RECLAMATION

Managing Water in the West

Hydrological, Chemical, and Biological Monitoring Plan

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

FINAL REPORT

Submitted To:

Bureau of Reclamation, Science and Technology Program

Project ID: 104 and 9589 Deliverable

Submitted by:

U.S. Department of the Interior

U.S. Geological Survey, Fort Collins Science Center, Denver CO

U.S. Geological Survey, National Research Program, Boulder CO

Bureau of Reclamation, Technical Services Center, Denver CO

Bureau of Reclamation, Oklahoma-Texas Area Office, Austin TX

Baylor University

Center for Reservoir and Aquatic Systems Research, Waco TX

In Partnership With:

Texas Water Development Board

U.S. Department of the Interior
Bureau of Reclamation **Revised January 2014**

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

Hydrological, Chemical and Biological Monitoring Plan

Joan S. Daniels[1], Katharine Dahm[2], Steffanie H. Keefe[3], Bryan W. Brooks[4], Larry B. Barber[3], Anna Hoag[5], and Collins Balcombe[5]

1. U.S. Geological Survey, Fort Collins Science Center, Denver, CO
2. Bureau of Reclamation, Technical Services Center, Denver, CO
3. U.S. Geological Survey, National Research Program, Boulder, CO
4. Center for Reservoir and Aquatic Systems Research, Baylor University, Waco, TX
5. Bureau of Reclamation, Oklahoma-Texas Area Office, Austin, TX

U.S. Department of the Interior
Bureau of Reclamation

Revised January 2014

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.

Contents

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. In fact, the National Research Council (2012) recently identified understanding the role of contaminant attenuation in environmental buffers as a research priority for advancing indirect potable reuse projects. In particular, the National Research Council recommended research on how well different environmental buffers function under various conditions, their potential weaknesses, and their impacts on water quality is crucial to the optimization of potable reuse systems and future decisions about their design.

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 Basin One, horizontal subsurface flow (HSSF) cells, stream channel, and hummock/habitat Basin Two) incorporating a sequence of specific features to promote photolysis, sorption, biodegradation,

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

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 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.

Site Description

The Brazos River Demonstration Wetland will be located along the Brazos River, northwest of the WMARSS on an adjacent property owned by the City. Figure 2 shows the demonstration wetland's location relative to the Brazos River and WMARSS. The area is within the Middle Brazos-Lake Whitney watershed, USGS cataloging unit 12060202.

This area is part of the Central Texas Blackland Prairie, the innermost sub-province of the Gulf Coastal Plains. The Blackland Prairie consists of chalks and marls weather to deep, black, fertile clay soils; shale in the Eagle Ford Group, Austin Chalk, and the Navarro Group (including the "Taylor marl") of Cretaceous age. These Cretaceous rocks are incised by several major stream systems. Quaternary stream terraces and alluvium are associated with the rivers, and drainage patterns are controlled by more resistant Cretaceous bedrock. The Blacklands have a gentle undulating surface, cleared of most natural vegetation and cultivated for crops. From sea level at the Gulf of Mexico, the elevation of the Gulf Coastal Plains increases northward and westward. In the Waco area the average elevation is 470-feet. Southeast of Waco at the wetlands site, the natural elevation varies from 375 to 385-feet, which was an advantage to site selection.

Waco experiences a humid subtropical climate (Köppen climate classification *Cfa*), characterized by hot summers and generally mild winters. The average annual temperature is 68° F (20°C). Temperatures of 90°F (32°C) have been observed in every month of the year, (NOAA, 2013). The record low temperature is −5°F (−21°C), set on January 31, 1949; the record high temperature is 112°F (44°C), set on August 11, 1969 (NWS, 2013). The average annual precipitation is 34-inches, with most of the rainfall occurring in spring and fall. However, periods with zero precipitation have also been observed in every month of the year (NWS, 2013).

Figure 1. Brazos River Demonstration Wetland Site Plan

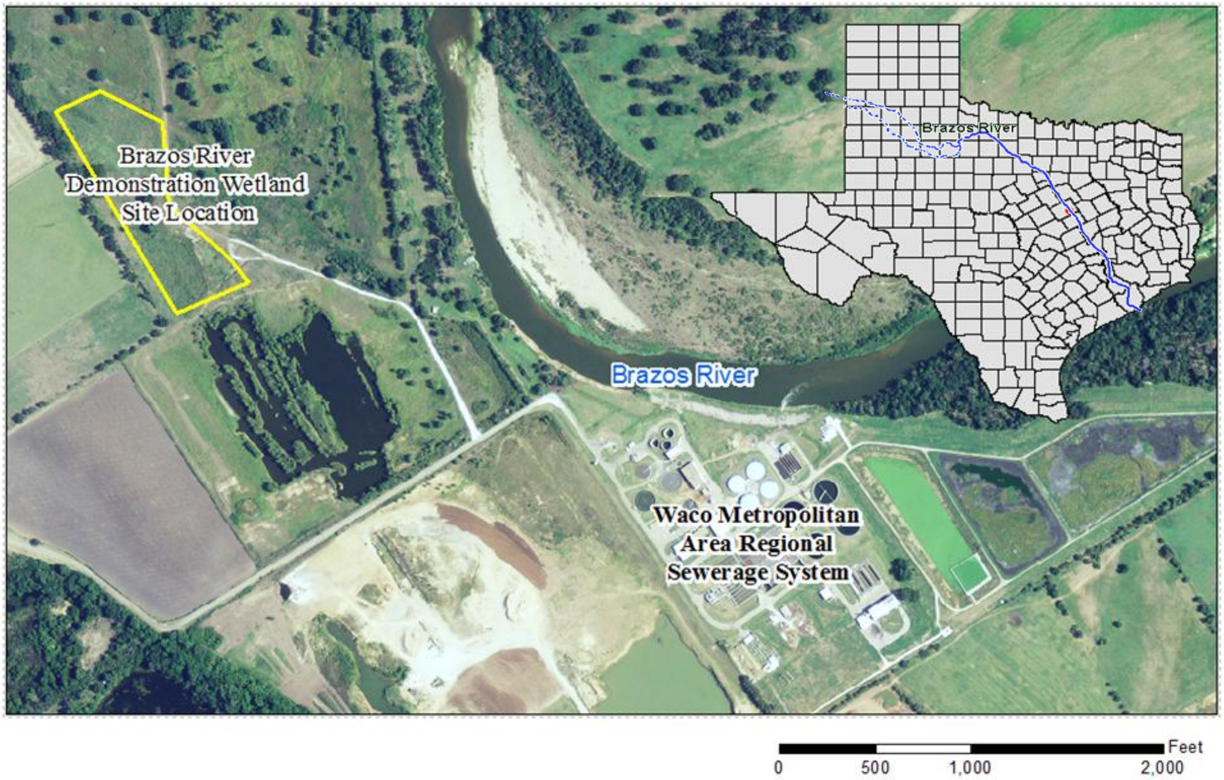

Figure 2: Proposed Brazos River Demonstration Wetland location relative to the Brazos River and WMARSS.

Groundwater Hydrology

Sandstone and carbonate layers in the Trinity Group are the principal aquifers in the Blackland Prairie. Falling water tables limit the use of this aquifer in Central Texas with a saturated thickness average of about 1,900 feet. Shallow ground water is scarce throughout this area, but along the river the Brazos River Alluvium is accessible. The alluvium aquifer is as much as 7 miles in width and extends along 350 river miles from southern Bosque County to eastern Fort Bend County. Groundwater is contained in alluvial floodplain and terrace deposits, although the latter is not an appreciable source of water. The floodplain alluvium consists of fine to coarse sand, gravel, silt, and clay. These deposits have a complex geometry, with beds or lenses of sand and gravel that pinch out or grade vertically into finer material. In general, finer sediments occur in the upper part of the aquifer, and coarser material is in the lower part. The thickness of the aquifer ranges from negligible to 168 feet, with an overall average of about 50 feet.

Water in the aquifer is very hard and fresh to slightly saline, generally containing less than 1,000 milligrams per liter of total dissolved solids but ranging to as much as 3,000 milligrams per liter in some wells. The aquifer is under water table conditions in most places and is used mainly for irrigation. The water table generally slopes toward the Brazos River, this direction indicating that the river is a gaining stream in most places.

Recharge to the aquifer occurs from rainfall infiltration into the aquifer and subsequent downward leakage to the saturated zone. Discharge from the aquifer occurs through evapotranspiration, discharge to the river, and withdrawals from wells. Some wells can yield as much as 1,000 gallons per minute, but the majority of wells yield from 250 to 500 gallons per minute. No significant water level declines have occurred in the aquifer.

The Brazos River Demonstration Wetland will be lined with a compacted clay liner and negligible groundwater interaction is anticipated. The Texas Water Development Board's Water Information Integration and Dissemination, Groundwater Database provides historical and existing well log data. Using the Groundwater Database, 15 surrounding wells were used to determine the minimum and maximum water level to be 13.2-ft and 34.8-ft respectively below surface elevations. The closest groundwater well recorded in the database to the site has a surface water elevation of 366.4-ft above sea level and a ground elevation of 383-ft. The existing site elevation would be excavated as much as 10-ft to 372-ft ground elevation for the wetland, allowing several feet before any groundwater interaction after the construction is complete.

Soils

The Brazos River Demonstration Wetland site consists of predominantly of Weswood silt loam (Wd), Weswood silty clay loam (We), and Ships clay (Sh). Because the site has an existing clay deposit, it has been used by Waco as a borrow pit for several decades for construction various projects. The Brazos River Demonstration Wetland will be lined with a compacted clay liner using site materials.

Biology

This area supports mixed tall and mid prairie grasses. Little bluestem is the dominant species. Indiangrass, big bluestem, switchgrass, tall dropseed, silver bluestem, sideoats grama, eastern gamagrass, and vine mesquite are the major herbaceous species. The plant community has many forbs, such as prairie clover, western ragweed, Maximilian sunflower, gayfeather, rattlesnake master, and Indian plantain. Areas along the major rivers and streams support savanna vegetation. Oak, elm, cottonwood, hackberry, and pecan trees produce a canopy cover of about 30 percent.

