2017 Project Abstract
For the Period Ending June 30, 2020

PROJECT TITLE: Assessment of Household Chemicals and Herbicides in Rivers and Lakes
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2017, Chp. 96, Sec. 2, Subd. 04a as extended by M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 19

APPROPRIATION AMOUNT: $ 236,000
AMOUNT SPENT: $ 235,871
AMOUNT REMAINING: $ 129

Sound bite of Project Outcomes and Results
The levels of quaternary ammonium compounds (QACs), which are used widely as disinfectants and for other purposes, were measured in Minnesota wastewaters and sediments. The QACs are present at microgram per liter levels in wastewater. While the QACs are slowly degraded in surface waters by bacteria and light, they accumulate in sediments. The QACs form specific suspected carcinogens during water disinfection in very low yield, and QACs are likely less important that other precursors for these toxins. The results provide information on current QAC levels and provide insights on how to lower them if desired.

Overall Project Outcome and Results
Quaternary ammonium compound (QACs) are ingredients in personal care products, fabric softeners, disinfectants, and herbicides. QACs, which are biologically active molecules, are unintentionally and intentionally released into the environment. QACs kill bacteria and may affect microbial communities in wastewater treatment and algal communities in surface waters. In this study, the levels of QACs in the effluent from 12 wastewater treatment plants were determined. Plants with more advanced treatment processes had lower levels of QACs. Sediment samples in a lake demonstrated potential inputs from both municipal wastewater effluent and agricultural sources for QACs. In sediment cores taken from lakes, two distinct trends over time were observed. In lakes with large watersheds and mixed domestic and industrial wastewater sources, peak concentrations of QACs were found at depths corresponding to deposition in the 1980s and decreases after this time are attributed to improved wastewater treatment and source control. In a smaller lake with predominantly domestic wastewater inputs, concentrations of QACs increased slowly over time. In surface waters, QACs were found to degrade by reaction with reactive species (hydroxyl radicals) generated by sunlight and by microbial processes. Even with these loss processes, QACs likely persist from days to weeks in the water, leading to their deposition in the sediments. QACs were found to form low levels of a carcinogenic class of compounds (nitrosamines) when reacted with a drinking water disinfectant (chloramine), but this would be of greatest concern in wastewater potable reuse scenarios. The overall results of the work indicate that QACs are being released by wastewater treatment plants. Once in the environment, degradation by bacteria and by sunlight can occur in surface waters, but accumulation in sediments, where the QACs are persistent, is likely the main removal process. During the wastewater disinfection process QACs can form a carcinogen, but QACs are not as important as other chemicals known to form nitrosamines. The findings allow more robust assessment of potential impacts of QACs and insight into wastewater treatment processes that lead to removal, which is important given the increasing use of QACs during the COVID-19 pandemic.

Project Results Use and Dissemination

Three papers were published: 1) the detection of QACs in wastewater and sediment (https://doi.org/10.1039/C9EM00554D; Data set https://doi.org/10.13020/ram6-m093); 2) Photolysis of QACs (https://doi.org/10.1039/D0EM00086H); and 3) Potential environmental impacts of elevated QAC usage during the COVID-19 pandemic (https://doi.org/10.1021/acs.estlett.0c00437 or https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7341688/). A public lecture that incorporated data for the project was also given at the U of MN, and it is available on YouTube (https://www.youtube.com/watch?v=BL-B_CPNPYY&feature=youtu.be).

Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2017 LCCMR Work Plan Final Report

Date of Report: July 24, 2020

Final Report

Date of Work Plan Approval: 06/07/2017

Project Completion Date: June 30, 2020

PROJECT TITLE: Assessment of Household Chemicals and Herbicides in Rivers and Lakes

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Location: Statewide

Total ENRTF Project Budget:		
	ENRTF Appropriation:	**$236,000**
	Amount Spent:	**$235,871**
	Balance:	**$129**

Legal Citation: M.L. 2017, Chp. 96, Sec. 2, Subd. 04a as extended by M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 19

Appropriation Language:
$236,000 the first year is from the trust fund to the Board of Regents of the University of Minnesota to quantify environmental levels of household chemical and herbicide ingredients in rivers and lakes and assess their potential to form toxic by-products.

Carryforward; Extension (a) The availability of the appropriations for the following projects is extended to June 30, 2020: (15) Laws 2017, chapter 96, section 2, subdivision 4, paragraph (a), Assessment of Household Chemicals and Herbicides in Rivers and Lakes.

I. PROJECT TITLE: Household chemicals as water pollutants and toxic precursors

II. PROJECT STATEMENT:
Personal care products, fabric softeners, disinfectants, and herbicides (both those used on land and to control aquatic plants) all have something in common. Each contains a type of chemical known as a quaternary ammonium compound (QAC or "quack"), which can react to form carcinogens in tap water. The overall goal of this project is to improve water quality, protect ecosystem health, and limit the production of carcinogens during tap water disinfection by 1) Quantifying current and historical levels of these pollutants by measuring concentrations in surface water and sediment samples, 2) Evaluating of the persistence of these compounds in surface waters through studies of biological degradation and indirect photolysis, and 3) Determining the levels of carcinogenic nitrosamines produced attributable to these contaminants during drinking water and wastewater disinfection.

Either intentionally (in use as herbicides as active or inactive ingredients) or unintentionally (via release in wastewater effluent due to incomplete removal in the treatment process), QACs enter the environment. They are biologically active molecules. They kill bacteria, and evidence suggests exposure to these chemicals may affect microbial communities in wastewater treatment and algal communities in surface waters. There is also evidence that exposure to QACs may lead to development of antibiotic resistance. There are also potential effects on plants, because there are herbicides that contain this moiety. Lastly, evidence suggests that if these common chemicals react with the disinfectant used in drinking water and wastewater treatment (chloramines) potent carcinogens known as nitrosamines are produced. *The types and amounts of QACs entering and present in Minnesota's lakes and rivers are unknown.* Our hypotheses are that levels of these chemicals in Minnesota's water and sediment and the carcinogenic nitrosamine formation potential are driven by household uses.

