Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2014 Work Plan

Date of Report: January 15, 2014

Date of Next Status Update Report: January 1, 2015

Date of Work Plan Approval:

Project Completion Date: June 30, 2017

Does this submission include an amendment request? _No_

PROJECT TITLE: Triclosan impacts on wastewater treatment

Project Manager: Timothy M. LaPara

Organization: University of Minnesota

Mailing Address: 500 Pillsbury Drive SE

City/State/Zip Code: Minneapolis, MN 55455

Telephone Number: (612) 624-6028

Email Address: lapar001@umn.edu

Web Address:

Location: Hennepin, Ramsey

Total ENRTF Project Budget:		
	ENRTF Appropriation:	**$380,000**
	Amount Spent:	**$0**
	Balance:	**$380,000**

Legal Citation: M.L. 2014, Chp. 226, Sec. 2, Subd. 03c

Appropriation Language:
$380,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to assess the role of the commercially used antibacterial agent triclosan in creating antibiotic resistant bacteria during the municipal wastewater treatment process. This appropriation is available until June 30, 2017, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Triclosan impacts on wastewater treatment

II. PROJECT STATEMENT:

An emerging paradigm for thwarting the spread of antibiotic resistance is to enhance the nation's municipal wastewater treatment infrastructure. The rationale for this paradigm is that people taking antibiotics will select for antibiotic resistant bacteria in their gastrointestinal tracts and then release these organisms upon defecation. This fecal material then coalesces at municipal wastewater treatment facilities where the treatment process could be used to eliminate antibiotic resistant bacteria. Because spent soap, toothpaste, etc., are washed down drains, however, municipal wastewater contains high concentrations of triclosan, thus exposing the antibiotic resistant bacteria from people's gastrointestinal tracts to yet another antimicrobial agent. Triclosan could, therefore, drive the selection of multiple antibiotic resistance during the wastewater treatment process, creating new bacterial strains that are resistant to numerous antibiotics — more commonly known as "super bugs" for their ability to resist many and potentially all antibiotics. Most troubling, however, is that treated wastewater with significant quantities of superbugs could be released to Minnesota's surface waters, creating a key route by which antibiotic resistance can spread to more people.

The proposed project will provide critically important information for the State of Minnesota as it considers future legislation to ban or to restrict triclosan use within the State. Triclosan (2,4,4′-trichloro-2′-hydroxydiphenyl ether) is an antibacterial agent used in numerous commercial products, including liquid hand soap, toothpaste, cosmetics, and children's toys. Triclosan, however, has become controversial. Scientific studies have suggested numerous adverse effects, including: reduced human immune function, bioaccumulation in the environment leading to algal toxicity, and the accumulation of triclosan-derived dioxins in lake and river sediments. In contrast, the American Cleaning Institute has maintained that triclosan-containing soaps have a decades-long track record of safety and play a beneficial role in the daily hygiene routine of millions of people. Recently, the State of Minnesota considered a bill to ban triclosan.

One of the primary concerns regarding the use of triclosan is that it selects for antibiotic resistance, not just to triclosan but also to a multitude of other antibiotics. Antibiotic resistance is a pending medical catastrophe. One example of antibiotic resistance (methicillin resistant Staphylococcus aureus – MRSA) is responsible for more deaths in the United States than emphysema, HIV/AIDS, Parkinson's disease and homicide (combined). In addition, the economic cost of additional medical treatments necessitated by antibiotic resistance is estimated to be $20 to $40 billion dollars each year.

III. PROJECT STATUS UPDATES:

Project Status as of January 1, 2015:

Project Status as of July 1, 2015:

Project Status as of January 1, 2016:

Project Status as of July 1, 2016:

Project Status as of January 1, 2017:

Overall Project Outcomes and Results:

IV. PROJECT ACTIVITIES AND OUTCOMES:

The project has been divided into three discrete activities. Activity 1 will be an experiment in which model bacterial communities will be grown at different concentrations of triclosan; these model communities are necessitated because we are unable to control triclosan concentrations at genuine wastewater treatment

facilities. Activity 2 will focus on characterizing the *known* antibiotic resistance genes in the model bacterial communities grown in Activity 1; these antibiotic resistance genes will be quantified by real-time PCR and by shotgun metagenomics. Activity 3 will look for novel antibiotic resistance genes using a functional metagenomic approach.

