# Environment and Natural Resources Trust Fund

2022 Request for Proposal

## General Information

**Proposal ID:** 2022-224

**Proposal Title:** Is the tire chemical 6PPDq killing Minnesota's fish?

## Project Manager Information

**Name:** Nicholas Phelps

**Organization:** U of MN - College of Food, Agricultural and Natural Resource Sciences

**Office Telephone:** (612) 624-7450

**Email:** phelp083@umn.edu

## Project Basic Information

**Project Summary:** A newly discovered and highly toxic tire-derived chemical (6PPDq) may be impacting Minnesota's fish populations - we will optimize detection methods, determine occurrence in the environment, and evaluate risk statewide.

**Funds Requested:** $491,000

**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.**

Last year Washington state researchers discovered that the previously unreported chemical 6PPDq (derived from the "parent" chemical 6PPD used in rubber tires) is associated with major fish kills (40-90% coho salmon mortality!). Understanding the occurrence and consequence of this contaminant of emerging concern (CEC) in Minnesota waters is a priority given the very high toxicity. Most CECs have subtle, sublethal effects that emerge upon long-term exposures. In contrast, 6PPDq has rapid effects, and even a short-term episodic exposure released to surface waters by stormwaters and road runoffs appear to be sufficient to cause severe fish kills. Given the ubiquitous use of 6PPD in tires, this CEC is suspected to be widespread in aquatic systems including Minnesota. Currently, there are no data about the occurrence of 6PPDq in Minnesota (and most other states), and the biological mechanisms by which it is rapidly killing fish are not understood. Our team is uniquely positioned to determine whether 6PPDq poses a threat to fish in Minnesota. We have researched and documented fish kills and factors driving those statewide. We also have specialized analytical expertise and a documented capacity to survey CECs, including 6PPDq, and to characterize their toxicity to inform management decisions.

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

Given the significance of the 6PPDq discovery and potential threat to Minnesota fish populations, it is imperative that baseline data are collected to guide appropriate management responses and future research plans. To that end, we have brought together a highly interdisciplinary team to conduct the first-ever research on this emerging CEC in Minnesota. First, we will identify and spatially define hypothesized risk-factors associated with potential 6PPDq exposure, such as proximity to major roads, rainfall and runoff patterns, and historical fish kills. The resulting risk map will be used to prioritize locations for surveillance, with special consideration for high-risk locations on tribal lands. Second, concurrent with the above we will develop and transfer methods for 6PPDq extraction and measurement in water and fish tissues to the Minnesota Department of Health. Third, we will collect samples using the locations and methods defined in the above steps and measure 6PPDq concentrations. Lastly, we will use cost-effective and rapid fish cell and larval toxicity tests to conduct pilot studies that will determine the biological basis of toxicity. Based on the above and complimentary toxicity information, we will determine whether 6PPDq poses a risk to Minnesota fish and it warrants further biological studies and monitoring.

**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?**

Identification of the risk factors and occurrence assessment will allow managers to understand sources and systemwide distribution of 6PPDq. The chemical extraction and analysis methods developed herein will be transferred to the Minnesota Department of Health, allowing Minnesota to monitor this highly toxic CEC as early as 2023. Determination of whether 6PPDq's association with fish kills extends to habitats/fish species outside Washington will inform and potentially incentivize the tire industry's efforts to design less toxic alternatives. Lastly, this research will fill knowledge gaps related to fish kills in Minnesota and inform science-based mitigation strategies.

## Activities and Milestones

### Activity 1: Develop methods for the detection of 6PPDq in water and fish tissue

**Activity Budget:** $113,800

**Activity Description:**
Evaluating the risks of 6PPDq and other CECs to aquatic organisms requires a well-developed and validated analytical method to measure their concentrations. While a basic analytical method exists at the University of Washington (and two private labs), it needs optimization to be sufficiently sensitive, accurate, and available for use in Minnesota. Working with the experts in 6PPDq detection, we will optimize existing analytical methods for Minnesota samples (e.g., snowmelt and roadway runoff) and develop protocols to transfer the technology to the Minnesota Department of Health. Briefly, we will optimize sample cleanup and C-18 or HLB solid-phase extraction procedures to measure concentrations of 6PPDq down to 10 ng/L, while minimizing interference from background organic matter and salts by selective elution and extract cleanup steps.

