Environment and Natural Resources Trust Fund
2010 Request for Proposals (RFP)

LCCMR ID: 011-A2

Project Title:

Fate and Ecological Impacts of Industrial Phytoestrogens

LCCMR 2010 Funding Priority:

A. Water Resources

Total Project Budget: $ $340,000

Proposed Project Time Period for the Funding Requested: 3 years, 2010 - 2013

Other Non-State Funds: $ $0

Summary:

Hormone mimics are present in some industrial effluents (e.g., biodiesel) and may damage human/ecological health. We must understand the fate and effect of these compounds to protect Minnesota waters.

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Sponsoring Organization: U of MN

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Location:

Region: Statewide

County Name: Statewide

City / Township:

_____ Knowledge Base _____ Broad App. _____ Innovation

_____ Leverage _____ Outcomes

_____ Partnerships _____ Urgency _____ TOTAL

I. PROJECT STATEMENT

Phytoestrogens are plant-based compounds that mimic estrogen and can therefore interfere with normal biological development. Our recent survey of effluent from 19 plant-processing industries showed that 9, including biodiesel and soy oil facilities, had effluent concentrations of phytoestrogens that could have adverse ecological effects. If biofuel production replaces our current use of 60 billion gal/yr of diesel fuel, the potential impact of phytoestrogens on surface water is enormous. **It is therefore critical to understand:**

- **The persistence of commonly discharged phytoestrogens and**
- **Their effect on aquatic life in receiving waters.**

This project will address these two needs. **With the strong commitment that Minnesota has to maintaining excellent water quality while also spearheading national biofuel production, this issue is of the utmost importance.** This research will facilitate thoughtful effluent treatment designs for current and new biofuel facilities and help determine where additional resources/attention should be focused to preserve Minnesota's water quality.

II. DESCRIPTION OF PROJECT RESULTS

Result 1: *Determine the chemical and biological fate of phytoestrogens in surface waters*
Budget: $190,000

We now know that phytoestrogens are discharged to surface water. Nevertheless, no research has been conducted on their biological or chemical fate in that environment. It is likely that these compounds will stick to particles in the receiving water and will undergo chemical and biological reactions. These processes will control the concentration of the phytoestrogens, and therefore, their ecological effect (see Result 2, below). Laboratory experiments will be performed with the two most-commonly observed phytoestrogens: genistein and daidzein. Single compounds and mixtures will be added to river water samples collected downstream of two soy-processing facilities in Minnesota (in Mankato and Brewster). The biological transformation of the phytoestrogens will be measured with time using liquid chromatography/mass spectrometry under different conditions (phytoestrogen concentration, biomass levels, oxygen levels). The estrogenicity of any byproducts formed will be determined as well, using a yeast estrogen screen assay. Experiments to determine photolysis (sunlight-driven reactions) rates of genistein and daidzein will also be conducted in river and pure water in both artificial and natural light. The effect of naturally-occurring ions and organic matter on photolysis rates will also be investigated. Again, the estrogenicity of the byproducts will be determined. Finally, the water-solid partitioning coefficients will be determined for both compounds.

After quantifying the appropriate rate constants, verification of the importance of these processes in the field is required. We will determine the concentration of genistein and daidzein at the point of discharge and in the rivers/streams downgradient of the two soy-processing facilities. A model for the concentration of phytoestrogens as a function of distance will be built, based on our experimental results above, and compared to the concentrations measured in the field.

Deliverables	Completion Date
1. Determine the biological transformation kinetics for genistein and daidzein	**6/30/12**
2. Determine kinetics of photolysis for genistein and daidzein	**6/30/12**
3. Determine estrogenicity of transformation products of genistein and daidzein	**6/30/12**
4. Measure the effluent concentrations and downgradient concentrations of genistein and daidzein in the field	**9/30/12**
5. Build and verify a model to determine the importance of various natural processes on phytoestrogen fate in the environment	**4/30/13**

