Detection and Quantitation of Estrogen in Watersheds

Aaliyah McCullough*, Jasmynn George* & Mintesinot Jiru**
* Graduating Senior, Department of Natural Sciences, Coppin State University, Baltimore, MD, USA
** Associate Professor, Department of Natural Sciences, Coppin State University, Baltimore, MD, USA

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Abstract- This paper focuses on the detection and quantitation of estrogen hormone in Baltimore's watersheds. Baltimore is the largest city in the U.S. state of Maryland, and the 30th-most populous city in the United States. Baltimore's drinking water primarily comes from three watersheds (Loch Raven, Prettyboy, and Liberty). Between 1999 and 2000, the U.S. Geological Survey sampled 139 surface waters throughout the U.S and they discovered that 80% of these waters contained endocrine disrupting chemicals (mostly estrogens). Fish, are changing sex due to exposure to excess estrogen. To check the presence of estrogen in water, we used an enzyme-linked immunosorbent assay (ELISA) kit and to quantify the concentrations, a microplate reader was used. Measurements were made during fall 2017 & winter 2018 to study the seasonal variations. Based on the measured optical density, we found traces of estrogen in the study watersheds during both seasons. The average estrogen levels in the Loch Raven sub-sheds was 0.074 ppb (Fall 2017) and 0.048 (Winter 2018) and for Liberty sub-sheds it was 0.046 ppb (Fall 2017) and 0.040 ppb (Winter 2018). In Prettyboy sub-sheds the level was 0.067 ppb (Fall 2017) and 0.042 ppb (Winter 2018). Overall, Loch Raven watershed has slightly higher levels of estrogen than the other two watersheds, both during the fall and winter seasons.

Index Terms- Estrogen, Watershed, ELISA, Microplate reader, Baltimore

I. Introduction

The past several years have seen a steady drumbeat of news reports and scientific studies which have raised concerns about the presence of estrogenic compounds (natural estrogens and synthetic chemicals that mimic natural estrogen) in waterways and drinking water, and potential harm to human health or aquatic life [1]. Three naturally occurring forms of Estrogen are Estrone (E1), 17 Beta Estradiol (E2) and Estriol (E3), which are all produced mainly in women. Estrone (E1) is the estrogen most commonly found in increased amounts in postmenopausal women. The body derives it from the hormones that are stored in body fat. Estriol is the weakest of the three major estrogens. Estriol (E3) is the estrogen that is made in large quantities during pregnancy and has potential protective properties against the production of cancerous cells.

Estradiol (17β-Estradiol) is the principal estrogen found in all mammalian species during reproductive years. High concentrations of E2 in source water can result in adverse health effects (kidney impairment, necrosis, and liver damage) on fish. Even concentrations as low as 25 ng/L (0.025 ppb) have been found to lead to reproductive impairment and feminization of fish resulting in skewed populations. The main sources of high E2 concentrations to the aquatic environment are sewage treatment waste water and livestock waste [2].

The effect of estrogenic compounds in the water supply from industry, agriculture, and other sources raises concerns about human health and deserves scrutiny. Estrogenic compounds are part of a larger category of chemicals known as endocrine-disruptors (EDCs), chemicals that can alter the hormonal and homeostatic systems enabling an organism—like a human being or other animal—to communicate with and respond to its environment [1], [2]. Given the demonstrated effects of EDCs on human reproductive health, it is important to examine the role played by EE2 in contributing to the presence of estrogenic compounds in our water. The good news is this: contrary to what has been stated or implied by media reports and anti-contraception advocates, synthetic estrogen from birth control pills is not the sole or primary source of endocrine-disrupting chemicals in water [3]. New findings from researchers at the University of California San Francisco (UCSF) Program on Reproductive Health and the Environment (PRHE) help explain why—and suggest a role for providers and women's health advocates in educating and empowering women to make informed choices about using contraception and limiting their exposures to harmful chemicals [2], [3].

