Project Abstract

Project Title: Establishing Guidelines for the use of Ozone-GAC for Control of Endocrine Disruptors and Related Compounds in Water

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Objectives:

1. To investigate effectiveness of ozone and biological filtration at removing selected endocrine disrupting compounds (EDCs), pharmaceutical and personal care products (PPCPs), their daughter products and residual endocrine activity from municipal water supplies.

2. To determine the same in twelve separate waters subject to a range of physicochemical treatment scenarios.

Expected Results: This research will make three major contributions to the field of drinking water treatment. First, it will provide information to decision makers on effectiveness of ozone/biofiltration for the removal of a wide range of EDCs, PPCPs, their daughter products and associated endocrine activity under a competitive scenario where a mixture of these chemicals are spiked prior to the treatment process. Second, it will provide engineers and operators with preliminary key information on how to design and run ozone/biofiltration systems for effective control of these compounds. Finally, it will provide utilities with information on likely removals of these compounds under a broad range of water qualities and treatment scenarios. The results of this study will be presented in a final project report as well as in published journal articles.

Approach: This work will be conducted in three major phases or tasks; and it builds upon some of the latest fundamental advancements in EDC and PPCP removal. All three major tasks are laboratory-based, and include bench-scale testing and trace organic analysis. Task 2 calls for collection of raw water field samples from the participating utilities with analysis of the target compounds. Task 3 concerns site-specific removal of the target compounds. This includes removal by the existing treatment systems (12 of them) as well as removal and formation of daughter products from conventional and alternative oxidation processes. A long-term ozone biofiltration study constitutes Task 4. This is viewed as one of the most promising technologies for removing EDCs, PPCPs and their daughter products. This study is carefully designed to yield high quality and well controlled data on efficiency under various conditions of process design and operation.

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Project Description

Introduction

There are a large number of chemicals that are widely used in households in the developed world that may have a substantial negative impact on water quality. Endocrine disrupting compounds (EDCs) and pharmaceutical and personal care products (PPCPs) incorporate a group of compounds that are widely used in modern society, most of which are not naturally-occurring. These compounds are a source of concern for several reasons. First, they have been shown to have detrimental impacts on aquatic organisms. Second, they have captured the public's imagination, and as a result there is intense interest in their possible presence in public water supplies. While it is generally accepted that the individual concentrations likely to exist in drinking water are so far below therapeutic doses that there should be no measurable effect on humans, the possibility exists that synergistic effects from multiple pharmaceuticals could render the aggregate biological impact greater than expected. Third, some pharmaceuticals (e.g., ranitidine) are believed to produce small amounts of highly toxic byproducts upon treatment with disinfectants (e.g., Schmidt et al., 2006). In addition, there are possibilities that PPCPs might be used as molecular markers for wastewater and their stereochemistry may even be able to indicate whether the wastewater had undergone biological treatment (e.g. Fono & Sedlak, 2005).

Background

The focus of the proposed research is on certain trace contaminants that have been recently found in US raw drinking waters. Our long-term objective is to determine the fate of EDCs, PPCPs, and N-Nitrosodimethylamine (NDMA) through potential treatment processes. One key element of this assessment will be to determine the daughter compounds created as a result of treatment steps and the hormonal activity associated with those daughter compounds. The preferred treatment train will incorporate combinations of oxidant doses and granular activated carbon filtration. The assessment will evaluate the constituents and their fates from influent raw water supply through the (intermediate) oxidation step and then after filtration.

Previous research efforts have demonstrated the effectiveness of treatment technologies to remove EDCs, PPCPs, and NDMA from raw-water supplies, but the resultant creation of daughter compounds and the effectiveness of processes to remove those compounds are less established. Consequently, the goal of this research will be to track the changes in selected compounds under varying treatment regimes. We anticipate this information to be of interest to utilities and to regulators, both of whom need to know what reasonable steps can be taken to control compounds that may be of concern to the public health.

EDCs have been a concern to the U.S. Environmental Protection Agency (US EPA) and state regulatory authorities throughout the past decade. More recently, PPCPs have generated similar concerns. These compounds are of interest because of their potential negative interactions with the human body, even at low concentrations. EDCs already have been identified in exposed non-human, wild populations to cause negative impacts, such as sexual development defects (known as "intersex") changes to the distribution of sex ratios (Vethaak et al., 2002). PPCPs, on the other hand, currently have not exhibited acute negative impacts on human health at the levels measured in source waters (Sedlak and Pinkston, 2005), but their long-term chronic effects are not known (Kiwa Water Research and Stowa 2004). Even though there is currently little evidence linking PPCPs to human health, the general public has been skeptical. Earth Tech has observed public concern in a case involving implementation of water reuse from wastewater containing PPCPs.

Research is actively underway to determine the ability of treatment processes to remove EDCs and PPCPs. Currently, several studies have shown relative success in removing EDCs and PPCPs when using treatment technologies commonly implemented in the water and wastewater industry. Membrane technologies tend to

be the most promising. Ultrafiltration (1,000 Dalton MWCO) and nanofiltration have shown greater than 70% removal, and reverse osmosis has shown even higher removals (Westerhoff 2003, Kiwa Water Research and Stowa, March 2004). Despite the treatment success of the processes themselves, there is still the question of how to dispose of the residuals. Other unit treatment processes have demonstrated varying abilities to remove these compounds depending on each compound's chemical properties (Westerhoff 2003, Kiwa Water Research and Stowa, March 2004). They include coagulation, powered activated carbon, biofiltration, chlorination, ultra violet irradiation, and ozonation.

In addition to the EDCs and PPCPs themselves (the "parent" compounds), there has been concern recently that the oxidation of parent compounds may yield similarly active "daughter" compounds. Consequently, this research proposal will include evaluation of daughter and parent compounds.

It is very likely that successful treatment and ultimate elimination of EDCs and PPCPs will require a multi-barrier approach. The most promising multi-barrier approach makes use of biofiltration preceded by ozone alone or ozone in turn preceded by hydrogen peroxide (Snyder et al., 2005, Westerhoff et al., 2005, Westerhoff 2003, von Gunten et al., 2003). This treatment could be implemented either at water or wastewater treatment facilities.

The proposed research will assess the ability of ozone/GAC biofiltration to remove the compounds listed in Table 1. Based upon the priority list developed by the Water Research Commission (2003) and the American Water Works Association Research Foundation (2005), these compounds are the EDCs and PPCPs most likely to cause adverse human impacts and/or are unlikely to be removed by conventional water and wastewater treatment processes. Note that, in addition to EDCs and PPCPs, N-Nitrosodimethylamine (NDMA) and perchlorate has also been included, largely at the request of Massachusetts Department of Environmental Protection. NDMA is a potential treatment byproduct formed during disinfection with chloramines. NDMA is not within the classification of EDC or PPCP compounds, but it is similarly new to researchers.

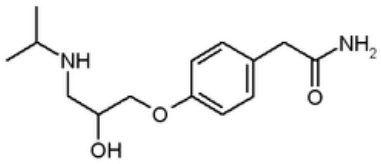
Table 1: Target Compounds

EDC	PPCP	WW associated
N-N-diethyltoluamide (DEET)	Atorvastatin or Gemfibrozil	N-Nitrosodimethylamine
Tris(2-chloroethyl)phosphate (TCEP)	Naproxen	
Estrone	Sulfamethoxazole	
	Trimethoprim	
Perchlorate	Atenolol	
	Ranitidine	
	Ciprofloxacin	

Compound Selection Criteria

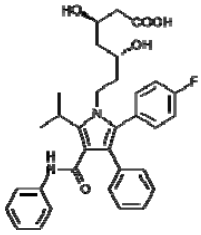
All of the compounds selected for this research are poorly removed by conventional coagulation and filtration. None is sufficiently hydrophobic to be strongly bound to alum or iron floc by hydrophobic forces. On the other hand, none has the functional group density to make binding by complexation or hydrogen bonding very strong either. Many of these are reactive with both free chlorine and ozone, leaving numerous daughter products. At least two are known to be relatively unreactive with ozone. Few, if any, have been tested for reactivity with chloramines with the exception of the recent report by Snyder et al. (Snyder et al., 2007). The goal of selecting these compounds was to select compounds that would be a model for a group of compounds and also could be used as indicator compounds which utilities could use as potential screening compounds.