ACTIVITY 1: Sequencing Batch Reactor operation and sample collection

Description:
This activity will involve the fabrication of model wastewater treatment bioreactors in the laboratory. These wastewater treatment bioreactors will be grown at different concentrations of triclosan, allowing us to directly test the ability to triclosan to select for antibiotic resistance genes. The outcome of Activity 1 will be bacterial biomass that will be queried for the presence of antibiotic resistance genes in Activity 2 (known genes) and Activity 3 (novel genes).

Summary Budget Information for Activity 1:

ENRTF Budget: $ 20,351
Amount Spent: $ 0
Balance: $20,351

Activity Completion Date:

Outcome	Completion Date	Budget
1. *Construct and Inoculate SBRs*	August 1, 2014	$15,351
2. *Collect samples*	January 1, 2015	$5,000

Activity Status as of January 1, 2015:

Activity Status as of July 1, 2015:

Activity Status as of January 1, 2016:

Activity Status as of July 1, 2016:

Activity Status as of January 1, 2017:

Final Report Summary:

ACTIVITY 2: Characterization of known antibiotic resistance genes by quantitative polymerase chain reaction and shotgun metagenomics

Description:

In this activity, the biomass grown in Activity 1 will be assayed for the quantity/presence of antibiotic resistance genes. Two different, but complementary approaches, will be used. The first approach will involve quantitative, real-time polymerase chain reactor (qPCR) to precisely quantify the amount of a handful of specific antibiotic resistance genes in this biomass. The second approach will use shotgun metagenomics to query the bacterial biomass for all known antibiotic resistance genes. The outcome of this Activity will be detailed knowledge of the precise quantities of a handful of specific antibiotic resistance genes as well as broad knowledge of the diversity of all known antibiotic resistance genes in these samples.

Summary Budget Information for Activity 2:

ENRTF Budget: $ 223,866
Amount Spent: $ 0
Balance: $223,866

Activity Completion Date:

Outcome	Completion Date	Budget
1. *DNA extraction and purification*	March 15, 2015	$22,500
2. *Community analysis of 16S rRNA genes*	July 1, 2015	$22,500
3. *qPCR targeting antibiotic resistance genes*	October 15, 2015	$58,866
4. *Shotgun metagenomics*	January 1, 2016	$60,000
5. *Data analysis*	January 1, 2017	$60,000

Activity Status as of January 1, 2015:

Activity Status as of July 1, 2015:

Activity Status as of January 1, 2016:

Activity Status as of July 1, 2016:

Activity Status as of January 1, 2017:

Final Report Summary:

ACTIVITY 3: Characterization of novel antibiotic resistance genes by functional metagenomics

Description:

In this activity, the biomass grown in Activity 1 will be assayed for the presence of novel, previously unknown antibiotic resistance genes using a functional metagenomic approach. The outcome of this Activity will be knowledge of the ability of triclosan to select for novel, previously-undiscovered antibiotic resistance genes.

Summary Budget Information for Activity 3:

ENRTF Budget: $135,783
Amount Spent: $ 0
Balance: $135,783

Activity Completion Date:

Outcome	Completion Date	Budget
1. *Fosmid library construction*	October 15, 2015	$25,783
2. *Screening of clones*	October 16, 2016	$85,000
3. *DNA sequencing of clones*	January 1, 2017	$25,000

Activity Status as of January 1, 2015:

Activity Status as of July 1, 2015:

Activity Status as of January 1, 2016:

Activity Status as of July 1, 2016:

Activity Status as of January 1, 2017:

Final Report Summary:

V. DISSEMINATION:

Description:

Findings will be disseminated and archived via reports to LCCMR, peer-reviewed publications, and presentations at conferences. We will also, when appropriate, disseminate results via press releases to the media. The audience is not only the scientific community, but also the public, policymakers, and practitioners. The work will also be of interest to the medical community and we will seek avenues to share the results with this community.

