Environment and Natural Resources Trust Fund
2011-2012 Request for Proposals (RFP)

LCCMR ID: 030-B

Project Title: Minnesota River: Occurrence and Potential Significance of Antibiotics

Category: B. Water Resources

Total Project Budget: $ $193,840

Proposed Project Time Period for the Funding Requested: 2 yrs, July 2011 - June 2013

Other Non-State Funds: $ 0

Summary:

We will examine the potential threat of antibiotics in the Minnesota River. We will measure antibiotic concentrations and antibiotic resistance and assess the contributions of farm runoff and wastewater treatment.

Name: Kristine Wammer

Sponsoring Organization: University of St. Thomas

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Location

Region: Central, SE

Ecological Section: Minnesota and NE Iowa Morainal (222M), North Central Glaciated Plains (251B)

County Name: Blue Earth, Le Sueur, Nicollet

City / Township:

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

2011-2012 MAIN PROPOSAL

PROJECT TITLE: Minnesota River: Occurrence and Potential Significance of Antibiotics

I. PROJECT STATEMENT

Pharmaceuticals and personal care products have gained significant attention in recent years as emerging contaminants in the environment. Numerous studies have reported the occurrence of pharmaceuticals at low levels in surface waters, and interest in this topic has moved beyond the scientific literature to the popular press. For example, a March 2008 story by the Associated Press highlighted the occurrence of pharmaceuticals in the drinking water supply of at least 41 million Americans. Extensive research is ongoing to determine the potential effectiveness of various treatment processes for removing pharmaceuticals in wastewater treatment plants. Legislation has been proposed both at the state and national levels to regulate use or disposal of pharmaceuticals; a bill recently passed by the MN House and Senate will regulate pharmaceutical disposal (H.F. 1217, S.F. 1568) while a current bill in the U.S. Congress would restrict the use of antibiotics for agricultural purposes (S. 619, H.R. 1549). While the environmental occurrence of these compounds has clearly spurred interest in both the scientific community and the public realm, major gaps still remain in our understanding of their significance and potential health and ecological impacts. Therefore, the critical question of which emerging contaminants are of the most concern is still largely unanswered.

The goal of this project is to efficiently identify emerging contaminants that pose a potential threat. We will focus here on antibiotics, and in particular on the development of antibiotic resistance due to the presence of antibiotics in farm runoff and in wastewater treatment plant effluents, which then subsequently impact surface waters. This project will study the Minnesota River, which has significant agricultural and municipal inputs, providing the opportunity to examine the relative importance of each input type. The project will assess current antibiotic concentrations, current antibiotic resistance levels, and the potential for future increases in resistance levels. The study will include four major classes of antibiotics that are used both for growth promotion in agriculture and in human medicine: tetracyclines, sulfa drugs, macrolides, and aminoglycosides.

II. DESCRIPTION OF PROJECT ACTIVITIES

Activity 1: Measure antibiotic concentrations at targeted MN River sites **Budget:** $84,208

Samples will be obtained from locations selected to allow comparison of primarily agricultural, primarily residential/industrial, and mixed inputs to the Minnesota River. See the attached map for proposed sample sites. We will analyze water samples for the presence of selected antibiotics using methods based on high performance liquid chromatography (HPLC) that have recently been developed in the laboratory of Dwight Stoll (one of the project partners). These methods have exceptional separation power that will allow us to accurately detect antibiotics even in complicated sample matrices such as those being considered in this work. For example, the Stoll group has successfully measured the concentration of phenytoin (a commonly used antiepileptic drug) in St. Peter, MN wastewater treatment plant effluent. We will use established solid-phase extraction (SPE) methods for sample pre-concentration prior to analysis to allow detection of antibiotics present at low levels in the river water and treatment plant effluent samples.

