July 1, 2005

Ms. Karlene Fine
Executive Director
North Dakota Industrial Commission
State Capitol
600 East Boulevard Avenue, Department 405
Bismarck, ND 58505-0840

Dear Ms. Fine:

Subject: EERC Proposal No. 2005-0324; "Investigating the Importance of the Mercury–Selenium Interaction"

Enclosed are the original and six copies of the subject proposal. The PDF of the file was e-mailed to you. Also enclosed is the $100 application fee.

If you have any questions or comments, please contact me by phone at (701) 777-5066 or by e-mail at nralston@undeerc.org.

Sincerely,

Nicholas V.C. Ralston
Project Manager

NVCR/sml

Enclosures

c/enc: Harvey Ness, Lignite Research Council

INVESTIGATING THE IMPORTANCE OF THE MERCURY–SELENIUM INTERACTION

EERC Proposal No. 2005-0324

Submitted to:

Ms. Karlene Fine

Executive Director
North Dakota Industrial Commission
State Capitol
600 East Boulevard Avenue, Department 405
Bismarck, ND 58505-0840

Amount of Request: $55,000

Submitted by:

Nicholas V.C. Ralston
Laura J. Raymond
Steven A. Benson

Energy & Environmental Research Center
University of North Dakota
PO Box 9018
Grand Forks, ND 58202-9018

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Nicholas V.C. Ralston, Project Manager

_____
Dr. Barry I. Milavetz, Interim Director
Research Development and Compliance

July 2005

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ABSTRACT

While mercury present in the environment or food sources may pose potential health risks, the protective effects of selenium is not adequately considered. Numerous studies report that vulnerability to mercury toxicity is inversely proportional to selenium status or level. However, selenium status has not been considered in the development of the reference dosage levels for mercury exposure. Experimental animals fed selenium-deficient diets are far more vulnerable to mercury exposure than animals fed adequate selenium, and animals fed selenium-rich diets are even more resistant to mercury exposure. Selenium's protective effects against mercury appear to be the result of the high binding affinities between mercury and selenium. In order to improve the understanding of the mercury issue, it is vital to study mercury's effects on selenium physiology.

This proposal describes a 2-year multiclient-funded research program that is aimed at showing that selenium status must be considered when evaluating the toxic effects of mercury. This project will determine the interactions between mercury and selenium in animal models as well as in mother and child pairs from the current Seychelles Study. The proposed studies will examine the effects of dietary intakes of methylmercury and the protective effects of dietary selenium in order to resolve important questions regarding the significance of mercury–selenium interactions in human health.

This series of complementary investigations will be performed in a research program costing a total of $385,000 ($55,000 from the North Dakota Industrial Commission; $200,000 in funds from cost-share partners; and $130,000 from the Energy & Environmental Research Center [EERC]–U.S. Department of Energy Jointly Sponsored Research Program) that will involve Dr. Nicholas V.C. Ralston, Dr. Laura J. Raymond, and Dr. Steven A. Benson of the EERC.

INVESTIGATING THE IMPORTANCE OF THE MERCURY–SELENIUM INTERACTION

PROJECT SUMMARY

The *primary objective* of our research is to clarify the biochemical mechanism for dietary selenium's protective effect against methylmercury (MeHg) toxicity. This is important since selenium status can vary and influence the sensitivity or protective robustness of a population to mercury exposure. Since the selenium status of most American citizens is quite good, they may be well protected against mercury exposure. This being the case, it is important to define the influence of selenium status on MeHg threshold levels (below which exposure is not considered harmful), so that decision makers are well informed when determining regulatory and legislative policy.

Selenium and mercury are known to influence one another's retention and distribution in tissues. However, experiments needed to characterize the nature of the interactions between dietary selenium and MeHg have not been done. The tasks detailed in this proposal describe experiments to determine the influences of mercury on selenium uptake, retention, distribution, and function.

The *specific objective* of this Energy & Environmental Research Center (EERC) proposal is to investigate and report selenium's influence on the effects of dietary MeHg exposure. This will be accomplished through neurofunctional studies in laboratory animals that will complement our collaboration with the Seychelles Child Development Study. Results from these tasks will be included in the expansion of our database and computational model of mercury–selenium interactions. Results from these studies will be presented in regional, national, and international meetings and published in peer-reviewed scientific journals.

The proposed research is *innovative* because we are examining the mechanism of mercury toxicity using the physiologically appropriate modes of mercury exposure while simultaneously investigating the natural physiological protective mechanisms of dietary selenium. Our *expectations* are that at the conclusion of this research, we will have confirmed that mercury doses that overwhelm the selenium-dependent physiology of vulnerable tissues are a required prerequisite of MeHg toxicity. We theorize that the molecular mechanism of MeHg involves depletion of selenium enzyme activity in the tissues of the endocrine and central nervous system. Therefore, unless chronically exposed to excessive amounts of MeHg, most populations will be protected by the selenium naturally present in the foods they eat, including ocean fish and seafood. The outcome of this research is expected to have a substantial *impact* on future assessments of risks associated with MeHg exposure. This information will be vital in identifying vulnerable or protected populations, will aid regulatory agencies when making their policy decisions, and will provide substantial nutritional information regarding consumption of fish and other selenium-rich foods.

PROJECT DESCRIPTION

Our *long-term goal* is to determine the biochemical mechanism and extent of selenium's involvement in MeHg toxicity. Our research tasks are designed to identify, quantify, evaluate, and report the effects of selenium in protecting against potential consequences of mercury exposure. As the expansion of a multiclient-funded research program, this project will explore the biochemical interactions between mercury and selenium through studies uniquely designed to reflect human patterns of MeHg exposure with various dietary levels of selenium. Since the major issue of public concern regarding mercury exposure is dietary MeHg present in fish consumed by humans, it is important to replicate this route of exposure in models that employ dietary selenium and mercury present at meaningful concentrations for determination of their interactions and effects. A

tremendous amount of work has been done with the intent of examining MeHg exposure and the protective influence of selenium. However, few studies have attempted to replicate the normal dietary exposure route of these elements or closely examine the effects of these elements on the distributions of one another in the tissues. Study 1 investigates the mercury–selenium interaction by examining the protective effects of selenium against mercury's toxic effects as determined by neurofunctional analyses. Study 2 extends this investigation by examining the potential therapeutic effects of selenium in reversing mercury's toxic effects. Study 3 involves compilation, evaluation, presentation, and publication of selenium–mercury interaction research in humans that the EERC is performing in collaboration with the Seychelles Child Development Study.

