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Hydrological, Chemical, and Biological Monitoring Plan

An Innovative Constructed Wetland Design for Attenuating Endocrine Disrupting Compounds from Reclaimed Wastewater

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Investigating an Innovative Constructed Wetland Design for Attenuating Endocrine Disrupting Compounds from Reclaimed Wastewater

Hydrological, Chemical and Biological Monitoring Plan

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Mission Statements

The mission of the Department of the Interior is to protect and provide access to our Nation's natural and cultural heritage and honor our trust responsibilities to Indian Tribes and our commitments to island communities.

The mission of the Bureau of Reclamation is to manage, develop, and protect water and related resources in an environmentally and economically sound manner in the interest of the American public.

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Introduction

Authority

This monitoring plan was developed under the Bureau of Reclamation's (Reclamation) Science and Technology (S&T) Program, as authorized by P.L. 92-149, the Reclamation Act of 1902 and P.L. 111-11, Omnibus Public Land Management Act of 2009. The S&T Program is a Reclamation-wide competitive, merit-based program focused on researching and identifying innovative solutions for complex water-related challenges faced by Reclamation and its partners. Over the past seven years, Reclamation has provided over $50 million for 800 research projects that have led to many important tools, solutions, and improvements in the way Reclamation manages its water and power infrastructure and related resources. Specific information about the S&T Program, including a list of awarded research projects, can be found at: http://www.usbr.gov/research/science-and-tech/.

Background

Growing demands on water resources will require the increased use of treated municipal wastewater to provide potable water supplies (National Research Council, 2012). Throughout the U.S., municipal, industrial, and agricultural wastewater is collected at wastewater treatment plants where it is treated prior to disposal into waterways. By further treating that wastewater and reusing it for beneficial uses, water management agencies can stretch existing drinking water supplies to help ensure that growing water demands can be met. Water reuse in Texas has been practiced since the 1800s, with initial uses primarily for irrigation of agriculture. The evolution of reuse in Texas has seen the range of beneficial uses grow extensively, including power plant cooling water, commercial and municipal irrigation, river and stream flow enhancement, natural gas exploration activities, and most recently, augmentation of drinking water supplies.

According to the 2012 Texas State Water Plan, approximately one million acre-feet (10 percent) of Texas 2060 water supply needs will need to be provided through the reuse of reclaimed wastewater. Most of this will be derived through "planned" indirect potable reuse projects which use environmental buffers, either surface or groundwater, to further enhance the quality of wastewater prior to discharging into a water supply source, where it will receive additional treatment before entering the drinking water distribution system. This strategy is not much different than "unplanned" indirect potable reuse projects, also known as "de facto" reuse, which already occur in any situation where a water user diverts and treats water that emanates from a water body which receives wastewater discharges from an upstream water user. With the case of "planned" indirect potable reuse, the user must obtain a water rights permit from the state and must adhere to Federal water quality regulations.

Constructed wetlands are widely recognized as excellent environmental buffers that enhance the quality of reclaimed wastewater through their complex interactions of physical, chemical, and biological processes that reduce concentrations of suspended solids, nutrients, dissolved organic carbon, volatile organic compounds, biochemical oxygen demand, and coliform bacteria (Walton et al., 2000; Barber et al. 2001; Keefe et al. 2004; Kadlec 2009). In the United States, it has been shown that many organic contaminants present in municipal wastewater treatment plant (WWTP) effluent are also widespread in surface and groundwaters that receive WWTP discharges (Kolpin and others, 2002; Barnes and others, 2008; Focazio and others, 2008). One of the issues of concern is the potential for estrogens and other endocrine disrupting chemicals (EDCs) in WWTP effluent to elicit adverse ecological or human health outcomes (Jobling and others, 1998; Sumpter and Johnson, 2005; Ankley and others, 2007; Vajda and others, 2008; Barber and others, 2011). A number of chemicals widely present in WWTP effluents (including steroidal hormones, alkylphenol nonionic surfactant degradation products, bisphenol A, natural products) have been shown to cause reproductive impairment in fish (Barber and others, 2007; Vajda and others, 2011). Likewise, it has been recently shown at the operational scale, that advanced treatment can remove EDCs as well as endocrine disrupting effects in exposed fish (Johnson and Sumpter, 2001; Barber and others, 2012).

It is important to address the issue of EDCs in treated WWTP effluents and evaluate the potential of using environmental buffers, such as treatment wetlands, as a resource management tool to further attenuate their concentrations and potential ecosystem and human health impacts. Funding was initially provided by the Bureau of Reclamation in Fiscal Year 12 to identify a preferred location to demonstrate an innovative treatment wetland designed to enhance the removal of EDCs and other biologically-active consumer product chemicals including pharmaceuticals. Five locations were evaluated using screening criteria which encompassed a wide range of technical and non technical issues, including costs, constructability, and sustainability. The preferred location recommended for implementation of a demonstration-scale project is at the City of Waco, Texas WWTP (Waco Demonstration Wetland)[1]. The City of Waco WWTP is part of the Waco Metropolitan Area Regional Sewerage System (WMARSS), a joint wastewater treatment effort by the cities of Bellmead, Hewitt, Lacy Lakeview, Lorena, Robinson, Waco and Woodway. This monitoring plan represents one of two products submitted to Reclamation's S&T Program Office as deliverables using the FY 12 funding. This monitoring plan also supports the proposal recently submitted to Reclamation for construction funding in Fiscal Year 13.