Outcome	Completion Date
1. Collect at least 2 sets of samples and screen them for the presence of 4 antibiotics: tetracycline, sulfamethoxazole (a sulfa drug), tylosin (a macrolide), and streptomycin (an aminoglycoside).	September 2011
2. Optimize our methods for the site matrices based on initial samples.	January 2012
3. Collect at least 5 additional sample sets by the end of September 2012. Quantify concentrations of the 4 antibiotics at all 7 sites for each sampling event.	June 2013

Activity 2: Measure antibiotic resistance levels at same MN river sites **Budget:** $134,002

Additional samples will be obtained at the same times from the same locations as described above to measure antibiotic resistance levels. Enumerating "antibiotic resistance" poses a unique challenge because of the diversity of microorganisms in nature and the diversity of antibiotics studied. Therefore, we will use two techniques that provide complementary data to give us the most accurate information. In the past, we have been successful using quantitative polymerase chain reaction (qPCR) as well as cultivation-based approaches. The qPCR technique involves concentrating the bacteria within the samples on filters and then extracting/purifying the DNA of any gene of interest. The qPCR technique allows us to quantify specific genes that encode antibiotic resistance, but the organisms that harbor the genes (and their characteristics) remain unknown. The benefit of the cultivation-based approach is that it provides bacterial isolates that can be analyzed further (for example, we will identify these organisms and determine their resistance to multiple antibiotics). We cultivate antibiotic-resistant bacteria using solid growth media amended with the target antibiotic.

Outcome	Completion Date
1. Cultivate bacteria from the initial sample sets on two different growth media (PYT80 for slow-growing and LB for fast-growing bacteria) amended with a range of concentrations of the 4 antibiotics.	September 2011
2. Quantify genes conferring resistance to tetracyclines, sulfa drugs, macrolides, and aminoglycosides in bacteria from the initial sample sets.	September 2012
3. Enumerate antibiotic-resistant bacteria from all 7 sites for each sampling event. Isolate resistant bacteria, and test their resistance to other classes of antibiotics.	September 2012
4. Quantify genes conferring resistance to the 4 classes of antibiotics at all 7 sampling sites for each sampling event.	June 2013

III. PROJECT STRATEGY

A. Project Team/Partners

Kris Wammer, University of St. Thomas, Chemistry. Responsible for coordinating sampling effort, getting samples to other researchers in timely fashion, and cultivation-based tests of antibiotic resistance levels including supervision of St. Thomas undergraduate students (Activity 2). Will coordinate project and make sure reports are filed on time and results disseminated. **Dwight Stoll, Gustavus Adolphus, Chemistry.** Responsible for analysis of concentrations of antibiotics and supervision of Gustavus undergraduate students and research technician (Activity 1). **Tim LaPara, University of Minnesota, Civil Engineering.** Responsible for gene-based tests of antibiotic resistance, including supervision of St. Thomas undergraduate students (Activity 2).

B. Timeline Requirements

As described in the activity outcomes, our plan is to collect a few sets of samples starting during the summer of 2011 and to collect at least 5 additional sample sets (varying seasonally and with rainfall events) by the end of September 2012. Analysis of samples will be completed by June 2013, therefore this project will be completed within 24 months.

C. Long-Term Strategy and Future Funding Needs

This project will help us understand the significance of an important class of emerging contaminants, antibiotics, as a potential threat in natural waters. By focusing on the selected portion of the Minnesota River, we will be able to investigate the relative importance of agricultural vs. municipal inputs. This work will inform future regulations related to wastewater, drinking water, and agriculture, and the advisability of the ever-increasing practice of using treated wastewater for non-potable applications (this is known as "water reuse").

