Environment and Natural Resources Trust Fund

M.L. 2024 Approved Work Plan

General Information

ID Number: 2024-086

Staff Lead: Lisa Bigaouette

Date this document submitted to LCCMR: June 5, 2024

Project Title: Fluorine Beyond PFAS: Pesticide and Pharmaceutical Degradation

Project Budget: $400,000

Project Manager Information

Name: William Arnold

Organization: U of MN - College of Science and Engineering

Office Telephone: (612) 625-8582

Email: arnol032@umn.edu

Web Address: https://cse.umn.edu/

Project Reporting

Date Work Plan Approved by LCCMR: June 20, 2024

Reporting Schedule: June 1 / December 1 of each year.

Project Completion: June 30, 2027

Final Report Due Date: August 14, 2027

Legal Information

Legal Citation: M.L. 2024, Chp. 83, Sec. 2, Subd. 04f

Appropriation Language: $400,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to identify fluorinated pesticides and pharmaceuticals that degrade into potentially persistent or toxic byproducts and to analyze the microbes, processes, and conditions involved to inform bioremediation and development of more sustainable chemistries.

Appropriation End Date: June 30, 2027

Narrative

Project Summary: The project will assess chemical and biological defluorination activities in environmental samples and identify the microorganisms, metabolic pathways, and intermediates resulting from degradation of fluorinated pesticides and pharmaceuticals.

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

Rachel Carson brought to light the dangers of persistent chlorinated chemicals in 1962, and we have spent decades dealing with these pollutants. Recently, persistent fluorochemicals, such as perfluorooctane sulfonate (PFOS), rose to prominence, and the poly- and perfluorinated chemicals (PFAS) class is under intense scrutiny by regulators and the public. Fluorine incorporation into organic chemicals is, however, much more ubiquitous than is generally known by the public. There are an increasing number of mass-produced chemicals containing one or more fluorine atoms, including pharmaceuticals, pesticides, and medical contrast agents. Yet, we understand little about the identity and fate of fluorinated byproducts produced in aquatic systems upon degradation of fluorinated pesticides and pharmaceuticals and their degradation products are mostly unknown. It is critically important to understand how the incorporation of fluorine into organic chemicals affects the full suite of potential biotic and abiotic degradation pathways in natural and engineered aquatic environments and what persistent fluorinated products (i.e., PFAS) form from both chemically and biologically driven natural and treatment processes. This information, in turn, will guide the development of molecules that require fluorine incorporation to obtain a desired effect, yet are able to break down into innocuous products.

What is your proposed solution to the problem or opportunity discussed above? Introduce us to the work you are seeking funding to do. You will be asked to expand on this proposed solution in Activities & Milestones.

There is a need to understand which fluorinated groups in chemicals lead to undesirable byproducts. The proposed work will use a screening assay to measure how much fluoride, which is added to toothpaste and is a benign degradation product, is produced from a suite of fluorinated chemicals via biological reactions that occur in the environment or during water and wastewater treatment. If all the fluorine initially in the chemical does not get converted to fluoride, that indicates unknown PFAS are forming. The next step will be to identify and quantify microorganisms and their enzymes degrading the fluorinated chemicals. This will also include identifying the specific processes leading to formation of potentially problematic byproducts and assessing how bacteria degrade the fluorinated pesticides and pharmaceuticals. Lastly, microbial responses to fluorinated compounds in wastewater sludge and soil samples will be assessed. This information will provide the information to assess which compounds and processes are most likely to form persistent fluorinated byproducts, leading to recommendations regarding compound use and water treatment/remediation options to limit these issues.

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?

We know that fluorinated pesticides and pharmaceuticals are entering Minnesota's waters. While some of these compounds are degraded into non-toxic byproducts via biological processes during wastewater treatment or in soils, there is the potential for a myriad of persistent, fluorinated, PFAS-like chemicals to form. By understanding which microorganisms effectively degrade these compounds and which compounds lead to problematic byproducts, recommendations about chemical use, water and wastewater treatment, and better chemical design can be made. This will lead to protection and enhancement of Minnesota's water resources.