Study 1. Examining Selenium's Protective Effect Against MeHg Neurotoxicity

Long Evans rats will be fed diets that have been prepared as deficient, adequate, or enriched in selenium and supplemented either with or without toxic concentrations of MeHg. At regular intervals throughout the study, motor coordination capabilities of rats in these feeding groups will be tested using sensitive instrumental methods. The blood, brain, pituitary, and liver will be collected at termination and analyzed for mercury and selenium content as well as selenium-dependent enzyme activity. The observed effects of MeHg on neurofunctional performance and selenium's protective effects will be statistically compared and evaluated. Results of this study will be presented at regional, national, and international meetings and published in peer-reviewed scientific journals.

Study 2. Examining Selenium's Role in Seychellois Mothers and Children

In parallel with Study 1, we will examine a potential means of supporting recovery from MeHg toxicity in exposed rats. Sets of rats from MeHg-fed groups showing compromised motor coordination will be switched to mercury-free diets containing either low or high selenium. The motor function of these sets of rats will continue to be monitored and the restorative effects of

supplemental dietary selenium will be assessed. The observed effects of selenium as a therapeutic treatment for alleviating signs and symptoms of MeHg toxicity will be statistically compared and evaluated. Results of this study will be presented at regional, national, and international meetings and published in peer-reviewed scientific journals.

Study 3. Computational Model of MeHg–Selenium Interaction Physiology

The EERC has performed the selenium analysis on blood samples from mothers and children from the current Seychelles Child Development Study headed by Dr. Thomas Clarkson. Significant findings of important distinctions in blood Hg and Se in these samples have been noted, and we have been asked to compile and evaluate the results. These results will be presented at regional, national, and international meetings and published in peer-reviewed scientific journals in collaboration with the other investigators of the Seychelles Child Development Study. Present plans are to prepare an initial publication that will report on the excellent selenium status in the Seychelles as the result of their seafood-rich diet and follow with further publications detailing important aspects of relationships between mercury and selenium in the blood samples.

STANDARD OF SUCCESS

The criteria for defining success in this research program will be the extent to which these tasks provide new information regarding the effect of mercury on selenium-dependent physiological processes and the effect of dietary selenium on mercury distribution and disposition in the body. The success of Study 1 will be determined by identifying the protective effects of selenium against the neurofunctional consequences of mercury exposure. Study 2 will be successful through determining the potential for selenium therapy as a treatment to remedy mercury toxicity. Study 3 will accomplish its goal through compilation and evaluation of new

information regarding Se and Hg distributions in blood samples of mothers and children of the Seychelles and presentation and publication of these highly interesting results.

The EERC is committed to delivering consistent, high-quality research results through performance of this project that will advance the scientific understanding of the relationship between mercury–selenium interactions and human health. Procedures and instrument calibrations in the analytical research laboratories follow nationally recognized or approved standards and methods. These laboratories have quality assurance and quality control protocols in place to ensure the assays applied in this project are properly implemented and high-quality data are obtained.

BACKGROUND

Recent research suggests selenium protects against potential negative consequences from mercury exposure (1–3). Since it was first described in 1967, selenium's protective effect against mercury toxicity has been demonstrated in all species investigated. In spite of these numerous studies, selenium's protective effect against mercury toxicity remains poorly understood. Because of selenium's extremely high affinity for mercury (4), it is thought that selenium sequesters mercury, preventing it from causing harm. However, selenium is essential to support normal enzyme activities (5). These enzymes are present in every cell of every creature. However, when excessive amounts of mercury are present, the mercury binds the selenium, making it biologically unavailable (6). Recent evidence indicates selenium prevents mercury toxicity even when mercury is many times more abundant than selenium. Thus selenium does not appear to act as a "tonic" that binds up mercury. Instead, selenium appears to be the "target" of mercury toxicity.

We hypothesize that selenium's protective effect against mercury toxicity is the result of supplying enough selenium to overcome the loss bound to mercury. If this is the case, ensuring adequate levels of dietary selenium may reduce the risks associated with mercury exposure. The health risks of MeHg exposure may then vary in response to individual and regional differences in selenium intake.

Quantitative determination of the risks of mercury toxicity requires more insight than can be gained by simply measuring mercury exposure. Concurrent assessment of selenium status and availability in the food must also be performed. Mercury-induced neurotoxicity appears to be the result of the negative balance of selenium relative to the amount of mercury acting in this biological equation.

Therefore, the apparent contrast in observations from the Faroes and Seychelles studies of the effects of methylmercury exposure may reflect complementary perspectives of the importance of selenium's protective effect against mercury toxicity. While the Faroe Island researchers reported neurological defects in children exposed to mercury in the womb (7), the Seychelles mercury study found no adverse health effects even at levels of exposure 10–20 times higher than is typical in the United States (8). The discrepancies between their conclusions appear likely to be due to differences in abundance of dietary selenium and mercury in the foods consumed by the study populations.

All fish contain some mercury, and predatory fish accumulate increasing amounts of mercury as they grow larger and older. However, mercury exposure can come from sources other than fish. In the Faroes, 90% of mercury exposure comes from eating whale meat, which contains 50 times as much mercury as the fish they eat (9). Whale meat is unusual since it is known to often contain more mercury than selenium (10). Meanwhile, eating ocean fish would

not cause decrements in selenium status since their mercury is present in much smaller quantities that are counteracted by the abundant amounts of selenium present. What had appeared to be conflicting results between the Faroes and the Seychelles studies may actually reflect complementary perspectives of the selenium-dependent protective effect.

This EERC proposal details a multiclient study of mercury's effect on selenium-dependent neurophysiology. Enhanced awareness of the importance of selenium physiology in relation to mercury exposure will provide policy makers with a more complete understanding of mercury exposure and enable them to make better-informed decisions. Understanding selenium's role in mercury toxicity will help resolve the differences in observations reported from the Seychelles' and Faroe Island studies.

QUALIFICATIONS

Dr. Nicholas V.C. Ralston worked in developing novel approaches to evaluating trace element physiology for over a decade at the Grand Forks Human Nutrition Research Center (GFHNRC) before returning for further training at Mayo Medical Center. After obtaining a Ph.D. in biomedical research biochemistry from Mayo Graduate School, he continued his studies of the molecular basis of disease at Bowman Gray Medical School at Wake Forest University. Dr. Ralston returned to trace element research at GFHNRC in 1998, where he investigated the biochemical basis of boron and selenium physiology. Since joining the EERC in 2002, Dr. Ralston has been involved in evaluating potential human health effects and risks resulting from environmental exposure to toxic metals including mercury. He has four U.S. Environmental Protection Agency (EPA)-sponsored projects currently under way as part of the Center for Air Toxic Metals® (CATM®) that are investigating basic aspects of the mercury–selenium

interaction, including collaborative work on assessing the selenium status of mercury-exposed mother–child pairs in the Seychelles Islands.