Further studies [3] note ongoing concern about possible links between chronic exposure to estrogens in the water supply and fertility problems and other adverse human health effects. Almost 12 million women of reproductive age in the United States take the pill, and their urine contains the hormone. Hence, the belief that oral contraceptives are the major source of estrogen in lakes, rivers, and streams. Knowing that sewage treatment plants remove virtually all of the main estrogen-17 alpha-ethinylestradiol (EE2) - in oral contraceptives, the scientists decided to pin down the main sources of estrogens in water supplies [4]. Their analysis found that EE2 has a lower predicted concentration in U.S. drinking water than natural estrogens from soy and dairy products and animal waste used untreated as a farm fertilizer. And that all humans, (men, women and children, and especially pregnant women) excrete hormones in their urine, not just women taking the pill [4], [5]. The study also suggests that animal manure accounts for 90 percent of estrogens in the environment. Other research estimates that if just 1 percent of the estrogens in livestock waste reached waterways, it would comprise 15 percent of the estrogens in the world's water supply.

In a South African water source, the first evidence of intersex fish was found. A research was conducted randomly from the Rietvlei Dam (RVD), and the Marais Dam (MD) in the Rietvlei Nature Reserve (RNR) in South Africa on one hundred catfish (Clarias gariepinus). Informal settlements, industrial sites, municipal treatment plants, and agricultural activities drains water streams into theses dams. Endocrine disruption verified intersex potential, in the fish through gonads being examined and blood drawn. In majority of the fish primary oocytes were found scattered in testicular tissues showing signs of intersex. In both dams 20% of fish showed intersexuality. Based off the sample feminization of male catfish was more likely the cause of the intersex fish. Further studies [4] have shown that these contaminants may pose a threat, but the detailed facts needed to establish the need for a regulatory standard have yet to be developed. There is emerging evidence that these hormones are finding their way into surface waters and sediments via ground water and surface waters from and the bio-solids that are be land applied.

Baltimore's water supply relies on surface water from rainfall and snowmelt, collected and stored in reservoirs outside the city. Three major impoundments (the Liberty, Loch Raven, and Prettyboy Reservoirs) derive water from two water sources (Gunpowder and Patapsco Watersheds) and one river (the Susquehanna). Water from the Liberty Reservoir and upstream sources is treated at the Ashburton Water Filtration Plant, while water from Loch Raven and Prettyboy Reservoirs is treated at the Montebello plant. EPA's Index of Watershed Indicators has determined that the Gunpowder and Patapsco Watersheds have less serious contamination problems but is highly vulnerable to contamination. The watersheds received an overall index rating of 4, on a scale of 1 to 6, with 6 being the worst rating [6].

The main goal of this study was to detect and quantify Baltimore's watersheds for any presence of estrogen. Once the concentration is measured, the levels will be compared with similar studies as there is no established standard by the Environmental Protection Agency (EPA). To our best knowledge, the distribution of estrogen hormones in Baltimore's water system is yet to be studied and we have selected 17 Beta-estradiol (E2) for this study as E2 is the principal estrogen found in all mammalian species during the reproductive years.

II. MATERIALS AND METHODS

2.1 Study area: The watersheds

Baltimore's water source is primarily surface water that feeds into the Liberty, Loch Raven and Prettyboy reservoirs. Three impoundments comprising two water sources and one river provide raw water to the City's water filtration plants [6]. Figure 1 shows Baltimore's watersheds and its reservoirs that supply the city's drinking water.

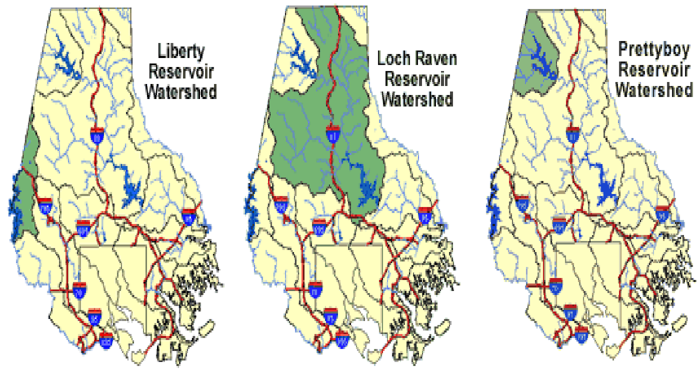

Figure 1. Map depicting watersheds and reservoirs supplying Baltimore City's drinking water.

Liberty Watershed is on the boundary between western Baltimore County and eastern Carroll County with the reservoir located on the North Branch of the Patapsco River (ref). It collects water from a 163.4 square mile drainage area that includes eastern Carroll County and southwestern Baltimore County. After traveling across seven sub watersheds (Beaver Run, Bonds Run, Liberty reservoir, Little Morgan Run, Middle Run, Morgan Run and North Branch), water from the reservoir flows by gravity through a 12.7-mile long, 10-foot diameter tunnel to the Ashburton Water Filtration Plant for treatment [6].

