Occurrence of Estrogen in Wastewater Treatment Plant and Waste Disposal Site Water Samples

by Katie McAvoy

Historical Perspective

The topic of pharmaceuticals in our water supplies first gained notice in Germany in the early 1990s when environmental scientists discovered clofibric acid, a cholesterol lowering drug in ground water below a water treatment plant (Pharmaceuticals in our Water Supplies, 1). This identical discovery of clofibric acid along with other pharmaceuticals in sewage water had been made in the US in 1970 by Wayne Garrison but gained no attention because research and legislation in that time period were focused on agrochemicals and pesticides (Daughton, 2). European concern and the resulting research was driven by the water delivery and treatment infrastructure in Europe. Europe has a higher density of urban life around their surface waters with a more antiquated sewer system and farming practices (Spotts, 3). Lower per-capita water use, smaller stream flows and higher confluence of sewage outfalls in municipal areas all resulted in higher discharge concentrations of pharmaceuticals and lower dilution by receiving waters (Daughton, 4). In the mid 1990s, Thomas Ternes, a German chemist, investigated what happened to pharmaceuticals after excretion. Up to 90 percent of pharmaceuticals pass through the human body to sewage treatment plants which are not designed to remove them (Kelli Whitlock, 2). Ternes found pharmaceuticals in treated and untreated sewage effluent, surface water, ground water and drinking water (Daughton, 1)

The US reacted and started developing new technologies to effectively test for this new contaminant group, pharmaceuticals and personal care products, or PPCP's. While the presence of PPCPs in water had most likely occurred with their first use, our scientific ability to measure their level in sub parts per billion has been only recently developed. The US Geological Survey (USGS) used gas chromatography/mass spectrometry analysis, or GC/MS, in its bench mark study of 139 US Streams in 1999-2000. In this study, chemicals from PPCPs were found in 80 percent of the streams sampled (Barnes, Kolpin, Meyer, Thurman, Furlong, Zaugg and Barber, 1).

Attention shifted from traditional pesticide and agrochemical concerns to this new class. Environmental Health Perspective estimated in 2002 that the amount of PPCPs released to the environment was equal to the amount of pesticides (Pharmaceuticals in our Water Supplies, 1). This release occurs from millions of unaware individuals. Pharmaceuticals pass virtually unchecked through sewage treatment plants (Spotts, 1) and approximately one million US homes send their sewage from septic tanks directly into the environment (Raloff, 2). It is not classified as hazardous waste (Emerging Contaminants in the Environment, 3). It is not tested for or treated for in wastewater (Reynolds, 1). There exists no maximum contaminant levels (MCL) for our drinking water. The EPA has not set standards for the majority of these pharmaceuticals (Woodhouse, 1), so drinking water plants make no effort to remove them (Male Fish Growing Eggs Found in Potomac, 2). Levels of estrone, a hormone, were between 45 and 80 parts per trillion in a study of Tulane tap water (Pharmaceuticals in Our Water Supplies, 2). The trillion dollar question is, "Is this too much?" The question becomes truly alarming considering PPCP use and subsequent contamination is on the rise world wide. This escalation is driven by a population which is increasing, living longer and consuming more drugs per capita. Television marketing is creating new potential consumers. Cosmetic drugs are a substantial new and growing category. Expiration of drug patents and the use of samples by physicians add to drug usage. All these increases will be further exacerbated by the increase in drug categories and types developed as mapping of the human genome unlocks medical mysteries (Daughton, 21), (Townsend, 3).

Origins of PPCPs in the Environment

"Sewage and domestic wastes are the primary sources of PPCPs in the environment" (Daughton, 8). Only a certain percent of pharmaceuticals are actually absorbed into the body; the rest are excreted. Pharmaceuticals enter sewage treatment plants and exit intact to surface water (Pharmaceuticals in Our Water Supply, 1). They also enter septic systems which are used by 25 percent of the US population where they have the potential to migrate into groundwater (Swartz, Reddy, Benotti, 1). Americans, innocently, dump expired or unused pharmaceuticals in the toilet and flush them into the water system. Unused drugs from households and hospitals alike are disposed of and taken to garbage dumps and landfills where leaching can occur contaminating ground and surface waters as a result (Daughton, 8). Cosmetics, toiletries, perfumes, sunscreens and detergents also enter the water system via washing and domestic use.

