Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2013 Work Plan

Date of Status Update Report: October 2, 2012

Date of Next Status Update Report: January 31, 2014

Date of Work Plan Approval: June 11, 2013

Project Completion Date: June 30, 2016 **Is this an amendment request?** __N__

PROJECT TITLE: Antibiotics in Minnesota Waters - Phase II Mississippi River

Project Manager: Kristine Wammer

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Location: Anoka, Dakota, Goodhue, Hennepin, Ramsey, Sherburne, Stearns, Wabasha, Wright

Total ENRTF Project Budget:		
	ENRTF Appropriation:	**$203,000**
	Amount Spent:	**$0**
	Balance:	**$203,000**

Legal Citation: M.L. 2013, Chp. 52, Sec. 2, Subd. 05h

Appropriation Language:
$203,000 the first year is from the trust fund to the commissioner of natural resources for an agreement with the University of St. Thomas to measure antibiotic concentrations and antibiotic resistance levels and assess the contributions of farm runoff and wastewater treatment in a portion of the Mississippi River. This appropriation is available until June 30, 2016, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Antibiotics in Minnesota Waters: Phase 2, Mississippi River

II. PROJECT STATEMENT:

Pharmaceuticals and personal care products have gained significant attention in recent years as emerging contaminants in the environment, including attention from legislative bodies. The MN legislature passed a bill in 2009 regulating human pharmaceutical disposal, and bills have been introduced in recent sessions of the U.S. Congress that would restrict the use of antibiotics for agricultural purposes due to concerns over harm to human health related to the development of antibiotic resistance. While the environmental occurrence of these compounds has spurred interest, major gaps still remain in our understanding of their significance and potential health and ecological impacts. The critical question of which, if any, emerging contaminants are of the most direct concern to human health is still largely unanswered. Because the threat of decreased efficacy of antibiotics due to increases in antibiotic resistance levels is such a significant human health threat, this class of pharmaceuticals is a priority for further study.

The goal of this project is to study the development of antibiotic resistance due to the presence of antibiotics and antibiotic resistance genes in farm runoff and in wastewater treatment plant effluents, which then subsequently impact surface waters. We will study a portion of the Mississippi River from north of St. Cloud to south of Kellogg. This project team is currently working on a similar ENRTF-funded study in a portion of the Minnesota River. Our findings to date suggest that wastewater treatment plant effluents are a potentially important path for both antibiotics and antibiotic resistance genes to reach surface waters. This is consistent with findings by a recent USGS study that reported elevated levels of pharmaceuticals, including one antibiotic (sulfamethoxazole) in wastewater treatment plant effluents throughout Minnesota. We now propose to address the pressing question of whether drinking water is being impacted, and whether this is observed on a larger scale.

Samples will be obtained from locations selected to allow comparison of primarily agricultural (including drainage ditches), primarily residential/industrial (including wastewater treatment effluents), and mixed inputs to the Mississippi River. In addition, we plan to collect samples near drinking water intakes and tap water from Minneapolis, St. Paul, and St. Cloud. The project will assess current antibiotic concentrations and antibiotic resistance levels for members of four major classes of antibiotics used in both human medicine and agriculture: tetracyclines, sulfonamides, macrolides, and fluoroquinolones. A unique strength of this project is that the project team combines expertise in cutting-edge analytical chemistry techniques with expertise in rigorous microbiology and molecular biology techniques to characterize each water sample. We intend to attempt to complete the bulk of the project work within the first two years; a project duration of three years is specified in case weather or other factors require more time for successful completion of the project.

III. PROJECT STATUS UPDATES:

Project Status as of January 2014:

Project Status as of October 2014:

Project Status as of March 2015:

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Collect samples and quantify cultivable antibiotic resistant organisms at targeted Mississippi 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 determine their resistance to multiple antibiotics). Samples will be obtained from locations selected to allow comparison of primarily agricultural, primarily residential/industrial, and mixed inputs to the Mississippi River. In addition, we will collect samples from areas near drinking water intakes and tap water samples. Samples will be collected from each site on several occasions, varying seasonally and with rainfall events as much as possible. Bacteria from these samples will be cultivated on two different types of solid growth media amended with a range of concentrations of each of four antibiotics: tetracycline, sulfamethoxazole (a sulfonamide), tylosin (a macrolide), and ciprofloxacin (a fluoroquinolone). Cultivable antibiotic-resistant bacteria will be enumerated and compared to the number of bacteria able to grow on non-amended growth media. Resistant bacteria will also be isolated and tested for resistance to other classes of antibiotics.

