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

Project Title: **ENRTF ID:** **030-B**

Antibiotics and Antibiotic Resistance Genes in Minnesota Lakes

Category: B. Water Resources

Total Project Budget: $ 338,000

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

Summary:

The historical relationship between antibiotics and antibiotic resistant bacteria in Minnesota lakes will be explored to determine if improved wastewater treatment is necessary to protect human and aquatic health.

Name: William Arnold

Sponsoring Organization: U of MN

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Minneapolis MN 55455

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Web Address

Location

Region: Statewide

County Name: Statewide, Douglas, Goodhue, Lake, St. Louis, Wabasha

City / Township:

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

PROJECT TITLE: Antibiotics and antibiotic resistance genes in Minnesota lakes

I. PROJECT STATEMENT

Pharmaceuticals are found in water bodies all across Minnesota. These compounds are biologically active and can disrupt the function of ecological communities or have other adverse effects. Of particular concern are antibiotics, one of the greatest inventions of the 20th century. The utility of antibiotics is at risk, however, due to resistance in clinical settings. The release of antibiotics and antibiotic resistance genes into the environment may also pose a threat to human health by encouraging broader development of antibiotic resistance or by leading to the harboring of elevated levels antibiotic resistance genes in environmental matrices. There is also potential for antibiotics to disrupt the proper functioning of ecosystems. While there is a background level of naturally occurring antibiotic resistance, elevated or persistent levels due to human activities have the potential to cause harm to human, veterinary, or ecosystem health. Our hypothesis is that the levels of resistance to particular antibiotics will be recorded in sediments and will correlate with the presence and/or usage trends of particular antibiotics.

The overall goal of this project is to improve water quality and to protect human and ecosystem health by

- Quantifying the current and historical levels of selected human and veterinary antibiotic compounds in lake sediments, and
- Determining the current and historical levels of genes that code for resistance to the selected human and veterinary antibiotics in lake sediments.

The results of this work will reveal if the environmental presence of human and veterinary antibiotics in Minnesota lake sediments leads to the retention of resistance genes.

If our hypothesis is confirmed, this would suggest that the presence of the antibiotics allows for resistance to be maintained in the environment and that additional measures should be taken to reduce the discharge of antibiotics and of antibiotic resistance genes to the environment from municipal, hospital, or agricultural sources. If the data do not support the hypothesis, the study would still provide the critical information that discharge of antibiotics and antibiotic resistance genes from municipal, hospital, or agricultural sources does not lead to persistence of resistance genes in aquatic systems.

II. DESCRIPTION OF PROJECT ACTIVITIES

Activity 1: Collection and dating of sediment cores **Budget: $49,000**

Based on our previous ENTRF sponsored work, we have identified three wastewater impacted sites (Lake Pepin, Duluth Harbor, and Lake Winona) for study. The control site will be Little Wilson Lake, which has no wastewater input. Using a piston corer, we will collect sediment cores from each site. We can use stored sediments for some analyses in Activity 2, but we will require unaltered cores samples for the microbiological characterizations of Activity 3. Cores will be dated using lead-210 and cesium-137 methods, and the organic matter content will be determined as a function of depth. Sediment deposition rates as a function of time will be calculated.

Outcome	Completion Date
1. Core collection	10/30/14
2. Core dating and determination of organic content and deposition rates	4/30/15

Activity 2: Measurement of sulfa, tetracycline, macrolide, and quinolone antibiotics as a function of depth/time in sediment cores **Budget: $134,000**

Sediments serve as integrators of the chemicals introduced into a lake over time. By analyzing the antibiotic

concentrations as a function of depth, it will be possible to assess the "dosage" each lake received as a function of time. The trends in antibiotic levels will be related to any trend in resistance determined in Activity 3. The sediment cores will be sectioned as a function of depth. A mass of ~10 g dry weight will be extracted and the concentration of the target antibiotics determined using liquid chromatography tandem mass spectrometry. The accumulation rates of each compound will be calculated.

Outcome	Completion Date
1. Optimize antibiotic extraction and analytical methods	12/31/14
2. Measure antibiotic concentrations in sediment samples	10/30/16
3. Calculate accumulation rates	1/31/17

Activity 3: Measurement of antibiotic resistance genes as a function of depth/time in sediment cores **Budget: $155,000**

Antibiotic resistance levels can be measured in sediment samples using techniques developed in previous ENTRF work. Sediment cores will be sectioned as a function of depth in parallel with Activity 2. Genomic DNA will be extracted and purified from these samples and then used as template to genetically determine the amount of antibiotic resistance in these samples. Results will be correlated to sediment age (Activity 1) and to antibiotic levels (Activity 2).