Activity Status as of January 1, 2015:

Activity Status as of July 1, 2015:

Activity Status as of January 1, 2016:

Activity Status as of July 1, 2016:

Activity Status as of January 1, 2017:

Final Report Summary:

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview:

Budget Category	$ Amount	Explanation
Personnel:	$ 267,771	For Drs. Donato and LaPara for directing the project; for a graduate student at the University of Minnesota; for 5 undergraduate students at the University of St. Thomas
Professional/Technical/Service Contracts:	$0	
Equipment/Tools/Supplies:	$110,729	General laboratory supplies ($5,000) DNA sequencing ($62,500) Bioreactors ($5,000) qPCR reagents ($10,000) Supplies for functional metagenomics/DNA extraction kits, cloning kits, competent cells, etc. ($28,229)
Travel Expenses in MN:	$1,500	Miscellaneous travel within MN. This will include travel to treatment facilities for bacterial inocula, travel between Universities for research duties and meetings, and travel to miscellaneous suppliers (e.g., hardware stores)
Other:	$	
TOTAL ENRTF BUDGET:	**$380,000**	

Explanation of Use of Classified Staff: N/A

Explanation of Capital Expenditures Greater Than $5,000: N/A

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 2.25

Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: N/A

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state			
University of St. Thomas	$67,500	$	In-kind contribution; indirect costs not charged to this project
State			
	$100,000	$	In-kind contribution; indirect costs not charged to this project
TOTAL OTHER FUNDS:	**$167,500**	**$**	

VII. PROJECT STRATEGY:

A. Project Partners: N/A

B. Project Impact and Long-term Strategy:

The goal of this proposed project is to understand the role of triclosan in selecting for antibiotic resistant bacteria during the municipal wastewater treatment process. This research will guide the State of Minnesota as it considers its future wastewater treatment needs; this could include limitations on the use of triclosan, improved wastewater effluent disinfection, better management of residual wastewater solids, or other initiatives to reduce the role of triclosan and municipal wastewater treatment in the spread of antibiotic resistance.

C. Spending History: N/A

VIII. ACQUISITION/RESTORATION LIST: N/A

IX. VISUAL ELEMENT or MAP(S): see attached visual

X. ACQUISITION/RESTORATION REQUIREMENTS WORKSHEET: N/A

XI. RESEARCH ADDENDUM: See attached

XII. REPORTING REQUIREMENTS:
Periodic work plan status update reports will be submitted no later than January 1, 2015, July 1, 2015, January 1, 2016, July 1, 2016, and January 1, 2017. A final report and associated products will be submitted between June 30 and August 15, 2017.

Environment and Natural Resources Trust Fund											
M.L. 2014 Project Budget											
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Project Manager: *Timothy M. LaPara*											
Organization: *University of Minnesota*											
M.L. 2014 ENRTF Appropriation: $ *380,000*											
3 Years, June 30, 2017											
Date of Report: *Fill in the date of report submission*											
ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	**Activity 1 Budget**	**Amount Spent**	**Activity 1 Balance**	**Activity 2 Budget**	**Amount Spent**	**Activity 2 Balance**	**Activity 3 Budget**	**Amount Spent**	**Activity 3 Balance**	**TOTAL BUDGET**	**TOTAL BALANCE**
BUDGET ITEM	***SBR operation and sample collection***			***Characterization of antibiotic resistance***			***Functional Metagenomics***				
Personnel (Wages and Benefits)	$13,851	$0	$13,851	$157,037	$0	$157,037	$96,883	$0	$96,883	$267,771	$267,771
Project Management, Timothy LaPara ($47,666;9.6% of time; 75% to salary, 25% to frine benefits)											
Co-Project Manager, Justin Donato ($30,851; 11.5% of time; 75% to salary, 25% to fringe benefits)											
Graduate Student at U of M ($123,222; 50% of time; 50% to salary, 50% to benefits)											
Undergraduate Students at UST ($66,032; 5 students; paid hourly; 100% to salary)											
Equipment/Tools/Supplies **(***General laboratory supplies ($5,000), DNA sequencing ($62,500), Bioreactors ($5,000), qPCR reagents ($10,000), Supplies for functional metagenomics/DNA extraction kits, cloning kits, competent cells, etc. ($28,229))*	$5,000	$0	$5,000	$66,829	$0	$66,829	$38,900	$0	$38,900	$110,729	$110,729
Travel expenses in Minnesota (*Miscellaneous travel to get samples and supplies; for vehicle rental or reimbursement of personal vehicles.)*	$500	$0	$500	$500	$0	$500	$500	$0	$500	$1,500	$1,500
COLUMN TOTAL	**$19,351**	**$0**	**$19,351**	**$224,366**	**$0**	**$224,366**	**$136,283**	**$0**	**$136,283**	**$380,000**	**$380,000**

Antibiotic Resistance and the Environment