Final Abstract

Final Report Approved on October 26, 2026

M.L. 2023 Project Abstract

For the Period Ending June 30, 2026

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

Project Manager: Kristine Wammer

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Funding Source:

Fiscal Year:

Legal Citation: M.L. 2023, Chp. 60, Art. 2, Sec. 2, Subd. 04f

Appropriation Amount: $279,000

Amount Spent: $277,619

Amount Remaining: $1,381

Sound bite of Project Outcomes and Results

Quinone outside inhibitors, common crop fungicides, can break down in the environment and during water treatment. We identified products generated via sunlight photolysis and during chlorination and assessed toxicity of both fungicides and transformation products. These findings help Minnesota regulators and water managers better understand potential risks of these fungicides.

Summary of Project and Outcomes

Quinone outside inhibitor (QoI) fungicides are widely used in Minnesota to protect crops from fungal disease. This project studied how these fungicides break down in the environment through sunlight or during drinking water disinfection with chlorine. Prior to this study, little was known about the breakdown products or whether they harm aquatic life.

We studied several common QoI fungicides, examining sunlight breakdown in water and on surfaces (seeds, leaves, soil) and chlorine breakdown under various conditions. We then compared toxicity of the fungicides and their products using cell-based assays, fish mitochondrial (cell energy) assays, and fathead minnow embryo assays.

Sunlight broke pyraclostrobin down into ten new products, several likely to stay biologically active. Sunlight also interconverted four forms of trifloxystrobin, possibly prolonging persistence of the most active form. Chlorine reacted differently depending on pH, changing both degradation speed during water treatment and the products formed. Pyraclostrobin, kresoxim-methyl, and azoxystrobin activated multiple cellular stress pathways in a dose-dependent manner and induced mitochondrial stress. The effects of chlorination on toxicity depended on where chlorination occurred in the molecule, with some transformation products retaining toxicity types similar to the parent compound (e.g., 8-cholropyraclostrobin). These findings show that disinfection can produce transformation products that remain toxic and may continue to affect nontarget species like fish. Neither the parent compounds nor their chlorinated transformation products affected fish embryo survival following a 96-h exposure at typical environmental baseflow concentrations (10-100 ng/L).

These results show that fungicide risk depends on the products formed, which vary with pH, sunlight, and surface. Regulators, water utilities, and land managers can use them to assess fungicide risk to Minnesota's waters and fish and to prioritize products for monitoring.

Project Results Use and Dissemination

The results from this project have been disseminated via five poster presentations and three oral presentations at national scientific meetings by the students involved over the course of the project; one additional poster presentation will occur next spring. One manuscript has been published and two more (one on the chlorination results and one on the photolysis results) will be submitted to scientific journals and will be available once they have completed the peer review process required prior to publication. A fact sheet on QoI fungicides will be posted to the Center for Health Effects of Environmental Contamination (CHEEC) website.

Environment and Natural Resources Trust Fund

M.L. 2023 Approved Final Report

General Information

Date: October 28, 2026

ID Number: 2023-107

Staff Lead: Tiffany Schaufler

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

Project Budget: $279,000

Project Manager Information

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Project Reporting

Final Report Approved: October 26, 2026

Reporting Status: Project Completed

Date of Last Action: October 26, 2026

Project Completion: June 30, 2026

Legal Information

Legal Citation: M.L. 2023, Chp. 60, Art. 2, Sec. 2, Subd. 04f

Appropriation Language: $279,000 the first year is from the trust fund to the commissioner of natural resources for an agreement with the University of St. Thomas to assess the ecological hazards associated with QoI fungicides and their major environmental transformation products.

Appropriation End Date: June 30, 2026

Narrative

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

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.

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: Evaluation of QoI environmental fate with identification of major transformation products

Activity Budget: $169,000

Activity Description:
Working with the QoIs 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 QoIs 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 QoIs. Moreover, we know QoIs are reactive toward chemical disinfectants like free chlorine, yielding products that are structurally similar to parent QoI 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 QoI 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	Approximate Completion Date
Measure rates of QoI chlorination during simulated water and wastewater treatment	July 31, 2024
Measure photodegradation rates of QoIs in water, bound to soils, and coated on seeds	June 30, 2025
Identify, scale up, and isolate photodegradation and chlorination transformation products for testing in Activity 2	June 30, 2025
Submit publication for peer review	June 30, 2025