Some of the major wildlife species in this area are white-tailed deer, javelina, coyote, fox, bobcat, raccoon, skunk, opossum, jackrabbit, cottontail, turkey, bobwhite quail, scaled quail, white-winged dove, and mourning dove. A description of the invasive species in the area and management activities is included in Section I.2.

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. A monitoring period of greater than three years would produce insight into how the wetland's treatment performance changes over time. For this project however the monitoring plan is designed to capture the highly dynamic initial start-up conditions (first three years), and to establish the infrastructure and baseline data for longer-term (multi decade) monitoring of performance required to optimize the operation and maintenance of the wetland. 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. Each sampling event will need to be coordinated by local researchers to capture "typical" conditions during different seasons. Field planning will attempt to identify optimal times, with contingency plans in the event of unacceptable hydrological conditions. Replicate samples for all analyses will be taken during start-up monitoring.
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. All aerial imagery will be geo-referenced and ground-truthed. 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:

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.

1. 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. Ethanol can be used to preserve the samples before they are identified. If they are to be analyzed for metal (or other) uptake, they are sorted in the field (all plant and soil matter removed) and then frozen.

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. This one month timeframe is flexible depending on the time required to establish full,

consist flows, and it may be prudent to wait more than a month after the wetland becomes fully operational to take baseline samples.

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. To observe water quality variation throughout the weekly sampling period composite samples will be collected to accompany certain sampling events as a point of comparison.

These composite sampling events will occur at the outset of the project when initial characterization occurs and during both bi-annual events each year as a point of comparison. Composite sampling will consist of 10 or more grab samples collected weekly and combined with equal parts into a single "composite sample" during the bi-annual monitoring event at the inlet and outlet of Basin One and Two.

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

Note: The *redox characteristics of the water will be evaluated by measuring dissolved oxygen.*

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). Currently, the intent is not to “replicate a natural environment.” Rather, caged organisms are used as biosensors and thus their ability to freely forage will be limited. They will largely graze on periphytic growth on cages and opportunistically on invertebrates

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. Quality Assurance Procedures

General Quality Assurance Quality Control (QAQC) protocols: Quality assurance protocols and documentation for water-quality sampling will follow the USGS National Field Manual guidelines. Equipment calibration, maintenance, and verifications will be performed according to USGS National Field Manual and Manufacturer guidelines. For the equipment being deployed during sampling all DO sensors will be calibrated on-site. The USGS National Water Quality Laboratory in Denver, Colorado is a NELAC accredited laboratory.

Quality Assurance Project Plan: In addition to the noted general QAQC protocols a formal document with the QAQC protocols linked to specific project activities and documentation will be created as a sampling guide for monitoring. The protocols will include documentation and procedures for each biannual monitoring event with information such as:

- Standard data collection forms
- Lab sample labeling information
- Calibration procedures and documentation forms
- Sampling protocol documentation
- Data review procedures and check sheets
- Database forms for collective data recording and presentation

Adaptive Management Plan: Finally, an adaptive management plan will also be created to cover adaptive operations alternatives and adaptive monitoring under various scenarios, such as extreme weather or presence of invasive species. The monitoring plan sampling areas, analyses types, and frequencies will also be subject to change based on adaptive strategies in order to better characterize the system.

H. Data Analyses

H.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.

H.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.
4. Non-parametric statistics (normality and homogeneity of variance) the analysis of mechanistic of biological compounds will also be employed.
5.

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.

H.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.

I. Other

I.1. Weather

Each sampling event will need to be coordinated by local researchers to capture "typical" conditions during different seasons. The daily monitoring by Baylor and Waco will be used to assist in monitoring if these events occur when scheduling difficulties cannot be avoided. Field planning will attempt to identify optimal times, with contingency plans in the event of unacceptable hydrological conditions. 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).

I.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 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. The preference is that no fish are stocked initially in order to limit the variables in determining whether this design is effective. Once research is complete and there is a need or desire to introduce other species into the system, then the project partners will need to coordinate with Texas Parks and Wildlife Department.

I.3. Waste 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. The WWTP effluent has a low total suspended sediment load and there should not be a significant sediment management issue. The elevated nature of the wetlands, and the limited drainage into the system (defined as what falls within the berms), will minimize storm water sediment loading. The accumulation of biomass can be managed in a number of ways including mechanical harvesting or burning. Most likely vegetation management will become necessary within three years. We will need to work with the City, Baylor University, and other local groups to determine how this should be addressed if it becomes an issue.

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.

Peer Reviewer Monitoring Expansion Suggestions

The peer review team consisted of a variety of experts in wetland design, wetland hydraulics, wetland vegetation, EDC monitoring, and wetland resources as a recreational benefit. The peer review team provided valuable comments that have been incorporated into Tier I monitoring procedures in this version of the monitoring plan. The peer review team also provided comments that would expand the scope of the project and are therefore recommended as part of Tier II monitoring for the wetland.

1. The focus of the project is the efficacy of the overall wetland design and the individual wetland components in reducing endocrine disrupting compounds. However, the wetlands themselves will be an ecosystem that will include fish, benthic macroinvertebrates, amphibians, insects and plants, and will likely serve as habitat for birds and mammals. While the function of the system is to attenuate EDCs, it will also likely attenuate metals and other chemicals present in the wastewater, and it follows that any chemicals that bioaccumulate will likely concentrate in the wetland sediments, plants and animals. Care must be taken to avoid inadvertently concentrating chemicals that will be harmful to wildlife, as occurred at the Kesterson Reservoir in California.

2. If the algae can be removed from the vegetation, it could be analyzed separately. The entire plant biomass will be removed from the quadrats, dried and weighed. There is no indication that algae will be separately removed from the quadrat or scraped from the leaves. Would it be helpful to distinguish between vascular plant above- and below-ground biomass and algae biomass?

3. Should soils be described in accordance with standard methodologies to determine at what point the wetland soils meet the definition for a jurisdictional wetland?

4. Sediments and soils are likely to sorb and concentrate EDCs; however, the monitoring plan does not appear to monitor any soil contaminants. While the routine monitored parameters will likely help interpret information about plant health, they seem unlikely to provide information about buildup of EDCs and other contaminants in the sediments. We recommend that in addition to routine parameters, sediments be monitored biannually for EDCs. This could provide some information about the environment of the benthic macroinvertebrate community, which is part of the food base for many fish. Attempts to understand fate and transport of EDCs may suffer from lack of this information.

5. What is the effect of the EDCs and other chemicals on wetland biota? What is the effect of the EDCs and other chemicals on wildlife that may utilize the wetland?

6. Monitoring macroinvertebrate tissue for EDCs would greatly contribute to understanding the fate and transport of the compounds and how they impact biota.

7. The measurement of algae counts should be performed during the study to assess they are not a factor and if present do they correlate with indicator EDC level trends.

8. Other indicator PPCPs are proposed to be performed during the Tier II study. But other treatment indicator PPCPs should be included to represent and isolate the differing

proposed removal processes: photolysis, sorption, biodegradation and volatilization. The selected indicator EDCs (Table 2) are could be similar in structure and if so more compound structural/reactivity diversity should be represented to represent the fate of other potential EDC compounds. It is unclear when Tier II activities would begin? After the 3 years or during? It would be ideal if it could be during as this is a valuable opportunity to include indicator PPCPs and other EDCs into the matrix and generate informative performance information, or else the PPCP samples will not be collected until sometime later, which would be unfortunate.

9. To assess the fate of sorption, which is likely important for a majority of these proposed indicator EDCs (due to their relatively higher log KOW), measuring the EDC levels on solid matrices (i.e., soil, vegetation) would be informative in regards to understanding their fate. However, this is probably out of the scope of Tier I monitoring, but could be included in Tier II monitoring.

10. I think only 7 temporal (biannual) EDC samples will be collected over the course of the 3 years. Considering this includes start-up, the data during start-up could differ to full performance data, and therefore, this could limit the number of full performance data (i.e., <7). To increase the statistical significance and quality of the full performance data the collection of indicator EDC needs to be increased to at least quarterly sampling.

11. Would consider adding monitoring of redox potential (ORP) to the proposed soils and sediments analyses.
12. I would suggest adding oxidation reduction potential (ORP) to the suite of water quality probes being deployed at the wetland site. This can be a useful and inexpensive measurement combined with dissolved oxygen for understanding and monitoring the redox conditions in the water.

Additional wetland research topics not mentioned above but identified as important by partners and stakeholders will be pursued through other funding mechanisms.

References

Ankley, G. T., Brooks, B. W., Huggett, D. B. and Sumpter, J. P. (2007). Repeating history: Pharmaceuticals in the environment. *Environ. Sci. Technol*. **41**, 8211-8217.

Barber, L. B., Brown, G. K. and Zaugg, S. D. (2000). Potential endocrine disrupting organic chemicals in treated municipal wastewater and river water. In *Analysis of Environmental Endocrine Disruptors*; Keith, L. H., Jones-Lepp, T. L., Needham, L. L., Eds.; Am. Chem. Soc., Symposium Series 747: Am. Chem. Soc., Washington, DC, 97-123.

Barber, L. B., Leenheer, J. A., Noyes, T. I., and Stiles, E. A. (2001). Transformation of dissolved organic matter in treatment wetlands. *Environ. Sci. Technol*. **35**, 4805-4816.

Barber, L. B., Keefe, S. H., Taylor, H. E., Antweiler, R. C., and Wass, R. D. (2006). Accumulation of contaminants in fish from wastewater treatment wetlands. *Environ. Sci. Technol*. **40**, 603-611.

Barber, L. B., Lee, K. E., Swackhamer, D. L. and Schoenfuss, H. L. (2007). Reproductive responses of male fathead minnows exposed to wastewater treatment plant effluent, effluent treated with XAD8 resin, and an environmentally relevant mixture of alkylphenol compounds. *Aquat. Toxicol*. **82**, 36-46.

Barber, L. B., Antweiler, R. C., Flynn, J. L., Keefe, S. H., Kolpin, D. W., Roth, D. A., Schnoebelen, D. J., Taylor, H. E. and Verplanck, P. L. (2011). Lagrangian mass-flow investigations of inorganic contaminants in wastewater-impacted streams. *Environ. Sci. Technol*. **45**, 2575-2583.