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 and In the Future

Activities and Milestones

Activity 1: Rapid, high-throughput screening method to determine chemical and biological defluorination capacity in environmental samples

Activity Budget: $100,000

Activity Description:
Because the inclusion of fluorine in pesticides and pharmaceuticals gives rise to favorable properties with minimal changes in molecular shape and potentially more potent activity, a wide variety of fluorinated groups have been and will continue to be incorporated in the synthetic organic compounds. Once present in the environment due to direct (pesticides) or indirect (pharmaceuticals) release, a range of chemical and biological transformations may (or may not) occur giving rise to new fluorinated (PFAS) transformation products. A rapid screening test would allow assessment of the extent of fluoride formation and compound stability. Fluoride (which is added to drinking water and toothpaste) is a desirable reaction product, and if all the fluorine in the molecules is not converted to fluoride, that would indicate other fluorinated organic molecules are forming. We will assess the extent of fluoride release from a suite of model fluorinated molecules and selected pesticides and pharmaceuticals under a variety of chemical and biological conditions to assess which molecules lead to formation of PFAS-like chemicals. We anticipate at least two publications/dissemination events prior to March 31, 2026.

Activity Milestones:

Description	Approximate Completion Date
Collection of water and soil samples	August 31, 2024
Develop fluoride assay	December 31, 2024
Testing control strains and enzymes	June 30, 2025
Enzymatic and microbial fluoride release	March 31, 2026
Publication/dissemination	June 30, 2026

Activity 2: Identification and quantification of microorganisms and their enzymes degrading fluorinated chemicals

Activity Budget: $150,000

Activity Description:
The experiments in Activity 1 will identify the environmental samples and conditions under which microbially catalyzed fluoride release can be quantified in the fluoride screening assay. Analysis of products containing fluorinated groups that show persistence will be performed using quantitative fluorine nuclear magnetic resonance spectroscopy (NMR) leveraging a separately funded US National Science Foundation project focusing on chemical product identification under biotic and abiotic conditions. This activity is aimed at identifying the microorganisms and their enzymes that can metabolize fluorinated pesticides and pharmaceuticals. Only a small number of bacteria have been found so far that can cleave the carbon-fluorine bond and degrade fluorinated compounds. The degradation pathways of some of these microorganisms have been elucidated but intermediates related to the degrading enzymes and functional genes have not been studied and tested. An improved exploration of the microorganisms, enzymes, and genes in charge of fluorinated pesticides and pharmaceutical biodegradation will bring multiple benefits for the development of effective bioremediation strategies. To achieve this, we will take advantage of advanced DNA and RNA sequencing technologies to identify the microorganisms and genes responsible for fluoride release from the pesticides and pharmaceutical studied in Activity 1.

Activity Milestones:

Description	Approximate Completion Date
DNA and RNA extraction	December 31, 2025
DNA and RNA (cDNA) sequencing	March 31, 2026
Sequence analysis: Identification of microorganisms	August 31, 2026
Sequence analysis: Enzymes and metabolic pathways	December 31, 2026
19F-NMR and mass spectrometry analyses of reaction products.	April 30, 2027
Publicaiton/dissemination	May 31, 2027

Activity 3: Quantify microbial responses to fluorinated pesticides and pharmaceuticals in the environment

Activity Budget: $150,000

Activity Description:
Biological degradation of fluorinated chemicals is particularly complicated in soils or wastewater, with multiple microorganisms potentially participating and making different fluorinated byproducts. To understand the diverse responses of environmental microorganisms to the presence of bio-transformable fluorinated pharmaceuticals and pesticides, it is important to determine when and under which conditions the microorganisms are active under environmentally relevant condition. By using fluorescent labels, cells that are actively degrading compounds can be detected, sorted, and then identified by sequencing. co-PI Behrens has recently developed a protocol to track microbial protein synthesis (i.e., biological activity) in activated sludge. Here we will apply the technique to label active microbial populations that show high fluoride release. Cell suspensions derived from activated sludge and soils will be spiked with selected fluorinated pharmaceuticals and pesticides. Cells will be disaggregated, stained, and sorted on a cell sorter calibrated to detect the fluorescence signal. We will extract DNA from the sorted cells, to identify which microorganisms are most “active” degrading fluorinated pesticides and pharmaceuticals in the respective environmental samples. Results from this Activity will also provide opportunities to develop targeted bioremediation strategies for selected fluorinated compounds in various contaminated ecosystems.

