# Environment and Natural Resources Trust Fund
# 2017 Request for Proposals (RFP)

**Project Title:** **ENRTF ID: 165-F**

Methods for Removing Problematic Pesticides from Minnesota Waters

**Category:** F. Methods to Protect, Restore, and Enhance Land, Water, and Habitat

**Total Project Budget: $** 344,000

**Proposed Project Time Period for the Funding Requested:** 2 years, July 2017 – June 2019

**Summary:**

We will develop, demonstrate, and disseminate, a simple, effective, innovative and inexpensive technology to remove toxic pesticides from Minnesota waters, increasing safety for Minnesotans health and environmental quality.

**Name:** Mikael Elias

**Sponsoring Organization:** U of MN

**Address:** 1479 Gortner Ave

St. Paul MN 55108

**Telephone Number:** (612) 626-1915

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**Web Address**

**Location**

**Region:** Statewide

**County Name:** Statewide

**City / Township:**

**Alternate Text for Visual:**

Many pesticides are stable in the environment for years. We will identify and apply new enzymes to break down pesticides. Enzymes are proteins, and proteins diversity is huge. We have new methods to identify proteins for pesticide break down, and we will use tham to degrade pesticides in engineered systems.

| | | | |
|---|---|---|---|
| _____ Funding Priorities | _____ Multiple Benefits | _____ Outcomes | _____ Knowledge Base |
| _____ Extent of Impact | _____ Innovation | _____ Scientific/Tech Basis | _____ Urgency |
| _____ Capacity Readiness | _____ Leverage | _____ TOTAL | _____% |

## I. PROJECT STATEMENT

We will develop, demonstrate, and disseminate, a simple, effective, and inexpensive technology to remove pesticides from Minnesota waters. This proposal falls under several funding priorities of the ENRTF fund, such as water resources protection and mitigation, as well as the methods to protect land or water, particularly for pollinators. **Natural systems will be used to bring bioremediation methods to bear on a large number of pesticides found in Minnesota waters by the Minnesota Department of Agriculture**, as published in their most recent water quality monitoring report. We will focus on the most problematic pesticides – Imidacloprid and Clothianidin, neonicotinoids linked to destruction of bee hives; Picloram – used in Agent White and an advanced form of Agent Orange during the Vietnam War; and Atrazine, that is found in waters throughout Minnesota and sometimes found above allowable drinking water limits. Pesticides are non-natural compounds, made to kill insects or plant pests but they often spill over and harm non-target species. These compounds are particularly problematic because of their high environmental stability. Therefore, innovative solutions are required to overcome the stability of the chemicals. Our methods identify bio-based systems that degrade and detoxify problem pesticides, taking advantage of systems developed over the last two decades, and those systems will be expanded. First, we will use and expand on the types of bioremediation databases we have developed in the past (http://eawag-bbd.ethz.ch) with a new database that can be used by us and will be made publicly available, to be shared with Minnesota State Agencies and others. Additionally, we will identify novel bioremediating systems using a rich source of natural degradation systems that can be rapidly screened for new capabilities to degrade the many pesticides in our waters. **The Minnesota group conducting this project has a unique collection of skills such that new tools will be generated to degrade pesticides most broadly**. The outcome will be systems that can be used in Minnesota and wherever they are needed. Near the end of the project period, we will hold a conference hosted by the Institute on the Environment with many invited stakeholders from around the state and the focus will be squarely on solutions for pesticides in Minnesota and beyond. The conference will serve to disseminate our findings and help arrive at the best practices for implementation in the state. Materials and information will be made available to all relevant state agencies. This project, if funded by the ENRTF, will develop, educate, and foster all best practices for treating pesticide contamination in Minnesota waters and can help institute a long-term, sustainable solution.

## II. PROJECT ACTIVITIES AND OUTCOMES

**Activity 1:** Obtaining biocatalysts **Budget: $ 79,000**

We will use University facilities at the BioTechnology Institute to efficiently and inexpensively produce the bio-component. The University provides facilities for producing bio-materials to faculty at well below market cost, as the BioTechnology Institute is supported by fees from Minnesota industry. This activity will be conducted by Profs. Elias and Wackett.

| Outcome | Completion Date |
|---|---|
| 1. Screen bioremediating material | November 1, 2017 |
| 2. Obtain bioremediating material | January 1, 2018 |

**Activity 2:** Develop bioremediation biocatalyst database **Budget: $ 76,000**

The project bioinformatics director (Wackett) will preside over the development of the database and database tools. He or she will direct a postdoctoral fellow and undergraduate computer science students. Note we have had many undergraduate volunteers in the past interested to learn database development.