Outcome	Completion Date
1. DNA extraction and purification	5/31/15
2. Quantify known antibiotic resistance genes	10/30/16
3. Data synthesis, reporting, and recommendations	1/31/17

III. PROJECT STRATEGY

A. Project Team/Partners : The project will be led by William Arnold and Timothy LaPara (University of Minnesota, Department of Civil Engineering). The team will consist of two graduate student researchers. Dr. Arnold has extensive experience quantifying chemicals in environmental matrices, and Dr. LaPara is an expert on the quantification of resistance genes. Daniel Engstrom at the Science Museum of Minnesota will perform the core collection and dating. Partnering with Dr. Engstrom provides additional education and outreach opportunities.

B. Timeline Requirements: The proposed project will be completed in a three-year period. The chemical and microbiological analyses are time consuming and require detailed quality assurance/quality control protocols.

C. Long-Term Strategy and Future Funding Needs: This project will provide an understanding of the historical levels of antibiotics used in human and veterinary medicine that have entered Minnesota lakes. Additionally, this will be the first study to investigate how the discharge of these chemicals has or is affecting the levels of resistance genes in the environment. This is information critical to protecting human and ecological health and may provide information relevant to antibiotic use and development. This study will reveal if additional treatment to remove antibiotics from wastewater or runoff is necessary or unnecessary in terms of proliferation of resistance genes. The results will be disseminated via the scientific literature and a publically available final report.

2014 Detailed Project Budget

Project Title: Antibiotics and antibiotic resistance genes in Minnesota lakes

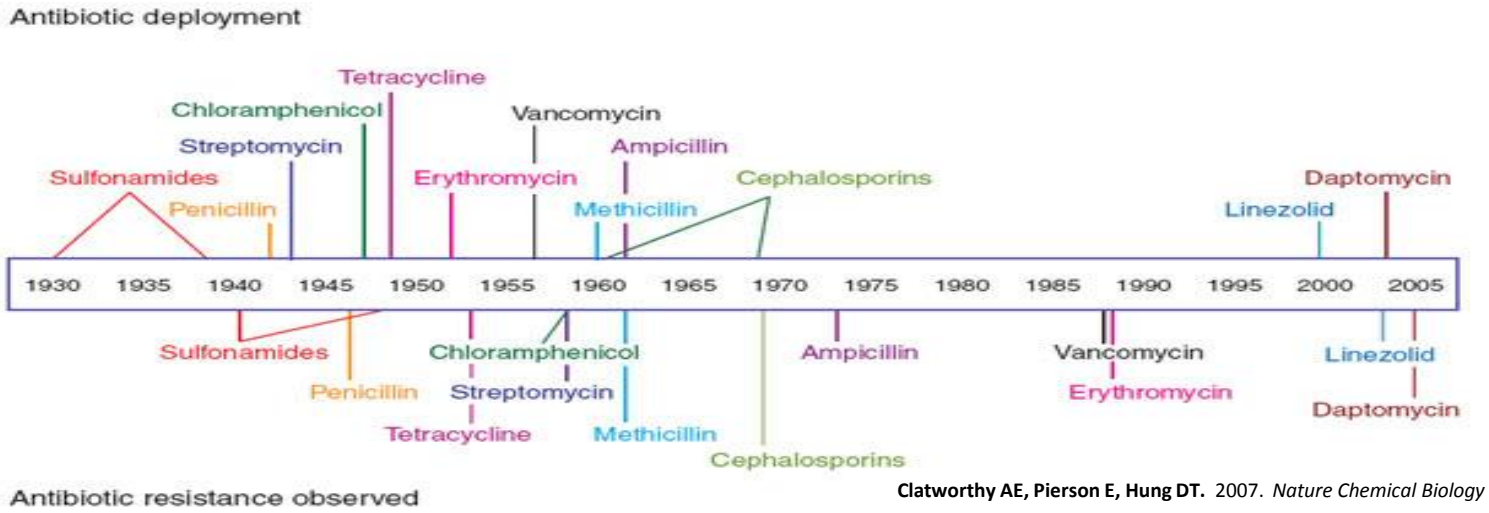
IV. TOTAL ENRTF REQUEST BUDGET *3* years

BUDGET ITEM *(See "Guidance on Allowable Expenses", p. 13)*	**AMOUNT**
Personnel: Arnold (PI, 6% time per year, salary 74.8% of cost, fringe benefits 25.2% of cost). Project supervision, supervision of graduate and undergraduate students and project reporting.	$ 36,100
Personnel: LaPara (co-PI, 6% time per year, salary 74.8% of cost, fringe benefits 25.2% of cost). Project supervision, supervision of graduate student #2 and and project reporting.	$ 28,600
Personnel: Graduate student #1 (50% time during academic year, 50% time in summer in Y1 and Y2; 25% time in Y3; 56% salary, 33% tuition, 11% fringe benefits). Sediment core collection and sectioning. Development of antibiotic extraction and analytical protocols. Determination of antibiotic concentrations in sediments.	$ 108,400
Personnel: Graduate student #2 (50% time during academic year, 50% time in summer in Y1 and Y2; 25% time in Y3; 56% salary, 33% tuition, 11% fringe benefits). Extraction and purification of DNA from collected sediment samples. Quantificaiton of resistance genes.	$ 108,400
Subcontractt: Science Museum of Minnesota for collection and dating of sediment cores. Costs include personnel (Dr. Daniel Engstrom, 2% effort $4688 salary, $1312 fringe) and analytical and dating costs ($11,500).	$ 17,500
Supplies: Supplies (chemical standards, isotope standards, microbiologial/DNA extraction kits, instrument/analytical time for antibiotic and DNA analysis, solvents, consumable supplies, notebooks, software licenses; $29,000 total).Maintenance and repair of laboratory equipment required for analyses and experiments ($5,000 total)	$ 34,000
Travel: Mileage charges and univeristy vehicle rental charges for trips to collect sediment cores. Hotel/meal charges if overnight stay required. Attendence for students at local conferneces to disseminate results.	$ 5,000
Additional Budget Items: none	
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 338,000