Activity Milestones:

Description	Approximate Completion Date
Set up microcosms and quantify fluorine release from pollutants	June 30, 2025
Identify conditions for microorganisms to actively degrade fluorinated compounds	June 30, 2026
Stain and sort active microbial populations	December 31, 2026
DNA sequencing and data analysis	June 30, 2027
Publicaiton/disseminaiton	June 30, 2027

Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
William Pomerantz	University of Minnesota, Department of Chemistry	consultant. Dr. Pomerantz is an expert in the synthesis and characterization of fluorochemicals. He will oversee the NMR analyses to obtain fluorine balances and interpret degradation pathways.	No
Sebastian Behrens	University of Minnesota, Civil, Environmental, and Geo-Engineering	co-Investigator: Dr. Behrens will lead the biodegradation studies and evaluate the microbial responses to organofluorine biotransformation.	Yes

Dissemination

Describe your plans for dissemination, presentation, documentation, or sharing of data, results, samples, physical collections, and other products and how they will follow ENRTF Acknowledgement Requirements and Guidelines.
For each of the activities, results will be disseminated by peer reviewed publications in archival journals. Data will also be archived in the open access data repository for the University of Minnesota. In both cases, this will make information from the project widely accessible to Minnesotans and other interested parties that are working fluorinated pesticides and pharmaceuticals. Results from the project will also be presented at local/regional conferences. We will also communicate key findings to scientists in the Minnesota Pollution Control Agency directly. The Environment and Natural Resources Trust Fund will be acknowledged through use of the trust fund logo or attribution language on project print and electronic media, publications, signage, and presentations.

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 work be funded?
The results will be disseminated to laboratories, agencies, stakeholders (including wastewater and drinking water plant operators) and practitioners through open access publications, direct meetings, and conference presentations. If additional work is needed, funding from federal sources will be sought.

Other ENRTF Appropriations Awarded in the Last Six Years

Name	Appropriation	Amount Awarded
Mapping Antibiotic Resistance in Minnesota to Help Protect Environmental, Animal, and Human Health	M.L. 2018, Chp. 214, Art. 4, Sec. 2, Subd. 04h	$750,000
Determining Influence of Insecticides on Algal Blooms	M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 04a	$350,000
Benign Design: Environmental Studies Leading to Sustainable Pharmaceuticals	M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 04b	$415,000
Improving Drinking Water for Minnesotans through Pollution Prevention	M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 04f	$345,000
Technology For Energy-Generating Onsite Industrial Wastewater Treatment	M.L. 2021, First Special Session, Chp. 6, Art. 5, Sec. 2, Subd. 04b	$450,000
Monitoring Emerging Viruses in Minnesota's Urban Water Cycles	M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 04c	$416,000

Microgeographic Impact of Antibiotics Released from Identified Hotspots	M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 04d	$508,000

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	$ Amount
Personnel								
Principal Investigator - Arnold		Lead project, supervise students			37.1%	0.09		$39,436
co-Principal Investigator - Behrens		supervise students, lead enzymatic and biodegradation studies			37.1%	0.18		$42,830
Graduate Student #1		Perform chemical degradation experiments and product analyses, build database			44.4%	1.11		$121,634
Graduate Student #2		Perform microbiological experiments, develop fluoride assay			44.4%	1.11		$121,635
							Sub Total	**$325,535**
Contracts and Services								
Core facilities at the University of Minnesota	Internal services or fees (uncommon)	Fees for NMR instrument time and microbial sequencing analyses				0.3		$25,738
							Sub Total	**$25,738**
Equipment, Tools, and Supplies								
	Tools and Supplies	Laboratory supplies	necessary chemicals, solvents, reagents, analytical supplies, and laboratory consumables needed for the experiments					$33,227
	Tools and Supplies	Equipment maintenance	Parts and service to keep necessary instruments functioning					$13,500
							Sub Total	**$46,727**
Capital Expenditures								
							Sub Total	**-**