Gathering information about QAC presence and reactivity will reveal the magnitude of any threats to human or ecosystem health posted by these chemicals, which include development of antibiotic resistance, disruption of plant/algal communities, and production of carcinogens in drinking water. This project will also make it possible to evaluate the major sources of these chemicals to Minnesota's waters. This knowledge will be critical in determining if regulations are needed or if risks can be ameliorated via alterations to/improvements in water treatment processes or product usage recommendations.

III. OVERALL PROJECT STATUS UPDATES:

Project Status as of January 1, 2018:
Efforts to date have focused on developing a liquid chromatography / mass spectrometry (LC/MS) method for targeted and untargeted analysis of QACs in water samples. The current method will allow quantification of 20 selected QACs down to the ng/L-range. Further optimization of solid-phase extraction procedures will be performed to decrease quantification limits in water samples and an accelerated solvent extraction procedure will be developed for analyzing sediment samples. Additionally, preliminary work has begun to assess persistence of QACs in sunlit surface waters, in particular looking at certain indirect photolysis mechanisms. The reactivity of a commonly used QAC, C-12 benzalkonium chloride, with hydroxyl radical was assessed in the solar simulator using hydrogen peroxide as a model sensitizer. Hydroxyl radical is one of the photochemically produced reactive intermediates (PPRIs) that dissolved natural organic matter can generate upon absorption of sunlight in natural waters. Further irradiation experiments with model sensitizers will be conducted for other PPRIs, such as singlet oxygen, and other QACs in the next few months.

Project Status as of July 2018: Work has continued to assess the reactivity and persistence of QACs in sunlit surface waters by evaluating indirect phototransformation of several QACs in the solar simulator and outdoors through sensitizer and quencher experiments and experiments in river water. Optimization of solid-phase extraction procedures was successfully completed leading to the possibility to quantify the 20 target QACs in water samples down to 36-506 ng/L. In the first wastewater effluent sample collected on June 13, 2018 from a

wastewater treatment plant in the Metro area, five QACs were detected with concentrations ranging between 76 and 423 ng/L. Future efforts will focus on collecting and analyzing more wastewater effluent and surface water samples to better assess the occurrence of QACs in the aquatic environment. Additionally, extraction procedures for sediment samples will be further optimized to increase recoveries and enable the quantification of QACs in sediment samples in the ng/g range.

Amendment Request (7/23/18): An amendment is requested to change the project completion date to June 30, 2020. No additional funds are needed, but the rate at which the compounds degrade is longer than anticipated, thus requiring additional experimental time and replication. It is recognized that such an extension requires approval via inclusion in the 2019 ENRTF bill and is not guaranteed.

Amendment Request (10/16/18): Supply costs have been lower than expected due to the fact that we did not need to purchase as many isotopically labelled standards are we originally thought. The method development for Activity, however has been significantly more complicated than we anticipated, and we have had to do much more testing and validation that we thought we would (based on the method descriptions in the literature). Thus, we will need additional analytical time to analyze the samples collected from the environment (Activity 1) as well as the samples generated in Activities 2 and 3. The overall budget line changes are as follows: supplies reduced from $18,000 to $9,000; analytical time increased from $9,000 to $19,000; travel reduced from $2,000 to $1,000. Specific changes by activity are in the budget spreadsheet. Amendment Approved by LCCMR 10/22/18.

Project Status as of January 1, 2019: The previously developed LC/MS method was applied to quantify QAC levels in 12 wastewater effluent samples collected in November 2018 from different treatment plants in southern Minnesota. Thirteen of the 22 target QACs were found at concentrations above limit of quantification (LOQ) in at least one of the samples with average concentrations between 1 and 1534 ng/L. The different effluent samples varied substantially in the number of compounds detected above LOQ and the cumulative QAC concentrations likely due to a combination of different input levels and different treatment processes. Sediment extraction procedures were successfully completed, and a first set of sediment samples were analyzed. Additional sediment samples will be analyzed in the coming month to obtain depth profiles of QACs in 4 different lakes. Work is ongoing for Activity 2. Sensitizer experiments to determine QAC reactivity with photochemically produced reactive intermediates are still being conducted and evaluation of QAC loss in natural waters will be continued. Biodegradation experiments to determine loss of select QACs in river water are in progress.

Project Status as of July 1, 2019: Hydroxyl radical sensitizer experiments were completed successfully with a series of QACs. Singlet oxygen sensitizer experiments were successfully completed except for one compound for which further tests are needed. From these experiments, second-order reaction rate constants between the respective QAC and photochemically produced radical species were calculated. River water experiments under simulated sunlight were successfully completed for selected QACs, and results indicate reaction with hydroxyl radical is important for QAC degradation in sunlit surface waters and indirect photolysis half-lives were estimated. Replicate outdoor photolysis experiments are ongoing. Biodegradation experiments were completed for a mix of QACs demonstrating degradation of the compounds over sequential spikes. Analysis of this data is ongoing.

Project Status as of January 1, 2020: Indirect and direct photolysis experiments in buffer and river water under either simulated or natural sunlight were completed for the QACs studied. The results have been written up in a manuscript to be submitted for publication soon. Biodegradation experiments are complete, though additional analysis is being performed for the sequencing data, and a manuscript is being drafted for the biodegradation data.

Overall Project Outcomes and Results: Quaternary ammonium compound (QACs) are ingredients in personal care products, fabric softeners, disinfectants, and herbicides. QACs, which are biologically active molecules, are unintentionally and intentionally released into the environment. QACs kill bacteria and may affect microbial communities in wastewater treatment and algal communities in surface waters. In this study, the levels of QACs in the effluent from 12 wastewater treatment plants were determined. Plants with more advanced treatment processes had lower levels of QACs. Sediment samples in a lake demonstrated potential inputs from both municipal wastewater effluent and agricultural sources for QACs. In sediment cores taken from lakes, two distinct trends over time were observed. In lakes with large watersheds and mixed domestic and industrial wastewater sources, peak concentrations of QACs were found at depths corresponding to deposition in the 1980s and decreases after this time are attributed to improved wastewater treatment and source control. In a smaller lake with predominantly domestic wastewater inputs, concentrations of QACs increased slowly over time. In surface waters, QACs were found to degrade by reaction with reactive species (hydroxyl radicals) generated by sunlight and by microbial processes. Even with these loss processes, QACs likely persist from days to weeks in the water, leading to their deposition in the sediments. QACs were found to form low levels of a carcinogenic class of compounds (nitrosamines) when reacted with a drinking water disinfectant (chloramine), but this would be of greatest concern in wastewater potable reuse scenarios. The overall results of the work indicate that QACs are being released by wastewater treatment plants. Once in the environment, degradation by bacteria and by sunlight can occur in surface waters, but accumulation in sediments, where the QACs are persistent, is likely the main removal process. During the wastewater disinfection process QACs can form a carcinogen, but QACs are not as important as other chemicals known to form nitrosamines. The findings allow more robust assessment of potential impacts of QACs and insight into wastewater treatment processes that lead to removal, which is important given the increasing use of QACs during the COVID-19 pandemic.

