Analytical Methods

A complex interdisciplinary project of this one involves many types of laboratory methods. Given the number of methods used, the uncertain nature of the final target compound list, and the limited space allowed for the project description, we have chosen to refer the reader elsewhere for this information. First, for all of our chemical analysis methods we default to the widely used standard reference, Standard Methods for the Examination of Water and Wastewater. There are some methods that are not "standard" and must either be developed (in some cases are currently being developed by others, or we must cite other references. Second, the analysis of target compounds will depend on the final list adopted in the early stages of this project. For now, the reader is referred to the initial proposed list in Table 7, where references can be found for available methods. Another innovative analytical method concerns TOX. Hua and Reckhow (2006) have refined these methods to allow easy analysis of halogen species (e.g., TOCl, TOBr and TOI). For information on the patellar instruments and equipment that is used in the various analytical tests, the reader is referred to the section on "Facilities".

5. EVALUATION CRITERIA

We propose the following evaluation criteria as linked to our research objectives:

#	Objective	Products & Criteria for Judging Success
1	Determine the rates of abiotic degradation of a group of non-regulated DBPs that is representative of some of the groups of greatest concern.	Experimental data supporting the determination of degradation rates for the selected or target compounds
2	Determine degradation products of these compounds when possible	Mechanistically-defensible byproducts, some should be verified with MS and commercial standards
3	Determine impacts of various conditions and characteristics (e.g., pH, temperature, concentrations of reactive solutes) of the water on these degradation rates.	Experimental data on degradation of the target compounds collected at varying pH values (5-10), varying temperature (5-25C), varying concentrations of ferrous iron, sulfite, and nitrite
4	Develop kinetic models for degradation.	Calibrated differential equations that lead to useable mathematical models for predicting degradation in #3. These will be in a format that can be used directly by mainstream commercial software
5	Develop similar models for DBP formation from the various drinking waters with their existing consortium of organic precursors	Calibrated differential equations that lead to useable mathematical models for predicting formation of the target compounds. These will be in a format that can be used directly by mainstream commercial software
6	Evaluate the importance and impact of	A scientifically-defensible assessment of the percent

	biodegradation in distribution systems for these compounds	of the target compounds that were lost to biodegradation vs all forms of abiotic degradation in each of the appropriate participating utilities. This comes from direct comparison of field test data with predictions of mathematic models (above) and from the bench-scale testing of samples containing DS solids.
7	Isolate and assess biodegradation from abiotic reactions with dissolved species, from abiotic reactions with particulate corrosion products in distribution systems.	A scientifically-defensible assessment of the percent of the target compounds that were lost to biodegradation vs heterogeneous abiotic degradation in each of the appropriate participating utilities. This comes primarily from the bench-scale testing of samples containing DS solids.
8	Combine the knowledge gained into a set of mathematical models incorporating rate laws and rate constants that can be used to help predict formation and especially degradation of the key non-regulated DBPs	Calibrated mathematical equations that lead to useable DBP models for predicting concentrations of the target compounds. These will be in a format that can be used directly by mainstream commercial software

VI. QA/QC

Sampling Methods

Water samples will be collected according to the *Standard Methods for the Examination of Water and Wastewater* (APHA, 2000) wherever applicable. Details of sample analysis for conventional analytes are provided in Table 4. All samples collected in the laboratory and field will be labeled clearly and legibly with the following information:

- Parameter
- Preservative agent
- Laboratory analyzing sample
- Date / time of sample collection
- Facility where sample collected
- Sample location within facility
- Sample number
- Replicate

Sample handling will utilize chain-of-custody forms to track sample handling. This will ensure that acceptable holding times are not exceeded, and allow reporting of sample conditions upon reception. The chain-of-custody forms will be filled out during sample collection, a copy of the chain-of-custody form will be filed for records on site, and the original chain-of-custody form will accompany the samples during transportation. Upon

sample reception at the designated laboratory, the integrity of the sample containers will be assessed, the chain-of-custody forms will be completed, and the samples will be stored at 4 ºC in darkness or processed immediately. No chain-of-custody forms will be required for analyses conducted onsite.