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

Activity Budget: $110,000

Activity Description:
First, the effects of QoIs 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 QoIs make them very likely to exert toxicity by affecting mitochondrial energy generation processes. Third, QoIs 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	Approximate Completion Date
Evaluate effects of QoIs and their transformation products on 70 molecular targets indicative of toxicity	June 30, 2025
Evaluate effects of QoIs and their transformation products on the fish mitochondrial function	June 30, 2025
Evaluate effects of QoIs and their transformation products on the physiology and behavior of fish	June 30, 2025
Submit publication for peer review	June 30, 2025
Production of fact sheet(s) through Center for Health Effects of Environmental Contamination (CHEEC)	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

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.
Findings will be disseminated and archived via reports to LCCMR, peer-reviewed publications, and presentations at conferences, including by undergraduate and graduate student contributors to the project. Acknowledgment of funding from the Environment and Natural Resources Trust Fund will be included on all presentations and all published manuscripts.

Dissemination efforts will also be led by the Center for Health Effects of Environmental Contamination (CHEEC), directed by Prof. Cwiertny at the University of Iowa. While funded by the state of Iowa, CHEEC is well known across the Midwest region for its work related to the links between environmental pollutants and public health effects. CHEEC frequently produces fact sheets and infographics for their research, and such documents will be developed and posted on the CHEEC website based upon findings from the proposed work. Project outcomes will also be shared via the CHEEC social media accounts, and through presentations given across Iowa and in neighboring states by CHEEC personnel. CHEEC will also be made aware of the responsibility to follow ENRTF Acknowledgement Guidelines.

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

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	$ Amount	$ Amount Spent	$ Amount Remaining
Personnel										
Dalma Martinovic-Weigelt		Co-Project Manager			7%	0.24		$35,955	-	-
Undergraduate research assistants (academic year)		Conduct lab experiments, analyze data			0%	1		$22,695	-	-
Undergraduate research assistants (summer)		Conduct lab and field experiments, analyze data			7%	1.4		$37,877	-	-
							Sub Total	**$96,527**	**$96,527**	**-**
Contracts and Services										
University of Iowa	Subaward	Personnel ($81,324, salary + fringe. 0.11 month effort for year for Dr. Cwiertny, 12 mo effort per year for PhD student.) Materials/supplies ($5,000). Tuition/Fees for Ph.D. student ($18,325) Perform reactions with free chlorine, identify and scale up transformation products.		X		2.02		$114,649	$114,649	-
							Sub Total	**$114,649**	**$114,649**	**-**
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					$41,540	$41,540	-
	Tools and Supplies	Miscellaneous disposable biological analyses lab supplies – cells, cell media and FBS, larvae, reagents, filters, buffers, sample processing supplies,	Used to set up transformation experiments, to conduct extractions and					$15,304	$14,778	$526

		animal microcosm setups, and molecular biology supplies.	fractionations and to conduct traditional toxicity analyses							
	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					$10,980	$10,125	$855
							Sub Total	**$67,824**	**$66,443**	**$1,381**
Capital Equipment										
							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	**$279,000**	**$277,619**	**$1,381**

Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request
Contracts and Services - University of Iowa	Subaward	Personnel ($81,324, salary + fringe. 0.11 month effort for year for Dr. Cwiertny, 12 mo effort per year for PhD student.) Materials/supplies ($5,000). Tuition/Fees for Ph.D. student ($18,325) Perform reactions with free chlorine, identify and scale up transformation products.	At the University of Iowa, Cwiertny and his research group have already conducted preliminary work to identify and isolate the transformation products generated from the reaction of quinone outside inhibitor (QoI) fungicides with free chlorine during simulated water/wastewater treatment. To the best of our knowledge, they are the only group to date to have developed methods to isolate sufficient quantities of these transformation products for use in ecotoxicity testing that we have proposed. Partnering with this group and leveraging their already established methodologies enables the research team to achieve all of the stated research goals within the 2-year project period and at the funding level requested. Developing these capabilities without the participation of the University of Iowa collaborators would add more time and require more financial support to complete the work as proposed. The work conducted at the University of Iowa will still directly benefit the state of Minnesota, as chemicals generated and isolated in the Cwiertny laboratory will be used in ecotoxicity testing at the University of St. Thomas. Moreover, data derived from these studies will be valuable to environmental fate and risk assessment associated with the use of QoI fungicides in Minnesota, where these chemicals are widely used and have been detected in Minnesota water resources.

Non ENRTF Funds

Category	Specific Source	Use	Status	$ Amount	$ Amount Spent	$ Amount Remaining
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 toxiciology experimental approaches....

