Environment and Natural Resources Trust Fund
2014 Request for Proposals (RFP)

Project Title: **ENRTF ID:** **032-B**

Does Triclosan Create Super Bugs During Wastewater Treatment

Category: B. Water Resources

Total Project Budget: $ 382,000

Proposed Project Time Period for the Funding Requested: 3 Years, July 2014 - June 2017

Summary:

This research project will assess the ability of triclosan, the widely used antibacterial agent, to create bacteria resistant to multiple antibiotics (a.k.a 'super bugs') during the municipal wastewater treatment process.

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Sponsoring Organization: U of MN

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Web Address

Location

Region: Metro

County Name: Hennepin

City / Township:

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

PROJECT TITLE: *Does Triclosan Create Super Bugs During Wastewater Treatment?*

I. PROJECT STATEMENT

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. The proposed research will add to our extensive knowledge that repeatedly has shown that wastewater treatment and surface water quality are critically important components of public health.

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.

II. DESCRIPTION OF PROJECT ACTIVITIES

Activity 1: *Establish wastewater bioreactors at four different triclosan concentrations.* **Budget: $70,000**

The proposed project will be performed as laboratory simulations so that the concentration of triclosan can be controlled. Four laboratory-scale bioreactors (working volume: 5 Liters) will be established as sequencing batch reactors to mimic the biological wastewater treatment process. The bioreactors will be operated for 6-9 months to establish a stable bacterial community. Samples will be collected and preserved throughout the duration of this activity for the subsequence two activities. All research (including Activities 2 and 3) will be performed under established biosafety guidelines to ensure the safety of all participants.

Outcome	Completion Date
1. Purchase supplies and initiate bioreactor operation	October 1, 2014
2. Bioreactor operation and sample collection	June 30, 2015

Activity 2: *Characterize and quantify antibiotic resistance levels in the laboratory-scale bioreactors.* **Budget: $150,000**

Genomic DNA will be purified from samples collected during Activity 1 and used to quantify the number of antibiotic resistance genes in each sample or for next-generation DNA sequencing. These methods are complimentary; one approach can detect very low concentrations of specific antibiotic resistance genes, while the other method can detect **all** known antibiotic resistance genes.

Outcome	Completion Date
1. DNA purification	October 1, 2015
2. Quantitative PCR targeting known antibiotic resistance genes	June 30, 2016
3. Next-generation DNA sequencing to detect all antibiotic resistance genes	June 30, 2017

Activity 3: *Characterize novel antibiotic resistance levels in the laboratory-scale bioreactors.* **Budget: $175,000**

The DNA from the entire bioreactor communities, harvested during Activity 2, will be cloned and introduced into *E. coli.* This collection of genetic material, termed a metagenomic library, will be selected for those fragments of DNA that confer antibiotic resistance to *E. coli*. This approach will allow us to detect genes that have never been previously detected/described in the scientific literature.

Outcome	Completion Date
1. Metagenomic library construction	*June 30, 2016*
2. Selection of metagenomic libraries for antibiotic resistance	*October 1, 2016*
3. Sequencing and characterization of antibiotic resistance genes	*June 30, 2017*

III. PROJECT STRATEGY

A. Project Team/Partners

Tim LaPara, University of Minnesota, Civil Engineering. Responsible for overseeing all tasks (including report submission and dissemination of results); specifically responsible for Activities 1 and 2.

Justin Donato, University of St. Thomas, Chemistry. Responsible for functional metagenomics (Activity 3).

B. Timeline Requirements

Establishing the laboratory-scale bioreactors is expected to take 3-6 months, after which the bioreactors will be operated for an addition 6-9 months. A substantial quantity of samples are anticipated to be collected, which will require 3-6 months of work (some of this will be concurrent to Activity 1). Quantitative PCR will require about 6 months to complete with an additional 3 months for data analysis. Next-generation DNA sequencing will require 6-12 months to complete, with an additional year required for data analysis (this study will generate terabytes of data). Construction of the metagenomic libraries will take approximately 6 months. The combined efforts to identify, sequence, and characterize novel resistance genes will take an additional 12 months.

C. Long-Term Strategy and Future Funding Needs

This project builds upon the prior expertise of the project personnel in wastewater treatment (LaPara), antibiotic resistance (LaPara, Donato), quantitative polymerase chain reaction (LaPara, Donato), next-generation DNA sequencing (LaPara), and functional metagenomics (Donato). The proposed project will fulfill a critical need in helping to define triclosan as detrimental chemical with serious collateral effects or as a useful component of personal hygiene. The project will be directly beneficial to Minnesota's legislation as it considers action on triclosan and to Minnesota's citizens who currently use this chemical extensively. Results will be widely disseminated, through various scientific conferences (e.g., the Minnesota Water Conference) and through press releases (if appropriate).