Beta-Blockers: Atenolol



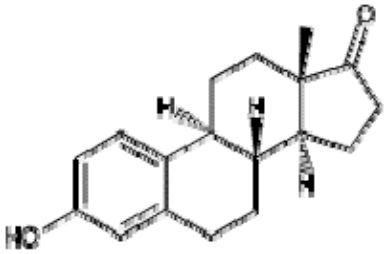
Atenolol is a representative of the group of Beta-Blockers, for treating cardiovascular disease. This particular drug has been in use since 1976. Sedlak and co-workers (2005) estimate a nationwide average raw municipal wastewater concentration of about 1,500 ng/L. This compound is rather unreactive with free chlorine, as it lacks activated aromatic structures as well as reactive nitrogen sites.

Statins: Atorvastatin



This compound is more commonly known as Lipitor, and it is representative of a larger group of cholesterol-reducing drugs called statins. It does not appear to have been tested for removal by coagulation or reaction with either chlorine or ozone. Based on its structure, we would expect it to be slightly reactive with ozone, but little affected by the other treatments.

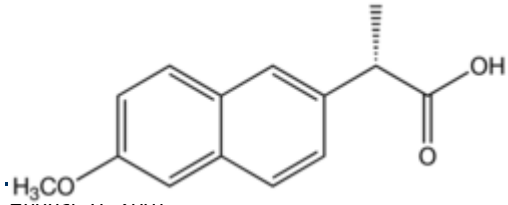
Reproductive Hormones Estrone



Estrone

Estrone is a naturally occurring human estrogen. It has the fundamental steroid skeleton, with many similarities in positioning of the functional groups to other widely studied estrogenic compounds (17β estradiol, estriol, ethynylestradiol). All of these compounds are rapidly destroyed by free chlorine (Westerhoff et al., 2005; Deborde et al., 2004). It's quite likely that the phenolic "A" ring is the initial site of attack and the most reactive structure within each of these compounds. Reaction with chlorine should results in large fragments that are partially oxygenated or even halogenated. For example, Estradiol has been found to produce at least 7 daughter products that persist in treated waters (Irmak et al., 2005; Hu et al., 2003)

Non-steroidal anti-inflammatory: Naproxen

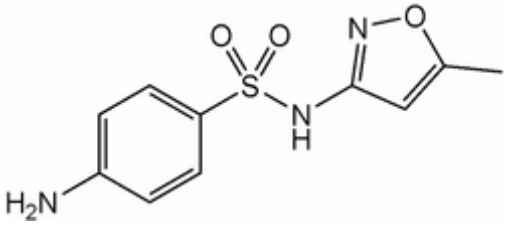


Naproxen is a common arthritis treatment, intended to reduce pain and inflammation. Its mean concentration in US wastewaters has been estimated to be about 2,400 ng/L (Sedlak et al., 2006). It is

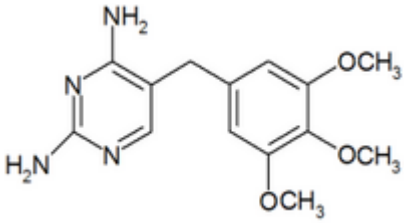
quite reactive with ozone and surprisingly reactive with chlorine too. Limited occurrence data has centered around 300 ng/L in US wastewaters.

Sulfonamide Antibiotic: Sulfamethoxazole

This antibiotic is a major component of Bactrim. Its median concentration in treated wastewaters has been measured at 1400 ng/L, a value quite close to its nationwide estimated level of 3,200 ng/L. This compound is moderately reactive with free chlorine and ozone.

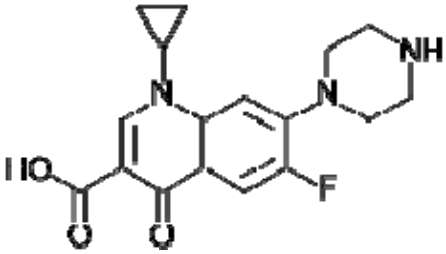


Bacteriostatic Antibiotic: Trimethoprim



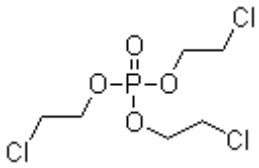
This particular antibiotic is widely used for treatment of urinary tract infections. It is also a member of the group of dihydrofolate reductase inhibitors. It is quite prevalent in US wastewaters (500 ng/L median; 1500 ng/L estimated nationwide). It is extremely reactive with free chlorine, as would be expected from its structure. It is quite likely that the molecule is extensively degraded and oxidized by chlorine or ozone treatments.

Fluoroquinolone Antibiotic: Ciprofloxacin



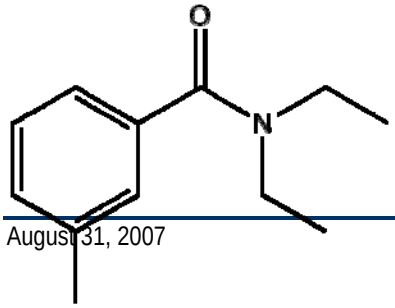
This is broad-spectrum antibiotic that often goes by the shortened name "Cipro". Like Trimethoprim, it is quite prevalent in US wastewaters (170 ng/L median, 1400 ng/L estimated nationwide). However, recent studies have shown this compound to be almost completely resistant to attack by ozone under normal treatment conditions (Vieno et al., 2007).

Flame Retardant: Tris(2-chloroethyl)phosphate (TCEP)



TCEP is an organophosphorus flame retardant that has been widely observed in natural waters. Kolpin and coworkers (2002) found it in about 58% of surface waters are a median concentration of 100 ng/L. It can be removed by high doses of GAC, but it is not substantially affected by ozonation (Westerhoff et al., 2005; Snyder et al., 2007).

Insecticide: N-N-diethyltoluamide (DEET)



DEET is a common ingredient in many commercial pesticides. It is widely used by travelers, campers, hikers and children. Kolpin and coworkers (2002) found it in 74% of the surface waters, and the median concentration was 60 ng/L. It is moderately susceptible to

removal by ozonation, but little affected by chlorination (Snyder et al., 2007).

Research Plan

Research Goals

The overall goals of this research project are (1) to determine the effectiveness of the ozone-GAC process in removing prototypical EDCs and PPCPs and (2) to establish preliminary design criteria for full-scale use in water and wastewater applications. The goals will be accomplished by spiking samples with known concentrations of compounds and then running pilot tests of treatment regimes. The effectiveness of treatment will be measured by analyzing and monitoring concentrations and hormonal activities of parent and daughter compounds. Task 2 and 3 will involve the participation of interested utilities. Table 2 below provides the type of work that will be performed for each individual utility. More details are covered under the individual tasks.

Table 2: Work to be Completed for Utilities

Analyses							
		Source Water		After Oxidation			
Participation Level	Cash Contribution Level	Table 1	Bioassay	Table 1	Daughter Compounds	Bioassay	Bench-Scale Removal*
A	$12,000	X	X	X	X	X	X
B	$6,000	X		X	X		X
C	$3,000	X					X

* Current unit process at the facility such as coagulation, etc.

Task 1: Literature Update

The research effort will begin with an update of the existing literature to determine the latest findings and to help clarify what is unknown regarding treatment efficacy for EDC, PPCP, compounds, and NDMA. For instance, two specific compounds, sulfamethoxazole (a PPCP antibiotic) and 17α-ethinylestradiol (an EDC from oral contraceptives) have been well studied in terms of their kinetic rates for breakdown of parent and some daughter compounds. By the time that this research project is initiated, there may have been more studies of this sort in the literature or data available in draft form. This literature review will culminate in a summary of available information, including known and proposed impacts of common treatment technologies on the parent and daughter products. This work will be conducted by Earth Tech and University of Massachusetts (UMass) researchers, and will occur in parallel with the early laboratory work.

Special attention will be paid to the formation and removal of oxidative daughter products of the target PPCP/EDC compounds. This will include bench, pilot and field studies where daughter products have been identified and quantified. It will also make use of current mechanistic knowledge of the oxidants so that the structure of likely daughter products may be proposed. This will facilitate the identification and quantification of daughter products in Tasks 3 and 4.

Task 2: Raw Water Occurrence Survey

The goal of this task is to perform a preliminary survey for the compounds listed in Table 1. The goal here is to 1.) Determine if they exist in the source water and 2.) Consider if the target compound list should be revised.

Task 2a: Raw Water Occurrence Survey

This is the only task for which ambient concentrations are to be determined, and it is designed to be completely anonymous. The utility survey will be conducted in parallel with the literature update and is intended to provide occurrence data on the target compounds (selected EDCs, PPCPs, and NDMA) at all participating utilities (groups A, B and C). One goal here is to determine if specific watershed characteristics can be linked to higher risks of EDC/PPCP occurrence. A large bulk sample of raw water will be collected from each utility for analysis by LC/MS. The timing of this sample collection will be made in consultation with the utility personnel. Attempts will be made to collect samples at a time when the raw water is most vulnerable and most likely to contain the target compounds.

