

2010 Environment and Natural Resources Trust Fund (ENRTF) Work Program

Date of Report: November 24, 2009
Date of Next Progress Report: January 31, 2011
Date of Work Program Approval:
Project Completion Date: June 30, 2013

I. PROJECT TITLE: Fate and ecological impacts of phytoestrogens

Project Manager: Paige J. Novak, Ph.D., P.E.
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Location: Minneapolis, Minnesota 55455 or St. Cloud, Minnesota 56301;
Additional work (sampling) will take place in Mankato, Minnesota
and Brewster, Minnesota. See attached map.

Total ENRTF Project Budget:	ENRTF Appropriation \$	340,000
	Minus Amount Spent: \$	0
	Equal Balance: \$	340,000

Legal Citation: ML 2010, Chap.[____], Sec.[____], Subd._____.

Appropriation Language:

II. PROJECT SUMMARY AND RESULTS:

Phytoestrogens are plant-based compounds that mimic estrogen and can therefore interfere with normal biological development. Research shows that phytoestrogens are discharged into surface water from wastewater treatment plants and certain industrial facilities. The biological effects of these compounds have not been well-studied, although it is known that they can feminize male fish. In addition, almost nothing is known about their environmental fate. When these compounds enter rivers and streams, it is likely that they will be degraded and may have a lessened impact on biota as a result, but this needs to be confirmed. We plan to determine the persistence of phytoestrogens by studying the reactions that transform these compounds in the environment (reactions with sunlight, naturally-occurring bacteria, and sediment) to predict their fate in natural waters. We will also perform exposure experiments at

realistic environmental concentrations to determine the impact of phytoestrogens on fathead minnows, an important component of the Minnesota aquatic food chain. This research will enable us to predict the concentrations of phytoestrogens in natural waters and their expected ecological impact. With this information, efforts can be directed to enhance wastewater treatment only at those industries (or during those seasons) where environmental degradation is not adequate to protect aquatic species. This will facilitate continued industrial development and production in Minnesota in an environmentally sensitive manner.

III. PROGRESS SUMMARY AS OF

IV. OUTLINE OF PROJECT RESULTS:

RESULT 1: *Determine the chemical and biological fate of phytoestrogens in surface waters*

Description:

We know that phytoestrogens are discharged to surface water from municipal wastewater treatment plants and from industrial facilities (including some dairies, meat processors, peanut processors, and soy processors). Nevertheless, no research has been conducted on their biological or chemical fate in the environment. It is likely that these compounds will adhere 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 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, and compared to the concentrations measured in the field.

Summary Budget Information for Result 1: ENRTF Budget: \$ 191,000
Amount Spent: \$ 0
Balance: \$ 191,000

Deliverable/Outcome	Completion Date	Budget
1. Determine the biological transformation kinetics for genistein and daidzein	6/30/12	56,000
2. Determine kinetics of photolysis for genistein and daidzein	6/30/12	56,000
3. Determine estrogenicity of transformation products of genistein and daidzein	6/30/12	21,000
4. Measure the effluent concentrations and downgradient concentrations of genistein and daidzein in the field	9/30/12	20,000
5. Build and verify a model to determine the importance of various natural processes on phytoestrogen fate in the environment	4/30/13	38,000

Result Completion Date: April 30, 2013

Result Status as of January 2011:

Result Status as of July 2011:

Result Status as of January 2012:

Result Status as of July 2012

Result Status as of January 2013

Final Report Summary:

RESULT 2: *Determine the impact of the phytoestrogens on fathead minnows*

Description:

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 (singularly 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.

