# Environment and Natural Resources Trust Fund
# 2015 Request for Proposals (RFP)

**Project Title:** **ENRTF ID:** **038-B**

Toxicity of Fragrances to Native Fish and Mussels

**Category:** B. Water Resources

**Total Project Budget: $** 219,572

**Proposed Project Time Period for the Funding Requested:** 3 years, July 2015 - June 2018

**Summary:**

Fragrances from household products are common contaminants in wastewater. This project will determine how UV disinfection can be used to remove endocrine disrupting activity and toxicity attributable to these fragrances.

**Name:** Kristine Wammer

**Sponsoring Organization:** University of St. Thomas

**Address:** 2115 Summit Ave

St. Paul MN 55105

**Telephone Number:** (651) 962-5574

**Email** khwammer@stthomas.edu

**Web Address** http://www.stthomas.edu/chemistry/faculty/kristine-h-wammer.html

**Location**

**Region:** Statewide

**County Name:** Statewide

**City / Township:**

**Alternate Text for Visual:**

A pictorial representation of the proposed experiments for Activities 1 and 2

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

**PROJECT TITLE:** Toxicity of Fragrances to Native Fish and Mussels

## I. PROJECT STATEMENT

This project will evaluate efficacy of UV treatment for removal of two contaminants found in wastewater treatment plant (WWTP) effluents and assess the toxicity of products that are formed. Tonalide and galaxolide have worldwide production volumes of over 6000 tons per year and account for 90% of the US market for polycyclic musks, which are used as fragrances in cosmetics and detergents. Examples of products containing tonalide and galaxolide (often in concentrations as high as several hundred parts per million) include perfumes, lotions, deodorants, shower gels, shaving creams, shampoos and conditioners, furniture polish, laundry detergents, and fabric softeners. In a 2011 monitoring study, the Minnesota Pollution Control Agency detected tonalide and galaxolide in 84% and 96% of MN WWTP effluent samples, respectively. Musks impair the first line of defense against toxicants (MXR defense) – this has been demonstrated in mussels. If MXR is impaired mussels cannot eliminate toxic chemicals commonly found in MN waters. This is of great concern as 25 of Minnesota's 48 native mussel species are listed as endangered, threatened, or of special concern. In addition to MXR toxicity in mussels and potentially fish, tonalide and galaxolide are also endocrine disrupting compounds (EDCs) – they can disrupt hormones and lead to impaired growth and reproduction, and are thus a potential threat to fish populations.

The goal of this project is to assess whether UV treatment of wastewater will effectively remove toxicity (MXR and endocrine) attributable to tonalide and galaxolide, which also includes assessing toxicity of products formed during UV treatment. We selected to evaluate efficacy of UV treatment because municipalities in various locations are conducting costly modifications of existing wastewater treatment processes to enhance removal of EDCs to protect surface waters, many of which serve as sources of drinking water. UV treatment is commonly considered because it can be used simultaneously to improve chemical removal and disinfect wastewater. UV treatment can be effective at reducing tonalide concentrations in effluent, but galaxolide is more recalcitrant to degradation. It is of particular concern that most galaxolide transformation products have been classified as very persistent and/or toxic. Therefore, there is an urgent need to further our understanding of the transformation products of these musks as it is very possible they 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 musks to endocrine and MXR toxicity of wastewaters, in addition to identifying target compounds and transformation products of particular interest for monitoring and further study.

## II. PROJECT ACTIVITIES AND OUTCOMES

**Activity 1: Quantify removal of the musks tonalide and galaxolide by UV treatment and measure toxicity of musks and UV treatment products** **Budget: $**144,275

Rates and extent of transformation with UV treatment will be quantified, and mixtures of UV treatment products will be generated for toxicity testing. The two musks and their UV transformation products will be screened to determine whether UV treatment can eliminate musk toxicity to fish and mussels. We will use two sets of assays:

- *MXR cell toxicity assays – test for impairment of organism's ability to eliminate contaminants*
  - If MXR cell 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 cell 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.

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

| Outcome | Completion Date |
|---|---|
| 1. Measure photolysis rates and quantum yields of the musks under UV light | December 2016 |
| 2. Determine if UV treatment can minimize toxicity to native fish and mussels | January 2018 |
| 3. Identify toxic musks and UV musk products | June 2018 |

**Activity 2: Measure the musks tonalide and galaxolide and their products in municipal wastewater** **Budget:** $75,297

Wastewater treatment plant effluents with and without UV disinfection will be analyzed for the presence of musks and products. The Stoll Laboratory is uniquely equipped with analytical technology and expertise that are well suited to the realization of the outcomes indicated below. Specifically, this currently consists of state-of-the-art equipment (referred to as 2D-LC/TOF-MS) that enables: 1) physical separation of a complex sample such as WWTP using liquid chromatography; 2) identification of unknown compounds and quantitation of compounds of interest using mass spectrometry. This instrumentation will be especially useful to this project because it enables the identification of most transformation products, and the measurement of the musks themselves and the transformation products at very low concentrations (akin to finding the 'needle in the haystack'). At this point there is no way of knowing what the relative concentrations of the musk transformation products will be in this mixture, however from previous work of this kind involving other pollutants we expect them to be very low. The Stoll group has extensive experience developing 2D-LC/TOF-MS analyses for a variety of other complex matrices.