Financial Capacity

File: b3193e12-e72.pdf

Supplemental Attachments

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

Title	File
Background check certification form	a7618245-d6d.pdf
Wammer 2023-107 research addendum	ca533211-7d3.pdf
Cooper 2024 ACS poster	d9773c0b-6f9.pdf
Deschamp 2024 ACS poster	c75abaf4-e56.pdf
Loisherua 2024 SETAC poster	48c480aa-d10.pdf
Carlin 2024 GRC poster	f13ff2c6-0ff.pdf
Stephens 2025 SETAC poster	2e5432a7-859.pdf
QoI chlorination paper 1	f68c7287-dc9.pdf
CHEEC fact sheet	fa22565f-fae.pdf

Difference between Proposal and Work Plan

Describe changes from Proposal to Work Plan Stage

No significant changes; small budget changes were made to meet new budget target ($3,000 reduction)

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 understand that travel expenses are only approved if they follow the "Commissioner's Plan" promulgated by the Commissioner of Management of Budget or, for University of Minnesota projects, the University of Minnesota plan?

N/A

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

Do you understand that a named service contract does not constitute a funder-designated subrecipient or approval of a sole-source contract? In other words, a service contract entity is only approved if it has been selected according to the contracting rules identified in state law and policy for organizations that receive ENRTF funds through direct appropriations, or in the DNR's reimbursement manual for non-state organizations. These rules may include competitive bidding and prevailing wage requirements

N/A

Work Plan Amendments

Amendment ID	Request Type	Changes made on the following pages	Justification For Amendment Request (word limit 75)	Date Submitted	Approved	Date of LCCMR Action
1	Amendment Request	• Other • Activities and Milestones • Budget - Personnel • Budget - Professional / Technical Contracts • Budget - Capital, Equipment, Tools, and Supplies	Note that I was unable to change the activity completion dates to after 6/30/25. We moved some student money ($11,985) to PI salary for work in Summer 2025 within Activity 2. We moved some student money ($15,000) and supplies ($9,000) from Activity 1 for some undergrad time and supplies at U Iowa ($10,000, Activity 1) and to run more biological samples in high-throughput assays ($14,000, Activity 2.)	January 21, 2025	Yes	February 5, 2025
2	Completion Date	Previous Completion Date: 06/30/2025 New Completion Date: 12/31/2025	We are proposing to extend the project for an additional six months, which will allow us to assess additional biological endpoints and/or parent chemicals and/or chemical degradation products. Overall, the project has been going well, but we have identified some new and very interesting degradation products recently and would like to have more time to address the biological effects thereof.	January 21, 2025	Yes	February 5, 2025
3	Completion Date	Previous Completion Date: 12/31/2025 New Completion Date: 06/30/2026	We can use some extra time to complete some experiments; this is within the appropriation end date so is not a legislative extension.	November 4, 2025	Yes	November 4, 2025

Status Update Reporting

Final Status Update August 14, 2026

Date Submitted: August 26, 2026

Date Approved: September 24, 2026

Overall Update

Our stated outcomes included providing a more comprehensive assessment of the ecological hazards associated with quinone outside inihibitor (QoI) fungicides and their major environmental transformation products. In Activity 1, we proposed to study two transformation processes (photolysis and chlorination) and to identify, scale up, and isolate transformation products. In Activity 2, we proposed to evaluate toxicity of fungicides and these major transformation products via molecular targets, fish mitochondrial function, and fish physiology and behavior experiments.
Current reporting period activities include:

- Establishing how common environmental variables (for example pH) influence the distribution and stability of transformation products generated during QoI fungicide chlorination.
- Evaluating how the distribution of products generated during QoI direct photolysis vary between their presence in water versus on an environmentally relevant surface (for example, a leaf, soil particle, or even in drywall, where certain QoIs are used for protection from mold).
- Assessing the relative environmental and human health risks associated with QoI transformation products relative to the parent fungicide species from which they are generated via either photolysis or chlorination.
- Continuing dissemination efforts, including preparing a fact sheet focused on QoI fungicides and drafting two manuscripts related to transformation products of QoI photolysis and chlorination.

Activity 1

Milestone 1: Focusing on QoI fungicides commonly used in seed treatments (pyraclostrobin, azoxystrobin, fluoxastrobin, and trifloxystrobin), we have conducted direct photolysis studies on seeds, model soils (e.g., aluminosilicate clays), leaves, and model surfaces (e.g., glass slides) to complement our prior work in aqueous systems.

Milestone 2: During chemical disinfection via chlorination, we have found that different types of chlorine species participate in QoI transformation at low pH (Cl2O the dominant reactant) versus high pH (HOCl dominates). This has implications for both how fast QoIs are degraded during water treatment and products generated via these reactions.