Barber, L. B., Brown, G. K., Nettesheim, T. G., Murphy, E. W., Bartell, S. E. and Schoenfuss, H. L. (2011). Effects of biologically-active chemical mixtures on fish in a wastewater-impacted urban stream. *Sci. Tot. Environ*. **409**, 4720-4728.

Barber, L. B., Vajda, A. M., Douville, C., Norris, D. O. and Writer, J. H. (2012). Fish endocrine disruption responses to a major wastewater treatment facility upgrade. *Environ. Sci. Technol*. **46**, 2121-2131.

Barnes, K. K., Kolpin, D. W., Furlong, E. T., Zaugg, S. D., Meyer, M. T. and Barber, L. B. (2008). A national reconnaissance of pharmaceuticals and other organic wastewater contaminants in the United States –I) Groundwater. *Sci. Tot. Environ*. **402**, 192-200.

Bistodeau, T. J., Barber, L. B., Bartell, S. E., Cediel, R. A., Grove, K. J., Klaustermeier, J., Woodard, J. C., Lee, K. E. and Schoenfuss, H. L. (2006). Larval exposure to environmentally relevant mixtures of alkylphenolethoxylates reduces reproductive competence in male fathead minnows. *Aquat. Toxicol*. **79**, 268-277.

Benotti, M. J., Trenholm, R. A., Vanderford, B. J., Holady, J. C., Stanford, B. D. and Snyder, S. A. (2009). Pharmaceuticals and endocrine disrupting compounds in U.S. drinking water. *Environ. Sci. Technol*. **43**, 1092-1098.

Boxall, A. B. A., Rudd, M., Brooks, B. W., Caldwell, D., Choi, K., Hickmann, S., Innes, E., Ostapyk, K., Staveley, J., Verslycke, T., Ankley, G. T., Beazley, K., Belanger, S., Berninger, J. P., Carriquiriborde, P., Coors, A., DeLeo, P., Dyer, S., Ericson, J., Gagne, F., Giesy, J. P., Gouin, T., Hallstrom, L., Karlsson, M., Larsson, D. G. J., Lazorchak, J., Mastrocco, F., McLaughlin, A., McMaster, M., Meyerhoff, R., Moore, R., Parrott, J., Snape, J., Murray-Smith, R., Servos, M., Sibley, P. K., Straub, J. O., Szabo, N., Tetrault, G., Topp, E., Trudeau, V. L., and van Der Kraak, G. (2012), *In press*. Pharmaceuticals and Personal Care Products in the Environment: What are the Big Questions? *Environ. Health Perspect.* http://dx.doi.org/10.1289/ehp.1104477

Bradley, P. M., Barber, L. B., Kolpin, D. W., McMahon, P. B., and Chapelle, F. H. (2007). Biotransformation of caffeine, cotinine, and nicotine in stream sediments: Implications for use as wastewater indicators. *Environ. Toxicol. Chem*. **26**, 1116-1121.

Bradley, P. M., Barber, L. B., Kolpin, D. W., McMahon, P. B., and Chapelle, F. H. (2008). Potential for 4-n-nonylphenol biodegradation in stream sediments. *Environ. Toxicol. Chem*. **27**, 260-265.

Bradley, P. M., Barber, L. B., Chapelle, F. H., Gray, J. L., Kolpin, D. W., and McMahon, P. B. (2009). Biodegradation of 17β-estradiol, estrone, and testosterone in stream sediments. *Environ. Sci. Technol*. **43**, 1902-1910.

Brix, H. (1994). Functions of macrophytes in constructed wetlands. Water Sci Tech. 29(4),71-78

Brooks, B. W., Foran, C. M., Weston, J., Peterson, B. N., La Point, T. W., and Huggett, D. B. (2003). Linkages between population demographics and municipal effluent estrogenicity. *Bull. Environ. Contam. Toxicol*. **71**, 504-511.

Brooks, B. W., Huggett, D. B., and Boxall A. B. A. (2009). Pharmaceuticals and personal care products: Research needs for the next decade. *Environ. Toxicol. Chem.* **28** 2469-2472.

Brooks, B. W., Chambliss, C. K., Sedlak, D. L., and Knight, R. L. (2011). Evaluate wetland systems for treated wastewater performance to meet competing effluent water quality goals. WateReuse Research Foundation, Project Number: WRF-05-006, Alexandria, VA, 127 p.

Dobbins, L. L., Brain, R. A., and Brooks, B. W. (2008). Comparison of the sensitivities of common in vitro and in vivo assays of estrogenic activity: Application of chemical toxicity distributions. *Environ. Toxicol. Chem.* **27**, 2608-2616.

Desbrow, C., Routledge, E. J., Brighty, G. C., Sumpter, J. P. and Waldock, M. (1998). Identification of estrogenic chemicals in STW effluent 1. Chemical fractionation and in vitro biological screening. *Environ. Sci. Technol*. **32**, 1549-1558.

Dickenson, E. V., Drewes, J. E., Sedlak, D. L., Wert, E. C. and Snyder, S. A. (2009). Applying surrogates and indicators to assess removal efficiency of trace organic chemicals during chemical oxidation of wastewaters. *Environ. Sci. Technol*. **43**, 6242–6247.

Dickenson, E. V., Snyder, S. A., Sedlak, D. L., and Drewes, J. E. (2011).Indicator compounds for assessment of wastewater effluent contributions to flow and water quality. *Water Res*., **45**, 1199-1212.

Focazio, M. J., Kolpin, D. W., Barnes, K. K., Furlong, E. T., Meyer, M. T., Zaugg, S. D., Barber, L. B., and Thurman, E. M. (2008). A national reconnaissance for pharmaceuticals and other organic wastewater contaminants in the United States – II) Untreated drinking water sources. *Sci. Tot. Environ*. **402**, 201-216.

Foreman, W.T., Gray, J.L., ReVello, R.C., Lindley, C.F., Losche, S.A., and Barber, L.B., (2012). Determination of steroid hormones and related compounds in filtered and unfiltered water by solid-phase-extraction, derivatization and gas chromatography with tandem mass spectrometry: U.S. Geological Survey Techniques and Methods, book 5, sec. B, chap. 9.

Hemming, J. M., Allen, H. J., Thuesen, K. A., Turner, P. K., Waller, W. T., Lazorchak, J. M., Lattier, D., Chow, M., Denslow, N., and Venables, B. (2004). Temporal and spatial variability in the estrogenicity of a municipal wastewater effluent. *Ecotoxicol. Environ. Saf.* **57**, 303–310.

Huggett, D. B., Foran, C. M., Brooks, B. W., Weston, J., Peterson, B., Marsh, K. E., La Point, T. W., and Schlenk, D. (2003). Comparison of in vitro and in vivo bioassays for estrogenicity in effluent from North American municipal wastewater facilities. *Toxicol. Sci.* **72**, 77–83.

Jobling, S., Nolan, M., Tyler, C. R., Brighty, G. and Sumpter, J. P. (1998). Widespread sexual disruption in wild fish. *Environ. Sci. Technol*. **32**, 2498-2506.

Johnson, A. C. and Sumpter, J. P. (2001). Removal of endocrine-disrupting chemicals in activated sludge treatment works. *Environ. Sci. Technol*. **35**, 4697-4703.

Kadlec, R. H. (2009) Wetlands for Contaminant and Wastewater Treatment, in The Wetlands Handbook (eds E. Maltby and T. Barker), Wiley-Blackwell, Oxford, UK.

Keefe, S. H., Barber, L. B., Runkel, R. L., Ryan, J. N., McKnight, D. M., and Wass, R. D. (2004a). Conservative and reactive solute transport in constructed wetlands. *Water Res. Res*. **40**, W01201, 12 p.

Keefe, S. H., Barber, L. B., Runkel, R. L., and Ryan, J. N. (2004). Fate of volatile organic compounds in constructed wastewater treatment wetlands. *Environ. Sci. Technol*. **38**, 2209-2216.

Keefe, S. H., Thullen, J. S., Runkel, R. L., Wass, R. D., Stiles, E. A., and Barber, L. B. (2010). Influence of hummocks and emergent vegetation on hydraulic performance in a surface-flow wastewater-treatment wetland. *Water Res. Res*. **46**, W11518, *doi:10.1029/2010WR009512.*

Kolpin, D. W., Furlong, E. T., Meyer, M. T., Thurman, E. M., Zaugg, S. D., Barber, L. B. and Buxton, H. T. (2002). Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000 - A national reconnaissance. *Environ. Sci. Technol*. **36**, 1202-1211.

Martinovic, D., Denny, J. S., Schmieder, P. K., Ankley, G. T., and Sorensen, P. W. (2008). Temporal variation in the estrogenicity of a sewage treatment plant effluent and its biological significance. *Environ. Sci. Technol.* **42**, 3421–3427.

Mitsch, W.J. and J.G. Gosselink. 2000.Wetlands, 3rd Ed. John Wiley & Sons, New York. 920 pp.

McGee, M. R., Julius, M. L., Vajda, A. M., Norris, D. O., Barber, L. B., and Schoenfuss, H. L. (2009). Predator avoidance performance of larval fathead minnows (*Pimephales promelas)* following short-term exposure to estrogen mixtures. *Aquat. Toxicol*. **91**, 355-361.

National Research Council. (2012). *Water Reuse: Expanding the Nation's Water Supply Through Reuse of Municipal Wastewater*. National Academy Press, Washington, DC.

Painter, M. M., Buerkley, M. A., Julius, M. L., Vajda, A. M., Norris, D. O., Barber, L. B., Furlong, E. T., Schultz, M. M., and Schoenfuss, H. L. (2009). Antidepressants at environmentally relevant concentrations affect predator avoidance behavior of larval fathead minnows *(Pimephales promelas*). *Environ. Toxicol. Chem*. **28**, 2677-2684.

Pawlowski, S., Ternes, T., Bonerz, M., Kluczka, T., van der Burg, B., Nau, H., Erdinger, L., and Braunbeck, T. (2003). Combined in situ and in vitro assessment of the estrogenic activity of sewage and surface water samples. *Toxicol. Sci.* **75**, 57–65.

