Estrogenic Compounds in the West River

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Introduction

Estrogenic compounds have been suspected to cause a host of negative effects, including increasing the likelihood of certain cancers, early puberty, and lower sperm counts. Evidence suggests that a wide variety of estrogenic compounds may lead to the disruption of the endocrine system in both humans and wildlife (Rudel 1997). An endocrine disrupter is an exogenous substance that alters the function of the endocrine system (Cooper and Kavlock, 1997). Endocrine disruption lead to a variety of adverse health effects, most of which are considered reproductive or developmental toxicities (Rudel 1997). These reported changes coincide with major changes in lifestyle and with the mass production of synthetic chemicals involved in the production of industrial and domestic commodities (Thompson *et al.*, 2009). Some of these synthetic chemicals have been shown to be weakly estrogenic (Safe 2000), while some merging contaminants such as estrogens and xenoestrogens may produce unforeseen effects at low concentrations affecting several aquatic organisms in natural waters as well (Page *et al.*, 2006).

There is a high potential that these chemicals will be found in the West River, with the highest concentrations expected to occur near urbanized or industrial areas (Chiari *et al.*, 2010). Chemicals originating from the plastics and detergent industries, such as alkyl-phenols and bisphenol-A, have been determined to be estrogenic (Chiari *et al.*, 2010). In the segments of the West River, the EPA does not support fish, shellfish, and wildlife propagation. Possible sources behind their decision include hydro-modifications, sedimentation, highway runoff, municipal & industrial discharge, combined sewer overflows, bacterial pathogens, nutrients, and algal blooms. (Deegan 2013) These have been all found the West River and flow out into the New Haven Harbor.

This study was undertaken to determine if a the West River, contains estrogenic compounds and thus may be a source of the contaminating estrogenic substances into New Haven Harbor.

Materials and Methods

To remove any organic material, all of the glassware was washed with soap and water, rinsed with deionized water, and then rinsed with methanol and acetone. The glassware was baked at 500°C for 5 hours to remove any organic compounds.

Sample Collection: One liter surface water samples were taken from five points along the West River; surface water samples were collected in amber glass bottles (Figures 9 and 10).

Filtration: Nalgene Rapid-Flow filters with 0.45 µm pore size membranes were used to filter out any large particles present in the water samples.

Solid Phase Extraction: Solid-phase extraction was performed following EPA Method 1694 using Hydrophilic-Lipophilic-Balance (HLB-H) Oasis disks. Minor modifications were made to the method to accommodate the possibly of river water adhering to the glass, including flow-through of 20 ml of deionized water after the sample. Analytes were eluted in 12 ml methanol (Figure 11). The methanol was then evaporated from the eluents under a gentle flow of nitrogen gas at a temperature of 50 - 55°C. The remaining sample was then re-dissolved in 1 ml of deionized water and transferred into a 4 ml glass vial.

Yeast Assays: Genetically engineered *Saccharomyces cerevisiae* containing a human estrogen receptor and a plasmid-based estrogen response element was used (Sanseverino *et al.*, 2009). *S. cerevisiae* BLYES and *S. cerevisiae* BLYR were grown in 30 ml of yeast minimal media overnight at 30°C and 200 rpm shaking. To make a standard curve, 17β-estradiol (E2) was serially diluted in methanol. Each of the sample solutions was also diluted and then added to a 96-well plate along with the yeast and deionized water. The plates were left to incubate at 30°C for four hours and the bioluminescence was measured using a SpectraMax plate reader (Figure 1).

Controls: 17β-estradiol were used as positive controls at different concentrations with the most concentrated being $1x10^{-5}$ to the least being $2.5x10^{-11}$; descending by serial dilutions (Fig. 1).

Results

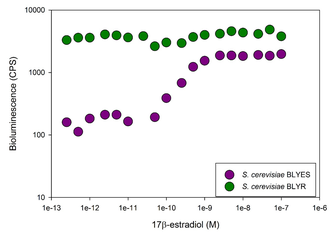

Figure 1: Standard curve in response to E2.