Soil practices and farming are sizeable secondary sources of PPCPs in the environment. Forty percent of the nation's antibiotics are currently fed to livestock as growth enhancers (Reynolds, 1). In 2001, the Union of Concerned Scientists estimated that 26.6 million pounds of antibiotics were fed to animals (Weir, 1). Hormones are given to farm animals to make them grow more quickly and become more fertile. Antibiotics and hormones which are unmetabolized are present in the animals' excreta which is washed from fields into rivers and streams. Higher concentrations of antibiotics in rivers are found near farms (Weir, 2). Animal manure and sewage treatment plant solids are used as fertilizers and soil amendments but they also add pharmaceuticals to the environment.

Antibiotics and estrogens are attracting a lot of attention due to their inability to naturally biodegrade and continued prevalence in the environment as a result of consistent sizeable release (Reynolds, 2). Scientists are concerned that this level of antibiotics in the environment will contribute to the spread of bacterial resistance and the ability of the antibiotics to treat infectious diseases (Townsend, 4). Estrogenic chemicals synthesized in pharmaceuticals are raising exposure levels in living things to the naturally occurring hormone estrogen. This compounded exposure has been associated with breast cancer, endomitriosis, birth defects, abnormal sexual development, lowered sperm counts and world-wide accounts of the feminization of male fish near sewage treatment plants. These chemicals have the potential of interfering with hormone production and are termed endocrine disrupting hormones.

Estrogen and Its Forms

Estrogen refers to any of several female sex hormones or synthetic compounds having similar composition or effect. The three

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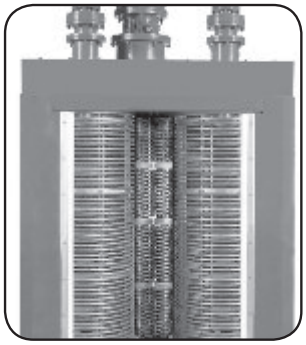

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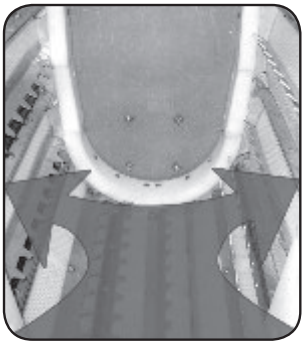

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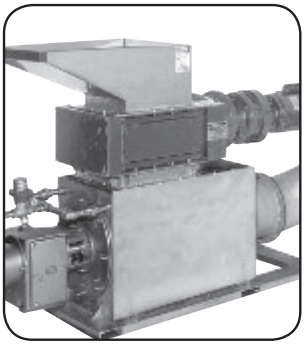

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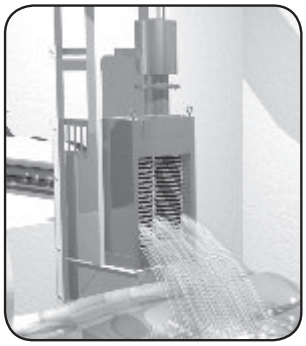

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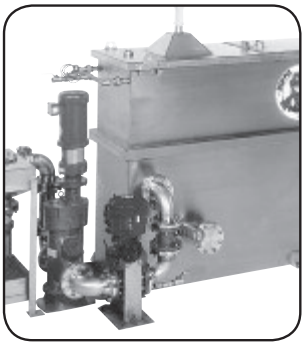

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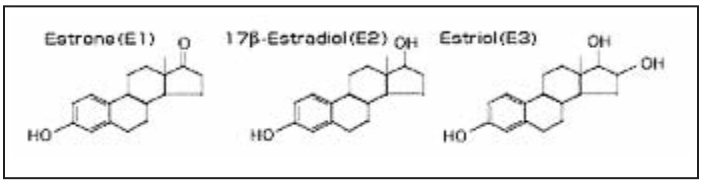
naturally occurring estrogenic hormones are: Estrone (E1) C18H22O2, Estradiol (E2) C18H24O2 and Estriol (E3) C18H24O3. Estrone is found in postmenopausal women. Estradiol is the principal estrogen found in mammals during reproductive years. Estriol is made in large quantities during pregnancy. Estradiol is the strongest, estriol the weakest. Estriol is water soluble, estrone and estradiol are not.



