



# Environment and Natural Resources Trust Fund

## 2021 Request for Proposal

### General Information

**Proposal ID:** 2021-121

**Proposal Title:** Monitoring Emerging Viruses in Minnesota's Urban Water Cycles

### Project Manager Information

**Name:** Sebastian Behrens

**Organization:** U of MN - College of Biological Sciences

**Office Telephone:** (651) 756-9359

**Email:** sbehrens@umn.edu

### Project Basic Information

**Project Summary:** This project will address the presence and fate of enveloped viruses (e.g. coronaviruses) and their survivability in aqueous environments with emphasis on wastewater and drinking water treatment processes.

**Funds Requested:** \$489,000

**Proposed Project Completion:** 2024-06-30

**LCCMR Funding Category:** Water Resources (B)

### Project Location

**What is the best scale for describing where your work will take place?**

Statewide

**What is the best scale to describe the area impacted by your work?**

Statewide

**When will the work impact occur?**

During the Project

## Narrative

### **Describe the opportunity or problem your proposal seeks to address. Include any relevant background information.**

Over the last month MPCA and Minnesota Department of Health have been receiving several questions related to the risk that COVID-19 poses to wastewater professionals. This project will develop new detection methods and quantitative risk assessment (QRA) models that MPCA and the Department of Health can use to better quantify future risks related to the role of the urban water cycle in the spread of enveloped viruses. Past research efforts have focused mainly on nonenveloped human enteric viruses such as human noroviruses. However, avian influenzas, SARS, MERS, and the ongoing COVID-19 pandemic, have been caused by enveloped viruses. These viruses have direct connection to wastewater and drinking water purification when they are excreted in feces or urine. Recent reports show that SARS-CoV-2 has been detected in stool samples of COVID-19 cases. Increasing circulation of viruses such as SARS-CoV-2 in a population will increase virus loads in to sewer systems of our cities. It is important to collect information about the occurrence and fate of enveloped viruses in sewage to understand if there is a risk to sewage workers, but also to determine if sewage surveillance could be used to monitor the circulation of enveloped viruses in our communities.

### **What is your proposed solution to the problem or opportunity discussed above? i.e. What are you seeking funding to do? You will be asked to expand on this in Activities and Milestones.**

SARS-CoV-2 (the virus that causes COVID-19), as well as any other disease-causing viruses in wastewater, are currently impossible to detect by wastewater professionals as they conduct their day-to-day work. The currently unknown role of the environment, specifically the urban water cycle, in the spread of enveloped viruses highlights the need for the development of rapid testing methods and risk assessment model proposed in this project. In order to effectively control outbreaks and pandemics of novel enveloped viruses in the future we need to understand what conditions influence their environmental persistence. In this project we will study the fate of enveloped viruses in the urban water cycle and identify locations of potential human exposure. In order to achieve this goal, we will develop new, cost-efficient, molecular screening methods that will allow the rapid detection and quantification of enveloped viruses in environmental water samples. The new testing methods will be useful to municipalities and state agencies, and health departments to estimate the risk of infection and illness when a population is exposed to enveloped viruses in the environment. Sewage surveillance can also serve as early warning of (re-)emergence of COVID-19 in the Twin Cities and local communities connected to central sewage treatment.

### **What are the specific project outcomes as they relate to the public purpose of protection, conservation, preservation, and enhancement of the state's natural resources?**

The proposed project will have the following specific project outcomes:

- (1) Development and optimization of methods for extracting, purifying, and quantifying enveloped and non-enveloped viruses and their nucleic acids from complex sample matrices such as wastewater, residual biosolids, and surface waters.
- (2) Quantification of presence and survivability of enveloped and non-enveloped viruses during wastewater and drinking water treatment processes, as well as when they are released into the environment via wastewater effluent or land-applied biosolids.
- (3) Quantitative risk assessment will be conducted to better characterize exposure and transmission pathways for enveloped viruses through the urban water cycle environment.