Summary Budget Information for Activity 1:

ENRTF Budget: $ 59,943
Amount Spent: $ 0
Balance: $ 59,943

Activity Completion Date:

Outcome	Completion Date	Budget
1. Develop any new necessary methods based on findings from the first several sampling events.	May 2014	$ 8,991
2. Collect samples from various sites, varying seasonally and with rainfall events.	April 2015	$ 14,986
3. Enumerate antibiotic-resistant bacteria for samples collected at each sampling event by cultivating bacteria on growth media amended with a range of concentrations of the 4 target antibiotics: tetracycline, tylosin (a macrolide), sulfamethoxazole (a sulfonamide), and ciprofloxacin (a fluoroquinolone) in addition to any new antibiotics identified as targets during the project. Isolate resistant bacteria and test their resistance to other classes of antibiotics.	June 2015	$ 35,966

Activity Status as of January 2014:

Activity Status as of October 2014:

Activity Status as of March 2015:

Final Report Summary:

ACTIVITY 2: Measure antibiotic concentrations at same Mississippi River sites

Description:

We will analyze water samples for the presence of selected antibiotics using methods based on two-dimensional high performance liquid chromatography 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. In the work currently funded by the ENRTF, the Stoll group has successfully measured the concentration of several antibiotics in drainage ditches, the Minnesota River, and wastewater treatment plant effluents with detection

limits in the parts per trillion range. An important aspect of the current work has been the development and implementation of online-Solid Phase Extraction (online-SPE) to reduce carryover, improve analyte recovery, and increase sample throughput. In Phase 2 of this project, we will continue development of our online-SPE approach coupled to two-dimensional HPLC with MS detection, with a focus on improving the sensitivity of the approach by reducing the dimensions of the analytical separation system. We anticipate that these improved levels of sensitivity will be required for work with tap water where the target compounds are unlikely to be present above the high parts-per-quadrillion or low parts-per-trillion range.

Summary Budget Information for Activity 2:

ENRTF Budget: $ 81,841
Amount Spent: $ 0
Balance: $ 81,841

Activity Completion Date:

Outcome	Completion Date	Budget
1. Screen samples collected throughout the first summer of the project for the presence of the 4 target antibiotics.	November 2013	$ 20,460
2. Optimize our methods for the samples of interest. Identify potential new target antibiotics based on initial results, and develop detection methods.	March 2014	$ 27,008
3. Quantify concentrations of the 4 target antibiotics plus any new target antibiotics for samples collected at each sampling event beginning in Spring 2014.	June 2015	$ 34,373

Activity Status as of January 2014:

Activity Status as of October 2014:

Activity Status as of March 2015:

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 (*tet*(A), *tet*(X), and *tet*(W)), sulfonamides (*sul1*), macrolide (*ermB*), and fluoroquinolone classes (*qnrA*). In addition, we will target Class 1 integrons (*intI1*), which are associated with multiple antibiotic resistance.

Summary Budget Information for Activity 1:

ENRTF Budget: $ 61,216
Amount Spent: $ 0
Balance: $ 61,216

Activity Completion Date:

Outcome	Completion Date	Budget
1. Isolate DNA from samples collected at each sampling event.	November 2014	$ 45,912
2. Quantify genes conferring resistance to the 4 original classes of antibiotics, in addition to Class 1 integrons, plus any new classes of interest for samples collected throughout the year.	June 2015	$ 15,304

Activity Status as of January 2014:

Activity Status as of October 2014:

Activity Status as of March 2015:

Final Report Summary:

V. DISSEMINATION:

Description: The results of this study will be disseminated through oral and poster presentations by the students and faculty involved in the project, briefings to the LCCCMR as requested, and peer-reviewed publication. We also intend to present progress on this 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 2014:

Status as of October 2014:

Status as of March 2015:

Final Report Summary:

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget:

Budget Category	$ Amount	Explanation
Personnel:	$ 55,963	$23,211 for principal investigator (Wammer), which includes 1.5 months of summer salary per year plus associated fringe benefits. $32,752 for undergraduate students: two working full-time each summer and three working 6 hours per week during the academic year.
Professional/Technical/Service Contracts:	$ 126,681	$44,840 to University of Minnesota (LaPara) includes 4 weeks of salary per year plus associated fringe benefits ($31,340), lab supplies ($12,500), and travel ($1,000). $81,841 to Gustavus Adolphus College (Stoll) includes 1 month of salary per year plus associated fringe benefits ($14,319), a research technician working 14 hours per week ($34,540), one student working full-time each summer ($12,512), one student working 8 hours per week during the academic year ($5,470), lab supplies ($6,000), instrument access ($8,000), and travel ($1,000)
Equipment/Tools/Supplies:	$ 16,356	General lab supplies (e.g. HPLC consumables, antibiotics, nutrient media, petri dishes)
Travel Expenses in MN:	$4,000	Mileage reimbursement and meals for approximately 20 sampling trips based on the plan of the Commissioner of Management of Budget.
TOTAL ENRTF BUDGET:	**$ 203,000**	

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: 1.7 FTE

Number of Full-time Equivalent (FTE) estimated to be funded through contracts with this ENRTF appropriation: 1.7 FTE