Phytoestrogens are naturally occurring estrogens in plants. They can be found in clover, soybeans, vegetables and whole grains. Mammals have evolved alongside these environmental estrogens. The emerging class of environmental estrogens are not naturally occurring; they are manmade estrogenic chemicals. These synthetic chemicals which mimic estrogen are found in pharmaceuticals (drug estrogens, i.e., birth control pills, hormone replacement therapy), pesticides, products associated with plastics, household products, industrial chemicals, and heavy metals. They are endocrine disrupting hormones.

Natural hormones: estrone, beta estradiol, estriol, and phytoestrogens are short lived. They stay in the blood stream for minutes to hours and then are broken down by enzymes in the liver into pieces which are either excreted or used to build other molecules. They are not bioaccumulative in fatty tissue. Estrogenic drugs such as ethynylestradiol found in birth control pills are more stable and remain in the body longer than natural estrogens. Two large classes of drug estrogens are birth control pills and hormone replacement therapy. In 2005, there were over 41 million prescriptions filled for these drug estrogens (as compiled using http://www.rxlist.com/top200.htm). Pill forms of estrogen enter septic and sewer systems at up to 90 percent effectiveness. Estrogen is an active ingredient in most oral contraceptives and finds its way back into surface waters through sewage systems (http://www.brightsurf.com/news/june_03/PNNL_news_060403.html). Patch and ring forms of birth control may enter landfills with up to 80 percent of the ethinyl estradiol unused. Estrogen shows up in water either through waste or birth control pills going down toilets (http://www.detnews.com/2005/project/0508/14/Z04-275435.htm).

The problem with pharmaceuticals is further exacerbated by synthetic environmental estrogens from pesticides, plastics, household products, industrial chemicals, and heavy metals. These can mimic the role of the estrogen hormone in the body. They enter our bodies and bioaccumulate around fatty tissues. They are not easily broken down and remain intact in the environment. They tend to work their way up the food chain. Chemicals that mimic the effects of estrogen wash off farmlands or are by products of manufacturing.

Environmental Impact of Estrogens

The synergistic effects of exposure to natural estrogens compounded with manmade estrogenic chemicals is being observed worldwide in many species (Environmental Estrogens Differ from Natural Hormones, 2). The most pronounced effect is occurring with aquatic species that make their homes in waters with elevated levels of estrogens. These generally occur downstream from wastewater treatment plants. Fish in these areas worldwide are being feminized.

In 2004, Vicki Blazer, a fish pathologist with the USGS, discovered inter-sex fish exhibiting both male and female characteristics in the Potomac River. Seven of 13 male bass tested positive for feminine characteristics; six of seven tested positive for the protein vitellogenin which starts egg production; three of six were actually carrying eggs ("Potomac 'intersex' fish worry scientists," 1). This occurred downstream from a sewage treatment plant (STP). Similar findings have been reported downstream from STPs in Las Vegas, Minneapolis (Townsend, 4), Boulder (Costello, 1), and the UK (Daughton, 16). A study at the Department of Energy's Pacific Northwest National Laboratory in 2003 indicates that when adult male fish are exposed to short term low concentrations of a synthetic estrogen, their fertility can drop as much as 50 percent ("Short-term exposure to estrogen cuts fish fertility," 1). This is significant because the 10 nanogram-per liter (ng/L) level used has been found in some surface waters.