The design of this wetland was developed based on an iterative hydrological/physicochemical process (Barber and others, in prep) to optimize natural attenuation mechanisms (Keefe and others, 2004a; Keefe and others,2004b; Bradley and others, 2007; Bradley and others, 2008; Bradley and others, 2010; Writer and others, 2011a; Writer and others, 2011b; Writer and others, 2012). The site design (figure 1) consists of a four compartment wetland (open-water cell A, subsurface flow cell B, turbulent stream flow cell C, and hummock/habitat cell D) incorporating a sequence of specific features to promote photolysis, sorption, biodegradation, volatilization, chemical transformations, solute mixing, and interactions with vegetation communities to optimize removal pathways. The sequence of independent cells allows for the determination of where, when, and how the specific functions occur in the natural wetland systems. While several different functions can occur in the same space at the same time (i.e., nitrification and photolysis), only by effectively monitoring the inflow and outflows of each of the wetland cells, as well as internally within the cells, can we gain insight into these hydrological and

[1] Bureau of Reclamation and USGS. 2012. Demonstration Project Alternatives Analysis – Innovative Constructed Wetlands for Attenuating Endocrine Disrupting Compounds from Reclaimed Wastewater

biogeochemical interactions. In addition, there are many interactions between vegetation, biota, climate, and hydraulic transport characteristics that also are important factors in determining how different types of chemical constituents are assimilated, broken down, and attenuated in the wetland water column. Overall, understanding the operative mechanisms associated with aquatic system conditions (physical configuration, biogeochemical interactions) in wetlands is essential to managing water reuse projects in a safe and sustainable manner.

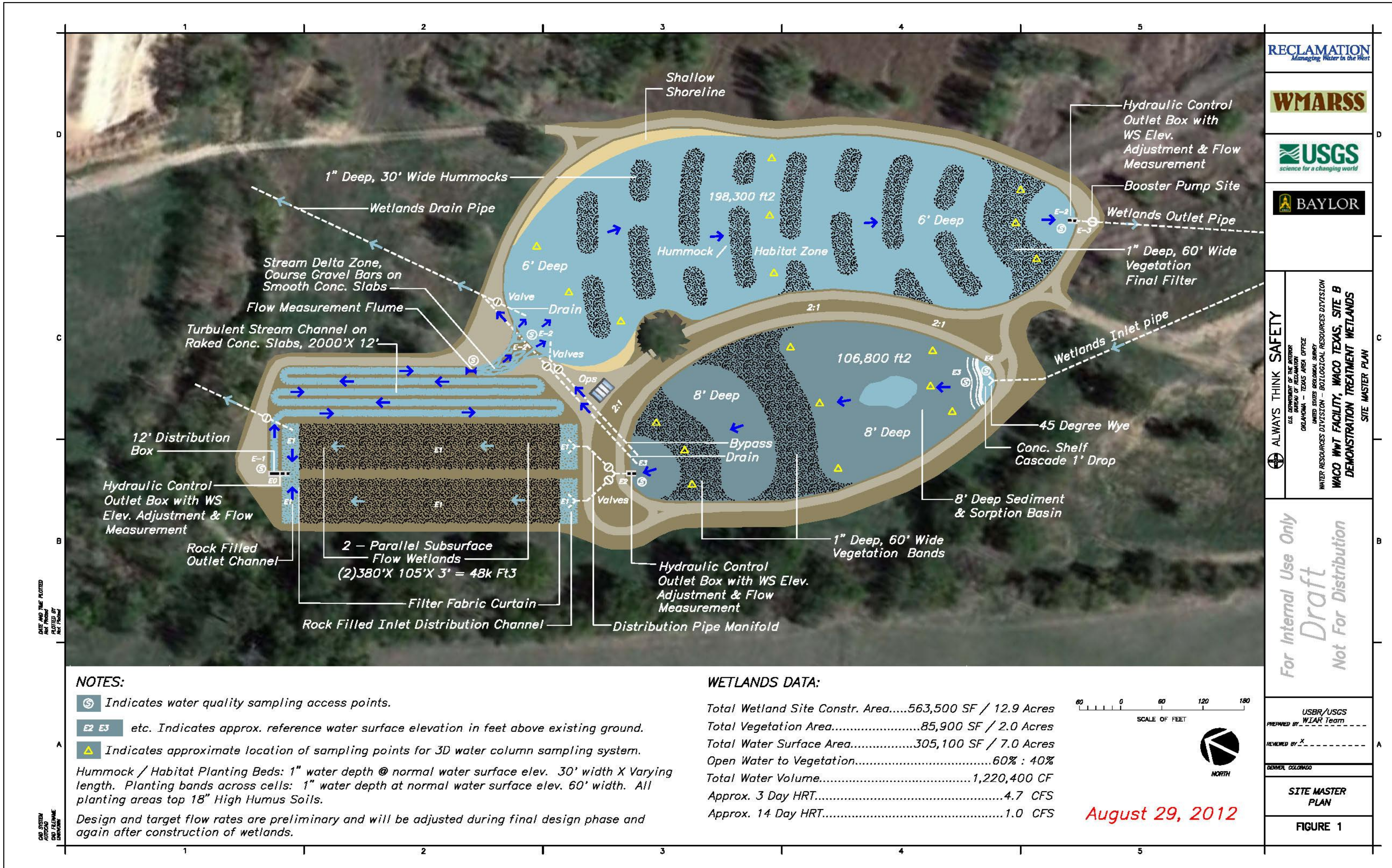

Figure 1. Waco Metropolitan Area Regional Sewerage System (WMARSS) Constructed Wetland Site Plan, 30 Percent Design Completion