2014 Detailed Project Budget

Project Title: *Does Triclosan Create Super Bugs During Wastewater Treatment?*

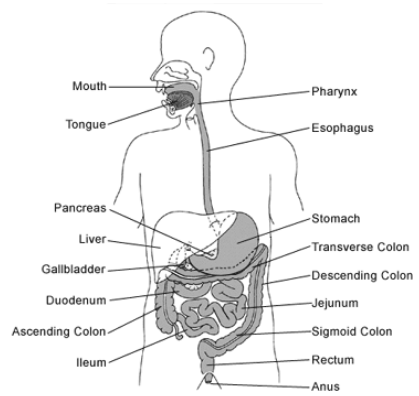
IV. TOTAL ENRTF REQUEST BUDGET *3* years

BUDGET ITEM	AMOUNT
Personnel: Timothy M. LaPara, *Project Manager, 5 weeks of salary per year plus associated fringe benefits (33.6%). Generally responsible for the successful completion of the project, specifically*	$ 48,000
Personnel: Justin J. Donato, *Principal Investigator 6 weeks of salary per year plus associated fringe benefits. Specifically responsible for Activity 3, will also actively collaborate on Activities 1 and 2.*	$ 37,000
Personnel: *Graduate Student, University of Minnesota, 50% effort plus fringe (including tuition). This student will perform the majority of the work described in Activities 1 and 2.*	$ 114,000
Personnel: *Undergraduate Students (5), University of St. Thomas. These students will perform the work for Activity 3. They will work full time during the summer and part time (5-10 hours per week)*	$ 60,000
Contracts: *In this column, list out proposed contracts. Be clear about whom the contract is to be made with and what services will be provided. If a specific contractor is not yet determined, specify the type of contractor sought. List out by contract types/categories - one row per type/category.*	$ -
Equipment/Tools/Supplies: This item will include expendable supplies like glassware, chemical reagents, DNA extraction kits, and other laboratory supplies. A substantial amount of funds ($62,500) is devoted to DNA sequencing.	$ 120,000
Acquisition (Fee Title or Permanent Easements): *In this column, indicate proposed number of acres and and name of organization or entity who will hold title.*	$ -
Travel: *A small amount of travel will be required for this project. It will include traveling to wastewater treatment plants to obtain inoculum, travel between Universities to meet with each other, and travel to local conferences to present the results.*	$ 3,000
Additional Budget Items: *In this column, list any additional budget items that do not fit above categories. List by item(s) or item type(s) and explain how number was reached. One row per type/category.*	$ -
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 382,000

V. OTHER FUNDS

SOURCE OF FUNDS	AMOUNT	Status
Other Non-State $ Being Applied to Project During Project Period: *Indicate any additional non-state cash dollars to be spent on the project during the funding period. For each individual sum, list out the source of the funds, the amount, and indicate whether the funds are secured or pending approval.*	$ -	*Indicate: Secured or Pending*
Other State $ Being Applied to Project During Project Period: *Indicate any additional state cash dollars (e.g. bonding, other grants) to be spent on the project during the funding period. For each individual sum, list out the source of the funds, the amount, and indicate whether the funds are secured or pending approval.*	$ -	*Indicate: Secured or Pending*
In-kind Services During Project Period: *Indicate any in-kind services to be provided during the funding period. For each type of service, list type of service(s), estimated value, and indicate whether it is secured or pending. In-kind services listed must be specific to the project.*	$ -	*Indicate: Secured or Pending*
Remaining $ from Current ENRTF Appropriation (if applicable): *Specify dollar amount and year of appropriation from any current ENRTF appropriation for any directly related project of the project manager or organization that remains unspent or not yet legally obligated at the time of proposal submission. Be as specific as possible. Describe the status of funds in the right-most column.*	$ -	*Indicate: Unspent? Not Legally Obligated? Other?*
Funding History: *Indicate funding secured prior to July 1, 2014, for activities directly relevant to this specific funding request, including past ENRTF funds. State specific source(s) of funds.*	$ -	

Antibiotic Resistance and the Environment



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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.

Justin J. Donato

Education: **B.S.** 1999, Chemistry, University of Delaware; **Ph.D**., 2006, Biochemistry, Cornell University.

Employment: **Assistant Professor**, 2010 - present, Department of Chemistry, University of St. Thomas. **Post-doctoral Fellow**, 2006 - 2010, Department of Bacteriology, University of Wisconsin - Madison.

Research
Dr. Donato's research focuses on identifying and characterizing new genes responsible for conferring important traits on their bacterial hosts. His group's current interests focus on the use of functional metagenomic techniques to analyze antibacterial resistance genes from diverse habitats.

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. Donato is in the Chemistry Department at the University of St. Thomas (St. Paul, MN).