Summary Budget Information for Result 1: ENRTF Budget: \$ 149,000
Amount Spent: \$ 0
Balance: \$ 149,000

Deliverable/Outcome	Completion Date	Budget
1. Determine the effects of phytoestrogens on embryonic and larval fathead minnow behavior	4/30/11	49,000
2. Determine the effects of phytoestrogens on the reproductive behavior of mature fathead minnows	4/30/12	50,000
3. Determine the effects of phytoestrogens on the physiology of mature fathead minnows (feminization, liver, and reproductive organ changes)	4/30/13	50,000

Result Completion Date: April 30, 2013

Result Status as of January 2011:

Result Status as of July 2011:

Result Status as of January 2012:

Result Status as of July 2012

Result Status as of January 2013

Final Report Summary:

V. TOTAL ENRTF PROJECT BUDGET:

Personnel: \$ 242,000
(William Arnold and Paige Novak, paid for 4% effort; 2 graduate students for 2.25 years (averaging 37.5% effort over 3 years))

Contracts:	\$ 58,000
<i>(Some of the work will be conducted at St. Cloud State University (Result 2). The subcontract amount will include Co-PI salary (Heiko Schoenfuss, paid for 2.5% effort (less paid effort because of summer teaching commitments)), 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.)</i>	
Equipment/Tools/Supplies:	\$ 39,000
<i>(Laboratory supplies and analytical costs)</i>	
Acquisition, including easements:	\$ 0
Travel:	\$ 1,000
<i>(Travel to sites for sampling)</i>	
Additional Budget Items:	\$ 0
TOTAL ENRTF PROJECT BUDGET:	\$ 340,000

Explanation of Capital Expenditures Greater Than \$3,500: *None*

VI. PROJECT STRATEGY:

A. Project 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 endocrine disrupting compounds on aquatic biota, will direct the biological impact research. Dr. William Arnold (UMN), an expert in photolysis of endocrine disrupting compounds 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. Project Impact and 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.

C. Other Funds Proposed to be Spent during the Project Period: The three PIs will each devote effort to the project that will be unpaid, 2% for both Novak and Arnold and 3.5% for Schoenfuss.