The drop in male fertility may not only be affecting aquatic life. In the UK a research study at Brunel University has linked a decline in male sperm count to low levels of birth control hormones in the environment (Daughton, 16). This decline is documented also by Niels Skakkcback, a Danish endocrinologist, who is considered a leading authority on the effects of estrogen levels in water. In 1991, he reported that due to exaggerated exposure to estrogen-like substances, men are exhibiting 50 percent lowered sperm counts and reduction in size of reproductive organs. In 2005, 100 scientists from fifteen European countries signed a report documenting concern for reproductive disorders in European males which called for a reduction of chemicals being released from sewage treatment plants into waterways and an acceleration of research (Schabath, 2).

Women are at no less risk in terms of escalating exposure to estrogen. As noted, synthetic estrogens are resistant to breakdown, entering water supplies and becoming stored in fatty tissue areas. Long Island has the highest incidence of breast cancer in the US and it is linked to estrogen mimics which were used in farming practices before regulation of the products and urbanization of the island (http://cancerresourcecenter.com/articles/article38.html). Estrogen overexposure can cause other cancers and endometriosis. Fetuses exposed to "high" levels of estrogen have higher incidence of birth defects and males show female tendencies (Pharmaceuticals in Our Water Supplies, 2). Ironically, while research and technology search for treatment for infertility and breast cancer, our own pharmaceuticals and innovations may be contributing factors.

Recommendations

Currently, American wastewater treatment plants do not, as a standard, test for estrogen levels or any other PPCP as "treated" water leaves the facility and re-enters the environment ("Drug Flush-Lake Mead," 1). There is no established maximum contaminant level to address and estrogenic chemicals and other PPCPs are not classified as hazardous waste. Yet there is increasing evidence that they pose a mounting environmental health risk. *The Journal of the American Medical Association* (July 2005), stated that there is "accumulating evidence" that the wide use of chemicals may have a hormonal affect on the body and long-term health risks (Schabath, 2).

There are many tactics which could be employed to control our exposure to estrogen. They can be broken down into consumer education, government regulation, medical innovation, proper disposal, and effective filtration in wastewater treatment facilities. These approaches would focus on limiting the introduction of estrogenic chemicals into the environment and efficient removal of those introduced.

Consumers need to be made aware that these chemicals pose a health risk and they require proper handling and disposal. The government needs to adopt regulations to support this end. Estrogenic chemicals must be classified as hazardous waste. Unused birth control pills, used birth control patches, and unused hormone replacement therapy drugs should be part of drug "take back" programs and either recycled or disposed of as medical waste by proper incineration (Emerging Contaminants in the Environment, 1).

Wastewater treatment plants must test for estrogen contamination before release of treated water back in the environment. If contamination exists, several filtration and treatment methods have proved effective. Researchers at Carnegie Mellon University have found that a rapid environmentally-friendly catalytic process involving Fe-TAML activator and hydrogen peroxide breaks down two types of estrogens ("Carnegie Mellon, USDA report that Fe-TAML catalysts degrade estrogenic compounds," 1). The catalyst interacts with hydrogen peroxide to form an intermediate compound that breaks down toxins via oxidation (Smith, 1). According to the Carnegie Mellon researchers, 95 percent of estradiol and ethinylestradiol (synthetic estrogen in birth control pills) is broken down within five minutes. Ninety percent of estrogen can be eliminated using a dose of activated carbon (10mg/L at a contaminant level of 3 ppb – Duff, DeAvila, 1). This process is costly, but has been used effectively in many European wastewater treatment plants (Raloff, 3). Advanced oxidation techniques, such as ozone and ultraviolet or ozone and hydrogen peroxide, are also effective as are membrane filtration and filtration with granular activated carbon for many PPCP-related pollutants. Chlorine, a standard US treatment ingredient, has not proved effective (Reynolds, 2).

Christian Daughton, chief of the EPA's Environmental Chemistry Branch, has also proposed a "Green Pharmacy" approach to pharmaceuticals. His approach would unite the medical community, environmental scientists and citizens to minimize the environmental release of PPCPs. This would involve drug companies developing better biodegradability of PPCP's on the market and before entering the market. It would re-educate medical professionals to prevent over prescribing pharmaceuticals. Drug companies would be encouraged to lower dosages contained within medicines to the 10 percent absorbed by the body (Woodhouse, 3). Drug take back programs would be initiated in pharmacies, instead of dumping unused portions in toilets (Daughton, 3).

