



Environment and Natural Resources Trust Fund

2023 Request for Proposal

General Information

Proposal ID: 2023-107

Proposal Title: Ecotoxicological Impacts of Quinone Outside Inhibitor (QoI) Fungicides

Project Manager Information

Name: Kristine Wammer

Organization: University of St. Thomas

Office Telephone: (651) 962-6012

Email: khwammer@stthomas.edu

Project Basic Information

Project Summary: This work will provide a more comprehensive assessment of the ecological hazards associated with quinone outside inhibitor (QoI) fungicides and their major environmental transformation products

Funds Requested: \$282,000

Proposed Project Completion: June 30, 2025

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 and In the Future

Narrative

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

Quinone outside inhibitors (QoIs) are widely used fungicides representing 15% of the fungicide market share. Due to their efficacy, QoIs are used extensively throughout the USA, particularly in the agricultural Midwest. With increasing concerns over antifungal resistance, fungicide use has rapidly increased in recent years; worldwide, QoI sales increased from \$620 million (1999) to \$1.636 billion (2007). In 2017, the most widely used fungicide in the world, azoxystrobin, was applied up to 16,000 kilograms per county in the USA.

Because QoIs are applied as seed treatments and/or aerially to crops as many as 10 times throughout the growing season, QoI-containing runoff and contamination of water resources is almost certain. Azoxystrobin was detected in 45 out of 103 water samples taken from 29 streams draining agriculture areas in the USA. QoI occurrence in water combined with the findings that azoxystrobin causes mortality and developmental malformations in fish has heightened concerns over ecological impacts. Recent reports indicating QoI exposure in pollinators and humans (70% of the children, 100% of the pregnant women studied) combined with findings that QoIs affect pollinator survival and increase markers associated with neurodegenerative diseases (i.e., Alzheimer's disease) bring further urgency to this issue.

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.

Because of their widespread occurrence and growing awareness of previously overlooked potential health impacts, additional investigation of QoIs is necessary. Moreover, recent research by our team has found that many QoIs degrade readily in the environment through reactions common in natural (e.g., surface water) and engineered (e.g., water and wastewater treatment) aquatic systems. These reactions with sunlight (i.e., photochemical processes) and chemicals used in water and wastewater treatment (i.e., disinfection with free chlorine) often produce new chemical species, or QoI transformation products. Because these transformation products retain structural similarity to their parent QoI compounds, we hypothesize that these QoI transformation products retain biological activity that pose new risks to ecosystem and human health and must also be investigated.

To address existing knowledge gaps over the safety of their widespread use, we propose to investigate adverse effects associated with common QoI fungicides and their major environmental transformation products. This work capitalizes on the unique capabilities of the research team, who collectively have previously identified common transformation products of QoI photochemical and chlorination reactions and have extensive expertise in the use of bioassays, for a variety of species and toxicological endpoints, to assess hazards of emerging pollutant classes.

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?

This work will provide a more comprehensive assessment of the ecological hazards associated with QoI fungicides and their major environmental transformation products. We will produce new knowledge of the adverse effects of this fungicide class used widely across Minnesota, including the first hazard assessment of their major transformation products. This should enable improved regulatory oversight of QoI use, as well as the disposal of fungicide-treated seeds, to better protect and enhance Minnesota's natural resources and by extension human health.

Activities and Milestones

Activity 1: Evaluation of Qol environmental fate with identification of major transformation products

Activity Budget: \$186,000

Activity Description:

Working with the Qols most predominantly used in Minnesota (e.g., fenamidone, azoxystrobin and pyraclostrobin, among others), we will evaluate two processes we have identified as critical determinants to their persistence and fate in aquatic environments. We have found that Qols degrade in sunlight, both by direct and indirect reaction pathways, yielding as yet unidentified transformation products. This process occurs readily enough it should be anticipated in sunlit Minnesota surface waters and on surfaces including soils and seeds treated with Qols. Moreover, we know Qols are reactive toward chemical disinfectants like free chlorine, yielding products that are structurally similar to parent Qol species. These may be present in wastewater effluents discharged into Minnesota surface waters. Here, we will conduct experiments that will allow us to quantify the timescales over which these processes occur and develop process descriptors (like rate constants) that can be used to better model and predict Qol behavior. We will also use advanced chemical identification methods to structurally identify the remaining transformation products not yet identified through our preliminary work. We will then conduct experiments at sufficient scale such that we can isolate these products, thereby allowing us to test their ecotoxicological impacts in Activity 2.