As a supplement to chemical analysis, a series of bioassays will be conducted on a subset of these tests (waters from group A utilities). These bioassays are intended to detect the overall activity from total estrogenic and antiestrogenic EDCs in water samples. These cell-based reporter assays and changes in gene expression in the freshwater fish, Japanese medaka are sensitive and useful for testing waters containing low levels of complex mixtures of potentially estrogenic and antiestrogenic EDCs. The short-term-exposure fish assays that we have optimized are, in general, more sensitive than cell-based reporter assays. For example, while the cell-based assays detect 17β-estradiol in the nanomolar range, the fish assays detect it in the picomolar range. The detection of low levels of 17β-estradiol is important since the normal concentration of this hormone in humans ranges from femtomolar (in children and men) to nanomolar (in women of reproductive age), and it is known that increases above these basal levels produce distinct physiologic responses, e.g., development of breast tissue. Similarly, picomolar levels of 17β-estradiol in streams are unlikely to alter the physiology of female fish, while these same levels are capable of eliciting gene induction in male fish resulting in the production of female proteins and sometimes eggs in genotypic males. Thus, changes in the expression of a gene in male fish that is normally induced only in female fish are a cause for concern. Since each bioassay will be conducted with several positive and negative controls, a "positive hit" would indicate that something in the water sample was physiologically active and action was warranted. The first step would be to compare the bioassay results with results from chemical analysis. Analytical measurement of an estrogenic compound would confirm the bioassay results and the water utility would be notified. However, lack of analytical confirmation would not eliminate the concern since the chemical analysis is limited to detecting the target compound(s), whereas the purpose of the bioassay is to detect the sum activity from both known and unknown compounds in the water. In the event of lack of agreement between a bioassay and chemical analysis, the bioassay would be repeated with additional water from the same collection to rule out any human error in the running of the assay. If the repeated bioassay indicated physiologic activity the water utility would be notified. At this point we would initiate a discussion with the water utility to decide whether further testing was warranted and possibly conduct bioassays with a series of diluted water samples to assess the range of the biological activity. While results with fish are not directly translatable to human health, they do directly relate to ecological health.

These will be handled in a confidential fashion. Sampling personnel will be distinct from the laboratory/analytical personnel. In many cases, the participating utilities will handle sampling and transport to UMass. All raw water samples will be delivered to the PI (Reckhow). He will assign each an anonymous code number and transmit them to the UMass research laboratory. The identity of each sample will remain sealed and will only be know to Dr. Reckhow. This information will not be disclosed to anyone (including the

UMass research team, Earth Tech or MA DEP) unless he receives permission in writing from a particular utility to release information identifying the analytical data for that utility.

The utility survey will assist in determining the occurrence of PPCPs and related compounds in raw drinking waters in the New England region. This will be a valuable database covering a broad range of compound types, representing different potential origins.

Task 2b: Review and Revision of Target Compound List Based on the Raw Water Survey

The purpose of this task is to review the results of the raw water utility survey. Consideration will be given to revising the list in Table 1 if a compound in Table 1 is not consistently detected in the source water. If this occurs, the research team will provide justification as why the chosen target compounds in Table 1 are believed to be the best compounds to pursue further research.

Task 3: Assessment of Site-Specific Removal

The effectiveness of conventional and existing treatment processes for removal of PPCPs and EDCs is not well characterized. This is because to date: (1) measurement techniques are have recently accommodated low levels of detection, (2) testing has been very limited and may be site specific, and (3) formation of daughter products or derivatives has not been generally considered. The purpose of task 3 is to work toward filling these gaps. In this task we will collect a large sample of raw water from each participating utility. This will be done independently of the Utility survey sampling so that anonymity for Task 2 is not compromised. These waters will be spiked with well-defined concentrations of the target compounds. They will not be analyzed for raw concentration levels. The spiked levels will be selected to match the 95 percentile reported for North American waters, as refined in the literature update (Task 1). Fortified raw waters will be subject to various bench-scale treatments as described on the following page.

Task 3a: Removal by Existing Treatment Systems

One of the objectives of this research is to assess the ability of existing and conventional treatment processes for removing the target compounds. With this in mind, all utilities (groups A, B and C) will be asked to submit a large raw water sample for bench-scale testing of treatment effectiveness. Fortified raw waters will be subject to bench-scale treatment modeled after the corresponding full scale process train. This may include pre-oxidation (chlorine, ozone) coagulation, flocculation, settling, intermediate oxidation, filtration, corrosion control and final disinfection. Adjustment of pH will follow full scale practice. Table 4 summarizes the lab-scale treatments as currently planned. Laboratory treated waters will be analyzed for persistence of the parent compounds. We will also measure DOC, DON, UVabs, pH and chlorine/chloramine residual on each raw and treated water. We will ask the utilities to measure alkalinity, pH, hardness and turbidity on each raw water collected. The goal is to assess treatment effectiveness of the participating utility's plant if it was ever to be challenged with high levels of the target compounds.

As a supplement to chemical analysis, a series of bioassays will be conducted on a subset of these tests (waters from group A utilities). These bioassays were previously described under Task 3. Bench-scale treatment analysis of the participating utilities will provide a wealth of data that will be valuable to a broad range of utilities. Not only does this group include several that practice biofiltration, but it also incorporates some of the most common treatment trains used in North America. There is also a broad range of raw water types from well protected ground water sources to vulnerable surface waters.

Task 3b: Removal by Oxidative Treatment

A subgroup of the utilities (groups A and B) will be asked to submit a larger raw water sample that will allow for testing by various oxidative treatments. The spiked raw waters will be dosed in the laboratory under different oxidation/disinfection scenarios including ozonation, chlorine dioxide, chlorination and chloramination. Proposed doses are shown in Table 5, although the final doses will be selected in consultation with the utilities so that they are appropriate given the scenario of interest. In each case, residual target compound will be analyzed. In addition, we will examine each water carefully for the presence of oxidative daughter products. This will be done using LC/MS/MS with our existing instrumentation (Acquity and Quattro Micro triple quadrupole). We also have GC/MS capabilities (ion trap and TOF) that can be used as needed. The preferred analytical protocols will be selected following Task 1 assessment. As a supplement to chemical analysis, a series of bioassays (as previously described under Task 2) will be conducted on for these tests.

Table 5: Proposed Criteria for Oxidant Doses in Task 3b

Oxidant	Dose
Ozone	0.5 mg-Ozone/mg-Carbon
Chlorine Dioxide	1.4 mg/L
Free Chlorine	Sufficient for 1.5 mg/L residual after 72 hrs
Chloramines	Sufficient for 3 mg/L residual after 72 hrs

Task 4: Biological Filtration: Assessment and Guidelines

The combination of preoxidation with ozone and biological filtration is well recognized as being extremely effective for removing trace organic contaminants from drinking water. This treatment scheme has been widely used in Europe and is now becoming more common in North America. One reason for its popularity is the lower cost versus competing technologies (GAC adsorbers, nano or ultrafiltration), the multiple benefits it brings, and the opportunity for producing biologically stable water. Despite its promise, ozonation and biofiltration has not been examined for PPCP removal in a well controlled way with acclimated filters. Nor have any operational or design guidelines been developed for use of biofiltration to control endocrine active compounds in drinking water. The purpose of this task is to establish such guidelines for the test compound and their daughter products.

This task will involve a long-term testing phase that will extend for one year following the establishment of a biologically active GAC filter. Mixtures of the test compounds (Table 1) will be injected at levels that are typical of contaminated raw drinking waters. The spiked concentrations will be directly measured, along with the ozone contactor effluent and the four filter effluents. Samples will also be collected for the various bioassays. The pilot testing phase for the proposed compounds will last one year. During this time we will examine removal performance for a range of temperatures, ozone doses, and filtration rates. The values explored will span the typical values encountered or used in drinking water treatment.

The pilot facility will be assembled and located in the UMass Environmental Engineering Laboratory. There will be four parallel process trains, each containing a GAC filter. Two of the trains will receive ozonated water. The other two will receive water that has not been ozonated. The anticipated flow rate to each filter will be 1.5 L/day. In addition, the ozone contactor will waste approximately 1.5 L/day at a constant rate throughout the test.