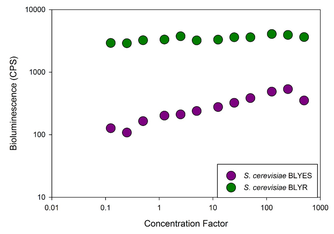

Figure 2: River Sample: Bradley Road & Route 69 6/23/15

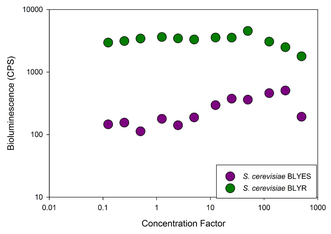

Figure 3: River Sample: Bradley Road & Route 69 7/27/15

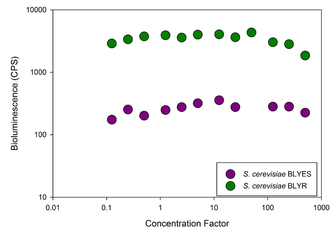

Figure 4: River Sample: Denny's on Route's 63 7/27/15

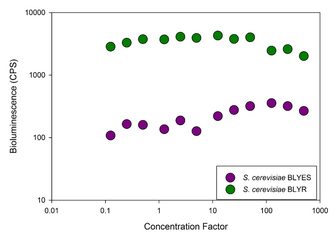

Figure 5: River Sample: Blake Street 6/23/15

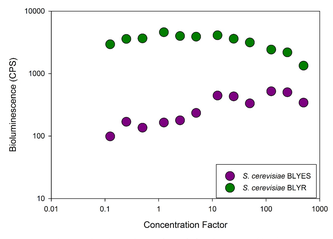

Figure 6: River Sample: Blake Street 7/27/15

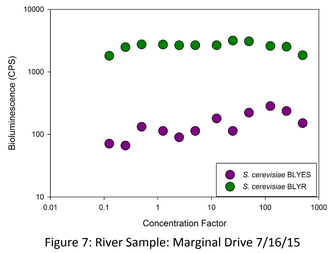

Figure 7: River Sample: Marginal Drive 7/16/15

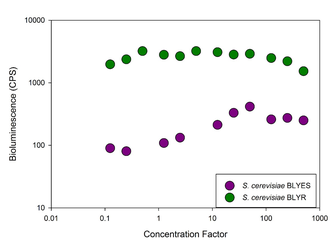

Figure 8: River Sample: West Cove Marina Cooperative 7/16/15

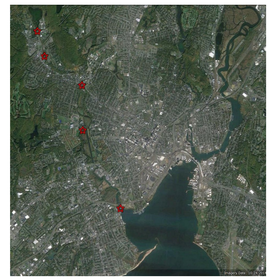

Figure 9: Five sampling locations used in this study: (starting from top) Intersection of Bradley Road & Route 69; Denny's on Route 63; Blake Street; Marginal Drive; West Cove Marina Cooperative.

Figure 10: Sample collection in the West River.

Figure 11: Solid-phase extraction equipment. This set up was used to extract and concentrate chemicals from natural samples.

Conclusions

The results of this experiment indicate that there are potentially estrogenic compounds present in the West River. *S. cerevisiae* BLYES measured the estrogenic compounds found in the water sample and *S. cerevisiae* BLYR measured the toxicity of the water sample. By comparing the curve of each sample to the positive control (Figure 1), the amount of estrogen substances found in the sample sites can be quantified. Estrogenic compounds were detected at all five of the sites tested. The water sample taken from Bradley Road & Route 69 (Figure 3) contained the highest recorded amount, followed by the surface water sample taken from West Cove Marina Cooperative (Figure 7 and 8). However, the concentrations of these substances were variable. One of the trials indicated that no estrogenic compounds were present (Figure 4). Due to the precision of the assay, it can be concluded that the West River is a potential source of estrogenic compounds in the New Haven Harbor. Future work will involve continued sampling and quantification of estrogen concentrations in these samples.

References

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David Deegan, West Haven, Conn. Fined for Illegal Sewage Discharges; Will Take Action Significantly Reduce Sewage Discharge to New Haven Harbor and Long Island Sound
http://yosemite.epa.gov/opa/admpress.nsf/d0cf6618525a9efb85257359003fb69d/2d3ca9376afcb97c85257c46007ac112!OpenDocument.

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