Rodgers-Gray, T. P., Jobling, S., Morris, S., Kelly, C., Kirby, S., Janbakhsh, A., Harries, J. E., Waldock, M. J., Sumpter, J. P., and Tyler, C. R. (2000). Long-term temporal changes in the estrogenic composition of treated sewage effluent and its biological effects on fish. *Environ. Sci. Technol.* **34**, 1521–1528.

Rostad, C. E., Martin, B. S., Barber, L. B., Leenheer, J. A., and Daniel, S.R. (2000). Effect of a constructed wetland on disinfection by-products - Removal processes and production of precursors. *Environ. Sci. Technol*. **34**, 2703-2710.

Sapozhnikova, Y., McElroy, A., Snyder, S., and Schlenk, D. (2005). Estrogenic activity measurement in wastewater using *in vitro* and *in vivo* methods. *In*: GK Ostrander (Ed.) Techniques in Aquatic Toxicology, Lewis Publishers/CRC Press, Boca Raton FL. pp. 465-476.

Sartoris, J. J., Thullen, J. S., Barber, L. B., and Salas, D. E. (2000). Investigation of nitrogen transformations in a southern California constructed wastewater treatment wetland. *Ecol. Engin*. **14**, 49-65.

Schlenk, D. (2008). Are steroids really the cause of fish feminization? A mini-review of *in vitro* and *in vivo* guided TIEs. *Marine Poll. Bull.* **57**, 250-254.

Schoenfuss, H. L., Bartell, S. E., Bistodeau, T. B., Cediel, R. A., Grove, K. J., Zintek, L., Lee, K. E. and Barber, L. B. (2008). Impairment of the reproductive potential of male fathead minnows by environmentally relevant exposures to 4-nonylphenol. *Aquat. Toxicol*. **86**, 91-98.

Schultz, M. M., Furlong, E. T., Kolpin, D. W., Werner, S. L., Schoenfuss, H. L., Barber, L. B., Blazer, V. S., Norris, D. O., and Vajda, A. M. (2010). Antidepressant pharmaceuticals in two

U.S. effluent impacted streams: Occurrence and fate in water and sediment, and selective uptake in fish neural tissue. *Environ. Sci. Technol*. **44**, 1918-1925.

Snyder, S. A., Villeneuve, D. L., Snyder, E. M., Giesy, J. P. (2001). Identification and quantification of estrogen receptor agonists in wastewater effluents. *Environ. Sci. Technol.* **35**, 3620–3625.

Sumpter, J. P. and Johnson, A. C. (2005). Lessons from endocrine disruption and their application to other issues concerning trace organics in the aquatic environment. *Environ. Sci. Technol*. **39**, 4321-4332.

Tanner, C. C. (2001). Plants as ecosystem engineers in subsurface-flow treatment wetlands. *Water Sci. Technol.* **44**(11-12), 9-17.

Texas Water Development Board. (2011). Water reuse research agenda. Alan Plummer Assoc., Inc., 47 p.

Thorpe, K. L., Cummings, R. I., Hutchinson, T. H., Scholze, M., Brighty, G., Sumpter, J. P., and Tyler, C. R. (2003). Relative potencies and combination effects of steroidal estrogens in fish. *Environ. Sci. Technol.* **37**, 1142–1149.

U.S. Environmental Protection Agency. (2005). Aquatic life ambient water quality criteria - Nonylphenol FINAL. *U.S. Environ. Protect. Agency 822-R-05-005*; U.S. Environ. Protect. Agency: Washington, DC.

Vajda, A. M., Barber, L. B., Gray, J. L., Lopez, E. M., Woodling, J. D. and Norris, D. O. (2008), Reproductive disruption in fish downstream of an estrogenic wastewater effluent. *Environ. Sci. Technol*. **42**, 3407-3414.

Vajda, A. M., Barber, L. B., Gray, J. L., Lopez, E. M., Bolden, A. M., Schoenfuss, H. L. and Norris, D. O. (2011). Demasculinization of male fish by wastewater treatment plant effluent. *Aquat. Toxicol*. **103**, 213-221.

Wehmas, L. C., Cavallin, J. E., Durhan, E. J., Kahl, M. D., Martinovic, D., Mayasich, J., Tuominen, T., Villeneuve, D. L., and Ankley, G. T. (2011) Screening complex effluents for estrogenic activity with the T47D-KBluc cell bioassay: assay optimization and comparison with in vivo responses in fish. *Environ. Toxicol. Chem.* **30**, 439-445.

Writer, J. H., Barber, L. B., Ryan, J. N. and Bradley, P. M. (2011). Biodegradation and attenuation of steroidal hormones and alkylphenols by stream biofilms and sediments. *Environ. Sci. Technol*. **45**, 4370-4376.

Writer, J. H., Ryan, J. N., and Barber, L. B. (2011). Role of biofilms in sorptive removal of steroidal hormones and 4-nonylphenol compounds from streams. *Environ. Sci. Technol*. **45**, 7272-7283.

Writer, J. H., Ryan, J. N., Keefe, S. H. and Barber, L. B. (2012), Fate of 4-nonylphenol and 17β-estradiol in the Redwood River of Minnesota. *Environ. Sci. Technol*. **46**, 860-868.

Xie, L., Sapozhnikova, Y., Bawardi, O., and Schlenk, S. (2005),Evaluation of wetland and tertiary wastewater treatments for estrogenicity using *in vivo* and *in vitro* assays. *Archiv. Environ. Contam. Toxicol.* **48**, 82-87.

Ying, G.-G. and Kookana, R. S. (2005), Sorption and degradation of estrogen-like-endocrine disrupting chemicals. *Environ. Toxicol. Chem*. **24**, 2640-2645.

Peer Review Comments and Responses

Documents Reviewed:

1. Wetland Site Design (Version: June 11th, 2013)
2. Demonstration Project Alternative Analysis
3. Hydrological, Chemical and Biological Monitoring Plan

Peer Review Schedule:

- Initial Reviewer Comments - Completed August 9th, 2013
- Distribution of Reviewer Comments to the Project Team August 12th, 2013
- Technical Team Comment Responses Complete by August 26th, 2013
- Technical Team Meeting to Discuss Comment Responses August 29th, 2013
- Responses and Updated Monitoring Plan Provided at Site Meeting Jan. 9th, 2014

Peer Reviewers:

- Nat Beal, PG
  Senior Hydrogeologist
  AQUI-VER, Inc.
- Eric Dickenson, PhD
  Project Manager, Research Development
  Southern Nevada Water Authority
- Nathan Kuhn
  Austin Team Leader
  Ecosystems Resources Program
  Texas Parks and Wildlife Department
- Scott O'Meara
  Botanist
  Environmental Applications and Research Group
  Bureau of Reclamation
- Loretta Mokry, PWS
  Environmental Scientist
  Alan Plummer Associates, Inc.

Peer Review Comments and Responses on the Wetland Site Design

General comments:

1. It is unclear how the wetland is to be constructed and inundated. I think a brief description of the wetland characteristics would be beneficial.
   We agree. Additional information on the wetland design, construction, and operation has been documented in the "Brazos River Demonstration Wetlands Design Considerations" to accompany the final design drawings.

   a. Is the wetland being constructed on native or fill soils or is the wetland to be lined with a hydraulically impermeable barrier.
   Yes. Basin 2 will be located in the footprint of an existing clay borrow area, with ample material still remaining. This basin will be excavated in to the clay borrow to shape the hummock plant beds. The clay material from the basin 2 excavation will be used as the 12" clay layer at the bottom of Basin 1 and the HSSF wetland cells. Surplus material will be used with site excavation to build the compacted embankments to contain parts of Basin 1 and the HSSF cells. The wetland is designed with a compacted clay liner with specified hydraulic conductivity < 1.0 x 10^{-7} cm/s, which will be achieve using the onsite clay material. See general comment 1.

   b. Does wetland inundation depend on water being pumped to the wetland and if so, what happens if the pump temporary ceases operation (e.g., does the wetland go dry)?
   Yes. The wetland will rely on pumps to deliver water. The revised wetland design bounds Basin A and Basin B with hydraulic control boxes and adjustable cipolletti weirs to maintain water storage in the main basins in the event of pump failure. The HSSF basins are bounded with fixed hydraulic controls to maintain saturation upon feed water interruption. The stream channel would run dry in the event of receiving water interruption, but is designed without vegetative aspects, so lack of inundation would not affect this design feature. See general comment 1.

   c. Has the wetland capacity been sized appropriately to handle short-term stormwater inundation and what would result if the wetland capacity was grossly exceeded during a large scale storm such as a 24 hour 100 year storm event (e.g., would instrumentation and vegetation be at risk of damage, is there risk of cross-contamination into other water bodies, etc)?
   Yes. We looked extensively at the effects of the water budget over a 60 year time period that was available. At the 90 percentile for precipitation and average evaporation, the increase in outflow (Q_{out}/Q_{in}) was 1%. For extreme flooding events two spillways are provided in revised design to direct overtopping water from Basin 1 towards the existing depression to the North. The depression is large enough to offer substantial retention and recharge time. The spillways will be located at the left and right ends of the HSSF cell array. The HSSF cells themselves will allow overtopping into the designed cascades to the stream channel. Basin 2 is an excavated cell in an

existing depression. Although a complete fill of this basin is unlikely, provisions will be made in the final design to pipe excess water to the depression to the north. See general comment 1.

2. In looking at the big picture, my interest here would be more focused on tweaking the design: Species composition, layout, planting density, etc. in order to amplify treatment effects and then examine associated costs. This is mentioned only briefly in the document, personally I would put more emphasis on this and less elsewhere (such as mechanisms) in Tier II.

   Plant specifications and planting plan are being developed and will be completed once the final design is completed. Many tweaks have been incorporated into the final design and work plan.