For the first three months following assembly of the pilot facility, the filters will be fed settled water from the Blackstone River to colonize and acclimate the filter biomass. Past experience indicates that acclimation should take place within three months. UMass graduate students will collect a bulk sample (approximately 130 L) of Blackstone River water each week and transport it to the pilot facility. Most of the sample will be

processed for isolation of its naturally-occurring organic matter, which will be used to maintain a consistent raw-water quality during the one-year test (see further discussion under "Test Waters" below). Approximately 25 L of each sample will be used for feeding the pilot system during the acclimation phase. In this way we will be seeding the filters with bacteria naturally present in the Blackstone and allowing them to reach some degree of steady state density. The fact that the raw water quality will change from week to week is not a problem; for the first three months, the objective will be to reach some level of acclimation rather than performing treatability testing. During the three-month acclimation phase, we will monitor removal of DOC and UV absorbance and the removal of selected ozone byproducts. Acclimation will have been achieved as soon as these water quality parameters have stabilized.

Once acclimated, the long-term testing will begin. Mixtures of the test compounds from Table 1 will be injected at levels that are typical of contaminated raw drinking waters. The spiked concentrations will be directly measured, along with the residual parent compounds and daughter products in the ozone contactor effluent and the four filter effluents. Samples will also be collected for the various bioassays. The pilot testing phase for the proposed compounds will continue for a full year from the beginning of the project. During this time we will examine removal performance for a range of temperatures, ozone doses and filtration rates. The values explored will span the typical values encountered or used in drinking water treatment.

Acclimation and breakthrough of the various parent and daughter compounds will be assessed based on measured influent and effluent concentrations. During the acclimation period, breakthrough of DOC (e.g., DOC_{eff}/DOC_{inf}) will be graphed versus bed volumes of water treated. After preliminary acclimation, the breakthrough calculations will be done on the filters run at differing flow rates resulting in differing empty bed contact times (EBCTs) as well as under differing temperature, and ozone doses. The variable EBCT runs will allow for the development of simple empirical models for biofiltration (e.g., Mitton et al., 1993; Huck et al., 1994). We will also explore the adaptation of quantitative structure activity relationships (QSARs) to the individual compound removals.

Task 5: Field Testing

Field testing will be conducted as part of a proposed follow-up study if any of the plants employing biological filtration are found to also have a measurable amount of EDCs in the raw water. The possibility of a field-testing phase of this work cannot be ascertained without results from the utility survey work, which is also a part of this study. Therefore, the field testing schedule is not fully defined at this point. We propose that it be done as part of a second year of work, with the full experimental design to be determined later.

Materials and Methods

Laboratory Pilot Setup

The small-scale laboratory pilot system will be assembled and configured as shown in Figure 1. The system will have four pilot filters. Two of the filters will receive ozonated water; and remaining two will receive water that was not pretreated with ozone. The entire system will be fed using test water (see Task 4 Test Water) from a single day tank that is continuously cycled through a constant-head reservoir. From this a portion of the water will flow by gravity through an ozonation column. The effluent of the column will be pumped through two test filter columns using a dedicated pump for each column. The remaining water will be pumped directly from the constant head tank through two control columns. The entire pilot system will be located in a constant temperature chamber so that temperature can be controlled and examined as a process variable.

Columns will be filled with fresh GAC media of about 1 mm in size prior to the start of the acclimation phase. Each column will be about 15 mm in diameter and 60 mm tall. A target EBCT of 10 minutes results in a required total flow of 6-7 L/day through the four pilot GAC columns. Half of this will be pre-ozonated, and an

ozonation column overflow must be incorporated to allow for collection of ozonated water without disturbing the GAC column rates. This overflow line will also allow the ozone contact time to be decoupled from the filtration rate.

The overall volume of test water needed to run the pilot for one year will be approximately about 2,800 liters. At 2 mg/L DOC, the total amount of NOM that is required will be about 5.5 g-Carbon.

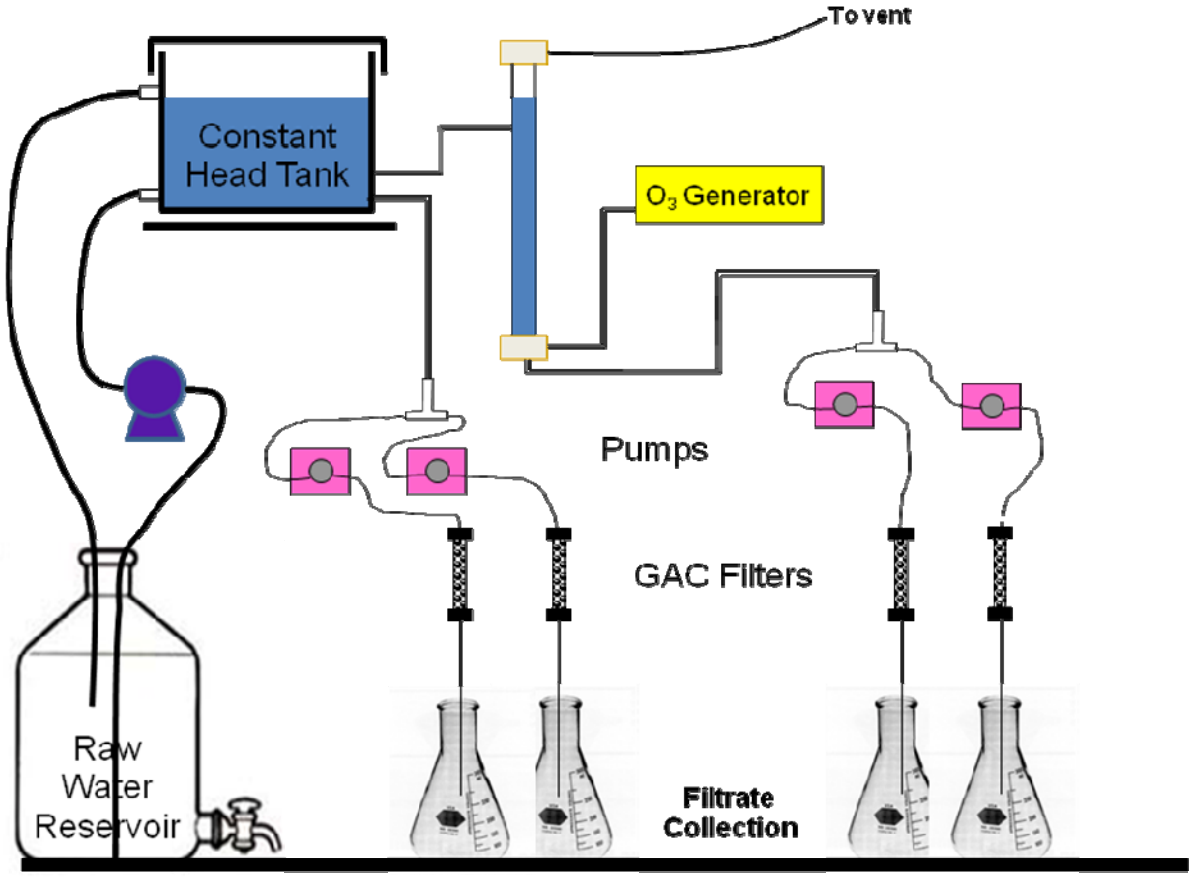


Figure 1. Pilot Laboratory Setup

Task 4 Test Water

The principal test water will be derived from the Blackstone River downstream of the Upper Blackstone Water Pollution Abatement District (UBWPAD) wastewater outfall. In order to have a one-year supply of this matrix that is of reliable and constant quality, we have decided to prepare freeze-dried extracts prior to starting the long term testing. A total of 1,100 liters of water will be collected in polyethylene carboys and transported to UMass where they will be stored at 4C until processing. These will be rotary evaporated in 20L batches down to 200 mL. Each will then be brought to dryness in a freeze dryer. This process will take about 11 weeks. Once the full volume is processed, the freeze dried material will be homogenized and stored in a freezer.

The pilot columns will be acclimated using unconcentrated, unaltered Blackstone River water with its natural consortium of bacteria. This will be stored for about one week at a time and re-collected as needed (anticipated frequency to be weekly). Based on past experience, we predict the acclimation period to be a maximum of three months. While the quality of this water will be variable, the purpose of the acclimation period does not require a highly uniform feed quality.

Once the acclimation period is complete, we will begin feeding re-constituted Blackstone River water prepared from the homogenized, freeze-dried extract. This material contains all of the NOM and salts that were in the Blackstone at the time of collection of the bulk sample.

Perchlorate Analysis

Low level perchlorate will be measured by Ion Chromatography, coupled with mass spectrometry. This is based on the IC/MS/MS method by Kroll. We use a Waters Alliance with an AS 14 column coupled to a Waters Micromass Quattro-Micro triple quadrupole mass spectrometer. Method detection limit for perchlorate by IC/MS/MS in our lab is about 0.1 µg/L. High level perchlorate is measured by the standard Ion chromatographic method. Method detection limit for perchlorate by IC in the UMass lab is about 2 µg/L.

