Environment and Natural Resources Trust Fund
2016 Request for Proposals (RFP)

Project Title: **ENRTF ID: 053-B**

Eliminating Contaminants to Protect Endangered Native Fish/Mussels

Category: B. Water Resources

Total Project Budget: $ 287,448

Proposed Project Time Period for the Funding Requested: 3 years, July 2016 to June 2019

Summary:

Tonalide and galaxolide are two of the most commonly detected wastewater contaminants. UV-treatment will be evaluated to remove these suspected endocrine disruptors and reduce toxicity to native fish and mussels.

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Sponsoring Organization: University of St. Thomas

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Location

Region: Statewide

County Name: Statewide

City / Township:

Alternate Text for Visual:

The visual is a graphical depiction of the experiments described in the project description.

_____ Funding Priorities	_____ Multiple Benefits	_____ Outcomes	_____ Knowledge Base
_____ Extent of Impact	_____ Innovation	_____ Scientific/Tech Basis	_____ Urgency
_____ Capacity Readiness	_____ Leverage	_____ TOTAL	_____%

PROJECT TITLE: Eliminating Contaminants to Protect Endangered Native Fish/Mussels

I. PROJECT STATEMENT

In 2009 the MPCA was directed by the legislature to monitor surface waters for endocrine disrupting compounds (EDCs) in the vicinity of at least 20 wastewater treatment plants (WWTPs); in the resultant study tonalide and galaxolide were detected in 84% and 96% of effluent samples respectively. This project will determine whether (a) UV disinfection would effectively remove these contaminants prior to discharge into surface waters and (b) whether the products formed when the contaminants break down would still be of concern.

Although tonalide and galaxolide are among the most commonly detected contaminants of emerging concern (CECs) in Minnesota WWTP effluents, the effects of these high production volume chemicals and their byproducts on the quality of Minnesota drinking water and aquatic life remain largely unknown. Municipalities in various locations are considering costly modifications of existing wastewater treatment processes to enhance removal of such CECs to protect surface waters, many of which serve as sources of drinking water, without adequate understanding of whether such treatments are effective and/or necessary. UV treatment is commonly considered because it can be used simultaneously to improve chemical removal and disinfect wastewater.

Both contaminants to be studied have worldwide production volumes of over 6,000 tons per year and account for 90% of the US market for polycyclic musks, which are used as synthetic fragrances in a wide range of products. Musks can impair the first line of defense against toxicants, known as MXR/PXR defenses. If detoxification ability is impaired, organisms cannot effectively eliminate other toxic chemicals found in MN waters - this has been demonstrated in mussels. This is of great concern as 25 of Minnesota's 48 native mussel species are listed as endangered, threatened, or of special concern. Tonalide and galaxolide are also known to induce other types of toxicity (e.g. liver damage, DNA/genetic damage) and are suspected EDCs, meaning they can disrupt hormones and impair growth and reproduction, and are thus a potential threat to mussel and fish populations.

This project will assess whether UV treatment of wastewater will effectively remove toxicity attributable to these common wastewater contaminants, including assessing toxicity of products formed during UV treatment. UV treatment can be effective at reducing tonalide concentrations in effluent, but galaxolide is tougher to break down. It is of particular concern that most galaxolide degradation products have been classified as very persistent and/or toxic. Therefore, there is an urgent need to further our understanding of what is formed when these contaminants are broken down by UV light as it is very possible these UV products could also be an important unknown source of toxicity for endangered native mussels and fish in MN waters. This work will provide valuable insight into the ability of UV treatment to mitigate contribution of these contaminants to toxicity of wastewaters, in addition to identifying contaminants and products of particular interest for monitoring and further study and enabling municipalities to make better informed decisions about the need for treatment upgrades.