Result 2: *Determine the impact of the phytoestrogens on fathead minnows* **Budget: $149,000**
The fathead minnow will be used as the biological model for this research, as this organism is used as a screening organism for EDCs by the US EPA and it is an important component of the Minnesota aquatic food chain. While two previous studies have indicated behavioral and physical changes in phytoestrogen-exposed fish, these studies did not use realistic compound concentrations, mixtures, or fish native to Minnesota. We propose to assess the effects of genistein and daidzein (singly and in mixtures) over a range of environmentally-relevant concentrations in controlled laboratory experiments. Three life stages (embryo, larva, adult) of the fathead minnow will be investigated to assess developmental, behavioral, and physical changes (including feminization) in the fish. For each life stage, fish will be exposed to three concentrations of each compound (spanning observed environmental concentrations) and to three mixtures of the two compounds. Following exposure, embryos and larvae will be assessed in their ability to perform innate predator avoidance behaviors. Adult fathead minnows (males and females) will be assessed for changes in their reproductive behavior. Fish will also be analyzed for vitellogenin concentrations (a precursor protein involved in egg production and a sign of feminization of male fish) and their livers and reproductive organs will be evaluated for changes. Finally, we will also perform in-stream experiments downstream of the discharge of the two soy-processing facilities to verify the results of laboratory experiments.

Deliverables	Completion Date
1. Determine the effects of phytoestrogens on embryonic and larval fathead minnow behavior	4/30/11
2. Determine the effects of phytoestrogens on the reproductive behavior of mature fathead minnows	4/30/12
3. Determine the effects of phytoestrogens on the physiology of mature fathead minnows (feminization, liver, and reproductive organ changes)	4/30/13

III. PROJECT STRATEGY

A. Project Team/Partners
Dr. Paige Novak (University of MN), an expert in the occurrence of phytoestrogens and their biological transformation, will lead the project and coordinate the research. Dr. Heiko Schoenfuss (St. Cloud State University), an expert on the impact of EDCs on aquatic biota, will direct the biological impact research. Dr. William Arnold (UMN), an expert in photolysis of EDCs and building kinetic models, will direct the studies on phytoestrogen fate with P. Novak. We have contacted personnel at the Mankato and Brewster wastewater facilities and have permission to sample their effluent.

B. Timeline Requirements
The proposed project will be completed in the allotted three-year period.

C. Long-Term Strategy
The proposed research fits into a larger research agenda centered at the University of Minnesota that is focused on the problem of environmental estrogens and endocrine disruptors in the State's surface waters. Although the proposed research will be completed in the allotted 3-year period with the requested financial resources, it complements current and prior research in this area. When taken together, the research performed or proposed by the University of Minnesota and its partners (e.g., St. Cloud State University) will provide a more complete picture of important sources and loads of estrogens/endocrine disruptors, the fate of these compounds in both engineered and natural systems, and potential strategies (communication or engineering) to mitigate the threat caused by these compounds.

Project Budget

IV. TOTAL PROJECT REQUEST BUDGET (*3* years)

BUDGET ITEM	AMOUNT
Personnel:	
Paige Novak, PI ($22,989 salary, $7,264 fringe, 31.6% fringe rate; total for 3 years; 6% effort, 2% unpaid (4% paid))	$ 30,000
William Arnold, Co-PI ($22,396 salary, $7,166 fringe, 31.6% fringe rate; total for 3 years; 6% effort, 2% unpaid (4% paid))	$ 30,000
Two Graduate Research Assistants ($107,798 salary, $74,225 fringe (includes healthcare and tuition); total for 2.25 years; additional 0.75 year will be supported through teaching assistantships)	$ 182,000
Subcontract: Some of the work will be conducted at St. Cloud State University (Result 2). The subcontract amount will include Co-PI salary (Heiko Schoenfuss, $7,000 salary, $2,000 fringe, 27% fringe rate; total for 3 years; 6% effort, 3.5% unpaid (2.5% paid); less paid effort because of summer teaching committments), one undergraduate research assistant ($12,000 for 3 years), supplies for experiments (fish, etc., $36,000), and half of the funds required for travel to the site for sampling and in-stream experiments ($1,000)). Graduate research assistants will work on both Result 1 and Result 2, but will be hired and paid through UMN.	$ 58,000
Equipment/Tools/Supplies: Laboratory supplies and analytical costs (includes chemicals for all analyses, supplies for fate studies, etc.)	$ 39,000
Travel: Travel to sites to pick up samples and monitor in-stream experiments (in state)	$ 1,000
TOTAL PROJECT BUDGET REQUEST TO LCCMR	$ 340,000