EDC & PPCP Analysis

Analysis of organic Endocrine Disrupters and PPCP compounds at trace levels commonly employs methods using both gas chromatography – mass spectrometry (GC/MS) and liquid chromatography – mass spectrometry (LC/MS). The methods used by Shane Snyder and colleagues (e.g., Vanderford et al., 2003; Westerhoff et al., 2005) are among the most advanced and widely accepted in the drinking water field, and these form the basis of the UMass methodologies used in this research. All of the organic EDC and PPCPs in Table 1 can be readily analyzed by a single LC/MS method. Samples are extracted with a polar SPE cartridge (Oasis) and eluted in methanol. Extracts are then concentrated prior to injection into a Waters Quattro Micro triple quadrupole, coupled with either an Alliance HPLC or an Acquity UPLC. If necessary, we can also analyze many of these by GC/MS using similar methodologies along with our Waters-Micromass time-of-flight instrument.

Matrix suppression can be a serious problem with all methodologies when examining ng/L levels amidst a large background of organic and inorganic solutes. Snyder and colleagues (Vanderford & Snyder, 2006) have recently developed an isotope dilution methodology that avoids this problem. We will use isotopically-labeled forms of the target compounds to the extent they are available.

NDMA Analysis

N-Nitrosodimethylamine (NDMA) and other nitrosamines are determined in the UMass lab by GC/MS with prior activated carbon concentration. We use the US EPA approved method that makes use of Restek coconut charcoal cartridges. For separation and analysis, we use a Waters GCT, which is a time-of-flight GC/MS. Method detection limit for NDMA in our lab is about 0.7 ng/L.

Tests for Biological Activity

Estrogenic and antiestrogenic activity will be assessed in a cell-based reporter assay and by measuring changes in gene expression in the Japanese medaka fish. These methods will allow us to detect total estrogenic activity produced by the four estrogenic compounds listed in Table 1 (17β-estradiol, Estriol, Estrone, 17α-ethinylestradiol), as well as their active metabolites and any other unidentified estrogenic compounds in the water.

For the cell-based assay, we will use a stably-transfected MCF-7 cell line which produces luciferase in response to exposure to estrogenic compounds. Cells will be seeded in 96-well plates and exposed to a concentrated water sample reconstituted in cell culture media. A control 17β-estradiol dose-response curve will be included on each plate and luminescence will be quantified using the Bright-Luciferase Assay System (Promega) and a Fusion Plate Reader (Packard Bioscience). The dose-response included one every plate includes two negative controls .

For the whole-animal assays we will measure changes in mRNA levels of vitellogenin, an egg yolk precursor protein. Fish will be exposed for 96 hours to 1 L of non-concentrated water collected either directly from the field or directly after treatment. This assay relies on the fish to concentrate the estrogenic compounds within 96 hours. The water will be exchanged every 24 hours, and after 96 hours, fish will be sacrificed and livers removed (other tissues, e.g. gonads and brain also will be removed, stored in RNAlater® and archived for potential future studies or examination of expression of other genes). Total RNA will be isolated from each liver and, using vitellogenin-specific primers, mRNA levels will be quantified using real time RT-PCR (Roche Light Cycler). Detection limit is typically 10 femtomolar.

This whole-animal assay has several advantages over the cell-based reporter assay. First, the fish concentrate the estrogenic pollutants in the water, so no sample preparation is needed. Second, the detection limit for estrogenic compounds is significantly lower, i.e, the detection limit in fish is at the same level that is physiologically relevant for fish and for humans... Third, the compounds in the water may be metabolized by enzymes in the fish, and their metabolites (if estrogenic) will be detected, thus providing a more accurate assessment of the potentially estrogenic compounds in water. Fourth and most importantly, this method can be adapted easily to detect other biologically active compounds in the water sample. For example, exposure to perchlorate, a disruptor of the thyroid endocrine system, can be detected by changes in mRNA levels of thyroid hormone receptor or thyroid transcription factor. Likewise, all of the compounds listed in Table 1 result in changes in the mRNA levels of specific genes, and assays to detect their presence can be developed. This approach is potentially valuable because tissue from a single group of fish exposed to a single water sample can be used to detect the exposure to multiple compounds.

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Applications Potential

This research will help to clarify which types of EDC or PPCP structures are likely to be poorly removed by conventional treatment, and the impacts of the organic and inorganic matrix on removal. It will also provide valuable information on the efficacy of almost a dozen different treatment scenarios on EDC and PPCP removal. A subset of these will be analyzed for residual endocrine activity, providing insights into removal of bioactive daughter products.

Most importantly, this research will determine the effectiveness of ozone and biofiltration on the removal of EDCs and PPCPs, their daughter products and the accompanying endocrine activity.

These results will be helpful to any utilities that are faced with concerns over EDCs and PCPPs in their raw water. In some cases they will assist with reassuring the public that the existing treatment systems will protect them from such compounds. In other cases, they will be given guidance as to how they might upgrade treatment to handle recalcitrant EDCs and PPCPs.

Summary of Related Research

There is a growing body of research on the removal of EDCs and PPCPs in drinking water treatment systems. A few research teams have conducted broad surveys of monitoring compound removal from a single water under a limited set of conditions (e.g., Westerhoff et al., 2005). These are very useful for the industry, but represent just the beginning of our understanding. They have also tended to focus on physical and chemical treatment. Very little has been done on biologically active filtration (e.g., see discussion in: Horst et al., 2006). This is largely due to the experimental difficulties inherent in running well controlled biofiltration studies. When it has been done, there is almost never a long-term, consistent, supply of water for treatment studies.

Many others have looked at one or two compounds in detail, and focused on mechanisms, byproducts or dependence on treatment conditions. Very few have considered the full suite of byproducts and associated endocrine activity. Nearly all of these studies have been concerned with one-step oxidation treatments, such as ozonation (Hashimoto et al., 2006) or chlorination (e.g., Bedner & MacCrehan, 2006).

Despite the lack of information on bioproceses, or on daughter product formation and removal, there is starting to emerge a substantial body of information on removal of selected EDCs and PPCPs by several types of physico-chemical water treatments. Coagulation (here defined as addition of a coagulant, often with flocculation and followed by a solid:liquid separation step) results in generally poor removal and its effectiveness for the compounds can often be disregarded (Ternes et al., 2000; 2002; Westerhoff et al., 2005). Experimental and model results suggest that most PPCPs are not sufficiently hydrophobic to be removed by organic partitioning (e.g., see Gallary and MacKay, 2005). Adsorption by GAC and PAC is much more effective for many EDCs and PPCPs. Some like clofibric acid and ibuprofen are not as well removed and would require extensive use of activated carbon.

Many oxidants are capable of reacting with EDCs and PPCPs producing daughter products most of which have not been investigated. Ozone is one of the most effective, yet even for this there are some compounds that are nearly unreactive (e.g., ibuprofen, iopromide, diazepam, clofibric acid) (Snyder et al., 2007). These less reactive compounds might still be attacked by hydroxyl radicals in processes engineered to produce such reactive species (e.g., ozone/UV, ozone/peroxide, UV/peroxide) (e.g., Seitz et al., 2006; Crosina et al., 2006). Chlorine dioxide is presumably less effective than ozone, however little data of this sort have been published. We do know that chlorine is only partially effective and results in many types of halogenated daughter products. Several specific PPCPs have been studied with regard to their degradation kinetics and daughter products (e.g., Kwon et al., 2006; Bedner & MacCrehan, 2006; Buth et al., 2006). However only a very few oxidative studies have actually looked at estrogenic activity in the treated water or the daughter products (Lee & von Gunten, 2006; Rosenfeldt et al., 2006).

High pressure membrane filtration (reverse osmosis, RO, and nanofiltration, NF) can be effective depending on the membrane type and the compound being removed. Anionic PPCPs were found to be almost completely rejected by polyamide membranes through charge repulsion (Kimura et al., 2003). Neutrals PPCPs were more subject to size-based rejection. Many others have shown excellent removals with tight NF and RO (Drewes et al., 2002; Heberer et al., 2002; Xu et al., 2005).

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Quality Assurance/Quality Control

General Approach

Quality assurance is an essential and integral part of a research study. The purpose of the QA plan is to insure that valid and reliable procedures are used in collecting and processing the research data. The procedures outlined are designed to eliminate or reduce errors in experiments, sample preparation and handling, and analytical methods. Emphasis will be given throughout the project to incorporate the plan into the research project by all research personnel.

