



Environment and Natural Resources Trust Fund (ENRTF) M.L. 2011 Work Plan

Date of Status Update:**Date of Next Status Update:** 1/1/2012**Date of Work Plan Approval:** 6/23/2011**Project Completion Date:** 6/30/2014**Is this an amendment request?** _____

Project Title: Assessment of Minnesota River Antibiotic Concentrations**Project Manager:** Kristine Wammer**Affiliation:** University of St Thomas**Address:** 2115 Summit Ave, OSS 402**City:** St Paul **State:** MN **Zipcode:** 55105**Telephone Number:** (651) 962-5574**Email Address:** khwammer@stthomas.edu**Web Address:** <http://www.stthomas.edu/chemistry/faculty/wammer.htm>

Location:**Counties Impacted:** Blue Earth, Le Sueur, Nicollet**Ecological Section Impacted:** Minnesota and Northeast Iowa Morainal (222M), North Central Glaciated Plains (251B)

Total ENRTF Project Budget:	ENRTF Appropriation \$:	190,000
	Amount Spent \$:	<u>0</u>
	Balance \$:	190,000

Legal Citation: M.L. 2011, First Special Session, Chp. 2, Art.3, Sec. 2, Subd. 05e**Appropriation Language:**

\$95,000 the first year and \$95,000 the second year are from the trust fund to the commissioner of natural resources for an agreement with Saint Thomas University in cooperation with Gustavus Adolphus College and the University of Minnesota to measure antibiotic concentrations and antibiotic resistance levels at sites on the Minnesota River.

I. PROJECT TITLE: Assessment of Minnesota River Antibiotic Concentrations

II. PROJECT SUMMARY:

While the environmental occurrence of antibiotics and other pharmaceuticals and personal care products (PPCPs) has spurred interest both in the research community and the public realm, major gaps still remain in our understanding of their significance and potential health and ecological impacts. Therefore, the critical questions of which antibiotics are of the most concern and which sources (human vs. agricultural) are the most significant are still largely unanswered.

The goal of this project is to efficiently identify classes of antibiotics that pose a potential threat. We will focus here on the potential for 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 and current antibiotic 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. A strength of this project is combining cutting-edge analytical chemistry techniques with rigorous microbiology and molecular biology techniques to characterize each site.

III. PROJECT STATUS UPDATES:

Project Status as of January 2012:

Project Status as of October 2012:

Project Status as of March 2013:

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Collect samples and quantify cultivable antibiotic-resistant organisms at targeted Minnesota River sites

Description: 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: quantitative polymerase chain reaction (qPCR, described in Activity 3), as well as the cultivation-based approaches of Activity 1. 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). Samples will be obtained from seven locations selected to allow comparison of primarily agricultural, primarily residential/industrial, and mixed inputs to the Minnesota River. In an initial sampling effort, at least two sets of samples will be obtained from each of the sites. Bacteria from these initial sample sets will be cultivated on two different growth media amended with a range of concentrations of each of four antibiotics: tetracycline, sulfamethoxazole (a sulfa drug), tylosin (a macrolide), and streptomycin (an aminoglycoside). For the main sampling effort at least five sets of samples will be obtained from each of the sites, varying seasonally and with rainfall events. Cultivable antibiotic-resistant bacteria will be enumerated from each sample. Resistant bacteria will also be isolated and tested for resistance to other classes of antibiotics.

Summary Budget Information for Activity 1:

ENRTF Budget: \$ 52,281
Amount Spent: \$ 0

Balance: \$ 52,281

Activity Completion Date:

Outcome	Completion Date	Budget
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: tetracycline, sulfamethoxazole (a sulfa drug), tylosin (a macrolide), and streptomycin (an aminoglycoside).	September 2011	\$ 6,767
2. Enumerate antibiotic-resistant bacteria from all 7 sites for each main sampling event.	September 2012	\$ 37,757
3. Isolate resistant bacteria, and test their resistance to other classes of antibiotics	December 2012	\$ 7,757

Activity Status as of January 2012:

Activity Status as of October 2012:

Activity Status as of March 2013:

Final Report Summary:

ACTIVITY 2: Measure antibiotic concentrations at same Minnesota River sites

Description: Samples from the initial sampling effort will be screened for the presence of the same four antibiotics listed in Activity 1. Based on these results, antibiotic detection methods will be optimized for the site matrices. New target antibiotics may also be added based on these results (especially if original target molecules are not detected.) Concentrations of the original and any other identified target molecules will then be measured in each sample collected as part of the main sampling effort. 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.