Loch Raven Watershed occupies northern Baltimore County, small parts of western Harford County and southern York County, Pennsylvania and its namesake reservoir is north of Baltimore City. The capacity of Loch Raven Reservoir, largest of the three supplying reservoirs, is approximately 23 billion gallons and the impounded area is roughly 2400 acres [7]. Raw water from this reservoir travels through a 7.3-mile long, 12-foot diameter tunnel for treatment at the Montebello Filtration Plants 1 & 2 in Baltimore City.

Prettyboy Reservoir is in the northwest corner of Baltimore County and its 80-square mile watershed lies in northern Baltimore County and small portions of northeastern Carroll County and southern York County, Pennsylvania. Prettyboy Dam was completed in 1932, has a spillway crest elevation of 520 feet of mean sea level, impounds about 19 billion gallons of water, and covers about 1500 acres. Prettyboy Reservoir water is transferred to Loch Raven Reservoir via Gunpowder Falls rather than directly to Baltimore. The dam releases water as needed into the river channel, which flows into Loch Raven reservoir [7], [8].

2.2 Water Sampling

From each watershed, four representative test points (Figure 2) were identified and surface water samples were collected using a five-foot long-handled dipper. To test the seasonal changes in estrogen levels, water samples were taken during fall (September) and winter (February) months over a period of a year (2017-2018). In situ analysis was on some physico-chemical characteristics (pH, nitrate-nitrogen, Biological Oxygen Demand, alkalinity and carbon dioxide concentration) using LaMotte test kits.

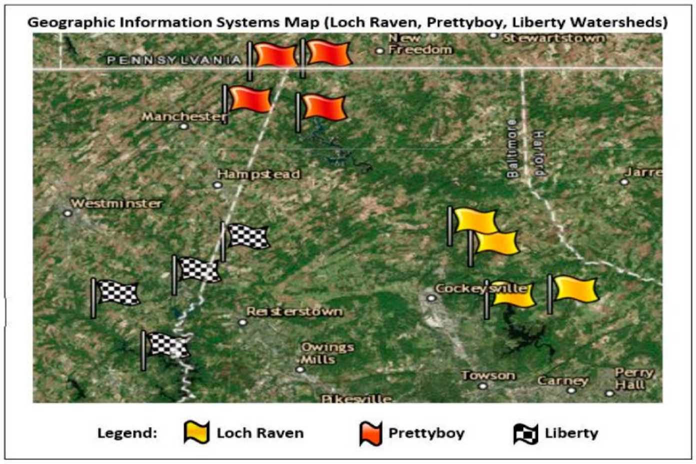

Figure 2. Map showing representative test points in the Liberty, Loch Raven and Prettyboy watersheds.

2.3 Estrogen analysis

2.3.1 Estrogen ELISA Kit

In this study, the Estrogen (E1/E2/E3) enzyme immunoassay kit licensed from Japan Enviro-Chemicals, Ltd. has been used for the determination of 17 Beta-estradiol (E2). A microplate reader was used to read the absorbance. The Total Estrogen (ES) ELISA test kit detects the estrogenic hormones estrone (E1), 17beta-stradiol (E2) and estriol (E3) with similar specificity (figure 3).

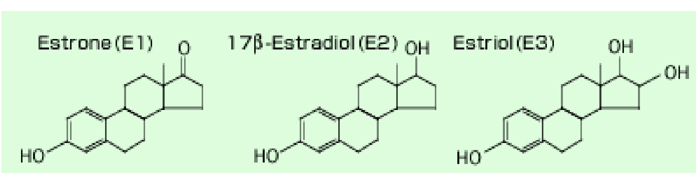

Figure 3. Chemical structure of the Estrogen hormone

The analysis is based on a competitive reaction where enzyme-labeled standard E2 competes with free estrogen in the sample for binding to a specific monoclonal antibody immobilized to the surface of the microplate well or tube. The amount of labelled E2 bound to the plate is determined by addition of a non-colored substrate which is converted into a colored product. The color intensity is measured at 450 nm wavelength (using a microplate reader) and is inversely proportional to the amount of estrogen in the sample. Figures 4 and 5 show color change in a 96 well plate after coloring reagent is added to the wells containing the standards and water sample.