Combining education, regulation, a "Green Pharmacy Approach," effective wastewater treatment, and prudent disposal, this concern could be sizably curtailed. The question is when.

The Experiment

The occurrence of estrogen in four water samples is tested in this study. Three samples were taken from a water pollution control plant at influent, post primary settling and post chlorination stages in the treatment process. One sample was taken from an estuary thought to possibly contain leached fluids from a 46-year-old landfill. Samples were then filtered in the laboratory. Acetone was added to filtered samples to extract the hormone. Then samples were analyzed using gas chromatography/mass spectrometry. Site selection was biased toward situations that research indicated were pathways for estrogen entering the aquatic environment. Synthetic estrogens from pharmaceuticals like birth control or hormone replacement are transported

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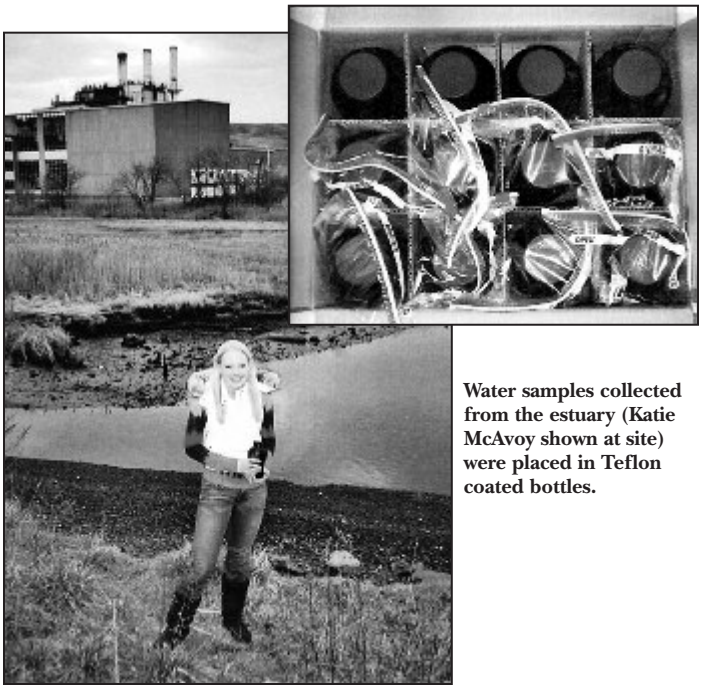
through septic systems and wastewater treatment plants back into surface water and potentially groundwater. Improper disposal of synthetic estrogens in landfills can leach back into surface water. Estrogen, in the forms of estrone, a-estradiol, b-estradiol or estriol, was detected in 100 percent of samples at levels ranging between .557ng/L upwards to 25.0 ng/L.

Hypothesis

My hypothesis is that the hormone estrogen would be present in all four samples: the estuary bordering the landfill and all three stages of the water reclamation process at the wastewater treatment plant. This study is designed to select sites where scientific research, literature and conversations with qualified professionals have indicated estrogen concentrations will be observed.



Water samples collected from the estuary (Katie McAvoy shown at site) were placed in Teflon coated bottles.