Specific Comments:

1. For open water cell A, I think the June 11, 2013 is a much better design than the one dated August 29, 2012. However, one concern is that the elevations indicate 18 inches of drop across the cell. With the reduction in cell width shown at the outlet end of the cell, water depths in the lowest portion of the cell, which is indicated to be vegetated with emergent vegetation, will be too deep to enable sustained growth of wetland plants; even the giant bulrush that Joan Daniels indicated would be used. We have experienced this situation at both the Richland-Chambers Wetland and the East Fork Wetland despite what hydraulic models have predicted. Current design for the full-scale build-out underway at Richland-Chambers Wetland includes flat-bottomed cells and cells with elevation drop limited to 6- inches. I would recommend that the elevation drop across the cell be minimal and open water areas be achieved through excavation of deeper areas within the bottom.

   For Cell A (now titled Basin One) we included a gradual slope with the shallowest 6-inch depth nearest the outfall, tapering down to the eventual depth of 6-foot to maintain some open water. Once the bulrush is established in the shallower areas, I expect it to fill into deeper depths up to 2½ to 3-foot via rhizome lateral growth, but since I have not used the local ecotype of the giant bulrush before we will need to adjust the heights of all of the planting beds.

2. Related to comment #1, I think it is very important to have a dense band of emergent vegetation immediately upstream of the outfall of open water cell A prior to the subsurface flow cells B to provide filtration of phytoplankton produced in the open water areas of cell A in order to reduce potential clogging of the media in the subsurface flow cells B.

   We agree, our current plan includes a dense band of emergent vegetation immediately upstream of Basin One's outfall with the exception of a small deep area to prevent the bulrush from overtaking the outlet structure. The water from Basin One, which will contain low levels of both DO and particulates, will go directly into the HSSF cells.

3. Elevations shown for top of media and bottom of media for the subsurface flow cells B indicate the proposed media depth is to be 3 foot deep. The wetland plants proposed for the subsurface flow cells B (giant bulrush and hardstem bulrush) will have most of their root mass within a 2-feet depth, providing opportunity for flows under the roots that will not have substantial contact with the living plant biomass. I would recommend limiting the media depth to two (2) feet.
   We agree that a 2-foot depth is more appropriate based on the anticipated root depth, however the 3-foot depth in our system is a compromise of treatment intensity as a true anaerobic condition for reductive dehalogenation rather than rhizosphere aerobic processes. A 3-foot depth would allow us to maximize the fraction of the wetland total flow that can be treated by this process.

4. I did not see information re: the materials proposed for construction of the turbulent stream channel; however, in order to enable extrapolation of the research results to natural and large-scale constructed systems, I would recommend using natural materials as much as possible rather than concrete (as is indicated for the outlet channels of the subsurface flow cells B). An earthen channel with riffle zones constructed of rocks, boulders, or large woody debris would be more comparable with natural stream systems in Texas.
   We agree, but were originally considering concrete to minimize maintenance. Using natural material is also more desirable from an economic stand point. The calculations for the turbulent stream channel were completed using a range of Manning's roughness coefficient. The results indicated that optimal volatilization was controlled more by bottom width and channel slope. In our design meetings, the consensus of the group was that the materials selected for the channel would most likely be a function of construction costs. We plan to line the channel with polyliner to eliminate seepage losses and strategically place stones to create the turbulence necessary for reaeration.

5. The design of the hummock/habitat cell D will provide a substantial preferential flow path along the north edge of the cells resulting in minimal effectiveness of the marsh zones shown in the design and potential dead water areas. If the idea is to create a hemi-marsh design with about 50% emergent marsh and 50% open water, I would recommend extending the marsh zones in a narrow, contiguous band across the width of the cell to minimize preferential flow paths. The open water areas can be created through excavation of deeper areas (3-4 feet deep) across the width of the cell in between the marsh zones. As shown in the proposed June 11, 2013 design, a contiguous band of marsh zone should be created above the outflow.
   We agree. The final design has rearranged the hummocks to prevent preferential flow pathways. Our paper regarding the contiguous bands verses hummocks, "Keefe, Steffanie H., Joan S. (Thullen) Daniels, Robert L. Runkel, Roland D. Wass, Eric A. Stiles, and Larry B. Barber. 2010. Influence of hummocks and emergent vegetation on hydraulic performance in a surface flow wastewater treatment wetland. Water Resources Research,

46, W11518, doi:10.1029/2010WR009512, 13 pp." discusses our decision for cell D to use hummocks to encourage mixing and longer HRT in order to enhance treatment.

6. Within the open water areas of both open water cell A and the hummock/habitat cell D, I would recommend the establishment of submerged aquatic vegetation (e.g., coontail (Ceratophyllum demersum), American pondweed (Potamogeton nodosus), wild celery (Vallisneria americana), or water stargrass (Heteranthera dubia)). The use of submerged aquatic vegetation has been demonstrated to improve removal of nutrients, especially phosphorus, and its effectiveness in removal of organic contaminants such as endocrine disrupting chemicals should also be evaluated.
   This is a good suggestion. We would love to evaluate SAVs' effectiveness in removing EDCs. In this design the open water areas are designed as photoreactors and this function would be impaired if we decrease any light penetration. In the vegetative areas we find it difficult to establish submerged aquatics, if the reviewer has proven methods for doing this, we would welcome any suggestions.

Peer Review Comments and Responses on the Demonstration Project Alternatives Analysis

General comments:

No general comments were made on this document.

Specific Comments:

1. P. 16. The description of the preferred alternative states that the wetland's discharge location will be pumped to the Brazos River or back to the treatment plant. Construction of an outfall structure in the Brazos River may require additional coordination with TPWD to determine direct and indirect (downstream) impacts. Depending on the proposed outfall design and location, TPWD permits may be required.
   This comment is well noted. If the wetland design proves successful proper permitting and monitoring will be acquired to support discharge into the Brazos River. This is a critical issue with respect to implementing this technology in water reuse planning.

Peer Review Comments and Responses on the Hydrological, Chemical, and Biological Monitoring Plan

General comments:

1. Overall, I thought the proposed Tier I Monitoring Plan was comprehensive and effective.

2. Overall, we believe that the project is significant and the monitoring plan is well thought out.

3. The sampling plan is well written, well thought out, and thorough.

4. I might say that the proposed sampling might be a bit excessive to get at the main goals. I understand this may be the "Cadillac" plan and it will likely be modified as the study continues, so I would not suggest changes at this stage.

5. The study is a worthwhile endeavor, in which is uses a U.S. wetland with diverse engineered cells in series for the treatment of EDCs. The Project Team has the capability, tools, expertise and experience to successfully complete this project.

6. I think the monitoring plan would benefit from a brief discussion on the geographic, hydrologic and physiographic setting. It is unclear where the wetland is located in relation to the Brazos River, what the soil types and geology are underlying the wetland site, and the depth to groundwater in relation to the wetland site. Obviously if the wetland is lined then interaction with groundwater can be ignored.
Additional context information on the site is now included in the introduction of the revised monitoring plan to describe characteristics, such as geographic, hydrologic, and physiographic setting.

7. The focus of the project is the efficacy of the overall wetland design and the individual wetland components in reducing endocrine disrupting compounds. However, the wetlands themselves will be an ecosystem that will include fish, benthic macroinvertebrates, amphibians, insects and plants, and will likely serve as habitat for birds and mammals. While the function of the system is to attenuate EDCs, it will also likely attenuate metals and other chemicals present in the wastewater, and it follows that any chemicals that bioaccumulate will likely concentrate in the wetland sediments, plants and animals. Care must be taken to avoid inadvertently concentrating chemicals that will be harmful to wildlife, as occurred at the Kesterson Reservoir in California.
The wetland is receiving effluent already being discharged to the Brazos River where all of the processes occur during de facto reuse as in-stream flow. Some of the issues raised above are important questions though and this is why we need to undertake a thorough investigation of the demonstration wetland. In addition to evaluating whether we can remove/attenuate emerging contaminates from municipal wastewater, we want to make sure we are also creating quality habitat for the native species that will use the wetland. This is an area that will require additional partners to assess potential impacts on wildlife.

8. The monitoring document does not mention preparation of a Quality Assurance Project Plan (QAPP). While this is not an EPA-funded project, it seems likely that data from the project will be used to make decisions related to how public monies are used to fund wastewater treatment. As such, it seems imperative that a QAPP be prepared and reviewed by an appropriate, experienced authority prior to any monitoring being conducted.

We agree that developing a QAPP and having it reviewed would provide a great opportunity to have a third-party provide comments and insight into making sure this project produces high-quality data. We will look into whether the City of Waco or Baylor University has an existing QAPP, since daily monitoring will be completed by them. We will work to pull funds to accomplish an appropriate version of a QAPP for this project.

9. It can take several years (decades or more in most cases) before restored or created wetlands reach close to comparable functionality with natural ones, particularly for biogeochemical functions, which will be critical for this system to operate. So, three years may not be sufficient time to see maximum functionality from these treatment wetlands.

   We agree a monitoring period of greater than three years would produce insight into how the wetland's treatment performance changes over time. For this project however the monitoring plan is designed to capture the highly dynamic initial start-up conditions (first three years), and to establish the infrastructure and baseline data for longer-term (multi decade) monitoring of performance required to optimize the operation and maintenance of the wetland.

Specific Comments:

1. Hydraulics Comments

1.1. The proposed hydraulic tracer testing is imperative. Adaptive management should be included within the plan to address detention times that are determined via the tracer testing to be substantially less than the designed nominal retention times of the different cells.

We agree. The tracer testing results as well as water quality criteria should be used to develop an adaptive management plan. We will need to discuss this with all of the project partners to determine how we can development an adaptive management plan for this project.

1.2. The water flow data collected at the hydraulic control boxes and the precipitation and evaporation data collected at the weather station should enable calculation of water balances across each of the compartments (open water cell A, subsurface flow cell B, turbulent stream flow cell C, and hummock/habitat cell D).

We agree. Water balance calculations are a critical component of treatment wetland operations. It is imperative that wetland operators know how much water is leaving the wetland through the wetland outlet, groundwater seepage, and evapotranspiration losses.

1.3. To facilitate monitoring water levels, staff gauges should be employed and staged within the cells where they can be readily observed (or vegetative around them kept trimmed where they can be observed). After staff gauges are set, water depths should be measured manually and calibrated with readings on the staff gauges. Adjustments to staff gauge settings should be made, as needed.

We agree and if possible to include staff gauges in the construction budget as an addition to the basins. Depth measurements will also be recorded in a variety of areas during sampling events using pressure transducers.

