cee 9/29/11

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MTSU Clean Energy Initiative Project Funding Request

There are five (5) sections of the request to complete before submitting. See http://www.mtsu.edu/sga/cleanenergy.shtml for funding guidelines. Save completed form and email to cee@mtsu.edu or mail to MTSU Box 57.

1. General Information	
Name of Person Submitting Request **Dr. Mary Farone**	
Department/Office **Biology**	Phone # (Office) **904-8341**
MTSU Box # **MTSU Box 0060**	Phone # (Cell)
E-mail **mfarone@mtsu.edu**	Submittal Date **September 30, 2011**

2. Project Categories (Select One)			
Select the category that best describes the project.			
x	Energy Conservation/Efficiency	x	Sustainable Design
	Alternative Fuels		Other
	Renewable Energy		

3. Project Information

a. Please provide a brief descriptive title for the project.
b. The project cost estimate is the expected cost of the project to be considered by the committee for approval, which may differ from the total project cost in the case of matching funding opportunities. **Any funding request is a 'not-to-exceed' amount. Any proposed expenditure above the requested amount will require a resubmission.**
c. List the source of project cost estimates.
d. Provide a brief explanation in response to question regarding previous funding.

3a. Project Title

Bioremediation of Barbiturates for Eco-Friendly Composting

3b. Project Cost Estimate

Bioremediation Project Total Request	$16846.00
Phenomenex C18 LC columns (2)	$1300.00
Strata X Solid Phase Extraction 100 ct 1 mL tubes	$ 600.00

Fisher Scientific HPLC Grade Acetonitrile (5 L)	$ 500.00
Fisher Scientific Certified ACS Methanol (2 L)	$ 190.00
Sodium acetate trihydrate	$ 70.00
Fisher HPLC grade acetic acid	$ 145.00
Millipore syringe microfilters 100 ct	$ 215.00
Isco Fraction Collector	$5500.00
Bacterial sequencing primers	$ 150.00
Bacterial sequencing reagent	$ 100.00
Disposable micropipette tips	$ 144.00
Set of adjustable volume micropipettes	$1000.00
Protein purification reagents	$ 800.00
Bacterial growth reagents and supplies	$ 800.00
Sodium pentobarbital	$ 100.00
Deuterated pentobarbital as standard	$ 105.00
HPLC Vials	$ 325.00
Centrifugal Concentrators	$ 350.00
Millipore Scepter 2.0 Automated Cell Counter	$3,295.00
Kelway soil pH/moisture meter	$130.00
Costar* Sterile Disposable Reagent Reservoirs/600 ct	$455.00
Thermo Scientific* Labquake* Tube Shaker/Rotator	$572.00

3c. Source of Estimate

USDA; MTSU Department of Agribusiness and AgriScience; Dr. David Whitaker; Fisher Scientific Co.; GE Healthcare; research publications

3d. If previous funding from this source was awarded, explain how this request differs?

4. Project Description

(Completed in as much detail as possible.)

a. The scope of the work to be accomplished is a detailed description of project activities.
b. The benefit statement describes the advantages of the project as relates to the selected project category.
c. The location of the project includes the name of the building, department, and/or specific location of where the project will be conducted on campus.
d. List any departments you anticipate to be involved. Were any departments consulted in preparation of this request? Who? A listing may be attached to this form when submitted.
e. Provide specific information on anticipated student involvement or benefit.
f. Provide information for anticipated future operating and/or maintenance requirements occurring as a result of the proposed project.
g. Provide any additional comments or information that may be pertinent to approval of the project funding request.

4a. Scope: Work to be accomplished

This work has two goals: (1) the development of a method to determine the amount of pentobarbital in soil and (2) the isolation and characterization of a barbiturate-degrading microorganism suitable for bioremediation of large-animal disposal sites.

In the development of a method to determine the levels of pentobarbital in soil, the first step will be the development of a liquid chromatograph/mass spectrometry (LC/MS) method to identify the presence pentobarbital in a sample. The pentobarbital will be identified based on its retention time and unique ion signature in the LC/MS. Next the detection limits of the instrument will be determined by injecting a series of samples of known concentration. Following the LC/MS work, a method to extract pentobarbital from soil will be explored and validated. Preliminary works indicates the pentobarbital can be extracted from the soil with methanol and the resulting solution concentrated by solid-phase extraction. The resulting sample is then quantitated by LC/MS. Once these methods have been validated, soil samples from large animal burial salts will be assayed for the presence of pentobarbital.