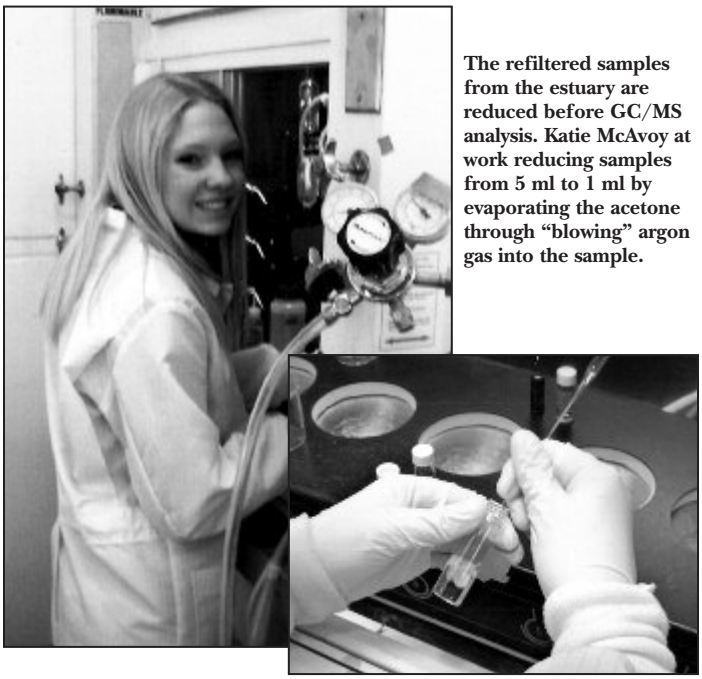
Sampling Sites and Methods

When selecting sampling sites, I wanted to choose those relatively close by, that would provide me with the best results. The Oceanside landfill was operated as a municipal landfill by the Town of Hempstead from 1961 to 1988. The site was chosen to check for possible leakage containing estrogens from improperly disposed pharmaceuticals that would flow into Barnum's Island Channel. The Cedar Creek Water Pollution Control Plant was placed into service in 1974 and treats domestic, commercial and industrial wastewater from Nassau County so as to protect the waters of Jones Beach, a major recreational and fishing area. It was chosen as a site to illustrate that estrogen is not removed effectively from wastewater.

Four sites were sampled: an estuary containing possible leached fluids at the Oceanside landfill; influent; post primary settling; and, post chlorination stages at Cedar Creek WPCP. In all cases, new surgical gloves were used when taking samples. Amber I-Chem guaranteed sterile sampling bottles equipped with Teflon neutral caps that were first rinsed with DI ultra pure water. DI water was recaptured in each case for integrity test sample 5, to ensure results. After rinse, two samples were obtained at each site. Each sample was then labeled, bagged in plastic, boxed and refrigerated for 24 hours.

Laboratory Preparation

In order to successfully separate the samples, certain steps were performed. Filtration was the first step. In order to filter the samples, it was necessary to wash all filtering apparatus with soap and water, then rinse them with DI ultra pure water, followed by a rinse with "pesticide grade" methanol in a vented booth. Then the apparatus were dried off in an oven. After that, the samples were run through a .7 µm (micrometer) filter via pressure pump and refrigerated. Then the samples were refiltered through a vacuum pump with a 6 nm (nanometer) filter. Extraction was next, in which the sample was reduced to two-thirds and acetone added to extract/elute (to remove from an adsorbed material by use of a solvent) the hormone. Then the samples were refiltered through a 6 µm filter, and 5 ml (milliliter) samples were stored in a refrigerator. After that, the samples were reduced from 5 ml to 1 ml by evaporating the acetone via "blowing" argon gas into the sample, and then were refrigerated until they were run through the GC/MS.



The refiltered samples from the estuary are reduced before GC/MS analysis. Katie McAvoy at work reducing samples from 5 ml to 1 ml by evaporating the acetone through "blowing" argon gas into the sample.

Analytical Methods

GC/MS is an analytical method which combines the features of gas-liquid chromatography and mass spectrometry to perform a specific test for a specific substance within a test sample. During the 1960s, the mass spectrometer was first used as the detector in gas chromatography. The gas chromatograph uses the difference in the chemical properties between different molecules in a mixture to separate the molecules. Different molecules take different amounts of time to exit the gas chromatograph which allows the mass spectrometer downstream to separately evaluate and identify these molecules. This is done by breaking down each separate molecule into ionized fragments. Identification can be performed by using their mass-to-charge ratio which is specific to the fragment. SIM, or selective ion monitoring, can then be used to look at certain peaks which are associated with a particular substance. In this case, peaks associated with E1- estrone, E2- beta estradiol and E3-estriol, will be compared with peaks resulting from samples taken from the four test samples.