Dr. Laura J. Raymond is a Research Manager in charge of the Natural Materials Analytical Research Laboratory (NMARL) and the Analytical Research Laboratory (ARL) at the EERC of the University of North Dakota (UND). She received her Ph.D. in Biochemistry and Molecular Biology from UND in 2002 and her B.S. in Microbiology from the University of Arizona in 1993. Prior to her position at the EERC, she worked in Biochemistry and Molecular Biology Research at the GFHNRC.

In addition to managing the NMARL and ARL, Dr. Raymond's research primarily focuses on examining biochemical and analytical approaches to evaluating potential human health effects and risks resulting from environmental exposure to air, water, and food toxins. Principal areas of interest and expertise include evaluating the effects of mercury exposure on selenium-dependent physiology, analyzing the effects of environmental toxins and particulates at the biochemical and molecular levels, and the impacts of pollutants on health and physiological processes. Responsibilities also include developing strategies of research involving the pathophysiological consequences of arsenic, vanadium, nickel, and asthma-related particulate materials. This includes analyzing the effects of environmental toxins, such as mercury, at the molecular, cellular, tissue, animal, and human population levels. In addition to these physiological studies, research also involves trace metal interactions in environmental systems.

Dr. Steve Benson (Ph.D., Fuel Science, the Pennsylvania State University) will be the Senior Manager for the project, responsible for overseeing program progress and communication with stakeholders. Dr. Benson has been a researcher at the EERC for the past 20 years, holding the positions of Associate Director for Research, Senior Research Manager of the Fuels and

Materials Science Group, Research Supervisor, and Research Chemist for the DOE Grand Forks Energy Technology Center, and the Director for CATM®. Dr. Benson is a member of several professional organizations and has been the technical coordinator, chairman, or cochairman for several national and international conferences.

VALUE TO NORTH DAKOTA

North Dakota power utilities burn coal fuels to drive their electricity generator plants, resulting in the release of elemental mercury into the atmosphere. Regulatory mandates are in response to mounting public concern regarding perceived risks of mercury exposure from fish consumption. However, natural mechanisms of protection against mercury exposure such as normal dietary selenium are not being considered. Therefore, enhancing our understanding of the effects of mercury on selenium physiology and the influence of selenium status on mercury exposure are important issues to address. The results of these studies will improve the understanding of the relationship between selenium and mercury and provide important information to regulatory agencies useful in making policy decisions.

If normal dietary intakes of selenium are found to be sufficient to protect against potential negative consequences of moderate mercury exposure, fish advisories in North Dakota may eventually be relaxed, enhancing the draw and appeal of sport fishing in our lakes and reservoirs. Since North Dakota farmers produce grains and beef that have higher selenium contents than those of many other regions, local agriculture will benefit from enhanced consumer appreciation for the health benefits accompanying the selenium present in their food products.

The results of this research effort will be published in peer-reviewed journals focusing on environmental, health, and nutritional topics. In order to further disseminate the results of this research, we will present our findings at national and international meetings.

MANAGEMENT

Dr. Steven Benson will be the Senior Manager for the project, responsible for overseeing program progress and communication with stakeholders. Dr. Nicholas Ralston will be Project Manager, responsible for guiding the design and performance of the scientific protocols of the study. Dr. Laura Raymond will oversee the performance and execution of the project's animal care, sample preparation, and analysis concerns. Further technical personnel supporting the project will be drawn from existing EERC research staff. These staff members are highly trained and have had substantial experience with evaluation of trace metal analysis and physiology.

The Environmental Health Research Group's mission is to evaluate environmental toxicity hazards as well as prevention and remediation strategies. Facilities at the EERC are among the best in the world and include state-of-the-art instrumentation and equipment for performing analytical research studies. Aside from two specialized instruments, one for assessing motor coordination and a dual detector system for online analysis of mercury and selenium that will be funded through this proposal, all other equipment needed to complete this project is currently available. Additional information regarding the programs, personnel, instrumentation, and expertise at these facilities are available upon request.

TIMETABLE

Figure 1 reflects the project schedule and milestones for this project.

BUDGET AND MATCHING FUNDS

The total estimated cost of the project is $385,000 over a 20-month period. Of the total $385,000, we are requesting $200,000 from industrial sponsors and $55,000 from the North Dakota Industrial Commission to complete the project as proposed. We will request the

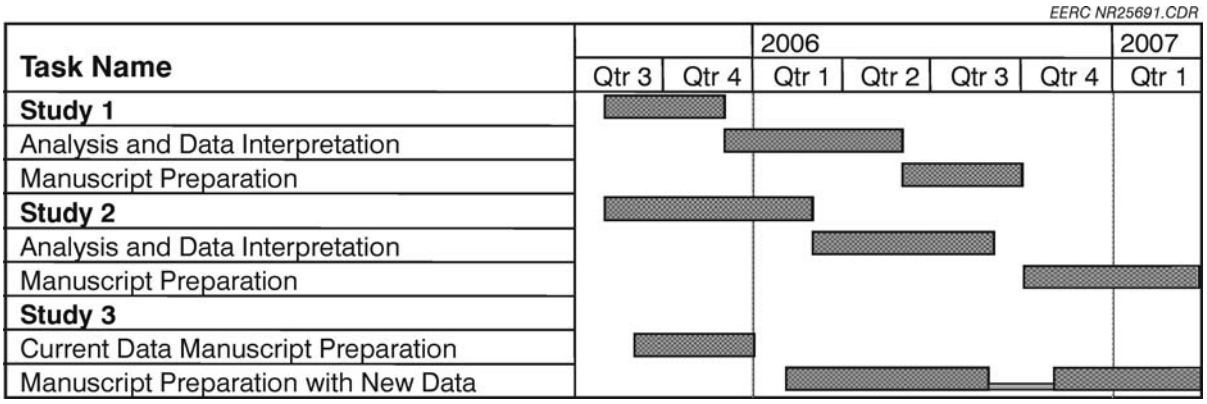

Figure 1. Project schedule.

project to be cofunded by the U.S. Department of Energy (DOE) through the EERC–DOE Jointly Sponsored Research Program (JSRP), which will provide approximately one-third of the project cost for a contribution of $130,000.

Once we have North Dakota Industrial Commission's firm commitment, we will submit the proposal to DOE, requesting approval of its share of the funding.

