memorizing for exams. I feel I have a more thorough understanding because of them.*

In the words of student Y:

> *The presentations allow us to see many real world situations that exams just can’t cover.*

While Student H said:

> *I do not prefer traditional exams. Paper/Presentation allows me to see the real world applications instead of just memorizing* facts.

Once again, the theme of deeper understanding of the course material is raised by Student CC. Additionally, both Students CC and H referred to exams as requiring “just memorizing”, indicating displeasure with the knowledge gained from the banking approach to education. “Real world” examples brought to the class through the presentations of research projects assisted students in developing a deeper understanding of the course material by highlighting its application in a variety of circumstances.

A final reason for the preference of research papers over exams was that the students could tailor the assignments their own interest by choosing environmental contamination sites from their home towns, or environmental contamination related to a hobby. For example, one student chose to do a research project on lead contamination in surface water near shooting ranges. Eight Students (F, KK, N, T, P, W, X, EE) specifically cited the ability to research a topic of interest to themselves as a reason for preferring research papers. Student F explained:

> *Projects allow research in an area of interest to me, and let me learn the material as a practical matter, not just to pass an exam.*

Student W stated:

> *I prefer the presentations because it gives me the chance to go in depth on a topic of my interest and learn a lot about it.*

While Student P said:

The method once again allows the student to narrow into an interest while still gaining an understanding of the class topics.

These statements reflect that the students preferred the research projects because of their ability to choose their own research subject. In fact, it would be impossible for any faculty member to tailor an assignment that would interest 51 students simultaneously. Allowing the students to exercise choice in their research subjects can result in assignments that captivate the interest of the whole class. As the class was composed of engineering students from two disciplines (civil and environmental engineering), this strategy allowed each student to focus on applying the course material to their own field, and may be a successful strategy when dealing with multidisciplinary classrooms.

Overwhelmingly, the students expressed a preference for the inclusion of the research paper and presentation over traditional exams. Students indicated a deeper conceptual knowledge gained of the application of course materials both from researching their own papers and from listening to each other's presentations. Students specifically valued the "real world" examples brought to the class from this assignment. The students indicated valuing the opportunity to choose research topics of interest to themselves. Additionally, students indicated displeasure at the memorization required for traditional exams.

4.4 Student Acceptance of Quizzes, Papers, and Presentation as Grade Components

The fourth question on the survey inquired whether grading based on quizzes, papers, and presentations appropriately measured student performance in the class. Only one student offered any criticism or complaints of the elements that made up the class grade. Student HH replied:

The quizzes are easy and the presentations/papers don't reflect all the course material.

Recall that Student HH earlier expressed a preference for exams over presentations. This student may perceive rote memorization as knowledge gained, and possess a reproducing orientation to learning. The aim of the papers and the quizzes as assessment tools was not to for students to demonstrate the memorization of facts, but for students to demonstrate a conceptual knowledge of the application of the course material. Thus, many details discussed in class would not be reflected in the quizzes.

In total, 40 of the 41 students surveyed approved of the course grade components. Overwhelmingly, the students praised weekly quizzes as a tool for enforcing attendance and reinforcing lecture material. Ten students (KK, E, Y, W, F, X, AA, DD, FF, Z) specifically mentioned useful aspects of the quizzes. Student E explains the incentive quizzes provide for attendance as follows:

You have to always attend class and keep up on your work.

While Student KK explained:

> *What is taught on Monday is quizzed on Friday. This helps (us to) know how much we retain.*

These students repeatedly cited retention and reinforcement of lecture material as results of the weekly quizzes. The quizzes also were considered a powerful motivator for weekly class attendance. It is not only important that class material captivate and engage students, but that they are motivated to participate in class. Weekly quizzes worked to prevent a pattern of falling behind in class material, or lack of attendance. As a result, quizzes proved a motivator for student involvement in the class.

Six students (Z, Y, F, X, AA, DD) specifically mentioned the ability of papers and presentations to develop and demonstrate a deeper knowledge of the class material. In the words of Student Y:

> *In order to write papers and give presentations you have to have a better understanding of the subject.*

While student DD explained:

> *Projects show applications of material learned.*

These students expressed that the papers were a place where more detailed knowledge and application of the course concepts could be conveyed.

