OFFICE OF WATER PROGRAMS

BUREAU OF CLEAN WATER

TOTAL ESTROGENICITY OF SPRINGS IN PENNSYLVANIA PILOT STUDY

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INTRODUCTION

Emerging contaminants such as pharmaceuticals, hormones, and other man-made products are a growing concern in fresh and salt waters of the world. Of particular concern are estrogenic compounds that act as endocrine-disrupting compounds (EDCs). Endocrine disruptors are compounds that can affect the endocrine system and often produce adverse effects in reproduction, the immune system, and development (National Institute of Environmental Health Sciences 2016). Both natural and man-made compounds can have endocrine disrupting effects. Most of these compounds are unregulated in both drinking water and for aquatic life uses.

Due to large portions of Pennsylvania having karst geology, there are many opportunities for contaminants to infiltrate into groundwater. Karst geology consists of soluble rock that is not only porous but has many fractures and caves as well. Therefore, surface contaminants can rapidly enter karst aquifers. A review of numerous studies of emerging contaminants in groundwater indicated that karst groundwater has lower concentrations and frequency of detections of contaminants than surface or shallow intergranular aquifers, but higher than other groundwater types (Reberski et al. 2022). Many studies have looked at the presence and concentrations of hormones and other EDCs in springs or groundwater across the world. Springs are areas where groundwater flows onto the surface and effectively becomes surface water. A study of bottled water from French springs looked at the presence of various emerging contaminant compounds and metabolites, including hormones, pharmaceuticals, pesticides, alkylphenols, phthalates, and perfluoroalkyl substances (PFAS) (Le Coadou et al. 2016). Pesticides and metabolites, PFAS, and alkylphenols were detected but hormones, pharmaceuticals, and phthalates were not. Another study sampled twenty shallow wells in Taiwan for fifty CECs, which included hormones, pharmaceuticals, and PFAS (Lin et al. 2015). Most compounds were detected at most sites. Out of the hormones, only beta-estradiol and estriol were not detected. Estrone and 17-alpha-ethynylestradiol were detected at 1.0 – 14.7 ng/L and 1822.2 ng/L, respectively. Livestock manure can also contribute hormones to the environment, for example, concentrations of 17-beta-estradiol in five springs in Arkansas near locations where poultry waste was applied as fertilizer ranged from 6 to 66 ng/L (Peterson et al. 2000).

Total estrogenicity via estimated estradiol equivalents (EEQ) is a test that characterizes the concentration of estrogenic compounds that can act as EDCs. The values reported are relative to 17-beta-estradiol (Luke Iwanowicz, 2014, personal communication). The result is the cumulative effect of all estrogenic chemicals in the sample, related back to 17-beta-estradiol. Values of EEQ show how much activity is in the water sample that could produce an estrogenic response – but not what individual compounds are in it. Regardless of the test limitations, it is still a very good way to quickly survey a site for potential endocrine-disrupting compound activity. Pennsylvania Department of Environmental Protection's (DEP) Water Quality Division (WQD) has been sampling streams and rivers for total estrogenicity since 2012. Samples are collected by discrete and passive water sampling protocols. So far, samples collected by WQD have only been collected in flowing surface waters. Neither springs nor groundwater have been sampled by WQD for total estrogenicity. Springs with a direct surface water connection may contribute contaminants found in groundwater to surface waters. Therefore, it was proposed to complete a pilot survey of Pennsylvania springs for total estrogenicity concentrations. The objective of this study was to complete a baseline survey of total estrogenicity in springs across Pennsylvania, and to determine if there are any patterns across seasons. There are currently no 25

Pa. Code Chapter 93 – Water Quality Standards § 93.7 water quality criteria for total estrogenicity, or any hormone, and this study may contribute data that could determine if criteria development for aquatic life or human health is necessary in the future.