1.4. Regarding the weather data to be collected from the Waco Weather Station, located 5.5 miles southwest of the site, the daily temperatures, and wind intensity and direction from the weather station should be adequate. However, I would strongly recommend also gathering precipitation data from onsite due to the extreme variability of precipitation in Texas. Based on experience at various sites across Texas, variation of more than an inch of rain is common for locations within a mile of one another.
Although the Sutrons would account for any significant hydraulic inputs to the system, we agree that it would be ideal to have a weather station onsite. We plan to work with Baylor and Waco to determine how a weather station onsite could be achieved.

1.5. Is the wetland located at a low or high point elevation in the watershed and how does the location of the wetland relative to the watershed relate to potential risks associated with stormwater effects on wetland operation and hydraulic control?
Basin 1 and the HSSF are located on an existing high point and will not be affected by the surrounding area's runoff. The site design was used to maximize elevation for gravity operation and discharge and to keep the entire system above the Brazos River maximum flood level. Direct rainfall could contribute to increased flows throughout the wetland, but the impact could be reduced by controlling the inflows as needed to maintain normalized flows. Operations will be discussed extensively with all partners to determine the best operating practices during these events. Also see response to general comment 1 and 1.c. for extreme flooding events.

1.6. If the wetland is free to hydraulically communicate with shallow groundwater then I think groundwater monitoring should be considered via a network of piezometers.
The lowest point on the site, basin 2, is well above the highest well levels recorded in the vicinity. Also see response to comment 1.a., the wetland is designed with a compacted clay liner, so interactions with the shallow groundwater should be minimal. The inflow water is currently permitted for land application in Waco, but if significant water loss occurs from the constructed site groundwater monitoring will be considered to track water movement.

Furthermore:

a. What is the depth to shallow groundwater?
The Texas Water Development Board's Water Information Integration and Dissemination, Groundwater Database provides historical and existing well log data. Using the Groundwater Database, 15 surrounding wells were used to determine the minimum and maximum water level to be 13.2-ft and 34.8-ft respectively below surface elevations. The closest groundwater well recorded in the database to the site has a surface water elevation of 366.4-ft above sea level and a ground elevation of 383-ft. The existing site elevation would be excavated as much as 10-ft to 372-ft

ground elevation for the wetland, and this should allow several feet before any groundwater interaction after construction is complete.

b. What is the local hydrostratigraphy and how will wetland inundation affect the groundwater system (e.g. are there low permeable layers that may result in perched groundwater)?
As previously noted the wetland is designed with a compacted clay liner, so interactions with the shallow groundwater should be minimal. Significant surface-groundwater interaction is therefore not expected beyond natural drainage in the area, so perched groundwater is unlikely to occur.

c. What affect will the wetland have on the local groundwater system and what hydrologic impacts, if any, are expected from wetland inundation (e.g., local increase in the groundwater table resulting in flooding in undesirable areas).
Please see previous comment 6.a. In summary, the amount of seepage from the wetland is expected to be insufficient to cause any appreciable increase in the water table and no hydrological impacts are expected.

d. If groundwater provides a component of base flow to the wetland, how will recharge and discharge to the groundwater system affect wetland hydraulics and water chemistry?
Please see previous comment 6.a. In summary, groundwater is not expected to contribute significantly to base flow due to the depth to groundwater and the compacted clay liner.

e. Is there a potential that EDCs may be released to shallow groundwater and if so, has contaminant fate and transport in groundwater been considered?
Please see previous comment 6.a. In summary, groundwater is not expected to contribute significantly to base flow due to the depth to groundwater and the compacted clay liner. This water is currently permitted for land application in the city of Waco, but if significant interaction occurs monitoring of EDCs in the groundwater may be a necessary component of a groundwater monitoring plan. The introduction of EDCs to groundwater would depend upon their presence in the influent to the wetland and the amount of EDC removal/transformation that occurred within the wetland prior to the location within the wetland where seepage is occurring. While the direction of groundwater flow at the site has not been measured, it is assumed groundwater is flowing in an easterly direction, towards the Brazos River, roughly 1800-ft from the site. Numerous unknown factors would affect the fate of any EDCs released, but it is expected that groundwater would transport the EDCs to the Brazos River if the EDCs are not naturally attenuate or retarded in the sub-surface before reaching the river.

f. If the wetland is in hydraulic communication with the groundwater then I feel that groundwater should be a component of the monitoring plan. For example, perimeter piezometers could be installed to monitor the effects of wetland inundation on groundwater levels and to monitor any releases of EDCs from the influent.

The potential for communication is minimal, but if significant water loss occurs from the constructed site then groundwater monitoring will be considered to track water movement and potentially water quality throughout the study. The use of perimeter piezometers would be an effective installation to measure groundwater interactions with the wetland. While the amount of seepage is expected to be small, a better understanding of the volume of seepage will be known from tracer tests, inflow and outflow volumes, and meteorological data recorded following completion of construction. There are no groundwater users between the wetland and the Brazos River, approximately 1800-ft from the wetland. If seepage is found to be significant, the installation of a groundwater monitoring well system would provide an opportunity to perform additional, valuable research into the subsurface fate of EDCs as a part of this project.

g. Also, if groundwater fate and transport is considered to be a potential issue then a sentry well or wells should be placed downgradient to evaluate downgradient transport of EDCs and other chemicals of concern in the groundwater aquifer(s).

Operation and monitoring of the site may be expanded in the future to include additional equipment and monitoring. The monitoring of groundwater on-site and along groundwater hydraulic gradients in the area will be considered as additional scope. Details of the potential for groundwater interaction and subsequent monitoring were added to the monitoring plan.

1.7. What are the range of acceptable hydraulic retention times (HRTs) and the related water depths and flow rates. What is the maximum amount of time the wetland could theoretically operate outside of the acceptable HRT range before being discovered through routine monitoring and what affect might this have on the water quality related to the hydraulic loading rates (HLR) and treatment effectiveness?

Our target HRT value was 7-days with a fixed flow rate of 1.5-cfs during the design process. Our target depths are 6-ft in open zones and 1-ft in vegetative zones. At other wetland sites, the treatment operators have incorporated the water quality collection at the outlet into their routine wastewater treatment plant monitoring (i.e. daily or weekly). If this approach is used, the time to discover a water quality concern would be equivalent to the time required to discover a concern with the wastewater treatment plant. These systems degrade at a slower pace, so most likely we would see trends in the routine water quality data leading to a change in operation.

2. Vegetation Comments

2.1. Assessment of vegetative cover from ground level measurements can be very problematic. Various methods can be used to obtain aerial photography of the cells, which I agree is much more accurate at measuring large wetland-scale vegetation growth patterns. Remote-controlled helicopters with mounted cameras can be effective, but are a bit tricky to operate. We have used aerial photography obtained from helicopter flights

for monitoring vegetative cover for large-scale wetland cells. Support for this might be obtained from local police department, county sheriff department, or regional water district, if they have helicopters for their operations. I would recommend aerial photographs at least 4 times per year to document changes seasonally.
Thanks for the recommendations, we will explore all of these opportunities.

2.2. Need to ensure that sampling methods for the vegetative biomass provides representation of all types of plant materials (e.g., floating species such as duckweed, submerged species, and emergent species).
We agree.

2.3. Need to identify what plant species are considered invasive, undesirable species prior to planting and starting monitoring.
We agree.

2.4. P. 9. B.2.5. The entire plant biomass will be removed from the quadrats, dried and weighed. There is no indication that algae will be separately removed from the quadrat or scraped from the leaves. Would it be helpful to distinguish between vascular plant above- and below-ground biomass and algae biomass?
It has been my experience that emergent vegetation growing in dense conditions does not have enough algae growing on it to be able to scrape it from the leaves (because the algae is light deprived). Algae growing along with, or on, submerged vegetation is a different story. If the algae can be removed from the vegetation, it could be analyzed separately as an aspect of the Tier II Monitoring.

2.5. P. 10. B.2.7. Will it be necessary to ground-truth aerial imagery, or will the imagery be assumed to correspond to the "as-planted" plan?
Yes, all aerial imagery will be geo-referenced and ground-truthed. This methodology has been added to the monitoring plan. One of the other reviewers provided some resources we investigated to take aerial photographs that we looked into and added to the monitoring plan in section B.

2.6. Plant detritus is an important component of the treatment capability of wetland cells. The litter layer developed provides both surface area and carbon substrate to support the microbial community which is very involved in the treatment process. Removal of detrital material should be limited to very infrequent events and only as needed to support the robust growth of the emergent plants.
We agree.

3. Macroinvertebrates and Aquatic Species Comments

3.1. P. 11. C.2. The 5 grams of macroinvertebrates in a bag, even cooled, will likely predate on each other if kept alive during transport. It would be advisable to check with the lab doing the analysis for suggestions on how best to ship these samples.

True. At other sites, we use ethanol to preserve the samples before they are identified. If they are to be analyzed for metal (or other) uptake, they are sorted in the field (all plant and soil matter removed) and then frozen. These specific procedures have been updated in section C of the monitoring plan.

3.2. P. 11. C.3. Macroinvertebrate communities have been shown to require several months to recover following drought. See http://www.sgmeet.com/sfs/sfs2012/viewabstract2.asp?AbstractID=7419. This suggests that, depending on the time required to establish full, consistent flows, it may be prudent to wait more than one month after the wetland becomes fully operational to take baseline samples, or that additional samples should be taken to demonstrate that the population is stable.
Good point. We will be having Baylor students collect the macroinvertebrate samples at the optimum times to track development once the wetland is established. These start-up considerations have been added to the monitoring plan in section C.

3.3. P. 11. C.3. One baseline sampling event does not seem adequate to characterize the macroinvertebrate community. If only one event can be scheduled, replicate samples should be obtained.
We agree. Replicate samples for all analyses have been noted in the monitoring plan, specifically at start-up.

3.4. Biannual sampling of macroinvertebrates may not be fully representative of the diversity of the macroinvertebrate community due to the substantial seasonality of invertebrate populations. I would recommend increasing the sampling of the macroinvertebrate community to at least quarterly to better document seasonal variations.
We agree and will change to quarterly sampling if funding permits.