| Outcome | Completion Date |
|---|---|
| 1. Develop website, data files, survey users and improve graphical interfaces | June 30, 2018 |
| 2. Develop computer-based tool to predict how to best biodegrade pesticides and other | June 30, 2019 |

| | |
|---|---|
| chemicals | |

**Activity 3:** Optimization of the bioremediating material **Budget: $ 189,000**

Optimization of the bioremediating material will be conducted using innovative methods that we have created and the unique set of skills on the team specifically assembled on the project. This will be directed by the project team (Elias, Wackett and Aksan) and conducted by the project manager (Elias).

| Outcome | Completion Date |
|---|---|
| 1. increase the efficiency of biocatalysts | January 1, 2019 |
| 2. Built a lab-scale water cleaning cartridge | June 30, 2019 |

**Budget:** not requested

**Activity 4:** Disseminating findings and informing stakeholders

We will organize and host a conference in the Institute on the Environment that we will be covered using funding that is already secured. The conference participants will include political leaders, people from relevant state agencies, concerned citizens, and the private sector. We will disseminate our findings from the project. We will also discuss the major state sites that are most impacted by pesticides and the best means for deploying the bioremediation, and any other technology that can help solve the problem. It is understood that pesticide contamination of waters has been a long- term problem in Minnesota and other agricultural states and so we seek to implement a long-lasting, sustainable solution.

| Outcome | Completion Date |
|---|---|
| 1. Disseminate information and move the best technology into practice in Minnesota | June 30, 2019 |

**III. PROJECT STRATEGY**

**A. Project Team/Partners**

The project will be carried out by a strong team that covers complementary areas. Collectively, the team covers environmental science, biology, and engineering expertise. The team has a specific and strong background in dealing with environmental chemicals, particularly pesticides, and engineering treatment systems to remove and degrade them. The teams consists of a Biochemistry assistant professor, Mikael Elias; a Fellow of the Institute on the Environment and professor, Lawrence Wackett; and a Mechanical Engineering professor, Alptekin Aksan. The team will also benefit from an already established collaboration with Computer Science assistant professor Dan Knights on the database component. Two project managers will handle the pesticide biodegradation component and the database component, respectively. Undergraduate biology, engineering and computer science students will be brought into the project. We have enlisted the aid of undergraduates to work with senior researchers on past projects. This advances the projects and provides an excellent educational experience for talented undergraduates who have gone on to high level careers in science and industry.

**B. Project Impact and Long-Term Strategy**

Focusing on the pesticides found in the Minnesota waters, the project will provide unique strategies to remediate these environmentally stable compounds, and provide a laboratory-scale proof-of-concept, demonstrating that innovative solutions can be used efficiently and inexpensively to treat water, and improve water quality for Minnesotans.

**C. Timeline Requirements**

This project will take 24 months to carry out as described above. Thereafter, it is expected that the products of the project to be handed off to state agencies and the private sector.

# 2017 Detailed Project Budget

**Project Title:** *Methods for Removing Problematic Pesticides from Minnesota Waters*

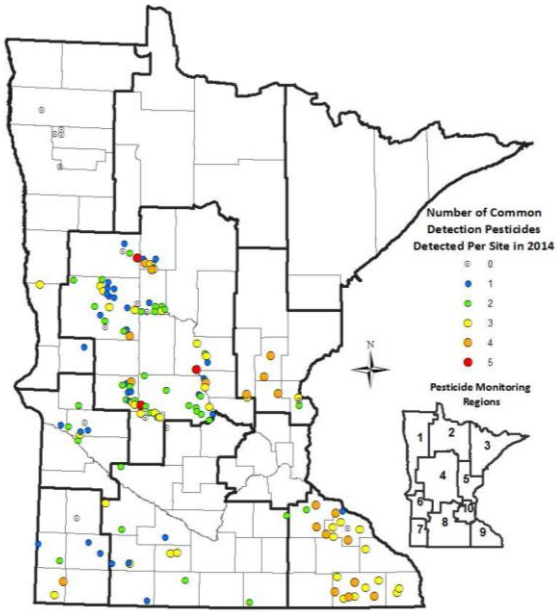
**IV. TOTAL ENRTF REQUEST BUDGET** *2* **years**