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: Quantify removal of contaminants by UV treatment and measure toxicity and endocrine disrupting activity **Budget: $**200,431

Rates and extent of removal with UV treatment will be quantified in the laboratory, and mixtures of UV treatment products will be generated for toxicity testing. Tonalide and galaxolide and their UV degradation products will be screened to determine whether UV treatment can eliminate adverse effects on fish and mussels. We will use multiple sets of assays (experiments that measure biological activity) including:

- *Detoxification assays – test for impairment of organism's ability to eliminate contaminants*
  - If toxicity is detected, we will conduct native fish and mussel tests to determine whether exposure to musks/their UV products increases toxicity of other contaminants.
- *Endocrine toxicity assays - test for disruption of reproductive hormones (e.g., testosterone, estrogen)*
  - If endocrine cell toxicity is detected, we will expose fathead minnow (an excellent model for MN natives) to musks/UV products and assess effects on fish health and reproduction.

- *General toxicity assays* – a series of widely recognized tests indicative of toxicity used for human and ecological hazard evaluation will be measured – positive hits in these assays will not require follow up studies with mussels and fish as those effects can be extrapolated based on the existing knowledge.

If biological assays suggest that some of these UV degradation products are toxic they will be characterized using a combination of chemical techniques and toxicity assays.

Outcome	Completion Date
1. Measure photolysis rates and quantum yields of tonalide and galaxolide under UV light.	December 2017
2. Perform biological screening tests and, where appropriate, follow-up fish and mussel studies to determine if UV treatment can minimize toxicity to native fish and mussels.	January 2019
3. Identify toxic products formed during UV treatment	June 2019

Activity 2: Quantify contaminants and their UV products in municipal wastewater **Budget: $**87,017

Effluents from wastewater treatment plants with and without UV disinfection will be analyzed for the presence of the two target contaminants and the products formed when they undergo degradation by UV light. Professor Stoll's laboratory at Gustavus is equipped with state-of-the-art equipment that enables: 1) separation of a complex sample such as WWTP effluent and 2) identification of unknown compounds and quantitation of compounds of interest. This instrumentation will be especially useful to this project because it enables the identification of most products formed after UV treatment, and the measurement of very low concentrations of target compounds and products (akin to finding the 'needle in the haystack'). The Stoll group has extensive experience developing analyses for complex matrices.

Outcome	Completion Date
1. Determine concentrations of target compounds and known UV products in at least six WWTP effluents and WWTP-impacted sites at least twice per year. Sites will be chosen to overlap significantly with those being studied in existing LCCMR-funded mussel-related work (e.g. Minnesota River basin, St. Croix River); the PI of the other study (Kozarek) will share sampling plans and field site locations.	June 2017
2. Identify additional UV degradation products in WWTP effluents based on major products observed during laboratory UV treatment studies.	June 2018
3. Measure concentrations of products determined to have endocrine activity or toxicity based on work carried out during this project.	June 2019

III. PROJECT STRATEGY

A. Project Team/Partners: **Kris Wammer, U. St. Thomas.** Will measure UV photolysis rates, generate product mixtures for activity assay testing, isolate suspected active products (Activity 1) and lead sampling (Activity 2). Will coordinate project and make sure reports are filed on time and results disseminated. **Dalma Martinovic-Weigelt, U. St. Thomas.** Will lead work on toxicity assays and fish and mussel studies (Activity 1). **Anthony Schroeder, U. MN – Crookston.** Responsible for molecular/physiological endpoints for fish and mussel studies (Activity 1). **Dwight Stoll, Gustavus.** Will assist with identification of products (Activity 1), and measure concentrations in WWTP effluents (Activity 2). All will supervise students and all will receive ENRTF funds.

B. Project Impact and Long-Term Strategy: In addition to disseminating our work through peer-reviewed scientific publications and presentations, we will communicate and work with the PI of an existing ENRTF mussel study and MPCA personnel involved in WWTP effluent survey work as appropriate during the project. If warranted by our findings, we will collaborate with WWTPs statewide to introduce appropriate UV technologies that will facilitate removal of the toxic contaminants and be protective of fish and mussel health.

C. Timeline Requirements: This project is expected to conclude within 36 months, by June 2019. Laboratory work and sampling of effluents will be performed in parallel throughout the duration of the project, with findings from each activity informing the work of the other.