Overall, the students approved of the course grade components. As mentioned earlier, the students expressed a perception of traditional exams as requiring "just memorization" rather than fostering a deeper learning approach. Despite the students' opinions of exams, the quizzes were praised for reinforcing the course material and enforcing class attendance. Thus, students not only valued an increased depth of knowledge, they also wanted to retain class information. Quizzes were valued for information retention, while the research papers were valued for the development and demonstration of a deeper understanding of course material.

5. Conclusion

As the results presented in Section 4 indicate, the students expressed an almost unanimous preference for the new format, including the following elements:

- No students expressed preference for the old class format when compared to the reduced lecture format.
- 40 of 41 students surveyed preferred inclusion of a weekly group discussion hour.
- 39 of 41 students surveyed preferred research papers and presentations over traditional exams.
- 40 of 41 students surveyed approved of the course grade components.

Reasons students cited for accepting this class format can be broken into two broad categories including: depth of knowledge and student interest.

The students indicated that preparatory assignments and discussion days were well coupled to add a greater understanding of each week's lecture topic. The students also stated that the discussion days were "more fun" and it was "easier to pay attention" on these days than in a traditional lecture.

The student response to the research papers and presentations indicated the wealth of "real world" examples brought to the through the presentations themselves and an increased depth of knowledge from researching the papers. Students also valued the papers for the ability to choose a subject of interests to themselves.

It should also be noted that there were almost as many comments purporting the increased depth of knowledge due to research projects (19 comments) when compared to group discussions and preparatory assignments (17 comments indicating increased depth of knowledge). Thus, students found both the research projects and discussions to be elements that increased their learning.

However, there were more than twice the comments related to preferring papers because of increased interest (8 comments) as compared to finding discussions interesting (3 comments). Students lead the discussion days, but did not specifically choose the topics to discuss, as these were based on each week's lecture. More choice was exercised over the content of the research projects than the discussions. This indicates that when it comes to capturing student interest, choice in content may be an important factor.

A few key alterations to the discussion days were suggested by the students that may increase motivation for preparation and quality of discussion. The preparatory research and reading assignments required for discussion days could be collected and included in the course grade component. This may provide greater incentive for students to attend discussion days prepared. Alternate discussion questions could be prepared by the instructor, to enrich the quality of discussions. However, these should be used sparingly, as student control over the discussion content was a key component that may foster a deep learning approach.

In conclusion, the students overwhelmingly preferred the reduced lecture format with the inclusion of active and collaborative learning techniques as opposed to a traditional banking education approach to the course. Students specifically noted an increased interest in the course and assignments and a deeper understanding of the application of course material provided by new class room methods.

Appendix A

CE4506 Survey

1. Have the Monday lectures been adequate to explain the weekly class topics? Would you prefer a tradition format of three lecture hours per week?

2. Do the Wednesday discussions improve your understanding of the topics? Discussion days usually require some student preparation prior to coming to class. Do you prefer these assignments to traditional homework?

3. This class replaces exams with two research papers and presentations. Please comment on the usefulness of these assignments and presentations as tools to enrich your understanding of the course material. Would you prefer traditional exams?

4. Do you feel that being graded on the quizzes, papers, and presentations adequately and appropriately measures your performance in the class? Explain.

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APPENDIX D:

Minority Student Enrollment in Environmental Engineering, General Student Perceptions of the Discipline, and Strategies to Attract and Retain a More Diverse Student Body

Abstract

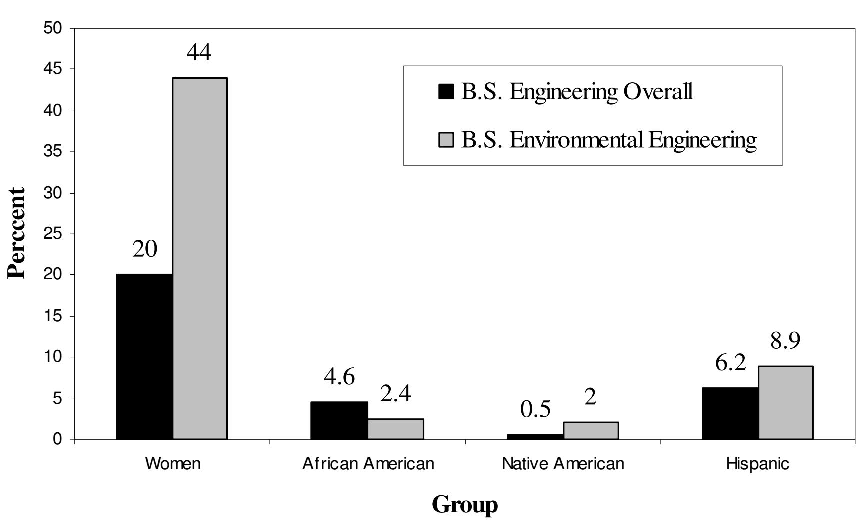
Environmental engineering, as a discipline has celebrated success at incorporating women into its ranks among undergraduate students. It appears that the discipline may also share a similar success at attracting Native American and Hispanic students. Data presented at the 2006 ASEE conference indicates that, across the nation, the discipline attracts more Native American and Hispanic students than engineering overall[1]. However, this paper takes a closer look at this data, which indicates that just a few schools across the nation are enrolling minority students within environmental engineering.