Any equipment and experimental procedure that are used to provide numerical data will be calibrated to the accuracy requirements for its use. Records shall be kept of all calibrations. Calibration schedules will be established for all aspects of physical and chemical measurements and will be strictly enforced. Physical standards and measuring devices will have currently valid calibrations, traceable to national standards. Chemical standards will be prepared using state-of-the-art analytical methods and materials of known purity (the highest purity available). Calibrations and standards obtained externally will adhere to the requirements for internal standards.

As a general rule, experiments will be replicated to assure reproducibility. All data reported will include a statement of its uncertainty, and the means for the determination and assignment of such limits. Standard reference materials will be used for this purpose where possible. Statistically established confidence limits and an analysis of sources of systematic error will be used in the absence of experimental demonstration of limits of inaccuracy.

Before a new method is used, or when an analyst performs a method for the first time, it is necessary to perform a set of tests called "initial demonstration of capability". For analytical methods these usually call for preparation of standard curves, method calibration and analysis of some test samples. Where available the test samples may be third party PT samples of known (or accepted) concentration in appropriate matrices. In other cases, these will be environmental samples and matrix spikes that have been prepared in a blind fashion.

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, meticulously detailed procedures, instrument and conditions of analysis, failed experiments, etc. Data output will be archived.

Weekly meetings (or more frequently if needed) will be held to review the results and project progress, and to plan further experiments. The results will be analyzed promptly and prepared for ultimate publication in scientific journals. The experimental and analytical procedures will be reviewed for their performances and changes will be made as necessary. The quality assurance program as described above will be strictly enforced.

Quality Assurance Objectives

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., 2005; USEPA-EMSL, 1990; ASTM, 1994). In addition to the analysis of sample replicates, approximately 10 percent of the time involved in analytical determinations will be devoted to quality control. The precision of each test is determined through analysis of sample replicates. These are commonly presented in the form of control charts (e.g. Section 1020B of APHA et al., 2005).

The accuracy of each analysis will be determined by measuring spike recoveries in the matrix of interest. The relative errors will be calculated and will be considered acceptable if they fall within the control limits determined for the particular test. For new methods developed under this research, we will have to establish criteria on acceptable control limits. In general, a test will not be deemed useful if its precision or accuracy is not within 25%. Where possible, external performance standards will also be run. This serves as a measure of accuracy both for the analysis and for standard preparation.
Data generated by the QA program will be incorporated into a Quality Control (QC) method that will monitor the fluctuations in precision and accuracy so that chance or assignable causes of error can be determined. Control charts such as X-charts for simple successive samples or cumulative sum techniques will be employed to record both precision and accuracy data (Taylor, 1987).

Procedures

General sample collection and handling will be in accordance with the guidelines of Section 1060 of Standard Methods (APHA et al., 2005). All previously established analytical methods used in the research will follow approved methods in the standard compilations (e.g., ,APHA et al., 2005; USEPA-EMSL, 1990, or ASTM, 1994).

Reagent grade chemicals or higher quality when needed will be used throughout the research. Super-Q water (purified by reverse osmosis, deionization, and carbon adsorption) or equivalent will be used for preparation of reagents, sample blanks, and dilution water. Where necessary, this water will be further purified using batch UV irradiation. Glassware used in the experiments and in analytical analyses are thoroughly cleaned, typically with a chromium-free sequence of detergent, oxidant and acid to prevent interferences from trace organics.

Chromatographic analyses will be standardized by the use of carefully prepared solutions of known standards.

In general, non-aqueous primary stocks will be kept in a -10^{o}C freezer and discarded after two months. Duplicate primary stocks will be prepared regularly, as a check against degradation of the primary stock. Data quality objectives for this work will be assured by: (1) use of blanks; (2) use of an internal standard; (3) analysis of duplicates; (4) determination of spike recovery; (5) analysis of a matrix standard; (6) monitoring of response factors; and (7) monitoring for spurious peaks.

Three types of blanks will be run daily or with each set of samples: (1) reagent blanks, composed of the extracting solvent(s) and internal standard; (2) Laboratory water blanks; and (3) Field blanks. This last type of blank is prepared by transporting laboratory reagent to the study site, and transferring it to a labeled sample vial at the time of general sample collection.

An internal standard will be used to control for solvent evaporation and variable injection volume. Most samples will be run in duplicate. If they differ by more than the acceptable range, additional GC or LC injections will be made. If a significant problem still exists, either the original sample will be re-extracted or the data will be discarded.

Spike recoveries will be determined for each analyte/method. Matrix standards will be prepared and analyzed with each method. These may be test-specific, but many types of DBP tests will make use of a bulk sample of raw drinking water (depending on the particular sample being studied). This would be treated with the oxidant of interest under well-defined conditions. Control charts are prepared and continually updated for this type of matrix standard. Data falling outside of the control limits require that the method be re-tested in order to bring it back under control. Calibration response factors will be monitored and compared from one day to the next. Significant changes in either these response factors or in the spike recoveries will be considered cause for method re-evaluation. Finally, general QA requires that all chromatograms be manually inspected for spurious

peaks. When such peaks are observed, potential sources must be investigated. If the problem cannot be corrected, the data may have to be discarded.

This outlines our general QA philosophy for chromatographic and other methods. Many specific details relating to the individual procedures may be found in the cited references, and other particulars must be adopted if new methods are developed.
A detailed set of standard operating procedure manual (SOPs) have been developed for use in the UMass environmental engineering laboratories. Some of these are in excess of 60 pages, so they are not attached with this proposal in the interest of space. Incorporated into these SOPs are a set of decision point controls requiring regular notification of the UMass QC officer (Reckhow) regarding QC data as they are generated or problems if they occur. These are intended to eliminate the possibility that analytical methods could fall out of control during the interval between weekly research meetings. Copies of the various SOPs are available from the PI upon request.

Sampling Custody

In most cases analyses will be performed immediately upon return from the field or after preparation or treatment of samples in the laboratory. Problems with sample custody will be minimized, because the same people will both receive (or sometimes, collect) the samples, and analyze them. In general sample collection, handling, and preservation will be in accordance with the guidelines of Section 1060 of Standard Methods (APHA et al., 2005).

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 by the PI to observe noteworthy trends or inconsistencies. These will be maintained in loose leaf notebooks for subsequent use in preparing quarterly or final reports. Major concerns and conclusions will be reported to the Project Officer via the project reports.

Laboratory data books will be backed up so that a file copy of raw data can be placed in safe storage in the event that the book is lost or destroyed. At the end of the project, all bound data books and any loose-leaf data will be stored by the project team for at least ten years. Summary data files will be put on magnetic or optical media so that statistical analysis of the data can be done.

Data quality objectives are selected so that both the stated project objectives and the QA criteria established for the particular analytical SOPs (non project specific) may be met. Because this project is largely one of an exploratory nature, there are very few clearly identifiable QA criteria that can be viewed as quantitative. Task 1 is aimed at summarizing existing knowledge and discovering potential utility partners. For this task to be completely successful, the project team must be able to find most of the relevant literature and produce a survey that yields high response rates and target data return. Task 2 is focused on a select group of utilities (about 12). This work will involve a substantial amount of sample analysis. Analytical functions focus on the EDCs and PPCPs as well as more standard parameters such as DOC and turbidity. The former are critical analysis (i.e., target organic pollutants). Task 3 incorporates laboratory process testing with the same type of analytical work as in Task 2. This requires analysis of oxidant doses and residuals. Finally, task 4, the long-term ozone-biofiltration lab pilot, involves most of the same analytical and process monitoring tests as in Task 3.

There are a few project-specific QA criteria that can be identified. Those that do exist pertain to the chemical and process analyses in Task 2-4. Numerical representations of these criteria can be found in the next section.

Quantitative QA Objectives

The quality criteria as proposed in the preceding section can be translated into numerical values for all of the analytical methodologies that are deemed critical as well as some that are non-critical. Table 5 contains a summary of those quantitative criteria. This information may be found in greater detail in the section on analytical methods and in the method SOPs found in Appendix A.

Table 5: QA Objectives for Critical Measurements

Measurement	Method	Reporting	QA Objectives[1]			
		Units	MDL	Precision[2]	Accuracy[3]	Completeness[4]
TOC and DOC	Combustion	mg/L	0.1	≤20	80-120	95
EDCs & PPCPs	LC/MS	µg/L	≤0.010	≤25	80-120	95
EDCs & PPCPs	GC/MS	µg/L	≤0.010	≤25	80-120	95

Other QA Objectives

The data collected in this study of any particular type are all being generated from a single laboratory and a single team of analysts. For example, all EDC chemical data are collected by the UMass Environmental Engineering team, whereas all endocrine activity is from the Arcaro lab. For this reason, questions of data comparability between laboratories are avoided.