Three items are required from North Dakota Industrial Commission's for inclusion in our proposal to DOE:

- A formal commitment to the project. This can be a letter of commitment, a purchase order, or a signed contract.
- A biographical sketch or resume for North Dakota Industrial Commission 's project manager or key technical contributor.
- A short overview of SDSP.

The EERC will submit a proposal to DOE for its approval upon receipt of North Dakota Industrial Commission's commitment and the information above.

A detailed budget and accompanying budget notes are attached.

TAX LIABILITY

The EERC is part of the University of North Dakota, a tax exempt entity.

CONFIDENTIAL INFORMATION

No confidential information is expected to result from performance of this project.

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APPENDIX A

RESUMES OF KEY PERSONNEL

DR. NICHOLAS V.C. RALSTON
Biomedical Research Scientist
Energy & Environmental Research Center (EERC)
University of North Dakota (UND)
PO Box 9018, Grand Forks, ND 58202-9018 USA
Phone (701) 777-5066 Fax (701) 777-5181
E-Mail: nralston@undeerc.org

Principal Areas of Expertise

Selenium's role in strategies for prevention, protection, and remediation of mercury toxicity and biochemistry, physiology, and analytical approaches to quantitative assessment of mercury exposure.

Qualifications

Ph.D., Biomedical Research Biochemistry, Mayo Medical Center, 1995.
B.S., Biology Composite, Mayville State University, 1978.

Professional Experience: Research Background, Experience, and Training

2002 – present	Biomedical Research Scientist, Environmental Health Research Group, Energy & Environmental Research Center, Grand Forks, ND. Lead scientist in investigations of heavy metal toxicity. Current studies are focused on selenium's effects on mercury bioaccumulation in the environment and mercury's effects on selenium-dependent enzyme physiology.
1998 – 2002	GS 12 Research Biochemist, Grand Forks Human Nutrition Research Center, USDA, Grand Forks, ND. Investigated new aspects of selenium physiology in inflammation, novel low-molecular-weight Se molecular species.
1996 – 1998	Research Fellow, Bowman Gray Medical School, Wake Forest University, Winston-Salem, NC. Investigated stereospecific biosynthetic pathway for lysosomal Bis-(monoacyl glycerol) phosphate (BMP) in Alveolar macrophages.
1989 – 1995	Biomedical Predoctoral Research Fellow; Mayo Graduate School, Mayo Medical Center, Rochester, MN. Investigated pulmonary inflammation and molecular effects of etiologic agents in industrial dusts.
1986 – 1989	GS-9 Research Biologist, Grand Forks Human Nutrition Research Center, USDA, Grand Forks, ND. Methods Development Section Head, designed studies to evaluate metal-dependent enzyme activities.
1979 – 1986	Chemist II, Chemist I, Laboratory Technician III, University of North Dakota, Grand Forks, ND. Developed and tested assays for human and animal study protocols.

Representative Publications (not including abstracts or technical reports)

Ralston, N.V.C. and Finley, J.W. (2005) Acute and Chronic Inflammation in Rats is Influenced by Source and Amount of Dietary Selenium. Biofactors. (Submitted for publication)

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Recent Presentations and Invited Talks

Selenium's interactions with mercury.
May 9, 2005. Seychelles child development study group.

Selenium's role in the mercury issue.
April 19, 2005. National Fisheries Institute annual meeting.

Mercury's effects on selenium metabolism.
April 12, 2005. Center for Food Safety and Applied Nutrition, US FDA.

Selenium modulation of toxicants in seafood.
April 11, 2005. Institute of Medicine: National Academy of Science.

Selenium as an indicator of susceptibility to mercury toxicity: The "tonic to target" paradigm shift.
October 2004. 20th Annual International Conference on Soils Sediments and Water.

The physiological and environmental significance of mercury selenium interactions.
Sept 2003. Mercury Roundtable (nationwide Internet presentation).

Potential mechanisms of mercury toxicity: impact of mercury on selenium physiology.
August 2003. Seychelles child development study group.

Selenium dependent physiology, mercury-selenium interactions, and mercury pathophysiology.
July 2003. Patuxent Wildlife Refuge, Patuxent, MD.

Mercury and selenium: balancing the biochemical equation.
Sept 2002. Air Quality III, Arlington, VA.

DR. LAURA J. RAYMOND
Research Manager
Energy & Environmental Research Center (EERC)
University of North Dakota (UND)
PO Box 9018, Grand Forks, ND 58202-9018 USA
Phone (701) 777-5066 Fax (701) 777-5181
E-Mail: lraymond@undeerc.org

Principal Areas of Expertise

Dr. Raymond is a Research Manager and manages the Natural Materials Analytical Research Laboratory (NMARL) and the Analytical Research Laboratory (ARL) at the Energy & Environmental Research Center (EERC) of the University of North Dakota (UND). Dr. Raymond's research primarily focuses on examining biochemical and analytical approaches to evaluating potential human health effects and risks resulting from environmental exposure to air, water, and food toxins. Principal areas of interest and expertise include evaluating the effects of mercury exposure on selenium-dependent physiology, analyzing the effects of environmental toxins and particulates at the biochemical and molecular levels, and the impacts of pollutants on health and physiological processes. This includes analyzing the effects of environmental toxins, such as mercury, at the molecular, cellular, tissue, animal, and human population levels. In addition to these physiological studies, research also involves trace metal interactions in environmental systems.

Qualifications

Ph.D., Biochemistry and Molecular Biology, University of North Dakota, 2002.
B.S., Microbiology, University of Arizona, 1993.

Professional Experience

2004 – Research Manager, EERC, UND. Dr. Raymond's research is a continuation of her postdoctoral research, examining biochemical and analytical approaches involved in evaluating potential human health effects and risks resulting from environmental exposure to air, water, and food toxins. Current research examines the physiological roles of trace elements in human health, in particular, the role and mechanisms of mercury exposure and its involvement in selenium physiology. This research also includes studies of the mercury-selenium interactions in environmental systems throughout the food web. In addition to mercury and selenium, future areas of research will involve the pathophysiological consequences of particulate materials and pesticides. Dr. Raymond also manages the Natural Materials Analytical Research Laboratory and the Analytical Research Laboratory at the EERC.

2002 – 2004 Postdoctoral Research Associate, EERC, UND. Dr. Raymond's research examined biochemical and analytical approaches involved in evaluating potential human health effects and risks resulting from environmental exposure to air, water, and food toxins, in particular, the role and mechanisms of mercury exposure.

