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

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Project Title:

Triclosan Occurrence and Bacterial Resistance in Minnesota Wastewater

Category: B. Water Resources

Total Project Budget: $ 51,992

Proposed Project Time Period for the Funding Requested: 1 Year, July 2014-June 2015

Other Non-State Funds: $ 0

Summary:

Triclosan and triclosan-containing products are being phased out by State agencies. This project aims to produce a snapshot of triclosan in wastewater; and bacterial resistance at this point in time.

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Location

Region: Statewide

County Name: Statewide

City / Township:

MP: 0613-2-213-proposa
Budget: 0613-2-213-bud
Qual: 0613-2-213-qualifi
Map: 0613-2-213-map-L
Resolution:
List:

_____ Funding Priorities _____ Multiple Benefits _____ Outcomes _____ Knowledge Base

_____ Extent of Impact _____ Innovation _____ Scientific/Tech Basis _____ Urgency

Capacity Readiness Leverage Employment TOTAL

PROJECT TITLE: Triclosan Occurrence and Bacterial Resistance in Minnesota Wastewater

I. PROJECT STATEMENT

Recently the State of Minnesota announced plans to discontinue purchasing the antimicrobial agent triclosan and products containing this agent, such as hand soap and other cleaners. Concerns regarding the discharge of this agent into wastewater streams, eventually reaching lakes, streams and other bodies of water have prompted the State to take this action. Concerns about resistance to antimicrobial agents have prompted research into triclosan resistance specifically, as it floods our waterways and watersheds. **At this point in time, when many agencies within the State are discontinuing use of triclosan-containing products, we have the opportunity to acquire a snapshot of triclosan occurrence from the wastewater stream, and partially quantify bacterial resistance in such water. This information, at the time when the State is severely reducing its contribution of triclosan into the environment, will allow for comparison in the future to similar studies.** The overall goal of this project is to collect information at a time when such information will not likely be available again in the identical manner. The specific outcomes of such an opportunity are to: **#1 –** Measure triclosan in samples from wastewater outflow obtained from numerous wastewater treatment facilities throughout the state; and **#2 –** Identify the presence of triclosan-resistance in bacterial populations from such waters. Collecting and testing wastewater from strategically selected facilities from throughout the State will provide a current, in effect baseline, snapshot of the occurrence and impact of triclosan.

Specific activities in this project:

1 – Collect wastewater outflow samples from around 50-75 treatment plants throughout the state.
2 – Measure triclosan occurrence in the wastewater sample.
3 – Culture bacterial colonies from wastewater and test as many as possible for triclosan resistance.
4 – Potentially identify any resistant bacteria through microbiological and genetic methods.

II. DESCRIPTION OF PROJECT ACTIVITIES

Activity 1: Collection of wastewater samples throughout the state — Budget: $1400.00

Obtain through postal mail samples collected by wastewater treatment plant operators. Sterile collection vials sent to wastewater treatment facilities would be returned by those facilities choosing to participate. Anonymity of specific treatment facility would be conferred to plant operator. This would be the most efficient manner of collection. After receiving samples from participating facilities and constructing a map of acquired samples, determine additional sites to result in a broad, evenly distributed set of sampling sites. Travel to additional sites to collect samples.

Outcome	Completion Date
1. Enlist participation of wastewater treatment plant operators in collection of samples and return of samples to project facility.	September 2014
2. Travel to additional sites in order to generate a full and broad snapshot of triclosan occurrence throughout the state.	November 2014

Activity 2: Measure triclosan in wastewater samples — Budget: $17830.00

Using an enzyme-linked immunosorbent assay (ELISA) specific for triclosan, samples would be measured and levels of triclosan ascertained. The ELISA is a specific biochemical assay utilizing antibodies specific for a target substance, and color development via actions of an enzyme on a substrate. Numerous samples can be assayed simultaneously and data generated relatively quickly.

Outcome	Completion Date
1. Quantitatively measure samples via commercial triclosan ELISA kits.	February 2015

Activity 3: Determine triclosan resistance in bacterial populations **Budget: $12835.00**

Wastewater samples will be cultured on several selective microbiological media to allow growth of as many species as possible. Colonies cultured on these media will be transferred to media supplemented with triclosan to determine whether resistance exists, and disk-diffusion tests will be performed. These disk-diffusion tests involve placing a filter paper disk impregnated with triclosan onto the media along with the bacterial culture and determining the severity of resistance. The disk-diffusion test is a common microbiological test for antibiotic resistance.