Milestone 3: We have concluded our comprehensive analysis of pyraclostrobin direct photolysis, identifying 10 transformation products, several of which we anticipate will retain bioactivity. For chlorination, we have collected clear evidence for distinct product species generated from certain QoIs (pyraclostrobin and dimoxystrobin) at high pH relative to lower pH systems, while also evaluating the stability of select chlorine transformation products.

Milestone 4: We have a draft manuscript (attached) nearing completion for submission that will acknowledge LCCMR support.
(This activity marked as complete as of this status update)

Activity 2

Milestone 1: Studies were performed using the Attagene cis-Factorial™ assay platform, which simultaneously evaluates the activation of 46 transcription factor signaling pathways associated with toxicity responses. Pyraclostrobin induced activation of multiple transcription factors across all tested concentrations and exhibited a clear concentration-dependent response whereas the isomer did not. Consistent with the prior mitochondrial toxicity findings (Milestone 2), these results demonstrate a markedly different toxicological profile between pyraclostrobin and its isomer, suggesting

that structural differences can substantially alter biological activity and downstream transcriptional regulatory responses.

Milestone 2: Results were summarized in last report; briefly, the assay showed pyraclostrobin caused mitochondrial stress and the isomer was less potent.

Milestone 3: The Fish Embryo Acute Toxicity test with fathead minnows was conducted to evaluate whether observed differences among chemicals and their chlorinated products were ecologically relevant when tested at environmentally realistic exposure concentrations. None of the parent compounds or transformation products produced significant effects at the lower concentrations tested (100 ng/L, 50 ng/L, and 10 ng/L).

Milestone 4: We have a draft manuscript (attached) nearing completion for submission that will acknowledge LCCMR support.

Milestone 5: We have prepared a fact sheet on QoI fungicides (attached).
(This activity marked as complete as of this status update)

Dissemination
We have attached two draft manuscripts nearing completion for submission that will acknowledge LCCMR support:

Abigail M. Carlin, Vincent H. Hesselink, Dalma Martinović-Weigelt, Maya I. Deschamp, Kristine H. Wammer, David M. Cwiertny, and James B. Gloer. Pyraclostrobin photolysis: Identification and toxicological assessment of a unique and unexpected isomeric rearrangement product. In preparation for submission to Environmental Science & Technology.

Abigail M. Carlin, Dalma Martinović-Weigelt, John D. Sivey, Kristine H. Wammer, James B. Gloer, David M. Cwiertny. Reaction of Quinone Outside Inhibitor Fungicides with Free Available Chlorine: Investigating the Active Oxidant and Transformation Product Toxicity. In preparation for submission to Environmental Toxicology and Chemistry.

We have prepared a fact sheet on QoI fungicides (attached) that will be posted to the CHEEC website in Fall 2026 as part of a broader educational initiative on fungal resistance and the expanding use of fungicides in the agricultural Midwest.

Status Update Reporting

Status Update April 1, 2026

Date Submitted: April 14, 2026

Date Approved: April 14, 2026

Overall Update
Our stated outcomes included providing a more comprehensive assessment of the ecological hazards associated with quinone outside inihibitor (QoI) fungicides and their major environmental transformation products. In Activity 1, we proposed to study two transformation processes (photolysis and chlorination) and to identify, scale up, and isolate transformation products. In Activity 2, we proposed to evaluate toxicity of fungicides and these major transformation products via molecular targets, fish mitochondrial function, and fish physiology and behavior experiments. Overall, the project has been going well and we are nearing completion of the project. Recent progress has continued to focus on identification and isolation of new and novel transformation products generated during the photochemical transformation of high use QoIs, as well as toxicity screening of these transformation products to improve risk assessment associated with QoI fungicides.

Activity 1
Milestone 1: Direct photolysis experiments have recently focused more extensively on pyraclostrobin including its photoisomer. We have also continued work to measure the rates of indirect photolysis of QoI fungicides, including for several QoI fungicides during indirect photolysis in the presence of nitrate and nitrite. Model surface experiments are also continuing. Results of these experiments will be described in our upcoming final report.

Milestone 2: We are continuing work on two different manuscripts that will report (i) the major products generated during the QoI chlorination and (ii) kinetic data to improve the modeling of QoI fungicide transformation during water/wastewater treatment. These manuscripts will include both fundamental insights into QoI reactivity and practical guidance to improve their management during treatment.