1996 – 2002 Predoctoral Research Fellow, Biochemistry and Molecular Biology Research, Human Nutrition Research Center, Agricultural Research Service, U.S. Department of Agriculture, Grand Forks, North Dakota. The location and function of the electron transport chain render the mitochondria the primary source for potentially damaging reactive oxygen species in aerobic cells. Normally, the concentration of superoxide anion in mitochondria (MnSOD) is controlled, at least in part, by the sequential action of superoxide dismutase and catalase. When the terminal steps of the electron transport are impaired or inhibited, superoxide anion production may rise to levels that overwhelm the normal protective enzymes. Studies have shown that copper deficiency causes a reduction in the activity of cytochrome-c oxidase, the copper-dependent, terminal respiratory complex of the electron transport chain, and also causes an increase in the transcriptional rate for MnSOD. It was hypothesized that under conditions of decreased copper, cytochrome-c oxidase function is impaired resulting in an increase in reactive oxygen species (ROS) production and the subsequent increase in MnSOD levels. Dr. Raymond's research was based on testing this hypothesis by analyzing molecular and biochemical properties of a copper-deficient vs. a copper-adequate cell line. In addition to this research, Dr. Raymond discovered an unusual characteristic of this cell line when certain antioxidants were added to the growing media. Under copper-deficient conditions, apoptosis was initiated. This was not seen in copper-adequate cells. These opposing effects of antioxidants have not been reported for the same cell system in current literature. Continued research involved assessing apoptotic characteristics in this cell line grown under the defined conditions. The overall results of the research suggest that copper deficiency reduces cytochrome-c oxidase function which, in turn, causes an increase in ROS production. The ROS affect the cell system through cell-signaling mechanisms and/or through oxidative damage. Presumably, the redox status of the cell is altered, rendering these cells more sensitive to apoptotic stimuli. This is the first time results such as these have been reported and may lead to novel areas of research in free radical signaling, antioxidant mechanisms, and understanding apoptosis.

1994 – 1995 Graduate studies, North Dakota State University, Fargo, ND. Including research-related courses such as biochemistry, genetics, and nutrition, leading to pursuit of an advanced degree in biochemistry/molecular biology with a major focus on nutritional metabolism.

1981 – 1992 Medic, United States Air Force (USAF) and USAF Reserve. From 1981 to1985, Dr. Raymond was an active-duty Air Force medic and served on an infection control committee. She remained on active reserve status as an Independent Mobilization Augmentee until July of 1990 at which time she was reactivated for 18 months because of the Persian Gulf War. During her entire Air Force service, she was trained and served throughout all areas of a hospital, but the majority of her time was spent as an emergency room medic. Training and experience were extensive, including in the following areas: emergency medicine, clinical, surgical, administration, medical laboratory, oncology, internal medicine,

intensive care, pediatrics, optometry, and obstetrics. As a medic, Dr. Raymond's responsibilities were all-inclusive and ranged from minor patient care to specialty tasks such as suturing, casting, minor surgery procedures, emergency cardiology procedures, drug administration, ambulance attendance, and postmortem care. She also gained field experience such as disaster preparedness, triage, and managing MASH units. Formal education was ongoing throughout years of service and included specific procedural education as well as basic knowledge courses, including earning an associates degree in biology through the USAF college.

Technical and Instrumental Experience

- Cell culture
- Column chromatography
- Spectrometry
- Fluorescence spectrometry
- Cytospin analysis
- Chemical synthesis
- Western analysis
- Northern analysis
- Southern analysis
- Gel electrophoresis
- Flow cytometry
- Enzyme assays
- Cell separation and purification
- Cellular organelle isolations and purification
- Animal dissection
- Phosphoimaging detection
- RNA/DNA Isolation
- Mini-preps
- Primer design
- PCR
- rtPCR
- Competitive PCR
- Internal standard synthesis
- Transformation of bacteria

Publications and Presentations

- Raymond, L.J.; Ralston, N.V.C. Mercury Selenium Interaction and Health Implications. Special Issue of *Seychelles Medical and Dental Journal* **2004**, *7* (1), 72–75.

- Ralston, N.V.C.; Jordan, C.R.; Raymond L.J. Selenium as a Bioindicator of Susceptibility to Mercury Toxicity: The "Tonic to Target" Paradigm Shift. *Soils Sediments and Water: An International Journal.* In Submission.

- Raymond L.J.; Johnson W.T. Copper-Deficiency Increases the Susceptibility of HL-60 Cells to Ascorbate and α-Tocopherol Induced Apoptosis. *Experimental Biology and Medicine* **2004**, 229: 885–894.

- Raymond, L.J.; Ralston, N.V.C. Environmental and Biological Significance of the Mercury–Selenium Interaction. In *Proceedings of the Air Quality IV: Mercury, Trace Elements, and Particulate Matter Conference*; Arlington, VA, Sept 22–24, 2003.

- Raymond, L. Copper Deficiency in HL-60 Cells Increases Their Susceptibility to Apoptosis When Treated with Antioxidants. Doctoral Dissertation, Department of Biochemistry and Molecular Biology, University of North Dakota, 2002.

- Sumner (Raymond), L.J.; Johnson W.T. Copper Deficiency in HL-60 Cells Increases Their Susceptibility to Apoptosis When Treated with Antioxidants. *FASEB J.* **2001**, *15*, A271.

DR. STEVEN A. BENSON
Senior Research Manager/Advisor
Energy & Environmental Research Center (EERC)
University of North Dakota (UND)
PO Box 9018, Grand Forks, ND 58202-9018 USA
Phone (701) 777-5000 Fax (701) 777-5181
E-Mail: sbenson@undeerc.org

Principal Areas of Expertise

Development and management of complex multidisciplinary programs focused on solving environmental and energy problems, including 1) technologies to improve the performance of combustion/gasification and associated air pollution control systems; 2) transformations and control of air toxic substances in combustion and gasification systems; 3) advanced analytical techniques to measure the chemical and physical transformations of inorganic species in gases; 4) computer-based models to predict the emissions and fate of pollutants from combustion and gasification systems; 5) advanced materials for power systems; 6) impacts of power system emissions on the environment; 7) national and international conferences and training programs; and 8) state and national environmental policy.

Qualifications

Ph.D., Fuel Science, Materials Science and Engineering, The Pennsylvania State University, 1987.

B.S., Chemistry, Moorhead State University (Minnesota), 1977.