Working from the basic tissue method described by Du et al. 2017, we will also develop our analytical capabilities in roadway-runoff exposed fish tissue deemed most suitable (e.g. plasma, liver, gill) to better measure bioavailable concentrations of 6PPDq and other roadway chemicals. Once this method is optimized, we can use it together with runoff water sampling to better understand how quickly and how much roadway chemicals, like 6PPDq, accumulate inside runoff exposed aquatic organisms.

**Activity Milestones:**

| Description | Completion Date |
|---|---|
| Validation of methods to determine 6PPDq concentrations in water and roadway runoff | March 31 2023 |
| Transfer water analysis methods to the Minnesota Department of Health | April 30 2023 |
| Adapt and validate methods for fish tissue analysis | June 30 2024 |
| Transfer fish tissue methods to the Minnesota Department of Health | June 30 2024 |

### Activity 2: Conduct a risk-based survey of water and fish for 6PPDq in Minnesota

**Activity Budget:** $279,163

**Activity Description:**
We know the potential exists for 6PPDq to be widespread in Minnesota lakes and rivers; however, the distribution and concentration of the chemical is unknown – critical information for risk assessment and effective management. As a first step, we will identify and spatially define risk-factors associated with potential 6PPDq exposure, such as proximity to major roads, rainfall and runoff patterns, and historical fish kill records. The resulting risk map will prioritize locations for surveillance, with special consideration for high-risk locations on tribal lands. We will strategically survey water and fish from high-risk locations and reference sites across Minnesota during rainfall and snowmelt events to maximize the detection of 6PPDq. In addition, we will actively collect samples from fish kill events to understand the frequency of 6PPDq-associated mortality. To ensure reliable and rapid response to potential 6PPDq exposure, we will create standardized sampling protocols to be used during our study and make them available for future management use. We will leverage existing fish kill response protocols and online reporting by the public and managers (http://z.umn.edu/fishkill). The results of this activity will be critical to informing an appropriate response by state and local managers to this emerging chemical contaminant.

**Activity Milestones:**

| Description | Completion Date |
|---|---|

| | |
|---|---|
| Create risk map of high-risk sites in Minnesota for 6PPDq exposure | April 30 2023 |
| Survey ~300 water and fish samples for the presence of 6PPDq in Minnesota | March 31 2025 |
| Actively sample fish kills following runoff events | June 30 2025 |

## Activity 3: Evaluate the hazard 6PPDq poses to Minnesota fish

**Activity Budget:** $98,037

**Activity Description:**

Fish cell and larval tests will be used for a rapid and cost-effective evaluation of toxicity. This portion of work is intentionally of limited scope because the chemical is not currently available/affordable. Cell and larval tests, unlike adult ones, require a small amount of chemical. Economically/ecologically important species, such as rainbow trout, bluegill and coho salmon, and chemical regulation-relevant fathead minnow will be used for cell tests. Effects of 6PPDq on cell death and mitochondria (cell "power plants" that generate energy essential for survival) will be measured because highly toxic chemicals often exert their toxicity by affecting mitochondrial function. In addition, live fish studies will be conducted with rainbow trout and fathead minnow larvae to determine whether cell/mitochondrial toxicity can lead to organismal effects (death and impairment of behaviors/physiological parameters associated with survival). If available, larvae of the additional native species (walleye and brook trout) may be tested in collaboration with the Grand Portage Band of Lake Superior Chippewa. Rapid assessment proposed here will allow us to determine if 6PPDq is likely to be toxic to a variety of Minnesota fish species and to hone in on the biological mechanisms underlying 6PPDq-associated fish kills in Minnesota.