QA/QC Procedure

For each batch of samples processed, the following steps are generally undertaken as part of Quality Assurance/Quality Control procedures and to assure defensibility of analytical results:

(a) Recovery of known additions:

The recovery of known additions will be part of regular analytical protocol. This will be used to verify the absence of matrix effects or the amount of interference. The sum of duplicates and known additions will be greater than 20% of the samples. The known addition will be between 1 and 10 times the ambient level. The procedure would not be used above the demonstrated linear range of the method. As part of this method, concentrated solutions will be used so that volume change in sample is negligible.

(b) Analysis of duplicates:

Duplicate samples will be processed on a routine basis. A duplicate sample is a sample that will be processed exactly as the original sample, including preparation and analysis. The duplicate samples will be used to determine precision. The sum of duplicates and known additions will be greater than 20% of the number of samples.

(c) Analysis of reagent blanks:

Reagent blanks will be analyzed whenever new reagents will be used or 5% of the sample load, whichever is greater. This will monitor purity of reagents and the overall procedural blank. A reagent blank will be run after any sample with a concentration greater than that of the highest standard or that might result in carryover from one sample to the next.

Table 4. Analysis – Container, Preservatives, Replicate Frequencies and Holding Times

Parameter	Collection Container	Preservative	Replicate Frequency	Maximum Sample Holding Time
Nitrite/nitrate	125-mL polyethylene bottle	None	1/10 samples analyzed in duplicate	Store at 4^0C, analyze within 2 days
pH	Not Applicable	None	Single	Store at 4^0C, analyze within 1 day
Temperature	Not Applicable	None	Single	Analyzed immediately onsite
Turbidity	500-mL polyethylene	None	Single	Store at 4^0C, analyze within 1

	bottle			day
Chloride	125-mL polyethylene bottle	None	1/10 samples analyzed in duplicate	Store at 4^0C, analyze within 14 days
Bromide	125-mL polyethylene bottle	None	1/10 samples analyzed in duplicate	Store at 4^0C, analyze within 14 days
Haloacetic acids and THMs	1-L borosilicate glass container	10 mg/L Ascorbic acid	samples analyzed in duplicate	Store at 4^0C, analyze within 14 days (chlorinated) Store at 4^0C, analyze within 1 day (unchlorinated)
TOC	125-mL borosilicate glass container	None	samples analyzed in duplicate	Store at 4^0C, start analyze within 1 day
DOC	125-mL borosilicate glass container	None	One sample analyzed in triplicate	Store at 4^0C, start analyze within 1 day

(d) Analysis of externally supplied standards:

As a minimum, externally supplied standards will be analyzed whenever analysis of known additions will not result in acceptable recovery, or once each analysis-day, whichever is greater. All attempts will be made to analyze laboratory control standards near sample ambient levels.

(e) Calibration with standards:

As a minimum, three different dilutions of the standard would be measured when an analysis is initiated. The standard curve would be verified each analysis-day by analyzing one or more standards within the linear range. Reportable analytical results would be those within the range of the standard dilutions used. Values above the highest standard would not be reported unless an initial demonstration of greater linear range has been made and the value is less than 1.5 times the highest standard. If a blank is subtracted, the result will be reported even if it turns out to be negative.

General sample collection and handling will be in accordance with the guidelines of Section 1060 of Standard Methods (APHA et al., 1995). Reagent grade chemicals or higher quality when needed will be used throughout the research. Milli-Q treatment of building RO water (purified by reverse osmosis, deionization, and carbon adsorption) will be used for preparation of reagents, sample blanks, and dilution water. Glassware used in the experiments and in analytical analyses will be thoroughly cleaned with a chromium-free sequence of detergent, oxidant and acid to prevent interferences from trace organics.