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Measurement of quaternary ammonium compound pollutants in river water, sediment cores, and surface sediments

Description: Because QACs stick to particles, the most likely environmental compartment to find them in is sediments. The target compounds will be selected based on 1) previous reports of QACs in the literature, 2) an evaluation of consumer products available in the Twin-Cities area, and 3) agricultural QAC sales data from the Minnesota Department of Agriculture. The Arnold lab has samples of sediment collected from four lakes and two rivers as part of an ongoing ENTRF project (Antibiotics and Antibiotic Resistance Genes in Minnesota Lakes) stored in freezers. We will evaluate previously reported methods using an accelerated solvent system to extract both wet and freeze-dried samples. We anticipate a clean-up step using solid phase extraction will be required. We will use a spike and recovery method to evaluate extraction efficiency of the QACs, and isotopically labelled surrogates to determine recovery.

Once extracted, the concentration of the target chemicals will be measured using liquid chromatography tandem mass spectrometry. The sediment cores will provide information about usage over time, and the surface sediments will reveal usage patterns across the state. The different QACs found will allow assessment of the loadings of these compounds to surface waters in Minnesota, as well as attribution to different sources/uses.

We will also collect wastewater effluents from several wastewater treatment plants and river water samples downstream of wastewater treatment plants and elsewhere along the Minnesota and Mississippi Rivers. These samples will be extracted using the solid phase extraction method developed for clean-up of the sediment samples and analyzed using the same mass spectrometry methods. The water samples will provide information about current environmental discharges. We will also attempt untargeted analysis to determine if any unanticipated quaternary ammonium compounds are present.

Summary Budget Information for Activity 1: **ENRTF Budget: $ 95,000**
Amount Spent: $ 94,871

Balance: $ 129

Outcome	Completion Date
1. Optimize extraction and analytical methods	12/31/17
2. Measure concentrations in sediment samples	10/31/18
3. Measure concentrations in water samples	4/30/19

Activity 1 Status as of January 1, 2018:
The initial focus was on the development of a targeted analytical method for quantifying 20 selected QACs spanning a wide range of hydrophobicity. The current method utilizes nanoflow-HILIC liquid chromatography coupled to accurate mass and high-resolution mass spectrometry (LC-HRAM-MS/MS) providing excellent sensitivity and appropriate accuracy with minimal amount of organic solvent consumption. The current method will allow quantification of the selected QACs in the ng/L-range in water samples and in the ng/g-range in sediments. The developed LC-HRAM-MS/MS method will also be applicable for untargeted screening of additional QACs. Optimization of the solid-phase extraction procedure, which will be used for both water and sediment samples, is still ongoing. Current recoveries are around 20% for the targeted QACs and will have to be increased to at least 50% before optimization of sediment extraction procedures will be pursued.

Activity 1 Status as of July 1, 2018:
The solid-phase extraction procedure for analyzing water samples was successfully completed. Absolute recoveries for the target compounds ranged from 14% to 129% in a wastewater effluent sample collected on June 13 resulting in limits of quantification (LOQs) between 36 and 506 ng/L. Five QACs were found in the effluent samples at concentrations above LOQ with concentrations ranging from 76 ng/L for C14-BAC to 423 ng/L for C18-DADMAC. Additional wastewater effluent and surface water samples will be collected and analyzed in the future to obtain a better picture of QAC occurrence in the aquatic environment. Optimization efforts for sediment extraction procedures are still ongoing and will enable the analysis of sediments samples in addition to water samples.

Activity 1 Status as of January 1, 2019:
Wastewater effluent samples were collected from 12 different treatment plants in the Metro area and southern Minnesota in November 2018 and analyzed with the previously developed method. Thirteen of the 22 target QACs were found at concentrations above LOQ in at least one of the effluent samples. For these compounds, average concentrations ranged between 1 and 1534 ng/L. In most effluent samples, the highest levels were found for C18-DADMAC, reaching up to 7194 ng/L. The different effluent samples varied substantially in the number of compounds detected above LOQ (3 to 11) and the cumulative total QAC concentrations (83-9909 ng/L). The main reasons for these differences are likely a combination of different input levels and different treatment processes. Sediment extraction procedures were successfully completed with final absolute recoveries ranging from 1.3-67% depending on the compound and type of sediment sample. In a first set of sediment samples analyzed, and nine QACs were detected above LOQ (1-436 ng/g) in at least one sample. Additional sediment samples will be analyzed in the coming month to obtain depth profiles of QACs in 4 different lakes. Further efforts will focus on establishing a nontarget analysis approach of the measured samples to identify the presence of additional QACs in both wastewater effluent and sediment samples.

Activity 1 Status as of July 1, 2019: Measurements for water and sediment samples are complete. The non-target approach identified additional compounds for which we did not have chemical standards. This allowed an estimate of overall QAC concentrations in environmental matrices. The analysis of the data is being finalized, and submission of two journal articles is expected soon.

Activity 1 Status as of January 1, 2020: Suspect screening protocols for identification of non-target analytes was finalized.