Figure 4: The color reagent is added to each well after wash

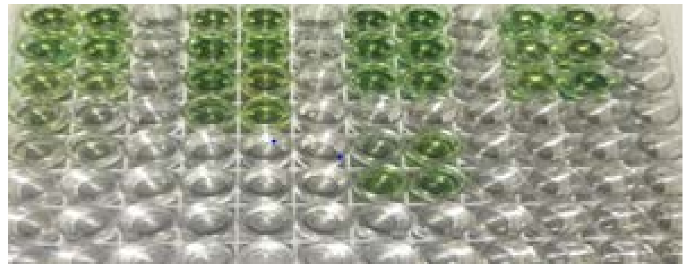

Figure 5: Color change after incubating for 30mins

Following the protocol suggested by “Ecologiena” (http://www.jechem.co.jp/eco/index-e.html), a standard curve was developed for concentrations ranging between 0.1 µg/L and 3 µg/L.

2.3.2 Quantitation of Estrogen using Microplate Reader

A microplate reader was used to measure the absorbance at 450 nm wavelength for each standard solution and a standard curve was generated. The quantity of Estrogen in each water sample was calculated from the absorbance reading and interpolated from the standard curve.

Table 1. Average Absorbance reading of the standards

Standard Conc. (µg/L)	Absorbance (nm)		Average Absorbance (nm)
0	1.182	1.466	1.324
0.05	0.052	1.175	0.6135
0.15	0.676	0.496	0.586
0.5	0.232	0.289	0.2605
3.0	0.116	0.133	0.1165

I.

II. Results and Discussions

3.1 Standard Curve for Estrogen

The standard curve was developed for concentrations ranging between 0.1 µg/L and 3 µg/L. Table 1 presents the absorbance reading results obtained from a microplate reader at 450 nm for each concentration of the estrogen standards. The standards were assayed in duplicates.

The average absorbance was plotted against the concentration of the standards to create the standard curve for estrogen (Figure 6). This curve was used to calculate the estrogen concentrations in each watershed.

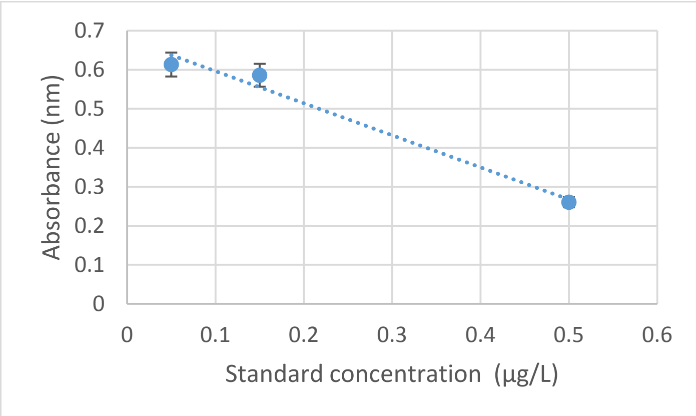

Figure 6. Standard curve for Estradiol (E2)

3.2 Quantitation of Estrogen

3.2.1 Mapping the wells

The table below presents the layout of standards and water samples in a 96-well plate. Well 1 & 2 contain five estrogen standards (0, 0.05 µg/L, 0.15 µg/L, 0.5 µg/L, and 3.0 µg/L) in two replications. Wells 3, 6 and 9 are blanks; Wells 4 and 5 contain Loch Raven samples; Wells 7 & 8 contain Prettyboy and Wells 10 and 11 contain Liberty water samples (all in duplicates).

Table 2. Plate layout of estrogen standards and water samples (LRW- Loch Raven watershed, PB – Prettyboy watershed and LW - Liberty watershed)

	Standards (µg/L)		Blank	Loch Raven Watershed		Blank	Prettyboy Watershed		Blank	Liberty Watershed	
	1	2	3	4	5	6	7	8	9	10	11
A	0	0		LRW 1	LRW1		PB1	PB1		LW2	LW2
B	0.05	05		LRW2	LRW2		PB3	PB3		LW3	LW3
C	0.15	.15		LRW3	LRW3		PB4	PB4		LW4	LW4
D	0.5	0.5									
E	3.0	3.0									

Tables 3 and 4 present the calculated estrogen concentrations using the standard curve and blank concentrations for the Fall 2017 and Winter 2018 seasons.