Analytical Procedures

Standard method protocols will be used to measure pH, ORP, conductivity, turbidity, alkalinity [APHA et al. 1998], chlorine, bromide, TOC and AOC. As no standard method is available to measure BDOC the Servais method will be used (Servais et al., 1989). pH and ORP will be measured using a bench top Thermo-Orion pH/ORP meter. Turbidity will be measured using a bench top Hach Ratio turbidimeter. Conductivity will be measured using Thermo Orion Model 105 conductivity meter. TOC and DOC will be measured using a Shimadzu 5000 TOC analyzer. Anion analysis will be accomplished using the methods outlined in Table 5. Method selection will depend on the sample matrix, expected concentration range, and required accuracy. The precision, accuracy and method detection limits will be evaluated, and where there are existing methods, held within the control limits set forth in the accepted references (e.g. APHA et al., 1995; USEPA-EMSL, 1990; ASTM, 1994).

Table 5. Anion analysis specifications.

Compounds	Specification	Method
Nitrate, nitrite, sulfate, sulfite	DX-500 IC system (Dionex, Sunnyvale, CA), Ionpac A14 column and AG14 guard column, with CD 20 conductivity detector. The eluent is 0.35M Na_2CO_3/1.0 mM $NaHCO_3$ at 1 mL/min with 100 □L injection loop. Chloride and Sulfate will be removed if required using on guard silver and barium cartridges respectively.	Dionex corporation, application note 135.
Haloacetic acids	Hewlett-Packard 5890 series GC, with DB-1701 analytical column and HP7673 autosampler	US EPA method 552.2
Metabolites	Colorimetric methods Hewlett-Packard 5890 series GC, with DB-1701 analytical column and HP7673 autosampler Hewlett-Packard 5890 series GC, with DB-5 analytical column and HP7673 autosampler	Friedman and Haugen (1943); Calkin (1943) US EPA method 551.1

Table 6. Microbiological Procedures

DNA extraction	Standard phenol/chloroform extraction method
PCR of 16S DNA from bacterial strains	PTC200 Peltier thermal cycler, MJ research
PCR product cloning	pGEM®-T easy vector system, Promega
Sequencing of bacterial clones	Applied Biosystems 137 using Bigdye® terminator v3.1 cycle sequencing kit

Standard aseptic techniques will be used for all applications involving microorganisms. (Leboffe and Pierce, 2002 Microbiology Laboratory Theory and application). All microbiological growth medium will be prepared using appropriate sterilization methods. (Standard Methods, 20th ed)

Data Reduction, Validation and Reporting

To ensure the accuracy and permanency of collected data, all research data will be recorded with permanent ink in a bound notebook and all QC data (precision, accuracy) will be recorded in instrument log notebooks. Summary QC graphs and tables will be reviewed at least quarterly Concerns and conclusions will be reported to the Project Officer via the project reports.

All data will be subject to review by the principal investigators before release. The analysts involved will sign reports as well as all who review them. All signers attest that the data and associated information contained in the report are believed to be correct and that all quality assurance requirements have been fulfilled, unless exceptions are approved and noted. Careful and detailed laboratory records by each analyst will be maintained, including source of reagents, detailed procedures, instrument and conditions of analysis, failed experiments, etc. Data output will be archived.

The documentation required for the project will include the following:

- project log books
- raw data log sheets for pilot-scale and bench-scale equipment operating data
- raw data log sheets for onsite laboratory results
- raw data log sheets for onsite analytical instrument calibration
- raw data log sheets for onsite equipment calibration and verification
- laboratory chain-of-custody forms
- laboratory reports
- initialed and dated printouts of verified electronic data.

Data Delivery

Data delivery requirements are listed in Table 7.