| **BUDGET ITEM** *(See "Guidance on Allowable Expenses", p. 13)* | **AMOUNT** |
|---|---|
| **Personnel**: Mikael Elias, project manager, PI: 8% time; 66.3% salary; 33.7% benefits, 1 month/year for 2 years . Prof. Elias is a new assistant professor (started 9/14) at the University of Minnesota. Dr Elias will be in charge of the completion of all project activities. | $ 26,000 |
| **Personnel:** Postdoc fellow (TBN): (77.6% salary, 22.4% benefits); 100% FTE for 1 year. Funds are requested for 1 year to support a postdoctoral fellow to fulfill activity 1. | $ 56,000 |
| **Personnel**: Postdoc fellow (TBN): (77.6% salary, 22.4% benefits); 100% FTE for 1 year. Funds are requested for 1 year to support a postdoctoral fellow to fulfill activity 2. | $ 56,000 |
| **Personnel:** Postdoc fellow (TBN): (77.6% salary, 22.4% benefits); 100%FTE for 2 years. Funds are requested for 2 years to support a postdoctoral fellow to fulfill activities 3 and 4. | $ 112,000 |
| **Professional/Technical/Service Contracts:** Funds are requested for service provided by the University of Minnesota's BioTechnology Institute Pilot Plant to prepare the pesticide-degrading biocatalysts used in this proposed research. | $ 30,000 |
| **Equipment/Tools/Supplies:** Funds are for producing and optimizing presticide-degrading materials for lab testing, as well as routine lab supplies (chemicals, flasks, pipetters). | $ 64,000 |
| **TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =** | $ 344,000 |

**V. OTHER FUNDS** *(This entire section must be filled out. Do not delete rows. Indicate "N/A" if row is not applicable.)*

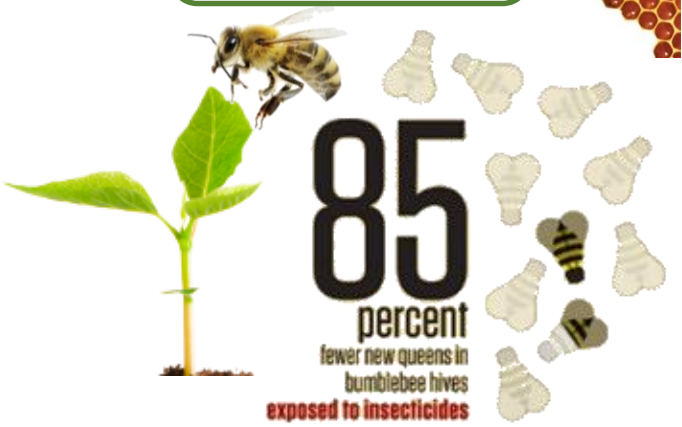
| **SOURCE OF FUNDS** | **AMOUNT** | **Status** |
|---|---|---|
| **Other Non-State $ To Be Applied To Project During Project Period:** *Indicate any additional non-state cash dollars secured or applied for to be spent on the project during the funding period. For each individual sum, list out the source of the funds, the amount, and indicate whether the funds are secured or pending approval.* | $ - | *Indicate: Secured or Pending* |
| **Other State $ To Be Applied To Project During Project Period:** MnDRIVE Initative project funded by State of Minnesota through the University of Minnesota to Mikael Elias and Lawrence Wackett for bioremediation projects. | $ 40,000 | *Secured* |
| **In-kind Services To Be Applied To Project During Project Period:** Faculty salary time paid by the University of Minnesota that will be devoted on the project over the rest of summer months and university indirect cost matching (52% mtdc in 2017; 53% in 2018) | $ 182,000 | *Secured* |
| **In-kind Services To Be Applied To Project During Project Period:** BioTechnology Institute Pilot Plant fee waiver. Since the PIs are members of the BioTechnology Institute, this project will have the entry fee waived for the use of the facilities to prepare pesticides-degrading biocatalysts to be used in this proposal. The project will only be charged for materials used in production and the hourly | $ 15,000 | *Secured* |
| **Funding History:**<br>**$738,000 -** *National Science Foundation - (Wackett, co-PI) - Project funded for 3 years through August 31, 2016 to engineer metabolic pathways to biodegrade triazine pesticides.* | $ 738,000 | Secured (spent by 2016) |
| **Remaining $ From Current ENRTF Appropriation:** *Specify dollar amount and year of appropriation from any current ENRTF appropriation for any directly related project of the project manager or organization that remains unspent or not yet legally obligated at the time of proposal submission. Be as specific as possible. Indicate the status of the funds.* | $ - | |

# Methods for Removing Problematic Pesticides from Minnesota Waters




**Pesticide contamination in Minnesota waters**
From the Minnesota Dpt of Agriculture 2014 water quality monitoring report

Bees can be contaminated by pesticides (e.g. neonicotinoids) through contacts with the plant