Perceptions of a discipline can alter career choice among first year students. This paper presents studies regarding the perceptions of the discipline among k-12 and first year students, and highlights the need for research regarding the perceptions of the discipline among minorities and factors influencing career choice of minority students. Finally, some suggestions are made for strategies which may increase the attraction and retention of minority students to the discipline.

Diversity within Environmental Engineering

Data from the American Society for engineering Education (ASEE) and the Engineering Workforce Commission (EWC) regarding enrollment and degrees awarded to women and minorities by engineering discipline for 2003 was compared and complied for a paper at the 2006 ASEE conference[1]. Figure 1 shows the percentage of bachelors degrees awarded to women and minorities for

Figure 1: B.S. Engineering Degrees Awarded in 2003[1]



environmental engineering and engineering overall. It can be seen that the discipline has successfully integrated women into its ranks, graduating a larger percentage of women (44%) than engineering overall (20%). In fact, environmental engineering graduated more female engineers than any other discipline in 2005[2]. Environmental engineering has emerged as a leader among the disciplines of engineering that attract women at higher percentages than engineering overall. The "pink collar" disciplines also include biomedical engineering, industrial engineering, and chemical engineering.

Environmental engineering is well on its way to closing the gender gap within the discipline. However, the enrollment statistics for minority students within the discipline are still woefully low.

At first glance, environmental engineering appears to exhibit a relative success in graduating Native American engineers, also shown in Figure 1. In 2003, the discipline graduated four times the percentage of undergraduate Native American students (2%) than engineering overall (0.5%)[1]. However, among 42 ABET-accredited environmental engineering programs in the nation, only 5 reported any Native American students in environmental engineering[1]. Thus, the national percentage of Native Americans enrolled in environmental engineering is misleading. It is not the discipline that is successful at attracting Native Americans, but just a few select programs. One could assume that these programs are likely to be in states with high percentages of Native Americans. The data in Table 1 compares the percentage of Native American students enrolled in each environmental engineering program, with the percentage enrolled in engineering overall, and the percentage of Native Americans among that state's population. No correlation exists between the two. The success of these programs must then be based on efforts of the individual programs.

Table 1: Percentage of Native Americans enrolled in engineering overall, environmental engineering, and state background population[1]

Institution	% Native American		
	All Engineering	Environmental Engineering	State Residents
North Carolina Agriculture and Technical Sate University	2	3	1.2
Montana Technological at University of Montana	0.9	5	0.8
MIT	2.2	9.1	0.2
Northern Arizona University	11.1	22.2	5
Southern Methodist University	10.2	33.3	0.6

In 2003, Hispanic students also represented a higher percentage of environmental engineering B.S. graduates (8.9%) than engineering overall (6.2%)[1], as shown in Figure 1. Among 42 ABET-accredited environmental engineering programs, 12 reported any Hispanic students in environmental engineering[1], shown in Table 2. Similar to the trend with Native American students, the success of the profession at attracting Hispanic students in greater percentages than engineering overall is not standard across the institutions. Table 2 also compares the percentage of Hispanic students enrolled in engineering overall, the percentage enrolled in environmental engineering, and the state background percentage of Hispanic residents. No relationship exists between Hispanic student enrollment in environmental engineering and either the state population or the enrollment of Hispanic students in engineering overall. Thus, the schools that did successfully attract Hispanic students into environmental engineering were not more likely to be in states with a large Hispanic population. The success of these programs must then, once again, be due to the individual efforts of those programs to attract minority students.