Possible Non-Attainment of QA Objectives

Non-attainment of the QA objectives for TOC and various measures of PPCPs and EDCs could substantially compromise the project goals. All efforts will be made so that this does not happen. Since these QA objectives have been shown to be generally obtainable, and have been obtained in the past by the UMass laboratory, there is no reason to believe that this will become a problem.

[1] MDLs for chromatographic methods are analyte specific, values shown here are typical of most analytes
[2] Relative percent difference of laboratory duplicates
[3] Percent recovery of matrix spike as compared to distilled water spike or external standard
[4] Percent of samples targeted for this analysis for which valid measurements are generated

Appendix A: Sampling Procedures

Field Sampling

Sampling will be conducted in accordance with accepted procedures for TOC, and other trace organic compounds (e.g., Standard Method 5710, EPA method 552.2, Standard Method 6232, and 5320).

Bulk samples required for task 2, 3 and 4 will require that air-tight high-density polyethylene containers be used. These must be thoroughly cleaned in accordance with the above procedures prior to use.

Laboratory Subsampling

Laboratory subsamples will be collected directly from the large vessels used for transport from the field. These will be manually shaken and inverted several times prior to collection of any subsamples. On each day for which subsamples are taken, UV absorbance will be measured on the subsample. In this way we will be able to monitor for possible changes in the bulk sample and unrepresentative subsampling.

Laboratory Treatments

Coagulation procedures

Samples intended for coagulation tests will be treated at bench scale in the UMass laboratory. A single coagulant (usually alum) dose will be added, followed by rapid mix, slow mix, and gravity settling. The alum dose will be selected so as to reproduce the full-scale process. Supernatant will be drawn off and filtered through a pre-cleaned GF/F glass fiber filter.

Chlorination procedures

Samples for chlorination (Tasks 2 & 3) are treated with a chlorine dose that will leave the prescribed residual at the end of the incubation period. Reactions are conducted in headspace-free glass vessels (chlorine demand free) in the dark at a fixed temperature (20 C). Additional details may be found in the UMass SOP for Chlorination (available upon request).

Ozonation Procedures

When required for Tasks 2 and 3, samples will be ozonated in a semi-batch system. Ozone is generated from pure oxygen by means of a laboratory corona discharge generator. The ozone/oxygen product gas is introduced into a 2-L glass reaction vessel containing the water to be treated. Flow is controlled with an electronic flow controller, and the ozone content is monitored by direct UV absorbance spectrophotometry. The gas is mixed with the sample by a porous quartz frit. Off-gas is re-directed through a spectrophotometer for determination of ozone content. A membrane ozone electrode (Orbisphere) is fitted into the side of the glass reactor so aqueous ozone concentration can be continuously monitored. Ozone transferred is determined from the flow rates and the differences in ozone content in the applied gas versus the off-gas. For the purpose of this study we will use a fixed ozone/TOC ratio that results in an ozone exposure (CT) representative of system using ozone for primary disinfection. Additional details may be found in the UMass SOP for Ozonation (available upon request).

Chemical Analysis: Validated Methods

Total Organic Carbon

Total organic carbon (TOC) will be measured on nearly all samples in this research. It will be measured by the high-temperature combustion method (APHA et al., 1999) using a Shimadzu 5000 analyzer. Additional details may be found in the UMass SOP for TOC Analysis (available upon request).

Residual Chlorine (Free and Combined)

Residual chlorine will be measured by titrimetric DPD methodology (4500-Cl, D and F: APHA et al., 1999). We will be measuring residual chlorine species on all samples collected for DBP analysis. Additional details may be found in the UMass SOP for chlorine residual (available upon request).

Bioassays: Gene Expression in Medaka

Dr. Arcaro has Institutional Animal Care Use Committees approval for maintaining a breeding colony of medaka and for conducting aquatic toxicology exposure studies with medaka. The medaka colony is monitored twice a day and measurements of water quality and fish health recorded. These logs are reviewed by the PI daily and if any problems with the health of the fish arise the University Veterinarian is notified. As mentioned in the methods for the medaka assays, negative and positive control groups will be run with each water extract. The need for controls is balanced by the need to conserve the number of animals tested. The control groups selected as well as the sample size (n = 4 per group) are based on our preliminary result and therefore are most likely appropriate. If we determine that the sample size or control groups are either insufficient or more than necessary, we will adjust he experiments accordingly. The quality of the RNA obtained from the fish will be assessed with A_{260}/A_{280} ratio, and only high quality RNA will be examined for gene expression.

Calibration Procedures and Frequency

Calibration procedures are generally outlined in detail in the preceding sections on chemical analysis or in UMass laboratory SOPs. Table A-1 contains a summary of the calibration methods for the critical measurements. This must be viewed as a simplified presentation. The reader is referred to the previously mentioned sections for full details.

Table A-1. Summary of Calibration Requirements for Critical Measurements

Measurement	Laboratory	Device	Calibration Procedure	Frequency	Acceptance Criteria
TOC & DOC	Env. Eng.	TOC analyzer	Analysis of phthalate standard solutions	≥5 times per day	±5%
PPCPs & EDCs by LC/MS	Env. Eng.	Liquid Chromatograph & Mass Spec	Analysis of Laboratory-prepared standard mix at several concentration levels	≥1 time per day	±10% for each based on linear standard curve
PPCPs & EDCs by GC/MS	Env. Eng.	Gas Chromatograph & Mass Spec	Analysis of Laboratory-prepared standard mix at several concentration levels	≥1 time per day	±10% for each based on linear standard curve

Data Validation

The project PIs are ultimately responsible for all data validation. Although they may delegate this task to the graduate research assistants and post-docs on the project, they are ultimately responsible for its accuracy. For example, in the UMass environmental engineering laboratory, there is a designated graduate QC officer who certifies that data quality have met method criteria. This person then transmits the decision and data to one of the PIs (Reckhow), who either confirms or reverses the decision. Dr. Reckhow will take this responsibility for analyses conducted in the UMass environmental engineering laboratory, and Dr. Arcaro will do the same for those performed in the her laboratory.

Data Reporting

The two project PIs (Drs. Reckhow and Arcaro) are ultimately responsible for all data reporting. Reports generally originate with the graduate research assistants and post-docs on the project. They then move on to the PI, who will make changes, deletions or amendments. The reports may go though several internal iterations. Eventually all reports will be centralized and submitted to the AWWARF project officer.

Units for data reporting are indicated in Table 1 and in specific methods SOPs. All samples will be held for at least a month beyond analysis, in case reanalysis is required. All data will be archived for at least 5 years.

Progress reports and a final report will constitute the deliverables to the sponsor (AWWARF). These will include all reduced data from all primary samples and some example raw data, where appropriate. In the interest of brevity, the only quality control data that will be regularly reported will be analyte recovery and precision data. The project advisory committee (PAC) may request additional QC data.

The final report will have a separate QA section that will document all QA/QC activities and results. These results will be correlated to the primary data and they will clearly indicate the limitations of the data and the range of validity of the conclusions. If QA objectives are not met, we will include an explanation of the impact of not meeting the project's QA objectives.

General Types of QC Checks

Replicates

All critical laboratory analyses will be conducted in duplicate. When possible, duplicate samples will be collected in a sequential fashion. If this is not practical, single samples will be split in the laboratory and treated as replicates. Statistics on these replicate analyses will be kept and reported.

Spikes

All critical measurements will involve analysis of matrix spikes and laboratory water spikes. These are used for assessment of analyte recovery, calibration, general method performance and calibration check.

Blanks

A wide range of analytical and experimental blanks will be analyzed as part of this work. These include laboratory water blanks, various types of method blanks (e.g. carbon blanks for the TOX analysis), experimental blanks, and field blanks. Most of these are specific to the particular analysis, so details may be found in the SOPs.

All field sampling will include a field blank. This is a sample of laboratory grade water that has been sent out into the field. At the time and place of sample collection, the vessel containing the laboratory water will be

opened and the contents transferred to a properly labeled sample bottle. This will be returned to the PI's laboratory along with the field samples, and analyzed as a field blank.

Others

Each analytical method has its own unique set of QC procedures. These include calibration standards, calibrations checks, instrument tuning (m/e for mass spectrometers), initial demonstration of capability tests, MDL determinations, etc. These are most efficiently presented on a method-specific basis (see sections on chemical analysis and SOPs).

Method-Specific QC Checks

Formal QC checks and the associated corrective actions have been documented for the conventional analyses that have been deemed critical to this project. Non-conventional analyses do not have the same well-developed QC checks. These techniques are not fully matured. As a result they still require the unique judgments of the PIs. These expert judgments are employed on an ad-hoc basis, and are not easily codified as yet.