D. Spending History: None.

VII. DISSEMINATION:

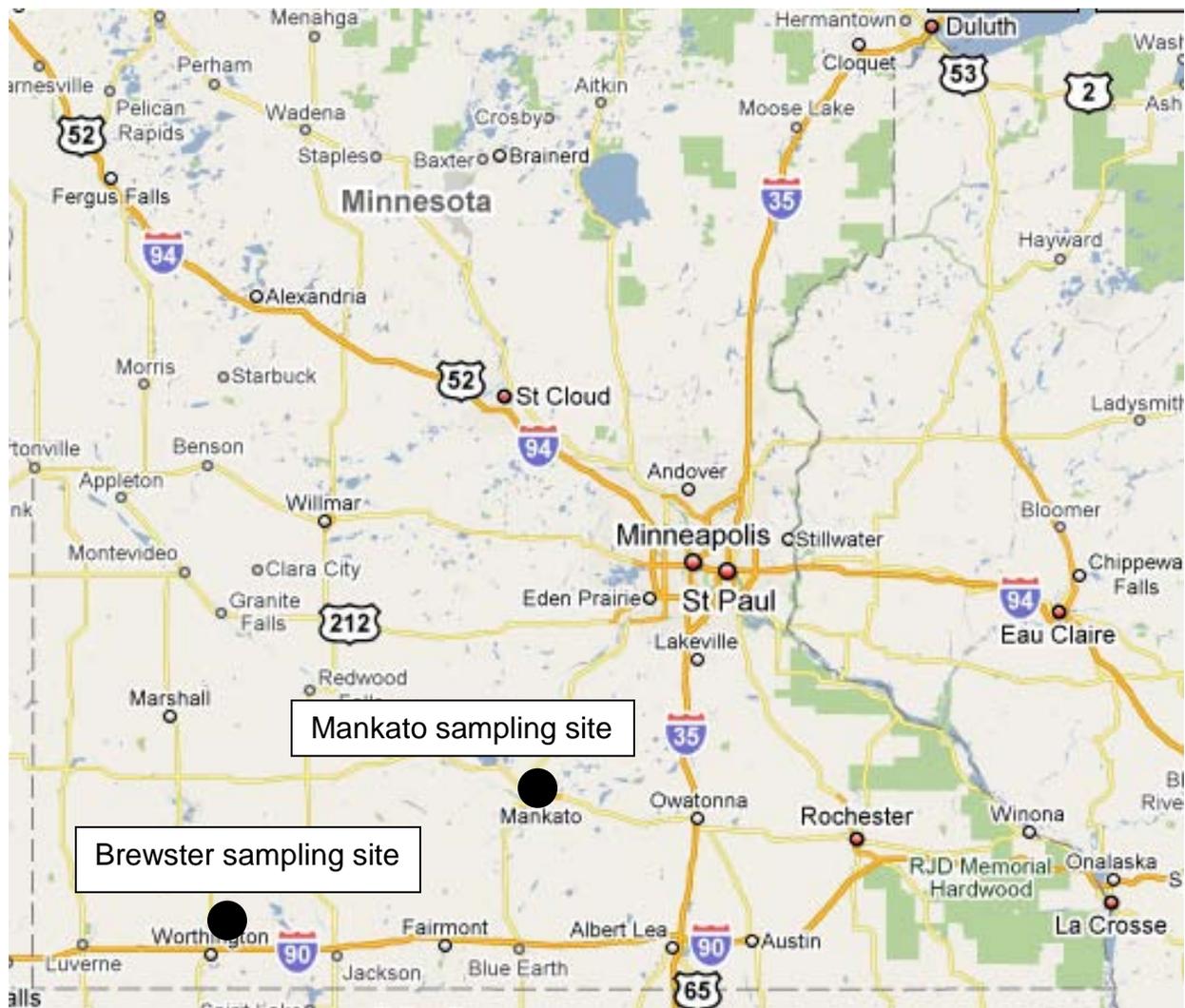
The target audience for results from this research will be professionals in the area of wastewater treatment, watershed management, and industry. Specific targets will be environmental engineers and scientists in academia, industry, state agencies such as the MDA and MPCA, and environmental consultants. Results will be disseminated through scholarly publications in peer-reviewed journals such as *Environmental Science and Technology*. Results from the research project will also be presented at regional conferences such as the *Minnesota Water* conference.

VIII. REPORTING REQUIREMENTS:

Periodic Work Program progress reports will be submitted not later than January 2011, July 2011, January 2012, July 2012, and January 2013. A final Work Program report and associated products will be submitted between June 30 and August 1, 2013 as requested by the LCCMR.

IX. RESEARCH PROJECTS:

See Attachment B.



Attachment A: Budget Detail for 2010 Projects									
Project Title: Fate and ecological impacts of phytoestrogens									
Project Manager Name: Paige Novak									
Trust Fund Appropriation: \$ 340,000									
2010 Trust Fund Budget	<u>Result 1 Budget:</u>	Amount Spent <i>(date)</i>	Balance <i>(date)</i>	<u>Result 2 Budget:</u>	Amount Spent <i>(date)</i>	Balance <i>(date)</i>	TOTAL BUDGET	TOTAL BALANCE	
	Determine the chemical and biological fate of phytoestrogens in surface waters			Determine the impact of the phytoestrogens on fathead minnows					
BUDGET ITEM									
PERSONNEL: wages and benefits									
Paige Novak (4%)	30,000						30,000		
William Arnold (4%)	30,000						30,000		
Graduate Research Assistant (37.5%)	91,000						91,000		
Graduate Research Assistant (37.5%)				91,000			91,000		
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, paid for 2.5% effort), 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			58,000		
Other direct operating costs									
Supplies (Laboratory supplies including, but not limited to, gas supply for the LC/MS, glassware, syringes, chemical standards, membrane filters, clean-up columns, and disposables; Analytical costs)	39,000						39,000		
Travel expenses in Minnesota	1,000						1,000		
COLUMN TOTAL	\$191,000	\$0	\$191,000	\$149,000	\$0	\$149,000	\$340,000	\$0	