Abstract

The goal of our research is to demonstrate that the rate of the synthetic estrogen, 17α-Ethinylestradiol (EE2), in the Denton Wastewater Treatment Plant (DWTP) effluent would be affected by the presence of college students. In the process of analyzing the thesis, we scanned the sample of sewage sludge through the liquid chromatography mass spectrometry (LC-MS) to detect the level of EE2, in which data was recorded throughout the school year and during the summer. We show in this report that the concentration of EE2 found in the waterways remains high during the time when students are on campus, specifically in April and October which means that students attending college has interfered with the water pollution caused by birth control pills. According to that, the result of the experiment proved our hypothesis to be incorrect due to the difference in the level of EE2.
Introduction

In the past few years, estrogentic compounds like 17α-Ethinylestradiol (EE2), which can be found in birth control pills, has been considered of having potential harm to human health and aquatic life since it can "bend the gender of pieces of animals living in or near water contaminated with the residue from oral contraceptives." The study of this biological dilemma shows that the body of a male fish reacting with EE2 leads to the condition called intersex, which will alter the hormonal and homeostatic system then change the animal’s ability to reproduce; meanwhile, it is not clear yet if the level of EE2 can affect mammal’s reproductive cycle. However, the process of effectively removing such compound from water is costly. Once the compound enters the waterways, it is very difficult to remove from the wastewater, which carries it into the natural water source that might become the water supply for agricultural, industrial or cooking uses. Although there are many components and issues that object to this research, there will not be a difference in the concentration of EE2 in Denton wastewater treatment plant effluent when college students are attending college in contrast to when they are not attending college. By doing this research, we want to suggest female students to consider in choosing among birth control methods and raise concerns about environmental impacts of hormonal contraception.

Materials

The list that follows includes the materials and methods that will be needed to conduct this experiment which will determine if the concentration of EE2 in the Denton wastewater treatment system is affected when college students are attending and not attending college.

- 1000 ml effluent samples of EE2
- d3-17B-estradiol
- 500 ml ethyl acetate
- 1.5 ml amber glass vials
- 100 µl of methanol
- Liquid Chromatography
- Nitrogen
- 50 µl aliquot
- Micromass Quattro Ultima Mass detector (quadrupole-hexapole-quadrupole instrument)
Methods

- Spike 1000 mL of EE2 with d3-17b-estradiol
- Liquid-liquid extract the samples twice with 500 mL of ethyl acetate
- Combine the ethyl acetate layers until dried under a stream of nitrogen
- Reconstitute the resulting residues in 1 mL of ethyl acetate
- Transfer the solvent to a 1.5mL amber glass vial
- Put results under a stream of nitrogen with residue resuspended in 100µl of methanol
- Take a 50 µl aliquot from the solvent
- Use the aliquot for estrogen quantifications using a dansyl chloride derivatization method

Data

The data that follows accounts for the concentration (ng/1) of EE2 in the Denton WWTP Effluent during the months of April, July and October.

![Graph 1: Concentration of EE2 in the Denton WWTP Effluent During April, July and October](image)

*Below levels of analytical detection, which is 0.5 ng/L*
Results

According to the data, it is apparent that the variance of the concentration of EE2 in the Denton WWTP effluent during the months of April, July and October shows that the waterways remain high during the time when students are on campus especially in April and October. When students are not attending school, which is during July, the levels of EE2 are below levels of analytical detection, which is under 0.5 ng/l. When students are attending college, during April and October, the levels of EE2 are from 2-5 ng/l. These findings demonstrate that student attendance interferes with the water pollution caused by birth control pills, therefore our hypothesis is proven to be incorrect.

Discussion

Our hypothesis stated that there would be no difference in the concentration of EE2 in the Denton waste water treatment plant effluent when college students are attending college and not attending college. The results in the table do not support this hypothesis because it is clear that rates of EE2 were higher in the months that students were present than when they were off during the summer. Replicated sample 1 shows 3 ng./L in April and 2 ng./L in October when students are present during school. When students are off during the summer, however, during July, the table shows a significant difference with a 0.5 ng./L, showing that the concentrations of EE2 were smaller during the summer. Results were similar for replicated samples 2 and 3, showing a low EE2 concentration in the water in July when students are absent and higher concentrations during the spring and fall when students are present. This is due to that college students increase the population of Denton and increase the consumption of birth control that contains the chemical EE2 that is in the water system of Denton. Decreasing the population decreases the likelihood that more people will buy birth control due to a lack of variety of different opinions and usage habits. However, because the team was more focused on the possibility that birth control was the cause of the spike of EE2 levels in Denton’s water supply, we didn't consider other possibilities that could be causing the pollution. I would suggest broadening the experiment in order to catch other possibilities that may cause rising levels of EE2 in the water supply. For future works on preventing higher amount of EE2 in the water supply, it would be suggested to lower birth control consumption as in lower EE2 levels, and increase injections to prevent pregnancy as well as promotions to increase practices of abstinence.
Conclusion

Based on the experiment and data that was collected for the concentration (ng/1) of EE1 in the Denton WWTP effluent in April, July and October, it can be concluded that the concentration of EE2 in the Denton wastewater treatment plant effluent varies when college students are attending and not attending college. The waterways remain high during the time that college students are attending college during April and October in comparison to July, when students are not attending college, which concludes that student attendance interferes with the water pollution caused by birth control pills. Our hypothesis was proven to be incorrect according to the data that was collected.
References


