Monitoring the Mississippi River for endocrine disruptors
by means of an estrogen-based piezoelectric biosensor

Recently, it has become apparent that a thorough chemical analysis alone cannot assess the ecological risks of contaminated waters and wastewaters.[i] For instance, there exist environmental contaminants known as endocrine disrupting compounds (EDCs) which produce adverse effects only after "interactions" have occurred. EDCs are a class of materials that remain undefined by chemical nature but can be classified by their biological impacts because they are capable of mimicking, antagonizing the effects of, or altering the synthesis and metabolism of endogenous hormones (such as estrogens and androgens).[ii] As studies progress there have been increasing amounts of synthetic chemicals common to both industry and household products such as phthalate plasticizers, surfactants, polychlorinated biphenyls (PCB)s, dioxins, alkylphenols (APs), bisphenol A, brominated flame retardants, parabens, polycyclic aromatic hydrocarbons (PAHs), and some pesticides that have all been found to be EDCs.

As a result of EDCs, male walleyes along with many other aquatic species in the Mississippi River near St. Paul have been found to have five times the normal level of estrogen and reduced amounts of testosterone in their blood.[iii] Knowing this, **we must develop adequate sensing technologies** because humans most likely will exhibit similar adverse effects from the EDCs as those experienced by other wildlife.

Due to the nature of EDCs, estrogen receptors (ERs) are an ideal receptor to have in a piezoelectric biosensor because many endocrine disruptors bind to estrogen as agonists or antagonists. Therefore, environmental toxicity can be tested by this binding capability. While optical and electrochemical[iv] biosensors have already been developed using the ER for evaluating environmental toxicity, none of them have been as small, or cheap, or as practical for onsite evaluation as the proposed estrogen-based piezoelectric biosensor.

An ideal biosensor is a small, portable, self contained, integrated device which provides analytical information. There exists a biological recognition element which is in contact with a transduction element that converts the recognition event into a usable output signal. Previously, a sandwich type monitoring assay system utilizing a piezoelectric biosensor has been developed and tested for its response to estrogen (See Figure 1).

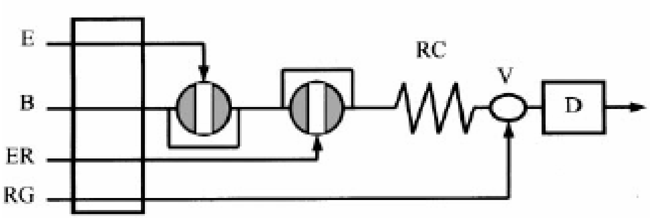

Figure 1. Schematic of the piezoelectric biosensor flow injection system for detecting estrogen.

The schematic of the biosensor includes: the buffer solution as carrier (B), the flow through detector mounted with the biosensor (D), estrogen sample solution (E), definite estrogen receptor solution (ER), reaction coil (RC), SDS regeneration solution (RG), and a valve switch (V). This preliminary sensor was found to have had a linear calibration graph in the 10-100 $nmol^{-1}$ detection limit of 7.8 $nmol^{-1}$ and a run time of 4 min for the detection of 17β-esteradiol. [v]

The research aims of this project revolve around further developing this piezoelectric biosensor for improved on-site testing. Previously, biotinylated 35-bp annealed oligonucleotide duplex containing an ERE was immobilized and used as the binding site to measure 17β-esteradiol binding. We propose to continue using this and also incorporate the biomarker vitellogenin. This will further display the effects of endocrine substances.

The current sensor works under the restricted laboratory condition of flowing 17β-esteradiol in distilled water. The only way to prepare this biosensor for real world applications would be to incorporate a chitosan nanoporous membrane which would allow the 17β-esteradiol molecules to flow while vastly restricting bio-foulings such as algae to enter the sensing mechanism.

A final aim is to further miniaturize the system using advanced microfabrication techniques that will facilitate on-site usage by the common user. Volunteers from the Water Resource Science center will test the biosensors and report whether the water they test is pure or not based on the sensors' readings. Within the center there already exists an Outreach and Public Engagement center and thus, one of broader impacts of this project is to work with this already existing facility.

This proposed research is of grave intellectual merit in terms of better understanding the nature of EDCs interactions with one specific estrogen receptor. EDCs are already dispersed amongst the environment and have been proven to accumulate in the tissues of plants and animals.[vi] As more and more sources are responsible for the accumulation of EDCs and being that their effects are already evident on wildlife there is no reason not to question the adverse effects that EDCs are having on both physiological processes and the reproductive health of humans including but not limited to increased cases of breast and testicular cancer, birth defects and declining sperm count.

---

[i] M. Castillo, M. C. Alonso, J. Riu et al. *Anal. Chim. Acta* 426, 265-277 (2001)
[ii] Rodriguez-Mozaz, Maria-Pilar, *Anal Bioanal Chem*. 378, 588-598 (2004).
[iii] Meersman Tom, Mississippi River Walleye Feminiaed. *Minneapolis Star Tribune* April 18, 1998.
[iv] Zhihong, Mo, Xiaohui, Long. *Anal Commun.* 36, 281-283 (1999).
[v] Rodrigues-Mozaz Sara, Marco Maria-Pilar, *Pure Anal Chem* 76, 723-752 (2004)
[vi] Endocrine-disrupting substances in the environment: what should be done? (1998) Environmental issues series, consultative report, Environmental Agencies (UK).