Activity Milestones:

| Description | Completion Date |
|---|-------------------|
| Measure rates of Qol chlorination during simulated water and wastewater treatment | July 31, 2024 |
| Measure photodegradation rates of Qols in water, bound to soils, and coated on seeds | December 31, 2024 |
| Identify, scale up, and isolate photodegradation and chlorination transformation products for testing in Activity 2 | March 31, 2025 |

Activity 2: Evaluation of toxicity of Qols and their major transformation products

Activity Budget: \$96,000

Activity Description:

First, the effects of Qols and their major transformation products on 70 molecular targets indicative of different toxicity types (including carcinogenesis, DNA damage, endocrine disruption, neurotoxicity) will be evaluated using cutting-edge, rapid, and cost-effective techniques where living cells are exposed to chemicals of interest and screened for changes in biological activity. Second, the effects on mitochondria (cell “power plants” that generate energy essential for survival) will be measured because chemical properties of Qols make them very likely to exert toxicity by affecting mitochondrial energy generation processes. Third, Qols and their major transformation products that initiate molecular responses and/or mitochondrial effects will be also evaluated for the effects on physiological and behavioral responses that are important for growth and survival of fish.

Activity Milestones:

| Description | Completion Date |
|---|-----------------|
| Evaluate effects of Qols and their transformation products on 70 molecular targets indicative of toxicity | March 31, 2025 |
| Evaluate effects of Qols and their transformation products on the fish mitochondrial function | March 31, 2025 |
| Evaluate effects of Qols and their transformation products on the physiology and behavior of fish | June 30, 2025 |

Project Partners and Collaborators

| Name | Organization | Role | Receiving Funds |
|--------------------------|--------------------------|--------------------|-----------------|
| Dalma Martinovic-Weigelt | University of St. Thomas | Co-Project Manager | Yes |
| David Cwiertny | University of Iowa | Co-Project Manager | 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 work be funded?

We anticipate significant interest of the public and managers in this work. Baseline data regarding occurrence/ hazards will aid with determining whether and which QoIs and transformation products need to be added to the monitoring list of Minnesota Pollution Control Agency and/or become a candidate for Minnesota Department of Health screenings of toxicity and exposure potential as a part of the Protecting Minnesota's Water Resources Initiative. Such review may result in the development of Health Based Values and aquatic life screening values that are important for human and ecosystem health. Raised awareness of the hazards should reduce non-essential uses.

Other ENRTF Appropriations Awarded in the Last Six Years

| Name | Appropriation | Amount Awarded |
|--|---|----------------|
| Assessing Techniques for Eliminating Contaminants to Protect Native Fish and Mussels | M.L. 2016, Chp. 186, Sec. 2, Subd. 04d | \$287,000 |
| Reassessing Toxicity of Petroleum Spills on Groundwater and Surface Water | M.L. 2017, Chp. 96, Sec. 2, Subd. 04e | \$300,000 |
| 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 |
| Microgeographic Impact of Antibiotics Released from Identified Hotspots | M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 04d | \$508,000 |

Project Manager and Organization Qualifications

Project Manager Name: Kristine Wammer

Job Title: Associate Dean, College of Arts & Sciences

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

Kris Wammer is a Professor of Chemistry and an Associate Dean in the College of Arts & Sciences at the University of St. Thomas. She has substantial experience in both environmental chemistry and environmental microbiology. Her research focuses primarily on the fate and transformation of biologically active molecules in natural and engineered water systems. Over the past seventeen years she has mentored over 40 undergraduate research students, most of whom who have worked in her lab for several years, and many of whom have gone on to enroll in top Ph.D. programs in the U.S. and abroad.

David Cwiertny is the William D. Ashton Professor in Civil and Environmental Engineering at the University of Iowa (UI). For over 15 years, he has researched the fate and effects of environmental transformation products, particularly those originating from widely used but understudied agrochemicals (e.g., neonicotinoids, steroidal growth promoters). His laboratory is ideally suited for this work, being the first to identify and isolate products of fungicide chlorination during

drinking water treatment. At UI, he also directs the Center for Health Effects of Environmental Contamination (CHEEC), which is nationally recognized for its work linking environmental pollution and public health.