Research Objectives

This document establishes the basic hydrological, chemical, and biological monitoring plan necessary to collect the performance data required to determine how well the innovative constructed wetland meets design objectives of attenuating EDCs and endocrine disruption effects. The monitoring plan described herein is necessary for acquiring the data needed to characterize wetland startup conditions, evaluate attenuation functions, and to generate a comparison dataset for the evaluation of treatment performance as the wetland system matures. Although more specific research studies to explore treatment mechanisms are warranted, they are beyond the scope of this monitoring plan, which lays out the minimum framework needed to answer the research questions:

1. What is the overall performance efficacy of the constructed wetland in attenuating EDCs from municipal wastewater treatment plant effluent?

2. How do different design features within the cells attenuate EDCs and other important constituents?

3. How does season and vegetation coverage affect water chemistry and biological activity, and thus attenuation?

The objectives of this project are intended to address priority research questions identified by the Texas Water Development Board (TWDB) as stated in the Texas Water Reuse Research Agenda (TWDB, 2011), namely “Understanding the role of environmental buffers in surface water indirect potable reuse projects” and “Effectiveness of treatment wetlands in improving reclaimed water quality”. The demonstration wetland design and monitoring plan were developed by the U.S. Geological Survey (USGS) and the Bureau of Reclamation, with support from Baylor University, based on their collective experience with wastewater treatment wetlands at various locations throughout the western U.S. (Rostad and others, 2000; Sartoris and others, 2000; Keefe and others, 2004a; Keefe and others, 2004b; Barber and others, 2001; Barber and others, 2006; Keefe and others, 2010). Two Tiers of monitoring are presented.

The Tier I monitoring plan focuses on evaluating the general performance of the innovative constructed wetland, and serves as the basis of the Fiscal Year 13 funding request to Reclamation’s S&T Program. The monitoring program begins with baseline sampling conducted at the completion of the construction phase and will continue for three years (i.e., three full growing seasons) as the initial vegetation establishes and matures. Tier I sampling includes studies on the effects of hydrology, chemistry, and biology in attenuating general wastewater constituents, surrogate parameters, and indicator EDCs and endocrine disruption. *In vitro* and *in vivo* bioassay responses will be targeted for advanced analysis of EDCs at the wetland’s inflow and outflow.

The Tier II monitoring plan identifies additional, more focused research opportunities that may be explored pending additional funding that are considered beyond the scope of the Tier I monitoring program. Potential Tier II investigation topics include contaminant fate and transport, wetland operations and treatment mechanisms, and wetland health and habitat.

Supplemental research may include additional biologically-active compounds, toxicity studies, macroinvertebrate analyses for EDC concentrations, as well as additional *in vitro* and *in vivo* bioassays. Tier I monitoring activities will be leveraged during Tier II activities to focus targeted research projects to better understand mechanisms of attenuation of EDCs by various wetland design features.

Tier I Monitoring

This section presents the general performance evaluation plan for the Waco Demonstration Wetland as conceived at the 30 percent design stage. The monitoring program begins with baseline sampling conducted at the completion of the construction phase and continues for three years (i.e., three full growing seasons) following the initial vegetation establishment. This is the minimum monitoring period required to evaluate treatment performance because of the length of time it takes for the vegetation and geochemical conditions to establish (Satoris and others, 2000; Keefe and others, 2010). The schedule of monitoring tasks is presented in Table 1, with specific descriptions of the monitoring task in the following sections.

It is important to note that uncertainties currently exist regarding the types and concentrations of EDCs present in the existing wastewater stream, so before Tier I monitoring begins, the following procedure will be performed to confirm a selected list of EDC indicators:

1. **Identify EDC Parameters using Historical Wastewater Data:** Evaluate existing records to determine which EDCs are detected and at what level/concentration.
2. **Identify Other Pollutants of Concern:** Evaluate existing records to verify that the indicators used in Tier I monitoring are representative of detected constituents and include priority pollutants identified in the Texas Surface Water Quality Standards or those that appear on the United State Environmental Protection Agency's Contaminant Candidate List 3 (USEPA CCL3).
3. **Validate EDC Occurrence with Preliminary Sampling and Analysis Program:** In order to determine the current concentrations and distributions of EDCs, it will be necessary to conduct preliminary sampling and analyses using currently available analytical methodologies. If the indicator list needs to be modified to include additional contaminants of emerging concern (CEC), then new activities need to be included as a Tier II study.

Table 1. Schedule for monitoring the Tier I parameters at the WMARSS Treatment Wetland. This illustrates the first year of the three year study, but subsequent years will be similar. Monitoring will be performed by WMARSS, USGS, Reclamation, and Baylor University.