METHODS

Spring sampling locations were chosen based on the following criteria:

- Volume of spring (larger springs will be easier to locate and will be greater surface water influences)
- Prior known pollution in spring (agricultural, bacterial, etc.)
- Documented or suspected use by local residents

Eleven springs were sampled across the state (Figure 1, Table 1). Field blanks and sequential replicates were collected according to DEP data collection protocols and Quality Assurance Project Plan (Lookenbill and Arnold 2023). One sequential replicate and one blank water sample were collected during each sampling effort, for a total of four sequential replicates and four blanks for the project. Springs were sampled four times, once in summer, fall, winter, and spring, through summer 2019 to spring 2020. Total estrogenicity samples were collected using a 1-L amber jar at each site in each season. Samples were preserved with HCl, placed on ice, and transferred as soon as possible to the United States Geological Survey (USGS) office in Leetown, WV for analysis. Samples were analyzed with a yeast estrogen screen (YES) assay.

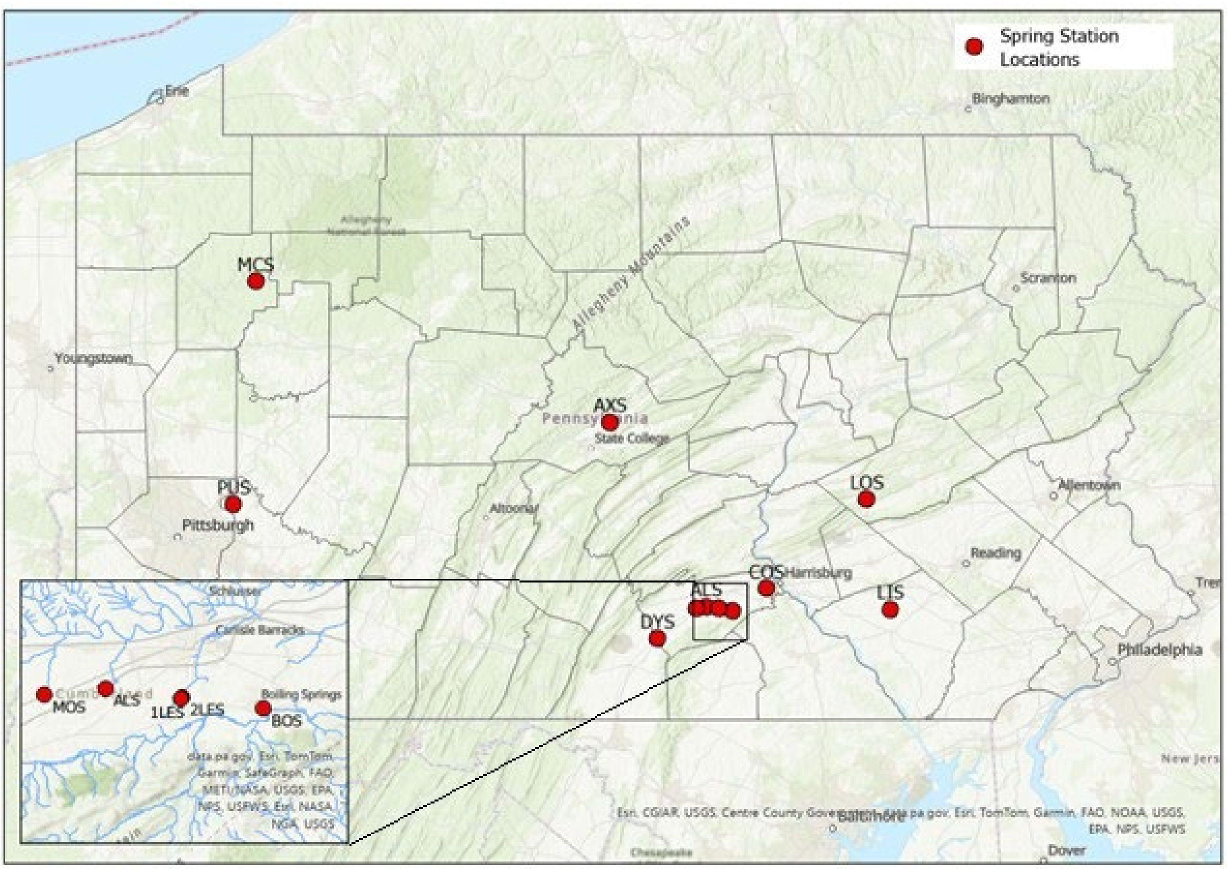

Figure 1. Spring Station Locations.

Table 1. Spring Station Locations.