Dalma Martinović-Weigelt co-authored three reports to MN Legislature (Pharmaceuticals and Chemicals of Concern in Rivers: Occurrence and Biological Effects (2017), tdr-g1-20; Wastewater Treatment Plant Endocrine Disrupting Chemical Monitoring Study (2011), lrp-ei-1sy11; Endocrine Disrupting Compounds (2008), lrp-ei-1syo8;) and circa 55 research manuscripts that assess occurrence and the effects of chemicals of emerging concern and other stressors on fish and aquatic ecosystems. She is a recipient of U.S. EPA's Scientific and Technological Achievement Awards and research funding from MN ENRTF, MN PCA, U.S. EPA, USGS, and NSF.

Organization: University of St. Thomas

Organization Description:

The University of St. Thomas was founded in 1885 and emphasizes values-based education and career preparation; it helps solve community problems through education and service-learning programs. 56% of St. Thomas students receive need-based scholarship or grant aid. The largest private university in Minnesota (11,000 students, 461 full-time faculty), it offers bachelor's degrees in 85 major fields of study and 45 graduate degree programs and is ranked as a National University. St. Thomas's Biology and Chemistry departments view student/faculty collaborative research as essential; over the past 10 years, the faculty in Biology and Chemistry received research grants from the NSF, NIH, NASA, USDA, USEPA, USGS, and multiple Minnesota agencies (e.g. DNR, MPCA).

Budget Summary

| Category / Name | Subcategory or Type | Description | Purpose | Gen. Ineligible | % Benefits | # FTE | Classified Staff? | \$ Amount |
|---|---------------------|--|---|-----------------|------------|-------|-------------------|------------------|
| Personnel | | | | | | | | |
| Dalma Martinovic-Weigelt | | Co-Project Manager | | | 7% | 0.16 | | \$23,970 |
| Undergraduate research assistants (academic year) | | Conduct lab experiments, analyze data | | | 0% | 1 | | \$34,680 |
| Undergraduate research assistants (summer) | | Conduct lab and field experiments, analyze data | | | 7% | 1.4 | | \$52,877 |
| | | | | | | | Sub Total | \$111,527 |
| Contracts and Services | | | | | | | | |
| University of Iowa | Sub award | The team at Iowa will perform chemical reactions of the most widely QoIs in Minnesota with free chlorine under conditions simulating water and wastewater, perform structural identification of transformation products, scale up reactor systems to isolate transformation products for subsequent toxicological testing, and assist with photoproduct identification/ isolation as needed. | | | | 2.44 | | \$107,874 |
| | | | | | | | Sub Total | \$107,874 |
| Equipment, Tools, and Supplies | | | | | | | | |
| | Tools and Supplies | High throughput assay supplies. Attagene assay runs and assay setup. \$3.64 per sample per endpoint. | To conduct comprehensive molecular toxicity evaluation | | | | | \$27,540 |
| | Tools and Supplies | Miscellaneous disposable biological analyses lab supplies – cells, cell media and FBS, larvae, reagents, filters, buffers, sample processing supplies, animal microcosm setups, and molecular biology supplies. | Used to set up transformation experiments, to conduct extractions and fractionations and to conduct traditional toxicity analyses | | | | | \$15,304 |

| | | | | | | | | |
|-------------------------------------|--------------------|--|--|--|--|--|--------------------|------------------|
| | Tools and Supplies | Miscellaneous disposable chemical analyses lab supplies - reagents, filters, buffers, sample processing supplies (disposable sampling containers, pipette tips, chemicals, extraction columns) and instrument time | Used to conduct photolysis experiments and generation of transformation products in Activity 1 | | | | | \$19,755 |
| | | | | | | | Sub Total | \$62,599 |
| Capital Expenditures | | | | | | | | |
| | | | | | | | Sub Total | - |
| Acquisitions and Stewardship | | | | | | | | |
| | | | | | | | Sub Total | - |
| Travel In Minnesota | | | | | | | | |
| | | | | | | | Sub Total | - |
| Travel Outside Minnesota | | | | | | | | |
| | | | | | | | Sub Total | - |
| Printing and Publication | | | | | | | | |
| | | | | | | | Sub Total | - |
| Other Expenses | | | | | | | | |
| | | | | | | | Sub Total | - |
| | | | | | | | Grand Total | \$282,000 |