Monitoring Tasks	1 Month				2 Month				3 Month				4 Month				5 Month				6 Month				7 Month				8 Month				9 Month				10 Month				11 Month				12 Month				
A. Hydrology and Hydraulic Transport																																																	
1. Hydrology																																																	
a. Inspect pump and weir boxes	D	D																																															
b. Inspect water depths in all cells	D	D																																															
c. Influent/effluent flow rates	D	D																																															
2. Hydraulic Transport																																																	
a. Baseline & Biannual tracer tests																																																	
B. Vegetation																																																	
Plant Inspection - general	D	D																																															
Plant stem density, diameters, lengths																																																	
Areal vegetation coverage																																																	
Plant biomass measurements																																																	
Plant uptake analyses																																																	
C. Macroinvertebrates																																																	
Collection for ID & enumeration																																																	
Macroinvertebrate uptake analyses																																																	
D. Soils and Sediments																																																	
Sample collection from cells A, B, and D																																																	
Soil analyses																																																	
E. Water Quality																																																	
In-situ water analysis - MiniSondes, if available	**D**	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	**D**	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	**D**
General wetland constituents																																																	
Surrogate parameters																																																	
Indicator EDCs																																																	
F. Endocrine Disruption Bioassays																																																	
In vitro assays																																																	
In vivo assays																																																	
G. Data Analyses																																																	
Data updates																																																	
Statistical testing																																																	
H. Other																																																	
1. Weather	D	D																																															
2. Wildlife management	D	D																																															
3. Nuisance vegetation removal	D	D																																															
4. Debris management	D	D																																															

Note: Schedule based on time since operational start up.

Colored Boxes indicate monitoring task to be performed during that month. Blank boxes indicate no monitoring.

D = Daily during that week.

A. Hydrology and Hydraulic Transport Monitoring

A.1. Purpose:

Monitoring of water levels and flow rates in treatment wetlands is essential to determine hydraulic retention times (HRT), hydraulic loading rates (HLR), and subsequent constituent removal rates. Maintaining appropriate water levels and flow rates is also critical for protecting the health and survival of wetland vegetation and to ensure that piping and structures are maintained. Quantitative hydraulic characterizations will be established using tracer experiments involving conservative (non-reactive) tracers to determine actual hydraulic retention times and critical transport properties for each treatment unit (Keefe and others, 2004a; Keefe and others, 2010). Required information includes design, construction, and initial fill volumes for the total system and the individual treatment units. Design volumes are calculated from design drawings (Figure 1). Construction volumes are collected from measurements of cut-and-fill soil volumes during wetland construction. The initial fill volume is a measurement of the initial volume of water needed to fill the wetland.

A.2. Methods:

Specific sampling locations and time intervals will be determined from the final design. Hydraulic tracer testing using a conservative and reactive tracer (i.e. bromide and rhodamine WT) will be done once the wetland is fully operational and the flow is at steady-state. Follow-up tracer experiments will be conducted at the beginning and end of each growing season.

Discrete time-series samples and in-situ fluorescence measurements will be collected at the outlet of each wetland cell compartment. Analytical measurements of tracers will be completed by ion chromatography and fluorescence at the USGS-National Research Program (USGS-NRP) in Boulder, Colorado. The duration of the tracer experiment will be approximately five times the calculated nominal hydraulic retention time. The number of samples collected will be dependent on the finalized plan but typically, samples are collected at one to four hour intervals. Methods will generally follow those employed by Keefe and others (2004a, 2010) and Brooks and others, (2011).

A.3. Sampling Frequency:

1. **Baseline:** Once the wetland is fully operational and the flow is consistent, the initial hydraulic characterization will be performed to determine actual hydraulic retention times and critical transport properties for each treatment unit. This initial step to characterize baseline conditions of the various treatment cells is essential for subsequent comparisons of Tier I results by season, year, and location within the wetland.
2. **Continuous:** Devices for continuously measuring water flow at hydraulic control boxes will be deployed, maintained, and data downloaded and collated on a regular basis.
3. **Daily:**
    a. Inspect inlet and outlet pumps and weir box to ensure proper water flow delivery. Clean and/or adjust as needed. Note observations and modifications.
    b. Check to ensure water level is maintained as designed for all sections, including the subsurface flow and stream channel cells; if water levels are too high or low,

adjust as appropriate. Improper water depth is often the principal culprit for the failure of constructed wetland systems. Note cause of instability and record adjustments.

4. **Biannual:** Biannual tracer experiments (timed to coincide with the beginning and end of the growing season) will be conducted for three years following initial vegetation establishment and will be coordinated with biannual vegetation sampling and Lagrangian water sampling (Barber and others, 2011).

B. Vegetation Sampling

B.1. Purpose:

Aquatic vegetation is an important component of any wetland because not only does it provide aesthetics and various wildlife habitat types, but it is actively involved in a number of complex water quality functions (Brix, 1994; Tanner, 2001). Vegetation characterization, health, coverage, growth, density, biomass and tissue analyses data are essential for evaluating whether a wetland system is working optimally. At this site, subsequent biannual or annual vegetation assessments will be made using baseline data for comparisons.

While sampling of vegetation biomass, density, and tissue analyses are important as quantitative measurements, percent vegetation coverage is important for evaluating plant establishment and determining growth patterns (Keefe and others, 2010). Percent coverage can be estimated at ground level by an individual to obtain general coverage data. However, for a more accurate method of measuring large wetland-scale vegetation growth patterns, aerial photography (bird's eye view) using Geographical Information System (GIS) analysis is necessary to quantify areal vegetation coverage data. Percent coverage data will then be compared by season and years throughout the project.