Milestone 3: We are completing work on elucidating stable photoproducts of the photolysis of QoIs, especially of pyraclostrobin. See Activity 2 Milestone 2 for an update on recent work on determining toxicity of the pyraclostrobin isomer.

Milestone 4: A manuscript on fate and transformation of QoIs via chlorination was published in December 2025: https://pubs.rsc.org/en/content/articlelanding/2026/ew/d5ew01004g

Milestone 5: The fact sheet is under development and will be posted by June 2026.

Activity 2
Milestone 1: Bioinformatics analyses are being completed for the molecular target toxicity experiments on 45 transcription factors linked to various toxicity pathways and 24 nuclear receptors. This includes effects of chlorinated and photolysis transformation products.

Milestone 2: The rainbow trout cell line (RTgill W1) was used to measure cellular oxygen consumption rate (OCR) under resting conditions and during exposure to pyraclostrobin and its isomer using the Seahorse® XFe24 Analyzer (Agilent Technologies, USA). We observed significant reduction in oxygen consumption rate (OCR) after 30 in exposure to 100, 400, and 800 µg/L pyraclostrobin ($p < 0.05$). Reduced oxygen consumption is consistent with the known mechanism of action of pyraclostrobin, a Complex III inhibitor of the electron transport chain (ETC), and is indicative of mitochondrial

stress. We found that the isomer was less potent - significant reduction in OCR was observed only in 800 µg/L treatment; there were no significant effects in 100 and 400 µg/L treatments ($p < 0.05$).

Milestone 3: Fish embryo toxicity experiments have been completed and results will be discussed in our upcoming final report.

Milestone 4: A draft of a manuscript on chlorine speciation is nearing completion and will be submitted by May.

Dissemination

A poster titled “Characterizing the Effects of Quinone Outside Inhibitor Fungicides and Their Metabolites in Human and Rainbow Trout Cell Lines” with lead author Kellenah Stephens (undergraduate student in the Martinovic-Weigelt group) and co-authored by members of the Cwiertny and Martinovic-Weigelt groups was presented at the national SETAC North America Annual meeting November 16-20, 2025 in Portland, OR.

An oral presentation titled “Aquatic Photochemical Fate, Transformation, and Toxicity of the Quinone outside Inhibitor Fungicides” with lead author Abigail Carlin (Ph.D. student in the Cwiertny group at the University of Iowa) and co-authored by members of the Wammer and Martinovic-Weigelt groups was presented at the national American Chemical Society Spring National meeting which was held March 22-26, 2026 in Atlanta, GA.

Status Update Reporting

Status Update October 1, 2025

Date Submitted: October 1, 2025

Date Approved: October 29, 2025

Overall Update
Our stated outcomes included providing a more comprehensive assessment of the ecological hazards associated with quinone outside inihibitor (QoI) fungicides and their major environmental transformation products. In Activity 1, we proposed to study two transformation processes (photolysis and chlorination) and to identify, scale up, and isolate transformation products. In Activity 2, we proposed to evaluate toxicity of fungicides and these major transformation products via molecular targets, fish mitochondrial function, and fish physiology and behavior experiments. Overall, the project has been going well and we plan to complete the project at the end of this calendar year. Recent progress has continued to focus on identification of new and novel transformation products generated during the photochemical transformation of high use QoIs, as well as toxicity screening of these transformation products to improve risk assessment associated with QoI fungicides, including the first set of molecular target toxicity screening experiments on chlorination products.

Activity 1
Milestone 1: We are continuing work on two different manuscripts that will report (i) the major products generated during the QoI chlorination and (ii) kinetic data to improve the modeling of QoI fungicide transformation during water/wastewater treatment. These manuscripts will include both fundamental insights into QoI reactivity and practical guidance to improve their management during treatment.

Milestone 2: Direct photolysis experiments have recently focused on unique photoisomerization pathways for two QoIs: pyraclostrobin and trifloxystrobin. We have also continued work to measure the rates of indirect photolysis of QoI fungicides, including for several QoI fungicides during indirect photolysis in the presence of nitrate and nitrite. Model surface experiments are also continuing.

Milestone 3: We are continuing to structurally elucidate products of the photolysis of QoIs. In addition to stable photoproducts (isomers for azoxystrobin and pyraclostrobin) we intend to include trifloxystrobin photoisomer mixtures for testing in the next round of samples analyzed on multiple molecular targets indicative of toxicity (see Activity 2 Milestone 1.)

Activity 2
Milestone 1: - We finalized the experimental design of the molecular target toxicity screening experiments; it was informed by a review of primary literature and mitochondrial stress data generated as described in Milestone 2. Molecular target toxicity experiments that evaluated the effects of five chlorinated transformation products on 45 transcription factors linked to various toxicity pathways and 24 nuclear receptors have been completed. Bioinformatics analyses are currently in progress to characterize these effects.