Final Report Summary: To assess the levels of QACs in aquatic environments, a liquid chromatography high-resolution mass spectrometry method using both target and suspect screening was developed. The water and sediment sample preparation, measurement, and data analysis workflow were optimized for 22 target compounds with a wide range of hydrophobicity, including ionic liquids that have potential use as solvents and QACs common in personal-care and sanitizing products. In wastewater effluents, average concentrations of all target and suspect QACs combined ranged from 0.4 μg L^{-1} to 6.6 μg L^{-1}. Various homologs of benzylalkyldimethylammonium (BAC) and dialkyldimethylammonium (DADMAC) as well as the ionic liquid butylpyridinium and 15 suspect QACs were detected in at least one wastewater effluent sample. A spatial profile of sediment samples in a lake demonstrated potential inputs from both municipal wastewater effluent and agricultural sources for BACs. In sediment cores, two distinct trends of temporal QAC accumulation were observed. In lakes with large watersheds and mixed domestic and industrial wastewater sources (Lake Pepin and Duluth Harbor), peak concentrations of QACs were found at depths corresponding to deposition in the 1980s and decreases after this time are attributed to improved wastewater treatment and source control. In a smaller lake with predominantly domestic wastewater inputs (Lake Winona), concentrations of QACs increased slowly over time until today. These data provide information about loadings to the environment and evaluation of the necessity of alteration of wastewater treatment practices. Additionlly, the data provide baseline levels present in Minnesota prior to the COVID-19 pandemic.

ACTIVITY 2: Evaluation of environmental persistence in surface waters

Description: The impact of QACs on algal or microbial communities will be a function of both their concentration and persistence. The persistence will be dictated by the (bio)availability as well as their reaction with reactive species produced by sunlight.

QACs, being positively charged, may associate with negatively charged dissolved organic matter. If associated with organic matter, the compounds may be less available to bacteria to degrade. Alternatively, because organic matter produces reactive species, such as hydroxyl radical, this association may enhance degradation via indirect photolysis. Using an established solid-phase microextraction method, the partitioning coefficients of the target QACs to dissolved organic matter (using standard materials, river water, and wastewater effluent) will be measured. By comparing how the chemical structure affects this partitioning, the potential effects on the susceptibility to degradation processes for various QACs will be evaluated.

The native microbiology in the river may be able to degrade the compounds. This will be tested by dosing river water samples collected from the Mississippi River with QACs and monitoring their loss over time using liquid chromatography. The detection methods will depend on the dosed concentration. The reactors will be 1-L Erlenmeyer flasks with a foam plug to allow air exchange. The reactor will be stirred and sampled as function of time. Biomass will be estimated via measurement of optical density and volatile suspended solids.

If associated with the dissolved organic matter in surface waters, QACs would be especially susceptible to reaction with reactive species, such as hydroxyl radicals. Because these reactive species are produced when organic matter is exposed to sunlight, the concentration of such species is elevated near the organic matter. The rate constants of the QACs with hydroxyl radical and singlet oxygen will be measured by generating these species in the laboratory. Experiments will then be performed in river water and wastewater effluent to measure 1) the concentrations of singlet oxygen and hydroxyl radical produced using chemical probes and 2) the loss rate of QACs due to indirect photolysis. Indirect photolysis will be quantified as the difference between direct photolysis (measured in distilled water) and that in the organic matter-containing water. Specific indirect photolysis processes will be confirmed with quenchers. Because the chemical probes measure the concentration of hydroxyl radical and singlet oxygen in bulk solution, a comparison between the predicted and actual QAC loss rates will reveal if the QACs experience higher than expected concentrations due to their association with organic matter.

The product of this activity will be rate constant and sorption parameters that will be of use to predict QAC lifetimes in lakes and rivers.

Summary Budget Information for Activity 2:

ENRTF Budget: $ 65,000
Amount Spent: $ 65,000
Balance: $ 0

Outcome	Completion Date
1. Measurement of partitioning to river water and wastewater dissolved organic carbon	6/30/18
2. Measurement of biodegradation rates in river/lake water	9/30/18
3. Quantification of indirect photolysis reaction rate constants	6/30/19

Activity 2 Status as of January 1, 2018:
Efforts have started with Outcome 3 instead of Outcome 1 due to development of the relevant protocols for another effort. The reactivity of C-12 benzalkonium chloride (BAC) with hydroxyl radical was assessed in the solar simulator. The compound was spiked into pH 7 buffer along with hydrogen peroxide, which is a model sensitizer that generates hydroxyl radical when it absorbs light. C-12 BAC was quantified using high-pressure liquid chromatography (HPLC) to track loss of the parent compound. Initial results indicate that the BACs will be susceptible to reaction with hydroxyl radicals. Additional experiments to quantify steady-state hydroxyl radical concentration, assess the reactivity of other QACs, and measurement of other photolysis processes will also be carried out.

Activity 2 Status as of July 1, 2018:
Work has continued on Outcome 3 to study indirect phototransformation of QACs. Additional solar simulator experiments were conducted using hydrogen peroxide to assess the reactivity of longer-chained BACs (C-14 and C-18) as well as of another QAC, benzethonium chloride (BZT), which is found in commercial products. These hydrogen peroxide solar simulator experiments were also conducted with the addition of a hydroxyl radical probe, para-chlorobenzoic acid (pCBA) to determine second-order reaction rate constants between the C-12, C-14, and C-18 BACs and BZT and hydroxyl radical via competition kinetics. Preliminary results indicate reactions occur at diffusion-controlled rates. Assuming a typical steady-state hydroxyl radical concentration in natural waters, the calculated second-order rate constants were used to estimate compound half-lives in river water. Based on these values, a prolonged exposure experiment was planned using river water.

Mississippi River water was collected and filter-sterilized to perform experiments assessing reactivity of some of the QACs in natural waters due to the presence of dissolved organic matter. Filtered river water was analyzed for dissolved organic carbon content, pH, and nitrite/nitrate concentrations. First, an experiment was conducted in the solar simulator. The BACs, BZT, C-10 alkytrimethylammonium bromide (ATMA), and C-12 dialkyldimethylammonium bromide (DADMA) were irradiated in the solar simulator over 15 hours in ultrapure water and river water with a hydroxyl radical probe; a chemical actinometer, p-nitroacetophenone/pyridine (PNAP-pyr), was also run in parallel to determine direct photolysis rate constants and quantum yields of the QACs studied. Rooftop exposure experiments were then conducted over the course of several days for a total of approximately 25 hours of sunlight irradiation with the BACs and BZT in river water. Samples containing a hydroxyl radical probe and quencher were exposed to determine second-order rate constants and assess the role of hydroxyl radical in contaminant loss.