V. OTHER FUNDS

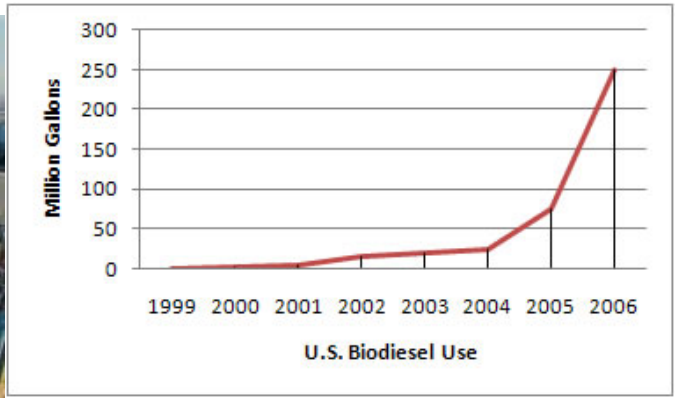
SOURCE OF FUNDS	AMOUNT	Status
Other Non-State $ Being Applied to Project During Project Period:	$ -	
Other State $ Being Applied to Project During Project Period:	$ -	
In-kind Services During Project Period:	$ -	
Remaining $ from Current Trust Fund Appropriation (if applicable):		
Funding History: Previous LCMR grant in 2005, "Unwanted Hormone Therapy: Protecting Water and Public Health"	$ 300,000	complete

The problem of phytoestrogens from plant-processing industries

Phytoestrogens are released into water at low levels from natural sources (decomposition of plant matter).

(from:http://www.fws.gov/Midwest/MiddleMississippiRiver/images/wetland_management.jpg)

As manufacturing of biodiesel and other plant material increases, high concentrations of phytoestrogens from point sources increases the concentration of these compounds in water.



(from: http://www.tradenote.net/images/users/000/031/118/products_images/Biofuel__Biodiesel__Ethanol__Gasoline__Sugar.jpg)
(from: http://www.oil2fuel.com/images/BioDieselUsageGraph.jpg)

This may cause feminization or intersex characteristics in fish, which could have wide-spread ecological consequences.

(from: http://www.appalachian-center.org/images/issues/fish_kill_poster.jpg)

Project Manager Qualifications and Organization Description

Paige J. Novak
Associate Professor, Environmental Engineering, Department of Civil Engineering and Founding Fellow of the Institute on the Environment, University of Minnesota

B.S., Chemical Engineering, 1992, The University of Virginia, Charlottesville, VA.
M.S., Environmental Engineering, 1994, The University of Iowa, Iowa City, IA.
Ph.D., Environmental Engineering, 1997, The University of Iowa, Iowa City, IA.

Dr. Paige Novak will be responsible for overall project coordination. She has been studying the fate and biological transformation of micropollutants for over ten years. Recent work has focused on the presence and fate of estrogenic compounds in wastewater, including wastewater flows from industrial facilities. Phytoestrogens have received little attention, yet Dr. Novak has found that these compounds are present in high concentrations in industrial effluents and is currently studying their fate under a variety of conditions. Dr. Novak was the 2007 recipient of the Paul L. Busch Award (Water Environment Research Foundation) for her research on industrial phytoestrogens. She, Dr. Michael Semmens, and Dr. Deborah Swackhamer recently completed an LCCMR-funded project on the presence and fate of estrogenic compounds across two Minnesota wastewater treatment plants. Two manuscripts will be submitted for publication from this work.

Dr. William Arnold (University of Minnesota) is an expert on the chemical transformation of organic chemicals in aquatic systems. For the past seven years he has focused on the photolysis of a wide range of pharmaceuticals. His current efforts are focused on tracking wastewater-derived compounds and their reaction products in the environment.

Dr. Heiko Schoenfuss (St. Cloud State University) has been studying the biological effects of EEs for the past 10 years. His laboratory has pioneered exposure systems at environmentally relevant concentrations in the ng/L range and has integrated field and laboratory studies over multiple levels of organsimal complexity.

Organization Description
The University of Minnesota is one of the largest, most comprehensive, and most prestigious public universities in the United States (http://www1.umn.edu/twincities/01_about.php). The laboratories and offices of the PI and co-PIs contain all of the necessary fixed and moveable equipment and facilities needed for the proposed studies.