Concurrent with the development of a suitable assay for sodium pentobarbital in the soil, a microorganism capable of degrading this drug will be isolated. Barbiturate-degrading microorganisms will be isolated by diluting 1 g of soil from various sites, including composting sites, into 99 mL of sterile water. Aliquots of the soil solution will be plated onto minimal agar containing 4 mM sodium pentobarbital. The plates will be incubated at temperatures ranging from 25-45°C. To assess potential degradation of sodium pentobarbital, bacterial cells from the plates will be grown in minimal broth containing sodium

pentobarbital as the only nutrient source. The degradation of sodium pentobarbital in the supernatants will be measured using an enzyme-linked immunosorbent assay (ELISA). Supernatants from microorganisms whose growth resulted in a decrease of sodium pentobarbital as measured by ELISA will be tested further for metabolites of sodium pentobarbital by gas chromatography/mass spectrometry. The identities of the barbiturate-degrading microorganisms will be determined by DNA sequence analysis. Preliminary work has identified at least one candidate microorganism which will undergo further testing.

4b. Scope: Benefit Statement

The ownership of horses, ponies, donkeys, or mules includes making inevitable decisions about end-of-life events and disposal of deceased animals. Proper disposal of these animals is required of all owners including the Horse Science Center at MTSU whether the cause of death was natural or humane euthanasia. Under the Tennessee Department of Environment and Conservation rules and regulations, carcasses should be disposed of within 48 hours of death. In Tennessee owners have had several disposal options including rendering, burial, composting, incineration, or landfills. Many of these options are no longer available. Because of new federal regulations, many rendering companies will no longer accept large animal carcasses, including horses. Many landfills do not accept large animal carcasses due to county laws or due to the regulations of the landfill operator. If the landfill does accept large animal carcasses, it charges a very large fee. Therefore as a practical matter most large carcasses must be disposed of by burial/composting or incineration. Incineration is a very energy-intensive process requiring large amounts of fossil fuels such as propane and the renting of large pieces of equipment. Burial/composting is a more practical, energy-efficient process. However since most horses are euthanized with large amounts of pentobarbital the possibility exists that the pentobarbital could leach into the soil and eventually make its way into water sources resulting in a potential environmental hazard.

The goals of this study are to determine the amount of sodium pentobarbital that could leach into the surrounding soil from a buried horse carcass and to use a barbiturate-degrading microorganism to reduce the amounts of barbiturate in the soil to environmentally safe levels. This would allow the on-farm composting and burial of large animal carcasses both environmentally sustainable and energy-efficient.

4. Project Description (continued)

4c. Location of Project (Building, etc.)

Davis Science Building Room 238

Davis Science Building Room 201 (Cold room for protein purification)

Davis Science Building Room 102

MTSU Horse Science Center

4d. Participants and Roles

Dr. Mary Farone MTSU Biology– Project Director and Isolation of Barbiturate-Degrading Microorganisms

Dr. Tony Farone MTSU Biology – Role of bacteria in bioremediation

Dr. Paul Kline MTSU Chemistry – Development of soil screening methods for presences of barbiturates and isolation and characterization of barbiturate degrading enzymes

4e. Student participation and/or student benefit

Matthew Rodgers – MS student – MTSU Department of Biology

Chasity Suttle – MS student – MTSU Department of Chemistry

Matthew, under the supervision of Drs. Mary and Tony Farone, would carry out the experiments necessary to isolate the bacteria involved in the bioremediation of barbitals. He would also be involved in the work necessary to identify these organisms and their characterization under different environmental conditions.

Chasity, under the supervision of Dr. Kline, would develop the techniques necessary to measure the amount of barbital present in soil samples. She will also be involved in the purification and characterization of the enzyme(s) responsible for the breakdown of barbital by the organisms identified by Matthew Rodgers.

The students will gain knowledge skills, and experience in scientific research. Included in the techniques the students will master in this work are the operation and data analysis of mass spectral data, enzyme-linked immunosorbent assay (ELISA), protein purification, and DNA sequencing.

The MTSU campus and the community at large will benefit by the reduced energy expenses incurred in the disposal of large animals, by the reduced contamination of soils and surrounding watersheds, and the development of a sustainable method for the disposal of large animal carcasses.