Professional Experience

1999 – Senior Research Manager/Advisor, EERC, UND. Dr. Benson is responsible for leading a group of about 30 highly specialized scientists and engineers whose aim is to develop and conduct projects and programs on power plant performance, environmental control systems, the fate of pollutants, computer modeling, and health issues for clients worldwide. Efforts have focused on the development of multiclient jointly sponsored centers or consortia that are funded by a combination of government and industry sources. Current research activities include computer modeling of combustion and environmental control systems, performance of selective catalytic reduction technologies for NO_x control, carbon-based NO_x reduction technologies, mercury control technologies, particulate matter analysis and source apportionment, the fate of mercury in the environment, toxicology of particulate matter, and in vivo studies of mercury-selenium interactions. The computer-based modeling efforts utilize various kinetic, thermodynamic, artificial neural network, statistical, computation fluid dynamics, and atmospheric dispersion models. These models are used in combination with models developed at the EERC to predict the impacts of fuel properties and system operating conditions on system efficiency and emissions. Dr. Benson is Program Area Manager for Modeling and Database Development for the U.S. Environmental Protection Agency (EPA) Center for Air Toxic Metals® (CATM®) at the EERC. He is responsible for identifying research opportunities and preparing proposals and reports for clients. 1994 – 1999 Associate Director for

Research, EERC, UND. Dr. Benson was responsible for the direction and management of programs related to integrated energy and environmental systems development. Dr. Benson led a team of over 45 scientists, engineers, and technicians. In addition, faculty members and graduate students from Chemical Engineering, Chemistry, Geology, and Atmospheric Sciences have been involved in conducting research projects. The research, development, and demonstration programs involve fuel quality effects on power system performance, advanced power systems development/demonstration, computational modeling, advanced materials for power systems, and analytical methods for the characterization of materials. Specific areas of focus included the development and direction of EPA CATM at the EERC (CATM, a peer-reviewed, EPA-designated Center of Excellence, is currently in its 12th year of operation and has received funding of over $12,000,000 from government and industry sources), ash behavior in combustion and gasification systems, hot-gas cleanup, and analytical methods of analysis. He was responsible for the identification of research opportunities and the preparation of proposals and reports for clients. Dr. Benson left this position to focus efforts on Microbeam Technologies' Small Business Innovation Research (SBIR).

1986 – 1994 Senior Research Manager, Fuels and Materials Science, EERC, UND. Dr. Benson was responsible for management and supervision of research on the behavior of inorganic constituents, including air toxic metals during combustion and gasification, hot-gas cleanup (particulate gas-phase species control), fundamental combustion, and analytical methods of inorganic analysis, including SEM and microprobe analysis, Auger, XPS, SIMS, XRD, and XRF. Responsible for identification of research opportunities, preparation of proposals and reports for clients, and publication.

1989 – 1991 Assistant Professor (part-time), Department of Geology and Geological Engineering, UND. Dr. Benson was responsible for teaching courses on coal geochemistry, coal ash behavior in combustion and gasification systems, and analytical methods of materials analysis. Taught courses on SEM/microprobe analysis and mineral transformations during coal combustion.

1984 – 1986 Graduate Research Assistant, Fuel Science Program, Department of Materials Science and Engineering, The Pennsylvania State University.

1983 – 1984 Research Supervisor, Distribution of Inorganics and Geochemistry, Coal Science Division, UND Energy Research Center. Dr. Benson was responsible for management and supervision of research on the distribution of major, minor, and trace inorganic constituents and geochemistry of coals and ash chemistry related to inorganic constituents and mineral interactions and transformations during coal combustion and environmental control systems.

1980 – 1983 Research Chemist, U.S. Department of Energy (DOE) Grand Forks Energy Technology Center. Dr. Benson performed research on surface and/or chemical analysis and characterization of coal-derived materials by SEM, XRF, and thermal analysis in support of projects involving SO_x, NO_x, and particulate control; ash deposition; heavy metals in combustion systems; coal gasification; and fluidized-bed combustion.

1979 – 1980 Research Chemist, DOE Grand Forks Energy Technology Center. Dr. Benson performed research on the application of such techniques as differential thermal analysis, differential scanning calorimetry, thermogravimetric analysis, and energy-dispersive XRF analysis with application to low-rank coals and coal process-related material. In addition, research was performed on the use of x-ray analysis to measure trace elements in fuels and conversion products.

1977 – 1979 Chemist, DOE Grand Forks Energy Technology Center. Dr. Benson performed analysis on coal and coal derivatives by techniques such as wavelength-dispersive x-ray analysis, argon plasma spectrometry, atomic absorption spectrometry, thermal analysis, and elemental analysis (CHN).

1976 – 1977 Teaching Assistant, Department of Chemistry, Moorhead State University.

Professional Memberships and Activities

United States Senate Committee on the Environment and Public Works

- One of three technical panelists invited to provide testimony on mercury control for the coal-fired power industry.
- American Chemical Society (ACS)
  - Chair – Fuel Division 2004 – Duties comprise coordinating all aspects of the division, including publications and national conferences.
  - Fuel Division – Participates on the Executive Committee involved in the coordination and direction of division activities, including outreach, programming, finances, and publications.
  - Councilor, Fuel Division – Represents the Fuel Division at the National ACS Council meeting.
  - Chair Elect, Fuel Division – August 2002 – Elected to be Chair of the Fuel Division.
  - Member, Committee on Environmental Improvement (CEI) – The committee provides advice and direction to the ACS governance on policies and programs related to the environment. Since becoming a member of the committee, we have developed policy statements on Global Climate Change, Reformulated Gasoline and MtBE, and Energy Policy. These policy statements are used to assist legislators in developing national environmental policy. Members of CEI also provide testimony on a variety of environmental issues.
- American Society for Mechanical Engineers (ASME)
  - Advisory Member, ASME Committee on Corrosion and Deposition Resulting from Impurities in Gas Streams. Developed several conferences through the International Engineering Foundation.

- ► Mercury Reduction Initiative – Minnesota Pollution Control Agency (MPCA)
  - Participated in meetings for the mercury reduction initiative and provided advice regarding mercury control technologies for electric utilities and MPCA for voluntary mercury reduction strategies.
- ► Elsevier Science, *Fuel Processing Technology*
  - Editorial board member whose role is to provide advice and direction for the journal.

Publications and Presentations

- Has authored/coauthored over 210 publications and is the editor of eight books and *Fuel Processing Technology* special issues.