| Outcome | Completion Date |
|---|---|
| 1. Determine concentrations of musk metabolites/transformation products known from previous work in three different WWTP effluents at four different times during the year | June 2016 |
| 2. Identify additional transformation products of musks in WWTP effluent based on major products observed during laboratory UV treatment studies | June 2017 |
| 3. Determine concentrations of musk transformation products known to have endocrine or MXR defense toxicity based on work carried out during this project. We will sample from three different WWTP effluents at four different times during the year | June 2018 |

## III. PROJECT STRATEGY

### A. Project Team/Partners

**Kris Wammer, University of St. Thomas, Chemistry.** Responsible for quantifying transformation rates, generating transformation product mixtures for activity assay testing, and isolation of suspected active transformation products for further testing (Activity 1). Will coordinate project and make sure reports are filed on time and results disseminated. **Dalma Martinovic-Weigelt, University of St. Thomas, Biology.** Responsible for MXR defense and endocrine toxicity assays (Activity 1). Both will supervise University of St. Thomas undergraduate students. **Dwight Stoll, Gustavus Adolphus, Chemistry.** Responsible for assisting with identification of active transformation products (Activity 1), and analysis of concentrations of musks and transformation products in WWTP effluents (Activity 2), including supervision of Gustavus undergraduate students and research technician. All are proposed to receive ENRTF funds.

### B. Project Impact and Long-Term Strategy

In 2009 the MPCA was directed by the legislature to monitor surface waters for EDCs in the vicinity of at least 20 WWTPs. The musk compounds proposed for analysis here were among the most frequently detected in WWTP effluents in the resultant study. This project will determine whether (a) UV disinfection would effectively remove these compounds prior to discharge into surface waters and (b) whether resultant transformation products would still be of concern as EDCs. In addition to disseminating our work through peer-reviewed scientific publications and presentations, we will communicate our findings to the relevant MPCA personnel and work with them 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 musks and be protective of fish and mussel health.

### C. Timeline Requirements

Analysis of samples is anticipated to be completed by June 2018, therefore this project is expected to conclude within 36 months. 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.

# 2015 Detailed Project Budget

**Project Title: Toxicity of Fragrances to Native Fish and Mussels**

**IV. TOTAL ENRTF REQUEST BUDGET 3 years**

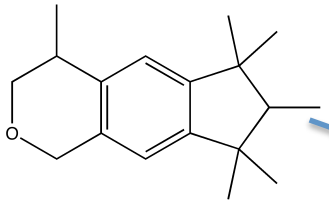
| BUDGET ITEM | AMOUNT |
|---|---|
| **Personnel:** | |
| Senior Personnel. Kristine Wammer: 1 month of salary first 2 years, 0.5 month third year. Duties: responsible for supervision of students, quantifying transformation rates, generating product mixtures for activity assay testing, and isolation of suspected active products for further testing. Coordinate project, compile and file reports, disseminate results. Dalma Martinovic Weigelt: 1 month of salary each year. Will supervise students, generate cell and MXR defense assay measurements, physiological data. Assist with coordinating project, compiling reports, and disseminating results. 93% salary, 7% fringe benefits. | $ 46,422 |
| Undergraduate students. 2 students during the academic year; one will work 5 hours per week and the other 10 hours per week for 32 weeks each year, $10 per hour. 2 students during each summer, will work 40 hours per week for 11 weeks each year, $10 per hour. Duties: will perform laboratory analysis associated with Activity 1. 95% salary, 5% fringe benefits. | $ 44,408 |
| **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. Duties: Responsible for assisting with identification of active transformation products (Activity 1), analysis of concentrations of musks and products in WWTP effluents (Activity 2), and supervision of Gustavus undergraduate students and reseach technician ($9,688, 86% salary, 14% fringe benefits). Research technician 10 hours per week in years 1 and 2, 7 hours per week in year 3 at $20 per hour ($31,381, 92% salary, 8% fringe benefits). 1 student during the first two summers, 40 hours per week for 10 weeks each year, $10.50 per hour, plus summer housing ($10,524, 81% salary,12% housing, 7% fringe benefits). 1 student during the academic year, 8 hours per week for 16 weeks, $10.50 per hour ($4,154).General lab supplies, e.g. solvents, vials, analytical standards ($7,500). LC/MS instrument access ($11,000). Travel for meetings with Wammer and Martinovic groups and some sampling trips ($1,050). | $ 75,297 |
| **Equipment/Tools/Supplies:** | |
| Sample collection, filtration, extraction and preparation for endocrine in vitro and MXR defense analyses | $ 3,500 |
| Cells/supplies, media, standards for endocrine in vitro asessments | $ 7,400 |
| Supplies for in vivo molecular/physiological endpoint assessment (primers, sybr mastermix, enzyme assays, hormone assays | $ 8,500 |
| Reagents and disposables for mussel MXR defense assays and nutrient chemistry | $ 5,600 |
| Miscellaneous lab supplies (pipette tips, culture plates, tubing, sterile syringes/containers, assay plates | $ 3,000 |
| Animals, animal 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 (56 cents per mile) for obtaining WWTP effluent samples several times per year. | $ 1,500 |
| **Additional Budget Items:** | |
| Shipping costs required to send effluent samples collected by UST senior personnel or students for analysis at Gustavus. | $ 360 |
| **TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =** | $ 219,572 |