Resulting Data

The results were surprising. The influent sample contained no estrone, but had 3.09 ng/L of alpha-estradiol, 7.22 ng/L of beta-estradiol, and 6.26 ng/L of estriol. The post primary settling sample was surprisingly higher in estrogen concentration containing 2.22 ng/L or estrone, 9.19 ng/L of alpha-estradiol, 6.05 ng/L of beta-estradiol and 25.0 ng/L of estriol. The sample after chlorination contained no estrone, 5.78 ng/L of alpha estradiol, 1.0 ng/L of beta-estradiol, and 5.38 ng/L of estriol. The last sample taken from a landfill contained no estrone, 2.08 ng/L of alpha-estradiol, 2.61 ng/L of beta-estradiol and .557 ng/L of estriol. These results were surprising to me because the second sample was so high in estrogen concentration, even surpassing the sample from the landfill (see Table below).

Estrogen Occurrence Levels in Parts Per Trillion

	estrone	a-estradiol	b-estradiol	estriol
Land Fill Estuary	–	2.08	2.61	.557
WWTP influent	N/D	3.09	7.22	6.26
WWTP post primary settling	2.22	9.19	6.05	25.0
WWTP post chlorination	N/D	5.78	1.00	5.38

Conclusions

Estrogen proved to be present in 100 percent of samples as originally hypothesized. Concentrations ranged between .557 ng/L and 25.0 ng/L, with mean level at 12.2 ng/L and median level at 5.38 ng/L. Studies vary as to what level causes adverse health effects to aquatic life. The DeMoines Waterworks study of 2005 found that levels at or above 25 ng/L cause kidney impairment, necrosis, liver damage, reproductive impairment and feminization to fish and aquatic life. The Department of Energy's (DOE) Pacific Northwest Laboratory's 2003 study indicates that even 10 ng/L of exposure to estrogens over a short term can cause a 50 percent decrease in sperm fertilization capacity. Research must address the issue of what should be considered a safe level for living things. Even qualified scientists classify these levels differently. Christian Daughton, chief of the USEPA's Environmental Chemistry Branch, said: "Concentrations are all in the low ng/L range," in this study. According to Bruce Brownawell, PhD, of the Marine Sciences Research Center at SUNY Stonybrook: "The measurements you report are high but not unbelievable."

Levels of all estrogens were extremely variable in the sewage treatment facility. Grab samples – one influent, two post primary settling, and three post chlorination – taken from the facility each represent only one point in time and space of the flow and are not controlled to represent the larger stream flow. Estrogens can, in theory, increase in the sewage treatment process when they become enzymatically released from the glucuronide and sulfate conjugates. Also, the estrone level can increase as it is oxidized from estradiol. These three effects may account for why levels of three estrogens: estrone, b-estradiol, and estriol, increase in the post primary settling test. Estradiol levels are higher at the sewage treatment plant than at the waste disposal site because natural estrogens and birth control estrogens are contained in excreted fluids which travel through the plant past chlorination and back into local surface waters. Levels of estradiol were significantly lower in the landfill estuary than the sewage treatment plant. The landfill had been out of operation since 1988 so any disposal of birth control pills would have to have

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occurred prior to that date with no consistent accumulation for almost two decades. Birth control and hormone replacement patches which would be disposed of in a garbage disposal site were developed after the site's closing. Estrone, a natural hormone from post-menopausal women, was not detected and this would make sense since urine is not a factor at this site. Estriol, a natural hormone made during pregnancy and excreted was found at a trace level.

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Katie McAvoy graduated with honors this year from West Hempstead High School, West Hempstead, NY, and is currently attending Fordham University. Her research paper, a two-year effort, won the Stockholm Junior Water Prize (SJWP) at the Long Island Science and Engineering Fair (LISEF) last year. Her project was selected as one of the state's finalists for the national Water Environment Federation's SJWP competition. Ms. McAvoy also earned the Ricoh Sustainable Development Award from the LISE Fair and the Legacy Award from the GCSAA Environmental Institute for Golf. She is the recipient of NYS Leaders of Tomorrow and Rotary Student of the Year scholarships.

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