APPENDIX B

DETAILED BUDGET AND BUDGET NOTES

INVESTIGATING THE IMPORTANCE OF THE MERCURY-SELENIUM INTERACTION
NDIC/INDUSTRY SPONSORS/DOE
PROPOSED START DATE: AUG 1, 2005
EERC PROPOSAL #2005-0324

SUMMARY BUDGET

CATEGORY		TOTAL HRS	TOTAL $COST	NDIC SHARE HRS	NDIC SHARE $COST	OTHER COST SHARE HRS	OTHER COST SHARE $COST	EERC JSRP SHARE HRS	EERC JSRP SHARE $COST
TOTAL DIRECT LABOR		3,473	$ 107,977	542	$ 17,730	2,371	$ 70,571	560	$ 19,676
FRINGE BENEFITS - % OF DIRECT LABOR	51%		$ 55,068		$ 9,042		$ 35,991		$ 10,035
TOTAL LABOR			$ 163,045		$ 26,772		$ 106,562		$ 29,711
OTHER DIRECT COSTS									
TRAVEL			$ 18,536		$ -		$ 18,536		$ -
COMMUNICATION - PHONES & POSTAGE			$ 364		$ 52		$ 237		$ 75
OFFICE (PROJECT SPECIFIC SUPPLIES)			$ 787		$ 432		$ 70		$ 285
SUPPLIES			$ 10,900		$ 8,000		$ 2,100		$ 800
EQUIPMENT > $5000			$ 83,930		$ -		$ -		$ 83,930
FEES			$ 1,020		$ -		$ 700		$ 320
TOTAL OTHER DIRECT COST			$ 115,537		$ 8,484		$ 21,643		$ 85,410
TOTAL DIRECT COST			$ 278,582		$ 35,256		$ 128,205		$ 115,121
FACILITIES & ADMIN. RATE - % OF MTDC		VAR	$ 106,418	56%	$ 19,744	56%	$ 71,795	47.7%	$ 14,879
TOTAL ESTIMATED COST			$ 385,000		$ 55,000		$ 200,000		$ 130,000

NOTE: Due to limitations within the University's accounting system, the system does not provide for accumulating and reporting expenses at the Detailed Budget level. The Summary Budget is presented for the purpose of how we propose, account, and report expenses. The Detailed Budget is presented to assist in the evaluation of the proposal.

INVESTIGATING THE IMPORTANCE OF THE MERCURY-SELENIUM INTERACTION
NDIC/INDUSTRY SPONSORS/DOE
PROPOSED START DATE: AUG 1, 2005
EERC PROPOSAL #2005-0324

DETAILED BUDGET

LABOR	LABOR CATEGORY	HOURLY RATE	TOTAL HRS	TOTAL $COST	NDIC SHARE HRS	NDIC SHARE $COST	OTHER COST SHARE PARTNERS HRS	OTHER COST SHARE PARTNERS $COST	EERC JSRP SHARE HRS	EERC JSRP SHARE $COST
RALSTON, N.	PROJECT MANAGER	$ 35.26	980	$ 34,555	240	$ 8,462	580	$ 20,451	160	$ 5,642
RAYMOND, L.	PRINCIPAL INVESTIGATOR	$ 29.62	990	$ 29,324	300	$ 8,886	660	$ 19,549	30	$ 889
BENSON, S.	PRINCIPAL INVESTIGATOR	$ 57.46	24	$ 1,379	-	$ -	24	$ 1,379	-	$ -
----------	SENIOR MANAGEMENT	$ 56.48	146	$ 8,246	-	$ -	-	$ -	146	$ 8,246
----------	RESEARCH SCIENTIST/ENGINEER	$ 30.99	468	$ 14,503	-	$ -	468	$ 14,503	-	$ -
----------	RESEARCH TECHNICIAN	$ 21.01	785	$ 16,493	-	$ -	609	$ 12,795	176	$ 3,698
----------	TECHNICAL SUPPORT SERVICES	$ 17.00	80	$ 1,360	2	$ 34	30	$ 510	48	$ 816
			3,473	$105,860	542	$ 17,382	2,371	$ 69,187	560	$ 19,291
ESCALATION ABOVE CURRENT BASE		2%		$ 2,117		$ 348		$ 1,384		$ 385
TOTAL DIRECT LABOR				$107,977		$ 17,730		$ 70,571		$ 19,676
FRINGE BENEFITS - % OF DIRECT LABOR		51%		$ 55,068		$ 9,042		$ 35,991		$ 10,035
TOTAL LABOR				$163,045		$ 26,772		$ 106,562		$ 29,711
OTHER DIRECT COSTS										
TRAVEL				$ 18,536		$ -		$ 18,536		$ -
COMMUNICATION - PHONES & POSTAGE				$ 364		$ 52		$ 237		$ 75
OFFICE (PROJECT SPECIFIC SUPPLIES)				$ 787		$ 432		$ 70		$ 285
SUPPLIES				$ 10,900		$ 8,000		$ 2,100		$ 800
EQUIPMENT > $5000				$ 83,930		$ -		$ -		$ 83,930
GRAPHICS SUPPORT				$ 1,020		$ -		$ 700		$ 320
TOTAL OTHER DIRECT COST				$115,537		$ 8,484		$ 21,643		$ 85,410
TOTAL DIRECT COST				$278,582		$ 35,256		$ 128,205		$ 115,121
FACILITIES & ADMIN. RATE - % OF MTDC			VAR	$106,418	56%	$ 19,744	56%	$ 71,795	47.7%	$ 14,879
TOTAL ESTIMATED COST				$385,000		$ 55,000		$ 200,000		$ 130,000

INVESTIGATING THE IMPORTANCE OF THE MERCURY-SELENIUM INTERACTION
EERC PROPOSAL #2005-0324

DETAILED BUDGET - FEES

			TOTAL
GRAPHICS SUPPORT	**RATE**	#	**$COST**
GRAPHICS (HOURLY)	$50	20	$ 1,000
SUBTOTAL			$ 1,000
ESCALATION		2%	$ 20
TOTAL GRAPHICS SUPPORT			$ 1,020

INVESTIGATING THE IMPORTANCE OF THE MERCURY-SELENIUM INTERACTION
EERC PROPOSAL #2005-0324

DETAILED BUDGET - TRAVEL

RATES USED TO CALCULATE ESTIMATED TRAVEL EXPENSES

DESTINATION	ECON AIRFARE	LODGING	PER DIEM	CAR RENTAL	REGIST.
Unspecified Destination (USA)	$ 900	$ 150	$ 51	$ 60	$ 525
Madison, WI	$ 700	$ 100	$ 43	$ 60	$ 525
Washington, DC	$ 900	$ 150	$ 51	$ 60	$ 525