3.5. P. 15. F.2. Will caged fathead minnows be able to forage among the plants and sediments for food? They are opportunistic feeders and will eat just about anything that they come across, such as algae, protozoa (like ameba), plant matter, insects (adults and larvae), rotifers, and copepods. The proposed design of comparing paired exposures of caged animals will provide good controls between seasons, but may not replicate a natural environment.
This is a good observation and relates to the scope of the current research plan. Currently, the intent is not to "replicate a natural environment." Rather, caged organisms are used as biosensors and thus their ability to freely forage will be limited. They will largely graze on periphytic growth on cages and opportunistically on invertebrates

3.6. P. 18. H.2.

a. With potential introduction of mosquito fish to control mosquito larvae, TPWD should be coordinated with on this project. A TPWD-issued freshwater stocking permit may be necessary.
If this is deemed necessary, then a TPWD-issued freshwater stocking permit will be applied for. The preference is that no fish are stocked initially in order to limit the variables in determining whether this design is effective. Once research is complete and there is a need or desire to introduce other species into the system, the City of Waco and project partners will coordinate with TPWD.

b. A plan for handling freshwater mussels should be considered in case they are found during debris removal or sediment sampling. Also, if found, the mussel meat should be tested for EDC's, etc. Please be aware of freshwater mussels during debris removal and construction of any outfall structures into perennial waters. The state-listed threatened Smooth pimpleback (Quadrula houstonensis) and Texas fawnsfoot (Truncilla macrodon) freshwater mussels have been documented in the Brazos River upstream of the WWTP near the Loop 6 bridge. Activities that may adversely impact state-listed threatened species, including the construction of outfall structures near mussel beds in the Brazos River, should be avoided. Please contact TPWD if mussels are encountered.
Thank you very much for this information. We were not aware of the state-listed mussels in that watershed. We will contact TPWD if we encounter any mussels

4. Soil and Sediment Comments

4.1. P. 12. D.1. and D.2. Baseline and biannual sediment contaminants samples should be obtained.
We agree that we need baseline and at least annual sediment sampling, see Table 1 of the monitoring plan.

4.2. P. 12. D.3. One baseline sampling event does not seem adequate to characterize the sediments. If only one event can be scheduled, replicate samples should be obtained.
We agree and will work with the monitoring budget to allow for this.

4.3. Should soils be described in accordance with standard methodologies to determine at what point the wetland soils meet the definition for a jurisdictional wetland? This can be an important component of proper wetland functionality.
We agree. Although monitoring the soils to see how quickly they transform to meet the definition of a jurisdictional wetland is not critical to the project, it would add an additional interesting and scientifically relevant layer of research to the work being performed. This would be an interesting consideration for the next phase of research.

5. Endocrine Disrupting Compound Monitoring Comments

5.1. What is the effect of the EDCs and other chemicals on wetland biota?

A better understanding of this complex issue is a primary objective of this project. Additional funding is required to explore aspects of this research area beyond bulk water quality measurements, but these aspects are critical parts of the next phases of wetland monitoring and will be included as a Tier II Monitoring recommendation.

5.2. What is the effect of the EDCs and other chemicals on wildlife that may utilize the wetland?
Please see response 1.

5.3. Sediments and soils are likely to sorb and concentrate EDCs, however, the monitoring plan does not appear to monitor any soil contaminants. While the routine monitored parameters will likely help interpret information about plant health, they seem unlikely to provide information about buildup of EDCs and other contaminants in the sediments. We recommend that in addition to routine parameters, sediments be monitored biannually for EDCs. This could provide some information about the environment of the benthic macroinvertebrate community, which is part of the food base for many fish. Attempts to understand fate and transport of EDCs may suffer from lack of this information.
Sorption of EDCs and other chemicals to the sediments is an important factor to consider (and will be) in order to obtain a mechanistic-based understanding of removal processes and to better predict potential adverse biological impacts. The scope of the monitoring plan is limited by funding availability to defining bulk removal through the various unit processes, with the primary question being whether final wetland effluent quality has been improved relative to the final WWTP effluent inflow. The team is currently pursuing additional funding in a number of research areas to expand the scope of the monitoring including the analysis of sediment EDCs that is detailed in the Tier II Monitoring Recommendations.

5.4. P. 11. C. Monitoring macroinvertebrate tissue for EDCs would greatly contribute to understanding the fate and transport of the compounds and how they impact biota.
We agree understanding bioconcentration of EDCs and other chemicals in macroinvertebrate tissue is an important factor to consider for organism exposure and potential adverse biological effects. Research proposals to acquire funding for Tier II monitoring include the collection and analysis of macroinvertebrate tissue for EDC analysis.

5.5. The most important issue that may prevent this project from being successful is assurance that the levels of the selected EDC indicators (Table 2) will be present and high enough to assess true wetland performance (i.e., 1 log removal). It is not clear if the EDCs in Table 2 is the list or as mentioned on page 6 EDCs will be identified initially? Occurrence data in the WW effluent was not presented in the proposal, therefore it is assumed it is not known? A recent study (Dickenson et al. 2011) examined various wastewater effluents (secondary and tertiary effluents) across the U.S. and deemed that the levels of 17alpha-estradiol, 17beta-estradiol, 17alpha-ethinylestradiol, and progesterone were not significant and would be inefficient to assess subsequent treatment performance. However, estrone proved to be a good potential indicator. If not done so already, this proposed EDC method should be applied to the Waco wastewater effluent to see if these compounds can be detected and at what levels respective to the reporting limits. The Project Team notes that they plan to do this (page 6), which needs to be done before the construction of the Wetland. If a majority of these compounds are proven not to be detected at significant levels, then other indicator PPCPs/EDCs should be included in the Tier I study and not the Tier II study.

Dickenson, E. V., Snyder, S. A., Sedlak, D. L., and Drewes, J. E. (2011).Indicator compounds for assessment of wastewater effluent contributions to flow and water quality. Water Res., 45, 1199-1212.

We agree. The need to characterize the effluent is the primary priority to updating the monitoring plan with appropriate indicators. We are familiar with the reference you have provided and appreciate your additional recommendations. If you are available, we will follow up and consult with you regarding the results of the initial effluent characterization.

5.6. Other indicator PPCPs are proposed to be performed during the Tier II study. But other treatment indicator PPCPs should be included to represent and isolate the differing proposed removal processes: photolysis, sorption, biodegradation and volatilization. The selected indicator EDCs (Table 2) are could be similar in structure and if so more compound structural/reactivity diversity should be represented to represent the fate of other potential EDC compounds. It is unclear when Tier II activities would begin? After the 3 years or during? It would be ideal if it could be during as this is a valuable opportunity to include indicator PPCPs and other EDCs into the matrix and generate informative performance information, or else the PPCP samples will not be collected until sometime later, which would be unfortunate.

Ideally additional measurements would be incorporated at the beginning of the study. This aspect is included as additional scope and will begin as soon as funding can be secured for this aspect of a Tier II study.

5.7. Note a fair amount of research (mainly from one research group in Europe) has been performed on this topic, but not necessarily on the EDCs in question. The literature needs to be scanned comprehensively and reviewed (include as a Task in the project). Below are some examples of relevant publications:

Matamoros V, García J, Bayona JM. Behavior of selected pharmaceuticals in subsurface flow constructed wetlands: a pilot-scale study. Environ Sci Technol. 2005 Jul 15;39(14):5449-54.

Matamoros V, Bayona JM. Elimination of pharmaceuticals and personal care products in subsurface flow constructed wetlands. Environ Sci Technol. 2006 Sep 15;40(18):5811-6.

Víctor Matamoros, Victòria Salvadó Evaluation of the seasonal performance of a water reclamation pond-constructed wetland system for removing emerging contaminants
Chemosphere, Volume 86, Issue 2, January 2012, Pages 111-117
Víctor Matamoros, Carlos A. Arias, Loc Xuan Nguyen, Victòria Salvadó, Hans Brix
Occurrence and behavior of emerging contaminants in surface water and a restored wetland Chemosphere, Volume 88, Issue 9, August 2012, Pages 1083-1089
Hijosa-Valsero M, Matamoros V, Martín-Villacorta J, Bécares E, Bayona JM. Assessment of full-scale natural systems for the removal of PPCPs from wastewater in small communities Water Research, Volume 44, Issue 5, March 2010, Pages 1429-1439
María Hijosa-Valsero, Víctor Matamoros, Ricardo Sidrach-Cardona, Javier Martín-Villacorta, Eloy Bécares, Josep M. Bayona Comprehensive assessment of the design configuration of constructed wetlands for the removal of pharmaceuticals and personal care products from urban wastewaters Water Research, Volume 44, Issue 12, June 2010, Pages 3669-3678

Thank you for the recommended studies. We are familiar with this literature and look forward to expanding on the results obtained in these studies and working at a larger scale site designed specifically to focus on these mechanisms in this study.

5.8. Hypotheses should be developed and formulated based on existing literature information and known chemical properties (i.e., structural activity relationships) for the project regarding the expected removal of the indicator EDCs for the multi-cell wetland. This will aid in the selection of appropriate indicator EDCs/PPCPs to assess performance removal by differing mechanisms.
We are developing a physiciochemically-based model for the removal mechanisms operating within each unit process to measure attenuation in the wetland design. We are creating a technical scientific report that will support the research at this site.

5.9. What is the design hydraulic retention time for the respective cells? This is likely known already and would be informative to know regarding expected design removal of indicator EDC as they could be biologically or photolytically mediated as a function of time.
The design hydraulic retention times for each treatment unit is listed in the table below.

Unit:	**HRT (days)**
Basin 1	2.26
Basin 2	3.74
Stream	0.04
HSSF	2.38
Total	**8.42**

5.10. What type of WWTP is the City of Waco, Texas? What is the wastewater effluent quality in regards to C/N/P nutrient levels? This should be known already and this will give some initial indication of the type of biological mechanisms (i.e., nitrification) that might be present in the system(s) that could affect performance.
The Waco WWTP is a conventional facility with secondary treatment including clarification, activated sludge, and anaerobic digestion. Treated water quality includes residual nutrients (nitrogen and phosphorous), which are targeted for removal in the wetland basins based on HLR and HRT. The briefing document that will supplemental the design specifications will include information on the effluent water quality provided by the city of Waco and design specifications targeting nutrient removal. The table below provides the average values of nutrients in the WWTP effluent in October of 2012.