Quencher experiments were conducted in the solar simulator for the BACs in river water in order to parse out which reactive intermediates generated from constituents in the river water are most responsible for compound degradation.

Activity 2 Status as of January 1, 2019:

Work has continued with Outcome 3, performing additional solar simulator sensitizer experiments to assess BAC and BZT reactivity with singlet oxygen. Rose Bengal is used as a sensitizer, furfuryl alcohol as a probe compound, and histidine as a quencher. Replicates of experiments are ongoing. Work has begun on Outcome 2 to assess whether QACs can be biodegraded by the native microbiological community in Mississippi River water. River water microcosms were set up in the lab and incubated aerobically at room temperature. The microcosms are dosed with a mixture of C-12 BAC, ATMA, and DADMA at 50 nM each, well below minimum inhibitory concentrations. Samples are collected over time for analysis. Compound loss is monitored by LC/MS. After 7 days, the average removal was about 84% for BAC, 73% for ATMA, and 56% DADMA. The reactors were re-spiked and kinetics monitored two additional times, exhibiting faster kinetics than the first spike. Outcome 2 will also explore whether sustained exposure to low levels of QACs induces changes in the microbial community and influences the prevalence of QAC resistance genes and class 1 integrons, clinically important mobile genetic elements that are ubiquitous in the environment and can acquire and express QAC and antibiotic resistance genes. The abundance of 16S rRNA, *qac*, and *int1* genes in temporal samples will be quantified by qPCR and DNA will be sequenced to determine community richness and composition changes.

Activity 2 Status as of July 1, 2019:
For Outcome 3, it was determined by sensitizer experiments that the QACs studied (C-12 and C-14 BAC, BZT, C-12 ATMA, and C-12 DADMA react with hydroxyl radical at or near diffusion controlled rates (second-order reaction rate constants on the order of $10^9 – 10^{10}$ $M^{-1}s^{-1}$). Structure did not seem to have a significant influence on the rate of reaction except for DADMA, which reacted at a rate ten times lower than the others. Half-lives in sunlit surface water were estimated using an expected hydroxyl radical concentration in natural waters under June mid-day sunlight conditions and assuming 7 hours of sunlight per day. It was calculated that compounds might persist for: 23 ± 5 days (C-14 BAC), 25 ± 3 days (C-12 BAC), 25 ± 1 days (BZT), 34 ± 13 days (C-12 ATMA), and 117 ± 29 days (C-12 DADMA). Mississippi River water experiments including quenched controls under simulated sunlight for BZT, C-12 BAC, and C-14 BAC were consistent with sensitizer experiments. The results indicated reactions with hydroxyl radical would dominate in natural systems. Using the same assumptions as for the sensitizer experiments, half-lives were estimated to be 30 ± 3 days (BZT), 24 ± 6 days (C-12 BAC), and 24 ± 7 days (C-14 BAC), showing agreement between the two experiments. Direct photolysis, however, also contributed significantly to the loss of BZT over the 30 hour irradiation period. The direct photolysis quantum yield for BZT was calculated to be 1.02×10^{-2} mol Ei^{-1}. Preliminary results from outdoor river water experiments appear to coincide with the results of the simulated sunlight experiments; replicate experiments are ongoing. Singlet oxygen sensitizer experiments demonstrated limited reactivity between C-12 BAC and singlet oxygen, as expected. BZT appeared to also have limited reactivity with singlet oxygen but possible reactivity with triplets. BZT could also be reacting with the sensitizer compound; therefore, further experiments are being conducted.

For Outcome 2, aerobic river water microcosms showed that sub-inhibitory concentrations of a mix of QACs (C-12 BAC, C-12 ATMA, and C-12 DADMA) were degraded by the bacteria present even after sequential spikes. 16S rRNA gene abundance results from qPCR eventually showed no significant change between the spiked microcosms and bottle control microcosms over the time of the experiment. Preliminary statistical analyses of DNA sequencing results from Illumina high-throughput sequencing suggest that there was no statistically significant shift in the overall bacterial community over the time of the experiment between the spiked and un-spiked microcosms (beta diversity). Despite this, the differential abundance of certain bacterial species changed between the spiked and un-spiked microcosms over the course of the experiment. Data analysis is ongoing to evaluate if an additional round of experiments is needed.

Activity 2 Status as of January 1, 2020: Photolysis experiments demonstrated that QACs react at or near diffusion-controlled rates with hydroxyl radical in surface waters and have limited reactivity with other PPRIs. For most of the QACs studied, indirect photolysis will be the predominant photolysis reaction mechanism, though benzethonium will also undergo slow direct photolysis. The submission of a journal article presenting these results is expected soon. Further statistical analysis of the biodegradation sequencing data is ongoing and a manuscript is being drafted of the results.

Final Report Summary: Kinetic studies of common QACs with and without aromatic groups under simulated and natural sunlight conditions were performed with model sensitizers and dissolved organic matter to estimate photochemical half-lives in the aquatic environment. All QACs investigated react with hydroxyl radicals at diffusion-controlled rates ($\sim 2.9 \times 10^9$ to 1.2×10^{10} M^{-1} s^{-1}). Benzethonium reacted *via* direct photolysis (quantum yield of 1.7×10^{-2}). Overall, reactions with hydroxyl radicals will dominate over direct photolysis due to limited spectral overlap of sunlight emission and QAC absorbance. Photolysis half-lives are predicted to be 12 to 94 days, indicating slow abiotic degradation in surface water. The biotransformation of a sub-inhibitory mixture of QACs was investigated, along with the impact that these compounds had on a subset of a native river water microbial population. The ability of short term, low-level exposures of QACs to increase the number of antibiotic resistance genes present or cause substantial microbial community compositional changes was specifically investigated. Environmentally relevant concentrations of QACs (e.g., 1 - 40 µg/L) were transformed over the course of multiple exposures, possibly via metabolic processes despite low concentrations and methanol addition. There was neither a statistically significant nor lasting impact of QAC exposure on the presence of antibiotic resistance genes when comparing river water microcosms amended with a QAC mixture and microcosms to which no QACs were added. Exposure to the QAC mixture did cause compositional changes in the community, though it was unclear whether this was the result of exposure to the QACs themselves or if methanol amended with the QACs caused these changes. Any association of QACs with dissolved organic matter was found to not impact the photolysis or biodegradation. These results demonstrate that QACs are degraded in surface waters, but not sufficiently rapidly enough to prevent deposition in sediments or continuous exposure of aquatic organisms downstream of wastewater treatment plants. These findings in part led to drafting of a review article regarding potential concerns regarding QAC usage during the COVID-19 pandemic.