## Activities and Milestones

### Activity 1: Development of molecular assays for extracting, purifying, and quantifying enveloped viruses in complex aquatic samples.

**Activity Budget:** \$158,876

#### Activity Description:

Detecting and quantifying viruses in environmental samples requires concentrating the virus in the samples into a smaller volume to improve detection limits. Most available methods for concentrating and extracting viral nucleic acids from aqueous environmental samples have been optimized for non-enveloped enteric viruses. SARS and influenza viruses are enveloped single-stranded RNA viruses. RNA is more challenging to extract from environmental samples than DNA. RNA also needs to be reverse transcribed into cDNA (complementary DNA) in order to allow detection and quantification with molecular assays such as quantitative PCR. In this activity we will develop assays for the reliable and fast detection of diverse groups of enveloped RNA viruses using reverse transcription quantitative PCR (RT-PCR). PCR assay will target specific viral nucleocapsid and envelope genes. Strict quality control protocols for RNA extraction and quantification using appropriate virus surrogates will be developed to ensure assay reliability and quantify the efficiency of virus detection in water and wastewater samples.

#### Activity Milestones:

| Description  | Completion Date |
|--|-----------------|
| Quality control and quality assurance of extraction and detection methods using appropriate virus surrogates | 2022-06-30      |
| Comparative metagenomic sequence analysis of virus populations in collected water and wastewater samples     | 2022-06-30      |
| Development of RT-PCR methods for the detection of enveloped viruses in environmental samples                | 2023-07-31      |

### Activity 2: Quantification of presence and survivability of enveloped viruses during wastewater and drinking water treatment processes

**Activity Budget:** \$163,988

#### Activity Description:

There is limited data on the concentration of enveloped viruses in raw sewage, wastewater effluent, surface waters, and drinking water. Enveloped viruses are generally considered unstable in the aqueous environment and have therefore not been emphasized in waterborne virus research, methods, or regulations. However, the group of enveloped viruses (including the virus that causes COVID-19) is extremely diverse and different viruses display a diverse range of environmental behavior and survivability. In this part of the project we will use the new tests developed in Activity 1 to answer specific questions regarding the fate of viruses in different compartments of the urban water cycle. The study will characterize how different stages of wastewater and drinking water treatment will impact virus particle partitioning between solids, liquid, and air and how engineering design of water treatment plants will impact viral detection in wastewater, in wastewater effluent, land-applied biosolids and through the different stages of the drinking water treatment process.

#### Activity Milestones:

| Description   | Completion Date |
|---|-----------------|
| Quantification of enveloped viruses in raw sewage, wastewater effluent, biosolids, surface waters, and drinking water   | 2023-07-31      |
| Comparison of different water treatment plant designs (continuous flow, sequencing batch reactors, anaerobic digestion) | 2023-12-31      |

|   |            |
|---|------------|
| Quantification of virus particle partitioning between solids, liquid, and aerosols during different water treatment processes | 2023-12-31 |
|---|------------|

### Activity 3: Quantitative risk assessment to characterize exposure and transmission pathways for enveloped viruses in the urban water cycle environment.

**Activity Budget:** \$166,136

**Activity Description:**

Potential exposure pathways for both wastewater workers and the general public to untreated wastewater are poorly characterized. In this activity we will characterize specific exposure scenarios, e.g. exposure to wastewater used for irrigation, combined sewer overflows, faulty plumbing systems, as well as risks associated with occupational exposures to wastewater during operation and maintenance of sewage and drinking water treatment infrastructure. Results from this project will inform QRA models to understand how much an impact different groups of enveloped viruses in the environment will have on the health of wastewater workers and the general public population of Minnesota. The QRA framework will consist of four steps, including 1) hazard identification, 2) dose-response, 3) exposure assessment and 4) risk characterization. The quantified virus gene copy numbers (Activity 2) will be analyzed to quantify their statistical signatures (mean, standard deviation, probability distribution). The estimated risk (step 4) will be evaluated using available EPA health benchmarks for microbial pathogen exposure scenarios. Monte Carlo simulations will provide a range of uncertainty in infection risks to human health from exposure to wastewater and other virus containing environmental water samples.