**V. OTHER FUNDS**

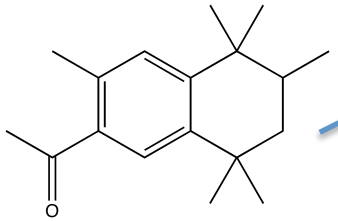
| SOURCE OF FUNDS | AMOUNT | Status |
|---|---|---|
| **Other Non-State $ To Be Applied To Project During Project Period:** | N/A | |
| **Other State $ To Be Applied To Project During Project Period:** | N/A | |
| **In-kind Services To Be Applied To Project During Project Period:** | N/A | |
| **Funding History:** | N/A | |
| **Remaining $ From Current ENRTF Appropriation:** Project manager has two current ENRTF appropriations, detailed below; the current proposal is unrelated to the work supported by these appropriations. | | |
| Assessment of Minnesota River Antibiotic Concentrations (M.L. 2011, First Special Session, Chp. 2, Art. 3, Sect. 2, Subd. 05e). Ends 6/30/14. | $8,032 | Unspent as of 3/1/14 |
| Antibiotics in Minnesota Waters - Phase II Mississippi River (M.L. 2013, Chp. 52, Sec. 2, Subd. 05h). Ends 6/30/16. | $181,588 | Unspent as of 1/31/14 |

## Activity 1

**PROJECT TITLE: Toxicity of Fragrances to Native Fish and Mussels**



galaxolide



tonalide

Measure UV photolysis rates; generate products for toxicity testing

Cell assays to screen for toxicity

MXR

If toxic then

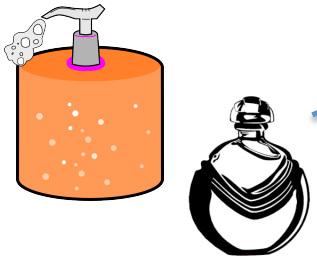
Native mussel/fish assays for contaminant defense toxicity

Endocrine

If toxic then

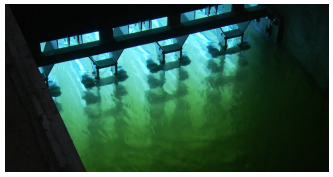
Fathead minnow assays for fish health and reproduction toxicity

## Activity 2

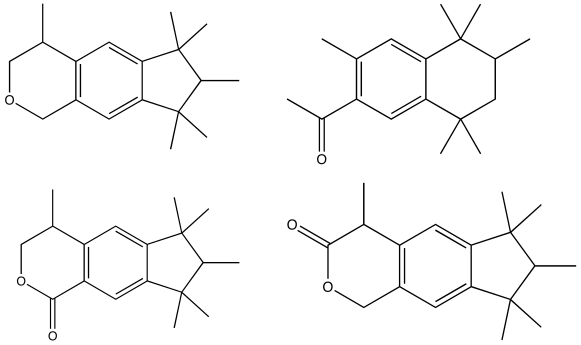


Products containing galaxolide and tonalide (e.g. perfumes, lotions, deodorants, shampoos, detergents)

Wastewater treatment plant



UV disinfection



Measure musks and products in municipal wastewater with and without UV treatment

**Project Title:** Toxicity of Fragrances to Native Fish and Mussels

**Project Manager Qualifications**

## Dalma Martinovic-Weigelt

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 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.

## Dwight R. Stoll

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

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.

See both the main proposal and the project budget for a description of the specific responsibilities of each project manager within this proposed project.

**Organization Descriptions**
Dr. Stoll is in the Chemistry Department at Gustavus Adolphus College (St. Peter, MN). Dr. Martinovic-Weigelt (Biology Department) and Dr. Wammer (Chemistry Department) are at the University of St. Thomas (St. Paul, MN). Both are private institutions of higher education that focus primarily on educating undergraduate students. All PIs have successfully managed multiple externally funded projects in collaboration with undergraduate students at these institutions.