STATION	DESCRIPTION
ALS	Alexander Spring Dickinson Township, Cumberland County Lat: 40.1679 Long: -77.2644
AXS	Axemann Spring Spring Township, Centre County Lat: 40.8900 Long: - 77.7603
BOS	Boiling Springs South Middleton Township, Cumberland County Lat: 40.1505 Long: -77.1289
DYS	Dykeman Spring Shippensburg Borough, Cumberland County Lat: 40.0419 Long: - 77.5156
1LES	Letort Spring (site 1) South Middleton Township, Cumberland County Lat: 40.1594 Long: -77.1999
2LES	Letort Spring (site 2) South Middleton Township, Cumberland County Lat: 40.1602 Long: -77.1991
LIS	Lititz Run Spring Lititz Borough, Lancaster County Lat: 40.1572 Long: - 76.3119
MOS	Mount Rock Spring West Pennsboro Township, Cumberland County Lat: 40.1623 Long: -77.3163
COS	Unnamed Spring (Conodoguinet Creek) Hampden Township, Cumberland County Lat: 40.2386 Long: -76.9522
PUS	Unnamed Spring (Little Pucketa Creek) Lower Burell City, Westmoreland County Lat: 40.5700 Long: -79.7045
LOS	Unnamed Spring (Lorberry Creek) Tremont Township, Schuylkill County Lat: 40.5891 Long: -76.435
MCS	Unnamed Spring (McCrea Run) President Township, Venango County Lat: 41.4366 Long: -79.5882

During the latest spring 2020 data collection effort, passive water samplers were also deployed at two spring locations, Mount Rock Spring (MOS) and Dykeman Spring (DYS). Polar organic chemical integrative samplers (POCIS) were used to sample for EEQ. These water samplers are placed in a waterbody for an extended period, normally around one month. They are excellent at detecting chemicals present in very low concentrations or those that occur periodically. As a result, many times there are detections in passive samplers that are not found in corresponding discrete samples. Raw results are received as ng/POCIS. If a sampling rate (Rs) is available, a time-weighted average concentration can be calculated. See blank correction descriptions and calculation information in Williams (2022).

RESULTS

EEQ discrete sample results are shown in Table 2. Out of 50 environmental samples, 44% had an EEQ detection. Concentrations ranged from 0.21 to 11.84 ng/L. The highest concentration, 11.84 ng/L, was at an unnamed spring (COS) near Conodoguinet Creek collected in February 2020. The remaining detections were low, with all below 1 ng/L except for one sample at Dykeman Spring (DYS) collected in June 2020 (1.47 ng/L). There were no visual patterns of detection noted by season collected. Average EEQ results varied at springs across the state. Highest average concentrations were around the urban areas of Harrisburg, Carlisle, and Chambersburg (Figure 2). All EEQ field blank samples were below the reporting limit. The Dykeman Spring (DYS) passive sampler EEQ time-weighted average concentration was 1.04 ng/L and the Mount Rock Spring (MOS) concentration was 0.36 ng/L. All field blank results were below the reporting limit.