ACTIVITY 3: Determination of carcinogen production during water disinfection

Description: When QACs react with the disinfectants used in drinking water and wastewater treatment, it is possible to produce carcinogens known as nitrosamines. Thus, there is the potential for release of carcinogenic nitrosamines to the environment or production in tap water and consumer exposure. Solutions of QACs in tap water and wastewater will be exposed to chloramines at various dosages. Waters will be collected from five drinking water systems and five wastewater systems to obtain a variety of water sources/chemistries.

The formation of nitrosodimethylamine (a known carcinogen) and total nitrosamines will be measured using liquid chromatography and gas chromatography mass spectrometry and a chemiluminescence method, respectively. Samples that are not dosed with QACs will be measured as well to measure background QAC production. The background QAC levels in these samples will be measured using the methods developed in activity 1 to determine the total nitrosamine production attributable to QACs. Results will demonstrate which QACs have the greatest potential to produce carcinogens.

Summary Budget Information for Activity 3:

ENRTF Budget: $ 76,000
Amount Spent: $ 76,000
Balance: $ 0

Outcome	Completion Date
1. Validation of analytical methods	9/30/18
2. Nitrosamine formation potential in drinking water	3/31/19
3. Nitrosamine formation potential in wastewater	6/30/19

Activity 3 Status as of January 1, 2018: Nothing to report.

Activity 3 Status as of July 1, 2018: Preliminary experiments with small QAC compounds have been performed to understand reaction kinetics with chlorine and chloramines.

Activity 3 Status as of January 1, 2019: Experimental design is underway and will commence shortly.

Activity 3 Status as of July 1, 2019: A selection of QACs were exposed to chloramines under uniform formation conditions, which are appropriate to determine realistic nitrosamine formation potential under water or wastewater treatment conditions. The experimental matrix consisted of 1) purchased QACs, 2) the purchased QACs treated via solid phase extraction to remove non-QAC impurities, 3) commercial consumer products containing QACs, and 4) the commercial products treated by solid phase extraction. Yields of total nitrosamines were low (0.003% to 0.03% on a mass basis), with BAC providing the highest yields. Analysis is ongoing to assess the maximum amounts of nitrosamines attributable to QACs based on the concentrations observed in wastewater samples.

Activity 3 Status as of January 1, 2020: Experiments are complete, and the assessment of NDMA formation potential in wastewater is being finalized.

Final Report Summary: Upon exposure to chloramine, the analytical standards of QACs and commercial products did product nitrosamines with yields of 0.003 to 0.03 percent on mass basis. The benzalkyl ammonium chlorides, which are common in many consumer products, had the highest yields. Compared to other known precursors, the QACs are likely a minor contributor to total nitrosamines, and the potential for human exposure to nitrosamines arising from QACs would be in direct or indirect potable water reuse situations.

V. DISSEMINATION:

Description: The results will be disseminated via peer reviewed publications in scientific journals, presentations at local/regional conferences, and via a publically available final report. Funds have been requested to pay fees for open access, so the articles will be available to the public and stakeholders without an embargo period.

Activity Status as of January 1, 2018: Nothing to report.

Activity Status as of July 1, 2018: Presentations were given at American Chemical Society National Meeting in March 2018.

Activity Status as of January 1, 2019: Papers on QAC detection method and levels in water and sediment are being drafted.

Activity Status as of July 1, 2019: Presentations were given at American Chemical Society National Meeting in March 2019. The paper on QAC levels in wastewater will be submitted soon. The papers on levels in sediment and the photochemistry are being drafted. We plan to deposit data in the Data Repository for U of M (https://conservancy.umn.edu/handle/11299/166578) in the near future.

Activity Status as of January 1, 2020: The paper describing the detection of QACs in wastewater and sediments has been peer reviewed and is being revised. The data are freely available in the Data Repository for U of M https://doi.org/10.13020/ram6-m093. A poster presentation on QAC photochemistry and biodegradation was given at the Conference on the Environment hosted by the Upper Midwest Section of Air & Waste Management Association and the Central States Water Environment Association in November 2019. The paper on photochemistry will be submitted soon.

Final Report Summary: Three papers were published and one is forthcoming. The first open access paper reports on the detection of QACs in wastewater and sediment and is available at https://doi.org/10.1039/C9EM00554D. The data are freely available in the Data Repository for U of M https://doi.org/10.13020/ram6-m093. The second open access paper focuses on the photolysis of QACs and is available at https://doi.org/10.1039/D0EM00086H. The third paper was not part of the original work scope, but

resulted from the recognition that the COVID-19 pandemic was likely to lead to dramatically increased use of QACs and took advantage of the knowledge gained from this project. The paper summarizes potential environmental impacts of elevated QAC usage (https://doi.org/10.1021/acs.estlett.0c00437 or https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7341688/). This paper was the subject of a brief news story on Minnesota Public Radio. The results were also presented at regional and national conferences as described above. The fourth paper will be submitted soon and presents the results of the biodegradation of the QACs. This paper and Dr. Priya Hora's thesis will be provided upon publication of this paper. A public lecture that incorporated data for the project was also given at the U of MN, and it is available on YouTube (https://www.youtube.com/watch?v=BL-B_CPNPYY&feature=youtu.be)

VI. PROJECT BUDGET SUMMARY:

A. Preliminary ENRTF Budget Overview:

Budget Category	$ Amount	Overview Explanation
Personnel:	$ 201,000	Arnold Project Manager (75% salary, 25% fringe benefits 7% FTE per year; $38,800). Postdoctoral Researcher, (82% salary, 18% fringe benefits, 75% FTE for year 1 and 50% year 2; %71,200). Graduate student Research Assistant and/or Temporary Casual Employee, (57% salary, 43% fringe benefits; 50% FTE for year 1 and year 2; $91,000). Costs include fringe benefits for all and tuition for the graduate student.
Equipment/Tools/Supplies:	$ 30,000	Chemical standards and reagents, instrument consumables ($18,000) and analytical time for quantification of target compounds and detection of reaction products ($9,000). Equipment operating and maintenance costs ($3,000)
Travel Expenses in MN:	$ 2,000	Sample collection and presentations at local conferences/workshops
Other:	$ 3,000	Publication fees for open access
TOTAL ENRTF BUDGET:	**$ 236,000**	