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 ($76,319)

Timothy LaPara, University of Minnesota, Department of Civil Engineering ($44,840)

Dwight Stoll, Gustavus Adolphus College, Department of Chemistry ($81,841)

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. In the first phase of this project, we focused on a selected portion of the Minnesota River that allowed us to investigate the relative importance of agricultural vs. municipal inputs. This work is still ongoing, but results to date suggest that wastewater treatment plants may be more significant sources of both antibiotics and antibiotic resistance genes than agricultural runoff. These results will hopefully be useful for informing future regulations related to wastewater, drinking water, and agriculture. A limitation of the first phase of the project, however, was that the selected portion Minnesota River is not used as a drinking water source. Phase 2 of the project will allow us to determine whether our findings from the Minnesota River are mirrored in a portion of the Upper Mississippi river, and will allow us to measure whether drinking water sources are impacted. This will allow us to more directly study the relevance of this issue as a human health concern.

C. Spending History:

Funding Source	M.L. 2007 or FY08	M.L. 2008 or FY09	M.L. 2009 or FY10	M.L. 2010 or FY11	M.L. 2011 or FY12-13
ENRTF					$190,000 Sub. 5(e)

VIII. ACQUISITION/RESTORATION LIST: N/A

IX. MAP(S): N/A

X. RESEARCH ADDENDUM: Peer reviewed in Phase 1 of project (Assessment of Minnesota River Antibiotic Concentrations, M.L. 2011)

XI. REPORTING REQUIREMENTS:

Periodic work plan status update reports will be submitted not later than January 2014, October 2014, and March 2015. If the project work continues beyond the original anticipated two-year time frame, additional reports will be filed (at least 2 per year). A final report and associated products will be submitted between June 30 and August 15, 2016 as requested by the LCCMR.

Attachment A: Budget Detail for M.L. 2013 Environment and Natural Resources Trust Fund Projects

Project Title: Antibiotics in Minnesota Waters: Phase 2, Mississippi River
Legal Citation: M.L. 2013, Chp. 52, Sec. 2, Subd. 05h
Project Manager: Kristine Wammer
M.L. 2013 ENRTF Appropriation: $ 203,000
Project Length and Completion Date: 3 years; June 30, 2016
Date of Update: October 2, 2012

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
BUDGET ITEM	**Collect samples and quantify cultivable antibiotic resistant organisms at targeted Mississippi River sites**			**Measure antibiotic concentrations at same Mississippi River sites**			**Quantify antibiotic resistance genes**				
Personnel (Wages and Benefits)	$39,587		$39,587.00				$16,376		$16,376.00	$55,963.00	$55,963.00
Kristine Wammer, Project Manager: $23,211 (92% salary, 8% benefits), 1.5 summer months effort.											
Undergraduate Research Assistants: $32,752 (95% salary, 4% benefits), 2 students full-time for 12 weeks each summer, 3 students working 6 hours per week for 32 weeks during the academic year.											
Professional/Technical/Service Contracts											
University of Minnesota: Timothy LaPara, Principal Investigator. 4 weeks of salary per year plus associated fringe benefits (34.9%). Duties: Responsible for some tests of antibiotic resistance levels including supervision of St. Thomas undergraduate students (Activity 2) ($31,340). General lab supplies (e.g. PCR primers, reagents ($12,500). Travel funds for a few targeted sampling trips independent of the main sampling events ($1000)							$44,840.00		$44,840.00	$44,840.00	$44,840.00
Gustavus Adolphus College: Dwight Stoll, Principal Investigator. 1 month of salary per year plus associated fringe benefits (16.48%). Duties: Responsible for analysis of concentrations of antibiotics and supervision of Gustavus undergraduate students and reseach technician (Activity 1) ($14,319). Research technician 15 hours per week at $20 per hour plus associated fringe benefits (9.48%) ($34,540). 1 student during each summer, 40 hours per week for 12 weeks each year, $10.50 per hour, plus associated fringe benefits and summer housing ($12,512). 1 student during the academic year, 8 hours per week for 32 weeks, $10.50 per hour ($5,470). General lab supplies, e.g. solvents, vials, analytical standards ($6000). LC/MS instrument access				$81,841.00		$81,841.00				$81,841.00	$81,841.00
Equipment/Tools/Supplies General lab supplies, e.g. antibiotics, nutrient media, petri dishes, solvents	$16,356.00		$16,356.00							$16,356.00	$16,356.00
Travel expenses in Minnesota Approximately 20 sampling trips, 300 miles round trip, based on the plan of the Commissioner of Management of Budget.	$4,000.00		$4,000.00							$4,000.00	$4,000.00
COLUMN TOTAL	**$59,943**	**$0**	**$59,943**	**$81,841**		**$0**	**$81,841**	**$61,216**	**$0**	**$61,216**	**$203,000**	**$203,000**