Table 2: Percentage of Hispanic students enrolled in engineering overall, environmental engineering, and state background population[1]

Institution	% Hispanic		
	All Engineering	Environmental Engineering	State Residents
Utah State University	0.5	20	14.2
Stanford University	7.2	50	32.4
MIT	11.9	9.1	6.8
Northern Arizona University	3.8	11.1	25.3
University of Southern California	8.6	20	32.4
North Carolina Sate University Raleigh	2.0	15	6.1
New Jersey Institute of Technology	13	50	13.3
University of Miami	29.1	33.3	19.1
Manhattan College	15.3	30	15.1
University of Florida	12.2	17.6	19.1
Cal Poly San Luis Obispo	9.3	5.3	32.4
University of Central Florida	10.6	11.8	19.1

A comparison of Tables 1 and 2 shows that only two schools, MIT and Northern Arizona University, reported enrollment of both Native American and Hispanic students in environmental engineering.

Table 3: Percentage of African American students enrolled in engineering overall, environmental engineering, and state background population[1]			
Institution	% African American		
	All Engineering	Environmental Engineering	State Residents
US Air Force Academy	3.4	16.7	12.2
Northwestern University	3.6	9.1	14.7
North Carolina Agricultural and Technical State University	78	71	21.6
Louisiana State University	7.5	8.3	32.5
University of Delaware	8.2	16.7	Not reported
University of Central Florida	7.2	5.9	15.1

The percentage of African Americans among undergraduate environmental engineering conferred (2.4%) in 2003 was below that of engineering overall (4.6%), as shown in Figure 1. Among the 42 ABET-accredited environmental engineering programs, only 6 reported any African American students enrolled in environmental engineering[1], as shown in Table 3. Again, no correlation existed between state demographics and enrollment of African Americans in environmental engineering programs within a state. Recall the previously mentioned trend of lower percentages of African Americans enrolled in environmental engineering when compared with engineering overall across the nation. Examination of Table 3 indicates that 4 of the 6 programs that attracted African American students did so at a higher rate than engineering overall at those schools. Additionally, the percentage of African American enrollment in environmental engineering at the 6 schools was much greater than the national average of 1.5%[1].

A comparison of Tables 2 and 3 show that only one school, University of Central Florida, included both Hispanic and African American students among their environmental engineering enrollment. Comparing Tables 1 and 3 shows that only one school, North Carolina Agricultural and Technical State University, attracted both Native American and Hispanic students to environmental engineering.

In the case of all three minority groups, enrollment of students from one of the groups in environmental engineering occurred at only a select few schools. Additionally, none of the schools managed to attract all three groups to environmental engineering, and only 4 schools(MIT, Northern Arizona University, University of Central Florida, and North Carolina Agricultural and Technical State University) managed to attract two of the minority groups to the discipline. Thus, enrollment of minority students in environmental engineering occurs within select programs, which tended to exhibit a unique success with regard to a specific minority population. It is likely that this success is based upon specific efforts to recruit and retain a target minority population on the part of the programs. The problem still remains, for the overwhelming majority of ABET accredited programs, of attracting minority students to the discipline.

However, among the schools that do manage to attract minority students into the discipline, they tend to be present at higher percentages than engineering overall. However, due to the relative lack of integration of minorities into the field across all the universities, it is impossible to determine if the field itself is actually more appealing to minority students. Further research is required to better understand the motivating factors behind minority student career choices. A better understanding of the values of these students could lead to more tailored and successful recruiting efforts.

Environmental engineering has been extremely successful at overcoming the gender gap existing in other disciplines. Women have traditionally been attracted in high numbers to serving or nurturing types of professions, such as nursing or teaching. Environmental engineering is often touted as a service profession, contributing to society and helping humanity. This image of the discipline has worked positively to attract women. In a recent study at the University of Colorado-Boulder, fist year 58% of females in a first year engineering course indicated an interest in serving society, compared with only 21% of the males[3]. Female students at this school also joined professional societies with a service focus, such as Engineers Without Boarders, at higher percentages than more traditional discipline specific societies, such as American Society of Civil Engineers.

Helping others matters to women. The success of the discipline in attracting female students may very well be that the primary goals of environmental engineering sync well with the altruistic desires of women. A deeper examination of the perceptions of the discipline and the factors which motivate discipline selection for minority students may provide some insight with regards to the comparative failure of environmental engineering to attract minority students. Recruiting efforts for minority students could be targeted to address their motivating factors behind career selection.