**Activity Milestones:**

| Description  | Completion Date |
|--|-----------------|
| Statistical data analysis of virus concentrations in aqueous samples   | 2023-12-31      |
| Quantitative risk assessment and development of guidelines to manage virus exposure risks through wastewater | 2024-06-30      |
| Characterization of occupational and public exposure scenarios   | 2024-06-30      |

## Project Partners and Collaborators

| Name                            | Organization   | Role   | Receiving Funds |
|---------------------------------|--|--|-----------------|
| Leisa Thompson & George Sprouse | Metropolitan Council, Environmental Services, Wastewater & Water | Facilitation and support of sample collection efforts. Data discussion and communication with policy-makers, planning agencies, and water service providers in the Twin Cities metropolitan region. Please also see attached letter of support from MCES.  | No              |
| Prof. Dr. Timothy LaPara        | University of Minnesota  | Dr. LaPara is a full professor in the Department of Civil, Environmental, and Geo-Engineering. His area of expertise are environmental engineering and environmental microbiology. Dr. LaPara will serve as co-PI on the proposed project. He will co-advise the graduate student and postdoc, and support data analysis and discussion. | Yes             |

## Long-Term Implementation and Funding

**Describe how the results will be implemented and how any ongoing effort will be funded. If not already addressed as part of the project, how will findings, results, and products developed be implemented after project completion? If additional work is needed, how will this be funded?**

The research questions addressed in this project are critical to prepare environmental engineers and public health workers in Minnesota in the event that enveloped viruses causing a deadly outbreak or pandemic enter the urban water cycle. Results will be published and shared with state agencies and health departments as soon as they become available to ensure instant public access to new testing methods and recorded water contamination data. Upon completion of the project publications and outcomes will be summarized in a final report downloadable from our University website. Talk and poster presentations will be given at local conferences.

## Other ENRTF Appropriations Awarded in the Last Six Years

| Name  | Appropriation                          | Amount Awarded |
|---|--|----------------|
| Engineered Biofilter for Sulfate and Metal Removal from Mine Waters | M.L. 2016, Chp. 186, Sec. 2, Subd. 04p | \$440,000      |
| Wastewater Treatment Process Improvements                           | M.L. 2016, Chp. 186, Sec. 2, Subd. 04k | \$398,000      |

## Project Manager and Organization Qualifications

**Project Manager Name:** Sebastian Behrens

**Job Title:** Assoc. Prof. Civil and Environmental Engineering/BioTechnology Institute

**Provide description of the project manager's qualifications to manage the proposed project.**

Dr. Behrens is an environmental microbiologist and an expert in detection, identification, and quantification of diverse microbial target sequences in environmental samples based on massively parallel sequencing technologies, quantitative (RT-)PCR, and flow cytometry. Dr. Behrens follows an interdisciplinary approach that combines the disciplines environmental engineering, and molecular biology to understand the basic ecological principles driving the bioremediation of contaminants and the transport and fate of pathogens in the environment. Dr. Behrens will advise and guide graduate students and postdocs working on this project. Dr. Behrens will guide the development of molecular assays to detect and quantify enveloped viruses. He will be responsible for the quantification of viruses in the collected water samples, facilitate the implementation of QRA modeling, and communicate project progress and results to the LCCMR.

**Organization:** U of MN - College of Biological Sciences

**Organization Description:**

The University of Minnesota is the State's main research and graduate teaching institution. The University partners with communities and governmental agencies across Minnesota to engage students, faculty, and staff in addressing society's most pressing issues. The Department of Civil, Environmental and Geo-Engineering focuses on collaborative and interdisciplinary research within critical areas such as managing and sustaining water and land-use infrastructure, mitigating disaster of the natural and built environments, engineering and developing earth resources, and designing renewable energy systems.