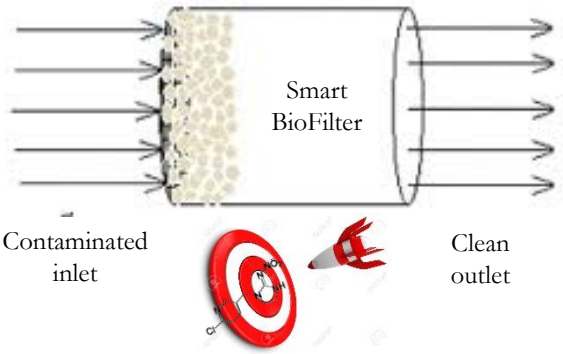

Pesticides can affect non-target beneficial species


Humans also exposed to pesticides

MANY PESTICIDES ARE STABLE IN THE ENVIRONMENT FOR YEARS

## We will identify and apply new enzymes to break down pesticides

- Enzymes are proteins
- Proteins are more numerous than stars in the galaxy (> 100,000,000,000)
- We have unique methods to sample nature's biodiversity of proteins
- Will identify many new proteins for pesticide break down
- New proteins can be used to breakdown pesticides in engineered systems




**Specific and efficient destruction of toxic pesticides**

**Management**: The research team will include Prof. Mikael Elias, Prof. Lawrence Wackett and Prof. Al Aksan. Prof. Elias will be the project manager. The team assembled has unique, and complementary, skills necessary to achieve the goals of the project. The specific expertise of each team member is described below.

**Prof. Mikael Elias, PI,** is an Assistant Professor in the Department of Biochemistry, Molecular Biology and Biophysics at the University of Minnesota. Elias has over 10 years of research experience on biodegradation enzymes, producing 4 patents and >10 articles on this topic alone, including in prestigious journals (*JACS*, *Nature*) and extensive know-how in protein engineering where he pioneered methods, such as the use of ancestral methods. He will invest most of his time on the project, and perform experiments and data analysis. Additionally, he reviews data and meets with laboratory personnel on a daily basis to promote the projects. He also prepares the dissemination of results, such as the proposed conference and publications. Mikael Elias has extensive experience with protein optimization methods, and X-ray crystallography. As the PI of the project, Dr. Elias will oversee the entire project, design the experiment plans, and draft the project reports.
**Prof. Lawrence Wackett, co-PI** is a Fellow of the Institute on the Environment and a professor in the Department of Biochemistry, Molecular Biology and Biophysics at the University of Minnesota. Prof. Wackett has extensive expertise in biodegradation, with over 20 years of cutting-edge research in the area, and numerous achievements that are available to the public such as the biodegradation database (http://eawag-bbd.ethz.ch). His expertise spans biodegradation, enzyme characterization, isolation and discovery of novel biodegradation material.
**Prof. Al Aksan, co-PI** is a professor in the Mechanical Engineering Department. Prof. Aksan is an expert in biodegradation, and pioneers new methods to produce cost-effective, efficient water and air filtration devices using biomaterials.
Collaboration: **Prof. Dan Knights** is a Professor in Computer Science and Engineering at the University of Minnesota. His expertise includes Bioinformatics and Computational Biology, and will be advisory for building the new database and prediction tools for the biodegradation of pesticides.

**Organization**: the University of Minnesota has several missions: improve lives through research, education, and outreach. The University possess extensive facilities that ensure high research performance. In particular, for this project:
• X-ray crystallography facility: houses four complete macromolecular X-ray data collection beam lines with three RigakuMSC Micromax 007 X-ray generators, three R-axis IV++ image plate detectors, and a Saturn 944+ CCD Camera. Crystallization robots and crystal growth monitoring systems are also available. • Minnesota Supercomputing Institute (https://www.msi.umn.edu/services/informatics). This resource will be useful for our bioinformatics research component.
• Biotechnology Resource Center: (http://www.bti.umn.edu/brc/index.html) A wide variety of bench-scale to pilot scale fermenters is available, up to 500L, and will be used in this project to produce cost-effective biomaterials.
Elias Lab: 1,800 sq. ft. of renovated research space is dedicated to Dr. Elias. This space is located on the 1st floor of the GortnerLab Building, on the St Paul campus. Elias's office space is adjacent to the laboratory. The lab contains all of the necessary equipment for molecular biology, biochemistry, protein production and purification, enzyme kinetics, and crystallography. Numerous facilities are available, such as microplate readers, spectrophotometers, scintillation counters, fplc, liquid nitrogen storage, -80 freezers, incubators/shakers, autoclave, as well as 4 and -20 rooms.
Wackett Lab: 3,600 sq. ft is adjacent to the Elias Lab, on the 1st floor of the GortnerLab Building, on the St Paul campus. The laboratory is equipped with the instruments that are essential for the biodegradation material discovery, isolation, purification, and characterization.
**Team**: Elias and Wackett's lab being adjacent, this ensures an efficient communication between the project members. All PIs and collaborator being from the UMN Twin cities campus, the coordination of tasks will be facilitated and ensure the success of this project.