Perceptions of the Discipline

It is well known that the demand for environmental engineers is on the rise. Despite the fact that it is among the fastest growing engineering professions, there is a shortage of students enrolling in environmental engineering. Market demand and image of a discipline are known to be among the major motivators for selecting a discipline. As the market demand for environmental engineers is high, the image of the discipline has been suggested as a cause for both low overall and low minority enrollment[4].

Because environmental engineering practitioners emerge from a variety of academic roots (civil engineering, chemical engineering, biochemistry, etc.), and work in multiple specialties (water, wastewater, air, remediation, etc.), environmental engineering lacks a common definition as a discipline[4]. There is even a lack of agreement within educational institutions regarding the relationship of environmental engineering to other disciplines. Some schools include environmental engineering as a sub-discipline of civil or chemical engineering, some schools have created multi-disciplinary degree programs, and some consider environmental engineering a stand alone discipline[4]. The nebulous nature of the discipline causes confusion, and potential students often do not understand exactly what environmental engineering entails.

If incoming students lack a strong concept of environmental engineering, they are making uninformed decisions about their major. Students that select environmental engineering as a major often are aware of current environmental issues, and have a strong desire to save the world[5]. One recent class introducing the discipline to fist-semester environmental engineering majors at Michigan Tech resulted in significantly less confidence in choice-of-major and satisfaction with the major among the students. Reasons students provided for their decreased happiness with the major included the discovery that environmental engineering work is not performed outside, but mainly done in an office setting; the amount of math and science classes required; and that the field did not focus on animals[5]. These results indicate that those students that had selected environmental engineering as incoming first year students had little to no understanding of environmental engineering coursework, and did not understand the primary focus of the field.

A recent study examined the perceptions of engineering disciplines among high school students taking STEM courses in Rolla, Missouri[6]. Students were asked to identify their familiarity with various engineering disciplines on a scale of 1-5. Students indicated less understanding of environmental engineering (1.8) than the mean of all disciplines (2.53). Students were also asked to provide one word or a phrase they associated with the various disciplines. The students could accurately identify the themes, materials, or technologies associated with the more traditional engineering disciplines. For example, students associated the terms “electricity, circuits, wires, wiring” with electrical engineering. On contrast, students associated the terms “environment, trees, tree huggers” with environmental engineering.

It has been shown that high school students and first year environmental engineering majors did not have accurate perceptions of the environmental engineering field[5-6]. The student’s perceptions of the discipline were more along the lines of environmentalism than engineering. In fact, environmental engineering requires the application of math, science, and technology to mitigate the impacts of human activities on the environment. However, students interested in engineering, may not view the disciple as technically rigorous as other engineering disciplines. To attract more students into the discipline, the “engineering” nature of the discipline needs to be more accurately conveyed to young students. What is missing in the reported views of the discipline is a sense of the design and application of technology. It is this author's belief that the simplest and most accurate definition of the environmental engineering is the design and implementation of pollution control technology. Most in-coming students do not share this concept of the discipline.

However there is hope for changing students' perceptions of the discipline. Recent hands-on engineering education programs aimed at first year engineering students and high school students in Virginia resulted in increased awareness of the interdisciplinary nature of engineering[7]. Additionally, the introduction of an NSF STEM teaching fellow into K-12 classrooms resulted in an increased understanding of the different fields of engineering, and the ability of students to portray more disciplines more accurately[8]. Thus, students' perceptions of an engineering discipline can be changed, resulting in first year engineering students who are more accurately informed about the discipline.

As minority students are still woefully underrepresented within the discipline, there is a need for educational outreach specifically aimed at introducing the discipline and its objectives to minority students. Additional research is needed to determine minority student motivators for career choice and perceptions of the discipline.

Existing Efforts for Increasing Diversity within Engineering Overall

Many schools are recognizing the need to increase diversity within engineering programs. Efforts to increase diversity include efforts to both attract and retain minority students to engineering. Lessons can be learned from efforts to attract minority students to general engineering programs that may be applied within the discipline.

In recent years, decline in engineering enrollment has led to efforts to educate students prior to college about the engineering profession. The short term goals of K-12 programs are to provide hands on engineering experiences to children, increase their knowledge of engineering as a profession, and create awareness of the different disciplines. The long term goal of K-12 engineering programs are to increase enrollment in engineering programs. K-12 programs targeting minority groups are among the existing efforts utilized to recruit minority students into engineering[9-10].