Explanation of Use of Classified Staff: not applicable

Explanation of Capital Expenditures Greater Than $5,000: not applicable

Total Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 3.25

Total Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: 0

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state			
	$ 103,000	$ 103,000	Because the project is overhead free, laboratory space, electricity, and other facilities/administrative costs (53-54% of

			direct costs, depending on FY, excluding permanent equipment and graduate student academic year fringe benefits) are provided in-kind by the University of Minnesota
State			
	$	$	
TOTAL OTHER FUNDS:	**$ 103,000**	**$103,000**	

VII. PROJECT STRATEGY:

A. Project Partners: The project will be led by William Arnold (University of Minnesota, Department of Civil, Environmental, and Geo- Engineering) who has extensive experience in quantifying pollutants in environmental matrices and studying the degradation of environmental contaminants. The team will consist of a postdoctoral researcher and one graduate student research assistant.

B. Project Impact and Long-term Strategy: This project will provide information regarding the usage of QACs in Minnesota and their distribution in the environment. Knowing the environmental fate of these pollutants will aid in assessment of environmental impacts, which include disruption of plant or algal function and development of antibiotic resistance. There potential for carcinogenic nitrosamines to be present in river and drinking water from the reaction of the target chemicals has important implications for the protection of human health. This study will reveal if action needs to be taken with regards to QACs in Minnesota's natural and engineered water systems. The results will be disseminated via the scientific literature and a publically available final report.

C. Funding History:

Funding Source and Use of Funds	Funding Timeframe	$ Amount
This project leverages samples and knowledge obtained from ENTRF project Antibiotics and antibiotic resistance genes in Minnesota lakes M.L. 2014, Chp. 226, Sec. 2, Subd. 03e	2014-2017	$ 300,000

VIII. REPORTING REQUIREMENTS:

- **The project is for 2 years, will begin on 07/01/2017, and end on 06/30/2019.**
- **Periodic project status update reports will be submitted 01/15 and 07/15 of each year.**
- **A final report and associated products will be submitted between June 30 and August 15, 2019**.

IX. VISUAL COMPONENT or MAP(S): See attached

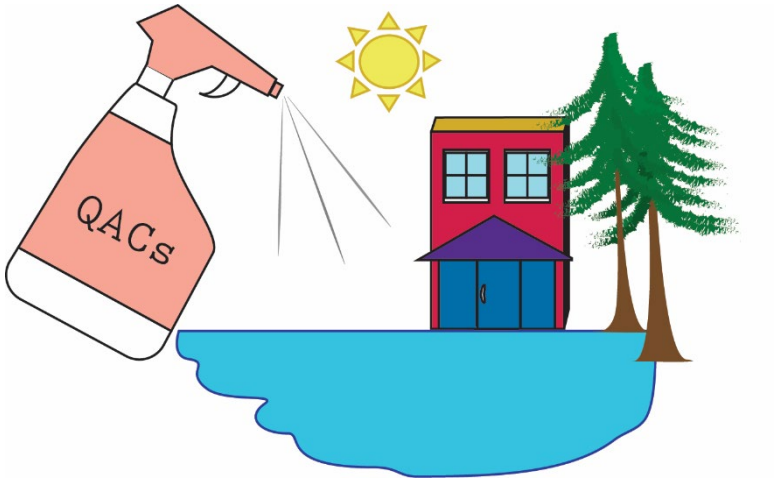
X. FEE TITLE ACQUISITION/CONSERVATION EASEMENT/RESTORATION REQUIREMENTS: Not applicable

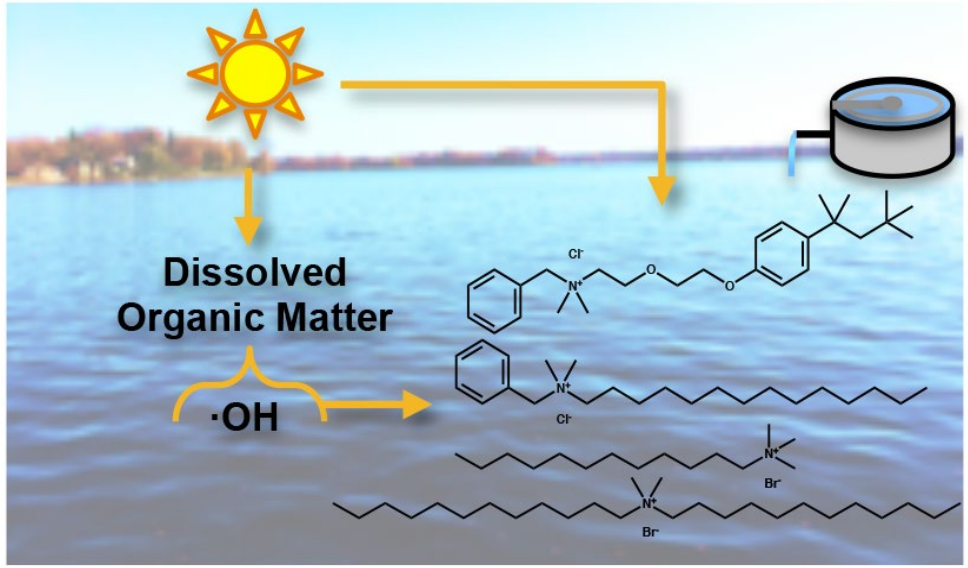
Environment and Natural Resources Trust Fund
M.L. 2017 Project Budget