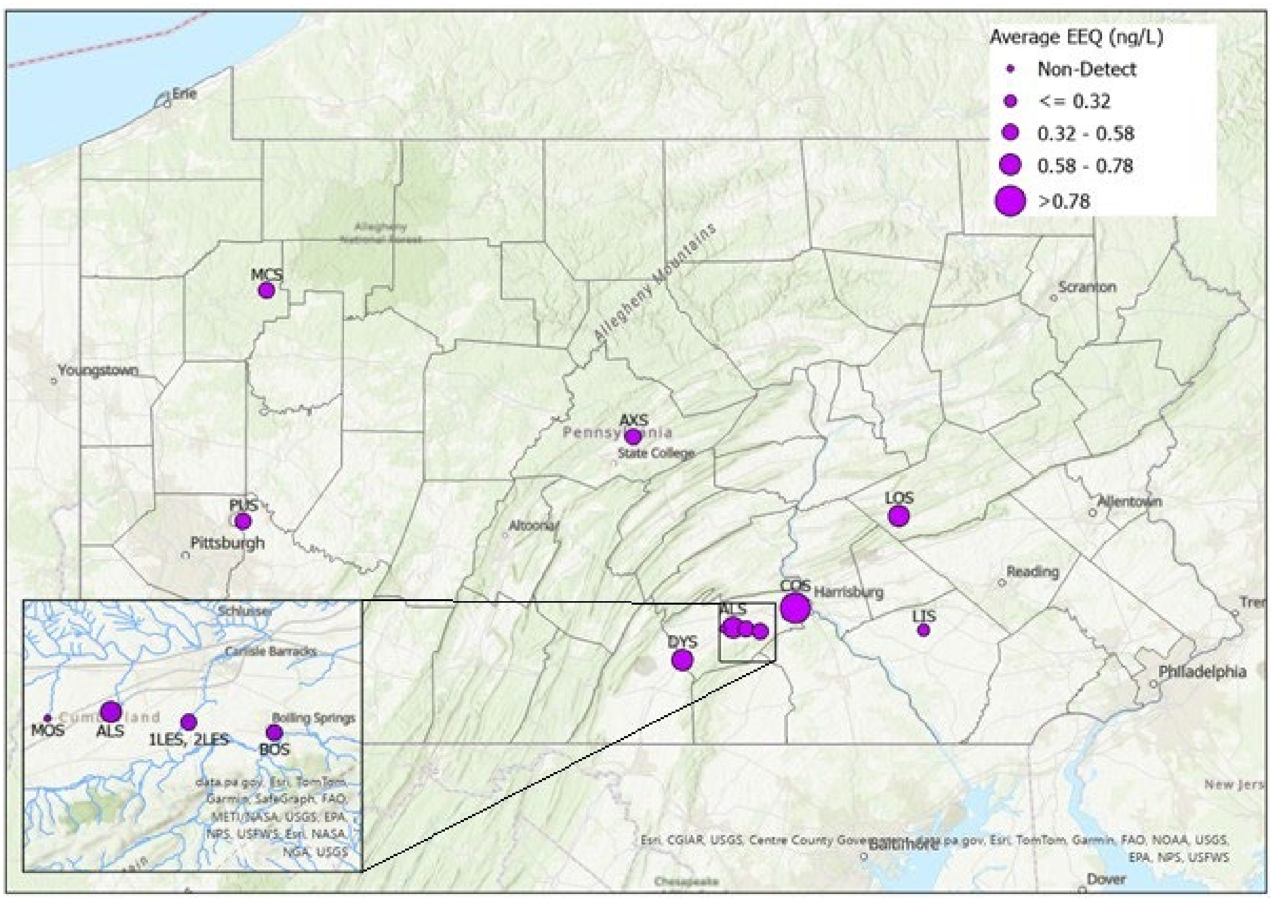

Figure 2. Average EEQ Results at Springs.

Table 2. EEQ Data.