Summary Budget Information for Activity 2:

ENRTF Budget: \$ 56,938
Amount Spent: \$ 0
Balance: \$ 56,938

Activity Completion Date:

Outcome	Completion Date	Budget
1. Collect at least 2 sets of samples and screen them for the presence of the 4 target antibiotics.	September 2011	\$ 10,441
2. Optimize our methods for the site matrices based on initial samples.	January 2012	\$ 6,357

3. Collect at least 5 additional sample sets by the end of September 2012. Quantify concentrations of the target antibiotics at all 7 sites for each sampling event.	June 2013	\$ 40,140
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Activity Status as of January 2012:

Activity Status as of October 2012:

Activity Status as of March 2013:

Final Report Summary:

ACTIVITY 3: Quantify antibiotic resistance genes

Description: Quantitative polymerase chain reaction (qPCR) will be used to provide complementary information to that obtained by the cultivation-based approaches of Activity 1. The qPCR technique involves concentrating the bacteria within the samples on filters and then extracting/purifying the DNA of any gene of interest. We will target genes that confer resistance to the antibiotics of the tetracycline, sulfa, macrolide, and aminoglycoside classes.

Summary Budget Information for Activity 3:

ENRTF Budget: \$ 80,781
Amount Spent: \$ 0
Balance: \$ 80,781

Activity Completion Date:

Outcome	Completion Date	Budget
1. Quantify genes conferring resistance to tetracyclines, sulfa drugs, macrolides, and aminoglycosides in bacteria from the initial sample sets.	April 2012	\$ 28,273
2. Quantify genes conferring resistance to the 4 classes of antibiotics at all 7 sampling sites for each sampling event.	June 2013	\$ 52,508

Activity Status as of January 2012:

Activity Status as of October 2012:

Activity Status as of March 2013:

Final Report Summary:

V. DISSEMINATION:

Description: The results of this study will be disseminated through oral and poster presentations by the students involved in the project, briefings to LCCMR as requested, and peer-reviewed publication. We will also present progress on the project periodically to relevant personnel who have been made aware of this project and may be interested in the results, specifically at the Minnesota Department of Health (Drinking Water Protection Section) and the Minnesota Pollution Control Agency.

Status as of January 2012:

Status as of October 2012:

Status as of March 2013:

Final Report Summary:

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget:

Budget Category	\$ Amount	Explanation
Personnel:	\$ 61,002	\$20,336 for principal investigator (Wammer), which includes 1.5 months of summer salary per year plus associated fringe benefits. \$40,666 for undergraduate students: two working full-time each summer and three working 10 hours per week during the academic year.
Professional/Technical Contracts:	\$ 120,650	\$63,712 to University of Minnesota (LaPara) which includes 5 weeks of summer salary per year plus associated fringe benefits (\$35,000), lab supplies (\$9,575), and services (\$19,137). \$56,938 to Gustavus Adolphus College (Stoll) includes 0.9 month of summer salary per year plus associated fringe benefits (\$11,881), a research technician working 20 hours per week (\$27,873), one student working full time each summer (\$10,714), lab supplies (\$3,170), instrument access (\$3,000), and travel (\$300).
Equipment/Tools/Supplies:	\$ 6,598	General lab supplies, e.g. antibiotics, nutrient media, petri dishes
Travel Expenses in MN:	\$ 1,750	Mileage reimbursement and meals for 9-10 total sampling trips
TOTAL ENRTF BUDGET:	\$ 190,000	

Explanation of Use of Classified Staff: Summer salary is included for the project manager (Wammer) and project partners (LaPara and Stoll) who are all on 9-month academic contracts.