B.2. Methods:

1. Examine plants for general health (i.e., height, robustness, color, flowers, etc.) and for stress (i.e., wilted, chlorotic) or damage by animals, insects, or disease. Record observations. If problems are noted, contact the wetland manager for remediation.
2. Vegetation percent coverage will be estimated from ground level by the same validated biologist each time, as objectively as possible.
3. Count the number of stems/culms within three randomly placed 0.0625 m^2 quadrats within vegetated zones in each of cells A, B, and D and record;
4. Measure the diameters and lengths of 10 representative stems/culms within each of the quadrats. The stem/culm diameters will be measured at their base as they emerge from the gravel substrate. Record all measurements.
5. Extract the entire plant biomass from within the quadrats. Separate the plant parts into above- and below-ground sections being careful to rinse off all sediment and rocks, place each section into separate labeled paper bags, dry for 48 hours at 38ºC or until no further weight loss occurs, weigh each portion separately, and record.

6. Separate three additional representative plants of each of the to be determined species, into above- and below-ground sections (similar to the biomass samples), place in labeled secure plastic storage bags; keep cool; send to an agricultural analytical laboratory such as Colorado State University's (CSU) Soil, Water and Plant Laboratory (SWPL) for plant elemental analysis. Plants will be analyzed for their nutrient concentrations as well as other constituents selected by project stakeholders.
    a. Send to an analytical plant laboratory, such as CSU's SWPL, for routine plant analysis of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), Iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), boron (B), and nitrate-nitrogen (NO_3-N) content.
7. Aerial photography may be taken biannually using true color and infrared-imagery contingent upon funding. The images will be plotted on geo-referenced maps using ArcGIS software to obtain aerial plant coverage. Data will be used in correlating plant coverage with plant biomass, water quality, and hydrology.

B.3. Sampling Frequency:

1. **Baseline:** Will occur at the time of plant installation into the wetland cells.
    a. Record plant spacing during planting. Typically plants are planted on 18-inch to 4-foot centers. Initial plant density will be calculated according to planting specifications, thereafter, as number of plants per square meter (# plants/m^2).
    b. All methods B.2.1-6 above will be performed for baseline monitoring with the exception that unplanted representative plants will be sampled. This will ensure data gathered represent the plants' initial health prior to the addition of WMARSS wastewater.
2. **Daily:**
    a. After plants are planted (installed) in the wetland planting beds, plant survival should be checked daily for the first two weeks as described in methods B.2.1 above.
    b. In addition, remove all invasive, undesirable plant species by pulling out the entire root system as soon as it is identified as undesirable. Delaying weed control efforts will increase the cost of removal significantly.
3. **Weekly**: Beginning two weeks after planting:
    c. Check to ensure water level is maintained as designed in all sections, including the subsurface and stream channel;
    d. All daily monitoring above (methods B.2.1) extends to weekly monitoring.
4. **Eight weeks after planting (the plant establishment period):**
    e. Plants will be inspected to determine whether 80 percent of each plant species installed has survived
    f. If less than 80 percent of the installed plants have survived, notify the plant contractor so dead plants can be replaced, as specified in the planting contract.

5. **Monthly:** After the eight-week establishment period, with at least 80 percent survival of each species, the following task must be done every one to two months (depending on need), until conclusion of the study:

    g. Perform vegetation management and/or detrital removal to prevent short circuiting and maintain a uniform flow pattern over the cells.

6. **Biannual:** Methods B.2.1-4 and B.2.7 above will be done biannually (spring and fall) during the same time as the hydraulic, water quality, macroinvertebrate, and endocrine disrupting bioassays monitoring. Timing of the sampling will coincide with the end of plant dormancy (spring) and at the end of the growing season (fall) until the conclusion of the study.

7. **Annual:** Methods B.2.5 and B.2.6 above will be done annually each fall when plants are mature.

C. Macroinvertebrate Sampling

C.1. Purpose:

Macroinvertebrate sampling provides an additional tool for evaluating the health and proper functioning of a wetland system. Taxonomic inventories can identify water quality issues by the species present, while chemical analyses of the macroinvertebrates can detect constituents of concern concentrating in the biota of the wetland.

C.2. Methods:

1. A composite sample, comprised of at least five sub-samples, will be collected from each wetland cell to characterize the initial macroinvertebrate community composition within each treatment unit. The individual sub samples should be collected from representative locations along the edges of the pond and vegetated areas, using the 1-minute kick method with a D-frame net (700-800 μm mesh). Each composite sample will be enumerated and identified to the lowest practical taxon by Reclamation's TSC for a taxonomic inventory.

2. Collect five grams (if possible) of macroinvertebrates from each wetland cell. Place in secure plastic storage bag labeled bags, keep cool, and send to an analytical laboratory (U.S. Geological Survey National Water Quality Laboratory, NWQL, or Baylor University) for analyses of constituents selected by stakeholders.

C.3. Sampling Frequency:

1. **Baseline:** One month after the wetland becomes fully operational and the flow is consistent, baseline samples of the initial macroinvertebrate community will be collected.

2. **Biannual:** macroinvertebrate sampling will occur concurrently with other biannual sampling and will use the same protocol as the baseline monitoring.

D. Soils and Sediments

D.1. Purpose:

Wetland soil is another important component of wetland function. Soil chemistry significantly impacts plant growth and establishment but also serves as a sink for sorption of phosphorus and various compounds, including EDCs such as alkylphenols, estrogen, and bisphenol A (Ying and Kookana, 2005). Therefore, comparing the initial soil characterization data with soil data collected over time helps to explain the pathways some constituents take within the system. At this site, annual soil assessments will be compared to initial baseline data.