STATION[1]	Date Collected	Time Collected	DEP Sample ID	Result (ng/L)
ALS	7/1/2019	11:15	0901-157	<0.23
ALS	10/8/2019	10:55	0901-213	0.78
ALS	2/10/2020	11:00	0901-225	<0.23
ALS	6/8/2020	14:20	0901-239	<0.23
AXS	7/30/2019	10:30	0901-167	<0.23
AXS	10/16/2019	9:50	0901-222	0.59
AXS	2/20/2020	10:40	0901-234	<0.23
AXS	6/9/2020	8:30	0901-247	0.37
BOS	7/1/2019	10:00	0901-156	0.64
BOS	10/8/2019	10:00	0901-212	0.34
BOS	2/10/2020	9:55	0901-224	<0.23
BOS	6/8/2020	15:45	0901-240	<0.23
BOS	6/8/2020	15:45	0901-241	<0.23
DYS	7/1/2019	13:57	0901-159	0.48
DYS	10/8/2019	12:45	0901-215	0.37
DYS	10/8/2019	12:46	0901-216	0.21
DYS	2/10/2020	13:15	0901-227	<0.23
DYS	6/8/2020	10:50	0901-237	1.47
DYS	9/28/2020	10:15	0901-260	<0.23
1LES	7/2/2019	14:30	0901-164	0.58
1LES	10/8/2019	14:20	0901-217	<0.23
1LES	6/8/2020	12:45	0901-238	<0.23
2LES	2/10/2020	14:45	0901-228	<0.23
LIS	7/2/2019	9:50	0901-160	0.32
LIS	7/2/2019	9:55	0901-161	<0.23
LIS	10/9/2019	8:50	0901-218	<0.23
LIS	2/11/2020	10:40	0901-230	<0.23
LIS	6/9/2020	11:30	0901-244	<0.23
MOS	7/1/2019	12:15	0901-158	<0.23
MOS	10/8/2019	11:40	0901-214	<0.23
MOS	2/10/2020	12:00	0901-226	<0.23
MOS	6/8/2020	9:25	0901-236	<0.23
MOS	9/28/2020	12:30	0901-261	<0.23
COS	7/1/2019	9:00	0901-155	0.93
COS	10/8/2019	8:50	0901-211	0.67
COS	2/10/2020	8:50	0901-223	11.84
COS	6/9/2020	8:55	0901-242	<0.23
PUS	7/29/2019	11:15	0901-165	0.24
PUS	10/15/2019	11:25	0901-220	0.4
PUS	2/19/2020	11:45	0901-231	<0.23
PUS	2/19/2020	11:50	0901-232	<0.23
PUS	6/8/2020	10:35	0972-342	0.88
LOS	7/2/2019	11:50	0901-163	0.89
LOS	10/9/2019	10:50	0901-219	0.75
LOS	2/11/2020	8:30	0901-229	0.79
LOS	6/9/2020	14:00	0901-245	0.61
MCS	7/29/2019	14:00	0901-166	0.45
MCS	10/15/2019	13:55	0901-221	<0.23
MCS	2/19/2020	14:45	0901-233	<0.23
MCS	6/9/2020	11:30	0901-246	<0.23

[1] Refer to Table 1 for Station Locations

DISCUSSION

Interestingly, the highest total estrogenicity result was 11.84 ng/L, which was much higher than the next-highest result (1.47 ng/L). The highest concentration was collected in February 2020 at an unnamed spring off Orrs Bridge Road (COS) in Camp Hill, PA. However, the other samples from this site, collected in July 2019, October 2019, and June 2020, were much lower, at 0.93 ng/L, 0.67 ng/L, and below detection, respectively. A stream gage is located on Conodoguinet Creek, which is the stream adjacent to the Orrs Bridge Road spring, approximately 8 river miles upstream of the spring site. Although a distance away, this gage provides stream flow data that could be applied to downstream sites. The February 10, 2019 sample occurs just after a high flow event – the daily mean discharge on February 8, 2019 was 1810 cfs, 1270 cfs on 2/9/19, and 962 cfs on February 10, 2019 (USGS 2025). This is in contrast to the other sampling events, where mean daily discharge was between 241 – 398 cfs and did not show elevated discharge in the days leading up to sampling. Although measuring the stream discharge and not groundwater, this could indicate a high rainfall or melt event that caused surface water contributions to the spring or a greater overland contribution of pollutants to groundwater through the spring itself. The Orrs Bridge Road spring is located in a mainly urban/suburban area. Of course, the groundwater may source from a variety of locations, so it is difficult to determine where this higher concentration originated. A variety of sources, such as agricultural runoff and wastewater effluent, can contribute to estrogenic compounds in water (Gordon et al. 2023). However, it is possible that other sources of runoff contributed to the higher EEQ concentration.

Generally, the total estrogenicity concentrations were low, and comparable or lower than what has been seen in other surface waters in DEP studies (Williams 2016, Williams 2022). EEQ discrete sample results in streams and rivers across Pennsylvania from 2012 to 2017 ranged from non-detect to 5.44 ng/L, with most results less than 1 ng/L (Williams 2016). The few surface water samples that were collected near the spring locations were non-detect. Field blanks of discrete EEQ samples were all below detection, indicating no field sampling contamination.

At the two locations where passive samplers were deployed and discrete samples were collected, EEQ concentrations were higher in the passive sample results than in the discrete results; in particular, at Mount Rock Spring (MOS), EEQ was non-detect in discrete samples but a concentration above the detection limit was documented in the passive sampler result. While WQD has not deployed passive samplers in springs before, these results follow similar patterns seen in stream and river samples.

This study offered a brief overview of EEQ in freshwater springs across Pennsylvania. More studies may be warranted to completely understand EEQ sources and concentrations.

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