## Budget Summary

| Category / Name                       | Subcategory or Type | Description  | Purpose   | Gen. Ineligible | % Benefits | # FTE | Classified Staff? | \$ Amount        |
|---------------------------------------|---------------------|--|---|-----------------|------------|-------|-------------------|------------------|
| <b>Personnel</b>                      |                     |  |   |                 |            |       |                   |                  |
| Faculty                               |                     | PI   |   |                 | 26.74%     | 0.33  |                   | \$54,588         |
| Faculty                               |                     | CO-PI  |   |                 | 26.7%      | 0.15  |                   | \$28,372         |
| 1 Graduate Student                    |                     | tbd  |   |                 | 43.61%     | 1.5   |                   | \$152,155        |
| Post Doctoral Researcher              |                     | TBD  |   |                 | 20.25%     | 3     |                   | \$187,885        |
|                                       |                     |  |   |                 |            |       | <b>Sub Total</b>  | <b>\$423,000</b> |
| <b>Contracts and Services</b>         |                     |  |   |                 |            |       |                   |                  |
|                                       |                     |  |   |                 |            |       | <b>Sub Total</b>  | -                |
| <b>Equipment, Tools, and Supplies</b> |                     |  |   |                 |            |       |                   |                  |
|                                       | Tools and Supplies  | \$15K of consumable and supplies per year , \$ 8K for each year for flow cytometry (dyes, standard beads, buffer, filters); \$ 5K per year for water chemistry analysis (nutrients, DOC, ions, metals); \$12K for each year for lab services to use UMGC DNA sequencing services \$ 5K for each year for lab services to run the flow sorter (100 hours over three years for \$150 per hour = \$15K) | for DNA/RNA extraction, cDNA synthesis, for flow cytometry, water chemistry analysis, lab services to use UMGC DNA sequencing services, lab services to run the flow sorter |                 |            |       |                   | \$60,000         |
|                                       |                     |  |   |                 |            |       | <b>Sub Total</b>  | <b>\$60,000</b>  |
| <b>Capital Expenditures</b>           |                     |  |   |                 |            |       |                   |                  |
|                                       |                     |  |   |                 |            |       | <b>Sub Total</b>  | -                |
| <b>Acquisitions and Stewardship</b>   |                     |  |   |                 |            |       |                   |                  |
|                                       |                     |  |   |                 |            |       | <b>Sub Total</b>  | -                |

|                                 |   |   |  |   |  |  |                    |                  |
|---------------------------------|---|---|--|---|--|--|--------------------|------------------|
| <b>Travel In Minnesota</b>      |   |   |  |   |  |  |                    |                  |
|                                 | Conference Registration<br>Miles/ Meals/<br>Lodging | meals and lodging for students                                | meals and lodging to allow students to participate in local conferences and meetings to present research on this grant. Travel would be to formally present of project findings(e.g. Minnesota Wastewater Operators Association or the Minnesota Section of the American Water Works Association). | X |  |  |                    | \$1,000          |
|                                 | Miles/ Meals/<br>Lodging                            | Mileage in Minnesota  | sample collection from wastewater treatment plants and drinking water facilities around the state  |   |  |  |                    | \$2,000          |
|                                 |   |   |  |   |  |  | <b>Sub Total</b>   | <b>\$3,000</b>   |
| <b>Travel Outside Minnesota</b> |   |   |  |   |  |  |                    |                  |
|                                 |   |   |  |   |  |  | <b>Sub Total</b>   | -                |
| <b>Printing and Publication</b> |   |   |  |   |  |  |                    |                  |
|                                 | Publication   | Sponsored Publications \$1500 per year based on current rates | Publication costs for research results on this project   |   |  |  |                    | \$3,000          |
|                                 |   |   |  |   |  |  | <b>Sub Total</b>   | <b>\$3,000</b>   |
| <b>Other Expenses</b>           |   |   |  |   |  |  |                    |                  |
|                                 |   |   |  |   |  |  | <b>Sub Total</b>   | -                |
|                                 |   |   |  |   |  |  | <b>Grand Total</b> | <b>\$489,000</b> |