D.2. Methods:

1. Collect composite surface soil samples (minimum of 5 subsamples composited into a single sample) from the top 10 cm within each major design feature and place in labeled sample containers; keep cool;
2. Send to an analytical soils laboratory, such as CSU's SWPL, for routine soils analysis of pH, electrical conductivity (EC), lime estimate, percent organic matter (%OM), sediment organic carbon, NO_3-N, P, K, Zn, Fe, Mn, Cu, B, Ca, Mg, S, Na, As, Se, and Hg content.

D.3. Sampling Frequency:

1. **Baseline:** samples of the surface sediment layer will be collected during the initial baseline period and analyzed.
2. **Annual**: samples will be done like baseline sampling each fall for the duration of the study.

E. Water Quality Sampling

E.1. Purpose:

Daily flow data and weekly chemical analysis results will be used to evaluate overall wetland performance. This frequency of sampling will capture variations in WWTP and wetland operation including inflow rates and individual treatment unit performance. Wetland treatment efficiency and contaminant removal can be calculated relative to changes in flow rate, inflow composition, hydraulic retention time, presence or absence of vegetation, topography of the wetland compartments, and seasonality.

Full monitoring of the fate of EDCs in the wetland system requires analyzing numerous compounds using a variety of analytical techniques. Samples will be analyzed for the specific indicator EDCs listed in Table 2 using methods described in Barber and others (2000) and Foreman and others (2012). These analyses include gas chromatography/tandem mass spectrometry, which provide a compound specific, part-per-trillion analysis for the predominant contaminants responsible for endocrine disruption.

To simplify the chemical analysis of EDCs and allow for a greater number of samples to be analyzed, surrogate parameters and other indicator chemicals will be used for this assessment (Dickenson and others, 2009; Benotti and others, 2009; Dickenson and others, 2011). Indicators and surrogates have been used in WWTPs to evaluate the relative occurrence of certain compounds and their behavior, reflecting the efficacy of a given type of treatment (Benotti and others, 2009). Using this approach supports potential collaboration with Tier II studies while offering sufficient information to assess the general fate and transport of EDCs through the wetlands.

The EDC indicators defined for this project are specific chemicals used to evaluate fate and transport through the engineered wetland system. Indicator compounds were selected due to their categorization as EDCs, availability of suitable analytical methods, and occurrence on the USEPA CCL3 list. Two additional EDCs not on the USEPA CCL3 List also will be monitored: Bisphenol A and 4-nonylphenol. The U.S. EPA has recently established toxicity-based aquatic life water quality criteria for 4-nonylphenol (U.S. Environmental Protection Agency, 2005), and it has been shown to have adverse effects on endocrine function (Bistodeau and others, 2006; Schoenfuss and others, 2008) and behavior (McGee and others, 2009).

A surrogate is a parameter that can serve as a performance measure of treatment processes that relates to the removal of specific contaminants and provide a means of assessing water quality characteristics without conducting difficult trace contaminant analysis (Dickenson and others, 2009). Surrogate analyses for this study will focus on the bulk characterization of dissolved organic matter in the system and will be conducted during baseline and biannual monitoring events.

E.2. Methods:

1. Hourly *in-situ* water quality parameters, including water temperature, pH, dissolved oxygen, and conductivity, will be measured at the wetland's inlet and at the end of each major treatment cell using on-line multi-probe water quality data loggers. The instruments will need to be serviced and their data downloaded weekly (at time of sample collection).
2. As part of the baseline water-quality determination, samples will be collected at the inlet and outlet of the wetlands during the initial planting period. The following steps include: 1) collect a grab water sample as it is delivered to the new plants; 2) preserve as needed for the various analyses and/or keep cool; and 3) take to analytical laboratory for analyses listed in Table 2.
3. Grab samples will be collected weekly from the wetland inflow and outflow and the Wetland General Constituents (listed in Table 2) will be analyzed. All samples will be collected on the same day of the week at the same time each day. Weekly sampling should continue throughout the entire monitoring period.
4. Endocrine disrupting chemicals will be assessed bi-annually in conjunction with tracer testing for evaluation of wetland performance to provide feedback for operating parameters and overall wetland design. Water quality samples will be collected from the inlet and after each major treatment unit Water-quality samples will be analyzed for the parameters listed in Table 2.

E.3. Sampling Frequency:

1. **Baseline:** Include all monitoring described above from wetland inflow and outflow.
2. **Continuous:** Multi-probe water-quality data.
3. **Weekly:** general constituents (see Table 2) will be collected at the wetland inflow and outflow locations.
4. **Biannual**: performed concurrently with the vegetation and hydraulic monitoring. Water samples will be collected from seven *in-situ* locations within the treatment wetland; at the inlet, after each major treatment unit, within each major treatment unit, and at the outlet, and analyses for Wetland General Constituents, Surrogate Parameters, and Indicator EDCs (see Table 2) will be performed. Bi-annual sampling should continue for a minimum of three full years.

Table 2. Baseline, weekly, and biannually collected water quality monitoring parameters.