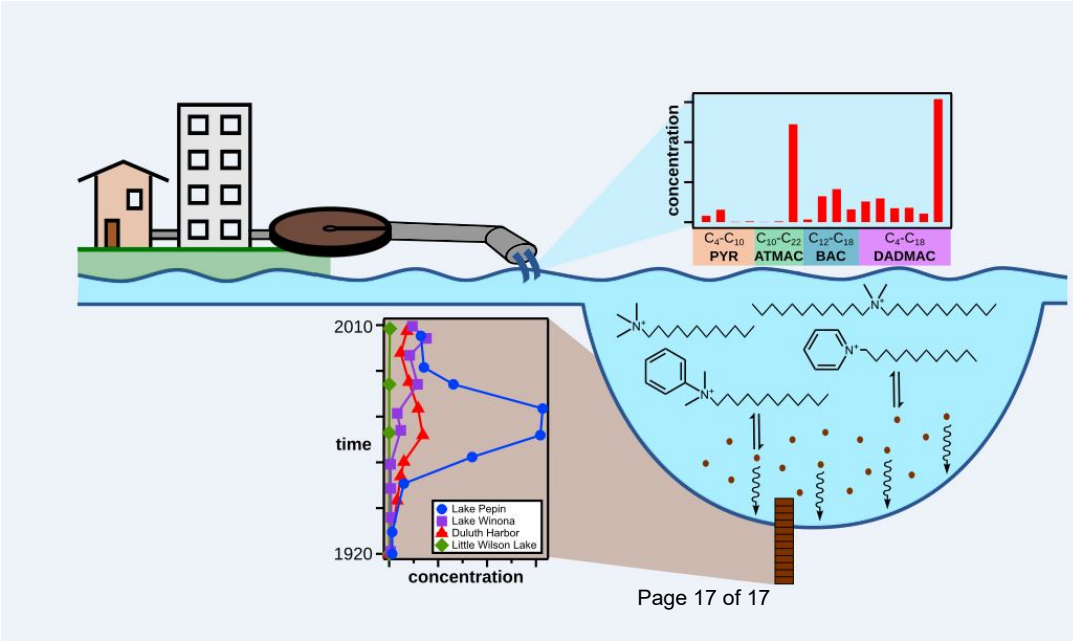
Project Title: Assessment of Household Chemicals and Herbicides in Rivers and Lakes
Legal Citation: M.L. 2017, Chp. 96, Sec. 2, Subd.04a
Project Manager: William Arnold
Organization: University of Minnesota
M.L. 2017 ENRTF Appropriation: $ 236,000
Project Length and Completion Date: 3Years, June 30, 2020
Date of Report: July 24, 2020

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Activity 1 Budget	Amount Spent	Activity 1 Balance	Activity 2 Budget	Amount Spent	Activity 2 Balance	Activity 3 Budget	Amount Spent	Activity 3 Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM	**Measurement of quaternary ammonium compound pollutants**			**Evaluation of environmental persistance in surface waters**			**Determination of carcinogen production during water disinfection**				
Personnel (Wages and Benefits)	$76,000	$76,000	$0	$56,000	$56,000	$0	$69,000	$69,000	$0	$201,000	$0
William Arnold, Project Manager (75% salary, 25% fringe benefits 7% FTE per year). Project supervision, design of experiments and data analysis of Activities 1 &2, supervision of postdoctoral researcher and graduate students and project reporting.	$13,260	$13,260	$0	$12,720	$12,720	$0	$12,820	$12,820	$0	$38,800	$0
Postdoctoral Researcher, (82% salary, 18% fringe benefits, 75% FTE for year 1 and 50% year 2). Extraction and analysis of water and sediment samples, development of analytical methods, photolysis experiments, initation of disinfection experiments with chloramines, data analysis and interpretation.	$42,720	$42,720	$0	$17,800	$17,800	$0	$10,680	$10,680	$0	$71,200	$0
Graduate student Research Assistant and/or Temporary Casual Employee, (57% salary, 43% fringe benefits; 50% FTE for year 1 and year 2) Assist with extraction and analysis of water and sediment samples and method development, photolysis experiments, sorption to organic matter experiments, biodegradation experiments, disinfection experiments with chloramines, data analysis and interpretation	$20,020	$20,020	$0	$25,480	$25,480.00	$0	$45,500	$45,500	$0	$91,000	$0
Equipment/Tools/Supplies											
Supplies $18,000 (chemical standards - $3500, isotopically labelled internal standards - $3000, chemical reagents for perisistence experiments and carinogen formation assays- $2500, necessary glassware-$2000, solvents - $1500, consumable supplies (autosampler vials, syringes, SPE cartridges, gloves)- $5000, laboratory notebooks-$250, software licenses-$250)	$5,000	$5,000	$0	$2,000	$2,000	$0	$2,000	$2,000	$0	$9,000	$0
Analytical time in mass spectrometry facility for QAC detection in sediment and water (250 analyses for method development, calibration standards, and samplse from sediment cores x $20 per sample = $5,000; appromiately 200 samples from product identification studies in Activity 2 and 3 x $20 per sample = $4000)	$11,000	$11,000	$0	$5,000	$5,000	$0	$3,000	$3,000	$0	$19,000	$0
Operating and maintenance costs for laboratory instruments required for analyses and experiments; costs portioned based on usage by project $3,000. Anticipated contributions to maintence costs include: seals and filters for HPLC pumps ($500, twice during project), HPLC detector replacement ($500), lamp replacement ($500), water purificaiton cartridges ($500), injector replacement ($500)	$1,000	$1,000	$0	$1,000	$1,000	$0	$1,000	$1,000	$0	$3,000	$0
Travel expenses in Minnesota											
charges and univeristy vehicle rental charges for trips to water samples. Hotel/meal charges if overnight stay required. Attendence for students at local conferneces to disseminate project findings to stakeholders and the public.	$1,000	$871	$129	$0		$0	$0		$0	$1,000	$129
Other											

Publication charges to make published journal articles (four) immediately available via open access to maximize data availability and dissemination	$1,000	$1,000	$0	$1,000	$1,000	$0	$1,000	$1,000	$0	$3,000	$0
COLUMN TOTAL	**$95,000**	**$94,871**	**$129**	**$65,000**	**$65,000**	**$0**	**$76,000**	**$76,000**	**$0**	$236,000	**$129**


QACs


Dissolved
Organic Matter
·OH
Cl⁻
Br⁻


concentration
C4-C10 PYR
C10-C22 ATMAC
C12-C18 BAC
C4-C18 DADMAC
2010
time
1920
concentration
Lake Pepin
Lake Winona
Duluth Harbor
Little Wilson Lake