Outcome	Completion Date
1. Growth of bacterial populations from wastewater samples and testing these existing bacteria for resistance to triclosan.	February 2015

Activity 4: Identify specific bacterial species exhibiting resistance **Budget: $5000.00**

Should any bacterial colonies present in the wastewater samples exhibit resistance to triclosan, these bacteria would need to be identified. Various microbiological methods and genetic methods of identifying bacterial species exist. Attempts would be made to identify the species of those bacterial populations which exhibit resistance to triclosan.

Outcome	Completion Date
1. Bacterial colonies exhibiting resistance to triclosan would be subjected to microbiological testing to begin the identification process.	June 2015
2. If needed, genetic methods of identification would be utilized to identify the species exhibiting resistance.	June 2015

III. PROJECT STRATEGY

A. Project Team/Partners

Stephen Bartell is a faculty member in the Biology department at Normandale Community College, Bloomington, MN and will be project leader. There will be the involvement of up to three undergraduate research students, working on the project in varied capacities. These students will be recruited by the faculty to minimize the amount of training which will have to take place. Most of the laboratory work will be carried out by these students under the supervision of Stephen Bartell.

B. Timeline Requirements

This will be a one year project, with some assumptions regarding the participation of the treatment plant operators in obtaining the samples at the beginning of the project. In the later phases of the project, it is anticipated that the number of samples requiring genetic identification may be such that some of the project will have to be curtailed or at least samples will have to be strategically selected for further analysis. In this event, the genetic testing will be funded through the end date of the project, June 30, 2015, and sample data may not be obtained until after this date, though it is anticipated that no additional funding will be required, just analysis of the data obtained.

C. Long-Term Strategy and Future Funding Needs

These findings will provide information regarding the current state of triclosan in Minnesota's wastewater. It is anticipated that in the future, another study could be conducted after several years of reduced triclosan discharge to see if there has been a change in triclosan levels or in the presence of resistant bacterial populations.

2014 Detailed Project Budget

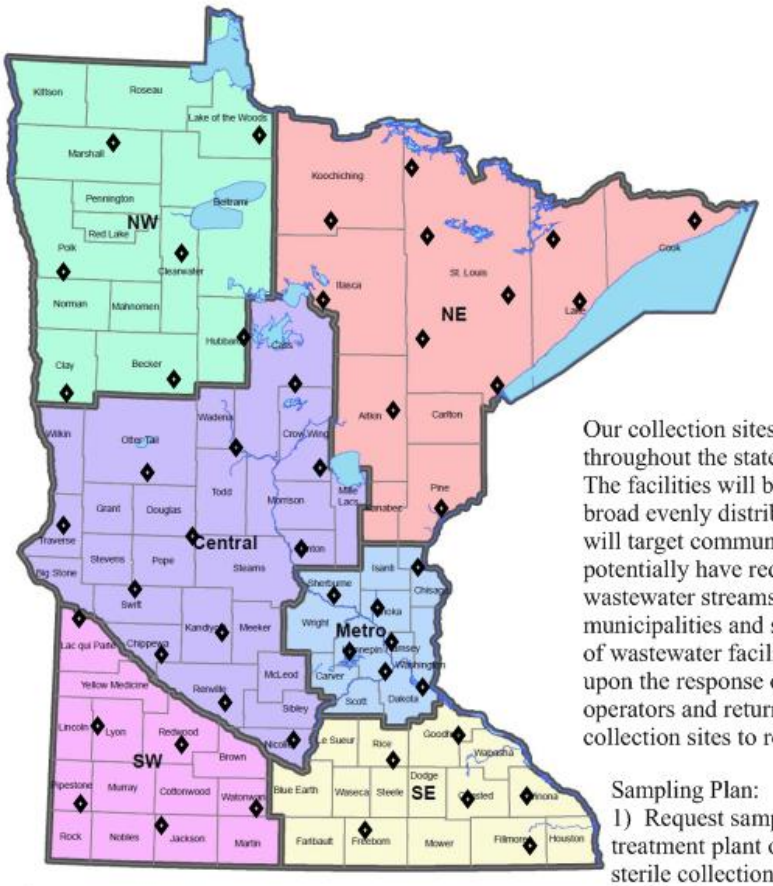
Project Title: Triclosan Occurrence and Bacterial Resistance in Minnesota Wastewater