2016 Detailed Project Budget

Project Title: Eliminating Contaminants to Protect Endangered Native Fish/Mussels

IV. TOTAL ENRTF REQUEST BUDGET 3 years

BUDGET ITEM	AMOUNT
Personnel:	
Senior Personnel. Kristine Wammer: 1 month of salary first 2 years, 0.5 month third year. Supervise students, coordinate project, compile and file reports, disseminate results. 93% salary, 7% fringe.	$ 20,866
Senior Personnel. Dalma Martinovic-Weigelt: 1 month of salary each year. Supervise students, assist with coordinating project, disseminating results. 93% salary, 7% fringe.	$ 28,003
Undergraduate students. 2 students during the academic year (one 5 hours per week, one 10 hours per week), 2 students each summer. 95% salary, 5% fringe benefits.	$ 44,117
Professional/Technical/Service Contracts:	
Gustavus Adolphus College. Dwight Stoll, Principal Investigator. 0.5 month of salary per year for first two years, 0.33 month salary for third year ($10,084, 86% salary, 14% fringe benefits). Supervise Gustavus undergraduate students and reseach technician. Research technician 10 hours per week in years 1 and 2, 7 hours per week in year 3 ($42,100, 75% salary, 25% fringe benefits). 1 student during the first two summers ($11,427, 80% salary, 12% housing, 8% fringe benefits). 1 student during the academic year, 8 hours per week for 15 weeks, ($3,856, 100% salary).General lab supplies, e.g. solvents, vials, analytical standards ($7,500). LC/MS instrument access ($11,000). Travel (meetings with other groups, some sampling) ($1,050).	$ 87,017
University of Minnesota – Crookston. Anthony Schroeder, Principal Investigator. 0.5 month of salary per year for first two years, 1 month of salary for third year (13,333, 100% salary). Supervise undergraduate student. One student during the first and second academic years, 8 hours per week for 16 weeks, ($5,376, 100% salary). One student during the first summer ($4,200, 100% salary). Lab supplies, e.g. disposable plastics, primers, Sybr green mastermix, RNA extraction kits, enzyme assays, hormone assays ($10,091).	$ 33,000
Equipment/Tools/Supplies:	
Sample filtration, extraction and preparation for all analyses - 20 samples @ $175/sample	$ 3,500
Cells/supplies, media, standards for endocrine in vitro asessments - 20 samples @ $220/sample	$ 4,400
50 general toxicity tests - parent/UV degradation compounds - 10 samples/ $54/sample/test	$ 27,000
Reagent supplies for in vivo molecular/physiological assessment (enzyme/hormone/ gene assays) -	$ 6,500
Reagents and disposables for mussel MXR defense assays and nutrient chemistry	$ 4,600
Miscellaneous lab supplies (pipette tips, culture plates, tubing, sterile syringes/containers, assay	$ 3,000
Fish and mussels, holding supplies, and food	$ 2,500
General photolysis and chromatography supplies (e.g. columns, quartz tubes, reagents, solvents)	$ 10,000
UV photoreactor (LuzChem ICH 2)	$ 11,085
Travel: Mileage costs (57.5 cents per mile) for obtaining WWTP effluent samples (6 treatment plants, at least twice per year; most sites in MN River basin or St. Croix River)	$ 1,500
Additional Budget Items: Shipping costs to send samples between institutions for analysis.	$ 360
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 287,448

V. OTHER FUNDS

SOURCE OF FUNDS	AMOUNT	Status
Other Non-State $ To Be Applied To Project During Project Period:	N/A	
Other State $ To Be Applied To Project During Project Period:	N/A	
In-kind Services To Be Applied To Project During Project Period: Indirect costs (waived)	N/A	71,200
Funding History: Project manager has had two ENRTF appropriations for a project unrelated to the current proposal (studying antibiotics in surface waters), one current (M.L. 2013, Chp. 52, Sec. 2, Subd. 05h) and one completed (M.L. 2011, First Special Session, Chp. 2, Art. 3, Sec. 2, Subd. 05e)	N/A	Will be done by 6/30/16
Remaining $ From Current ENRTF Appropriation:	N/A	

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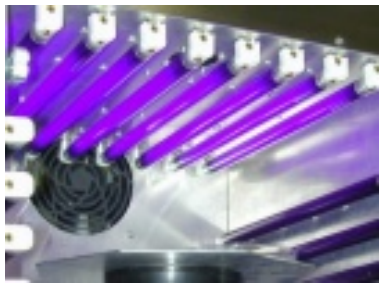
Lab studies