PURPOSE/DESTINATION	NUMBER OF TRIPS	NUMBER OF PEOPLE	NUMBER OF DAYS	AIRFARE	LODGING	PER DIEM	CAR RENTAL	MISC.	REGIST.	TOTAL
Presentation/Unspecified Dest. (USA)	2	1	3	$ 1,800	$ 600	$ 306	$ 360	$ 120	$ 1,050	$ 4,236
Presentation/Madison, WI	1	2	5	$ 1,400	$ 800	$ 430	$ 300	$ 200	$ 1,050	$ 4,180
Washington, DC	2	2	5	$ 3,600	$ 2,400	$ 1,020	$ 600	$ 400	$ 2,100	$ 10,120
TOTAL ESTIMATED TRAVEL										$ 18,536

DETAILED BUDGET - EQUIPMENT

DESCRIPTION	$COST
Dual Millenium System	$ 76,900
Microprocessor Controlled Rota Rod	$ 7,030
TOTAL ESTIMATED EQUIPMENT	$ 83,930

BUDGET NOTES

ENERGY & ENVIRONMENTAL RESEARCH CENTER (EERC)

Background

The EERC is an independently organized multidisciplinary research center within the University of North Dakota (UND). The EERC receives no appropriated funding from the state of North Dakota and is funded through federal and nonfederal grants, contracts, or other agreements. Although the EERC is not affiliated with any one academic department, university academic faculty may participate in a project, depending on the scope of work and expertise required to perform the project.

The proposed work will be done on a cost-reimbursable basis. The distribution of costs between budget categories (labor, travel, supplies, equipment, subcontracts) is for planning purposes only. The principal investigator may, as dictated by the needs of the work, reallocate the budget among approved items or use the funds for other items directly related to the project, subject only to staying within the total dollars authorized for the overall program. Escalation of labor and EERC fee rates is incorporated in the budget when a project's duration extends beyond the current fiscal year. Escalation is calculated by prorating an average annual increase over the anticipated life of the project. The current escalation rate of 5% is based on historical averages. The budget prepared for this proposal is based on a specific start date; this start date is indicated at the top of the EERC budget or identified in the body of the proposal. Please be aware that any delay in the start of this project may result in an increase in the budget.

Salaries and Fringe Benefits

As an interdisciplinary, multiprogram, and multiproject research center, the EERC employs an administrative staff to provide required services for various direct and indirect support functions. Direct project salary estimates are based on the scope of work and prior experience on projects of similar scope. Technical and administrative salary charges are based on direct hourly effort on the project. The labor rate used for specifically identified personnel is the current hourly rate for that individual. The labor category rate is the current average rate of a personnel group with a similar job description. For faculty, if the effort occurs during the academic year and crosses departmental lines, the salary will be in addition to the normal base salary. University policy allows faculty who perform work in addition to their academic contract to receive no more than 20% over the base salary. Costs for general support services such as grants and contracts administration, accounting, personnel, and purchasing and receiving, as well as clerical support of these functions, are included in the EERC facilities and administrative cost rate.

Fringe benefits are estimated on the basis of historical data. The fringe benefits actually charged consist of two components. The first component covers average vacation, holiday, and sick leave (VSL) for the EERC. This component is approved by the UND cognizant audit agency and charged as a percentage of direct labor for permanent staff employees eligible for VSL benefits. The second component covers actual expenses for items such as health, life, and unemployment insurance; social security matching; worker's compensation; and UND retirement contributions.

Travel

Travel is estimated on the basis of UND travel policies which can be found at: http://www.und.edu/dept/accounts/employeetravel.html. Estimates include General Services Administration (GSA) daily meal rates. Travel includes scheduled meetings and conference participation as indicated in the scope of work.

Communications (phones and postage)

Monthly telephone services and fax telephone lines are generally included in the facilities and administrative cost. Direct project cost includes line charges at remote locations, long-distance telephone, including fax-related long-distance calls; postage for regular, air, and express mail; and other data or document transportation costs.

Office (project-specific supplies)

General purpose office supplies (pencils, pens, paper clips, staples, Post-it notes, etc.) are provided through a central storeroom at no cost to individual projects. Budgeted project office supplies include items specifically related to the project; this includes duplicating and printing.

Data Processing

Data processing includes items such as site licenses and computer software.

Supplies

Supplies in this category include scientific supply items such as chemicals, gases, glassware, and/or other project items such as nuts, bolts, and piping necessary for pilot plant operations. Other items also included are supplies such as computer disks, computer paper, memory chips, toner cartridges, maps, and other organizational materials required to complete the project.

Instructional/Research

This category includes subscriptions, books, and reference materials necessary to the project.

Fees

Laboratory, analytical, graphics, and shop/operation fees are established and approved at the beginning of the university's fiscal year.

Laboratory and analytical fees are charged on a per sample, hourly, or daily rate, depending on the analytical services performed. Additionally, laboratory analyses may be performed outside the University when necessary.

Graphics fees are based on an established per hour rate for overall graphics production such as report figures, posters for poster sessions, standard word or table slides, simple maps, schematic slides, desktop publishing, photographs, and printing or copying.

Shop and operation fees are for expenses directly associated with the operation of the pilot plant facility. These fees cover such items as training, safety (protective eye glasses, boots, gloves), and physicals for pilot plant and shop personnel.

General

Freight expenditures generally occur for outgoing items and field sample shipments.

Membership fees (if included) are for memberships in technical areas directly related to work on this project. Technical journals and newsletters received as a result of a membership are used throughout development and execution of the project as well as by the research team directly involved in project activity.

General expenditures for project meetings, workshops, and conferences where the primary purpose is dissemination of technical information may include costs of food (some of which may exceed the institutional limit), transportation, rental of facilities, and other items incidental to such meetings or conferences.

Facilities and Administrative Cost

The facilities and administrative rate (indirect cost rate) included in this proposal is the rate that became effective July 1, 2002. Facilities and administrative cost is calculated on modified total direct costs (MTDC). MTDC is defined as total direct costs less individual items of equipment in excess of $5000 and subcontracts/subgrants in excess of the first $25,000 for each award.

United States Tuna Foundation
ONE TUNA LANE • SAN DIEGO, CA 92101
(619) 233-6407 • Fax (619) 233-8336

Mr. Nicholas V.C. Ralston
Research Scientist
Energy and Environmental Research Center
PO Box 9018
Grand Forks ND

July 12, 2005

Dear Nick:

The U.S. Tuna Foundation has agreed to fund the sum of $150,000 in support of EERC Proposal No. 2006-0002, entitled Investigating the Importance of the Mercury-Selenium Interaction. We view the role of selenium as being a critical part of the ongoing scientific investigation into the effects of mercury exposure through fish and seafood consumption.
We will wait for your instructions regarding payment of the committed amount.

D. G. Ramey
Executive Director
U.S. Tuna Foundation