2011-2012 Detailed Project Budget

IV. TOTAL TRUST FUND REQUEST BUDGET 2 years

BUDGET ITEM	AMOUNT
Personnel:	
Kristine Wammer, Principal Investigator. 1.5 months of salary per year plus associated fringe benefits. Duties: responsible for coordinating sampling effort, getting samples to other researchers in timely fashion, and cultivation-based tests of antibiotic resistance levels including supervision of St. Thomas undergraduate students (Activity 2). Will coordinate project and make sure reports are filed on time and results disseminated.	$ 20,336
University of St. Thomas undergraduate students. 3 students during the academic year, will work 10 hours per week for 32 weeks each year, $10 per hour. 2 students during each summer, will work 40 hours per week for 12 weeks each year, $10 per hour, plus associated fringe benefits. Will perform experiments associated with Activity 2.	$ 40,666
Contracts:	
University of Minnesota: Timothy LaPara, Principal Investigator. 5 weeks of salary per year plus associated fringe benefits. Duties: Responsible for gene-based tests of antibiotic resistance, including supervision of St. Thomas undergraduate students (Activity 2) ($35,000). General lab supplies, e.g. PCR primers, reagents ($10,000). Services associated with gene sequencing ($20,000).	$ 65,000
Gustavus Adolphus College: Dwight Stoll, Principal Investigator. 1 month of salary per year plus associated fringe benefits. Duties: Responsible for analysis of concentrations of antibiotics and supervision of Gustavus undergraduate students and research technician (Activity 1) ($13,201). Research technician 20 hours per week at $12 per hour plus associated fringe benefits ($27,873). 1 student during each summer, 40 hours per week for 12 weeks each year, $10 per hour, plus associated fringe benefits ($10,714). General lab supplies, e.g. solvents, vials, analytical standards ($3,000). LC/MS instrument access ($3,000). Travel for meetings with Wammer and LaPara groups, once each year ($300).	$ 58,088
Equipment/Tools/Supplies: General lab supplies, e.g. antibiotics, nutrient media, petri dishes	$ 8,000
Travel: Approximately 10 total sampling trips, 250 miles round trip, 50 cents per mile for mileage reimbursement. $50 per trip for meals for students/faculty.	$ 1,750
TOTAL ENVIRONMENT & NATURAL RESOURCES TRUST FUND $ REQUEST	$ 193,840

V. OTHER FUNDS

SOURCE OF FUNDS	AMOUNT	Status
Other Non-State $ Being Applied to Project During Project Period: Summer salary and fringe benefits for one undergraduate student will be provided by the University of St. Thomas each summer	$ 10,541	Pending
In-kind Services During Project Period: The PI will contribute an additional 0.5 months effort each year with associated salary and fringe as in-kind services.	$ 13,829	