**Activity Milestones:**

| Description | Completion Date |
|---|---|
| Evaluate effects of 6PPDq on fish cell survival and mitochondrial function | August 31 2023 |
| Evaluate effects of 6PPDq on fish survival and behavior | August 31 2024 |
| Integrate chemical occurrence and effects data to estimate risk to fish | May 31 2025 |
| Disseminate results to diverse audiences including regulatory and natural resources managers and citizens. | June 30 2025 |

## Project Partners and Collaborators

| Name | Organization | Role | Receiving Funds |
|---|---|---|---|
| Dalma Martinovic | University of St. Thomas | Environmental Toxicologist – Environmental sample extraction, toxicity method development and toxicity assessment. Assistance with sampling strategy design, analytical method development and data analyses associated with all the above. Student supervision, coordination of sample transfer and research activities across groups. Outreach and preparation of reports and manuscripts. | Yes |
| Seth Moore | Grand Portage | Director of Biology and Environment - will provide biological and ecological expertise, environmental sampling, logistics, and staff time for field sampling. | No |
| Mark Ferrey | MN Pollution Control Agency | Environmental scientist – will work in an advisory role to the project, contributing to the development of the project sampling plan, quality assurance, and the analysis of chemistry results. | No |
| Ed Kolodziej | University of Washington | Environmental Chemist - No lab in MN has a basic 6PPDq method developed. To assess the presence of the 6PPDq rapidly Dr. Kolodziej was identified as an essential out-of-state collaborator. Roles: analytical method development, sample analysis, data analyses, technology transfer, contribute to manuscript and report writing. | Yes |
| Mark Jankowski | US Environmental Protection Agency | Environmental toxicologist - will serve as a technical consultant for the project, providing his toxicological perspective and expertise to aid in the interpretation of the project chemical concentration data. He will serve in this role as an in-kind contribution to the project. | No |

## Long-Term Implementation and Funding

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

We anticipate significant interest in the results of this work and long-term impacts. With baseline data and reliable methods, we expect 6PPDq will be added to the regular monitoring list by Minnesota Pollution Control Agency and may also become a candidate for Minnesota Department of Health (MDH) screenings of toxicity and exposure potential as a part of the MDH CEC - 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.

## Other ENRTF Appropriations Awarded in the Last Six Years

| Name | Appropriation | Amount Awarded |
|---|---|---|
| Identification of Chemicals of Emerging Concern in Minnesota Fish | M.L. 2017, Chp. 96, Sec. 2, Subd. 04g | $400,000 |

## Project Manager and Organization Qualifications

**Project Manager Name:** Nicholas Phelps

**Job Title:** Assistant Professor

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

Dr. Nick Phelps is an Assistant Professor in the Department of Fisheries, Wildlife and Conservation Biology at the University of Minnesota and the Director of the Minnesota Aquatic Invasive Species Research Center (MAISRC). His research focuses on emerging threats to the health and sustainability of aquatic ecosystems, which lie at the intersection of animals, humans and the environment. Dr. Phelps has managed over $24M in competitive grant funding,

led large complex collaborations, held numerous outreach and public engagement events, and published ~50 peer-reviewed manuscripts and book chapters. Dr. Phelps has earned a BS in aquatic biology (Bemidji State University), an MS in aquaculture/fisheries (University of Arkansas-Pine Bluff), and a PhD in veterinary medicine (University of Minnesota).

**Organization:** U of MN - College of Food, Agricultural and Natural Resource Sciences

**Organization Description:**
The University of Minnesota's abbreviated mission statement: The University, founded in the belief that all people are enriched by understanding, is dedicated to the advancement of learning and the search for truth; to the sharing of this knowledge through education for a diverse community; and to the application of this knowledge to benefit the people of the state, the nation, and the world. The University's mission, carried out on multiple campuses and throughout the state, is threefold: Research and Discovery, Teaching and Learning, and Outreach and Public Service.