Milestone 2: We completed a series of concentration-response intensive mitochondrial stress experiments for pyraclostrobin, kresoxim-methyl, azoxystrobin, and five of their chlorinated transformation products. Analyses indicate that all three parent compounds can induce mitochondrial toxicity in rainbow trout cell lines, with pyraclostrobin being the most potent. Among the five chlorinated transformation products tested, 8-chloro-pyraclostrobin was the most toxic. Acute toxicity tests for these chemicals confirmed no toxicity under a 30-minute exposure scenario. This suggests that the observed reduction in cellular respiration was due to mitochondrial stress rather than compromised cell

viability.

Milestone 3: Fish embryo toxicity experiments with kresoxim-methyl were repeated. We confirmed acute toxicity in fathead minnow embryos, with a significant increase in mortality observed at the 100 µg/L treatment level. Transformation product studies are underway.

Dissemination

An abstract titled “Characterizing the Effects of Quinone Outside Inhibitor Fungicides and Their Metabolites in Human and Rainbow Trout Cell Lines” with lead author Kellenah Stephens (undergraduate student in the Martinovic-Weigelt group) and co-authored by members of the Cwiertny and Martinovic-Weigelt groups was accepted for presentation at the national SETAC North America Annual meeting which will be held November 16-20, 2025 in Portland, OR.

An abstract titled “Aquatic Photochemical Fate, Transformation, and Toxicity of the Quinone outside Inhibitor Fungicides” with lead author Abigail Carlin (Ph.D. student in the Cwiertny group at the University of Iowa) and co-authored by members of the Wammer and Martinovic-Weigelt groups was submitted for presentation at the national American Chemical Society Spring National meeting which will be held March 22-26, 2026 in Atlanta, GA.

Status Update Reporting

Status Update April 1, 2025

Date Submitted: April 1, 2025

Date Approved: April 24, 2025

Overall Update
Our stated outcomes included providing a more comprehensive assessment of the ecological hazards associated with QoI fungicides and their major environmental transformation products. In Activity 1, we proposed to study two transformation processes (photolysis and chlorination) and to identify, scale up, and isolate transformation products. In Activity 2, we proposed to evaluate toxicity of fungicides and these major transformation products via molecular targets, fish mitochondrial function, and fish physiology and behavior experiments. Overall, the project has been going well and is on track (details for each activity are described below), Recent progress has focused on identification of new and novel transformation products generated during the photochemical transformation of high use QoIs, as well as toxicity screening of these transformation products to improve risk assessment associated with QoI fungicides.

Activity 1
Milestone 1: We are currently drafting two different manuscripts that will report (i) the major products generated during the QoI chlorination and (ii) kinetic data to improve the modeling of QoI fungicide transformation during water/wastewater treatment. These manuscripts will include both fundamental insights into QoI reactivity and practical guidance to improve their management during treatment.

Milestone 2: We are establishing the rates of indirect photolysis of QoI fungicides. For example, we have measured rate constants for several QoI fungicides during indirect photolysis in the presence of nitrate, which we expect to co-occur with QoI fungicides in agricultural runoff. Insights from these indirect photolysis studies is guiding ongoing photolysis studies on model surfaces that are representative of their application in seed treatment, on plants, and as protection in wall board.

Milestone 3: We are structurally elucidating products of the photolysis of QoIs. For example, we have tentatively identified several products generated from the direct photolysis and indirect photolysis (with nitrate) of pyraclostrobin. Notable products include a novel isomeric species, which we believe forms through a ring closure reaction, and, in nitrate systems, several oxidized products that exhibit greater environmental mobility than pyraclostrobin while preserving the bioactive portion of the molecule.

Activity 2
Milestone 1: The Martinović-Weigelt team completed review of the primary literature and publicly available databases and identified molecular targets and effective concentrations of the QoIs azoxystrobin, kresoxim-methyl, pyraclostrobin and trifloxystrobin. We are currently investigating effects of kresoxim-methyl and pyraclostrobin metabolites on the molecular targets associated with oxidative stress.

Milestone 2: To elucidate whether QoIs and their transformation products act as mitochondrial toxicants in fish the Martinovic-Weigelt lab evaluated an additional QoI - pyraclostrobin. We completed toxicity screening experiments with pyraclostrobin and one of its chlorinated transformation products. Both the parent and its chlorinated product inhibited mitochondrial function in a rainbow trout cell model in a concentration-dependent manner. Additional concentration response intensive experiments are underway to quantify toxicity of the chlorinated transformation product relative its parent.