K-12 programs could be utilized to educate students early and accurately about the discipline. Every parent is familiar with the kindergarteners' obsession with poop-based humor. This is a perfect age to introduce the concepts of sewage collection systems and wastewater treatment. As students age and are introduced to the concepts of picking up litter and recycling, solid waste management can be addressed. Basic concepts behind landfill design, such as design life and liners could be covered. High school students can be exposed to the more complex issues of chemical pollutants and remediation techniques. Age appropriate minority outreach should attempt to create a new generation of incoming college students that are familiar with the discipline.

K-12 programs are often aimed at increasing the diversity of incoming freshman classes. However, efforts can also be made to increase diversity beyond the first year of college. Thus, some schools facilitate community college transfers into engineering programs, as a means of increasing minority student enrollment[9-10]. Outreach should also be addressed to community college populations. Students enrolled in math and science classes should be targeted for their interest in core concepts. Additionally, educational materials about the discipline could be made available to enrollment counselors.

Funding is often an issue for many first generation college students. Increasing diversity in engineering programs means attracting students whose parents often did not attend higher educational institutions. Thus, some schools are increasing their efforts to identify funding for minority students[9]. Efforts should be made to identify funds specifically for minority students within the discipline.

Graduation is not ensured just because minority students are enrolled and their education is funded. In fact, individual schools that successfully attract minority students often have issues with the retention of minority students[9]. Thus, many schools are developing new programs aimed at increasing retention rates. First year introduction to engineering courses are being offered by many schools as an effort to increase the retention of engineering students[10]. The goal of these courses are to provide students with the basic skills for success as engineering students, familiarize students with the various disciplines of engineering, assist students in selecting a major, increase student sense of identity and belonging as engineers, increase student interest in engineering, and provide hands on engineering problem solving and design experiences earlier in the educational time line[11]. Recognizing the importance of hands-on learning for attracting a wider diversity of students to engineering, many programs are restructuring these classes to include more experiential learning techniques in an effort to increase retention among engineering students.

Early evidence exists that first year experiential learning based engineering courses increase retention. One recent study of a first semester engineering technology course at Old Dominion University found increased retention of students within the college, and more students transferred from engineering to engineering technology[12]. It is possible that students that may normally be averse to the traditionally reflective math and science course work of engineering, may be attracted to the more active and applied classes offered in engineering technology. If the loss of these students was due to the desire for more experiential learning, the implementation of these techniques within engineering classes could increase retention.

Another introduction to engineering course at the University of Virginia focused on teaching engineering in context (EIC), through a semester long project. EIC emphasizes the "application of the engineering problem-solving method to a current challenge or opportunity, coupled with more focused consideration of problem identification and definition and the potential impact of a solution." The EIC class replaced a traditional class based on the engineering science model, which focuses on the classroom presentation of technical knowledge and skills out of the context in which they are applied. Students taking this class provided a higher overall course rating and exhibited a slightly higher retention rate than previous students under a more traditional format[13].

First year experiential learning courses should address the various disciplines in an effort to aid student selection of a major. Students interested in environmental engineering should be engaged with hands-on projects as early in their academic career as possible, sustaining their interest through the more fundamental math and science classes of the early years.

Many schools also recognize the importance of industry partnerships that provide minority students with engineering co-op and intern experiences prior to graduation[9-10]. Industry partnerships with companies that value diversity can provide essential mentors and role models for minority students, in addition to the valuable hands on work experience.

Mentors can also be provided within the university setting. Some schools are recognizing the importance of mentoring and advising for increasing the retention of minority students[10]. Engineering educators are increasingly called upon to initiate dialogue about students' development and skills. Educators can provide essential advice to facilitate student success.

Another tool for increasing student retention is to encourage undergraduate research projects for minority students[9-10]. These projects also provide valuable hands on experiences that increase student identity as engineers and interest in engineering. They also provide ample opportunity for developing deeper mentoring relationships with students.

Conclusion

Environmental engineering has been comparatively successful at attracting female students. However, significant efforts need to be made to increase minority enrollment within the discipline. Currently only a handful of institutions are attracting minority students into environmental engineering programs. Minority recruitment and retention efforts practiced by general engineering programs should be further employed by individual environmental engineering programs. Additional research is needed to understand the factors influencing career selection among minority students.

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