Table 7. Data Delivery Requirements

Type of Data	Delivery Requirements
Bench-scale analytical results, operating	Raw data forms (copies for subcontractors)

results, and experimental conditions	
Bench-scale calibration and verification records	Raw data forms (copies for subcontractors)
Outside laboratory analytical results	Reports of analytical results and QC results Copies of chain-of-custody forms
Bench-scale project log books	Original log book (copies for subcontractors)
Electronic data	Spreadsheet format, similar in organization to raw data forms

Assessment and Response Actions

All calibration and QC data will be reviewed by the PIs and they will be responsible for assuring that all verifications and calibrations have been conducted on bench-scale equipment, pilot-scale equipment and analytical instruments at the beginning of the project. The graduate students working on this project will be responsible for ensuring that instrument systems are in control (i.e., they meet the acceptance criteria specified) and that QA objectives for method detection limit, precision, accuracy, and completeness are being met. If any QC data are outside of the acceptance criteria, the lead investigator on that task together with the PI (Dr. Park) will investigate the cause of the discrepancy. If the discrepancy is due to an analytical problem, the sample will be re-analyzed or another sample will be collected and analyzed. If there is any other problem, the data will be flagged, another sample will be collected, or the steps outlined in the Corrective Action Plan in Table 9 will be implemented.

Precision

The precision of duplicate samples will be assessed by calculating the relative percent difference (RPD) according to:

$$RPD = \frac{|S - D|}{(S + D)/2} \times 100$$

where S is the sample concentration and D is the duplicate sample concentration
If calculated from three or more replicates, the precision will be determined using the relative standard deviation (RSD):

$$RSD = \frac{SD}{Average} \times 100\%$$

where SD is the standard deviation for the replicate samples.

Accuracy

For Measurements where matrix spikes are used, the accuracy will be evaluated by calculating the percent recovery (R):

$$R(\%) = \frac{S - U}{C_{SA}} \times 100\%$$

where S is the measured concentration in spiked sample, U is the measured concentration in the unspiked sample and C_{SA} is the calculated concentration of spike in the sample.
When a standard reference material (SRM) is used, the percent recovery is determined by:

$$R(\%) = \frac{C_m}{C_{SRM}} \times 100\%$$

where C_m is the measured concentration of SRM and C_{SRM} is the actual concentration of SRM.

Method Detection Limit (MDL)

To determine the MDL, at least seven replicates of a laboratory fortified blank at a concentration of three to five times the estimated instrument detection limit is analyzed through the entire analytical method. The MDL is calculated using the following equation:

MDL = (t) x (SD)

where t is the student's t value for 99 percent (t for 7 replicates = 3.14).

Completeness

Completeness is a measure of the amount of valid data obtained compared to the amount of samples collected. The degree of completeness is the number of acceptable analyzed samples divided by the number of samples collected, multiplied by 100. Completeness is defined by the following equation:

$$\%C = 100 \times \left(\frac{V}{T} \right)$$

where %C is the percent completeness, V is the number of measurements judged valid and T is the total number of measurements

The acceptable criteria for QA objectives are listed in Table 6. The acceptance criteria and corrective action plan for pilot-scale and bench-scale routine water quality parameters are summarized in Table 8.

Table 8. Acceptable Criteria for QA Objectives

Measurement	Reporting Units	MDL Goal	Precision (% RPD)[1]	Accuracy (%Recovery or % Bias)[2]	Completeness[3]
pH	pH units	0.1	± 10	90 – 110	90
Chloride	µg/L as Cl^-	100	± 20	80 – 120	90
Bromide	µg/L as Br^-	100	± 20	80 – 120	90
Haloacetic acids	µg/L	≤1	≤20	80 – 120	95
TOC	mg/L	0.1	≤20	80 – 120	95
DOC	mg/L	0.1	≤20	80 – 120	95
BDOC	µg/L	≤0.2	≤25	75 - 125	95
AOC	µg acetateC/L	10	≤17.5	75 - 125	95

Notes:

1. Given as Relative Percent Difference (RPD) of laboratory duplicates
2. As percent recovery of matrix spike
3. Based on the number of valid measurements compared to total number of measurements

Table 9. Corrective Action Plan

Parameter	Acceptance Criteria	Steps for Corrective Action
Any duplicate analysis	See Table 8, Precision	Duplicates Check instrument calibration; re-

		calibrate instrument
Any method blank	See Table 8, Accuracy	Perform procedures specific to each analysis as determined by the laboratory performing the analysis