



# Environment and Natural Resources Trust Fund

2027 Request for Proposal

## General Information

**Proposal ID:** 2027-253

**Proposal Title:** Impact of “Forever Chemicals” on Blanding’s Turtle Health

## Project Manager Information

**Name:** Jeanine Refsnider

**Organization:** University of Toledo

**Office Telephone:** (419) 530-2664

**Email:** jeanine.refsnider@utoledo.edu

## Project Basic Information

**Project Summary:** We will test Blanding’s turtles, a state-Threatened species, for PFAS (“forever chemicals”) and quantify turtles’ health along a PFAS-contamination environmental gradient to understand how PFAS affect Minnesota’s rare wildlife.

**ENRTF Funds Requested:** \$283,000

**Proposed Project Completion:** May 31, 2029

**LCCMR Funding Category:** Small Projects (G)

**Secondary Category:** Fish and Wildlife (D)

## Project Location

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

Region(s): Metro, SE,

**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.**

Per- and polyfluoroalkyl substances (PFAS), also known as “forever chemicals,” are common contaminants in surface and groundwater. They do not break down in the environment, are easily transported over long distances in the atmosphere and waterways, and tend to bioaccumulate in wildlife. Even at low concentrations, PFAS can be harmful to human health, and they likely also have adverse effects on the health of exposed wildlife.

Some wildlife, such as the state-Threatened Blanding’s turtle, may be particularly vulnerable to adverse effects of PFAS because chemical concentrations can build to high concentrations over their long lifespan, they may be exposed to contamination from both terrestrial and aquatic habitats used throughout the year, and PFAS may bioaccumulate in their tissues from their carnivorous diet. Blanding’s turtles have declined throughout their historical range due to extensive habitat loss and fragmentation, and their low reproductive rates mean that populations are unlikely to recover without extensive intervention. If environmental contaminants such as PFAS impact the health of this species, then they likely have similar effects on other wildlife, including other vulnerable and common species. Thus, improving the health of state-listed species to enhance population persistence would be additional impetus to clean up PFAS-contaminated sites.

### **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.**

This project will validate methods of measuring PFAS concentrations and quantifying metrics of individual health in wild turtles using the state-Threatened Blanding’s turtle as a model species. We collect blood samples from three populations of Blanding’s turtles along a gradient of PFAS contamination near the Twin Cities. For each turtle sampled, we will measure the concentration and composition of PFAS in its blood plasma, and we will then correlate PFAS concentrations with several metrics of individual health (including white blood cell profiles, plasma biochemistry, and competency of the innate and constitutive immune systems). We predict that 1) Blanding’s turtles from areas with higher PFAS contamination will exhibit greater concentrations of PFAS in their blood plasma than turtles from less-contaminated sites, and 2) turtles with higher PFAS concentrations in their blood will exhibit poorer immune responses and altered biochemistry profiles compared to turtles with lower PFAS concentrations.

In addition to elucidating a potential role of PFAS in contributing to species declines, this research will establish a protocol by which wildlife species of concern can be screened over a broad spatial scale to assess the potential threat posed by PFAS to individuals’ health.

### **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?**

First, this project will provide timely information on PFAS exposure in Blanding’s turtles, a state-Threatened species of high conservation priority. Second, this project will determine whether PFAS contamination is associated with adverse health impacts in Blanding’s turtles. Third, this project will develop and validate a protocol that can be used to screen other wildlife for PFAS concentrations and predict likely impacts of PFAS on individual health. Our PFAS-screening protocol for wildlife will help to inform decisions on where PFAS decontamination efforts on protected land are likely to be most beneficial for enhancing the health and persistence of vulnerable wildlife populations.

## Activities and Milestones

### Activity 1: Turtle capture and collection of blood samples

**Activity Budget:** \$69,178

**Activity Description:**

In late spring 2028, when Blanding’s turtles are most active, we will capture up to 20 wild Blanding’s turtles in each of three populations along a gradient of environmental PFAS contamination around the Twin Cities. Tentative sampling sites include Crow-Hassan Park Reserve (Hennepin County), Murphy-Hanrehan Park Reserve (Scott County), and the Weaver Dunes area (Wabasha County). Turtles will be captured using a combination of fyke nets, baited traps, and basking traps placed in shallow wetlands known to be inhabited by Blanding’s turtles. At some of the proposed study sites, a few Blanding’s turtles have been outfitted with radio-transmitters by park district biologists, and these individuals can be radio-tracked and captured by hand for sampling.