Explanation of Capital Expenditures Greater Than \$3,500: N/A

Number of Full-time Equivalent (FTE) funded with this ENRTF appropriation: 2 FTE per year

B. Other Funds:

Source of Funds	\$ Amount Proposed	\$ Amount Spent	Use of Other Funds
Non-state			
University of St. Thomas	\$ 10,541	\$	Salary and fringe benefits for one undergraduate student each summer.
State			
N/A	\$	\$	
TOTAL OTHER FUNDS:	\$ 10,541	\$	

VII. PROJECT STRATEGY:

A. Project Partners:

Kristine Wammer, University of St. Thomas, Department of Chemistry (\$69,350; includes salary, students, general supplies, and travel)

Dwight Stoll, Gustavus Adolphus College, Department of Chemistry (\$56,938; includes salary, research technician and students, general supplies, instrument access, and travel)

Timothy LaPara, University of Minnesota, Department of Civil Engineering (\$63,712; includes salary, supplies and services)

B. Project Impact and Long-term Strategy: 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”).

C. Spending History: none

VIII. ACQUISITION/RESTORATION LIST: N/A

IX. MAP(S): N/A

X. RESEARCH ADDENDUM: See Research Addendum

XI. REPORTING REQUIREMENTS:

Periodic work plan status update reports will be submitted not later than January 2012, October 2012, and March 2013. A final report and associated products will be submitted between June 30 and August 1, 2013 as requested by the LCCMR.

Attachment A: Budget Detail for M.L. 2011 (FY 2012-13) Environment and Natural Resources Trust Fund Projects

Project Title: Assessment of Minnesota River Antibiotic Concentrations

Legal Citation: \$95,000 the first year and \$95,000 the second year are from the trust fund to the commissioner of natural resources for an agreement with Saint Thomas University in cooperation with Gustavus Adolphus College and the University of Minnesota

Project Manager: Kristine Wammer

M.L. 2011 (FY 2012-13) ENRTF Appropriation: \$ 190,000

Project Length and Completion Date: 2 years; June 30, 2013

Date of Update: May 12, 2011

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Activity 1 Budget	Amount Spent	Balance	Activity 2 Budget	Amount Spent	Balance	Activity 3 Budget	Amount Spent	Balance	TOTAL BUDGET	TOTAL BALANCE
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BUDGET ITEM	Collect samples and quantify cultivable antibiotic resistant organisms at targeted Minnesota River sites			Measure antibiotic concentrations at same Minnesota River sites			Quantify antibiotic resistance genes				
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Personnel (Wages and Benefits)

Kristine Wammer, Project Manager: \$20,336 (93% salary, 7% benefits), 1.5 summer months effort	\$20,336.00									20,336	20,336
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Undergraduate Research Assistants: \$40,666 (96% salary, 4% benefits), 3 students full-time for 12 weeks each summer. 2 students working 10 hours for 32 weeks during the academic year.	\$23,597.00						\$17,069.00			40,666	40,666
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Professional/Technical Contracts

University of Minnesota: Timothy LaPara, Principal Investigator. 5 weeks of summer 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 (\$9,575). Services associated with gene sequencing (\$10,127)							\$63,712.00		63,712	63,712	63,712
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Gustavus Adolphus College: Dwight Stoll, Principal Investigator. 0.9 month of summer 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) (\$11,881). 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 (\$3,170). LC/MS instrument access (\$3,000). Travel for				\$56,938.00		\$56,938.00				56,938	56,938
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Equipment/Tools/Supplies

General supplies, e.g. antibiotics, nutrient media, plates, etc.	\$6,598.00									6,598	6,598
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Travel expenses in Minnesota Mileage and meals for approximately 10 sampling trips.	\$1,750.00									1,750	1,750
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COLUMN TOTAL	\$52,281.00	\$0.00		\$56,938.00	\$0.00		\$80,781.00	\$0.00		\$190,000.00	\$190,000.00
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