IV. TOTAL ENRTF REQUEST BUDGET 1 year

BUDGET ITEM	AMOUNT
Personnel: Project Leader - Stephen Bartell - 20% of full-time employment (51% salary; 49% fringe benefits; July 1st, 2014 through June 30th, 2015) - 1 person	$ 12,650
Personnel: Undergraduate Research Students - Tuition paid for 2 research credits for up to 3 students; the involvement of the students will be sufficient for a two-credit course equivalent.	$ 2,277
Contracts: Contracted facility/individual capable of genetic identification of bacterial coloines exhibiting resistance to triclosan. The certain need of this service is not yet known. Faculty at the project facility will likely be contracted if this need exists.	$ 5,000
Equipment/Tools/Supplies: Postage for mailing collection vials and return mail.	$ 400
Equipment/Tools/Supplies: Triclosan ELISA kits for measuring triclosan.	$ 6,830
Equipment/Tools/Supplies: Microplate reader for ELISA	$ 5,600
Equipment/Tools/Supplies: Automated plate washer	$ 4,800
Equipment/Tools/Supplies: Bacterial incubator for dedicated environmental bacterial culturing.	$ 4,600
Equipment/Tools/Supplies: Bacterial incubator capable of elevated CO2 for dedicated environmental bacterial culturing.	$ 7,000
Equipment/Tools/Supplies: Microbiological media and general laboratory supplies, such as petri dishes, microcentrifuge tubes, pipette tips.	$ 1,835
Travel: In the event that limited samples are returned by treatment plant operators, additional sites would be chosen in order to create a full and broad set of collection sites. This could potentially include travel to all four corners of the state, to include as many regions of the state as possible. Estimates for the maximum travel: Gas: $200; Lodging 5 nights - $400; Vehicle Rental: $400/week	$ 1,000
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 51,992

V. OTHER FUNDS

SOURCE OF FUNDS	AMOUNT	Status
Funding History: None	N/A	



Our collection sites are proposed to be spread throughout the state, at wastewater treatment facilities. The facilities will be chosen initially to produce a large, broad evenly distributed set of sites. Initially, the sites will target communities where state agencies potentially have reduced outflow of triclosan through wastewater streams. Both a collection of larger municipalities and smaller communities, with a variety of wastewater facilities will be selected, and based upon the response of wastewater treatment plant operators and return of samples, we will travel to collection sites to round out the collection site map.

Testing Plan:

1) After collection of all samples, the first round of testing will be triclosan levels via ELISA testing methods.
2) Bacteria from the site samples will be grown on several selective microbiological media.
3) The bacteria which are cultured will be tested for triclosan resistance on triclosan-supplemented media and via disk-diffusion test methodology.
4) Bacteria which exhibit triclosan resistance will be identified as much as possible using microbiological methods as well as pertinent genetic tests.
5) An overall snapshot of triclosan levels in wastewater from around the state, at this time of the state's phasing out of triclosan will be produced, along with a list of resistance to triclosan among inhabitants of wastewater.

Sampling Plan:

1) Request sample collection from wastewater treatment plant operators, sending the plant operator sterile collection vials, with return shipping materials.
2) Based upon returns of samples, construct a site collection map which gives a broad sampling from across the state, determining additional collection sites to fully round out the sites.
3) Travel to the additional sites to perform sample collection from wastewater treatment facilities, after consulting with site operator.

Stephen Bartell is a faculty member in the Biology department at Normandale Community College, Bloomington, MN and will be project leader. Mr. Bartell teaches anatomy, physiology and microbiology. The department serves several thousand students per year, and Normandale Community College regularly serves around ten thousand students per year. Mr. Bartell has research affiliations with the Aquatic Toxicology Laboratory at Saint Cloud State University, and currently has around ten publications in the aquatic toxicology field, including a recent article involving triclosan in the environment. He is an expert in ELISA development and has five years of experience teaching microbiology. In his affiliations with the Aquatic Toxicology Laboratory at Saint Cloud State University, Mr. Bartell has supervised numerous graduate students in the methodology and practice of ELISA protocols measuring emerging contaminants in the environment.