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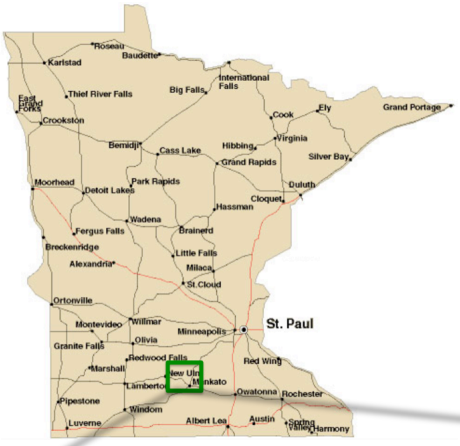
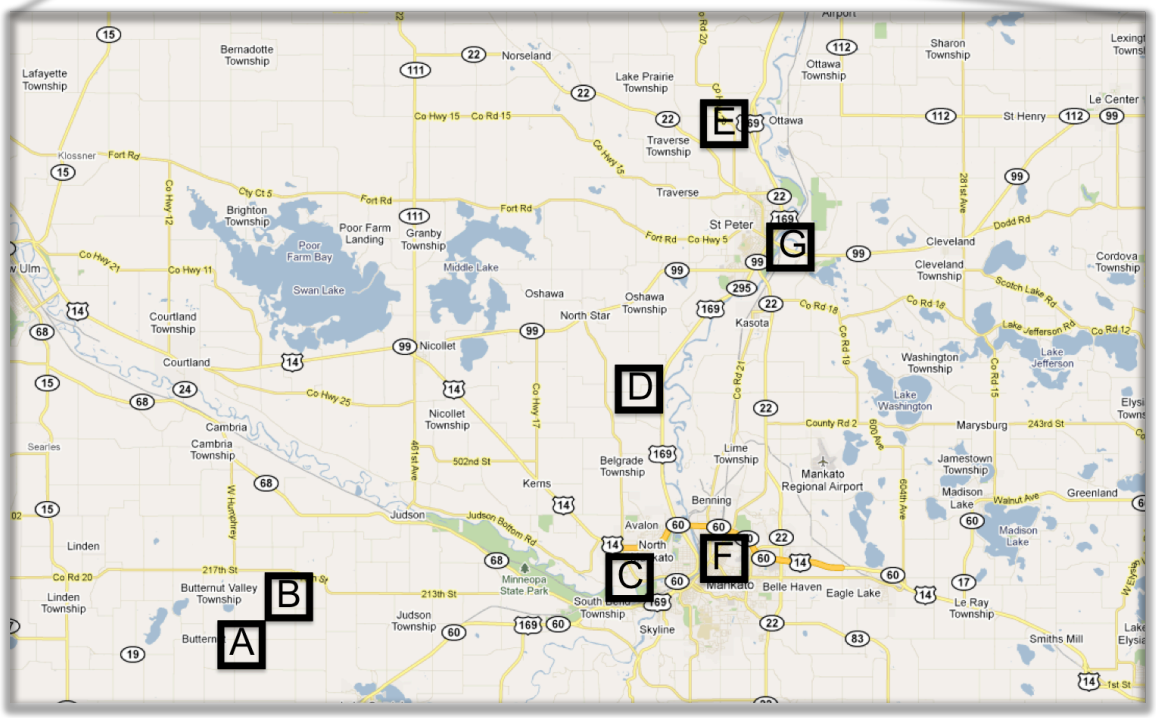


Proposed Sampling Site Descriptions

- **A and B:** Drainage ditches receiving input primarily from tile drains; feed the MN River eventually via Minneopa Creek
- **C:** MN River downstream of Minneopa Creek, just upstream of Blue Earth River
- **D:** MN River between St. Peter and Mankato
- **E:** MN River downstream of St. Peter
- **F:** Mankato wastewater treatment plant effluent
- **G:** St. Peter wastewater treatment plant effluent



Sample Collection at Each Site

→ **Stoll lab, Gustavus Adolphus College**
Measure antibiotic concentrations

→ **Wammer lab, University of St. Thomas**
Cultivate antibiotic-resistant bacteria

→ **LaPara lab, University of Minnesota**
Measure antibiotic resistance genes

Project Title: Minnesota River: Occurrence and Potential Significance of Antibiotics

Project Manager Qualifications

Timothy M. LaPara

Education: **B.S.C.E.,** 1995, Civil Engineering, University of Notre Dame; **Ph.D**., 1999, Environmental Engineering, Purdue University.

Employment: **Associate Professor**, 2006-present, Department of Civil Engineering, University of Minnesota; **Assistant Professor**, 2000-2006, Department of Civil Engineering, University of Minnesota; **Post-doctoral Research Associate**, 2000, Department of Biological Sciences, Purdue University.

Research
Dr. LaPara's research is focused on the role of municipal and industrial wastewater treatment plants in preserving environmental quality and in protecting public health. His research has a strong interdisciplinary nature, stemming from his unique background in both environmental engineering and microbiology.

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: **Assistant Professor***,* 2005-present, 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 microbiological processes affecting fate of organic contaminants in the aquatic environment. Her group's current interests include the environmental photochemistry and potential biological impacts of certain classes of pharmaceutical and personal care products.

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. LaPara is in the Civil Engineering department at the University of Minnesota-Twin Cities, which is the state of Minnesota's largest institution of higher education. Dr. Stoll and Dr. Wammer are both in Chemistry departments that educate undergraduate students at Gustavus Adolphus College (St. Peter, MN) and the University of St. Thomas (St. Paul, MN).