We will collect a blood sample from each turtle, which will be used to measure PFAS concentrations and health metrics for each individual turtle. Most Blanding’s turtles at the proposed study sites have been individually marked during previous conservation research. During sampling efforts for this project, we will identify any previously marked individuals, and individually mark any new individuals, and provide all capture data to park managers for their records. All turtles will be released at their capture site immediately following collection of a blood sample.

**Activity Milestones:**

Description	Approximate Completion Date
completion of blood sample collection from wild turtles	July 31, 2028

### Activity 2: Measuring PFAS concentration from blood samples

**Activity Budget:** \$119,500

**Activity Description:**

Per- and polyfluoroalkyl substances (PFAS) are a class of human-made chemicals that have been widely used in commercial products and industrial processes since the 1950s. There are thousands of different types of PFAS, and they have been detected in water, soil, and air around the world, including in otherwise pristine environments. They do not break down in the environment, and are therefore commonly known as “forever chemicals.” PFAS are also easily transported over long distances in the atmosphere and waterways. They have been detected in both wildlife and humans, and even at low concentrations PFAS can cause adverse impacts on individual health.

We will measure the total PFAS concentration, as well as the composition and types of PFAS detected, in plasma from each of the blood samples collected from wild Blanding’s turtles. Analysis of PFAS concentration and composition in turtle blood plasma samples will be conducted by Dr. Matt Simcik at the University of Minnesota.

**Activity Milestones:**

Description	Approximate Completion Date
completion of PFAS analysis	December 31, 2028

### Activity 3: Quantifying metrics of individual health from blood samples

**Activity Budget:** \$41,040

**Activity Description:**

We will use several assays commonly employed in conservation physiology to quantify metrics of individual health in each of the Blanding’s turtles from which blood samples were collected. First, we will characterize each turtle’s white blood cell profile from blood smears made using whole blood. The ratio of different types of white blood cells can indicate baseline physiological stress levels and leukocyte proliferation important for mounting an immune response. Second, we will separate packed blood cells from blood plasma and analyze plasma biochemistry for twelve different analytes, which have not previously been characterized for Blanding’s turtles. White blood cell profiles and plasma biochemistry will be analyzed by EA Engineering, Science, and Technology, Inc., PBC.

We will measure each individual turtle’s immune competency using two different assays. First, we will use a bacteria-killing assay to quantify the competency of each turtle’s blood plasma to kill E. coli bacteria, a measure of an individual’s innate immunity, or its first line of defense against an infection. We will also measure each individual’s constitutive immune competency using an assay that measures the capacity of antibodies to agglutinate to and destroy foreign cells. Both immune competency assays will be conducted at the University of Toledo.

**Activity Milestones:**

Description	Approximate Completion Date
completion of all health assays	December 31, 2028

**Activity 4: Analyze data and prepare final reports**

**Activity Budget:** \$53,282

**Activity Description:**

We predict that PFAS concentrations in wild Blanding’s turtles will increase along the sampling gradient from northwest of the Twin Cities, where we predict the lowest PFAS concentrations, to southeastern Minnesota, where we predict the highest concentrations due to downstream transport of PFAS. We also predict that individual turtles’ health will decline as PFAS concentrations increase, such that turtles with higher PFAS concentrations will exhibit altered white blood cell profiles and lower immune functioning than turtles with lower PFAS concentrations. We will analyze data by correlating individual PFAS concentration and health metrics, and mean population PFAS concentration with PFAS levels in environmental samples, collected at each of the three study sites.

Following data analysis, final project deliverables will include a final report to the Minnesota DNR, LCCMR, and all project partners and stakeholders at the study sites. In addition, this project will result in a Master’s thesis in Ecology by a University of Toledo graduate student.