Table 3. Fall 2017 Estrogen analysis data

	Standards (µg/L)		Blank	Loch Raven Watershed(ppb)		Blank	Prettyboy Watershed (ppb)		Blank	Liberty Watershed (ppb)	
	1	2	3	4	5	6	7	8	9	10	11
A	0	0		0.068			0.069			0.049	
B	0.05	.05		0.079			0.073			0.043	
C	0.15	.15		0.076			0.058			0.046	
D	0.5	.5									
E	3.0	3.0									

Table 4. Winter 2018 Estrogen analysis data

	Standards (µg/L)		Blank	Loch Raven Watershed (ppb)		Blank	Prettyboy Watershed (ppb)		Blank	Liberty Watershed (ppb)	
	1	2	3	4	5	6	7	8	9	10	11
A	0	0		0.051			0.024			0.05	
B	0.05	.05		0.05			0.051			0.031	
C	0.15	.15		0.043			0.051			0.041	
D	0.5	.5									
E	3.0	3.0									

3.3 Interpretation of the data

We calculated the average estrogen for each watershed per season (Table 5) to determine if the detected concentrations are comparable with other studies in a similar setting.

Table 5. Average Estrogen levels for each watershed in µg/L

Watershed	Fall 2017 (E2), (ppb)	Winter 2018 (E2), (ppb)
Loch Raven	0.074	0.048
Prettyboy	0.067	0.042
Liberty	0.046	0.040

As can be seen from the table, all calculated estrogen values range between 0.4-0.074 ppb. A similar study in the Raccoon River watershed (Des Moines, Iowa) detected estrogen levels between 0.003 to 0.007 ppb in Des Moines water treatment plant [9]. Even concentrations as low as 25 ng/L (0.025 ppb) have been found to lead to reproductive impairment and feminization of fish resulting in skewed populations [2]. Intersex has been recorded in Japanese medaka (O. latipes) exposed to 100 ng/L 17β-oestradiol [10]. Other authors have also reported intersex in medaka; for example [12] reported ovotestes in fish exposed to 10 ng/L. In the zebrafish (D. rerio) all early life stage fish exposed to 100 ng/L developed female-like reproductive ducts with 75% showing this condition at lower concentrations, e.g. 25 ng/L.

Although the detected concentration difference among watersheds is insignificant, Loch Raven has relatively higher concentration than Pretty boy and Liberty watersheds during both fall and winter seasons. To better understand why Loch Raven has the higher levels, we looked into the bio-chemical watershed characteristics data and found out that the dissolved oxygen (DO) values for Loch Raven are higher (9.2 ppm) than Pretty boy (4 ppm) and Liberty (2.1 ppm) suggesting that there is a lot of biological activity in this watershed which could possibly be a sources of estrogen [6].

On the other hand, seasonal comparison reveals that there is relatively higher estrogen concentration in the fall than winter season across all the samples measured. This is mainly due to high river flow making movement of the contaminants possible whereas during winter (due to the freezing condition) contaminants remain localized.

III. CONCLUSIONS

One of the emerging water contaminants that is raising serious concerns on aquatic life health is coming from estrogenic compounds. Studies have shown that fish are changing sex due to exposure to excess estrogen.

The main goal of this study was to detect and quantify estrogen levels in Baltimore's watersheds and to see if the concentration warrants serious concerns. Our finding revealed the average estrogen levels in the Loch Raven sub-sheds was 0.074 ppb (Fall 2017) and 0.048 (Winter 2018) and for Liberty sub-sheds it was 0.046 ppb (Fall 2017) and 0.040 ppb (Winter 2018). In Prettyboy sub-sheds the level was 0.067 ppb (Fall 2017) and 0.042 ppb (Winter 2018).

Overall, Loch Raven watershed has slightly higher levels of estrogen than the other two watersheds, both in the fall and winter seasons. Seasonal comparison indicated that more estrogen is found during fall than winter seasons. Future studies will focus on the impacts of estrogen levels on aquatic life and/or humans.

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AUTHORS

First Author – Aaliyah McCullough is a graduating senior at the Department of Natural Sciences, Coppin State University

Second Author - Jasmynn George is a graduating senior at the Department of Natural Sciences, Coppin State University

Third Author – Mintesinot Jiru is an Associate Professor at the Department of Natural Sciences, Coppin State University; MJiru@coppin.edu

Correspondence Author – Mintesinot Jiru, MJiru@coppin.edu, Mintesinot@gmail.com 410-951-4139 (USA)