Monitoring Parameter	**Monitoring Frequency**
Wetland Inflow and Outflow to Each Design Feature	
Water flow rates	Continuous from start of wetland operation
Continuous Water Quality Monitoring – On-line multi-probe data logger	
pH	Hourly during baseline and biannual sampling events or continuously from start of wetland operation, if instruments are available.
Temperature	
Dissolved Oxygen (DO)	
Specific Conductance	
Wetland General Constituents – Weekly	
Total Dissolved Solids (TDS)	Once at the designated time of baseline sampling, then weekly at wetland inflow and outflow. For biannual sampling, samples will be collected at all sampling locations.
Total Suspended Solids (TSS)	
Alkalinity ($CaCO_3$)	
Turbidity, in NTU	
Total Organic Carbon (TOC)	
Unfiltered UV transmittance (UFT), in %	
Nitrogen series – NO_2-N, NO_3-N, TKN, NH_3-N	
Orthophosphate	
Boron	
Surrogate Parameters – Biannually	
Dissolved Organic Carbon (DOC)	Once at the designated time of baseline sampling, then biannually at all sampling locations.
Full Scan UV transmittance (200 – 800 nm)	
3-D Fluorescence Spectroscopy	
Indicator EDCs – Biannually	
Bisphenol A	Once at the designated time of baseline sampling, then biannually at all sampling
4-Nonylphenol	

Equilenin	locations.
Equilin	
17α-Estradiol	
17β-Estradiol	
Estriol	
Estrone	
17α-Ethinylestradiol	
Mestranol	
Norethindrone	
Progesterone	

F. Endocrine Disruption Bioassays

F.1. Purpose:

Each biannual sampling event will be incorporated into targeted bioassays to: (1) evaluate the endocrine disruption and modulation potential of the treatment wetland influent and effluent, (2)assess attenuation of endocrine disruption and modulation potential as a function of treatment units, and (3) define initially the influence of season on endocrine disruption and modulation potential (Rodgers-Gray and others, 2000; Brooks and others, 2003; Hemming and others, 2004; Martinovic and others, 2008). For example, *in vitro* and *in vivo* assays of estrogenic activity have been widely employed to assess effluent quality (Brooks and others, 2003; Huggett and others, 2003; Pawlowski and others, 2003; Sapozhnikova and others, 2005; Schlenk, 2008; Wehmas and others, 2011; Xie and others, 2005). Sensitivity of various *in vitro* and *in vivo* assays inevitably can differ among various EDCs (Thorp and others, 2003; Dobbins and others, 2008). However, several *in vitro* assays of estrogenicity (e.g., Yeast Estrogen Screen (YES), MCF-F, T47D-KBluc) appear quite useful for monitoring effluent activity for diagnostic purposes within different components of the wetland system proposed here, though each assay inherently possesses strengths and weaknesses (Sapozhnikova and others, 2005; Dobbins and others, 2008; Schlenk, 2008).

F.2. Methods:

A robust *in vitro* assay (e.g., YES, MCF-7) was selected for more routine monitoring of each component of the wetland system during each season. *In situ* studies with sexually mature fathead minnows represent robust approaches to identify *in vivo* responses to EDCs. In the present study, we propose to expose *in situ* adult fathead minnows at the inflow and outflow of the wetland system for a 7-day period during late summer and late winter. These two study periods are intended to characterize conditions with differential macrophyte biomass and temperature regimes. Following this exposure period, plasma vitellogenin, 11-ketotestosterone and 17B-estradiol levels, gonadal and hepatic somatic indices, secondary sexual characteristics, and gonadal histopathology will be assessed following common methods (Brooks and others, 2003; Vajda and others, 2008, 2011).

F.3. Sampling Frequency:

1. **Baseline:** This approach will allow us to provide baseline information to evaluate the efficacy of internal attenuation processes, and is consistent with the recent review of *in vitro* and *in vivo* monitoring assays by the National Research Council (2012) report on Water Reuse. Further, it is anticipated that information collected in Tier I will provide a reasonable baseline for more intensive monitoring and targeted research studies in Tier II
2. **Biannual:** Though it is ideal to couple such *in vitro* assays with *in vivo* responses through caged or other exposure designs (Schlenk, 2008), the scope of this baseline monitoring effort will only allow for coupling *in vitro* and *in vivo* measures of endocrine function in fish models twice per year (spring, fall) for the wetland influent and effluent.

G. Data Analyses

G.1. Purpose:

The creation and maintenance of a monitoring database is necessary for the documentation of monitoring data results and observations. A monitoring database will be created to archive the results from the monitoring program. It will document general observations and include data from flow meters, in-line water-quality monitors, as well as sampling events performed during targeted research studies. It will be used as a library to store and provide information for wetland performance and records of wetland events. The monitoring database will be used to assess wetland performance over the monitoring period through a number of statistical evaluations.

G.2. Methods:

1. **Data Quality:** Monitoring data will be compared to previous monitoring events and historical city data to address laboratory/operator error in analysis or entering data into the monitoring database. Data tagged in this quality assessment and quality control review will be excluded or noted in the statistical assessments.
2. Statistical methods are categorized and proposed to answer the proposed research questions:
    a. What is the performance efficacy of constructed wetlands in attenuating EDCs from municipal wastewater treatment plant effluent?
        i. To assess the overall wetland performance, data will be evaluated and compared to the null hypothesis scenario, which is the current Waco WWTP effluent. Statistical methods that can be employed in this assessment include:
            1. Descriptive statistics to describe the individual WWTP effluent and wetland effluent data sets (such as the mean, min, max, sum, etc.)
            2. Direct comparison through the use of empirical statistics such as t-tests to verify the null hypothesis.