Parameter	Units	C_i
NO_2-N	mg/L N	0.1±0.0
NO_3-N	mg/L N	7.7±0.7
TKN	mg/L N	1.8±2.3
TN=NO_2-N+NO_3-N+TKN	mg/L N	9.5±3.0
TP	mg/L P	1.7±1.5

5.11. I think only 7 temporal (biannual) EDC samples will be collected over the course of the 3 years. Considering this includes start-up, the data during start-up could differ to full performance data, and therefore, this could limit the number of full performance data (i.e., <7). To increase the statistical significance and quality of the full performance data the collection of indicator EDC needs to be increased to at least quarterly sampling.

We agree. The goal is to augment the minimum Tier I monitoring plan with additional dedicated studies related to this area for more frequent and diverse measurements.

5.12. The measurement of algae counts should be performed during the study to assess they are not a factor and if present do they correlate with indicator EDC level trends.
This is a good suggestion. We have included this as an additional area of emphasis for Tier II to accompany and improve the EDC monitoring.

5.13. To assess the fate of sorption, which is likely important for a majority of these proposed indicator EDCs (due to their relatively higher log KOW), measuring the EDC levels on solid matrices (i.e., soil, vegetation) would be informative in regards to understanding their fate. However, this is probably out of the scope of Tier I monitoring, but could be included in Tier II monitoring.
We agree and will be reassessing the Tier II monitoring scope.

5.14. Grab samples (synoptically?) are proposed. EDC levels in the wetland influent could be variable (this could be assessed before the wetland is operational) due to carry over of

diurnal or weekly patterns present in raw wastewater inputs into the WWTP. If this is the case, composite sampling should be attempted.
This is a good suggestion and will be considered for biannual monitoring as well as perhaps as a comparison for the initial water quality characterization that will be performed in the next few months.

6. Routine Maintenance Comments

6.1. How will the project characterize and dispose of sediments and plants that result from routine maintenance? It seems imperative that the design include a waste management plan and an invasive plant and animal control plan, since aspects of the design will likely serve as a prototype for other facilities in the future.
The WWTP effluent has a low total suspended sediment load and there should not be a significant sediment management issue. The elevated nature of the wetlands, and the limited drainage into the system (defined as what falls within the berms), will minimize storm water sediment loading. The accumulation of biomass can be managed in a number of ways including mechanical harvesting or burning. Most likely vegetation management will become necessary within three years. We will need to work with the City, Baylor University, and other local groups to determine how this should be addressed if it becomes an issue. These aspects have been further defined in section I.3 of the monitoring plan.

6.2. Under wildlife management, no specific reference was made for nutria and feral hogs, the two most destructive animals to constructed wetland systems. It is imperative that a management plan for these two species be developed PRIOR to construction of the wetland system, as IMMEDIATE action should be taken upon observation of these animals within (or near) the wetland cells. Shooting nutria is the most effective control method for protection of the wetland cells. Feral hogs can sometimes be effectively trapped, but shooting can also be effective. Both of these species are exotic, invasive species with no regulated hunting seasons (i.e., they are legal to kill year round), so no coordination with US Fish and Wildlife Service or Texas Parks and Wildlife Department is required. However, public safety and concerns should always be considered. If shooting can be conducted safely, a suppressor or silencer may be employed to reduce noise impact. These modifications also improve efficiency of the hunting program.
Initially we asked about nutria or other local pests and was told they didn't have a problem in the area. Since, you are saying that nutria and feral hogs will be an issue, we will discuss this issue with WMARSS or the City of Waco to address the control plan based on the local laws.

6.3. What considerations if any have been made with respect to periodic ice formation in the winter months in areas of stagnant or slow moving water in the wetland and how might this affect the hydrological monitoring equipment if deployed in these areas and the overall treatment effectiveness?

We agree, but do not anticipate ice formation at the inlet or outlet v-notched weir. Most WWTP effluent has elevated temperatures relative to the ambient outside temperature. In addition, the water flowing across a v-notched weir would be moving rather quickly limiting ice formation. The effect of freezing conditions on removal will be evaluated using the chemical data.

7. Equipment Calibration and Measurements Comments

7.1. There is no mention of periodic equipment calibration, maintenance, and verification throughout the course of Tier 1 monitoring.

Equipment calibration, maintenance, and verifications will be performed according to USGS National Field Manual and Manufacturer guidelines. This has been specifically mentioned this in section G of the revised monitoring plan.

7.2. There is no mention of probe calibration and maintenance throughout the course of Tier 1 monitoring.

Equipment calibration, maintenance, and verifications will be performed according to USGS National Field Manual and Manufacturer guidelines. This is specifically mentioned in section G of the revised monitoring plan.

7.3. Section E.2., page 13.

a. Will sondes be routinely calibrated?

Yes. Equipment calibration, maintenance, and verifications will be performed according to USGS National Field Manual and Manufacturer guidelines. We will specifically mention this in the monitoring plan as part of our Quality Assurance Procedures *(section G).*

b. Will samples be analyzed at NELAC accredited laboratories?

Yes, the USGS National Water Quality Laboratory in Denver, Colorado is a NELAC accredited laboratory.

c. What quality assurance protocols and documentation will be in place?

Quality assurance protocols and documentation for water-quality sampling will follow the USGS National Field Manual guidelines. To further expand on this area the monitoring plan will be updated with specific QAQC protocol for each area of emphasis to standardize protocols between sampling and data analysis efforts.

7.4. Would consider adding monitoring of redox potential (ORP) to the proposed soils and sediments analyses.

Developing an understanding of the redox conditions at the sediment/water interface is an important factor to consider and will be added as a focus area to support tier II monitoring. In Tier I we will plan to evaluate the redox characteristics of the water by measuring dissolved oxygen.

7.5. I would recommend adding ORP, total volatile solids (TVS), and total phosphorus (TP) (in addition to orthophosphate) for the water quality parameters analyzed. Also, orthophosphate should be reported as phosphorus (e.g, PO4-P).
We are currently planning to evaluate the redox characteristics of the water by measuring dissolved oxygen. We are still refining what can be measured during Tier I monitoring efforts and appreciate your recommendations. TP and PO4-P are within WMARSS' lab capabilities and will be added to our list.

7.6. During a mosquito population dynamics study conducted during the development of the East Fork Wetland, seven mosquito predators, including both vertebrate and invertebrate species, were caught during monitoring for every mosquito larvae caught. As part of the invertebrate sampling, identification of invertebrate mosquito predators should be included.
We agree.

7.7. Where and what type of devices are planned for monitoring water column depth and flow rates in the wetland?
Flow will be measured using 3 weir mounted flow meters (located at the basin 1 inlet, basin 1 outlet, and basin 2 outlet) and 4 in-pipe flow meters. These devices will be used to control flows within each component and the entire wetland.

a. With respect to water depth measurements, my suggestion would be to deploy pressure transducers either gauged or non-gauged.
Thank you for the recommendation. At this time depth measurement will only be monitored and controlled at the outlet weirs for each basin and through in-pipe flow control for the HSSFs. As the project moves forward and specific research is identified that requires the use of fine-tuned depth measurement devices for monitoring or experiments this suggested method will be considered.

b. Measurement of flow and water depth should be done in areas that are sensitive to fluctuations caused by variations in influent flow and periodic stormwater recharge.
Wetland flow rates will be measured continuously through measurement devices described in the responses above. Hydraulic tracer tests performed biannually will include evaluation of water depth in a number of regions throughout the wetland to record fluctuations.

7.8. There should be flexibility in scheduling baseline and biannual sampling so that potential impacts from recent heavy rains or scouring events can be avoided.
We agree. Each sampling event will need to be coordinated by local researchers to capture "typical" conditions during different seasons. The daily monitoring by Baylor and Waco will be used to assist in monitoring if these events occur when scheduling difficulties cannot be avoided. Field planning will attempt to identify optimal times, with

contingency plans in the event of unacceptable hydrological conditions. This has been documented in section I.1 of the monitoring plan.

a. What is the course of action if routine sampling is scheduled during a storm event where the wetland is outside the acceptable range of HRT?
We agree. The team will rely on local information to plan to avoid storm events. The daily monitoring by Baylor and Waco will be used to assist in monitoring if these events occur when scheduling difficulties cannot be avoided.

b. It would be my recommendation to postpone sampling until water levels and flow return to within the designed HRT range.
We agree. The team will rely on local information to plan to avoid storm events. The daily monitoring by Baylor and Waco will be used to assist in monitoring if these events occur when scheduling difficulties cannot be avoided.

7.9. I would suggest adding oxidation reduction potential (ORP) to the suite of water quality probes being deployed at the wetland site. This can be a useful and inexpensive measurement combined with dissolved oxygen for understanding and monitoring the redox conditions in the water.
Currently the only deployed probes that we will have on site will be during sampling events to reduce the cost of long term monitoring. Weekly monitoring parameters with handheld meters could easily include ORP. If the budget permits, ORP will be added to handheld measurements and mention of this parameter will be made in the monitoring plan.

7.10. I would suggest using an optical dissolved oxygen (DO) sensor rather than a wet cell. In my experience the wet cell technology is unreliable and requires frequent maintenance and re-calibration (e.g., every 30 days).
Wet cells are the technology our probes are currently outfit with for DO measurements. Optical DO sensors are more robust than wet cell DO sensors especially in environments where biofouling may be a concern. If funding can be obtained for their purchase, optical dissolved oxygen monitors will be deployed. For the equipment being deployed during sampling all DO sensors will be calibrated on-site. See section G of monitoring plan for revisions.

7.11. Since biological data regularly do not meet the assumptions of parametric statistics, namely normality and homogeneity of variance (even after transformation), we'd advise the authors to be prepared to use non-parametric statistics for their analyses if these assumptions are not met.
We agree. The analysis of mechanistic removal through the use of non-parametric statistics will be employed in data analysis for the system. See section H of monitoring plan for revisions.