Milestone 3: Kresoxym-methyl and its chlorinated metabolites were identified as the priority. Fish toxicity experiments with kresoxim-methyl indicated that it is acutely toxic to fathead minnows, but high mortality rates observed in controls are problematic so these experiments will need to be repeated before toxicity metrics can be reported with high confidence. We are in the process of starting an on-site fish culture and plan to switch to locally generated embryos to minimize mortality.

Dissemination

Research poster titled “Role of photosensitizers in enhancing environmental photolysis of quinone outside inhibitor fungicides” with lead author Maya Deschamp (undergraduate student in the Wammer group at St. Thomas) and co-authored by members of the Cwiertny group was presented at the American Chemical Society Spring 2025 National Meeting held March 23-27 in San Diego, CA.

Additional Status Update Reporting

Additional Status Update January 8, 2025

Date Submitted: January 15, 2025

Date Approved: February 5, 2025

Overall Update

Our stated outcomes included providing a more comprehensive assessment of the ecological hazards associated with QoI fungicides and their major environmental transformation products. In Activity 1, we proposed to study two transformation processes (photolysis and chlorination) and to identify, scale up, and isolate transformation products. In Activity 2, we proposed to evaluate toxicity of fungicides and these major transformation products via molecular targets, fish mitochondrial function, and fish physiology and behavior experiments. Overall, the project has been going well and is on track (details for each activity are described below), but we have identified some new and very interesting degradation products recently and would like to have more time to address the biological effects thereof. Therefore, we are submitting this additional status update in preparation for an upcoming amendment and extension request to complete the project.

Activity 1

Milestone 1: Over the past project period we have studied the chlorination of QoI fungicides under a variety of different water quality conditions. Generally, we find that the rate of QoI chlorination increases with decreasing pH and increasing chloride concentration. To date, these studies have yielded reaction rate constants and insights into the nature of the reaction driving QoI transformation during chemical disinfection. Milestone 2: In addition to continuing to measure rates of QoI photolysis in the presence of nitrate and nitrite, recent work has focused on the direct photolysis of trifloxystrobin. During direct photolysis we are observing unique interconversion between the active ingredient (which is the most bioactive isomer) and three other isomers, which we suspect may prolong the environmental persistence of the most bioactive isomer. Moreover, preliminary work studying the photolysis of trifloxystrobin on model surfaces (e.g., glass) suggests that the product distribution of the different isomers varies from that observed in water.
Milestone 3: We continue to structurally identify and isolate novel transformation products. To date, we have elucidated the structure of transformation products generated from the photolysis of trifloxystrobin and the chlorination of four QoIs, with particular recent focus on the primary pyraclostrobin chlorination product.

Activity 2

Milestone 1: The Martinović-Weigelt team completed review of the primary literature and publicly available databases and identified molecular targets and effective concentrations of the QoIs azoxystrobin, kresoxim-methyl, pyraclostrobin and trifloxystrobin. Based on these findings we completed the design of the high-throughput molecular target tests planned for metabolites for Spring 2025.
Milestone 2: To elucidate whether QoIs and their transformation products act as fish mitochondrial toxicants the Martinovic-Weigelt lab completed: 1) confirmatory experiments with two chlorinated metabolites of kresoxim-methyl, 2) assessment of the azoxystrobin and two of its chlorinated metabolites characterized and isolated as a part of Activity 1. Experiments with chlorinated metabolites of kresoxim-methyl confirmed our pilot finding that one of the metabolites affected mitochondrial function. Experiments with azoxystrobin found mitochondrial toxicity similar to that reported in mammalian systems. Chlorinated metabolites of azoxystrobin were not toxic to rainbow trout gill cell mitochondria.
Milestone 3: Based on the Milestone 1 and 2 outcomes we identified kresoxym-methyl and its chlorinated metabolites as the priority for the fish tests. We completed fish assay transfer and adapted it to our needs and have started fish embryo experiments.

Dissemination

Research poster titled “Environmental Fate, Transformation, and Toxicity of Quinone Outside Inhibitor (QoI) Fungicides”

with lead author Abigail Carlin (doctoral student in the Cwiertny group at the University of Iowa) and co-authored by the members of the Wammer and Martinović-Weigelt groups was presented at the 2024 Gordon Research Conference on Environmental Sciences: Water held June 23-28, 2024 in Plymouth, NH.