3. Cumulative frequency to describe the number of events in which the wetland attenuates EDCs when compared to the WWTP effluent.

b. How do different design features within the cells attenuate EDCs and other important constituents? And how does season and vegetation coverage affect attenuation?

i. To assess the attenuation potential of individually designed wetland cells and wetland conditions, monitoring data will be evaluated between subsequent inter-wetland sampling points. For instance, hydraulics, vegetation coverage, surrogates, and bioassays in conjunction with EDC indicator measurements, can be used to evaluate conditions in which the wetland system has favorable potential for EDC removal. These conditions could be evaluated by a number of statistical methods including:

1. Data set comparison tests to observe variations between wetland cell attenuation such as chi square or ANOVA tests.

2. Data correlations and similarities using statistical methods such as spearman correlations, simple/multiple regressions, and factor analysis.

3. Additional grouped indications of attenuation may also be observed through factor or cluster analyses.

ii. By classifying the optimal wetland conditions through the combined areas targeted in the wetland monitoring protocol, potential mechanisms for each treatment cell may be evaluated. Information on design, operation, and maintaining these conditions can be used to evaluate the current engineered system and be transferred to various wetland system designs.

G.3. Frequency:

1. **Baseline:** Baseline observations and sampling results will be recorded at the time of baseline monitoring. The template in which baseline results are entered will serve for the template for weekly and biannual data additions.

2. **Weekly/Biannually:** The monitoring database will be updated monthly with continuous monitoring data and biannually to record data collected during comprehensive biannual sampling events. The database will be assessed for consistency in sampling and monitoring plan parameters after biannual events. Data analysis will be performed on the database upon completion of data entry for both weekly and biannual time points.

H. Other

H.1. Weather

During the monitoring period, daily temperatures, wind intensity and direction, and precipitation data will be collected from the Waco Weather Station, located 5.5 miles southwest of the site at

Cottonwood Creek Golf Course (5200 Bagby Avenue Waco, TX 76711) (see: http://texaset.tamu.edu/date.php?stn=85&spread=7).

H.2. Wildlife

Due to the wetland location and its proximity to the Brazos River, wildlife, especially birds, will be attracted to it. If nests of migratory or other Federally-protected bird species are present on the wetland aggregate or among the desirable wetland vegetation, then particular care must be made to allow the eggs to hatch and fledglings to fly before destructive sampling, cleaning out, or otherwise maintaining the area occurs.

If the wildlife, or their activities, is found to interfere with the proper operations of the wetland cells or to significantly impact the water, then care must be taken to discourage them from using the area. Possible techniques for deterring bird or wildlife usage could include netting the area, using sound systems, or trapping and removing. The US Fish and Wildlife Service (USFWS) should be informed regarding all techniques used to deter wildlife. At least weekly, check areas surrounding the wetland for evidence of animal activity.

Mosquito abatement either through the use of mosquito fish and/or the periodic application of a biological larvicide to surface waters of the habitat zone should be considered on an as needed basis if mosquitoes become an issue. Chemical pesticides will be forbidden due to the impact the chemicals will have on evaluating wastewater chemicals moving through the wetland system.

H.3. Debris Management

The wetland will be inspected weekly, particularly at the inflow and outflow areas, for problem accumulations of detritus and debris and dispose of appropriately (record the frequency of cleaning and amount removed each time). Evidence of internal clogging, such as water ponding on the surface of the subsurface flow beds, will be recorded.

If the wetland site is used as an educational facility providing public tours, then weekly monitoring and clean-up of trash and waste products should be performed.

Tier 2 Monitoring

As noted above, Tier II monitoring identifies additional, more focused research opportunities that may be explored pending additional funding that are considered beyond the scope of the Tier I monitoring program. Tier II activities represent an important consideration because the treatment wetland design also should attenuate other biologically-active EDCs, such as pharmaceuticals and personal care products (Boxall and others, 2012; Brooks and others, 2009; Brooks and others, 2011; Painter and others, 2009; Schultz and others, 2010). However, studies to investigate such contaminants are beyond the scope of this preliminary Tier I monitoring program. Additional focused studies will be pursued through other funding agreements and partnerships with local utilities, and state and national agencies. These studies organized by major topic, might include, but are not limited to, the following:

Contaminant Fate and Transport

1. Contaminant fate and cycling coupled with treatment model rate calibration.
2. Nutrient fate and removal.
3. Perform comprehensive toxicity identification evaluations of EDC and other CEC removal throughout the wetland system using *in vitro* and *in vivo* bioassays (Desbrow and others, 1998; Snyder and others, 2001; Brooks and others, 2003; Huggett and others, 2003).

Wetland Operations and Treatment Mechanisms

1. Supplement this study with additional research on the wetland system to develop an advanced understanding of factors (e.g., photolysis, adsorption, biotransformation) influencing the seasonal fate and transport of specific EDCs and other CECs.

2. Engineered wetland design modifications to further optimize various functions.

Wetland Health and Habitat

1. Plant tissue analysis to look at EDCs, and other CECs (including Se, As, and Hg as potential toxins to wildlife) accumulation in plants.

2. Adult macroinvertebrate emergence from specific wetland zones and tissue analysis for bioaccumulation and trophic transfer of EDCs and other CECs (including Se, As, and Hg as potential toxins to wildlife) in various macroinvertebrate instars.

Additional wetland research topics not mentioned above but identified as important by partners and stakeholders will be pursued through other funding mechanisms. Potential partners include the City of Waco, Baylor University, USGS, Reclamation, U.S. Environmental Protection Agency, WateReuse Research Foundation, and Texas Water Development Board.

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