## Classified Staff or Generally Ineligible Expenses

| Category/Name              | Subcategory or Type                               | Description                    | Justification Ineligible Expense or Classified Staff Request   |
|----------------------------|---|--------------------------------|--|
| <b>Travel In Minnesota</b> | Conference<br>Registration<br>Miles/Meals/Lodging | meals and lodging for students | meals and lodging to allow students to participate in local conferences and meetings to present research on this grant. Travel would be to formally present of project findings(e.g. Minnesota Wastewater Operators Association or the Minnesota Section of the American Water Works Association). |

## Non ENRTF Funds

| Category         | Specific Source   | Use  | Status                     | Amount           |
|------------------|---|--|----------------------------|------------------|
| <b>State</b>     |   |  |                            |                  |
|                  |   |  | <b>State Sub Total</b>     | -                |
| <b>Non-State</b> |   |  |                            |                  |
| In-Kind          | Indirect costs for this proposal, though not allowed, are listed as in-kind contribution of 55% MTDC which is the Federally Negotiated rate with the U of MN. The indirect is proportionate to the awarded funds at a rate 55% so if the award is reduced the F&A would be reduced. | To pay for administrative and facility expenses for this project | Secured                    | \$241,744        |
|                  |   |  | <b>Non State Sub Total</b> | <b>\$241,744</b> |
|                  |   |  | <b>Funds Total</b>         | <b>\$241,744</b> |

## Attachments

### Required Attachments

#### *Visual Component*

File: [2c30b554-09f.pdf](#)

#### *Alternate Text for Visual Component*

The fate of infective viruses in the urban water cycle and locations of potential human exposure. Viruses that are excreted in feces, urine, and vomit enter the sewage system. Toilet flushing or problems with indoor plumbing systems may form virus-laden aerosols that could result in human exposure. Viruses are transported through the municipal sewage system to the wastewater treatment plant (WWTP). Workers servicing sewage systems could be exposed to infective viruses. Combined sewage overflow events lead to the release of infective viruses in untreated sewage to surface waters. Viruses that enter the municipal WWTP are exposed to physical, biological, and chemical treatment processes. WWTP employees may be exposed to infective viruses present in the untreated and treated wastewater, as well as residual biosolids. Wastewater effluent can carry viruses that have survived treatment to surface waters. Residual biosolids from WWTP are disposed, often via land-application. Workers or others in close contact with the biosolids may be exposed to infective viruses that have survived the solids treatment processes. Recreational activities can lead to exposure to infective viruses present in surface waters. Leaky sewage pipes can lead to contamination in the underground drinking water distribution systems. Intake water at drinking water treatment plants can contain infective viruses. The water is treated with a range of physical and chemical treatment processes to remove contaminants, including viruses. Municipal drinking water consumers are exposed to viruses that either maintain infectivity through drinking water treatment and distribution or enter the distribution system through leaks in underground pipes.

### Optional Attachments

#### *Support Letter or Other*

| Title                  | File                             |
|------------------------|----------------------------------|
| Letter of support MCES | <a href="#">3d489c91-23e.pdf</a> |

## Administrative Use

**Does your project include restoration or acquisition of land rights?**

No

**Does your project have patent, royalties, or revenue potential?**

No

**Does your project include research?**

Yes

**Does the organization have a fiscal agent for this project?**

Yes, Sponsored Projects Administration



Environment and Natural Resources Trust Fund (ENRTF)  
2021 Main Proposal VISUAL  
Project Title: Monitoring emerging viruses in Minnesota's urban water cycles

### The fate of viruses in the urban water cycle and locations of potential human exposure

|  |                   |
|--|-------------------|
|  | Infected person   |
|  | Waterborne virus  |
|  | Wastewater worker |
|  | Healthy persons   |