Further a part of this project will be the development of a high school biology laboratory experiment to illustrate the process of bioremediation. The laboratory will be demonstrated by MTSU Biology and Chemistry graduate students at Rutherford County high schools. This aspect of the project will impact large number of both MTSU and high school students.

4f. Future Operating and/or Maintenance Requirements

4g. Additional Comments or Information Pertinent to the Proposed Project

5. Project Performance Information

Provide information if applicable.

a. Provide information on estimated annual energy savings stated in units such as kW, kWh, Btu, gallons, etc.
b. Provide information on estimated annual energy cost savings in monetary terms.
c. Provide information on any annual operating or other cost savings in monetary terms. Be specific.
d. Provide information about any matching or supplementary funding opportunities that are available. Identify all sources and explain.

5a. Estimated Annual Energy Savings (Estimated in kW, kWh, Btu, etc.)

Incineration of one horse carcass requires 2 million BTU's of fossil fuel, mainly propane. In addition each carcass requires the use of heavy equipment for transportation to the cremation site.

Burial of a horse carcass requires 30 min of diesel tractor time which consumes approximately 0.25 gallon of diesel fuel. This translates to approximately 60,000 BTUs. (http://www.provehicles.co.uk/john-

deere-310g-backhoe-loader).

Therefore burial of each horse carcass would conservatively 1,940,000 BTU's over incineration. Each year MTSU euthanizes 5 horses, while in the United States approximately 90,000 horses are euthanized. (http://www.avma.org/advocacy/federal/legislative/110th/issue_briefs/ahspa.asp) Therefore MTSU would save approximately 9.7 million BTUs, while the United States would save 174.6 billion BTUs.

5b. Annual Energy COST Savings ($)

Burial of the 5 horses MTSU euthanizes each year would cost approximately $10.00, the cost of 2-3 gallons of diesel fuel. The cost of incineration of a single horse is estimated to be between $600-$1000 depending on the current price of propane. (http://www.extension.org/pages/20164/horse-disposal-options) Therefore the cost to MTSU of disposing of 5 horse per year would be between $3000-$5000. This results in an annual savings to MTSU of between $2990-$4990.

The annual cost to the United States of composting 90,000 horses per year versus incineration would be $1,400,000 versus $54,000,000 at the low end and $90,000,000 at the upper end.

5c. Annual Operating or Other Cost Savings. Specify. ($)

5d.Matching or Supplementary Funding (Identify and Explain)

Dean Allen-Vice Provost for Research and $8000.00

Dean of College of Graduate Studies

Funds to collect preliminary data on the feasibility

of the project

Subject: FW: Clean Energy Fund Proposal
From: Danny Kelley <Danny.Kelley@mtsu.edu>
Date: Thu, 29 Sep 2011 15:16:33 +0000
To: "Linda Hardymon (lhardymo@mtsu.edu)" <lhardymo@mtsu.edu>

Can you add this one to the others, please?

Thanks,

Dan

Danny R. Kelley, Ph.D
Assistant Vice President for Student Affairs
Center for Student Involvement and Leadership (CSIL)
Middle Tennessee State University
Keathley Univeristy Center 326S
P.O. Box 39
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Center for Student Involvement and Leadership (CSIL) Offices:
* Greek Affairs
* Intercultural and Diversity Affairs Center
* June Anderson Center for Women and Nontraditional Students
* Leadership and Service
* Student Government Association

-----Original Message-----
From: mfarone@mtsu.edu [mailto:mfarone@mtsu.edu]
Sent: Thursday, September 29, 2011 10:15 AM
To: Danny Kelley
Subject: Clean Energy Fund Proposal

Dear Dr. Kelley,

Attached is a Clean Energy Fund proposal. The proposal is a collaboration between myself, Dr. Tony Farone, and Dr. Paul Kline in Chemistry.

Thank you for considering the proposal and we look forward to your response,

Mary

Mary B. Farone, Ph.D.
Biology Department
Middle Tennessee State University
Murfreesboro, TN 37132
615-904-8341

Barbiturate_CleanEnergyProjectGrant_Farone.doc	**Content-Description:** Barbiturate_CleanEnergyProjectGrant_Farone.doc **Content-Type:** application/msword **Content-Encoding:** base64