**Activity Milestones:**

Description	Approximate Completion Date
completion of data analysis	March 31, 2029
completion of final report	May 31, 2029
submission of thesis by MS student	May 31, 2029

## Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
Matt Simcik	University of Minnesota	Sub-awardee	Yes
David Lee Haskins	EA Engineering, Science, and Technology, Inc., PBC	Service Contractor	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.**

This project will result in a Master’s thesis at the University of Toledo, at least one peer-reviewed publication in a scientific journal, and presentations by the MS student and the PIs at regional and/or national wildlife conferences. We will also produce a final report with site-specific data on PFAS concentrations and turtle health metrics, data analyses, and management recommendations. The MS thesis, peer-reviewed publication(s), and final report containing management recommendations will be provided to the LCCMR, Minnesota DNR, and land managers for all study sites. Upon publication, all data will be publicly available in a repository such as Dryad.

## 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?**

This project will correlate PFAS concentrations detected in blood plasma samples with metrics of an individual’s health in a threatened turtle species. If high PFAS concentrations reliably indicate poor health in Blanding’s turtles, this would provide proof-of-concept for future work that PFAS contamination is associated with adverse health effects in reptiles and other wildlife. Subsequent conservation projects will be able to use and improve upon the protocols validated here to screen other vulnerable wildlife populations for PFAS concentrations to determine where targeted PFAS decontamination efforts are most needed to improve the health of rare species.

## Project Manager and Organization Qualifications

**Project Manager Name:** Jeanine Refsnider

**Job Title:** Associate Professor

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

Dr. Refsnider (Associate Professor, Department of Environmental Sciences, University of Toledo) will oversee this project and serve as the primary advisor of the graduate student leading field data collection. Dr. Refsnider has over 20 years of field experience trapping, marking, and collecting blood samples from both aquatic and terrestrial turtles, including common and endangered species, and has advised six MS and two PhD students in reptile-based ecological research. In particular, Dr. Refsnider has previously conducted extensive radio-telemetry research on Blanding’s turtles in Minnesota, and is familiar with the study species and potential sampling sites proposed for this research.

Research in the Refsnider Lab is focused on understanding how animals, especially reptiles, are affected by and respond to human-caused changes to the environment. We have published over 60 peer-reviewed papers in the scientific literature and our research has been funded by the National Science Foundation, U.S. Fish and Wildlife Service, Ohio

SeaGrant, and the Ohio and Michigan Departments of Natural Resources. Previous research has included projects on the effects of climate change, harmful algal blooms, oil spills, salinization, fungal disease, and urbanization on wildlife populations, with specific focus on how these disturbances impact individuals' health and fitness, as well as population demography and persistence. This research has involved numerous species around the world, including tuatara in New Zealand, turtles in Australia, frogs in California, lizards in Utah and New Mexico, snakes in Michigan and Ohio, songbirds in Ohio and Minnesota, and freshwater turtle species across the U.S. Many of these studies have used the same immunological assays proposed here to assess the health of individual animals under various environmental challenges. Ultimately, our goal is to enact science-based conservation that takes advantage of species' natural abilities to respond to environmental change to enhance species resilience in a rapidly changing world.

**Organization:** University of Toledo

**Organization Description:**

The University of Toledo is a public R1 research university and academic medical center in Toledo, Ohio. The university is known nationally as a leader in solar energy innovation, water quality research, cellular research, and astrophysics. With a growing research enterprise, the University of Toledo tackles the challenges of today while engaging students as early as their first year to participate in undergraduate research.

The College of Natural Sciences and Mathematics is home to the Department of Environmental Sciences, which is an interdisciplinary group of ecologists and geologists whose research and teaching interests address human impacts on the environment, earth surface processes and ecosystem science. The Department of Environmental Sciences offers graduate degrees (MS and PhD) in both Ecology and Environmental Sciences, with research strengths in aquatic ecology, organismal biology, and conservation biology.

## Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	\$ Amount
<b>Personnel</b>								
Jeanine Refsnider-Sterby/Associate Professor		Principal Invenstigator			32%	0.07		\$10,519
Graduate Student		Graduate Student			2%	0.46		\$29,053
2 Undergraduate Students		Undergraduate Student			2%	0.25		\$11,750
							<b>Sub Total</b>	<b>\$51,322</b>
<b>Contracts and Services</b>								
University of Minnesota	Subaward	Dr. Simcik (15% effort, salary plus benefits \$41,953) to the collection, analysis and interpretation of results and supervision of the graduate assistant (GA Salary \$38,301). He will be in charge of the PFAS analysis and reporting. Graduate student 50% effort salary plus benefits \$38,301.				0.15		\$112,000
EA Engineering Science and Technology, Inc	Service Contract	Dr. Haskins (15% effort, salary plus benefits \$49,119) will be in charge of blood plasma analysis and reporting. Also includes \$12,400 for field sampling supplies, transportation, and lodging; \$8205 for laboratory analysis supplies and labor; and 10% service fee (\$7551 on \$75,507 pre-fixed fee).				1		\$83,057
							<b>Sub Total</b>	<b>\$195,057</b>
<b>Equipment, Tools, and Supplies</b>								
	Tools and Supplies	Field sampling supplies include 4 fyke nets to capture turtles plus bait. Laboratory supplies include sample storage and reagents for 60 blood samples.	Traps and bait are needed to capture wild turtles from which to collect blood samples. Laboratory supplies and reagents will be used in					\$5,669

			measuring health metrics of individuals from which blood was collected. Health metrics will include innate immune competency measured with a bacteria-killing assay, and constitutive immune functioning measured with a natural antibody agglutination assay.					
							<b>Sub Total</b>	<b>\$5,669</b>
<b>Capital Equipment</b>								
							<b>Sub Total</b>	<b>-</b>
<b>Acquisitions and Stewardship</b>								
							<b>Sub Total</b>	<b>-</b>
<b>Travel In Minnesota</b>								
	Miles/ Meals/ Lodging	Rental car to transport PI, graduate student, and 2 field technicians among sampling sites daily for up to 8 weeks, plus fuel for estimated 150 miles/day. Also included is lodging for PI (for two weeks) and graduate student and 2 field technicians for up to 8 weeks.	Turtle traps must be checked daily when set, so field crews will need to travel among two or three sampling sites daily during fieldwork, which is estimated to take up to 8 weeks, to check traps and collect blood samples from trapped turtles. The field sampling crew will need daily transportation and accommodation during the field sampling period.					\$12,100
							<b>Sub Total</b>	<b>\$12,100</b>
<b>Travel Outside Minnesota</b>								
							<b>Sub Total</b>	<b>-</b>
<b>Printing and Publication</b>								
							<b>Sub Total</b>	<b>-</b>
<b>Other Expenses</b>								

		Tuition remission for Graduate student, includes General fees and Medical insurance	Graduate student will receive tuition remission, general fees, and medical insurance during the following semesters: Summer 2028, Fall 2028, and Spring 2029. The student will be supported on a departmental teaching assistantship during the other semesters they are working on this project (estimated Spring 2028, Summer 2029, and Fall 2029).					\$18,852
							<b>Sub Total</b>	<b>\$18,852</b>
							<b>Grand Total</b>	<b>\$283,000</b>

Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request
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Non ENRTF Funds

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

**Total Project Cost: \$283,000**

**This amount accurately reflects total project cost?**

Yes

## Attachments

### Required Attachments

#### *Visual Component*

File: [5015cb41-ba8.pdf](#)

#### *Alternate Text for Visual Component*

We will measure PFAS concentrations and individual health metrics in wild Blanding's turtles from three sites along a gradient of low-high environmental PFAS contamination....

### Supplemental Attachments

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

Title	File
Letter authorizing submission	<a href="#">4fc636cc-91f.pdf</a>
Support Letter - Three Rivers Park District	<a href="#">0a6e7052-3ef.pdf</a>

## Administrative Use

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

No

**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?**

Yes, I understand the Commissioner's Plan applies.

**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?**

Yes, University of Toledo

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

No

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

No

**Provide the name(s) and organization(s) of additional individuals assisting in the completion of this proposal:**

Christina Don Bosco, University of Toledo Research and Sponsored Programs

**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**

Yes, I understand