Research poster titled "Characterizing Effects of Quinone Outside Inhibitor Fungicides and Their Metabolites on Mitochondrial Function in Rainbow Trout Cell Lines" co-authored by Martinović-Weigelt and Cwiertny teams was presented at the North American Annual Meeting of the Society for Environmental Toxicology and Chemistry (SETAC) held in Oct, 2024 in Ft. Worth, TX. Two undergraduate students from U of St. Thomas were the lead presenters.

A research poster will be presented by Maya Deschamp (U St. Thomas) at the upcoming American Chemical Society spring national meeting in March.

Status Update Reporting

Status Update October 1, 2024

Date Submitted: September 18, 2024

Date Approved: September 19, 2024

Overall Update
This status update is waived per guidance from LCCMR staff provided on August 13, 2024.

Activity 1
This status update is waived per guidance from LCCMR staff provided on August 13, 2024.

Activity 2
This status update is waived per guidance from LCCMR staff provided on August 13, 2024.

Dissemination
This status update is waived per guidance from LCCMR staff provided on August 13, 2024.

Status Update Reporting

Status Update April 1, 2024

Date Submitted: March 28, 2024

Date Approved: May 8, 2024

Overall Update

This is our first status update on this project, so progress since the beginning of the project is described. Our stated outcomes included providing a more comprehensive assessment of the ecological hazards associated with QoI fungicides and their major environmental transformation products. In Activity 1, we proposed to study two transformation processes (photolysis and chlorination) and to identify, scale up, and isolate transformation products. In Activity 2, we proposed to evaluate toxicity of fungicides and these major transformation products via molecular targets, fish mitochondrial function, and fish physiology and behavior experiments. We are on track with our stated milestones including completion of Milestone 1 under Activity 1: "Measure rates of QoI chlorination during simulated water and wastewater treatment."

Activity 1

For studies of fungicide photolysis at the University of St. Thomas (Milestone 2), we have focused initial efforts on determining photolysis rates to inform which compounds are most likely to yield photoproducts in water under environmentally relevant timescales. We have measured quantum yield values in water for azoxystrobin, fenamidone, pyraclostrobin, dimoxystrobin, fluoxastrobin, and kesoxim methyl. We have also worked to develop methods for performing photolysis on surfaces. For studies of fungicide chlorination at the University of Iowa (Milestone 1), we have surveyed the reactivity of several QoI fungicides to determine those most prone to transformation under the conditions used in water and wastewater treatment. We have prioritized fenamidone, kresoxim methyl, dimoxystrobin and axozystrobin based on their relatively high reactivity toward free chlorine and likely transformation during chemical disinfection processes. For these fungicides, we have established their rate of reaction and resulting product distribution. We have also isolated and structurally elucidated major chlorination products (Milestone 3), which have been shared with researchers at the University of St. Thomas for ecotoxicological evaluation; see Activity 2 update for details.

Activity 2

The focus was on fulfilling Milestone 2 - Evaluation of the effects of QoIs and their transformation products on the fish mitochondrial function. To elucidate whether QoIs and their transformation products act as fish mitochondrial toxicants Martinovic-Weigelt lab: 1) established rainbow trout gill cell line in UST lab, 2) developed assay for mitochondrial toxicity using this cell line, 3) completed a series of experiments with QoIs pyraclostrobin and kresoxim-methyl, and 4) completed pilot experiments with two chlorinated metabolites of kresoxim-methyl characterized and isolated as a part of Activity 1. Both pyraclostrobin and kresoxim-methyl reduced oxygen consumption rates upon acute exposure; pyraclostrobin was more potent than kresoxim-methyl. These responses are congruent to mammalian literature and in vivo fish findings. A pilot experiment with chlorinated metabolites of kresoxim-methyl indicates that one of the metabolites affected mitochondrial function at concentrations comparable to or lower than the parent chemical kresoxim methyl. Additional trials with kresoxim-methyl metabolites are underway to increase sample size and determine if the findings of the pilot experiment can be repeated and confirmed. Next set of experiments will focus on azoxystrobin and its metabolites. Milestone 2 data will be used to identify products for the Activity 2 milestones 1 and 3.

Dissemination

Cooper Larson, an undergraduate student from St. Thomas, presented a poster at the American Chemical Society spring national meeting:

Larson, C.J., C.D. Knellwolf, J.H. Nickowski, A.M. Carlin, D.M Cwiertny, K.H. Wammer. “Environmental photolysis of quinone outside inhibitor (QoI) fungicides.” Poster presentation at the American Chemical Society National Meeting, New Orleans, LA, March 17-21, 2024.