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Short Communication

The Determination of the Amount of Estrogenic Compounds in the Birmingham Water Supply

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Introduction

The environment contains many endocrine disrupting compounds (EDCs). EDCs include pesticides, polychlorinated biphenyls (PCBs), dioxins, furans, alkyl phenols, steroid hormones (natural and synthetic), and other contents [1]. It is important to note that a recent comprehensive literature survey of 48 endocrine disrupting chemicals (EDCs) revealed that 79% of these EDCs were also carcinogenic or mutagenic and 52% were also immunotoxic [2]. The amount of estrogen in the water has become a concern in the recent years. Many people are worried about their health. It has also raised the question in the community: is the water harmful?

Previous studies have looked at the various endocrine disrupting compounds (EDC) in the water supply. The goals of this research was to asses and quantify only the natural and synthetic estrogen content in the waste water treatment plants (effluent, 50 meters upstream and downstream from the effluent) in the Birmingham area The samples used were taken from the Five Mile Creek, Cahaba, Leeds, Valley, Village, and Turkey waste water plants in the months of August, September, and October. The estrogenic compounds that were analyzed from the samples were estriol, ethinylestradiol (EE2), beta-estradiol (b E1), alpha-estradiol (aE1), and estrone. LC/MS/MS was used to analyze the presence of the estrogen compounds.

Methods

The steps below were completed for all of the cartridges.

Phase extraction

100 microliter of internal standard was added to 1L of river water passed over the bond elute cartridges at UAB.

- **Step 1**: Washed all cartridges with 6mls of 40% methanol and then discarded and used the light vacuum. Washed all cartridges with 6mls of distilled water and then discarded and used the light vacuum. Pulled vacuum through cartridges to dry.
- **Step 2:** Eluted analytes off the cartridges. Added 6mls of 10% Methanol in TBME and the collected that in tubes and used the light vacuum. Pulled the vacuum through cartridges to collect everything.
- **Step 3:** Transferred approximately 3mls of the collected solution at a time to small culture tubes. Dried under stream of nitrogen at 60 degrees Celsius.

Dansyl Chloride Derivatization and TBME Extraction

- **Step 4:** Dissolved the residue in 100 microliter of 100mM Sodium Bicarbonate buffer, pH 10.5. Then, vortexed each tube. Added 100 microliter of 1mg/ml Dansyl chloride solution. Vortexed again for 1 minute. Then, heated the tubes to 60 degrees Celsius for 4 minutes.
- **Step 5:** Added 1ml of TBME and vortexed well. Transferred the top layer (TBME) to a clean culture and dried down. Redissolved in 400 mircoliters of 80/20 ACN/ 5mM
- **Step 6:** Transferred to limited volume to auto sample vials and analyze. LC/MS/MS was used to analyze the presence of the estrogen compounds.

Results

	Estriol	EE2	b E1	a E1	Estrone
Sample Name	**Conc (ng/L)**	**Conc (ng/L)**	**Conc (ng/L)**	**Conc (ng/L)**	**Conc (ng/L)**
Five mile up	No IS (No Peak)	No IS (No Peak)	No IS (No Peak)	No IS (No Peak)	No IS (peak was detected)
Five mile down	No Peak	No Peak	No Peak	No Peak	BQL
Five mile eff	No Peak	No Peak	No Peak	No Peak	No Peak
Cahaba up	No Peak	No Peak	No Peak	No Peak	BQL
Cahaba down	No Peak	No Peak	No Peak	No Peak	BQL
Cahaba eff	No Peak	No Peak	No Peak	No Peak	BQL
Turkey up	No Peak	No Peak	BQL	No Peak	BQL
Turkey down	No Peak	No Peak	No Peak	No Peak	BQL
Turkey eff	No Peak	No Peak	No Peak	No Peak	No Peak
Leeds up	No Peak	No Peak	No Peak	No Peak	BQL
Leeds down	No Peak	No Peak	No Peak	No Peak	BQL
Leeds eff	No Peak	No Peak	No Peak	No Peak	BQL
Village up	No Peak	No Peak	BQL	No Peak	BQL
Village down	No Peak	No Peak	No Peak	No Peak	0.811
Village eff	No Peak	No Peak	No Peak	No Peak	0.612
Valley up	No Peak	No Peak	No Peak	No Peak	0.831
Valley down	No Peak	No Peak	No Peak	No Peak	1.00
Valley eff	No Peak	No Peak	No Peak	No Peak	0.856
BQL = below quantitation limit (0.5 ng/mL)					

Table 1: Data from August and September Sample.

	Estriol	EE2	b E1	a E1	Estrone
Sample Name	**Conc (ng/L)**	**Conc (ng/L)**	**Conc (ng/L)**	**Conc (ng/L)**	**Conc (ng/L)**
Leeds up 10/24/13	No Peak	No Peak	BQL	No Peak	BQL
Leeds down 10/24/13	No Peak	No Peak	No Peak	No Peak	BQL
Leeds eff 10/24/13	No Peak	No Peak	BQL	No Peak	No Peak
Turkey Creek Up 10/21/13	No Peak	No Peak	BQL	No Peak	BQL
Turkey Creek down 10/21/13	No Peak	No Peak	No Peak	No Peak	BQL
Turkey Creek eff 10/21/13	No Peak	No Peak	No Peak	No Peak	No Peak
Village up 10/30/13	No Peak	No Peak	BQL	No Peak	BQL
Village down 10/30/13 *	No Peak	No Peak	No Peak	No Peak	BQL

Village eff 10/30/13	No Peak	No Peak	BQL	No Peak	BQL
Cahaba up 10/30/13	No Peak	No Peak	No Peak	No Peak	BQL
Cahaba down 10/30/13**	No Peak	No Peak	No Peak	No Peak	BQL
Cahaba eff 10/30/13	No Peak	No Peak	No Peak	No Peak	No Peak
Five Mile up 10/21/13	No Peak	No Peak	No Peak	No Peak	BQL
Five Mile down 10/21/13	No Peak	No Peak	No Peak	No Peak	0.605
Five Mile eff 10/21/13	No Peak	No Peak	No Peak	No Peak	BQL
Valley up 10/23/13	NS	NS	NS	NS	NS
Valley down 10/23/13	No IS (No Peak)	No IS (No Peak)	No IS (No Peak)	No IS (No Peak)	No IS (peak was detected)
Valley eff 10/23/13	No Peak	No Peak	No Peak	No Peak	0.617

Table 2: Data from October Samples.

Discussion and Conclusion

LC/MS/MS was used to analyze the presence of the estrogen compounds. The samples were compared to the known standards. Only Five Mile Creek Up and Valley Down samples did not have the internal standard in the cartridges. Estrone and b E1were the main analytes that were seen in the samples, but majority of the peaks detected were below the quantitation limit (BQL). Seven out of the thirty- six samples had measurable amounts of estrogenic compounds in the samples analyzed. Estrone was the only compound that had amounts above the quantitation limit of 0.5 ng/mL. Among all of the sites that had measurable estrogenic analytes, they were mostly found in the effluent and downstream samples. The data showed that majority of the estrogens are being removed from the water by the waste water plants in the Birmingham area. Overall, the amount of estrogenic compound detected in water may not be harmful and is considered safe to drink.

Bibliography

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