## Budget Summary

| Category / Name | Subcategory or Type | Description | Purpose | Gen. Ineligible | % Benefits | # FTE | Classified Staff? | $ Amount |
|---|---|---|---|---|---|---|---|---|
| **Personnel** | | | | | | | | |
| Nicholas Phelps | | Project Manager; Oversee all aspects of the project, coordinate team meetings and external communication, prepare written reports and presentations, etc. | | | 36.5% | 0.15 | | $25,806 |
| Post-doctoral associate | | The post-doctoral associate will be involved in all aspects of the project, with primary responsibilities for sample collection, data management, project team coordination, and scientific and stakeholder communication. | | | 25.4% | 3 | | $188,500 |
| | | | | | | | **Sub Total** | **$214,306** |
| **Contracts and Services** | | | | | | | | |
| University of St. Thomas | Professional or Technical Service Contract | Dalma Martinovic (University of St. Thomas, UST) will be involved with all aspects of the project, with primary responsibilities for Activities 1 and 3, including method development, sample collection and processing, student technician supervision, and coordination with the University of Washington for analytical testing and technology transfer. | | | | 0.6 | | $256,224 |
| | | | | | | | **Sub Total** | **$256,224** |
| **Equipment, Tools, and Supplies** | | | | | | | | |
| | Tools and Supplies | Field supplies for sample collection (bottles, bags, necropsy tools, etc.) | Supplies are requested to support the sample collection of water and fish tissues from high-risk sites in Minnesota. | | | | | $8,000 |
| | Tools and Supplies | Office supplies (e.g., lab notebooks, pens, etc). | Supplies are requested for project team members to record data, procedures, and share information. | | | | | $150 |
| | Equipment | One computer | One laptop computer is requested for the post-doctoral associate for use only on this project. The ability to track | X | | | | $2,000 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | samples, record data, and prepare publications/reports (sometimes from field locations) is an essential component of this project. | | | | | |
| | | | | | | | **Sub Total** | **$10,150** |
| **Capital Expenditures** | | | | | | | | |
| | | | | | | | **Sub Total** | **-** |
| **Acquisitions and Stewardship** | | | | | | | | |
| | | | | | | | **Sub Total** | **-** |
| **Travel In Minnesota** | | | | | | | | |
| | Miles/ Meals/ Lodging | 3250 total miles at a rate of $0.56/mile to collect water and fish samples. | Project team members must travel across Minnesota to collect water and fish samples. The total number of trips is TBD, but we estimate a total of 3250 total miles. | | | | | $1,820 |
| | Miles/ Meals/ Lodging | Project team coordination meeting with travel for three collaborators. The meeting will be held over two days in St. Paul, MN. Funds requested for travel costs and facility fees. | It is important to hold face-to-face project team coordination meetings to share results, discuss analysis, and plan for stakeholder communication. The meeting will be held in St. Paul (local for most project team members) and include travel for three collaborators from Grand Portage (Moore), University of Washington (Kolodziej), and the US EPA in Seattle (Jankowski). A second team meeting will be hosted by Grand Portage with non-state funds. | | | | | $3,000 |
| | | | | | | | **Sub Total** | **$4,820** |
| **Travel Outside Minnesota** | | | | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Conference Registration Miles/ Meals/ Lodging | Travel and registration for one scientific conference (Ecological Society of America), location within United States TBD. | We expect the results of this project to be of high interest to the scientific community. It is therefore prudent that one project team member (post doctoral associate) travel to attend a conference in Year 3 of the project to present. | X | | | | $2,500 |
| | | | | | | | **Sub Total** | **$2,500** |
| **Printing and Publication** | | | | | | | | |
| | Publication | Publication of two peer-reviewed manuscripts | We will publish two peer-reviewed manuscripts and make all methods and data associated with this project publicly available through open access journals and/or the UMN Data Repository. | | | | | $3,000 |
| | | | | | | | **Sub Total** | **$3,000** |
| **Other Expenses** | | | | | | | | |
| | | | | | | | **Sub Total** | **-** |
| | | | | | | | **Grand Total** | **$491,000** |

## Classified Staff or Generally Ineligible Expenses

| Category/Name | Subcategory or Type | Description | Justification Ineligible Expense or Classified Staff Request |
|---|---|---|---|
| **Equipment, Tools, and Supplies** | | One computer | One laptop computer is requested for the post-doctoral associate for use only on this project. The ability to track samples, record data, and prepare publications/reports (sometimes from field locations) is an essential component of this project. |
| **Travel Outside Minnesota** | Conference Registration Miles/Meals/Lodging | Travel and registration for one scientific conference (Ecological Society of America), location within United States TBD. | We expect the results of this project to be of high interest to the scientific community. It is therefore prudent that one project team member (post doctoral associate) travel to attend a conference in Year 3 of the project to present. |