Expose contaminants to UV light, measure how fast they are broken down, generate products for toxicity testing

General toxicity cell assays

MXR/PXR cell assays

If toxic then

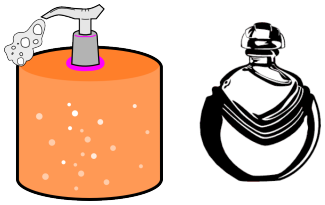
Native mussel/fish assays for contaminant defense toxicity

Endocrine cell assays

If toxic then

Fathead minnow assays for fish health and reproduction toxicity

Wastewater analysis



Products containing contaminants (galaxolide and tonalide)

Wastewater treatment plants with and without UV disinfection

Measure contaminants and UV breakdown products in municipal wastewater

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Project Manager Qualifications

Dalma Martinovic-Weigelt, Department of Biology, University of St. Thomas

Education: **B.S.,** 1994, Ecology, University of Zagreb; **M.S.,** 1999, Biological Sciences, University of Mississippi; **Ph.D.,** 2005, Fisheries Science and Water Resources, University of Minnesota.
Employment: **Assistant Professor**, 2009-present, Department of Biology, University of St. Thomas; **Post-Doctoral Research Associate**, 2005-2008 National Academies, U.S. Environmental Protection Agency.
Research: Dr. Martinovic-Weigelt has co-authored two reports to the MN Legislature (Endocrine Disrupting Compounds, lrp-ei-1syo8; Wastewater Treatment Plant Endocrine Disrupting Chemical Monitoring Study, lrp-ei-1sy11) and circa 40 research manuscripts that assess occurrence and the effects of chemicals of emerging concern and other stressors on fish and aquatic ecosystems.

Anthony Schroeder, Math, Science, and Technology Department, University of MN - Crookston

Education: **B.S.,** 2006, Biology, University of North Dakota; **Ph.D.,** 2012, Biology, University of North Dakota.
Employment: **Assistant Professor**, 2015-present, Math, Science, and Technology Department, University of Minnesota-Crookston; **Post-Doctoral Research Associate**, 2013-2015, University of Minnesota in Cooperative Agreement with U.S. Environmental Protection Agency.
Research: Dr. Schroeder's research has focused on developing and utilizing approaches to better and more rapidly assess the biological effects of complex environmental mixtures on fish and aquatic ecosystems.

Dwight R. Stoll, Department of Chemistry, Gustavus Adolphus College

Education: **B.S.,** 1999, Plant Biology, and **B.S.,** 2001, Biochemistry, Minnesota State University, Mankato; **Ph.D**., 2007, Analytical Chemistry, University of Minnesota.
Employment: **Assistant Professor**, 2008-present, Department of Chemistry, Gustavus Adolphus College*;* **Post-doctoral Fellow**, 2007-2008, Departments of Biochemistry, Molecular Biology, and Biophysics, and Medicine, University of Minnesota; **Instructor**, 2005-2006, Department of Chemistry, St. Olaf College.
Research: Dr. Stoll's research is focused on the development of novel separations based methods for the determination of trace level compounds in complex matrices such as environmental and biological samples. He uses multidimensional separations coupled with detection methods that include mass spectrometry and UV absorbance spectroscopy.

Kristine H. Wammer, Department of Chemistry, University of St. Thomas

Education: **B.A**., 1997, Chemistry, St. Olaf College; **Ph.D**., 2003, Civil and Environmental Engineering, Princeton University.
Employment: **Associate Professor**, 2012-present and **Assistant Professor**, 2005-2012, Department of Chemistry, University of St. Thomas; **Post-doctoral Fellow**, 2003-2005, Departments of Environmental Health Sciences, Chemistry and Civil Engineering, University of Minnesota.
Research: Dr. Wammer's research focuses on elucidating the chemical and biological processes affecting fate and impacts of organic contaminants, especially pharmaceutical and personal care products, in the aquatic environment.

Organization Descriptions
All three organizations are institutions of higher education that focus primarily on educating undergraduate students. All four investigators will manage pieces of the project while supervising undergraduate students; see the main proposal for a description of the specific responsibilities of each project manager within this proposed project.