Classified Staff or Generally Ineligible Expenses

| Category/Name | Subcategory or Type | Description | Justification Ineligible Expense or Classified Staff Request |
|---------------|---------------------|-------------|--|
|---------------|---------------------|-------------|--|

Non ENRTF Funds

| Category | Specific Source | Use | Status | Amount |
|------------------|-----------------|-----|----------------------------|--------|
| State | | | | |
| | | | State Sub Total | - |
| Non-State | | | | |
| | | | Non State Sub Total | - |
| | | | Funds Total | - |

Attachments

Required Attachments

Visual Component

File: [bf9a4d95-9d4.pdf](#)

Alternate Text for Visual Component

The figure depicts estimated use of a commonly applied QoI fungicide, a picture of seeds treated with QoIs, and a schematic overview of the proposed chemistry and toxicology experimental approaches....

Financial Capacity

File: [b3193e12-e72.pdf](#)

Administrative Use

Does your project include restoration or acquisition of land rights?

No

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

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

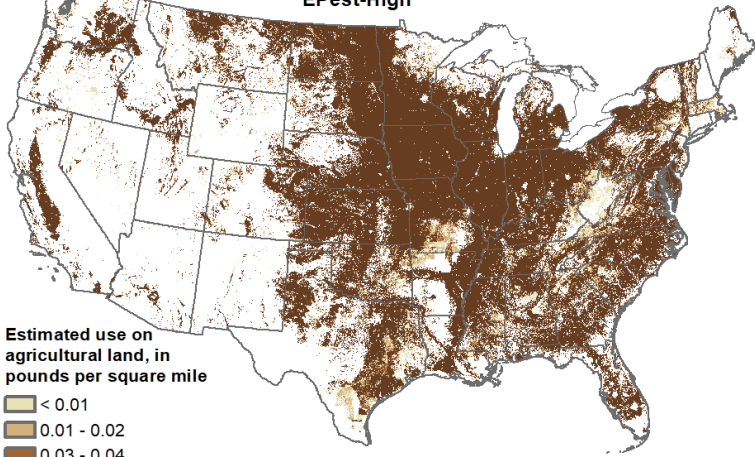
Does your project include original, hypothesis-driven research?

Yes

Does the organization have a fiscal agent for this project?

No

Estimated Agricultural Use for Azoxystrobin , 2019 (Preliminary)
EPest-High



Estimated use on agricultural land, in pounds per square mile

- < 0.01
- 0.01 - 0.02
- 0.03 - 0.04
- > 0.04
- No estimated use

Use Of Commonly Applied Qoi

Azoxystrobin. Source: USGS- National Water-Quality Assessment (NAWQA) Project – Estimated Annual Agricultural pesticide Use.

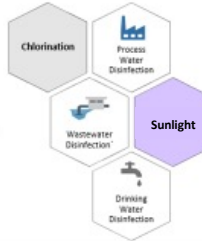
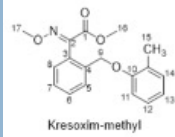


Chemistry

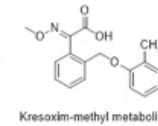
Generate transformation products of QoI commonly used in Minnesota

Identify and quantify QoI transformation products

QoI



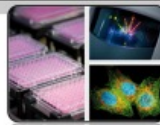
QoI Transformation Products



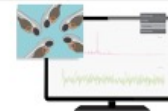
Toxicology

Test above chemicals for 70+ molecular toxicity indicators using cell assays

Identify toxic outcomes of concern and confirm those in larval fish tests



Cell assays



Fish testing – physiological function, behavior and growth

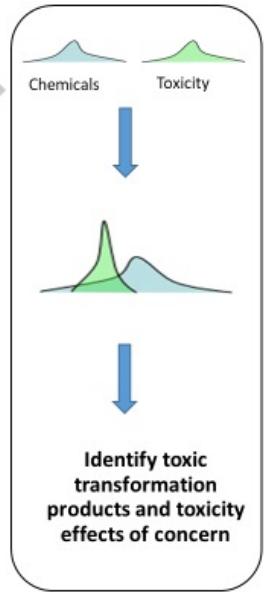


Image sources: Adobe Stock and noldus.com

OVERVIEW OF THE PROJECT APPROACH