## Non ENRTF Funds

| Category | Specific Source | Use | Status | Amount |
|---|---|---|---|---|
| **State** | | | | |
| | | | **State Sub Total** | **-** |
| **Non-State** | | | | |
| In-Kind | The University of St. Thomas | Waived indirect cost recovery (41.4%) for the professional service contract to the University of St. Thomas. | Secured | $97,797 |
| In-Kind | University of Minnesota | Waived indirect cost recovery (55% + 25% for UST subcontract) for the University of Minnesota. | Secured | $141,226 |
| Cash | Grand Portage Band of Lake Superior Chippewa | Sample collection, sample analysis, travel and personnel time. | Secured | $75,000 |
| In-Kind | Grand Portage Band of Lake Superior Chippewa | Sample collection, sample analysis, travel and personnel time. | Secured | $75,000 |
| | | | **Non State Sub Total** | **$389,023** |
| | | | **Funds Total** | **$389,023** |

## Attachments

### Required Attachments

*Visual Component*

File: 87d1c729-ebc.pdf

*Alternate Text for Visual Component*

The infographic provides a visual description of the project, including the problem, question and plan.

The problem: In late 2020, researchers discovered that an analog of a common tire chemical was responsible for significant salmon mortality events in the Pacific Northwest. This highly toxic chemical is now known as ”6PPDq”. The research was highlighted in the popular press and concerns have quickly grown across the country, including Minnesota.

The question: Is the tire chemical 6PPDq ki...

## 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?**

Yes, Sponsored Projects Administration

# THE PROBLEM

In late 2020, researchers discovered that an analog of a common tire chemical was responsible for significant salmon mortality events in the Pacific Northwest. This highly toxic chemical is now known as "6PPDq". The research was highlighted in the popular press and concerns have quickly grown across the country, including Minnesota.

Science
RESEARCH

ECOTOXICOLOGY

**A ubiquitous tire rubber–derived chemical induces acute mortality in coho salmon**

Tian *et al.*, *Science* **371**, 185–189 (2021) 8 January 2021

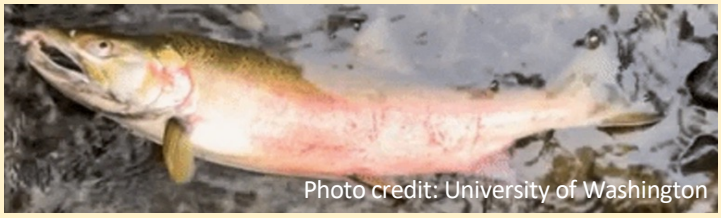

Photo credit: University of Washington

The New York Times

***How Scientists Tracked Down a Mass Killer (of Salmon)***

Something was decimating the salmon that had been restored to creeks around Puget Sound.

Dec. 3, 2020

CNN

**Salmon have been dying mysteriously on the West Coast for years. Scientists think a chemical in tires may be responsible**

Updated 4:11 PM ET, Thu December 3, 2020

THE NATIONAL LAW REVIEW

**Scientific Study Links Tire Preservative Chemical 6PPD to Fish Deaths**

Friday, December 4, 2020

# THE QUESTION

## Is the tire chemical 6PPDq killing Minnesota's fish?

An estimated 500 fish kills occur in Minnesota each year (Phelps et al. 2020), the vast majority of which go undiagnosed. Given the association of many local fish kills with hypothesized risk factors (e.g., proximity to major road, rain/snow runoff) for 6PPDq exposure, the concern for our fish populations is justifiably high. However, reliable detection methods are not available, and no baseline data exists, making the evaluation of risk and informed management decisions difficult.

# THE PLAN

Complete In progress Pending

1

**Assemble the team**

This proposal has already brought together an interdisciplinary team of scientific experts and key stakeholders to ensure rigorous and actionable results.

2

**Develop technology**

We will build on past work by project team members to fully validate 6PPDq detection assays and transfer the technology to the MN Dept of Health for future use.

3

**Determine occurrence**

We will conduct risk-based sampling of water and fish tissues to evaluate the occurrence of 6PPDq, with an emphasis on high-risk locations selected by tribal partners.

4

**Assess risk in Minnesota**

We will perform exposure trials using cell lines and larval fish to evaluate the mechanisms of 6PPDq toxicity. These critical data will help to inform risk assessments and next steps.

5

Communication We anticipate significant interest in the results of this work and long-term impacts. We will broadly disseminate our findings to diverse audiences throughout the project and ensure the translation of the science to end-users through active research engagement.