Listed below are guidelines and formulae for calculating data quality indicators. These will be used for such calculations over the course of this project.

Common Data Quality Indicators

Precision

Precision may be expressed as the relative percent difference (RPD) from duplicate measurements (C_1 and C_2) of the same sample:

$$RPD = \frac{|C_1 - C_2| x100\%}{(C_1 + C_2)/2}$$

When three or more replicates are available, the relative standard deviation (RSD) should be used:

$$RSD = \left(\frac{s}{\bar{y}}\right) x100\%$$

where the standard deviation (s) is determined from:

$$s = \sqrt{\sum_{i-1}^{n} \frac{(y_i - \bar{y})^2}{n-1}}$$

Accuracy

Accuracy is best assessed by analysis of a standard reference material (SRM) prepared in the matrix of interest. It is quite rare that such materials are available, so two possible compromise may be used instead. These are the laboratory-prepared matrix spikes, and the independent SRM prepared in a standard matrix. One or both may be analyzed and the percent recovery (%R) calculated as a measure of accuracy.

$$\%R = \left(\frac{S - U}{C_{sa}}\right) x100\%$$

where:

S = measured concentration in spiked aliquot
U = measured concentration in unspiked aliquot
C_{sa} = actual concentration of spike added

$$\%R = \left(\frac{C_m}{C_{srm}} \right) x100\%$$

and:

C_m = measured concentration of SRM
C_{srm} = actual concentration of SRM

Completeness

Percent completeness (%C) is defined as follows:

$$\%C = \left(\frac{V}{T} \right) x100\%$$

where:

V = number of samples for which valid measurements have been made
T = total number of samples for which measurements are to be made

Method Detection Limit (MDL)

The method detection limit (MDL) will be defined as:

$$MDL = s_7 \bullet t_{(n-1,1-\alpha=0.99)}$$

where:

s_7 = standard deviation of 7 replicate analyses where the mean is no more than 10 times the MDL
$t_{(n-1,1-\alpha=0.99)}$ = Students' t-value for a one-sided 99% confidence level and a standard deviation estimate with n-1 degrees of freedom.

Linearity

The calibration curve linearity (L) is defined as the ratio of the slope using the highest standards (S_U) divided by the slope determined from the lowest standards (S_L) as follows:

$$L = \frac{S_U}{S_L}$$

The highest standards shall be all those that fall within the top 50% of the calibration range including the 50% standard if it is used. If only one standard falls within that range, the S_U shall be calculated based on the top two standards. The lowest standards are all those that fall within the bottom 50% of the calibration range including the 50% standard if it is used. Least squares linear regression is used to determine slopes.

QC Reports to Management

For most analytical activities in this project, there will be three tiers of QC reporting. These involve communication and reporting up the QC chain of command as follows:

- Analyst
- Graduate QC officer
- Principal investigator
- Project officer (PO)

Reporting will be done by means of printed reports (all levels), computer files (mostly internal), and email (mostly internal).

Analyst reports to Graduate QA Officer

The analyst must report on all QA data collected as soon after analysis as possible. The initial recipient of these data is the graduate QA officer designated to oversee that particular method. The purpose of this report is to allow the graduate QA officer to compile all QA data on a particular analysis as they are generated throughout the laboratory by the various analysts. This person compiles these data in a computer spreadsheet and updates a standard set of control charts. The graduate QA officer must then determine if the QA data meet the method criteria. If they do not, the graduate QC officer meets with the analyst in an effort to determine the source of the problem. An initial corrective action plan is then developed.

Changes in any method SOP can be proposed at this point. These remain proposals until approval by the PI (for minor changes) or the PO (for major changes).

Graduate QA Officer reports to PI

The next step is for the graduate QA officer to report on the QA performance to the project's principal investigator. The PI either confirms or refutes the determination of the graduate QA officer. The PI also makes a final decision on any proposed corrective action.

PI reports to PO

Quarterly report prepared by the PIs will be submitted to the project officer. These reports will contain key QA data (e.g., accuracy and precision data). Minor changes in method SOPs and the QA project plan will be presented.

If major changes are proposed in either a method SOP or the overall QA project plan, this will be made known to the PO as soon as possible. Implementation of these changes will only occur after approval by the project officer.

Literature Cited:

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ASTM (1994) *Annual Book of ASTM Standards, Part 31: Water*. American Society for Testing and Materials, Philadelphia, PA.

Taylor, J.K. (1987) *Quality Assurance of Chemical Measurements*. Lewis Publishers, Inc., Chelsea, MI.
USEPA-EMSL. Methods for the Determination of Organic Compounds in Drinking Water - Supplement I. 1990. Washington, D.C., National Technical Information Service.

Abbreviations/Definitions

A_{260}/A_{280}:	Absorbance ratio to determine if there is protein or excessive RNA contamination
APHA:	Standard Methods for the examination of water & wastewater
Aromatic:	Six carbon ring characteristic of the benzene series & related organic group.
ASTM:	American Society for Testing & Materials
Bench Top Study:	Laboratory scale testing and experiments
Biofiltration:	Biological assisted filtration usually using granular activated carbon
Byproduct:	Compounds formed from the interaction of an oxidant with the parent compound. Byproduct is same as daughter products, but usually used in the context of disinfection with a negative connotation.
CT:	Concentration of disinfectant (C) multiplied by Residence time (T). Used by USEPA & States for determining microbial inactivation
Daughter Compounds:	Secondary compounds created as a result of treatment steps such as the addition of an oxidant
DBP:	Disinfection byproduct
DNA:	A nucleic acid that carries the genetic information in the cell and is capable of self replication and synthesis of RNA
DOC:	Dissolved organic carbon
DPD:	N,N-Diethyl-P-Phenylenediamine: Chemical used to measure residual chlorine
EBCT:	Empty bed contact time
EDC:	Endocrine disrupting compounds
Field Study:	Pilot test performed in field with the goal to mimic full scale
Full Scale:	Actual size of treatment system
Functional Group:	An atom or group of atoms that replaces hydrogen in an organic compound and that defines the structure of a family of compounds and determines the properties of that family
GAC:	Granular Activated Carbon
GC:	Gas Chromatograph

Hormonal Activity:	The ability of a compound to elicit a physiological response in an organism which in this study uses freshwater Japanese Medaka fish
Hydrophobic:	Compounds generally of a non-polar character with limited water Association
IC:	Ion Chromatograph
LC:	Liquid Chromatography
MassDEP:	Massachusetts Department of Environmental Protection
MDL:	Method detection Limit: The statistical lower limit of a specified method
Mechanistic:	The physical pathway in which a parent compound changes to a daughter compound
mRNA:	Messenger Ribonucleic acid
MS:	Mass Spectroscopy
Multi-barriers:	Consecutive unit treatment operations used remove or transform a target compound
NDMA:	N-Nitrosodimethylamine
NOM:	Natural organic matter
PAC:	Project advisory committee
Parent Compounds:	The original starting compound before transformation in this research by an oxidant or biological means
PI:	Principal Investigator
Pilot Study:	Small scale testing, sometimes performed in field, to mimic full scale
PO:	Project Officer
PPCP's:	Pharmaceutical & personal care products
QA/QC:	Quality Assurance/Quality Control: A plan to ensure that valid and reliable procedures are used in collecting & processing data
QSAR:	Quantitative structure activity: The process of relating the structure of the compound with removal of the compound
Reagent Grade:	Chemicals suitable for use in general laboratory applications; meets in-house established limits in the absence of any compendial reference for the given compound. In-house established limits & their associated test methods are in many cases derived from common compendial methods such as USP, NF, ACS, etc.

RNA:	A single stranded chain of alternating phosphate and ribose units. The structure & base sequence of RNA are determinants of protein synthesis & the transmission of genetic information
RNAlater®:	An aqueous tissue storage reagent that rapidly permeates most tissues to stabilize and protect RNA in fresh specimens
RPD:	Relative percent difference
RT-PCR:	Real Time Polymerase Chain Reaction: A biochemistry technique for isolating or amplifying a fragment of sequence of DNA
SC:	Special Collaborator
SOP:	Standard Operation Procedures: An accepted standardized method of performing a task
SRM:	Standard Reference Material
TOC:	Total Organic Carbon
TOX:	Halogenated total organic compounds
US EPA:	United Sates Environmental Protection Agency
USEPA-EMSL:	Methods for the determination of organic compounds in drinking water-Supplement I
UMass:	University of Massachusetts at Amherst
US:	United States
UV:	Ultraviolet (Wavelength)