V. OTHER FUNDS

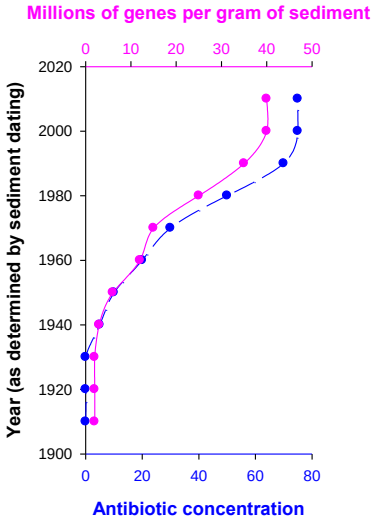
SOURCE OF FUNDS	**AMOUNT**	**Status**
Other Non-State $ Being Applied to Project During Project Period: none	$ -	
Other State $ Being Applied to Project During Project Period: none	$ -	
In-kind Services During Project Period: Arnold and LaPara will also devote 1% time per year in kind ($10,700). Because the project is overhead free, laboratory space, electricty, and other facilities/adminstrative costs (52% of direct costs excluding permanent equipment and graduate student academic year fringe benefits) are provided in-kind ($130,800)	$ 141,500	secured
Remaining $ from Current ENRTF Appropriation (if applicable): no current project directly applicable	$ -	
Funding History: none	$ -	

The problem: Antibiotic use causes antibiotic resistance



Clatworthy AE, Pierson E, Hung DT. 2007. *Nature Chemical Biology* 3(9):541-548.

The question: Do antibiotics released into aquatic systems lead to the retention or development of resistance?



Benefits

- Evaluation of antibiotic resistance reservoirs in aquatic systems
- Determination of need for additional wastewater treatment
- Protecting human and ecological health
- Provide information relevant to antibiotic use and development

Project Manager Qualifications and Organization Description

William A. Arnold

Joseph T. and Rose S. Ling Professor and Associate Head, Environmental Engineering, Department of Civil Engineering, University of Minnesota

B.S., Chemical Engineering, 1994, Massachusetts Institute of Technology, Cambridge, MA.
M.S., Chemical Engineering, 1995, Yale University, New Haven, CT.
Ph.D., Environmental Engineering, 1999, The Johns Hopkins University, Baltimore, MD.

Dr. William Arnold will be responsible for overall project coordination. He has been studying the fate of pharmaceutical and pesticide compounds in aquatic environments for fourteen years. The main focus has been the photolysis rates of pharmaceuticals and personal care products in surface water to determine the persistence of these compounds in the environment. As part of these efforts, reaction products have been identified to determine if photolysis leads to a loss of biological activity of the compounds and/or if reaction products are of additional environmental concern. Recent work in Dr. Arnold's group funded by the Environmental Natural Resource Trust Fund revealed that triclosan and its associated dioxins are accumulating in Minnesota Lakes. He has published over twenty peer-reviewed papers on pharmaceutical fate since 2003, and he is the co-author of a textbook on water chemistry published in 2011. Dr. Arnold is a Resident Fellow of the University of Minnesota Institute on the Environment, an Associate Fellow of the Minnesota Supercomputing Institute, and a member of the graduate faculty in Water Resources Science. He won the *Arcadis/Association of Environmental Engineering and Science Professors Frontier in Research Award* in 2012 and the University of Minnesota College of Science and Engineering *George W. Taylor Award for Distinguished Research* in 2011.

Timothy M. LaPara

Professor, Environmental Engineering, Department of Civil Engineering, University of Minnesota

B.S.C.E., Civil Engineering, 1995, University of Notre Dame, Notre Dame, IN
Ph.D., Civil Engineering, 1999, Purdue University, West Lafayette, IN

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.

Organization Description

The University of Minnesota is one of the largest, most comprehensive, and most prestigious public universities in the United States (http://www1.umn.edu/twincities/about/index.html). The laboratories and offices of the PI contain the necessary fixed and moveable equipment and facilities needed for the proposed studies.