Treated wastewater discharged from municipal wastewater treatment plants (WWTPs) contains estrogenic chemicals that may disrupt normal endocrine function in animals. These chemicals may threaten uses of reclaimed water in Arizona and the health of ecosystems that the water supports. New advanced WWTPs may remove these chemicals more effectively but more information is needed. Sensor technology to provide rapid, field-ready, inexpensive testing of these chemicals in wastewater is also needed.

**PROJECT TEAM**

**Investigators**
- Catherine Propper (NAU) – Lead
- Timothy Vail and Jani Ingram (NAU)
- Eduardo Saez and Martin Karpiscak (UA)
- Paul Westerhoff (ASU)

**Research Assistants**
- Bingfeng Dong and Sandra Tseske (UA)
- Silke Buschmann, Fiyori Melaki, Larissa Jatho, Geertje Tulipani and Melissa Malvar (NAU)

**External Partners**
- Patricia Adler (ADHS Laboratory)

**PROJECT FUNDING CYCLE**
- 2007

**PROJECT GOALS**

This project had two goals: 1) sample modern, high-performance WWTPs in Arizona to determine how effectively treatment removes estrogenic chemicals, and 2) develop sensor technology for the rapid measurement in wastewater of two key estrogenic chemicals, 17ß-estradiol (E2) and 17α-ethynylestradiol (EE2).

**BACKGROUND/RESEARCH METHODS**

Research indicates that traditional secondary WWTPs—those that rely on sedimentation and simple aeration—are not very effective in removing estrogenic chemicals from effluent. Chemicals not removed by treatment are released in the wastewater, which often supports riparian growth, is reused for irrigation and other purposes, or is recharged to offset groundwater pumping. Estrogenic chemicals are of concern because research shows that their presence in aquatic ecosystems has caused adverse impacts, including feminization of fish and amphibians.

In Arizona new WWTPs must employ “tertiary” treatment processes. These advanced plants radically improve the overall quality of the treated wastewater compared to secondary plants. However, we do not know how effective these plants are at removing estrogenic chemicals specifically. Because reclaimed water from these plants will play an increasing role in Arizona’s future water budget, it is important to know how effective they are at removing these chemicals. The team collected influent, effluent, and sludge (bio-solids) from six modern, high-performance treatment plants in metropolitan and smaller urban settings.

The team tested for estrogenic activity using the Yeast Estrogen Screen (YES), and for E2 and EE2 using the liquid chromatography tandem mass spectrometer (LCMS/MS) at the Arizona Department of Health Services (ADHS) Laboratory. The team also used the ELISA (Enzyme Linked Immunoassay) method to test for E2. E2 is the primary estrogen in vertebrate animals and EE2 is an active ingredient in oral contraceptives.
This project also supported the development of rapid, inexpensive, yet sensitive sensors to measure E2 and EE2 in wastewater using lateral flow assay and immunochromatographic techniques. The team used the sensors to analyze for E2 and EE2 in wastewater as a proof of concept demonstration.

**KEY SCIENCE FINDINGS**

Due to the presence of extraneous substances in the samples, analyses did not provide consistent results across the different analytical methodologies. (Wastewater samples are notoriously prone to interference problems). Although it was possible to partially rank the facilities as to relative estrogen levels in the treated wastewater, accurate calculation of estrogen removal efficiencies was not possible. The project team is investigating this issue and will submit an addendum to the final report to describe their findings.

On the other hand, the team made excellent progress on the second goal of this project: developing lateral flow assay sensors for E2 and EE2. They obtained good calibration curves using actual wastewater samples, and the assays are sensitive enough for detection of extracted E2 and EE2 in wastewater.

**KEY STAKEHOLDER ENGAGEMENT and OUTCOMES**

This project benefited from the partnership of the three universities and the ADHS State Laboratory in a concurrent AWI project, *Collaborative Approach to Analyzing Emerging Contaminants in Water* (AWI 07-28), which funded development of LCMS/MS analytical methods for E2 and EE2. These collaborations led to a follow up project, *Operation of the Activated Sludge Process for Removal of Estrogenic Activity During Conventional Wastewater Treatment* (AWI-08-36), with several of the same investigators. AWI-08-36 will investigate estrogen removal efficiencies in a more controlled laboratory environment and support work to perfect the E2 and EE2 sensors.

**CONCLUSIONS and IMPLICATIONS**

Sample problems proved to be an obstacle to obtaining estrogen removal efficiency values for the advanced WWTPs studied. The lack of consistent results across the assays suggests that each assay may be sensitive to interferences from substances in the samples, and the interferences are different for the different types of assays. The project team is investigating the nature of these interferences, which may yield more consistent results among methods and allow calculation of estrogen removal efficiencies.

The team needs to further test and optimize the sensor technology to lower the detection limit of E2 and EE2, which will allow direct measurement of the estrogen levels without extraction.

This research ultimately will translate to better WWTP design and operation so as to achieve greater removal of estrogen chemicals and, hence, produce treated wastewater that maximizes the safety of reclaimed water reuse.

**FIND OUT MORE**

*AWI and the project team express their thanks to Pima County Wastewater Management, the cities of Phoenix, Flagstaff, and Prescott, and the Northern Gila County Sanitary District for their assistance and cooperation in this project.*

*Project Final Reports and other information available at www.azwaterinstitute.org*