Acquisitions and Stewardship								
							Sub Total	**-**
Travel In Minnesota								
	Conference Registration Miles/ Meals/ Lodging	Project team to attend MN Water Conference	disseminate results to Minnesota audience	X				$2,000
							Sub Total	**$2,000**
Travel Outside Minnesota								
							Sub Total	**-**
Printing and Publication								
							Sub Total	**-**
Other Expenses								
							Sub Total	**-**
							Grand Total	**$400,000**

Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request
Travel In Minnesota	Conference Registration Miles/Meals/Lodging	Project team to attend MN Water Conference	Attendance and presentation of the results at conferences in Minnesota is a key avenue to present to the results to stake holders. Attendance is to participate in formal presentation of project findings. Up to 3 people each year will attend the Minnesota Water Resources conference or another conference held in Minnesota. Covered costs will include conference registration ($325 for senior personnel, $100 for students), parking at the venue, and mileage costs.

Non ENRTF Funds

Category	Specific Source	Use	Status	$ Amount
State				
			State Sub Total	**-**
Non-State				
In-Kind	Indirection costs not charged	U of MN facilities and administrative support	Secured	$176,871
Cash	National Science Foundation	Support of complementary research efforts in terms of personnel, laboratory supplies, and out of state travel	Secured	$100,000
Cash	NSF project and discretionary funds	publication fees, if needed	Secured	$5,000
			Non State Sub Total	**$281,871**
			Funds Total	**$281,871**

Attachments

Required Attachments

Visual Component

File: 172f360d-25c.pdf

Alternate Text for Visual Component

Fluorinated pesticides enter surface waters from (sub)urban and agricultural land. Fluorinated pharmaceuticals enter the environment via wastewater treatment. Biological, sunlight-driven, and other reactions can convert these pollutants into PFAS or fluoride. This work will assess biological defluorination and potential persistent products....

Supplemental Attachments

Capital Project Questionnaire, Budget Supplements, Support Letter, Photos, Media, Other

Title	File
UMN authorization to submit	c400855e-c2f.pdf
2024-086_Research_Addendum_Arnold_Behrens_revised_2023-11-28	aa0bc8f2-0d3.pdf

Difference between Proposal and Work Plan

Describe changes from Proposal to Work Plan Stage

Because we received National Science Foundation Funding for a portion of the proposed work, a revised plan more focused on microbiological aspects with a reduced budget was presented to LCCMR. The changes in Activities 1 and 2 reflect the scope of work discussed during the presentation, and Activity 3 has been expanded.

Additional Acknowledgements and Conditions:

The following are acknowledgements and conditions beyond those already included in the above workplan:

Do you understand and acknowledge the ENRTF repayment requirements if the use of capital equipment changes?

N/A

Do you agree travel expenses must follow the "Commissioner's Plan" promulgated by the Commissioner of Management of Budget or, for University of Minnesota projects, the University of Minnesota plan?

Yes, I agree to the UMN Policy.

Does your project have potential for royalties, copyrights, patents, sale of products and assets, or revenue generation?

No

Do you understand and acknowledge IP and revenue-return and sharing requirements in 116P.10?

N/A

Do you wish to request reinvestment of any revenues into your project instead of returning revenue to the ENRTF?

N/A

Does your project include original, hypothesis-driven research?

Yes

Does the organization have a fiscal agent for this project?

No

Does your project include the pre-design, design, construction, or renovation of a building, trail, campground, or other fixed capital asset costing $10,000 or more or large-scale stream or wetland restoration?

No

Do you propose using an appropriation from the Environment and Natural Resources Trust Fund to conduct a project that provides children's services (as defined in Minnesota Statutes section 299C.61 Subd.7 as "the provision of care, treatment, education, training, instruction, or recreation to children")?

No